2014 - 2020 Interreg V-A

Italy - Croatia CBC Programme

Call for proposal 2019 Strategic

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

D3.3.1

DESIGN OF THE OPTIMAL OBSERVING SYSTEMS FOR MARINE COASTAL ENVIRONMENT CHARACTERIZATION

PP in charge: PP4 – Environmental Protection Agency of Friuli Venezia Giulia

Final version

Public document

2022

Project acronym	**CASCADE**
Project ID number	**10255941**
Project title	**CoAStal and marine waters integrated monitoring systems for ecosystems protection AnD managemEnt**
Priority axis	**3 - Environment and cultural heritage**
Specific objective	**3.2 - Contribute to protect and restore biodiversity**
Strategic theme	**3.2.1 - Marine environment**
Word Package number	**WP3**
Word Package title	**Coastal Marine Environment characterization of (species and) ecosystems**
Activity number	**Activity 3.3**
Activity title	**Report on design the optimal observing system for marine coastal environment characterization**
Partner in charge	**PP4 – Environmental Protection Agency of Friuli Venezia Giulia**
Partners involved	**All PPs**

Table of contents

Chapter 1 Introduction

ArpaFVG is in charge of the deliverable 3.3.1: 'Report on design of the optimal observing systems for marine coastal environment characterization for each Pilot', which is part of the activity 3.3 'Design of the optimal observing systems for marine coastal environment characterization' and Work Package 3 'Coastal Marine Environment characterization of (species and) ecosystems'.

To design an optimal observational system for transitional and coastal areas, all involved partners contributed through target actions for each pilot area as follows:

- P1: in situ sampling campaign of benthos, biological and chemical analyses
- P2: integration of new physical observing system, with an additional focus on ecosystem component
- P3: plans for ocean observations and analysis of heavy metals, microplastics, biological, toxicity
- P4: design of environmental studies
- P5: plans of the optimal observing system
- P6: design of environmental studies
- P7: ecosystem assessment through bioindicators
- P8: design of the optimal observing system for threatened ecosystem
- P9: design of monitoring activities involving innovative techniques (e.g. underwater drones)
- P10: ecosystem assessment through bioindicators
- P11: design of optimal observing system

Local stakeholders were involved in each Pilot design when necessary. The description of each pilot area is organized in five chapters.

Introduction	• General introduction, including why the pilot area was chosen.
Description of the pilot area	• Geographic frame of each pilot area, identification of pressures and other critical issues.
Target action and final goals'	• Actions needed in each pilot area. • Goals/system/results expected.
Material and methods	• Detailed account of reaserches, procedures and tools used and planned for further actions.
Personnel and resources involved	• Brief description of the general work effort needed to bring on the activities described in the other chapters
Closing remarks	• Focus on the main points discussed in the document.

Chapter 2 Report on design the optimal observing system for marine coastal environment characterization on each pilot

2.1. P1. Grado and Marano Lagoon and Gulf of Trieste (IT); PP: ArpaFVG, UNIBO

2.1.1. Introduction

ARPA FVG is the managing authority of the Pilot area P1 (Figure 1). P1 includes both the coastal marine area and transitional waters, respectively represented by the Gulf of Trieste and the complex of Marano and Grado Lagoon. In these two areas, different activities are being conducted in order to characterize the coastal-marine environment and to evaluate the conservation status of endangered species and ecosystems.

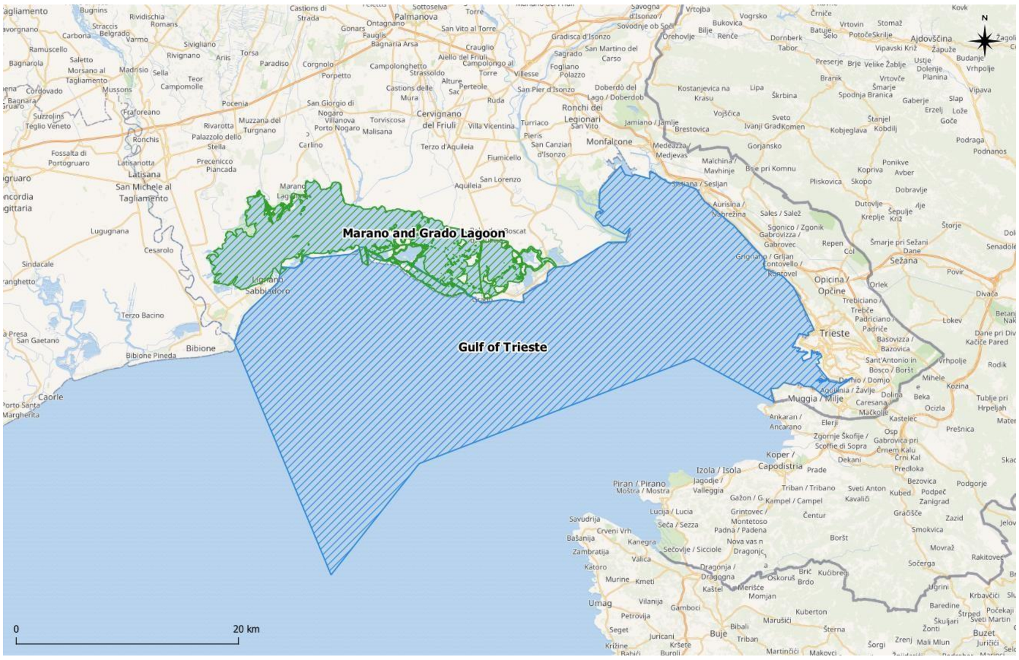

Figure 1. Pilot area P1

Regarding the marine-coastal area, the rocky-mesolittoral and the underneath infralittoral zone are being investigated. The study area is placed in the easternmost area of the Gulf and it extends, along the Carso region. These habitats are characterized by a relatively poor conservation status inasmuch as strongly threatened by several natural forces and anthropic impacts. In addition, the last studies on these biocenosis had been carried forward during the 1960s but subsequently abandoned. A few exceptions in Italy are made by macroalgae populations, which are currently studied by the university of Trieste and the zoo-benthonic and fish communities, partly investigated by the MPA of Miramare. In contrast, on the Slovenian coast, updated research is being conducted by the Marine Biology Station of Piran.

Accordingly, the Marano and Grado Lagoon has been included in the Pilot area P1 due to the typical characteristics of this environment. The region is very rich both in biomasses and species and it offers many significant ecological services. Nevertheless, at the same time, this transitional environment can be considered incredibly fragile due to its exposure to anthropogenic pressures from both land and sea. The multiple environmental stressors must be increasingly investigated through the use of semi-autonomous technologies that allow to obtain high-frequency data over the whole 24h. The ability to remotely monitor the trends of physical-chemical parameters allows to quickly provide a cognitive frame of the area and support any decisions and management activities to be implemented in the area. Furthermore, these data will provide a useful knowledge to carry out the benchmarks on the physico-chemical models that will also be used within this project.

2.1.2. Description of the Pilot Area

Gulf of Trieste

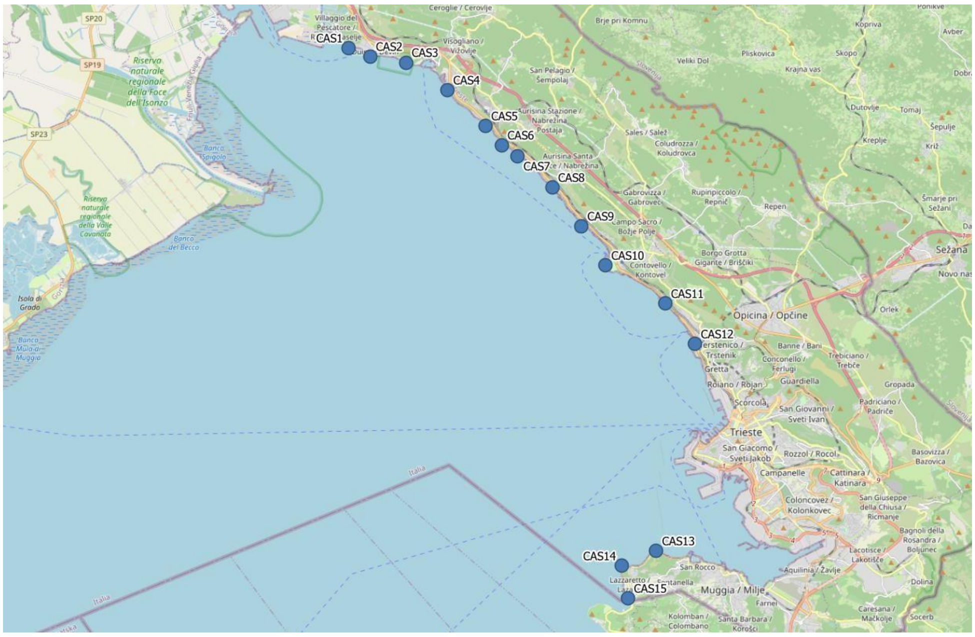

Figure 2. The sites of the mesolittoral and infralittoral monitoring system.

The Gulf of Trieste is a shallow marginal sea of the Adriatic Sea. It is extended from Cape Savudrija (Croatia) to Grado (Italy) with a coastline about 130 km long (Orlando-Bonaca et al., 2013) and defined by the further characteristics: high variations of salinity (32 to 38 PSU) and temperature (6,5°C in winter and 28°C in summer), a northward wind-driven water circulation, high productivity and a tidal range of approximately 90 cm. Moreover, a study of the reduction of chlorophyll-a concentrations all over the basin (Mozetič et al., 2009) underlined the decrease in

concentrations of phosphate and ammonia (Solidoro et al., 2009). While during the rest of the year the water column is well layered, in winter the Gulf of Trieste is characterized by considerable vertical homogeneity due to autumnal cooling processes and wind mixing (Mozetič et al., 1998), mainly caused by Bora, and influenced also by the strong freshwater input.
Besides, the coastal morphology of the Italian sector of the Gulf of Trieste has been heavily modified by human activity, and these modifications become more evident while approaching the city of Trieste (Brambati and Catani, 1988).

Marano and Grado Lagoon

The Marano and Grado Lagoon (Figure 3) covers approximately 160 km^2 between the Tagliamento and Isonzo River deltas and is formally subdivided in six basins: Lignano, Sant'Andrea, Buso, Morgo, Grado and Primero. The western sector of the lagoon has few areas above the sea level and several channels connecting the plain spring rivers flowing into its internal edge towards the sea. The eastern sector is shallower (<1 m, on average), it has a complex network of tidal flats, tidal channels and subtidal zones and the connection with the open sea occurs through several inlets.

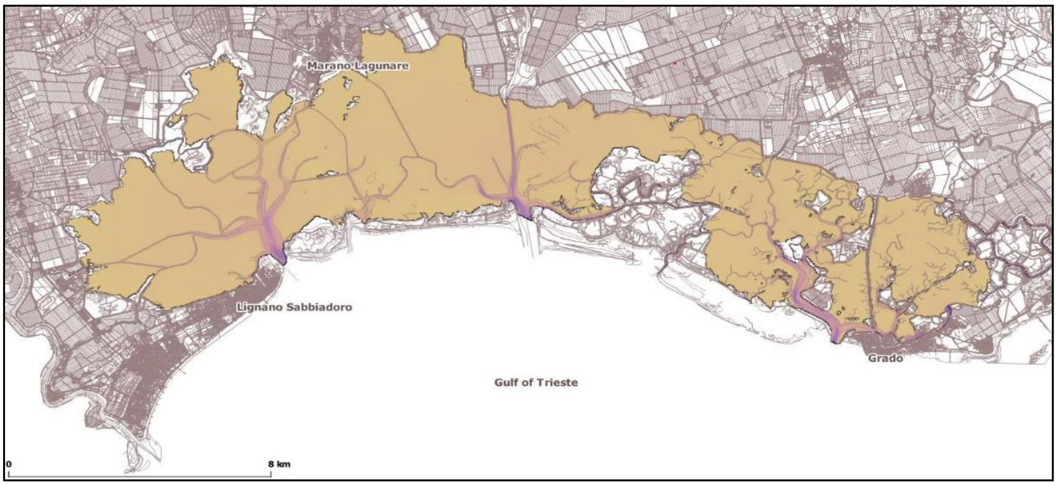

Figure 3. Marano and Grado Lagoons, the bathymetry map realized by FVG Region, the main canals enlighten.

Several rivers and a complex system of drainage pumps are responsible for the freshwater inputs and the Stella River (western sector) is the main contributor (Ret, 2006). However, some areas, especially in the inner part of the lagoon, suffer for scarce water renewal (Ferrarin et al., 2010). Tide and wind represent the main forcing factors for water circulation and renewal. Tide generates an important daily salinity gradient (more than 10 psu) in the western part of the lagoon and close to the mouths of the Aussa-Corno and Natissa. The Lignano, Buso and Grado inlets are responsible for about 35, 30 and 22 % of the total water discharge between the lagoon and the sea. When the wind action occurs, the lagoon circulation changes radically (especially in case of ENE Bora) and the tidal exerts a minor role.

Since 1971, the system is protected by the Ramsar Convention and, following the implementation of the Habitats Directive (92/43/EC), it was also identified in the state-sponsored ‘‘Natura 2000’’ survey as a Site of Community Importance (SCIs – IT3320037). The lagoon hosts economic, touristic and industrial activities, thus experienced some remarkable impacts. Large amounts of anthropogenic nutrients, mainly in form of nitrogen, are released from inland due to intense agricultural activities and urban wastewaters and, in the past, abnormal growth of nitrophilous macroalgae and extensive red microplanktonic algae were observed.

2.1.3. Targeted actions and final goals to achieve

The study aims to achieve two main goals. On one side, the neglected macrozoobenthic communities, living on shallow rocky substrates in the Gulf of Trieste, must be investigated and, consequently, the conservation status.

Therefore, another goal will be to upgrade the physical and ecosystem observing system of P1. ArpaFVG will develop and validate modelling tools for coastal and marine waters that will be tested in P1. Simulations for the past and short-term forecasting capability will be provided.

2.1.4. Material and Methods

The fieldwork was carried out *una tantum* within April and September 2021, between Villaggio del pescatore and Lazzaretto (nearby the Slovenian boarder) with a total number of sites was 15 (Figure 2), resulting in more than 130 samples. To reach the sampling site, a vessel and a small support boat with oars were used to transport all the equipment directly near the rocky coast.

The collection of samples in the two mediolittoral belts was done by snorkeling, while in the upper- infralittoral was used scuba gears to remain for the time needed on the sea bottom. All the organisms were collected and scraped from the rocks or the bottom by a rasp, within a metal square 20x20 cm that is the reference sampling area. The sampled material was funneled inside a collecting bag that will be changed before collecting the next sample. In the infralittoral belt the collection was carried on using also a small sorbona. Each sample consists of 3 subsamples of 400 cm^2 (20x20cm), collected in the upper-mediolittoral, lower-mediolittoral and upper-infralittoral belt (total of 9 samples for site), at the maximum depth of 1.5 m. The vertical and horizontal extension, and distinction among the upper- and lower-mediolittoral and the upper-infralittoral belt was done mainly through the identification of sessile organisms.

During the main monitoring activities other two objectives were pursued on the same sites:

1. A visual census of *Fucus virsoides* noting the presence/absence;
2. Identification of the main fish species of littoral by the means of non-invasive methods, locating video

cameras on the sea bottom or moving them inside the ravines. In particular, it will be investigated the presence/absence of adults and/or juvenile of the *Gobius cobitis.*

Photos of the surface of each square were taken with a digital camera, before (non-invasive method) and after (invasive method) organism's collection, in order to obtain data about the substrate features and to monitor the accurateness of the sampling operation. Once the samples had been collected and moved on the vessel, they were immediately fixed with ethanol diluted to 70% in seawater into labelled plastic bottles.

All sampled organisms will be counted and identified to the higher taxonomic level under a stereomicroscope and an optic microscope. The animals counted must been alive at the time of collection. Identification of the taxa will bring to a faunal list that will be used to calculate all the univariate indexes used to study benthic communities (Pitacco et al., 2013). It is also possible to apply an index developed by researchers of the Piran Marine Biology Station (Orlando-Bonaca et al., 2012) and published in the Elsevier journal. This index called BIRS (acronym for Benthic Index for Rocky Shore) evaluates the probability of presence of each species or taxon for each identified hydromorphological stress class (HM). Thus, for each site, the BIRS index allows to detect the predominant species (in terms of presence and abundancy) that better represent a natural or an altered condition and all the other conditions between the two extremes.

Consequently, density (n.ind/area), taxon richness, Shannon diversity (Shannon and Weaver, 1949) and Pielou evenness (Pielou, 1969) of communities will be calculated. Univariate and multivariate analysis will be performed on the resulting matrix. A PERMDISP analysis will be used to test the null hypothesis of equal dispersions among area and to detect differences among all taxa. In case of significance level <0.05 the null hypothesis will be rejected. Spatial differences on the species composition will be explored by performing a two-way PERMANOVA routine on the corresponding resemblance matrices and a pair-wise comparison right after (Franzo and Del Negro, 2019). A multidimensional scaling ordination (nMDS) will be performed to visualise spatial patterns of species composition. Eventually, the main influencing environmental variables will be found using a distance-based linear model (DISTLM) routine (McArdle and Anderson, 2001).

Regarding the upgrade of the observing system, the SHYFEM (Umgiesser et al., 2014) finite element hydrodynamic model has been identified as the state-of-the-art numerical tool most suitable for the CASCADE project objectives.

The horizontal discretization of the physical state variables is carried out with the finite element method, with the subdivision of the numerical domain in triangles varying in form and size. Such a method has the advantage of representing in detail complicated bathymetry and irregular boundaries in coastal areas. Thus, it can solve the combined large-scale oceanic and small-scale coastal dynamics in the same discrete domain by using unstructured meshes. The model has been already applied to simulate hydrodynamics in the Mediterranean Sea (Ferrarin et al., 2018), in the Adriatic Sea (Bellafiore et al., 2018), and in several coastal systems (Umgiesser et al., 2004). The numerical computation is performed on a spatial domain that represents part of the northern Adriatic Sea and the lagoon of Marano and Grado (Figure 4). To adequately resolve the river-sea continuum, the unstructured grid also includes the lower part of the other major rivers flowing into the considered system. The use of elements of variable sizes, typical of finite element methods, is fully exploited, in order to suit the complicated geometry of the basin, the rapidly varying topographic features, and the complex bathymetry of the lagoon systems (Figure 5). The numerical grid consists of 33,100 triangular elements with a resolution that varies from 4 km in the open-sea to a few hundred meters along the coast and tens of meters in the inner lagoon channels. The bathymetry of the northern Adriatic Sea and the Marano and Grado lagoon was obtained by merging several datasets, having different spatial resolution and obtained using different measurement approaches.

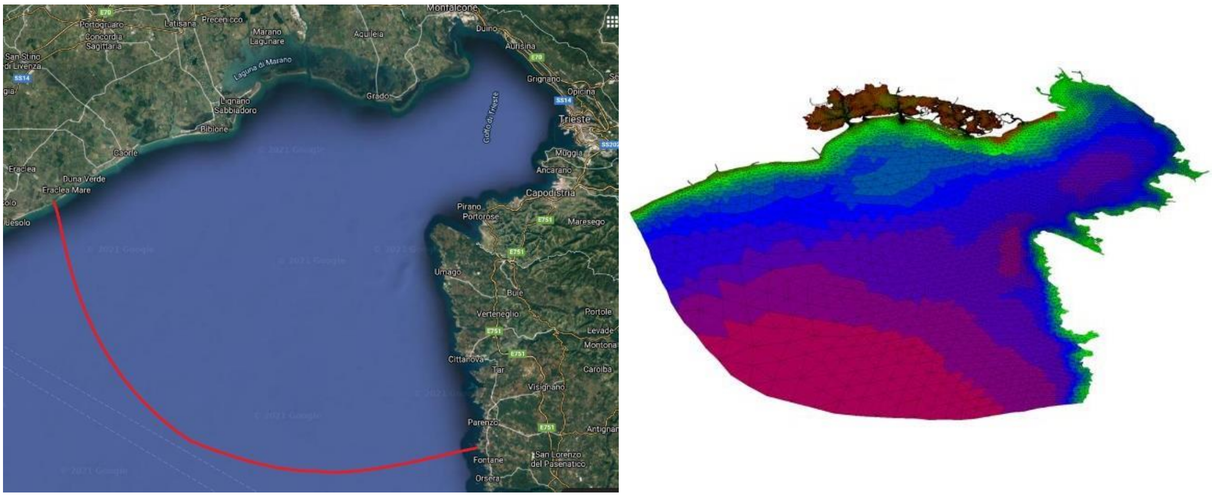

Figure 4. The computational domain that has been adopted for CASCADE optimal observing system (left) and an overview of the model grid resolution, which is variable across the domain (right).

The boundary conditions for stress terms (wind stress and bottom drag) follow the classic quadratic parameterization. Heat fluxes are computed at the water surface. Water fluxes between air and sea consist of the precipitation and runoff minus evaporation computed by the SHYFEM model. Smagorinsky's formulation (1963) is used to parameterize the horizontal eddy viscosity. For the computation of the vertical viscosities, a turbulence closure scheme was used. This scheme is adapted from the k-ε module of GOTM (General Ocean Turbulence Model) described by Burchard and Petersen (1999).

Boundary conditions from Copernicus Marine Service (COPERNICUS, 2021) are going to be used for the oceanographic physical fields while, for meteorological forcing, the 2 km resolution dynamical downscaled WRF model reanalyses of ARPA FVG (NAUSICA, 2018; NAUSICA, 2019) will

be the inputs. Tidal data will be added too. Concerning water inflow contribution at the river outlets, time series for the Isonzo and Tagliamento rivers at hourly base will be used.
The quality of the model simulation assessment will be by means of hindcast runs, that is the application of the model to the past. Then the model outputs are going to be compared with measurements, which are already available in the Project Partners data sets. Operational short-term forecasting capability will be evaluated too. To this end, three multi-parameter probes will be placed in three key points in the Marano and Grado lagoon. The probes will acquire hydrological and physico-chemical data in continuous so they will add information required for te model validation in the most resolved domain area.

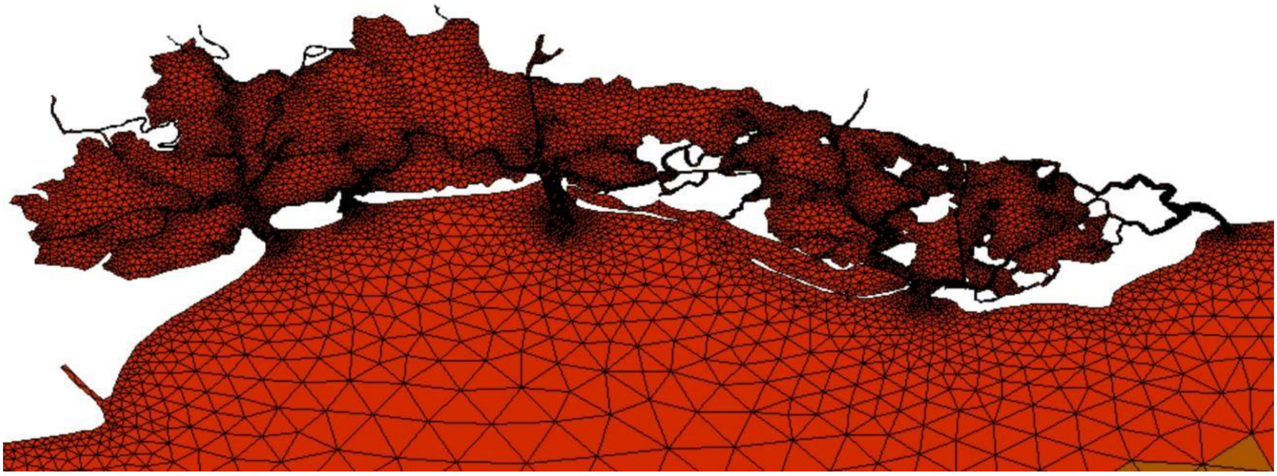

Figure 5. The model grid resolution in the Marano and Grado lagoon has been defined with the aim to match the spatial details of the available bathymetry.

2.1.5. Personnel and resources involved

The fieldwork is carried out by at least 5-6 operators each time: 1 or 2 people are needed to drive the support vessel while four technicians are engaged in the actual field work. Research equipment comprehends five complete snorkeling gears, including long and short wetsuits, masks, boots and gloves as well as a dive breathing set (one octopus, two long hoses, two regulators and one 10L reserve tank for rent). Several diving tools will be used in order to ease the activities, e.g. two 120m-long lanyards, underwater whiteboards, one sorbona (an underwater pump used to adsorb mixed material) and a set of sampling rust-proof devices made for the project. Moreover, in order to record and photograph the interested elements, a complete photo and video-camera kit are used. The camera, used in combination with a water-proof case, is equipped with the necessary items - as diving spotlights, flash and macro lens. The action cameras are used to record the icthyofauna for at least 20 minutes and equipped with tripods and camera sticks. Eventually, water samples are collected by one operator and preserved in case of need for

further analyses.
To recognize all macrozoobenthic specimens, the needed books for identification and taxonomy of polychaetes, molluscs, echinoderms and the other taxa are used for this purpose by the involved technicians. Multivariate analyses of biological and environmental data were performed using PRIMER v7 software package (Clarke and Warwick, 2001) with the PERMANOVA add-on (Anderson et al., 2008).
Finally, the measurements made by the monitoring system are support the modelling study of hydrological and biogeochemical features through data acquired in the Grado-Marano Lagoon.
The continuous monitoring system consists of 3 autonomous multi-parameter probes, equipped with all the most recent technologies regarding mooring instruments:

- Pressure, temperature, conductivity, optical dissolved oxygen and pH sensors (specifically develop for long-term deployment);
- Internal data logger;
- GPS antenna;
- Remote data transmission system;
- Communication software for mobile devices.

Data are being recorded by the probes every 15 minutes 24/7 and transmitted to the ARPA's server daily. The rechargeable battery pack provides at least 3 months' non-stop measurements before needing any maintenance.
The computational power required by numerical model runs and the storage needed to archive the big data amount, which are the simulation outputs, are available through a High Performance Computing (HPC) structure which is supporting the PP4 in the CASCADE computational activities. The structure is a medium size cluster composed by 11 computing nodes with 24 cores each (Intel(R) Xeon(R) CPU E5-2697 v2 @ 2.70GHz) and a storage capacity of 30 TB.

2.1.6. Closing Remarks

This study aims to reach two goals: to provide a more accurate characterization of the marine zoo benthic communities in the considered habitats, which were barely considered until now, and to realize a connection with the data collected in Slovenia, ensuring geographic continuity. This purpose is fully coherent to WP3 targets which consist also of the use of monitoring systems and data collections for protecting biodiversity and ecosystems.
The collected results will be part in the analysis of the efficiency of a lagoon stress operational forecasting system and, in turn, in the evaluation of the usefulness of its operational implementation. It is expected the pilot action could stimulate the roadmap towards an operational warning system for hazardous events in lagoon ecosystems. Besides the forecast implementation, that reliable modeling system would be useful in the management of the ecosystem and in planning actions for its preservation, limiting the vulnerability to environmental

stresses.

2.2. P2: Transitional (e.g. Goro area and Bevano Mouth) and coastal areas in Emilia Romagna (IT); PP: ARPAe; UNIBO; CMCC; Delta2000

2.2.1. Introduction

Sacca di Goro is the chosen area of the P2 pilot area for developing the integration of observing systems and ecosystem data. It represents an important site for clam cultivation on which is based the economy of the nearby coastal zone; however, the clam stock has faced a decreasing trend with time. Phytoplankton and the particulate organic matter deriving from its deposition can be the source of food for these organisms and it is important to monitor changes within its population, in addition it is important to detect the presence of alien species or of species which can cause harmful blooms or anoxic events. The area is routinely monitored but, although its extension is not wide, it is possible to recognize subzones having different chemical and physical characteristics. It is thus important to implement a model of the area which combines the hydrodynamic and ecological processes taking into account the different chemical-physical characteristics of the different zones: a "box model" as described in "***D.3.4.1 Report on the model design for marine coastal environment characterization for each Pilot"***. Data measured by the observing system will be used to validate and calibrate the model.

2.2.2. Description of the Pilot Area Sacca di Goro

Sacca di Goro is a roughly triangular, shallow-water lagoon of the Southern Po River Delta covering an area of approximately 26km² with an average depth of 1.5m. At its Northern portion, the lagoon is surrounded by embankments while it is connected to the Adriatic Sea in its Southern opening. Important freshwater inputs are represented by the Po di Volano River (approximately 350.106 m^3 yr^{-1}), the Canal Bianco and Giralda (both with similar discharge rates of around 20-55 x 106 $m^3 yr^{-1}$). Freshwater inlets are also located along the Po di Goro River and are regulated by sluices plus a large channel with unregulated input close to the local lighthouse. There are no direct estimates of the freshwater input from Po di Goro, which is usually assumed to be equivalent to that of the Po di Volano. The fresh water or hydraulic residence time oscillates monthly between 2.5 and 122 days with a mean value of 24.5 days, whereas the water exchange time ranges from 2 to 4 days. The tidal amplitude is ca 80 cm. In terms of sediment and geomorphology, the lagoon is mostly flat containing alluvial muds with high clay and silt content in its 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 currently implemented observing systems, Arpae maintains a network of six multiparametric probes providing hourly *in situ* measurements of water temperature, salinity, pH, oxygen dissolved and oxygen dissolved (% sat). The two main areas covered by this network are the Goro Lagoon and the Comacchio Valleys with the measured data being processed and made available online in the Dext3r platform (https://simc.arpae.it/dext3r/). Additionally, one integrated meteo-marine station and one wave buoy provide site-specific measurements in two localities of the regional coast (Porto Garibaldi and Cesenatico). More details in terms of the currently implemented observing system can be found in the deliverable "***D.3.1.1 Report on the review of existing observing and modelling systems***".

In what refers to modeling, Arpae has two basin scale oceanographic models currently implemented (AdriaROMS - Chiggiato and Oddo, 2008 - and Adriac) providing daily forecasts of sea-level, *u* and *v* current components, temperature, salinity and waves (for Adriac only). The spatio-temporal coverage refers to the whole Adriatic Sea basin with resolutions varying from 1km to 2km and forecasts of +72h (after a simulation using meteorological analysis that comprehends from -24h to 0h of the issuing day). Additionally, the modeling system also involves applications of the Simulating WAves Nearshore (SWAN) model covering the whole Mediterranean, the Italian territory and specific regions such as Emilia-Romagna and Marche (Valentini et al., 2007). More details in terms of the currently implemented modeling system can be found in the deliverable "***D.3.1.1 Report on the review of existing observing and modelling systems***".

2.2.3. Targeted actions and final goals to achieve Sacca di Goro

Arpae's foreseen activities in the context of CASCADE involve two main branches of upgrades: one involving the observing system and another the modelling component. In what refers to the former, the project foresees new multiparametric probes that aim on upgrading the physical observing system of the area. The new probes are necessary as the spare components of the currently implemented ones are now out of production so they can be repaired only until stocks last. Their essential role for monitoring chemical and physical parameters to be used in long-term, seasonal and extreme event response evaluations make them indispensable for continuous monitoring activities.

As for the modelling upgrade, a new implementation combining the finite element model SHYFEM (System of HYdrodynamics Finite Element Modules; Umgiesser et al., 2004, Cucco and Umgiesser, 2006) and a biogeochemical model (BFM) targets to combine the hydrodynamic and ecological processes through an offline coupling scheme. The final goal is to have a better modeling strategy that provides precious information for local stakeholders on physico-chemical parameters that strongly influence the clam production in the area. Additionally, a better understanding on how physical and biogeochemical variables interact is central to better develop management plans and strategies to help both short- and long-term decision making processes.

2.2.4. Materials and Methods Sacca di Goro

In order to implement the integrated monitoring actions of WP4 UNIBO will carry out further sampling activity at the Sacca di Goro, as it was done under the WP3 activities. Physical, biotic and abiotic data are collected from the seawater surface in 4 different stations of the Sacca di Goro, chosen to represent areas of the same sub-basins having different hydrographic characteristic (Porto Gorino – Gorino – Foce Volano – Bocca Mare), reached on a seasonally base with a fishing boat. Physical parameters such as temperature (°C) and dissolved oxygen (mg/L and saturation %) are measured in each station using a multiparameter water probe HQ30d (Hach-Lange GmbH). Salinity is determined with a refractometer Atago S-10 properly calibrated with distilled water. Depth (m) is determined using a tape measure equipped with a ballast.

Water samples are collected at surface in triplicate in polyethylene sample bottles of 1 L each, carried to the laboratory and processed in order to evaluate the following data:

- **Chlorophyll-*a* and phaeopigments**: a direct measure of the active and inactive phytoplankton biomass, respectively, and a good accepted criteria used for trophic status classification of water bodies.
- **Dissolved Inorganic Nitrogen (DIN) and Reactive Phosphorus (RP):** the essential nutrients for phytoplankton biomass and eutrophication assessment tool.
- **Particulate Organic Carbon and Nitrogen (POC and TPN):** the concentration of organic matter and relative atomic ratios, which affect the nutrient cycle and the subsequent incorporation through the food web.
- **Phytoplankton abundances and composition:** an important component in the functioning of aquatic ecosystems, of which structure and dynamics are influenced by physical, chemical, biological and hydrological factors.

Macrobenthos and sediment are collected only once during the sampling period related to WP4 to represent, together with already performed 3 samplings, a whole sampling year and dataset. Benthic macrofauna samples are sampled using a Wildo Box-corer (15×15 cm2), sieved through a 500 µm mesh size, collected into clean plastic jars and preserved with 90% Ethanol. A sediment sub-sample from the same Wildo Box- corer was simultaneously collected into a small plastic vial. Samples were collected in four replicates, carried to the laboratory and processed in order to evaluate **granulometric composition and organic matter** of sediment and the **benthic macrofauna abundances and composition**. More details regarding the specific method and materials involved can be found in the deliverable "***D.3.2.1 Ecosystem Pilot characterization report***".

Furthermore, and as previously mentioned, in terms of the observing system upgrading, new multiparametric probes will be added to the already implemented system in the region. These probes can be installed with an integrated depth sensor to compensate for the atmospheric pressure.
Referring to the modeling strategy, in recent years a joint effort between Arpae, Unibo and CNR-ISMAR made possible the development of a hydrodynamic model of the Goro lagoon called GOLFEM (GOro Lagoon Finite Element Model; Maicu et al., 2021). The system is based on the unstructured grid ocean model SHYFEM. This new hydrodynamic system will be coupled with the BFM biogeochemical model. For the biogeochemical simulations and considering the high number of nodes in the hydrodynamic grid, it seems unrealistic to simulate the ecological processes on all nodes. This is especially true as many calibration and validation runs will have to satisfactorily describe the involved processes.
A box model approach has been chosen where only some macro-areas will be covered by the ecological model. The hydrodynamic fluxes between the boxes will be used to simulate the biogeochemical reactions inside each box. Every box has been designed to comprehend the location of at least one monitoring station as *in situ* measurements allow for calibration and validation procedures. Moreover, the boxes have been designed to divide the Goro lagoon in already well established areas with already known hydrodynamic and ecological characteristics. A more detailed description of the modelling system approach to be implemented for Sacca di Goro can be found in the deliverable ***"D.3.4.1 Report on the model design for marine coastal environment characterization for each Pilot"***.

2.2.5. Personnel and resources involved

The sampling activity will be carried out by 2-3 operators each time in addition to the person that will drive the vessel; 2 people are usually enough to perform measurements of seawater parameters and to fill the bottles with surface water, one more person is needed to collect benthic samples and to sieve them. Research equipment for field work is very basic consisting in portable instruments, containers and a box- corer. Analysis in the laboratory will be performed by means of instruments such as a spectrophotometer, elemental analyzer, ionic chromatography, microscopes.
Phytoplankton and benthic organisms will be identified by means of manual and for identification and taxonomy.
The modelling activity will be carried out by 2-3 people. An operator will run the simulations in a coupled way with the box model for the biogeochemical part. Another operator will support the hydrodynamical part of the modelling activity on the Arpae cluster. One more operator will provide information about the biogeochemistry in the Goro lagoon and will help the coupling

between the hydrodynamical module and the biogeochemical module. The Hydrodynamic model used for these simulation is Shyfem (Umgiessere et al., 2004) while the biogeochemistry is simulated with the BFM (Vichi et al., 2015) model.

2.2.6. Closing Remarks

This study has the aim to provide a more accurate characterization of the ecological characteristic of the Goro Lagoon, specifically linking seawater parameters, detailed analysis of phytoplankton abundance / composition, and variation within years, and benthic communities. This purpose is in continuity with WP3 actions and with the aim of using observing systems for protecting biodiversity and ecosystems. The collected results will be used to calibrate and validate the coupled hydrodynamic-biogeochemistry model. The modelling system would be useful to understand the interactions between the physics and the biogeochemistry in the Goro lagoon. Sensitivity experiments can be done to evaluate the influence of each forcing on the most important biogeochemical parameters.

The collected results will be utilized in the biogeochemical model (BFM) which will be implemented in each macro-area (as described above). The biogeochemical model will compute the evolution of relevant biogeochemical properties in dependence of biological and chemical processes and of the exchange processes of properties among boxes governed by the circulation dynamics as simulated by the SHYFEM model.

It is expected that the pilot action could result in a new methodology that shows how different processes that have different characteristics and time scales can be modelled efficiently. The box model combines high resolution hydrodynamic information together with slower varying ecological parameters and allows an easy application, calibration, and validation of the system.

The modeling system could be useful also in other areas that are similarly structured (other lagoons with complex hydrodynamics) and shows a way to apply a highly complex model as the BFM to these areas without dedicating too much of computer resources. The tight linking of the hydrodynamics with the box model allows for an easy application of this methodology to other areas.

2.3. P3: Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT); PP: Regione Puglia; CMCC; UNISALENTO

2.3.1. Introduction

CMCC, UNISALENTO and Regione Puglia are carrying out a series of activities for the characterization of the pilot area P3, which includes different areas selected with stakeholders and, in particular:

- Marine Protected Area (MPA) of Torre Guaceto,
- Protected land of Salina Punta della Contessa,
- The coastal marine area of Melendugno Municipality
- Torre Guaceto Marine Protected Area is shown in Figure 6.

In particular, CMCC is going to upgrade a buoy in MPA, integrating several oceanographic sensors managed by a low-cost electronic system. In addition, as a spatial integration of the buoy measures, an oceanographic survey is planned to acquire data for numerical model validation. All these activities will be supported by the Marine Protected Area facilities.

Figure 6. Torre Guaceto Marine Protected Area

Regarding the marine-coastal area, the Marine Protected Area, falling within the municipalities of Brindisi and Carovigno, is about 8 km long from the zone of Apani till the littoral of Punta Penna Grossa.

The soil use of maritime national properties and adjacent areas may impact on natural resources, such as: impacts on aquatic populations deriving from the collection of coastal benthic organisms by visitors of the Reserve; impacts on the abiotic compartment of the aquatic environment, etc. In addition, non-controlled discharges of undetermined organic and inorganic pollutants have impact on the soil of the coastal zone.

Marine pollution in the area could be due to the beaching of solid inorganic reject and to the organic charge transported along the littoral from the Northern Adriatic and from Albania due to winds and currents. A potential area of accumulation is near the promontory where a no-take zone is present.
As to the chemical status, the review of the available assessments (D3.2.1) showed the importance to increase the sampling points next to Canale Reale river output and Salina Punta della Contessa as different priority substances exceeded the thresholds. Along the coast of the Melendugno municipality a gas pipeline has been recently built by Trans Adriatic Pipeline AG. As a result, within the Pilot P3 and with the agreement of the relevant stakeholders, a transect parallel to the coast and north to the pipeline has been devised to assess the chemical status of water column and sediments. UniSalento organized a sampling activity to collect sediment and seawater in the Pilot area P3. This sampling plan defines the sampling locations, stations, and quantities to be analyzed in relation to the activities foreseen in the Cascade project. The aim is to evaluate the presence of pollutants as microplastics, heavy metals and chlorinated pesticides both in water column and superficial sediments and in determining the horizontal distribution of the selected pollutants. The sampling activities will take place in the summer season of 2021.

2.3.2. Description of the Pilot Area

Torre Guaceto Marine Protected Area

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 coast line, 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 stations for chemical analyses community responses

The number of sampling stations is dependent on the survey objective. UniSalento carried out an in situ survey of the areas which is to be sampled. Sampling of water column and sediments from the proposed sites should be representative of the horizontal distribution.

a. TORRE GUACETO

The sampling plan in Torre Guaceto marine protected area (MPA) has been organized 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. As a result, the coastal tract where Canale Reale flows into the sea (between Apani's cliff and the south boundary of the A zone) is the ideal point for the sampling station next to the shoreline.

Two transects starting from the outlet of Canale Reale (TG1) have been devised to determine the horizontal distribution of pollutants in the area (Figure 7, table 1). One transect develops towards the north, while the other one develops towards the north-east direction and both follow the most probable dispersal directions of Canale Reale output. Both these transects are about 1 km long and, as agreed with LP, avoid crossing the ARPA monitoring point (Torre Guaceto 500). The bay2 point is common between UniSalento and CMCC. According to the bathymetric map, all the sampling stations have a depth less than 5 m.

Figure 7. Picture of Torre Guaceto with the UniSalento sampling points that are those found along the red transects

In the laboratory, the metabolic rate responses of benthic model amphipod species to chemical pollutants and expected water temperature increases according to the most recent IPCC scenario will be assessed through flow-through techniques. The data will be used to calibrate models of biodiversity and C fluxes in the animal guilds the study area.

sampling point	Latitude	Longitude
TG1	40°42'17.84"N	17°48'25.68"E
TG2	40°42'35.70"N	17°48'23.26"E
TG3	40°42'59.90"N	17°48'17.79"E
TG2b	40°42'21.25"N	17°48'50.51"E
bay2	40°42'27.05"N	17°49'10.52"E

Table 1. Geographical coordinates of proposed sampling stations in Torre Guaceto.

b. 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.

The shoreline next to the outlet of Foggia di Rau (point S1) is the first sampling station in the area from which two transects start: one develops towards the north, while the other develops towards the east (S2-S2B and S3-S3B, respectively) Table 2). These transects, required to determine the horizontal distribution of pollutants in the area, measure almost 1 km and remain far from the ARPA monitoring point which is in the south of this area. According to the bathymetric map, all the sampling points are located inside the bathymetric line of 5 m.

Figure 8. Proposed sampling stations in Salina Punta della Contessa

sampling point	Latitude	Longitude
S1	40°36'17.94"N	18° 2'8.70"E
S2	40°36'32.07"N	18° 2'10.79"E
S3	40°36'48.56"N	18° 2'12.47"E
S2b	40°36'19.29"N	18° 2'28.93"E
S3b	40°36'20.31"N	18° 2'51.26"E

Table 2. Geographical coordinates of proposed sampling points in Salina Punta della Contessa

At the study site in the coastal wetland of the Saline Punta della Contessa pilot area, a chemical analysis of water and sediment and the ecological responses of the biotic community will also be assessed, considering the high relevance of some components of the fauna, with the main focus on the bird fauna, for conservation purposes, and the potential spill-over of perturbative responses to other Mediterranean coastal wetlands through the migratory bird fauna. To this aim, the food web networks characterising the coastal wetlands will be described using stable isotope techniques, the distribution patterns of pollutants will be followed and the ecological responses of species at the low level of the food web networks will be assessed.

In the laboratory, will be carried out the same assessment protocol of metabolic rate responses of benthic model amphipod species to chemical pollutants and expected water temperature increases already described with reference to the Torre Guaceto study case.

Figure 9. Proposed sampling stations along the coast of Melendugno Municipality next to Torre Specchia Ruggeri

c. <u>MELENDUGNO</u>

In the area north of San Foca there is no important industrial activity, so run-off from agricultural operations represents 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 and this allows both to remain far from the sampling station of TransAdriaticPipeline and ARPA and next to naturalistic important coralline reef. The transect is about 1 km long, it is parallel to the shoreline, and the sampling stations are 250 m far from the coast and inside the bathymetric line of 5 m Table 3).

sampling point	Latitude	Longitude
SF1	40°19'20.26"N	18°22'56.57"E
SF2	40°19'14.55"N	18°23'0.68"E
SF3	40°19'8.74"N	18°23'4.84"E

Table 3. Geographical coordinates of proposed sampling stations along the coast of Melendugno Municipality next to Torre Specchia Ruggeri

2.3.3. Targeted actions and final goals to achieve

The study aspires to achieve a continuous monitoring (based on the buoy data) of the main characteristics of marine waters, a spatial synoptic characterization of the area (also exploiting the data from oceanographic campaign) and the validation of numerical models for Apulia Pilot designed in Deliverable 3.4.1.

As to the chemical status, the WP3 activities aim to research the lesser known aspects and collect new data of the coastal-marine environment by upgrading the observing systems. The collected results described in D3.2.1 are the starting point of the chemical status of the pilot area and permitted the implementation of the observing system as described in the present D3.3.1. The aim is to sample water column and sediments along transects to detect significant patterns in the horizontal distribution of selected priority substances. Moreover, an accurate characterization of the microplastics down to few microns in all water column and sediment samples will be provided in order to fulfill the lack of information existing in this topic. A more effective monitoring systems is important to have a deeper knowledge of the presence and impact of microplastics in the pilot area P3, as a result, collected samples for chemical analysis will be characterized for microplastics down to the low micron range. The determination of microplastics in the same samples used for chemical analysis will also permit to evidence, if any, correlation among microplastics pollution and chemical status of water bodies.

2.3.4. Materials and Methods

Upgrade of the buoy instrumentation and oceanographic survey

The upgrade of the buoy instrumentation (Figure 10, 12 with photo and position) with new sensors and the oceanographic survey will be done during summer 2021.

In this first stage, the new sensors installed on the buoy will measure the temperature, conductivity (salinity, density), dissolved oxygen and turbidity; moreover, a set of hydrophones will be installed, measuring the marine noise as well as the sounds produced by marine species, with a focus on mammals. During the project period the instrumentation will be furtherly integrated with other sensors thanks to the support of MPA personnel and facilities.

Figure 10. Instrumented Buoy and position in Torre Guaceto MPA.

The data acquired by the buoy will be processed and validated and will be able to be shared with the project community and on MPA website in order to have a useful time series of the main parameters which will allow to analyse the seasonal trends and potential variations.

The system is based on low-cost electronics and will allow to manage analog and digital sensors and will acquire data with a frequency of 10 minutes. The data are transmitted to a server in .csv format. The first installation will integrate the following sensors: AML Xchange CT for conductivity and temperature; Atlas Scientific EZO DO OEM for dissolved oxygen; Pt 100 class A for temperature; SeaTech for turbidity.

- AML Oceanographics:
  - CT XChange OEM:

    Range: 0 ÷ 90 mS/cm (conducibilità)

    -5 ÷ 45°C (temperatura)

    Accuracy: ±0.01 mS/cm

    ±0.005 °C

    Precision: ±0.003 mS/cm

±0.003°C

Resolution: 0.001mS/cm

0.001°C

Stability: 0.003 mS/cm/mese

- Atlas Scientific
  - Lab grade DO DO

    Ezo OEM: Range:

    0 ÷ 100 mg/L

    Accuracy: ±0.05 mg/L
  - Pt100 Wika classe A + scheda RTD Ezo OEM:

    Range: -50 ÷ 260 °C (4 fili)

    Accuracy: ±0.03 °C

    Resolution: ±0.001°C
- SeaTech
  - Turbidimeter #116

    Range: 33 mg/L o 100 mg/L (High o

    Low Gain) Output : 0 ÷ 5V analogico

    Resolution: ±0.01% FS (3µg/L)

The survey will be performed along the MPA coast, as reported in Figure 8. At the stations vertical profiles of the following parameters will be performed through a multiparametric probe: pressure, temperature, conductivity (salinity, density), dissolved oxygen, pH, turbidity, fluorescence of chlorophyll a. The probe used for the survey is an Idronaut 316 Plus (Figure 11), which can be used in auto-acquisition mode thanks to the integrated memory and batteries; the probe is able to acquire at 20Hz sampling rate:

Figure 11. Multiparametric probe Idronaut 316 plus

Figure 12. In situ survey: location of sampling stations.

The data acquired by the multiparametric probe will be processed in order to have the vertical distribution of the measured variables in all the stations and in addition will be processed by Ocean Data View software in order to obtain different layers which will allow to analyse the distribution of parameters at different depth.

All the data will be used for the validation of the numerical model (based on SHYFEM) implemented the Apulia Pilot. In Figure 9 we report the geographical domain, the bathymetry and grid of the very high- resolution coastal based on unstructured grid, which has the advantage of representing in detail complex bathymetry and coastlines (Figure 13). A full description of model setting is detailed in Deliverable 3.4.1.

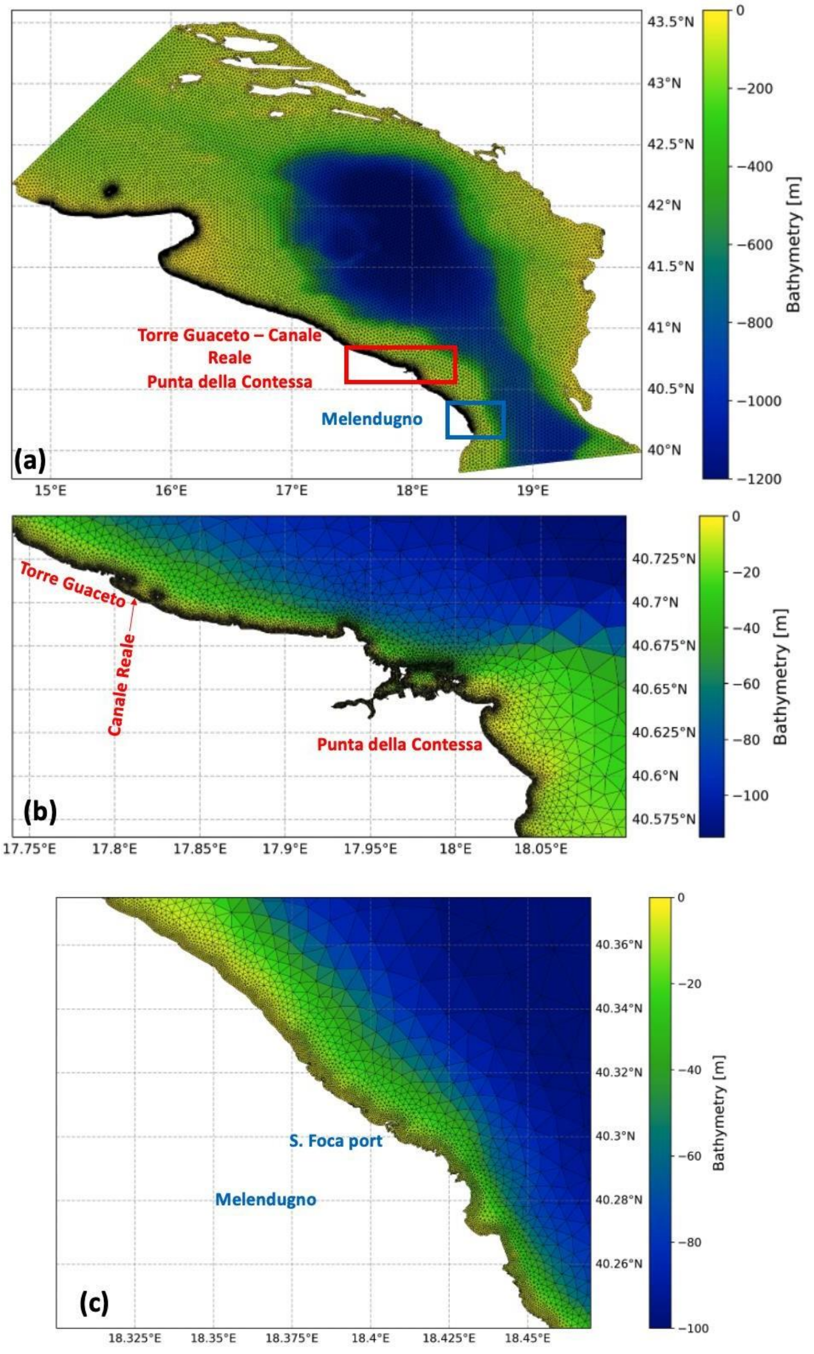

Figure 13 Geographical domain, bathymetry, and grid of very high-resolution coastal modelling for Apulia Pilots

The instrumentation (hydrophones) used for measuring the sound pressure level in the area of interest are characterised by sensors for humidity, temperature, ambient light and atmospheric pressure; moreover, they have an optional and configurable online calculation of ACI Metrics (index of biodiversity) (Figure 14).

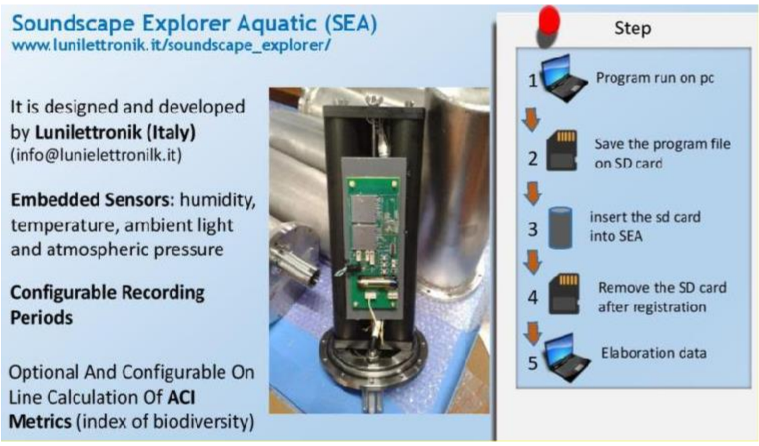

Figure 14. Hydrophone for measuring the sound pressure level in the area of interest.

The use of hydrophones, placed both on the surface and in depth on the buoys, will allow both to evaluate the anthropic impact in terms of underwater noise and to characterize the existing marine community through the analysis of the soundscapes.

Update of the chemical status of water column and sediments

Sampling Methods

For the sampling stations located in the shoreline or near it (Figure 12, 15), sampling is envisaged to take place from land. The sampling activities in Torre Guaceto will be performed using the MPA authority ship. For sampling of sediments and water column in the other stations, a tender will individuate a shipping company which will assist UniSalento for any logistic requirements.
All the sampling stations will be evaluated in situ during the sampling activity with a side scan sonar and, if necessary, with sub immersions, in order to verify the nature of the seabed (whether it is sandy or rocky): it is important to avoid any algal species present, because they could be protected species (i. e. *Posidonia oceanica*) or, in general, they could affect the sediment sampling.
Sediment sampling will be performed with a box corer or a grabber according to the seabed, whereas water column will be sampled by a Ruttner bottle. Further to the sampling process, the samples are stored and sealed in clean environments to ensure no cross-contamination occurs.

Manual handling of samples will be avoided. The samples are stored in glass or polyethylene containers and vials as appropriate. All samples are photographed and kept in a cool environment at a temperature <8°C. Samples are shipped to the laboratory for analysis within 12 hours.

Chemical Analyses

Chemical analyses will be carried out on the fine fraction sample (<2mm) since this is the fraction in which contaminants tend to concentrate. The sediment chemical analyses will be carried out in the Laboratories of Analytical Chemistry at DISTEBA, UniSalento.
The laboratory will carry out chemical analyses on the parameters listed in the table 4.

Microplastics
Arsenic (As)
Cadmium (Cd)
Copper (Cu)
Mercury (Hg)
Zinc (Zn)
Chromium (Cr)
Lead (Pb)
Nickel (Ni)
Chlorinated pesticides

Table 4. List of the analytes to be determined

This plan has been designed on the basis of information available. Modifications to this plan can be necessary in dependence of actual conditions of sampling points when observed during sampling campaign as well as meteorological conditions different from expected ones.

Autonomous technologies

During summer 2022 an Autonomous Surface Vehicle (ASV) will be available to carry out a series of surveys to characterize the different pilot areas. The ASV is a highly portable survey platform that sits on the sea surface recording oceanographic data across a range of variables. The ASV can be equipped with a multipayload system and can be managed both manually and autonomously through a remote control system, making it a safely and cost effectively way to collect data at sea.

Figure 15. Example of survey performed by the ASV in the Torre Guaceto pilot site

The ASV will be equipped with instrumentation able to acquire data about the bottom morphology and substrate types (such as the presence of *Posidonia oceanica*) and water column characterization, which will be useful for model validation. A market research was conducted to determine the best solution both for the vehicle and integrated instrumentation and during the next months the ASV acquisition will be finalized.

2.3.5. Closing Remarks

This study aims to provide a more accurate and in-continuous characterization of the marine water in an area characterized by important biocenosis and habitats, such as Torre Guaceto MPA.
It also aims to update the chemical status in the pilot P3 using the optimal monitoring system and relevant results will be reported in D4.1.2.

2.4. P4: Neretva river mouth (HR); PP: IOF, DNC

2.4.1. Introduction

The main objectives of the activities defined by the work plan of the Interreg Italy-Croatia project "CoAStal and marine waters integrated monitoring systems for ecosystems proteCtion AnD managemEnt" (CASCADE), in particular the design of environmental studies carried out by Work Package (WP) 3, are:

- The development of measures to improve and protect the environment.
- To provide sufficient knowledge about environmental degradation - inherent or induced changes in organisms and environmental damage caused by anthropogenic influence to the environment.

The study area is located in Croatia, more specifically in Neretva River (P4, Figure 16). Croatia is a largely mountainous country with a land and sea area of over 56 000 km². It has great natural resources, such as protected land and clean and abundant fresh water resources.

The Neretva River was included in Pilot Area 4 because it has a great diversity of habitats, mainly aquatic and wetlands. Also in Croatia, the delta area is recognized as very important for the protection of nature and biodiversity, so in recent years' activities have been undertaken by the government to protect the Neretva Delta and its population from further destruction as a future Nature Park.

2.4.2. Description of the Pilot area Neretva River

One of the hinterlands is in the southern part of Dalmatia, commonly called Neretva. Naron is the Roman name for Neretva, the largest tributary (225 km) which flows into the Adriatic Sea and occupies 20% of the total watershed of Bosnia and Herzegovina. Due to its characteristics, Neretva is a typical mountain river with strong hydropower potential. 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 of Hutovo blato belongs to Bosnia and Herzegovina.

In the Pleistocene, the region around the present mouth of the Neretva looked markedly different. It was occupied by the then middle course of the Neretva River, whose bed stretched along the present 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 the shortening of the river and the formation of a new mouth, which was located approximately at the place of today's river mouth and near three triangular widenings. These widenings were not the result of erosive activity of the river, but of tectonic predisposition. Since then, the eroded material in the upper course of the Neretva was deposited there and formed the present delta.

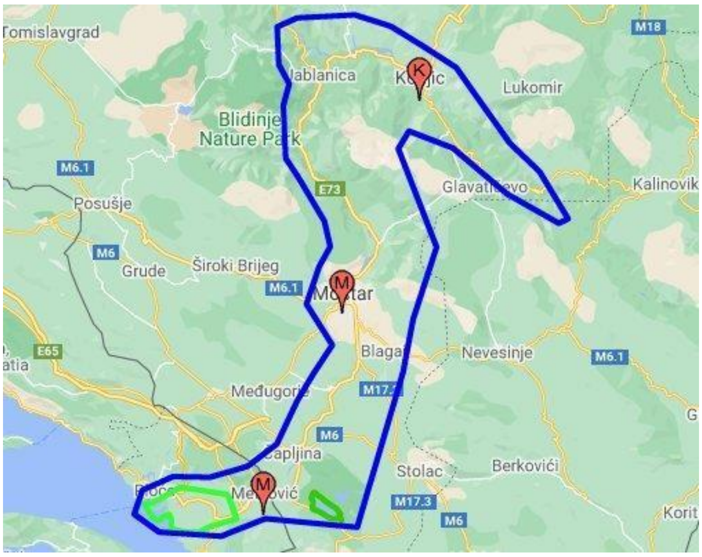

Figure 16. P4 Pilot Area.

Changes in the delta are also indicated by the existence of Vid, i.e. the former Narona, which was mentioned in the 4th century BC. During the Roman Empire there was a port, and today it is a wetland. The Neretva had 12 natural branches through which it flowed into the Adriatic Sea. Regulation of the Neretva River began in 1881 from the mouth of the river to the port of Metković, while intensive land reclamation began after World War II from Opuzen to the mouth of the river and gradually extended to the entire area of the delta.

In the past, the Neretva Delta was known for the abundance and diversity of bird and fish fauna, as well as for the hunting and fishing activities practiced by almost all the inhabitants of the region. The dense marshes were covered with hydrophilic vegetation that provided excellent conditions for fish spawning and bird nesting. This explains why this region was historically home to several species of herons, cormorants, ducks, and other waterfowl, as well as colonies of the now regionally extinct species of ruffed pelicans. In the last 100 years, 310 species of birds, 115 of which are breeding birds, have been recorded in this region. About 35 species are water birds (Mrakovčić, 2001). The central and Northeastern European bird populations use the delta for wintering. The shoals and shallows of the Neretva estuary were of great importance for the passage of waders, terns and gulls, as well as the reed beds and water areas for the passage and wintering of geese and ducks.

Today, the Neretva is the only river in this region that has a delta at its mouth. Neretva Delta is surrounded by karst hills rich in underground water that feeds numerous springs, streams and lakes. In the areas where the Neretva flows into the sea, sandy sediments are deposited and smaller, muddy particles are carried to a deeper area and deposited. Furthermore, the entire project area, especially its fragile karst and wetland ecosystems, is threatened by various activities, such as: negative human impacts on freshwater ecosystems and global climate change. The most significant transformation of the Delta has occurred in recent decades through intensive, long-term melioration aimed at creating agricultural land and protecting the region from flooding. A significant attack on the marshes was the drainage of Lake Modrič and the entire lagoon. A large part of the wetland was thus lost to migratory birds and spawning fish. The further development of the port and the settlement of Ploče, the construction of holiday homes, industry and the pollution of water from Mostar and other sources endanger the marshy valleys of the Neretva River to this day.

In addition, global climate changes have had and continue to have a strong impact on Neretva River and its flora and fauna. Constant fluctuations in water levels, lack of water in summer, increased water temperatures, etc. have led to disruptions in the reproductive cycle of mussels and fish. Unsuccessful spawning and increased breeding mortality due to climate change impacts have reduced overall recruitment. It is a contradiction to Neretva Delta that this region, although highly endangered, is home to many protected "objects of nature." According to the law Nature Protection, the protected areas (cultural and historical heritage) occupy an area of 1724 ha (13% of the total region) and are divided into five scattered protected areas. There are more than 3,000 ha of wetlands that are not yet protected by law.

The water of the Neretva River is also of A-class quality and it is one of the coldest rivers in the world. The average annual water temperature is 8-10 ^{o}C. The dissolved oxygen concentration is between 9 and 10 mg/L, which is why the Neretva and its tributaries are exceptionally rich in fish species. Out of almost 150 species that use the watercourse and/or estuary at some stage of their life, 49 are freshwater fish and of them as many as 19 are endemic to the catchment area of Eastern Adriatic, while 4 are endemic to Croatia.

Despite the large-scale disturbance of birds and fish and the destruction and degradation of wetlands in the past and today (Mužinić, 1993), Neretva River still represents an ecologically unique area.

2.4.3. Targeted actions and final goals to achieve

The main goals of environmental studies are:

- Chemical monitoring of water to determine the presence and possible source of pollutants in water environmental sphere providing important support for environmental protection agencies and research institutions.
- Chemical monitoring of water to determine the presence and source of pollutants in water environmental an important support for environmental protection agencies and research

institutions.

- To understand how the unpolluted environment functions, which chemicals are naturally present and in what concentrations, and what their effects are.
- To study exactly what anthropogenic effects have on the environment through the release of chemicals.
- To determine the effects of environmental degradation on marine organisms, their habitats and the biodiversity of the ecosystem.
- Assessment of the state of the environmental habitat and general monitoring of the biodiversity of marine ecosystems in order to maintain the biodiversity, integrity of habitat types and target species of the Natura2000 ecological network and other protected areas.

2.4.4. Materials and methods

The Croatian Regulation on the Standard of Water Quality includes the requirements of the Water Framework Directive (WFD) regarding monitoring of substances in sediment and biota. Monitoring of sediment and/or biota is carried out in order to obtain a coherent and comprehensive picture of the status of water bodies within each river basin district. Directive 2008/105/EC (Environmental Quality Standards Directive (EQS)) defines the good chemical status to be achieved by all Member States and, together with WFD 2000/60/EC, provides the legal basis for monitoring priority substances in sediment and biota.

For the monitoring, sediment or alternatively suspended particulate matter (SPM) and biota are the most appropriate matrices for many substances as they integrate pollution in a given water body over time and space (see Table 5). Changes in pollution in these compartments are not as fast as in the water column and long-term comparisons can be made. Directive 2008/105/EC provides guidance on the substances that should be considered in trend monitoring and the frequency of monitoring of these substances (Guidance document No. 25, 2010).

The substances in red are those suggested by Directive 2008/105/EC for sediment and biota trend monitoring. The values of the log KOW are taken from the Chemical Monitoring Guidance n.19. The values of Bioconcentration Factor (BCF) are taken from the datasheets of the priority substances in the public section of the CIRCA forum.

Priority Substance	BCF	Log K_{ow}	Water	Sediment/SPM	Biota
Alachlor	50	3.0	P	O	N
Anthracene	162-1440	4.5	O	O	O
Atrazine	7,7-12	2.5	P	N	N
Benzene	13	2.1	P	N	N
Brominated diphenyl ethers [a]	14350-1363000	6.6	N	P	P
Cadmium and its compounds		n.a.	n.a.	n.a.	n.a.
C10-13-chloroalkanes	1173-40900	4.4-8.7	N	P	P
Chlorfenvinphos	27-460	3.8	O	O	O
Chlorpyrifos (-ethyl, -methyl)	1374	4.9	O	O	O
1,2-Dichloroethane	2-<10	1.5	P	N	N
Dichloromethane	6,4-40	1.3	P	N	N
Di(2-ethylhexyl)phthalate (DEHP)	737-2700	7.5	N	O	O
Diuron	2	2.7	P	N	N
Endosulfan	10-11583	3.8	O	O	O
Fluoranthene	1700-10000	5.2	N	P	P
Hexachlorobenzene	2040-230000	5.7	N	P	P
Hexachlorobutadiene	1,4-29000	4.9	O	O	P
Hexachlorocyclohexane [b]	220-1300	3.7-4.1	O	O	P
Isoproturon	2,6-3,6	2.5	P	N	N
Lead and its compounds		n.a.	n.a.	n.a.	n.a.
Mercury and compounds [c]		n.a.	N	O	P
Naphthalene	2,3-1158	3.3	O	O	O
Nickel		n.a.	n.a.	n.a.	n.a.
Nonylphenols [d]	1280-3000	5.5	P	P	O
Octylphenol [d]	471-6000	5.3	P	P	O
Pentachlorobenzene	1100-260000	5.2	N	P	O
Pentachlorophenol	34-3820	5.0	O	O	O
Polyaromatic Hydrocarbons [e]	9-22000	5.8-6.7	N	P	P
Simazine	1	2.2	P	N	N
Tributyltin compounds	500-52000	3.1-4.1	O	O	P
Trichlorobenzenes	120-3200	4.0-4.5	O	O	O
Trichloromethane	1,4-13	2.0	P	N	N
Trifluralin	2360-5674	5.3	N	P	O
DDT (including DDE, DDD)		6.0-6.9	N	P	P
Aldrin		6.0	N	P	P
Endrin		5.6	N	P	P
Isodrin		6.7	N	P	P
Dieldrin		6.2	N	P	P
Tetrachloroethylene		3.4	O	O	N
Tetrachloromethane		2.8	P	N	N
Trichloroethylene		2.4	P	N	N

[a] Including Bis(pentabromophenyl)ether, octabromo derivate and pentabromo derivate
[b] HCH (all isomers) - BCF (lindane)
[c] methylmercury
[d] Nonyl- and Octylphenols do not follow the classical K_{ow} partition, because they can establish hydrogen bonds by the phenolic hydroxyl.
[e] Including Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(g,h,i)perylene, Benzo(k)fluoranthene, Indeno(1,2,3-cd)-pyrene. For these compounds the metabolisation in higher trophic levels should be taken into account.

P = preferred matrix - monitoring should be performed in this matrix;

O = optional matrix - monitoring can be performed in this matrix, but also in other compartments/matrices; the choice will also be made on the basis of the degree of contamination of a particular matrix;

N = not recommended - monitoring in this matrix is not recommended unless there is evidence of the possibility of accumulation of the compound in this matrix;

n.a. = not applicable

Table 5 Monitoring matrices for the priority substances and some other pollutants listed by the EQS Directive.

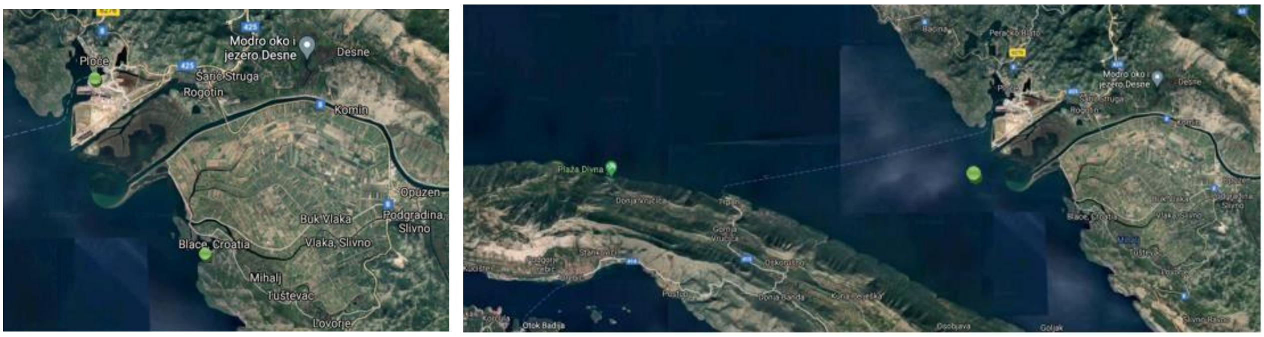

Figure 17 Maps of the sampling sites.

Sampling of seawater, sediment, and shellfish is conducted at various sampling sites at Neretva River during the winter and summer of the year (Figure 17, Table 6).
Mussel and sediment samples are collected in plastic sampling bags and refrigerated until arrival at the processing laboratory. At the laboratory, specimens of *M. galloprovincialis* were opened, stripped of their shells, and washed with fresh water to remove debris. After that, each mussel sample was homogenized (cca 100 g) with a blender and stored at -20°C until analysis. For trace metals analysis, mussel samples from whole body tissue are freeze-dried while sediment samples are freeze-dried and sieved (fractions <2 mm) to further digest them.

Sampling points	**Longitude (East)**	**Latitude (Nord)**	**Depth (m)**
Neretva river (Point A)	43,039641	17,414490	15
Neretva river (Point B)	43,006527	17,470675	Mediolittoral zone
Neretva river (Point C)	43,005181	17,448217	25
Neretva river (Point D)	43,056125	17,424808	13

Table 6 Longitude and latitude of the sampling points.

Water samples are collected in the bottle at three relevant depths (usually 0, 5 and 10 m).
Quantitative chemical analysis is an important component of environmental chemistry as it provides the data that frame most environmental studies.

IOF uses the analytical technique Atomic Absorption Spectrophotometry (AAS) to determine trace metals. The atomic absorption spectroscopy method (graphite furnace or flame atomization technique) is used for trace metal analysis in digested samples. The cold vapor atomic absorption technique is used for mercury analysis. The methods are validated by determining precision (reproducibility, repeatability), trueness and detection limits. The quality and accuracy of the analytical procedure is controlled by the analysis of Certified Reference Materials (CRMs). PAHs and PSP marine biotoxins, are measured using Ultra High Performance Liquid Chromatography (UHPLC). UHPLC is an advanced technique of liquid chromatography in which it takes advantage of innovations in various technologies. It operates at higher pressure and provides high quality data with reproducible and robust methods compared to conventional High Performance Liquid Chromatography (HPLC).

Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) is used as the official analytical method for the measurement of lipophilic marine biotoxins (okadaic acid, pectenotoxin, yessotoxin and azaspiracid groups) according to Regulation (European Community, 2011) No. 15/2011. LC-MS/MS is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. LC-MS system contains an interface that efficiently transfers the separated components from the LC column to the MS ion source.

Furthermore, the dissolved oxygen content of seawater samples is determined titrimetrically using thiosulfate (Marasović et al., 2013). Titrimetric analysis is a method of analysis in which a solution of the substance to be determined is treated with a solution of a suitable reagent at a precisely known concentration. The reagent is called the titrant. The reagent is added until the analysis is determined on the basis of its stoichiometric reaction with a reagent of the specified concentration.

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

For the organic matter content, the samples are treated with H2O2 and heated at 450°C for 6 hours. The weight loss after this treatment is attributed to the organic matter content. The carbonate content, expressed as CaCO3, is determined as the weight loss after treatment with 4M HCl.

The temperature, salinity and depth of the seawater are measured using a CTD sonda (conductivity, temperature, and depth) instrument. The main function of a CTD device is to determine how the conductivity and temperature of the water column change with depth. Conductivity is a measure of how well a solution conducts electricity, and it is directly related to salinity. By measuring the conductivity of seawater, salinity can be derived from the temperature and pressure of the same water. The depth is then derived from the pressure measurement by calculating the density of the water from the temperature and salinity.

Concentrations of nutrient salts of nitrogen, phosphorus and silicon are determined photometrically on a SEAL Analytical AutoAnalyzer III using the automated seawater method

(Grasshoff, 1976) and on a Shimadzu UV mini 1240 spectrophotometer using the methods according to Parsons et al. (1985) and Ivančić and Degobbis (1984). Total dissolved inorganic nitrogen is determined as the sum of concentrations of nitrates, nitrites and ammonium salts, while total dissolved nitrogen and phosphorus were determined as nitrates and orthophosphates after decomposition and oxidation of the samples in an autoclave or UV oxidizer. The concentrations of dissolved organic nitrogen and phosphorus are determined from the difference between the concentrations of total nitrogen and nitrate and total phosphorus and orthophosphate (Marasović et al., 2013).

To assess the status of ecological habitat, fish stock and general monitoring of marine ecosystem biodiversity (habitat mapping and zoning, inventory of existing status, monitoring of invasive species, deployment of fishing gear, marine litter monitoring), the non-invasive method is used due to the specific area of the Neretva estuary (shallow silty/sandy sediments), where it is easy to disturb the physical characteristics (turbidity of the water column) of the habitat and disturb flora and fauna.

The main objective of the environmental studies is to assess and prevent potential pollution and ecological status of the Neretva estuary. The data obtained will contribute to the conservation of the ecosystem of the Adriatic Sea and especially the estuaries. Anthropogenic impacts of industrial and other economic activities in coastal areas lead to significant input of pollutants such as metals (Cd, Cu, Cr, Pb, Zn, Hg) and PAHs into the marine environment. In addition, the ecological parameters of the marine environment are changing, 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.

2.4.4. Personnel and resources involved

Field work will be carried out by at least 10 personnel (scientists): 3 or 4 people will collect samples of seawater, sediment and shellfish and measure temperature and salinity of seawater.

In addition, at least 5 or 6 people are needed for sample preparation and for determining the concentration of metals, nutrient, PAHs, marine biotoxins, and oxygen saturation in the samples. To protect the shoreline from potential spills, Dubrovnik-Neretva County (DNC) will deploy the vessel and vessel equipment (Figure 18). A minimum of 2 operators will be required for normal operation of the vessel and a minimum of 5 operators will be required in the event of an accident. DNC will also use underwater drones to explore marine depths waters that are difficult to access and/or inaccessible to humans for physical and safety reasons and to monitor underwater flora and fauna.

Figure 18. Ship for prevention water pollution.

The research equipment includes:

1. Chemical equipment such as instruments (UHPLC and AAS, mercury analyzer, laser granulometry particle size analyzer), pH-meter, centrifuge, pipette, certified referent materials and standards, hot plate, wash bottle, test tube rack, syringe, water bath, water deionizer, freezer, laboratory glassware, analytical balance, etc.;
2. Standard marine equipment - outboard engine 20 hp - 4 stroke, 3.5 m auxiliary boat - aluminum flooring, spotlight system (4 pieces 24 V - 2 fixed, 2 portable), "echo sounder";
3. Fire equipment – communication equipment, cleaning chemicals in case of sea pollutions, compressor (blower) for inflating the dam segment, installation of BOMPET fire extinguisher (2 pcs);
4. Special equipment - equipment for combating heavy marine pollution (sets and systems

of buoys and suction systems, skimmers), equipment for combating marine pollution – dry suit for 2 persons (set with equipment), high gumboot for flood for 5 persons, diving equipment, storage area container, aluminum path for firefighters to descend and lower the equipment from the ship to shore, an aluminum cage (as a part of a crane) for firefighters to descend and lower the equipment from the ship to shore;
5. Underwater drone.

2.4.5. Closing remarks

The environmental studies are intended to achieve the following objectives:

- Determine the levels of hazardous substances, organic compounds and marine biotoxins in samples from the Neretva estuary area in order to assess the impact of water pollution on aquatic life and ecosystems;
- To provide a critical overview of the impact of water pollution in terms of global challenges, threats and climate impacts, also focusing on various possible preventive measures;
- To contribute to the conservation of particularly important coastal and marine areas in Pilot area 4 (Neretva River).

The above objectives are fully consistent with the objectives of WP3 to conserve biodiversity and ecosystems.

2.5. Coastal area in Veneto (IT); IUAV with the support of CORILA;

2.5.1. Introduction

Università IUAV di Venezia is coordinating the activities at P5 area, including the Gulf of Venice with a specific focus on the Tegnùe di Chioggia area. In the Tegnùe di Chioggia SCI area, different monitoring activities will be conducted in order to characterize the coastal-marine environment, to evaluate the conservation status of endangered species and ecosystems, and test the applicability of different monitoring techniques. Monitoring activities will be carried out with the support of Corila (Consortium for the Coordination of Research Activities concerning the Venice lagoon system), and in collaboration with UNIBO, for what concerns the scuba diving activities.

2.5.2. Description of the Pilot Area

The marine area of "Tegnùe di Chioggia" 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 marine area of the Tegnùe di Chioggia includes a very representative type of Mediterranean marine environment present in the Northern Adriatic Sea.

In a very large area of the north-western Adriatic, between 15 and 40 meters deep from the Isonzo mouth to the northern area of the mouth of the Po (Caressa et al., 2002;), there are rock formations known in the Veneto with the name of tegnùe (term with which the fishermen of Chioggia indicate a grab that holds and/or damages the fishing nets (ARPAV, 2010)), trezze and grebeni in Friuli Venezia Giulia, scagni along the Istrian coast. With extensions ranging from a few to several hundred square meters (Mizzan, 1994), elevations up to 2-3 meters from the seabed and very diversified morphologies (some outcrops are characterized by large horizontal surfaces, whereas others are composed of scattered conglomerates of small rocks (Falace et al., 2015)), they represent the main natural hard substrates in the Adriatic.

These are bio-concretioned rocky calcareous build-ups formed in the last 3-4000 years, often superimposed on older substrates, which host a rich and diverse flora and fauna (ISPRA, 2010); their origin appears complex and in some cases can be traced back to an initial carbonate cementation of sandy sediments mixed with shells and exoskeletons of echinoderms and crustaceans which constitute the more or less thick base layer (Ponti e Mastrototaro, 2006; Bertasi, 2007).

The presence of these natural rocky outcrops surrounded by sandy/muddy sediments of detrital type, of varied and complex morphology, together with the naturally eutrophic and not excessively deep waters, locally creates areas rich in micro-environments and ecological gradients that favour an increase in the specific diversity of both benthic and pelagic populations (Ponti et al., 2011; Falace et al., 2015). The Tegnùe are in fact “biotopes of exceptional ecological value, for environmental and fishery purposes, as well as for natural repopulation, reproduction, nursery and refuge for numerous valuable fish species” (ARPAV, 2010; Melli et al., 2017).

The rocky outcrops located along the coast of the Veneto Region, in particular from the mouth of the river Brenta to the city of Grado, are unevenly distributed (Figure 19), but seem to be positioned along parallel bands at 3–5 miles, 10-12 miles and 20 miles off the coast, at depths ranging from 8 to 40m (Mizzan, 1994).

The dimensions can be very different, ranging from a few square meters to several thousand square meters in the larger ones, with elevations from the seabed ranging from a few decimetres in the low and flat formations, sometimes called "lastrure", to a few meters in the higher ones, often located at greater depth (ARPAV, 2010). The most extensive, highest from the bottom and best known Tegnùe are present between 3 and 5 nautical miles in front of Chioggia (Figure 19): here the rocks extend seamlessly for hundreds of meters, taking on complex shapes often meandering which recall the course of deltaic mouths and natural lagoon channels. (ISPRA, 2010).

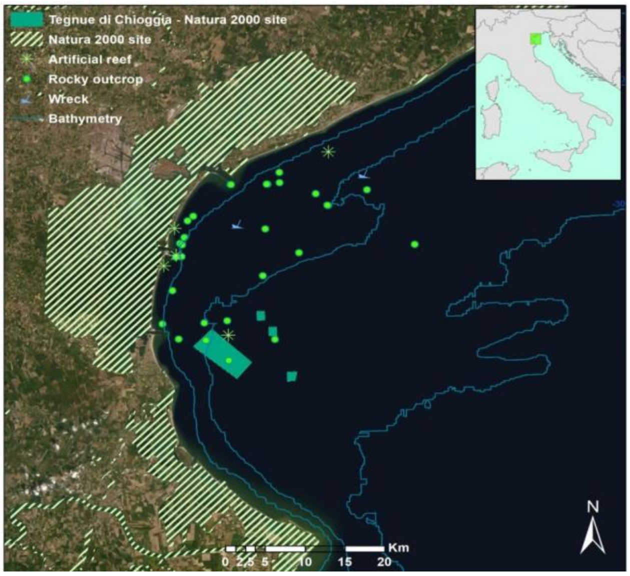

Figure 19. Overview of the subtidal rocky outcrops and hard structures surrounding the Tegnue di Chioggia area (Modified from Nesto et al. 2020).

2.5.3. Targeted actions and final goals to achieve

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 to be carried out between June 2021 and March 2022 was designed. This includes the following four 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. Two campaigns with gillnets targeting fish fauna (November and March 2021);
D. Three campaigns implementing an active bioacoustics survey, targeting fish community present in the area, and carried out from fall 2021 until March 2022.

2.5.4. Materials and Methods

This paragraph provides details with respect to the four specific actions (A-D) presented in paragraph 3:

A) Video recording survey with ROV

Investigation with ROV (Remotely Operated System, Figure 20) instrumentation for the acquisition of HD videos and images for the study of fish populations and macro-benthic communities is carried out in the survey areas, without taking physical or biological samples.
Monitoring activities are conducted using a VideoRay Pro 4 Plus model ROV (shown). This model can be equipped with an umbilical cable over 300 m in length, it has two separate motors and it's particularly compact (37.5, 28.9, 22.3 cm), weighs less than 10 kg and it can reach a maximum operating depth of 305 m. During the investigations, the ROV is equipped with two cameras, one SD navigation and one HD high definition (for shooting as detailed as possible), two headlights with LED lights, a gyrocompass, and a depth gauge. The ROV is therefore small and suitable for filming environments such as the Tegnùe, often partially covered with fine sediments that with a larger ROV equipped with more powerful engines would certainly be resuspended creating considerable turbidity (which is already normally present in the area). The field activities will be concentrated in 1 working day and will cover 4 areas within "IT3250047 - Tegnùe di Chioggia". The videos that will be recorded will be subject to the subsequent analysis phase and to draw up the list of detected species. The reference method (with appropriate modifications determined by the particularity and three-dimensionality of the investigated outcrops) follows the indications present in the methodological sheets drawn up by the Ministry of the environment for the protection of the territory and the sea, as part of the Monitoring programs for the Marine Strategy: Module 7; Coralligenous habitat. For each of the 4 transects, a route was chosen, which tends to coincide with the coast-wide gradient (east direction in our case), following both the modest gradient and the morphology of the outcrops being sampled, filming the outcrops also laterally, from the bottom to their upper part. For each transect of acquisition of HD video images and photographs, lasting about thirty minutes and not less than 100m in length, the ROV must be kept at a distance as constant as possible from the bottom of about 1m with a speed never exceeding 1,5 knots. The subsequent video analysis will allow to estimate the specific and / or taxonomic richness, i.e. the total number of megabenthic taxa, identified in the greatest possible taxonomic detail, found along the transept, as well as the observed fish fauna. The species will be reported with an indication of their ecological characteristics or will be divided into sessile benthic or vagile benthic as regards megabenthic organisms while they will be divided into benthic, bentonectonic and nectonic as regards fish fauna.

Figure 20. VideoRay Pro 4 Plus model ROV.

B) Photographic sampling survey by scuba diving

Species composition and abundances of the epibenthic assemblages on coralligenous biogenic reefs were investigated in July 2021, on 6 selected sites within the no-take zone off Chioggia (Figure 19). Assemblages were sampled using a non-destructive photographic sampling method as in Ponti et al. (2011). Photographic samples (21 × 28 cm, ~0.06 m2) were collected using a Canon PowerShot G15 digital camera (12 Megapixel; Canon, Tokyo, Japan), equipped with an aluminum underwater case, S-TTL strobe (Inon D-2000), and a custom stainless-steel frame. Ten randomly selected photos were analysed at each site. Organisms were identified to the lowest possible taxonomical level according to a reference collection of specimens previously photographed and withdrawn from the same sites. Percent cover of sessile organisms was quantified by superimposing a grid of 400 cells (i.e., 0.25% each) using the free available photoQuad software (Trygonis & Sini, 2012). Slight differences among sampling areas, due to dark and blurred zone or portions covered by vagile organisms, were accounted by standardizing to the total readable area of each image (Ponti et al., 2011). The endolithic bioeroder bivalve *Rocellaria dubia* (Pennant, 1777) was identified and quantified by counting its typical '8-shaped' calcareous siphon holes.

C) Gillnets sampling

This sampling campaign aims at estimating of the fish fauna associated with the Tegnùe of the Biological Protection Zone, including both the hard substrate and the nearby soft bottom areas. The sampling will be carried out in the same areas sampled between June 2005 and December

2007 by the ISPRA researchers (ISPRA, 2010) in order to compare the data on a time scale. The sampling will be carried out in two areas, Area 1 and Area 3 of the ZTB, in the same points sampled by ISPRA personnel, in the months of November 2021 and March 2022, and will be carried out with the support of a professional fisherman who has already carried out the sampling previously and who operates with artisanal fishing (gill nets). The sampling will be carried out using barracuda-type gillnets with these characteristics: length 400 m (10 pieces of 40m) with 30 mm of mesh (side), 1 piece of 30 m with a mesh of 20 mm (side) to also sample the smaller individuals; lead file of 80 gr / m. For each area, the sampling will be carried out in one night. Upon arrival at the site, the nets for soft bottom sampling will be lowered (within and outside the ZTB). The nets will be left to fish for 4-5 hours and then set sail. At the end of the sampling on the soft bottom, the nets will be lowered after reconnaissance with an echo sounder on the spiral rocky bottom and then set sail immediately. For each sampling the meteo-marine data, times and coordinates of laying and sailing of the nets will be recorded. After the windlass, all the catches will be identified, measured (total length with an ichthyometer; total weight with dynamometer or field scale), identified the sex if evident. If the sample is too large to be processed on board or for individuals of doubtful identification, some samples will be brought ashore, frozen and subsequently analyzed in the laboratory. The data will be inserted in excel sheets and analyzed, using the R software, with univariate and multivariate analyzes and compared with the data collected with the same methods by ISPRA.

D) Bioacoustics survey

The bioacoustics survey will be aimed at carrying out monitoring campaigns of the nekton community, at regular intervals, in the area of the Tegnùe di Chioggia, with the use of active acoustic instruments equipped with technology suitable for the discrimination of single targets (Scientific Echosounder), and for the quantitative determination of the biomasses (Anelli Monti et al., 2020, 2021). The 3 acoustic sampling campaigns covering the seasonal variability within the project period, are expected to take place between September 2021 and March 2022. Sampling will be carried out from the boat in movement. Each campaign will include one sampling for each of the 4 separate areas of interest above the Tegnùe di Chioggia SCI (4 sampling in total for each campaign). The samplings will have to be distributed in order to cover the entire tidal cycle and potential variations connected to the day/night cycle. The sampling sites are disposed through a transect orthogonal from the coast, with the aim of obtaining information about the entire extension of the area. Echosounder Scientific model Simrad WBT Mini is used for the elaboration of the data collected, the estimation of the observed biomasses, the fluxes transiting above the Tegnùe and the characterization of the species found.

2.5.5. Personnel and resources involved

The fieldwork will be carried out by CORILA, which will make use of specialized companies, in possession of the necessary tools for the purpose of the specific activities, and the collaboration of experts in the sectors both for the in the field activities and for the production and analysis of data.

As for the investigation with ROV instrumentation, it is necessary to have a boat with pilot and GPS, to accurately identify the diving points of the instrument, an operator with extensive experience in driving the ROV and 1 expert researcher for the first analysis of the images that are rendered in real time on the screen of the PC connected to the machine. For the secondary video analysis phase, 1 or 2 biologists expert in the identification of benthic, bentonectonic and neptonic organisms in the Tegnùe di Chioggia area will be involved. As regards the catches for the estimation of fish fauna, it is necessary to have a motor trawler, with standard crew, one or two researchers who are experts in this activity; it is important to rely on a professional fisherman who has already carried out samplings of this type and who operates with artisanal fishing (gill nets). As concerns Photographic sampling survey by scuba diving, it will be necessary to have a boat and 3-4 expert divers, diving equipment, camera. In the picture analysis phase specific software and expert researcher will be required. As for the bioacoustics survey is concerned, a boat and a specific Echosounder is required. In the analysis phase trained personnel will be required for the estimation of the biomasses and the fluxes transiting above the Tegnùe.

2.5.6. Closing Remarks

The proposed sampling design aims at achieving two main goals: integrating existing knowledge within the Tegnùe di Chioggia area, ensuring a continuity with monitoring efforts carried out in the framework of previous research initiatives, and assessing complementarity, applicability and cost-effectiveness of different monitoring techniques, applicable in the area. These objectives will be functional to the development of effective management plans for the study area, by integrating the existing knowledge supporting the characterization phase of the plan, and identifying appropriate tools for designing monitoring in the area.

2.6. P6: Miljašić Jaruga river mouth, Nin bay (HR); IOF

2.6.1. Introduction

The Miljašić Jaruga watercourse flows into the sea in the area of the Nin Bay. The last section of the watercourse passes through the urban area of the City of Nin. The bottom of a watercourse is partially covered with sediment, overgrown with grass and low vegetation. Furthermore, the riverbed stone facing is damaged and partially ruined, on some parts even totally ruined. The collapsed riverbed structures and deposited sediment reduce the flow profile of the watercourse and in some parts of the watercourse slow down the water flow. The last section of the watercourse, from the mouth 450 m upstream, is included in the other project of the rehabilitation of the Miljašić Jaruga. The mouth of the Miljašić Jaruga itself, which is going to be restored through this pilot project, consists of two groynes, east and west, both in very poor condition. The seabed between these two groynes and next to them, is also filled with silty sediment. The inner side of the eastern groyne is partially formed as a vertical maritime wall, on which an occasional (summer) berth is enabled (approximately 30 m long), and partially as a slope made of hand-folded stone (36 m long). The crown of the groyne is also made of hand-folded stone, but is partially collapsed. On the outside of the eastern groyne there is a rubble mound in rather poor condition. The western groyne was constructed as a stone embankment lined with rubble mound, but over time it has largely disappeared.

2.6.2. Description of the Pilot Area Climatic features

According to Köppen's climate classification, the islands and coastal area of Croatia belong to the areas where the olive climate prevails - moderately warm rainy climate (Csa) in which the dry period is in the warm part of the year, the driest month has less than 40 mm of precipitation and less than a third of the rainiest month in the cold part of the year (s), with two precipitation maxima (x”). Moderately warm rainy climate corresponds to the average temperature of the coldest month higher than -3°C and lower than 18°C.
The mean monthly temperature measured at Zadar station Is 14.9°C, with the minimum monthly mean temperature 7.3°C in January, and the maximum 23.9°C in July. The absolute minimum temperature was measured in January and is -7.9°C. The absolute maximum temperature was measured in August and is 36.1°C. The average annual rainfall is 879.2 mm, with the minimum average monthly rainfall of 30.4 mm in July, and the maximum average monthly rainfall of 106.7 mm in October.

Extreme precipitation during the storm that hit Zadar and its surroundings on September 11, 2017 caused torrential floods and overflow of watercourses in the Miljašić Jaruga catchment area, which resulted in great material damage in the town of Nin. From Sunday night (10.9.2021.) until Monday (11.9.2021.) in the evening, about 305 mm of rainfall fell in Zadar, and in Zemunik airport about 325 mm (Cvitan et al., 2017.). Recorded 10 to 50-minute maximums represent record values in a series of ombrometer measurements since year 1961. Recorded precipitation maximum for duration from 60 minutes to 24 hours, as well as daily and multi-day (up to 5 days) maximums in September 2017 are the second largest (behind the maximum of 1986) ever recorded maximum in the same long-term sequence for Croatia. The precipitation quantities for

certain durables were two to four times higher than the corresponding medium (1961 - 2015). Such maximums represent very rare phenomena and according to the theoretical estimate of the expected extremes can be expected once in more than 100 years.
According to DHMZ simulation the largest changes in mean air temperature in Croatia in period 2011 - 2040 are expected in summer, when temperatures could rise by around 0.8°C along the inner Adriatic coast, as well as in the central and southern Adriatic (Đurin & Muhar, 2017.). These changes will act unfavorable to water resources or human activity. It will increase the need for water from the population as well as for irrigation purposes. Since the evapotranspiration is associated with the temperature, the shortage of water will increase, which, with the negative impact on agriculture, can lead to disorders in the circulation cycle of water, or to a slower renewal of aquifers and watercourses. The biggest changes in the seasonal amount of precipitation in the near future in the P1 period are projected for autumn when the rainfall can be expected to reduce precipitation mostly between 2% and 8%. In other seasons, the ENSEMBLES model projects an increase in precipitation (2% - 8%), except in the spring on the Adriatic, where the reduction of the rainfall from 2% to 10% can be expected. Reduction of precipitation on the Adriatic in the fall and spring is reflected in changes in precipitation on an annual basis - on parts of the northern and middle Adriatic in the near future, 2-4% less precipitation can be expected.

Geological features

The Nin area is built of Cretaceous, Paleogene and Quaternary deposits. Quaternary deposits, among other, are alluvial sediments which are in correlation with the Miljašić Jaruga watercourse. The area of the project consists of Holocene, at the same time the youngest deposits of alluvium (al). Alluvium is a sediment of flowing waters deposited with stronger occasional flows, and it was formed by the wear of primary rocks, especially clastic alluvium, and by the redepositing of Quaternary sediments, especially diluvium and red soil. The sea sediment in the wider project area according to the granulometric composition largely (70%) belongs to the sandy fraction of the medium diameter of 0.1 mm, followed by powder fraction (25%) of a medium diameter 0.01 mm.

Water

The project area is part of the following areas of special water protection:

- Areas of water protection intended for human consumption: “Adriatic basin – mainland”
- NATURA 2000 areas: “SZ Dalmacija i Pag, Ninski zaljev” and “Privlaka – Ninski zaljev – Ljubački zaljev”
- Areas of poor water exchange by coastal waters: sensitive area basin and eutrophic

area “Ljubački i Ninski zaljev”

Groundwater from the wider project area belongs to the groundwater body JKGI_09 - Bokanjac-Poličnik (Figure 21), which is characterized by fracture-cavernous porosity. The condition of the groundwater body is poor. The poor chemical status of the water body is due to the intrusion of salt water. Poor quantitative status is a consequence of the depletion of renewable groundwater reserves during prolonged summer drought periods at the Bokanjac water intake. Watercourse Miljašić Jaruga represents a surface waterbody JKRN0052_001, Miljašić Jaruga (Figure 21), which is in a moderate state.

The coastal sea of the Nin Bay, into which the Miljašić Jaruga flows, belongs to the coastal water body Southern part of Kvarnerić O423-KVJ (Figure 21). It is a coastal waterbody of the type "euhaline coastal sea of fine-grained sediment". This coastal waterbody is in good condition.

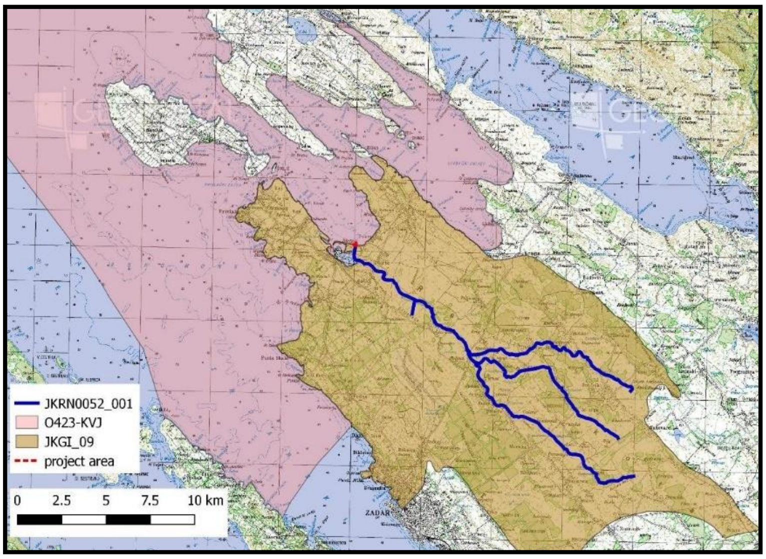

Figure 21. Waterbodies in wider project area (Hrvatske vode, 2021)

Long-period sea oscillations

Long-period sea level oscillations (periods greater than 1 min) are largely caused by the action of tidal force as well as by the action of atmospheric forces, primarily air pressure and wind. The tidal force has a periodic character, with the noticeable half-day and daily components. The influence of the tidal force can change the level of the Adriatic Sea, which represents a semi-grained pool, from 30 cm in the southern Adriatic to 1 m in the northern Adriatic. In the area of Nin and Privlaka, the average daily oscillation of sea levels (medium amplitude) is 29.5 cm. On the annual and multiannual time scale the most significant oscillation in the Adriatic has a seasonal character, caused by meteorological and climatic processes in the atmosphere as well as the processes in the sea. Increasing the time scale, the sea level is also subject to geo-tectonical changes of the sea bottom, as well as anthropogenic influence.

The nearest tide gauge station for the project area is the one in Zadar. Extreme fluctuations of the sea level registered with a tide gauge set in Zadar are presented in Table 7.

	[m]
HWLRP=50g.	1,1
HWLRP=2g.	0,8
HWLRP=1g.	0,6
MWL	0
LWLRP=2g.	-0,5
LWLRP=50g.	-0,7

HWL-high level
LWL-low level
MWL-mean water level

Table 7. Extreme sea level fluctuations (until year 2000) according to the recorded data of the tide gauge station in Zadar

Biodiversity

Project area belongs to the Natura 2000 conservation area significant for birds 2HR1000023 SZ Dalmacija i Pag" and conservation areas significant for species and habitats types "HR3000176 Ninski zaljev" and "HR4000005 Privlaka - Ninski zaljev - Ljubački zaljev" (Figure 22).

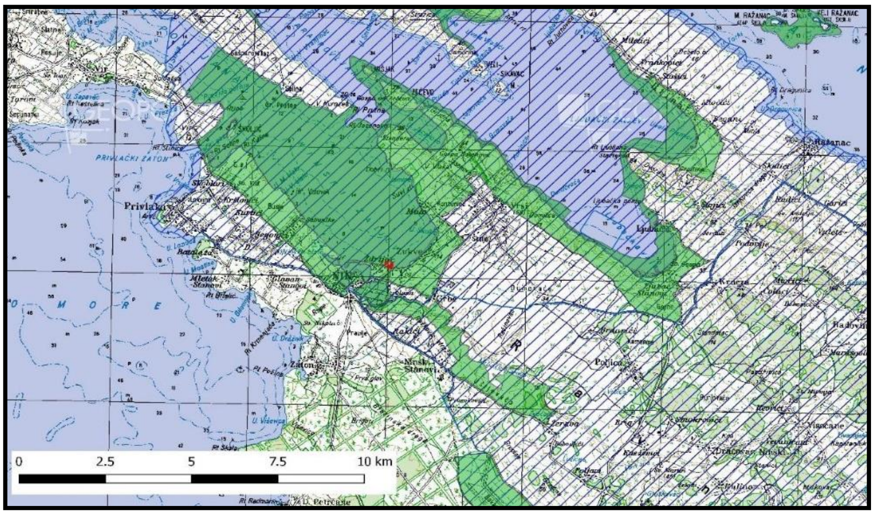

Figure 22. Natura 2000 sites in wider project area (Bioportal, 2021)

The SPA HR1000023 SZ Dalmacija i Pag covers NW part of Dalmatia near the Zadar and also includes the island of Pag. The SPA covers 59893 ha and 39.89% of it is marine area. This is the most important wintering area for waders, divers, Sandwich Tern, sea ducks and grebes, and the most important breeding site of Kentish Plover in Croatia. Nin saltpans is important wintering area for many birds that need such habitat as well as important stopover site during migration.

The SCI HR3000176 Ninski zaljev is marine site characterized by shallow water and a low sandy shore. Maritime domain in the city of Nin is specific by the shallowest and sandiest coast in the county, which resulted in the construction of salt pans. In recent years, plateaus terrace for drying salt has been neglected, and their coast is being covered with various waste materials. The depth of the lagoon is steadily reducing due to the sand deposits, which is limiting navigability. This marine site id one of the most representative sites for Mudflats and sandflats not covered by seawater (code 1140) and Sandbanks which are slightly covered by sea water all the time at low tide (code 1110). Important habitat type of the site is also totally or partially submerged sea caves (code 8330). The site covers 2259 ha.

The SCI HR4000005 Privlaka - Ninski zaljev - Ljubački zaljev is important site for Mediterranean and thermo- Atlantic halophilous scrubs (Sarcocornetea fruticosi; code 1420) and one of the most representative sites of Mudflats and sandflats not covered by seawater at low tide (code 1140). In addition, the habitat types present on the site are Salicornia and other annuals colonizing mud and sand (code 1310), Embryonic shifting dunes (code 2110), Mediterranean salt meadows (Juncetalia maritimi; code 1410) and Caves not open to the public (code 8310). The area of this ecological network site is 2002 ha.

According to the official Habitat map of the Republic of Croatia, the project area covers the following habitat types (Figure 23):

- A.2.3. Permanent watercourses
- F.3.2/C.3.5.1./I.1.2. Supralittoral gravels and stones / Sub-Mediterranean rocky pastures of Eastern Adriatic / Mediterranean weeds and ruderal vegetation
- G.3.2. Infralittoral fine sands with more or less mud

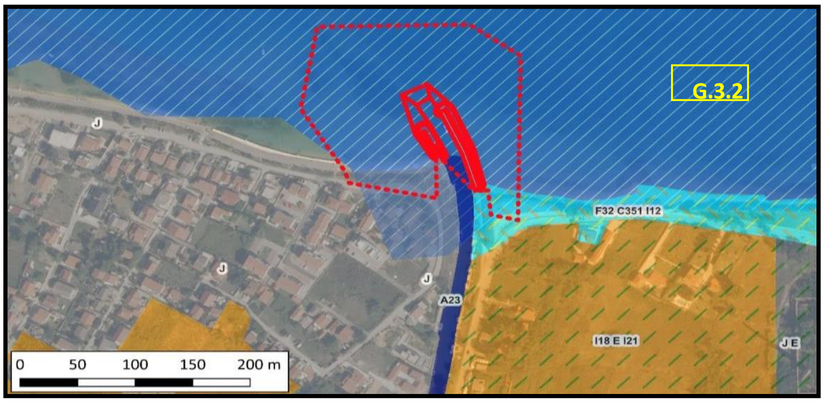

Figure 23. Habitat types in the project area according to Habitat Map of the Republic of Croatia (Bioportal, 2021)

Cultural heritage

Life in the Nin area began in the Neolithic era, as evidenced by the discovery of the remains of a Neolithic settlement at the position of Čvrljevića ograda, in the south-east part of the inner Nin lagoon. At the transition from the Bronze Age to the Iron Age, the Nin area was occupied by the Liburnian community, which established a significant settlement on it as the centre of the territorial community.

Fifteen archaeological sites and zones and ten monuments of architectural heritage have been recorded in the area of Nin, most of which refer to churches, and then to the medieval walls, the Upper and Lower Bridges. Most of the monuments are located in the centre of the city, on the island of Nin, while archaeological sites and zones are located both on the island and in the surrounding area.

In the wider area of Miljašić Jaruga, at the position of Čvrljevića ograda in the south-east part of Nin salt pans, on an area of about 40x100 m the remains of settlements from the early Neolithic were found, consisting of ceramic fragments decorated with *impresso* ornaments, fragments of grindstones made of sandstone, flint tools, sea shells and animal bones.

Cemeteries from prehistoric, ancient and early medieval times were discovered on the Ždrijac peninsula, and the remains of two medieval ships were found in the shallow sea at its western end. In addition, along the northern coast of the peninsula in the shallow sea, rows of wooden pylons possibly used as fishing traps have been recorded, but this assumption needs to be confirmed by additional arguments. About 400 m west of the mouth of the Miljašić Jaruga, at a depth of 3.4 to 4 m, about 150 m from the shore, the cluster of Roman roof bricks and gutters was discovered, under which a group of metal tools, a copper bucket, a glass balsamarium, a ceramic jug and several larger fragments of amphorae, dating to the 6th or 7th century AD. Since the finding was only protectively explored, it was not possible to determine whether it was a sunken ship or only part of the dumped cargo.

At the place where the peninsula separates from the mainland, a group of 60 amphorae were traced by protective archaeological research, in which, in most cases, their throats and shoulders were removed, after which they were buried in the vertical position in the sand. Amphorae laid in this way were discovered on several occasions along the northern side of the peninsula, during extremely low water levels. Although several ways of secondary use of amphorae have been recorded in the literature and in the field, which may be related to the situation in Nin (geotechnical or hydrogeological construction, planting vessels, funeral gardens), none of the assumptions can be confirmed with certainty.

Sediment granulometry

In the sediment of the Nin Bay in the wider area of the project, the sand size fraction with a medium diameter of 0.1 mm prevails with dominance percentage of 70%, and is followed by the silty fraction with a medium diameter of 0.01 mm and 25% dominance.

2.6.3. Targeted actions and final goals to achieve

The project area covers about 2.5 ha of sea and coastal area. The project is planned to regulate the mouth of the Miljašić Jaruga in the Nin Bay, taking into account the valuable Natura 2000 areas to which the mouth belongs, the rich cultural heritage of the city of Nin and the protection of water bodies in the area.

2.6.4. Materials and Methods

Coastal and marine habitats

In order to explore habitats of the project area, in July 2021, a submarine diving inspection was performed.

An inspection within the final part of the constructed canal (space between two groynes) at the mouth of the Miljašić Jaruga confirms the presence of species typical of coastal and pelagic seaport habitats. Anthropogenic influence on mediolittoral biocenoses within the canal itself and the immediate exit to the sea is visible. The mediolittoral zone is dominated by the presence of snails Patella rustica and *Chthamalus stellatus*. The concrete slope at the end of the canal 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 town of Nin. In the infralittoral zone, in the final part of the canal, the bottom of the silty type is covered with a thick layer of green algae of the genus Ulva spp. The depth is about 2 m in the middle of the channel. This indicates eutrophication and the anthropogenic impact of the canal, as these algae are characteristic of shallow polluted seas (Turk, 2011). Green algae of the genera *Codium* and *Ulothrix*, brown algae *Zanardinia typus*, *Padina pavonica* and *Dictyota dichotoma* appear on the rocky base along the edges of the canal. On the outer part on the side of the canal (towards the north side) on a silty and sandy bottom, a meadow of little Neptune grass Cymodocea nodosa has developed. The benthos fauna of silty sediments is dominated by sea cucumbers of the genus *Holothuria* and *Hexaplex trunculus*. Also, the population of the noble pen shell *Pinna nobilis* was observed, but a more detailed examination did not reveal live individuals but only shells in the sediment. This is a consequence of the occurrence of mass mortality in the Mediterranean and the Adriatic during 2019 and 2020 (Šarić et al., 2020). By moving away from the canal on both sides, the bottom changes from silty to sandy, and is occasionally interrupted by smaller and larger rocks overgrown with brown algae. The dominance of purple sea urchin Paracentrotus *lividus* and sea cucumbers of the genus *Holothuria* was observed on the sand. The rocks and larger stones are dominated by the European flat oyster Ostrea *edulis* and the Noah's ark shell Arca noae. The gastropod Hexaplex trunculus sporadically appears on the sand. Regarding marine sponges, the species Crambe crambe and *Aplysina aerophoba* were observed. Regarding sea anemone, the species *Condylactis aurantiaca* and *Anemonia viridis* are present. The presence of the seagrass *Posidonia oceanica* was not observed. On both sides of the canal there are sandy shores that are used as beaches during the tourist season, in some places flooded with outgrowths of the species *Cymodocea nodosa*.

Cultural heritage

In order to explore the project area regarding possible archeological findings, in August 2021, a submarine diving inspection was performed. The inspection began in the interior of the river mouth, in the area where the Miljašić Jaruga flows into the sea. In this area, the eastern and western groynes are built as slopes made of hand-folded stone, the seabed is silty and partly covered with seagrass, and visibility in the water is extremely limited. Miljašić Jaruga brings silty material to the estuary in which no archaeological findings are observed. After that, the inspection continued along the eastern groyne built as vertical maritime wall, and along the ruined rubble mound on its outside. Visibility in that area was also limited, but slightly better than in the Miljašić Jaruga itself. Rare fragments of recent construction waste have been observed on the surface of the seabed, but no archaeological findings have been recorded. A similar situation was repeated on the west groyne, in the area of the ruined rubble mound, where there are a large number of people during the summer months. A slightly denser concentration of seagrass was observed on the seabed, but without traces of material that could indicate historical traces of human presence.

Content of hazardous substances in sediment

Since the project includes excavation of the silty material from the mouth of the Miljašić Jaruga (deepening), in order to determine if hazardous substances are present in sediment, in July 2021, sampling and analysis of marine sediment was performed.

2.6.5. Personnel and resources involved

Inspection was performed by autonomous diving with photo and video recording. Biocenological analysis of seabed communities was performed according to the Brown - Planket method, and the nomenclatures Peres and Picard (1964) and Bakran - Petricioli (2007) were used for terminology. Habitat names were determined using the National Habitat Classification of the Republic of Croatia (version 5).
Analysed parameters are defined by the Ordinance on methods and conditions of waste disposal, categories and operating conditions for landfills (OG 114/15, 103/18, 56/19). Results of the analyses showed that silty material at the mouth of the Miljašić Jaruga belongs to non-hazardous waste.

2.6.6. Closing Remarks

Thanks to the mentioned activities, operative plans for the study and protection of the rich cultural heritage and water bodies will be developed.

2.7. P7: Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC) (IT)¸ PP: UNIMOLISE

2.7.1. Introduction

UNIMOL (PP13) implemented observing systems on the Pilot area P7 (Molise coast) and P10 (Torre Cerrano MPA coast) including coastal transitional areas. We focused monitoring activities on native salt marsh vegetation (wet brackish mosaics of the Biferno mouth) and on coastal dunes vegetation (of the Torre Cerrano Marine Protected Area). In these two areas, several monitoring activities will be conducted in order to characterize the coastal environment and to evaluate the conservation status of endangered species and ecosystems.

2.7.2. Description of the Pilot Area Biferno mouth wet brackish area

Unimol implemented an observing system for coastal environment characterization on Pilot area P7 (comprising 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; Figure 24). In these area, several activities are conducted in order to characterize the coastal environment and to assess the conservation status of endangered species and ecosystems using bio-indicators. Specifically, we focused on Biferno wet brackish area, located in the central part of Molise coast, 1.5 km south of the mouth of the Biferno river. The area is part of the S.A.C. IT7222216 "Foce Biferno - Litorale di Campomarino" and presents plant communities typical of brackish wetlands which constitute residual strips of ecosystems widespread in the past and which are of great interest for the conservation of biodiversity.

The mouth of Biferno river is characterized by the presence of humid interdunal depressions, with salty soils, which host a complex mosaic of habitats of European conservation concern (Council Directive 92/43/EEC of 21 May 1992 on the Conservation of natural habitats and of wild fauna and flora) which includes:

- 1310 Salicornia and other annuals colonizing mud and sand;
- 1410: Mediterranean salt meadows (Juncetalia maritimi);
- 1420: Mediterranean and thermo-Atlantic halophilous scrubs (Sarcocornietea fruticosi);
- 1430 Halo-nitrophilous scrubs (Pegano-Salsoletea);
- 1510* Mediterranean salt steppes (Limonietalia);
- 2230: Malcolmietalia dune grasslands;
- 2240: Brachypodietalia dune grasslands with annuals;
- 3170*: Mediterranean temporary ponds;
- 6420: Sub-pannonic steppic grasslands.

For a detailed description of these habitats see Angelini et al 2016.

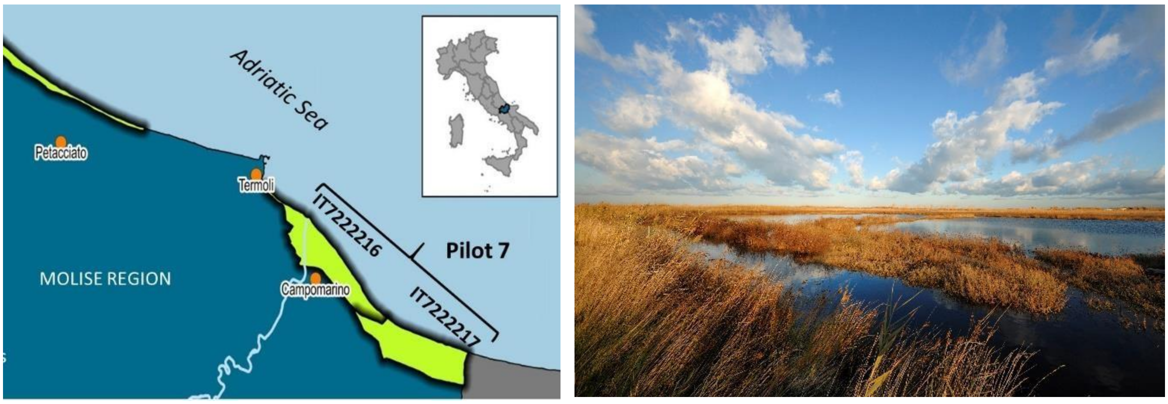

Figure 24. P7 Pilot Area is part of the Long Term Ecological Research network (LTER - https://deims.org/088fe3af-c5bb-4cc8-b479- fe1ea6d5be80) and includes two N2k sites: Biferno river mouth-Campomarino Coast (IT7222216), and Saccione mouth Bonifica Ramitelli (IT7222217). Here we focused on Biferno mouth wet brackish vegetation mosaic. Photo: Angela Stanisci.

The integrity and the survival of such hot spot of salty biodiversity is impinged by the surrounding touristic settlements, by agricultural and recreational areas and by the wrong management and abandonment of hydraulic structures and drainage channels. In this area, reclamation works, intensive agriculture and touristic settlements have greatly reduced the extent of natural vegetation, leading to a trivialization of the natural flora and the introduction of invasive alien species (Stanisci et al. 2008). Furthermore, the entire Molise coast is crossed by the railway and motorway lines, which in some sections run very close to the dune and back-dune environments and which constitute a further element of habitat fragmentation, as well as a preferential route for the diffusion of exotic species (Carranza et al. 2018). Another disturbing factor affecting these coastal and river mouth environments is marine erosion, which in Molise has produced deep changes in the shore line with setbacks that in some coastal stretches have reached 250 m in 30 years (Iannantuono et al. 2004; Aucelli et al. 2008).
Two technicians of "Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA)" visited on 26 and 27 April 2021 the area for its ecological relevance and endangered in Italian Adriatic coast (Figure 25, http://envixlab.unimol.it/visita-di-tecnici-dellispra-monitoraggio-della-biodiversita-sulla-costa-molisana/).

Figure 25: UNIMOL researchers and ISPRA technicians during a field monitoring campaign. (http://envixlab.unimol.it/visita-di-tecnici- dellispra-monitoraggio-della-biodiversita-sulla-costa-molisana/) Photo: Marco Varricchione.

2.7.3. Targeted actions and final goals to achieve

The implementation of monitoring activities in the P7 salt marshes mosaic, are useful to characterize the coastal environment and to assess the conservation status of endangered species and ecosystems using bioindicators. Furthermore, with field monitoring campaigns and data elaboration we will increase the database of field monitoring data on saltmarshes and we will analyze how it have changed focusing on the role of vascular plants as bio indicators for ecosystem assessment.

2.7.4. Materials and Methods Data collection

The monitoring campaigns on wet brackish vegetation on the Biferno mouth river started in the year 2010.

Such monitoring work was carried out by the EnvixLab scientific research team of the University of Molise in collaboration with colleagues of the University of Roma Tre (Professors Maria Laura Carranza, Angela Stanisci and Giovanni Salerno).

The floristic monitoring design follows a stratified random frame which use a detailed land cover map (1: 5000 scale) (AA.VV. 2008) and the high-resolution color digital orthophotos (Flight 2007, granted by the Civil Protection) for identifying the strata to be sampled. During the growing season (April-October), 37 georeferenced floristic surveys (4x4m plots) were collected in EU Habitats of Community Interest (Council Directive 92/43/EEC) 1410, 1420, 1510*, 2230, 2240, 3170* and 6420.

For each georeferenced sampling plot all plant species and their cover/abundance according with the Braun- Blanquet scale (1932) were registered using the classic phytosociological approach.

Species nomenclature follows the updated checklist of "Flora d'Italia" (Pignatti 1982 and revised versions).
Ten years later, after an intervention of naturalization in the study area carried out by the project Life Maestrale NAT / IT / 000262 (http://lifemaestrale.eu/), the Biferno brackish areas was resampled to explore the temporal trends on the structure and composition of the vegetation in EU Habitats mosaic.
For this purpose, in the year 2020-21 (hereafter T2), we resampled the georeferenced vegetation plots of 16 m^2 (4 x 4 m) in the EU Habitats 1410, 1420, 1510* and 3170*. We resampled the same areas of the 2010. Data collection was performed following the same sampling protocol of 2010 (hereafter T1).

Data analysis

To investigate species composition over time (T1: 2010, T2: 2020.21) we focused on the role of vascular plants as bio indicators (Santoro et al. 2012), subdividing the different taxa in diagnostic, ruderal and alien species. Diagnostic species are those taxa that help guarantee the functionality and existence of the entire habitat and that are particularly sensitive to a range of threats. The diagnostic species can be considered as "keystone species". In nature conservation and management, the identification of diagnostic species is of great value for the drafting and application of specific environmental legislation. In fact, the EC Directive 92/43/EEC (Habitats Directive) (EEC 1992), one of the major steps towards a European strategy for nature conservation, lists a series of diagnostic species for the habitats of conservation interest. For example, diagnostic species indicated in the Directive for coastal dune habitats play a major role in determining the structure and functioning of these systems as, directly or indirectly, they control the availability of resources for other species. Moreover, they can cause significant changes to their environment allowing the creation, modification or maintenance of the surrounding habitat (Interpretation Manual of EU Habitats – European Commission 2007). Diagnostic species of the analyzed coastal habitats were assigned following the "Vegetation Prodrome" and the Italian Manual of the 92/43/EEC Directive Habitats. Ruderal species which are well adapted taxa to disturbance, and so, excellent indicators of ecosystem alterations.
is first to colonize disturbed lands. Alien species are those taxa introduced into a natural environment where they are not normally found, with serious negative consequences for their new environment. They represent a major threat to native biodiversity in Europe, causing damage worth billions of Euros to the European economy every year. Ruderal species and aliens were identified according with the Flora d'Italia" (Pignatti 1982, and successive editions; Conti et al. 2005; Celesti-Grapow et al 2009).
We compared the species richness patterns of the overall species pool and of diagnostic species

on considered time steps (T1: 2010, T2: 2020.21) by plot-based rarefaction curves. We also assessed the contribution of each species to temporal changes (T1 and T2) analyzing floristic data by a similarity percentage procedure (SIMPER; Clarke 1993).

Furthermore, to detect temporal changes in the ecology and the structure of the analyzed vegetation, we used Ellenberg bioindicator values (Ellenberg, 1974) of temperature (T), moisture (U), soil nutrients (N), luminosity (L) and salinity (S) and the life-form categories of Raunkiaer (Raunkiaer, 1934). Ellenberg's indicator values is a numerical system to classify species' (on a simple ordinal classification) according to the position of their realized ecological niche along an environmental gradient (e.g. temperatures, moisture, nutrients, luminosity, salinity, etc.). The Raunkiaer life forms system categorize plants using resting buds position and depict the structure and function of the different species present in a plant community.

We compare the ecology (Ellenberg values) and structure (Raunkiaer life forms) in T1 and T2 using Mann- Whitney test. All analyses will be performed in the R statistical computing program (R Core Team 2020) using Rarefy package and PAST (paleontological statistics software for education and data analysis; Hammer et al. 2001).

2.7.5. Personnel and resources involved

The field work is carried out by at least 2 operators. First, plots are identified by the GPS, in order to collect data in the same area of the previous field campaign. Then a plot of 4x4 m is laid out and as 1 researcher fill the field sampling grid, the other observe and measure all vascular plants present in the plot (Figure 26). Collected data will be gathered and stored in a unique database of coastal ecosystems as part of LTER data monitoring data. Such information will allow to describe ecological changes on the analyzed area and to compare them with similar ecosystems occurring on areas with different management and disturbance regimes.

Figure 26: field work carried out by UNIMOL (P13) CASCADE team in the wet brackish vegetation mosaic characterizing the Biferno mouth river (P7). Photo: Marco Varricchione.

2.7.6. Closing Remarks

The proposed and implemented ecosystem assessment through Bioindicators, is fully coherent to WP3 targets which consist also of the use of monitoring systems and data collections for protecting biodiversity and ecosystems. Furthermore, it offers a sound support for implementing WP4. The collected data and the obtained results of statistical analysis will offer new insights on ecosystems conservation status, using some species functional groups as indicators. The monitoring campaigns and the analysis of the turnover of some "bio indicator species" could help to: better understand the ongoing ecological processes, identify early warning signs of ecosystems degradation and build empirical models of ecosystem change facing global change (WP4). Furthermore, based on monitoring data we could propose conservation actions and improved management approaches of the analyzed ecosystems.

2.8. P8: Northern-eastern Adriatic in Croatia (HR); IRB

2.8.1. Introduction

Ruđer Boškovic Institute Center for marine research is the managing authority of the Pilot area P8 (Figure 27). P8 includes coastal, transitional and open sea -marine areas. In these areas different activities will be conducted in order to characterize the coastal-marine environment and to evaluate the conservation status of endangered species and ecosystems.

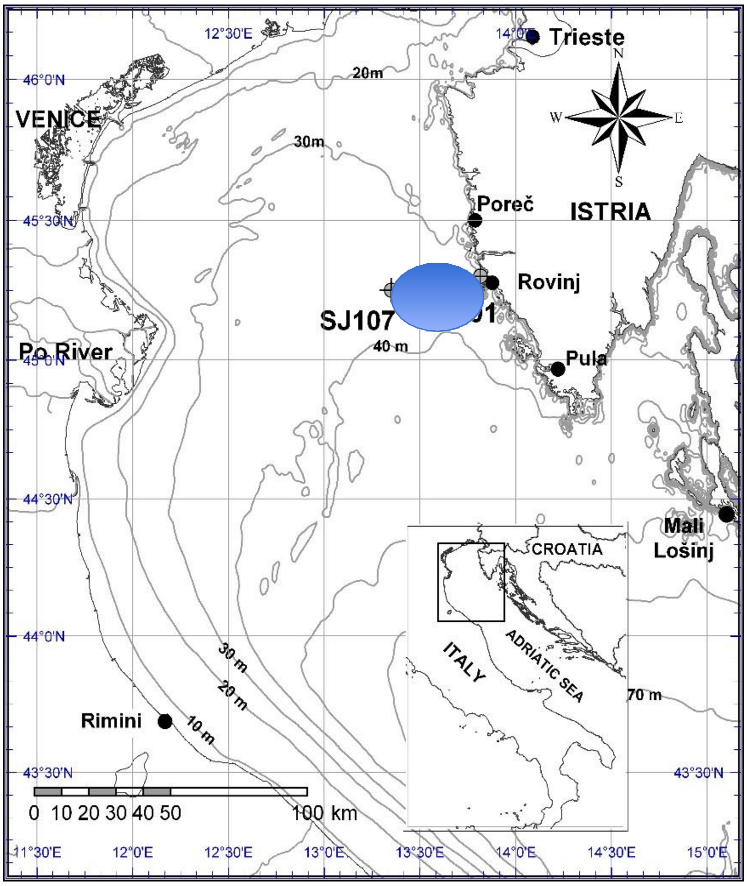

Figure 27. P8 Pilot Area is depicted in blue.

2.8.2. Description of the Pilot Area

The Northern Adriatic Sea

The northernmost part of the Adriatic Sea, north to the Rimini-Pula section is a shallow (up to 50 m), landlocked area. It receives freshwater inputs from the Po River, one of the Mediterranean's largest freshwater sources. Main winds in the region are dry bora, blowing from NE, and jugo, moist wind blowing from SE (Cushman-Roisin et al., 2001[1]). The basic hydrographic conditions of the region, temperature, salinity and density, are influenced by air-sea interaction and Po River discharge (Supić and Ivančić, 2002). Although wind induced currents of the region are pronounced (Kuzmić et al., 2007[2]) the geostrophic component presumably plays a major role in redistributing freshened waters and organic/inorganic substances across the NA (Supić et al., 2012[3[). Processes in the ecosystem of the region depend on sea conditions and are thus dependent on changes in air-sea and hydrological (mainly Po River) forcing.

The NA has been monitored since 1920, when continuous bi-weekly measurements of temperature and salinity started in the coastal zone off Rovinj. With few data gaps the measurements continued until today and the dataset obtained is among one of the longest time series in the Mediterranean area, enabling a good insight into climatic changes of basic hydrographic conditions of the region. More comprehensive monitoring of various physical, chemical and biological data in the northern Adriatic started in 1972 and included six stations at section between the Po River delta and Rovinj, monitored seasonally or monthly. Based on that long-term monthly dataset the basic characteristics of the NA ecosystem were described in numerous publications. The pronounced variability in year-to-year cycles in it were observed and related to interannual changes in atmospheric, hydrologic and sea dynamic conditions.

Considering the long term research of thermohaline properties carried out in front of Rovinj (CRM data set from 1921), station RV001 (1 Nm west off Rovinj) could be used as an adequate reference for the yearly average of these properties (Figure 28; seasonal changes in sea temperature (t), salinity (s) and reduced density (γ) for some depths during the period 1972-2006).

Approximately 520 microphytoplankton species (including several cryptic species complexes) are catalogued for the NA. Their identification relies on light- and electron microscopy. State of the art in microphytoplankton monitoring is the measurement of chlorophyll a concentrations or the light microscopic identification and quantification of groups and several selected species. The exhaustive dataset of the CIM Rovinj for the NA however contains microphytoplankton quantifications at species level and thus already exceeds the state of the art. The further inclusion of molecular taxonomy and ultrastructural analysis allows the identification and quantification of species, subspecies and populations, which spearheads microphytoplankton.

2.8.3. Targeted actions and final goals to achieve

Therefore, the main circumstances that allow us to address some of the question posed through the review part are that half way up the Istrian coast to the NA and a mile off the coast, the regularly investigated sampling station RV001 is in alternating cycles under the influence of a variety of different ecological conditions. A 40 years spanning set of monitoring data delivered a sound background for a modern and in depth functional analysis of planktonic systems. At the aforementioned point of convergence, we can sample alternatively engrossing water originating from the central Adriatic, residual water from the NA, water under strong influence from the River Po (the largest freshwater and nutrient source in the area) and water under the strong influence of coastal effects like surface runoff or anthropogenic influences. We therefore, at this one point, can investigate snapshots of the largest variety of ecological states of the water column. Those snapshots will deliver a good and integrated picture of the metabolic and systematic capacities of the NA. The aim of this study is to document *in situ*, how the biological and chemical systems function under the largest variety of ecological conditions observable at one point. This is also a great spot to track new and invasive species.

2.8.4. Materials and Methods

The fieldwork is carried out from June 2020 to spring 2021, on RV001 and RV004 (1 AND 4 nm from Rovinj (Table 8).

STATION	LATITUDE	LONGITUDE
RV004	45.06185	13.549164
RV001	45.08000000	13.61000000

Table 8. Station Let and Long

Sampling and in situ measurements will be performed monthly with the RBIs' research vessels "Burin" or "Vila Velebita", resulting in more than 48 samples of diverse oceanographic parameters.

Sea water measurements will be performed with CTD profiler and sampled with Niskin bottles with reverse digital thermometers. Routine oceanographic methods will be used to determine the dissolved oxygen content, nutrients and chlorophyll *a* concentrations, pH and alkalinity (Parsons et al., 1985[4[4]).

Sea water samples for the composition and structure of phytoplankton community and an estimate of phytoplankton biomass will be collected with the standard oceanographic method during the field studies in the coastal waters of the eastern Adriatic at stations RV001 and RV004. Biodiversity analysis will encompass the following activities: Water samples will be analyzed microscopically (LM) to determine microphytoplankton diversity. On the background of the aforementioned microscopical analysis and on the pre-existing long term data, molecular biodiversity analysis (metabarcoding) will further complete the knowledge on planktonic biodiversity of the NA. DNA-extractions for all samples will done and analysed via next generation sequencing analysis methods. **This will dramatically enhance the knowledge on molecular biodiversity of the NA** and reveal hidden biodiversity especially in groups which cannot be distinguished microscopically.

For phytoplankton metabarcoding live net and Niskin sample will be filtered on polycarbonate membranes no later than 2 hours after sampling and stored immediately in -80°C freezer until analyses. Samples for the determination of phytoplankton composition and relative abundance will be preserved in formaldehyde for later analysis in laboratory on land [67].

For whole community genomic DNA extraction (DNA metabarcoding), filters stored on -80ºC, community genomic DNA extraction will follow a non-commercial method based on Sigma-Aldrich GenElute™-LPA DNA precipitation. Two DNA barcodes will be amplified for every sample;18S rDNA (V4 region) and rbcL plastid gene (5' region of a large subunit), both of ~300-bp sequencing length. Sample libraries construction and NGS sequencing will be performed by the IMGM Laboratories GmbH (Germany) using the MiSeq Illumina technology. All the fastq files will then be treated together following the bioinformatics process using the Mothur software. Bioinformatics process (pipeline) has been adapted and modified in LEE (CIM) for high quality diversity assessment of marine samples. Phytoplankton molecular inventories will be obtained using the R- syst:diatom library, Silva and NCBI GenBank database (Schloss PD [6]).

Phytoplankton biomass will be estimated by chlorophyll a measurements with the fluorometric method (Strickland and Parsons, 1972 [5]) on the fluorometer Turner TD700. The composition and structure of phytoplankton community will be analysed by the method of sedimentation and counting after Utermöhl method. [6] (1958) on an inverted research microscope (Zeiss Axiovert 200).

2.8.5. Personnel and resources involved

The fieldwork will be carried out by at least 5-6 operators each time: 1 or 4 people are needed to drive the support vessel while four technicians will be engaged in the actual field work.Most of the resources will be invested in the acquisition of data about the molecular diversity in the region and the establishment of high throughput analyses in the frame of biodiversity monitoring. Additionally, the resources are invested in the acquisition of moored sensor platforms that will allow continuous data acquisition and near real time access to digitalized measurements of oceanographic parameters. Oceanographic buoys equipped with two groups of sensors: a sensor group for the air and a sensor group for the sea will be positioned 1 and 4 miles off the coast respectively. Sensors for the air will be at an altitude of 6 m above sea level, while the sensors for the sea are at a 1.5 m depth and throughout the water column. The sensor packages will cover all autonomously and automatically measurable, physical, chemical and biological oceanographic parameters. The continuous measurements of meteorological and oceanographic parameters will allow the continuation of 40 years' long-term measurements of basic hydrographic conditions in the area and it will improve the temporal resolution significantly. Real time remote access to the measured parameters will improve the quality of operational oceanography in the area, safety at sea and quick insights into ecological changes in the area. The time series of collected data will be presented and compared to existing long-term data series and used in climatology studies (Dadić et al., 2006).

pH (SeaFET) and active fluorescence sensors will be installed and calibrated. pH, a master variable of the CO_2 system, will show the changes and trend in sea surface buffer capacity. The active fluorescence measurement will describe how well phytoplankton experiencing different environmental conditions, such as stressful temperature or nutrient availability, can assimilate light or photosynthesize. These two sensors will enable a study of ocean acidification and its impact on the physiology and metabolism of marine organisms. Intranet availability of all data from the oceanographic buoy in real time in addition to regular bi-weekly sampling and measurements of main oceanographic/chemical (temperature, salinity, dissolved oxygen, nutrients-nitrate, nitrite ammonia, orthophosphate, total phosphorous, orthosilicate, chlorophyll *a*, pH and alkalinity) and biological parameters (plankton counts and composition and enzymatic activities) will be used to evaluate the health of the ecosystem in general and to identify variables (the main stressors) that indirectly or directly affect plankton physiology. According to identified stress conditions an event adapted sampling regime will be performed, i.e. diel variations of oceanographic, biological, physiological and chemical parameters to evaluate the actual coverage of ecological capacity of the system. During events seawater will be sampled after attainment of vertical gradients by CTD profiler. The microbial community structure and physiology will be studied at sensor and gradient depths.

For microphytoplankton diversity samples will be analyzed microscopically using light (LM), microscopy types following an improved and adapted Uttermoehl method (Godrijan, Marić et al. 2013). The knowledge on plankton biodiversity will be completed by molecular biodiversity analysis (metabarcoding).

Environmental sequencing of established barcode regions will deliver information on the overall biological diversity in the investigated plankton community. For that purpose, DNA-extractions for all the samples will be done, processed using molecular methods and analysed via next generation sequence analysis.

The characterization and the quantification of picoplankton with flow cytometry will provide insight into changes of picoplankton abundance and community composition in response to environmental forcing. The analysis will be obtained using the flow cytometer ACURI C6 plus. The different subpopulations of autotrophic picoplankton are going to be distinguished according to their autofluorescence of chlorophyll a (FL3) and phycoerythrin content (FL2) as well as by the cells' side-angle light scatter (SSC) as a proxy of their size as previously described. The different subpopulations of heterotrophic picoplankton (high-DNA and low-DNA bacteria) are going to be distinguished after a staining procedure with SYBR-Green (Marie et al., 1997). The data is going to be collected in list mode files using FL3 for autotrophic picoplankton and FL1 for heterotrophic picoplankton as a trigger parameter and processed with FloMax software (Partec, Germany).

2.8.6. Closing Remarks

This study aims to reach two goals: to provide a more accurate characterization of the marine phytoplankton communities in the considered area revealing the hidden biodiversity. This purpose is fully coherent to WP3 targets, which consist also of the use of monitoring systems and data collections for protecting biodiversity and ecosystems.
The collected results will be part in the analysis of the efficiency of operational forecasting system and, in turn, in the evaluation of the usefulness of its operational implementation. It is expected the pilot action could stimulate the roadmap towards an operational warning system for hazardous events like harmful algal blooms. Besides the forecast implementation, that reliable modelling system would be useful in the management of the ecosystem and in planning actions for its preservation, limiting the vulnerability to environmental stresses.

2.9. P9: Cetina river mouth (HR); PP: Sea and Karst

2.9.1. Introduction

PP15, which is Public Institution for the Management of Protected Areas in the area of Split-Dalmatia County “Sea and Karst” (Sea and Karts), is responsible for the management of Natura 2000 ecological network areas and protected areas of the Split-Dalmatia County. Currently, the Institution manages 39 protected areas and 148 areas of the Natura 2000 ecological network. The area of the Natura 2000 ecological network in the Split- Dalmatia County is 280,164.37 hectares (approximately 20% of the County's area), of which 180,882.53 hectares belong to the land and 99,281,840 hectares of marine area. Pilot Area Cetina Estuary is 100% marine area.
Ecosystem pilot characterization report show 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, 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. Probably there are changes in usual patterns of the water column salinity and temperature however there is no systematic monitoring. Also state of the ecosystem in relation to EU biodiversity directives is not carried out.
Accordingly, Sea and Karts will develop monitoring (observation) system for pilot site involving innovative techniques (e.g. satellite images, photogrammetry) and test it for benthic habitats (e.g. *Cymodocea nodosa* meadows*, Cladocora caespitosa* banks). In addition, equipment for

monitoring environmental conditions in the sea (temperature, salinity, oxygen, etc.) will be installed in the pilot area.

8.2. Description of the Pilot Area

Cetina estuary is a site of Natura 2000 ecological network (code: HR3000126) proclaimed as such in 2013. The site is located in south of Croatia, in front of the town Omiš and includes the mouth of river Cetina. The river Cetina is historically, economically and ecologically one of the most important rivers in the Adriatic basin. The length of its course is 104 km, while the area of the entire basin to the mouth of the Adriatic Sea is about 4,145 km2. The coastal belt that is under the hydrological influence (groundwater) of the river Cetina stretches from Vrulja in the southeast to the river Jadro in the northwest. It is a coastline about 30 km long. In the central part of this area is the mouth of the river Cetina protected as Natura 2000 site which key habitat types are 1110 - Sandbanks which are slightly covered by sea water all the time, 1130 - Estuaries, and 1140 - Mudflats and sandflats not covered by seawater at low tide.

Cetina estuary is a marine area of 6.67 km2. Lithostratigraphic unit represented around this area are cretaceous rudist limestone and eocene flysch sediments. 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 estuaries and for Petromyzon marinus reproduction (http://www.bioportal.hr/gis/) . Its' geographical coverage is from 4808485 on south, to 4811749 on the north, 511486 on west and 517597 on east (measures in HTRS96).

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 (UNEP/MAP, 2000). 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.)

SITE DISPLAY

Figure 28 Map of Natura 2000 site Cetina estuary

According to Water Framework Directive (WFD 2000/60/EC) this area has 3 water bodies of transitional water that as such were defined in 2012. Transitional water areas are "transitional waters" of inland waters near estuaries that are partly saline due to the proximity of coastal waters. The transitional type of surface water occurs between freshwater and coastal water, where the boundary with freshwater in the upper part of the watercourse is defined by the occurrence of salinity greater than 0.5 PSU, and in the estuary by a link between opposite estuaries or the appearance of a pronounced horizontal salinity gradient. Starting from the one with the hight influence of the fresh water, P1_2-CEP encompassing estuarian part of the mouth with total surface of 0.17 km2, then P2_2-CE with a surface of 2.18 km2 and the largest one which is partly included in Natura 2000 site P2_3-CE with its surface of 13.5 km2. Transitional water of river Cetina makes 10% of the total area of all transitional waters of the Republic of Croatia and is among those that have the largest number of water bodies and the greatest diversity of types, and thus the associated ecosystems.

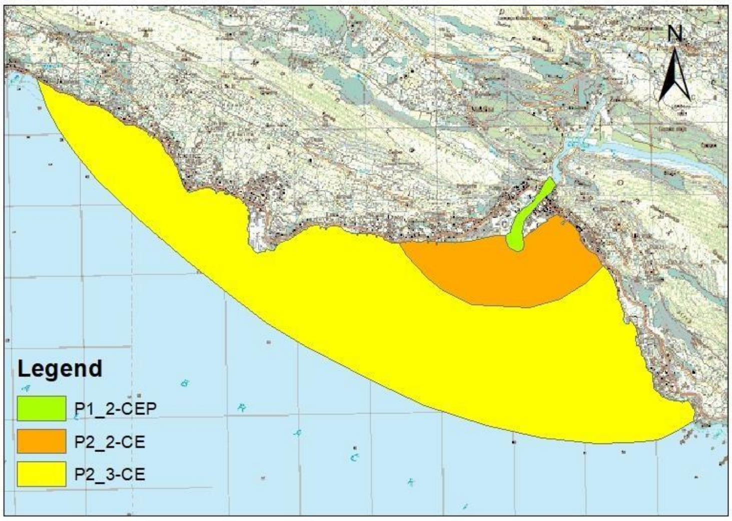

Figure 29. Cetina river transitional water bodies

8.3. Targeted actions and final goals to achieve

Final goal of setting-up an optimal observing system at Natura 2000 site Cetina estuary is to improve knowledge on the state of its 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, we will focus 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 include:

- Establishment of high-resolution monitoring of environmental conditions in the sea (temperature, salinity, oxygen, etc.);
- Assessment of a dominant benthic habitat i.e. *Cymodocea nodosa* meadows;
- Testing of a monitoring system focusing on key benthic habitat formers (e.g. *Cymodocea nodosa, Pinna nobilis*);
- 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;
- 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;
- Capacity building of managing authority for carrying out monitoring and conservation activities;

- Identification of risks and threats to the Pilot Area;
- Identification of conservation measures for the Pilot Area.

Besides informing local management, spatial information on the distribution and conservation status of Cymodocea nodosa meadows obtained by the methodology proposed herein will contribute to:

- The fulfilment of the national reporting obligations on the status of 1110 habitat type according to the EU Habitat Directive 92/43/EEC;
- The fulfilment of the national reporting obligations on the status of infralittoral sedimentary bottoms – Cymodocea nodosa meadows according to the Integrated Monitoring and Assessment Programme (IMAP) of the Barcelona Convention;
- The future evaluation of goods and services provided by this ecosystem (e.g. Casas et al. 2021), to be used (besides legally bounding strict protection status), as an additional argument to enforce effective conservation measures within the Natura 2000 site when addressing other sectors (e.g. tourism, fisheries) which may exert pressures on this habitat.

In general, such information is scarce across the Mediterranean Sea. Moreover, to our knowledge, CymoSkew index is going to be applied in the Eastern Adriatic Sea for the first time.

8.4. Materials and Methods

Continuous, high-resolution seawater temperature monitoring along a depth gradient

The proposed method for seawater temperature monitoring is based on scientific and field experience gathered within the T-MEDNet network (https://t-mednet.org/) over the period of almost 20 years. It is designed to acquire long-term, high-resolution information on temperature conditions along the depth gradient in coastal waters. According to this protocol sea water temperature conditions are characterized on the basis of hourly data records by HOBO Onset data loggers deployed and recovered over an annual or a semi-annual period. Data loggers are placed every 5 m from surface down to a chosen depth (usually 40 m but can be also placed shallower if such depth is not available in the selected coastal area) in order to acquire information about seasonal stratification dynamics and temperature conditions at depth. In the case of the Natura 2000 site Cetina Estuary dataloggers will be placed down to the maximum depth of 30 m at the site Mala luka and down to a 20 m depth at the site Shallow mark (Figure 30). In the long-term, these data series will build robust baselines and track hydrological changes (e.g. warming, heat waves, shifts in seasonality, stratification) to better understand the impacts of climate warming on marine coastal biodiversity (Garrabou et al. 2018). As an example, dataloggers at the site Shallow mark will be set next to the *Cymodocea nodosa* meadow, whereas dataloggers at the site Mala luka will be set in the vicinity of the *Cladocora caespitosa* banks. Assessment and future monitoring of conservation status of both habitats is envisaged by the proposed monitoring programme for the Natura 2000 site Cetina Estuary. Further information on the implementation of the protocol is available from the T-MEDNet web platform in form of video tutorials and presentations (https://t-mednet.org/observation-system/videotutorials).

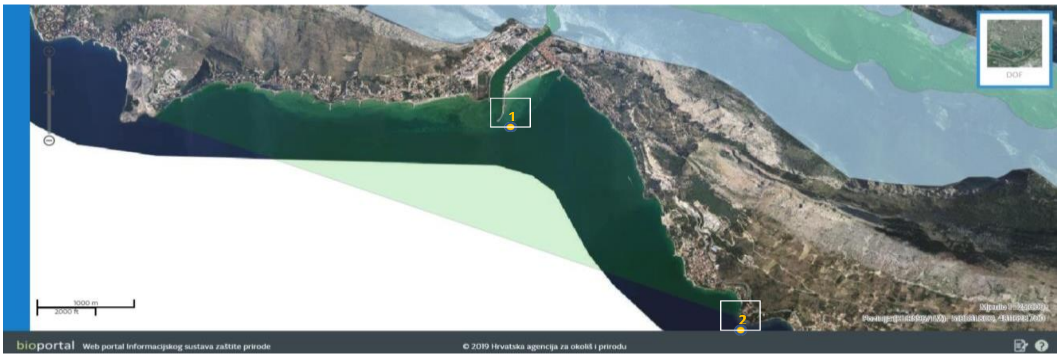

Figure 30. Proposed sites for the establishment of continous, high-resolution monitoring of seawater temperature: 1) Shallow mark (down to 20 m depth) and 2) Mala luka (down to 30 m depth). Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu.

MATERIAL

- HOBO® Onset data loggers: HOBO® Pendant temp/light
- Fixation kit – depending on a method employed e.g. Inox rings, ankles Colson, two component putty for underwater sealing, plastic gloves and bag, plastic screws, plastic ties, drill-in anchors (note: fixation method will be selected after the *in situ* inspection of the environmental conditions and a substrate type at the chosen site)
- Tool to scratch the rock prior fixation (e.g. diving knife) and a cutting pliers or scissors
- Hoboware software, base station and the appropriate coupler to launch the temperature data loggers

MONITORING TIME FRAME

- Continuous (filed download of data one/twice a year)

Continuous, high-resolution seawater, salinity and oxygen monitoring

In order to acquire information on the hydrodynamics between freshwater and coastal water an In-situ multiparameter probes will be placed at the Shallow mark at 5m depth (Figure 30). It will acquire data on temperature, salinity and dissolved oxygen. Data will be continuously transmitted to Public Institution Sea and Karst through a set of elements for telemetric data download from an in-situ multiparameter probe. In the long-term, these data series will build robust baselines and track hydrological changes (e.g. warming, heat waves, shifts in seasonality, stratification) to better understand the impacts of climate warming on marine coastal biodiversity (Garrabou et al. 2018). For installation of the in situ multiparameter probe on the Shallow mark, a permit shall be obtained from the Croatian Ministry of the Sea, Transport and Infrastructure.

MATERIAL

- Aqua TROLL 600,
- Aqua TROLL 600 sensors for temperature, salinity and dissolved oxygen,
- VuSitu Mobile App,
- Fixation kit – depending on a method employed e.g. Inox rings, ankles Colson, two component putty for underwater sealing, plastic gloves and bag, plastic screws, plastic ties, (note: fixation method will be selected after the in situ inspection of the Shallow mark and following instructions form the Ministry of the Sea, Transport and Infrastructure).

MONITORING TIME FRAME

- Continuous (no field work if equipment operational)

Monitoring of noble pen shell (*Pinna nobilis*) recruitment

Given the recorded mass mortality events of the noble pen shell in the Croatian part of the Adriatic Sea from May 2019 onwards, and the conclusions of the working group adopted at a meeting of the Directorate for Nature Protection, it is necessary to take all possible measures to protect this strictly protected and now critically endangered species. There are already elaborated methods of collecting juvenile noble pen shells and breeding them in the natural environment (Kersting et al., 2019), which we will use. They are also recommended as conservation measures by the International Union for Conservation of Nature (IUCN; https://www.iucn.org/news/mediterranean/202001/mediterranean-noble-pen-shell-crisis-pinna-nobilis- january-2020-update).
Before the appearance of mass mortality event, significant populations of noble pen shell were

recorded at Cetina estuary. A total of 57 specimens of noble pen shell (*Pinna nobilis*) were recorded in the area of 100m^2 at Water polo playground site which was characterized as area of high-density population. The species was present in a greater or lesser density within the entire surface of the 1110 habitat type, most often within the settlement of the seagrass *Cymodocea nodosa*. At the end of December 2019, all individuals recorded at Water polo playground site died (Kurtović Mrčelić J., 2019). One individual was recorded near the Shallow mark in July 2020 however he did not survive the summer. Now all specimens are considered dead. In order to follow eventual recovery of impacted populations, larval collectors will be installed at 3 sites of Cetina estuary, namely Water polo playground, Shallow mark and Mala luka bay (Figure 31).

Figure 31. Proposed sites for the establishment of monitoring of noble pen shell (Pinna nobilis) recruitment: 1) Water polo playground (4m depth), 2) Shallow mark (6m depth) and 3) Mala luka bay (12m depth). Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu.

Collectors would be installed at the mentioned locations in June at the latest. We would collect the collectors at the beginning of November at the latest and take out juvenile noble pen shells from them, measure them and transport them to the Pula Aquarium, where noble pen shells are currently grown *ex situ*. The possibility of repopulation in the sea is possible at target intervals by monitoring the saturation of pathogens in reservoirs.

<u>MATERIAL</u>

- Anchor screw for fixing mooring line;
- Mooring buoy to ensure floating of the mooring line;
- Mash bags and plastic ties for collectors' preparation;
- Working tools.

MONITORING TIME FRAME

- Once every year (field work in May and November).

Assessment and monitoring of *Cymodocea nodosa* meadows

Habitat distributional range/habitat extent of *Cymodocea nodosa* meadows

Habitat distributional range will be assessed from the satellite imagery (acquired for example through the Copernicus Open Access Hub that provides complete access to Sentinel-1, Sentinel-2, Sentinel- 3 and Sentinel-5P user products) or high quality aerial photography. Ground truthing may be carried out by scuba diving at selected locations, if needed. Ideally, as a reference point a year 2013 will be taken (or the one nearest to it with satisfactory image quality) since that was the year of the official Croatian Natura 2000 network proclamation. This baseline will be compared with the present state. Furthermore, the period of late 2016-early 2017 will be investigated to check if the impacts of sand extraction (carried out in March 2016, putatively also affecting *Cymodocea nodosa* meadows) within the Natura 2000 site Cetina Estuary can be tracked and quantified.

MONITORING TIME FRAME

- Once every 6 years (no field work)

Condition of key habitat-forming species Cymodocea nodosa and its meadows

Assessment at the population level

To assess the current condition of *Cymodocea nodosa* habitat, a recently updated CymoSkew index Orfanidis et al. 2020) will be used which provides a quantitative expression of *C. nodosa* photosynthetic leaf length (PLL) asymmetry. It has been used as an early warning response indicator of coastal ecosystem status and trends (Orfanidis et al. 2010) and so far, it has been applied successfully within the WFD (2000/60/EC) and Habitat (92/43/EEC) Directives in different Greek and Cypriot coasts (see Orfanidis et al. 2020 and references therein).
Since we are dealing with an endangered and strictly protected species, destructive sampling will be kept to a minimum – at each of 3 sites situated along the isobath of 4 m (close to the lower distributional depth limit of the meadow: 1 – Omiš, Shallow mark; 2 – Omiš, camp; 3 – Duće) and approximately 500 m apart from each other (Figure 32), 5 replicates will be collected. A replicate will consist of all *Cymodocea nodosa* shoots thriving within 25 x 25 cm quadrat. They will be collected in previously marked individual plastic bags and stored at -20 °C until further analysis.

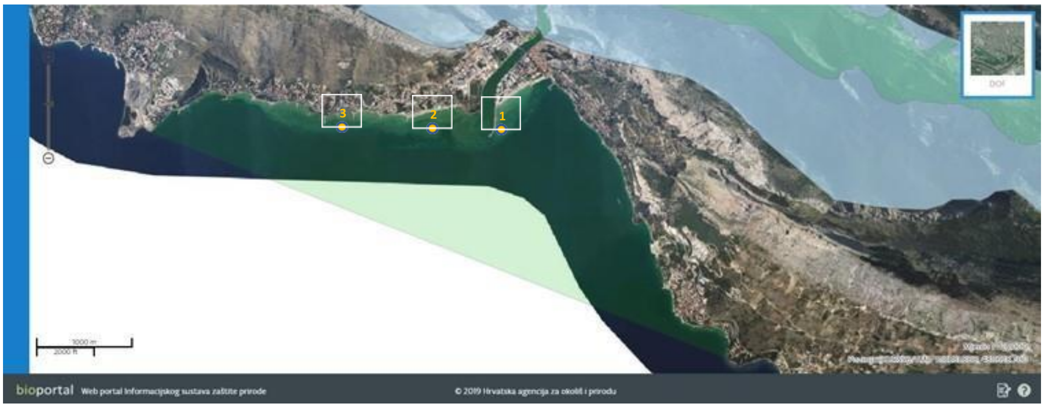

Figure 32. Selected sites within the Natura 2000 Cetina Estuary for monitoring of Cymodocea nodosa meadows: 1 – Omiš, Shallow mark; 2 – Omiš, camp; 3 – Duće. Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu.

The CymoSkew index will be estimated following the formula (Orfanidis et al. 2010): Skewness index = N * M3/ [(N - 1) * (N - 2) * s3].

MATERIAL

- Plastic or metal quadrates 25 x 25 cm (2)
- Knife/scissors
- Plastic bags (previously marked)
- gloves
- Underwater camera
- Portable fridge (1-2)
- Dive computer

MONITORING TIME FFRAME

- Once every 3 years (field work in July)

Assessment of *Cymodocea nodosa* habitat structure

In addition to CymoSkew index as a population metric used as an indicator of ecosystem status and trends, *Cymodocea nodosa* habitat structure in the Natura 2000 site Cetina Estuary will be assessed. Such assessment will be based on the habitat structure index (HIS; Irving et al. 2013) which enables rapid assessment and direct comparison of seagrass habitat structure using scores of 0 (poor) to 100 (excellent) achieved through integration of five habitat variables: area, continuity, proximity, percentage cover and species identity. For that purpose, *in situ* video transects 50 m long and 1 m wide (at least 3 per site, georeferenced) will be recorded (pointing the camera perpendicular to the substrate) and necessary data (i.e. percentage cover estimations, seagrass species identities) will be extracted during desktop video analysis for each 1 m^2 of the transect.

MATERIAL

- Underwater camera (e.g. GoPro)
- Marked transect 50 m long
- GPS and a buoy
- Dive computer
- Compass

MONITORING TIME FRAME

- Once every 3 years (field work at any time)

Assessment of passive restoration of C. *nodosa* meadow at a site of former sand extraction

At one site (Omiš, Shallow mark, Figure 32), which was exposed to sand extraction during 2016, we plan to monitor the passive restoration of the *C. nodosa* meadow, measured as a recolonization of the affected area by the seagrass rhizomes and shoots from the moment of the initial assessment of the meadow border till the autumn of 2022. For that purpose, several small underwater buoys will be placed along the current limit of the meadow, bordering with the affected area (currently hosting bare sand). A border marked in such a way will be video recorded and a total length of the surveyed border will be measured *in situ*. During the second field visit, the border will be recorded again, and potential elongations (i.e. beyond the previously marked limit of the meadow, towards the once affected area) of the seagrass rhizomes will be measured *in situ* and new shoots recolonizing the area will be counted.

MATERIAL

- Underwater camera
- Small marked buoys to be placed underwater

- Measuring tape/marked transect
- Rulers
- Plastic slates for writing and pencils
- Dive computer
- Compass

MONITORING TIME FRAME (tentative)

- Once every year (field work in autumn or spring before vegetation growth)

Assessment and monitoring of the *Cladocora caespitosa* bank

Cladocora caespitosa is an endemic species and the sole zooxanthellate scleractinian reefbuilder in the Mediterranean. Nowadays, extensive bioconstructions of this coral (i.e., banks) are very rare and are severely threatened by global change-related disturbances, such as the marine heat waves (resulting in prolonged and/or more frequent exposure to the elevated seawater temperature) and the presence of invasive species (Kersting et al. 2013, 2014). Yet unrecorded and undescribed *C. caespitosa* population forming a remarkable, relatively deep (between 25-30 m), bank just outside of the southern border of the Natura 2000 site Cetina estuary (Figure 33) merits further investigation in terms of its characterization, mapping and assessment of current conservation status. Obtained data will provide baselines for future monitoring of this important bioconstruction and it will inform potential decision on the extension of Natura 2000 site borders to encompass also this valuable 1170 Reefs habitat and to promote its effective conservation.

Figure 33. Location of the yet undescribed Cladocora caespitosa bank, next to the southern border of the Natura 2000 site Cetina estuary. Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu.

Habitat distributional range/habitat extent

The *C. caespitosa* bank will be mapped both by using the classic *in situ* techniques as well as by using an innovative approach enabled by the underwater photogrammetry, as one of the latest trends in survey and mapping of coral reefs. It uses a non-metric camera in 3D reconstruction of coral reefs that finally produce photo maps in 3D. 3D reconstructions can provide highly accurate measures of the surface area and volume of coral colonies and skeletons across a broad range of morphologies. Furthermore, temporal comparisons can provide reliable estimates of change in surface area and volume to document fine-scale patterns of structural change in individual coral colonies and skeletons (Ferrari et al. 2017). Reliable quantification of the effects of sustained and ongoing disturbances on the structure and function of coral habitats, such as *C. cladocora* banks, is urgently needed and call for more widespread implementation of this method. To date, such technique has been rarely applied on the Mediterranean temperate reefs (see for example Palma et al. 2018) and we found no published record on its application on the banks constructed by the cushion coral *C. caespitosa.* The classic *in situ* mapping technique will follow Kružić & Benković (2008). Transect lines marked at 5-m intervals will be used to make 25 m^2 frames and all coral colonies inside the frame will be counted and outlined. Moving the frame, the whole coral bank will be mapped. The work flow of underwater photogrammetry will to the largest extent follow the one described in Ferrari et al. (2017).

Population structure and conservation status of *Cladocora caespitosa*

To assess the condition of the bank, photographs of 50 x 50 cm quadrats will be taken along at least 3 random transects, each 10 m in length. In addition, separate colonies not fused into a bank but occurring nearby will be photographed. All of these photographs will be used to depict the areas affected by necrosis. Hence, a percentage of the colony area affected by necrosis will be expressed in increments of 10% and recent and/or old necrosis will be differentiated. Necrosed areas below 10% will not be considered to prevent confusion with other sources of natural mortality, such as those eventually induced by depredation by gastropods (Kersting et al. 2013, Kružić et al. 2013). Moreover, for each surveyed colony (i.e. those ones not fused into a bank) the following data will be obtained:

1. Depth of occurrence
2. The size of the colony as its: length (maximum diameter, D1), width (D2) and height (H) Morphology of such colonies near the coral bank (but not connected to it) will be described using the Is-index (Riegl 1995), as a degree of sphericity: Is-index = maximal height of colony ⁄ maximal diameter of colony.

MATERIAL

- GoPro or similar action camera, 2 underwater video lights
- Coded targets and scale bars for photogrammetry
- Transects of at least 10 m length
- Plastic plates for writing and pencils
- Rulers
- Dive computer
- Compass

MONITORING TIME

FRAME

- Once every year (field work in September or October)
- Once every 3 years (field work for photogrammetry)

8.5. Personnel and resources involved

For the purpose of establishing observation system Public Institution Sea and Karst contracted a Scientific coordinator, dr. Silvija Kipson from SEAFAN – marine research and consultancy company. She has more than 10 years of experience working on assessment, monitoring and conservation of habitat and species that are subject of Natura 2000 site Cetina estuary observation system. During CASCADE project duration she will train and capacitate Public Institution oceanographer, Jelena Kurtović Mrčelić, for carrying out assessment and monitoring activities. They will carry out all filed related activities for which two certified scuba divers (advanced level or higher) - working in couple are needed. Further logistical support will be provided by local diving centre (boat, boat operator, tanks, additional divers, etc.). Additional expertise will be needed in terms of satellite images analyses and photogrammetry which will probably be subcontracted or provided (in-kind) by long-term associates of the Public Institution Sea and Karst. All assessment and monitoring activities are relatively easy to employ, low-cost methods, which are to the largest extent executable by the staff of the Public Institution Sea and Karst. During the second year of activities implementation, professors and students from the University Department of Marine Studies in Split as well as associates from the Natural History Museum in Split will be trained to carry out certain activities in order to minimise risks related to expertise.

8.6. Closing Remarks

Natura 2000 site Cetina estuary observation system will function only if it is part of a national monitoring system, because as such it will receive adequate human, financial and technical resources. As there is a national reporting obligation on the status of all Natura 2000 habitat types according to the EU Habitat Directive 92/43/EEC, line institutions are working on setting-up a monitoring system similar to the one existing for the Water Framework Directive (WFD, 2000/60/EC). CASCADE project outputs will serve as best practice examples and guidelines that Public Institution Sea and Karst will provide to line institutions in order to facilitate setting-up of such national level monitoring system.

2.10. P10: Torre del Cerrano, Pineto Abruzzo (IT); PP: UNIMOLISE

2.10.1. Introduction

UNIMOL (PP13) implemented observing systems on the Pilot area P7 (Molise coast) and P10 (Torre Cerrano MPA coast) including coastal transitional areas. We focused monitoring activities on native salt marsh vegetation (wet brackish mosaics of the Biferno mouth) and on coastal dunes vegetation (of the Torre Cerrano Marine Protected Area). In these two areas, several monitoring activities will be conducted in order to characterize the coastal environment and to evaluate the conservation status of endangered species and ecosystems.

2.10.2. Description of Pilot area

In the Pilot area 10 which is the Torre Cerrano Marine Protected area (MPA), we focused on coastal dunes hosting valuable relicts of well-preserved natural ecosystems (Figure 34).

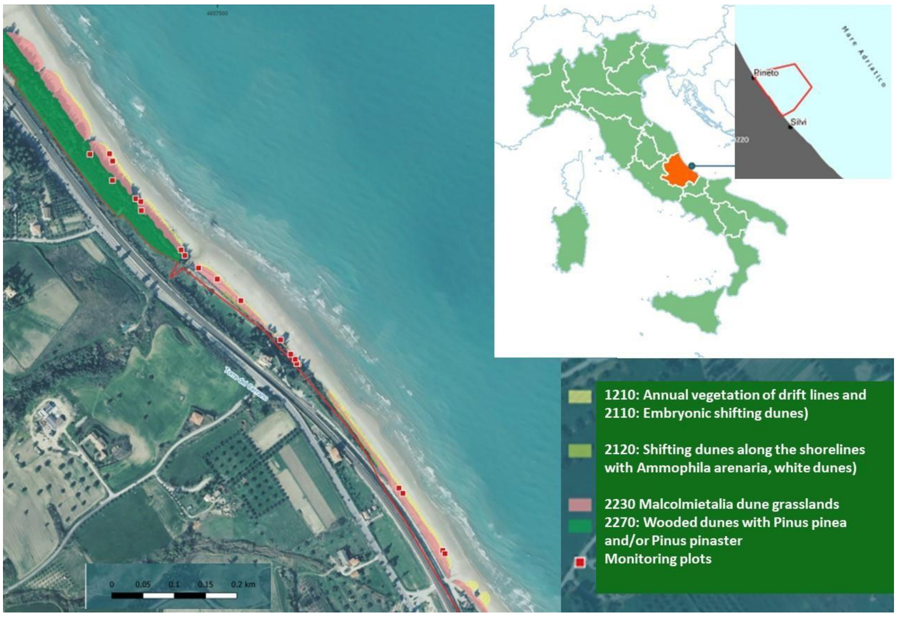

Figure 34. P10 Pilot area is a Marine Protected area (https://www.torredelcerrano.it/area-marina-protetta.html) along with coastal dune habitats (Habitats Directive 92/43/EEC). Some monitoring plots on coastal dunes are reported in red.

The sandy seashore of Torre Cerrano MPA includes well preserved coastal dune ecosystems representative of the Central Adriatic coast for a 6 km long coast tract. The coast of Torre Cerrano hosts several species and habitats of European conservation concern (Habitats Directive 92/43/EEC) as:

1. Annual vegetation of drift lines (EU code 1210),
2. Embryonic shifting dunes (EU code: 2110),
3. Shifting dunes along the shorelines with Ammophila arenaria white dunes (EU code 2120),
4. Malcolmietalia dune grasslands (EU code 2230),
5. Mediterranean salt meadows - Juncetalia maritime (EU code 1410),
6. Wooded dunes with Pinus pinea and/or Pinus pinaster (EU code 2270)

For a detailed description of the habitats see Carranza et al 2008; Stanisci et al 2014 and Angelini et al. 2016. Coastal dune ecosystems integrity and diversity are impinged by several anthropic pressures as urban expansion, increasing road infrastructures and massive beach tourism which in turn led to pollution, biological invasions, over-exploitation of the natural resources and to the loss and degradation of natural ecosystems.

Unfortunately, local administrators and tourists consider coasts only as a place for vacation and leisure sports; thus, coastal sand is often cleaned mechanically and dunes are critically trampled. In particular, human trampling and recreational activities especially on summer present extremely negative effects including substrate erosion, decreasing of plant community's diversity and wildlife dismissal from no longer suitable habitats (Prisco et al 2021).

2.10.3. Targeted actions and final goals to achieve

The implementation of monitoring activities in the P10 coastal dunes, are useful to characterize the coastal environment and to assess the conservation status of endangered species and ecosystems using bioindicators. Furthermore, with field monitoring campaigns and data elaboration we will increase the database of field monitoring data on coastal dunes and we will analyze how they change focusing on the role of vascular plants as bio indicators for ecosystem assessment. Such information could support the planning of soft conservation actions on AMP Torre Cerrano coastal dunes.

2.10.3. Materials and Methods Data collection

The monitoring campaigns of coastal dunes of central Adriatic coast started approximately 20 year ago. Such monitoring work is carried out by the University of Molise in collaboration with colleagues of the University of Roma Tre and started out in the context of the LTER monitoring and observation frame (IT20 - Central Italy coastal dunes – Italy DEIMS.iD https://deims.org/6d7ffd99-40e1-4f0d-ad26-6904581dbe9b).

During the last years, we extended the monitoring area to wider areas of the Abruzzo coast, including MPA Torre Cerrano. We decided to adopt a re-visitation procedure consisting on the periodical sampling of permanent random plots distributed across the coastal dune sea-inland gradient (Figure 34 - study area figure red dots). We will focus on sampling coastal dunes vegetation to identify areas that urgently need of conservation actions, offering the needed support for panning them in the next future. Specifically, we started a prelaminar data collection phase aimed at recording control data before the conservation actions implementation (e.g. boardwalks' installation to reduce trampling disturbance and the banalization of biodiversity and dune ecological functioning) on areas of intervention and outside. We generated random plots in a GIS environment (ArcGIS v. 10.2.2; ESRI 2012), located across coastal dune zonation and in the area in which conservation actions could be implemented and on other areas in which no conservation actions are planned in the next future. We have sampled the dune vegetation (T1) in random plots that will be resampled before conservation actions implementation (T2).
All random plots are 16 m2 area (4 m × 4 m) are carried out between April and June in each fieldwork session (Figure 35). In each plot we record the complete list of vascular plants as well as their cover percentage, using the Braun-Blanquet scale of abundance/dominance (Braun-

Blanquet 1964; Westhoff and van der Maarel 1973). We adopt Conti et al. (2005) and Celesti-Grapow et al. (2009) as a taxonomic reference list for, respectively, the native and exotic plant species.


Figure 35: Data collection on MPA Torre Cerrano coastal dunes (P10) carried out by UNIMOL experts (PP13) for ecosystem assessment through Bioindicators. http://envixlab.unimol.it/monitoragigo-dune-torre-cerrano/. Photo: Francesco Pio Tozzi.

Data analysis

Specifically, we will focus on diversity patterns of some species guilds (e.g. focal, ruderals and aliens' species) in relation to anthropic disturbance (trampling) on coastal dune herbaceous vegetation. To analyze the effects of conservation actions (e.g. boardwalks) on coastal dunes vegetation we will compare species richness over time through rarefaction curves (Gotelli and Colwell 2001). This method widely used in ecological studies for estimating diversity, compute the cumulative number of species deriving from the sequential input of the sampled plots randomized 100 times (Colwell and Coddington 1994). We will compare rarefaction curves for overall the species pool and for specific functional groups as:

1. Focal species: a pool of native and characteristic species which identify the habitats (diagnostic species), determine the structure and guarantee the functionality of the habitats (Santoro et al. 2012). Furthermore, as particularly sensitive to disturbance, focal species can be considered reliable indicators of a good conservation status. Focal species will be identified based on the Italian Interpretation Manual of Habitats Directive (Biondi et al. 2009);
2. Ruderal species, which are well adapted to living in different habitats. They are usually associated with disturbance and a poor conservation state;
3. Exotic species which are species are introduced from another geographical area and that

may become invasive. Exotic species may cause change in biotic interactions and community functioning, as well as the alteration of ecosystem services. Ruderal species and aliens will be identified according with the Flora d'Italia" (Pignatti 1982, and successive editions; Conti et al. 2005; Celesti-Grapow et al 2009).

As proposed on brackish vegetation analysis, also for coastal dunes we can analyze temporal changes in the ecology and the structure using Ellenberg bioindicator values (Ellenberg, 1974) of temperature (T), moisture (U), soil nutrients (N), luminosity (L) and salinity (S) and the life-form categories of Raunkiaer (Raunkiaer, 1934). Ellenberg's indicator values is a numerical system to classify species' (on a simple ordinal classification) according to the position of their realized ecological niche along an environmental gradient (e.g. temperatures, moisture, nutrients, luminosity, salinity, etc.). The Raunkiaer life forms system categorize plants using resting buds position and depict the structure and function of the different species present in a plant community.
We will the ecology (Ellenberg values) and structure (Raunkiaer life forms) on plots collected over time using Mann-Whitney test.

2.10.4. Personnel and resources involved

The field work is carried out by at least 2 operators. Before the identification of sampling locations with the support of the GPS, plots of 4x4 m are laid out. As 1 researcher fill the field sampling grid, the other observe and measure all vascular plants present in the plot (Figure 3). Collected data will be gathered and stored in a unique database of coastal ecosystems (including data collected on P7) as part of LTER data monitoring data. Such information will allow to describe ecological changes on the analyzed area and to compare them with similar ecosystems occurring on areas with different management and disturbance regimes.

2.10.5. Closing Remarks

The proposed and implemented ecosystem assessment through Bioindicators, is fully coherent to WP3 targets which consist also of the use of monitoring systems and data collections for protecting biodiversity and ecosystems. Furthermore, it offers a sound support for implementing WP4 and identifying conservation actions. The collected data and the obtained results of statistical analysis will offer new insights on coastal dunes ecosystems conservation status, using some species functional groups as indicators. The monitoring campaigns and the analysis of the turnover of some "bio indicator species" could help to: better understand the ongoing ecological processes, identify early warning signs of ecosystems degradation and build empirical models of ecosystem change facing global change (WP4). Furthermore, based on monitoring data we could propose conservation actions and improved management approaches of the analyzed ecosystems.

2.11. P11. Marche coastal area (IT); Regione Marche

2.11.1. Introduction

WP3 activities aim to research the lesser known aspects and collect new data of the coastal-marine environment by upgrading the observing systems. First, the collected results will be part of the analysis of the efficiency of a lagoon stress operational forecasting system and, in turn, in the evaluation of the usefulness of its operational implementation. Secondly, an accurate characterization of the neglected marine zoo benthic communities will be provided in order to fulfil the lack of information existing in this topic.

Last December 2019, MARCHE REGION approved the REGIONAL ICZM Plan, which is the official planning and programming act that regulates the use and management of the entire coastal area of MR territory. It complies with:

- EU Floods Directive 2007/60/EU,
- National Guidelines for the defense of the coast from erosion and the effects of climate change (MATTM and ISPRA, 2017)
- PROTOCOL on Integrated Coastal Zone Management in the Mediterranean connected with the sustainability strategies towards resources ecosystem and coastal resilience.

ICZM Plan is the new planning and programming tool for coastal defense interventions in Marche region, an important aspect of which is the environmental characterization based on three issues: quality of the rear reef sediments, bathing and biocenosis, analysed by ARPA Marche (Regional Environmental Protection Agency) and revised and integrated with the scientific support of CNR IRBIM of Ancona and the Universities of Camerino, Urbino and Ancona.

2.11.2. Description of Pilot area

MARCHE REGION is in charge of the *pilot area P11*. P11 includes the entire coastal 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, consisting of an alternation of beautiful pebble, rocky and sandy beaches, responds to all the needs of visitors of our land and confirms an ancient tradition of hospitality. On the northern coast, long and thin beaches alternate here and there interrupted by a promontory, by small coves or by the mouth of a stream. The famous seaside resorts of Gabicce Mare, Fano, Pesaro characterized by wide sandy beaches offer a quiet seaside life even to the inexperienced and small bathers. From Ancona, capital of Marche Region, you can see Monte Conero, a promontory of extraordinary beauty overlooking the blue of the Adriatic Sea. 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. From the same southern gates of Ancona, there are suggestive tourist resorts: the Portonovo Oasis, the award-winning Sirolo overlooking the sea, Numana with its equipped and functional tourist port and Marcelli the most modern with tourist villages, residences and adequate accommodation facilities.

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. Furthermore, we cannot forget the "verde Riviera Picena", which extends between Porto Sant'Elpidio, Lido di Fermo, Porto San Giorgio and Pedaso, and the exotic "Riviera delle Palme" between Cupramarittima, Grottammare and San Benedetto del Tronto, with its 7000 palm trees that also grow on the very fine and white beach that slopes into the sea characterized by shallow waters.

Geographical context

The Adriatic Sea is characterized by a prevalent anticyclonic circulation, more evident in winter and spring, while in summer and autumn, a series of anticyclonic and cyclonic gyres prevail. The heading north eastern current is called East Adriatic Current (EAC). The heading south western current is called Western Adriatic Current (WAC).

In summer the Adriatic Sea hydrodynamic slows down creating a general stratification of the water column. In the Adriatic Sea also dense waters are present; in winter they are formed by the Bora and, in spring, they flow southward.

The sea level oscillations in the Adriatic Sea are influenced by both tides and meteorological factors: the maximum tide height occurs, in fact, in low pressure conditions and with winds from Levante-Scirocco that accumulate near the coast the waters for over a meter of height, with increasing values from South to North. Along the Marche coast the “reigning sea” is the sea coming from Scirocco (SSE) while the “dominant sea” is that from Bora or Grecale.

2.11.3. Targeted actions and final goals to achieve

The P11 Pilot site characterization is ongoing and it is part of the monitoring programme of the regional ICZM plan ed the pilot action is composed by two main tasks:

- "Analysis of biocenosis emerged and submerged along the entire coastal strip"
- "Service of coastal engineering of technical-scientific deepening and verification with consequent proposal of re-perimeter of coastal areas subject to marine flooding of five pilot sites of the Marche region already perimeter in accordance with EU Directive 2007/60/EC and Legislative Decree no. 49 of 23/2/2010 and included in the ICZM Plan"

The proposed tasks are part of the 2022-2023 REGIONAL ICZM Programme of intervention that will have final formal approval before December 2021.

2.11.4. Materials and Methods

In the ICMZ Plan it is implicitly pointed out that some Sustainability Guidelines (SDGs) and related Indicators can be achieved through a unique action aiming at the complete and homogeneous knowledge of the coastal biodiversity, and especially at the perimeter of the emerged and submerged biocenosis and habitats along the whole coastline of the Marche Region without interruption.

The results of this service will be included in the Costa SIT (Sistema Informativo Territoriale - Territorial Information System) for its updating provided for by the L.R. 15/2004; this information system is made up of vector files type shp organised both individually and in personal geodatabases. All the information collected is structured in mxd projects. The choice of ESRI software is the result of the specialisation and training of the staff working in this field and of the SIT coast activated and set up about 25 years ago. Similar organisation of information is also required in qgz projects. With regard to the software version, reference will be made to ARCMAP version 10.3 or higher for mxd projects, and to QGIS version 3.20 or higher for qgz projects.

This objective constitutes the correct basis for subsequent environmental comparisons and thus for monitoring the quality and quantity of the Marche's coastal biological resources.

Therefore, the achievement of this objective will also allow the implementation of some indicators referred into the Program, in particular those relating to the following SO:

- OS2 (Fratino nesting sites);
- SO5 (Pinna nobilis and Lithophaga lithophaga locations)

- SO10 (location of emerged and submerged coastal habitats);
- OS14 (computerized data on submerged habitats and protected species and inclusion in the Costa SIT);
- OS15 (computerised data on Fratino nesting and inclusion in the coastal SIT)

“Service of coastal engineering of technical-scientific deepening and verification with consequent proposal of re-perimeter of coastal areas subject to marine flooding of five pilot sites of the Marche region already perimeter in accordance with EU Directive 2007/60/EC and Legislative Decree no. 49 of 23/2/2010 and included in the ICZM Plan".

The objective is to investigate from a technical-scientific point of view the sea flood perimeters related to the Tr20, Tr100 and Tr>100 (Tr= return period) flooding scenarios of five coastal pilot sites in the Marche Region, already perimeterised according to the European Directive 2007/60/CE and the Legislative Decree no. 49 of 23/2/2010 and included in the ICZM Plan. The Tr20, Tr100 and Tr>100 perimeters already realized by the Marche Region will be verified and a new proposal of Tr20, Tr100 and Tr>100 marine flooding perimeter will be produced according to the European Directive 2007/60/CE and the Legislative Decree no. 49 of 23/2/2010.

The 5 survey sites were selected taking into account the presence or absence of rigid coastal defence structures and according to the different granulometry of the beach sediments (sand or gravel or sand/gravel beaches). The pilot sites contracted are:

1) Pesaro, built-up area - SITcosta (Coastal Informative System) “Transetti” (coastal stretch between two successive sections perpendicular to the coastline) of ICZM Plan: from no. 86 to no. 98 included - Defence structure: submerged/emerged reefs - Beach sediments: sand;
2) Fano, Sassonia beach - SITcosta “Transetti” of ICZM Plan: from no. 137 to no.146 included; Defence structure: free/submerged/emerged reefs - Beach sediments: gravel;
3) Montemarciano - SITcosta “Transetti” of ICZM Plan: from no. 278 to no. 287 included - Defence structure: free/brushes -Beach sediments: gravel;
4) Porto Recanati - SITcosta “Transetti” of ICZM Plan: from no. 511 to no. 521 included - Defence structure: emerged reefs, brushes/free - Beach sediments: sand/gravel;
5) Fermo - SITcosta “Transetti” of ICZM Plan: from no. 625 to no. 631 included - Defence structure: emerged reefs - Beach sediments: sand.

Both tasks will be realized through two public tenders are planned to be launched before the end of January 2022 with an estimated budget of 150.000 €

2.11.5. Personnel and resources involved

Marche Region established an innovative framework for Interreg ITALY-CROATIA Strategic projects. With DGR 1396/2019: the coordination of 4 strategic project was assigned to **Productive Activities, Labour and Education Dep.t – EU Strategic Project Office (EuSPO)** in collaboration with the following technical departments:

- **Coastal & Soil Defense, Water Protection Dep.t** **(COAST) for CASCADE project**
- **Waste and Energy Dep.t** **(WASE)** for **ADRIACLIM** project
- **Civil Protection Dep.t** **(CIPRO)** for **STREAM** project
- **Fishing Economy Dep.t** **(FISHE)** for **ARGOS** projects

Moreover, in order to share information and create internal synergies, cr internal experts of COAST, CIPRO and WASE Departments are involved in a cross-topic working group between the projects ADRIACLIM, CASCADE, in order to realize the following sub tasks

- "Analysis of biocenosis emerged and submerged along the entire coastal strip"

2.11.6. Closing Remarks

This pilot action aims to provide a more accurate information on biocenosis emerged and submerged along the entire coastal strip and provide a more accurate information of re-perimeter of coastal areas subject to marine flooding of five pilot sites of the Marche region.

References

1. AA.VV., 2008, Sito d'interesse comunitario "Foce Biferno - Litorale di Campomarino" (IT7222216). Convenzione stipulata tra la Regione Molise e la Società Botanica Italiana per la realizzazione del "Progetto di ricerca per la cartografia CORINE Land Cover e la distribuzione dei siti Natura 2000 del Molise degli habitat e delle specie vegetalied animali di interesse comunitario". URL:http://www.regione.molise.it/web/grm/ambiente.nsf/0/4A4D333C181C6E63C125757C003EFE54?Open Document.
2. Anelli Monti M., Caccin A., Stocco A., Pranovi F., 2021. Applications of 'new' technological solutions for analyzing nektonic communities in the deepest areas of the Venice lagoon. Book of Abstracts, XVII incontro dei dottorandi e dei giovani ricercatori in ecologia e scienze dei sistemi acquatici.
3. Anelli Monti M., Cavraro F., Caccin A., Pessa G., Pranovi F., 2020. First assessment of the fish assemblage's biomass in the Venice Lagoon inlets by means of active acoustics. EuroLag 9 International Congress. Future vision and knowledge needs for coastal transitional environments, 20-24 gennaio 2020. Venezia.
4. Angelini P., Casella L., Grignetti A., Genovesi P. (ed.), 2016. Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: habitat. ISPRA, Serie Manuali e linee guida, 142/2016.
5. Angelini P., Casella L., Grignetti A., Genovesi P. (ed.), 2016. Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: habitat. ISPRA, Serie Manuali e linee guida, 142/2016.
6. ARPAV e Fondazione Musei Civici Venezia (2010). Le tegnùe dell'Alto Adriatico: valorizzazione della risorsa marina attraverso lo studio di aree di pregio ambientale. ISBN 978-88-7504-151-9
7. Aucelli P.P.C., Iannantuono E., Rosskopf C. M., 2008, Evoluzione e dinamica della costa molisana con particolare riguardo all'equilibrio e alla salvaguardia delle dune, in Mastantuono A. (ed.) Lontano dal Paradiso: Le Dune del Molise, Il Melograno, pp. 63-85.
8. Bakran – Petricioli. 2007. Manual for the determination of marine habitats in Croatia according to the EU Habitats Directive (in Croatian). Državni zavod za zaštitu prirode Zagreb, 2011
9. Bellafiore, D., Mc Kiver, W., Ferrarin, C., Umgiesser, G. (2018). The importance of modeling nonhydrostatic processes for dense water reproduction in the southern Adriatic Sea. Ocean Model. 125, 22–28. doi:10.1016/j.ocemod.2018.03.001.
10. Bertasi Elisa, 2007. Distribuzione spaziale e variazione temporale di stadi planctonici di

invertebrati sulle Tegnùe di Chioggia. Tesi di laurea specialistica in biologia marina, Università degli Studi di Padova

11. Biondi E., Blasi C., Burrascano S., Casavecchia S., Copiz R., Del Vico E., Galdenzi D., Gigante D., Lasen C., Spampinato G., Venanzoni R., Zivkovic L., 2009, Italian interpretation manual of the 92/43/EEC directive habitats. Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma. [online] URL: http://vnr.unipg.it/habitat
12. Biondi, E, C. Blasi, S. Burrascano, S. Casavecchia, R. Copiz, E. Del Vico, E. Galdenzi, et al. 2009. "Italian interpretation manual of the 92/43/EEC Directive Habitats." Ministero dell'Ambiente e della Tutela del Territorio e del Mare. Roma. http://vnr.unipg.it/habitat/.
13. Bioportal. Web portal of the Nature Protection Information System. Available at: http://www.bioportal.hr/gis/. Accessed 12/07/2021
14. Brambati, A. et Catani, G. (1988). Le coste e i fondali del Golfo di Trieste dall' Isonzo a Punta sottile: aspetti geologici, geomorfologici, sedimentologici e geotecnici. Hydrores Inf. 5:13 – 28.
15. Braun Blanquet J., 1932, Plant sociology: the study of plant communities, New York. McGraw-Hill, 439 pp
16. Braun-Blanquet J 1964. Pflanzensoziologie. Grundzüge der Vegetationskunde. 3rd edn. Springer, Wien.
17. Burchard, H. & Petersen, O. (1999). Models of turbulence in the marine environment—a comparative study of two equation turbulence models. J. Mar. Sys. 21, 29–53.
18. Caressa S., Gordini E., Marocco R., Tunis G. (2002). Caratteri geomorfologici degli affiornamenti rocciosi del Golfo di Trieste (Adriatico settentrionale). Gortania – Atti Muse Friul. diStoria Nat., 23 (2001), 5-29, ISSN: 0391- 5859
19. Carranza M.L., Drius M., Malavasi M., Frate L., Stanisci A., 2018. Assessing land take and its effects on dune carbon pools. An insight into the Mediterranean coastline. Ecological Indicators 85: 951-955
20. Carranza, M. L., A.T.R. Acosta, A. Stanisci, G. Pirone, and G. Ciaschetti. 2008. "Ecosystem Classification for EU Habitat Distribution Assessment in Sandy Coastal Environments: An Application in Central Italy." Environmental Monitoring and Assessment 140 (1–3): 99–107. doi:10.1007/s10661-007-9851-7.
21. Casas E, Martín-García L, Otero-Ferrer F, Tuya F, Haroun R, Arbelo M (2021) Economic mapping and assessment of Cymodocea nodosa meadows as nursery grounds for commercially important fish species: A case study in the Canary Islands. One Ecosystem 6: e70919. https://doi.org/10.3897/oneeco.6.e70919
22. Celesti-Grapow L, Alessandrini A, Arrigoni PV, Banfi E, Bernardo L, Bovio M, Brundu G, Cagiotti MR, Camarda I, Carli E, Conti F, Fascetti S, Galasso G, Gubellini L, La Valva V,

Lucchese F, Marchiori S, Mazzola P, Peccenini S, Poldini L, Pretto F, Prosser F, Siniscalco C, Villani MC, Viegi L, Wilhalm T, Blasi C 2009. Inventory of the non- native flora of Italy. Plant Biosystems 143(2):386-430.

23. Celesti-Grapow L, Alessandrini A, Arrigoni PV, Banfi E, Bernardo L, Bovio M, Brundu G, Cagiotti MR, Camarda I, Carli E, Conti F, Fascetti S, Galasso G, Gubellini L, La Valva V, Lucchese F, Marchiori S, Mazzola P, Peccenini S, Poldini L, Pretto F, Prosser F, Siniscalco C, Villani MC, Viegi L, Wilhalm T, Blasi C (2009) Inventory of the non- native flora of Italy. Plant Biosyst 143(2):386–430
24. Chiggiato, J. and Oddo, P., 2008. Operational ocean models in the Adriatic Sea: a skill assessment. Ocean Science, 4(1), pp.61-71.
25. Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Austral Ecol. 18:117–143.
26. Colwell, R. K., and Coddington, J. A. (1994). Estimating Terrestrial Biodiversity through Extrapolation. Phil. Trans. R. Soc. Lond. B 345 (1311), 101–118. doi:10.1098/rstb.1994.0091
27. Conti F, Abbate G, Alessandrini A, Blasi C 2005. An Annotated Checklist of the Italian Vascular Flora. Palombi Editore, Roma.
28. Conti F, Abbate G, Alessandrini A, Blasi C 2005. An Annotated Checklist of the Italian Vascular Flora. Palombi Editore, Roma.
29. Conti F., Manzi A. Pedrotti F., 1997, Liste Rosse Regionali delle Piante d'Italia, Ed. Società Botanica Italiana, WWF.
30. Cucco, A. and Umgiesser, G., 2006. Modeling the Venice Lagoon residence time. Ecological modelling, 193(1- 2), pp.34-51.
31. Cushman-Roisin B, Gačić M, Poulain PM, Artegiani A (2001) Physical oceanography of the Adriatic Sea: Past, present and future. New York: Springer. 304 p.
32. Cvitan, L., T. Renko, K. Cindrić Kalin, T. Kozarić & P. Mikuš Jurković. 2017. Obilna oborina u Hrvatskoj u rujnu 2017. Hrvatska vodoprivreda, 221: 19-24
33. Đurin, B. & A. Muhar. 2017. Predviđene promjene klime u hrvatskoj i utjecaj na vodne resurse. Hrvatska vodoprivreda,
34. Ellenberg, H. 1974, Indicator Values of Vascular Plants in Central Europe. Scr. Geobot. 9, 7–122.
35. European Commission. (2007). Interpretation manual of European Union habitats, Eur 27. European Commission, DG Environment, Strasbourg, 142pp. Available from http://ec.europa.eu/environment/nature/legislation/habitatsdirective/docs/2007 07 im.pdf
36. Falace, Annalisa et al. 2015. "Calcareous Bio-Concretions in the Northern Adriatic Sea: Habitat Types, Environmental Factors That Influence Habitat Distributions, and Predictive Modeling." PLoS ONE 10(11): 1–21.

37. Ferrarin, C., Bellafiore, D., Sannino, G., Bajo, M., Umgiesser, G. (2018). Tidal dynamics in the inter-connected Mediterranean, Marmara, Black and Azov seas. Prog. Oceanogr. 161, 102–115. doi:10.1016/j.pocean.2018.02.006.
38. Ferrarin, C., Umgiesser, G., Bajo, M., Bellafiore, D., De Pascalis, F., Ghezzo, M., Mattassi, G., Scroccaro, I. (2010). Hydraulic zonation of the lagoons of Marano and Grado, Italy. A modeling approach. Estuar Coast Shelf Sci, 87, 561–572.
39. Fidon Ltd. 2021. Environmental protection study for the reconstruction of the watercourse Miljašić Jaruga mouth
40. Franzo A. and Del Negro P. (2019) Functional diversity of free-living nematodes in river lagoons: can biological traits analysis (BTA) integrate traditional taxonomic-based approaches as a monitoring tool?
41. Garrabou J, Bensoussan N, Azzurro E (2018) Monitoring Climate-related responses in Mediterranean Marine Protected Areas and beyond: FIVE STANDARD PROTOCOLS. Institute of Marine Sciences, Spanish Research Council ICM-CSIC, Barcelona, Spain (ed.), 36 p. Available at:
42. Gotelli NJ, Colwell RK. 2001 Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness, Ecol. Lett., vol. 4 (pg. 379-391)
43. Guidance document No. 25 Guidance on chemical monitoring of sediment and biota under the Water Framework Directive; 2010
44. Hammer, Øyvind, Harper, David A.T., and Paul D. Ryan, 2001. Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica, vol. 4, issue 1, art. 4: 9pp., 178kb. http://palaeo-electronica.org/2001_1/past/issue1_01.htm
45. Hrvatske vode. Excerpt from the Register of Water Bodies, River Basin Management Plan 2016-2021. Prepared: April 2020
46. http://dx.doi.org/10.20350/digitalCSIC/8612 (last access on September 30 2021)
47. Iannantuono E., Rosskopf C.M., Stanisci A., Aucelli P.P.C., 2004, Effetti della dinamica costiera sull'evoluzione dei sistemi dunali presenti lungo la costa molisana (Italia meridionale), Atti dei Convegni dei Lincei, 205, pp. 321-332
48. Irving AD, Tanner JE, Gaylard SG (2013) An integrative method for the evaluation, monitoring, and comparison of seagrass habitat structure. Marine Pollution Bulletin 66:176-184
49. ISPRA. 2010. "Valutazione Degli Effetti Della Zona Di Tutela Biologica Di Chioggia Sui Popolamenti Demersali e Bentonici e Sulle Possibilità Di Ripopolamento Di Specie Di Interesse Commerciale."
50. Ivančić, I., Degobbis, D., 1984. An optimal manual procedure for ammonia analysis in 417 natural waters by the indophenol blue method. Water Research 18, 1143-418 1147.

51. Kersting D. K., Hendriks I. E. (2019) Short guidance for the construction, installation and removal of Pinna nobilis larval collectors. IUCN. 6 str.
52. Kersting DK, Ballesteros E, De Caralt S, Linares C (2014) Invasive macrophytes in a marine reserve (Columbretes Islands, NW Mediterranean): spread dynamics and interactions with the endemic scleractinian coral Cladocora caespitosa. Biological Invasions, (16): 1599–1610
53. Kersting DK, Bensoussan N, Linares C (2013) Long-Term Responses of the Endemic Reef-Builder Cladocora caespitosa to Mediterranean Warming. PLoS ONE 8(8): e70820. doi:10.1371/journal.pone.0070820
54. Kurtović Mrčelić, J. (2019) Monitoring populacija plemenite periske (Pinna nobilis) u Natura 2000 području Ušće Cetine, Izvješće JU More i Krš. 10. str.
55. Kuzmić M, Janeković I, Book JW, Martin PJ, Doyle JD (2007) Modeling the northern Adriatic double-gyre response to intense bora wind: A revisit. Journal of Geophysical Research C: Oceans 112.
56. Maicu, F., Alessandri, J., Pinardi, N., Verri, G., Umgiesser, G., Lovo, S., Turolla, S., Paccagnella, T. and Valentini, A., 2021. Downscaling With an Unstructured Coastal-Ocean Model to the Goro Lagoon and the Po River Delta Branches. Frontiers in Marine Science.
57. Marasović I., Krstulović, N., Leder, N., Lončar, G., Precali, R., Šolić, M., Lončar, G., Beg-Paklar, G., Bojanić, N., Cvitković, I., Dadić, V., Despalatović, M., Dulčić, J., Grbec, B., Kušpilić, G., Ninčević-Gladan, Ž., P. Tutman, Ujević, I., Vrgoč, N., Vukadin, P., Žuljević, A. Coastal cities water pollution control project, Part C1: Monitoring and Observation System for Ongoing Assessment of the Adriatic sea under the Adriatic sea Monitoring Programme, Phase II. Interim report (IR), December, 2013.
58. McArdle B.H., Anderson M.J. (2001). Fitting multivariate models to community data: a comment on distance- based redundancy analysis, Ecology, 82, pp. 290-297.
59. Melli Valentina, Michela Angiolillo, Francesca Ronchi, Simonepietro Canese, Otello Giovanardi, Stefano Querin, Tomaso Fortibuoni (2017). The first assessment of marine debris in a Site of Community Importance in the north-western Adriatic Sea (Mediterranean Sea). Marine Pollution Bulletin, Volume 114, Issue 2, 30 January 2017, Pages 821-830
60. Mizzan Luca (1994). Malacocenosi in due stazioni altoadriatiche a substrati solidi: Analisi comparativa fra popolamenti di substrati naturali ed artificiali. Società Veneziana di Scienze Naturali, Lavori vol. 19. ISSN 0392- 9450
61. Mozetič, P., Solidoro, C., Cossarini, G., Socal, G., Precali, R., Francé, J., Bianchi, F., De Vittor, C., Smodlaka N. et Umani S. F. (2009). Recent trends towards oligotrophication of

the northern Adriatic: evidence from chlorophyll a time series. Estuar. Coast. 33: 362 – 375.
62. Mozetič, P., Umani, S. F., Cataletto, B. et Malej, A. (1998). Seasonal and inter-annual plankton variability in the Gulf of Trieste (northern Adriatic). ICES Journal of Marine Science, 55, 711-722.
63. Mrakovčić M. Identification of relationship between hydrological dynamics and biodiversity values of the Neretva river delta [in Croatian]. Zagreb: Regional Center for Environment Protection in Middle and Eastern Europe (REC – office in Croatia); 2001. A document in possession of author.
64. Mužinić J. Neretva valley – threatened area in Croatia [in Croatian]. Proceedings of the fourth congress of Croatian biologists. Zagreb: Croatian biological society; 1993.
65. National Habitat Classification of the Republic of Croatia (5th version)
66. Ordinance on the methods and conditions of waste disposal, categories and working conditions for landfills (OG 114/15, 103/18, 56/19)
67. Orfanidis S, Papathanasiou V, Mittas N, Theodosiou T, Ramfos A, Tsioli S, Kosmidou M, Kafas A, Mystikou A, Papadimitriou A (2020) Further improvement, validation, and application of CymoSkew biotic index for the ecological status assessment of the Greek coastal and transitional waters. Ecological Indicators 118, 106727
68. Orlando-Bonaca M., Mavric B. & Urbanic G. (2012). Development of a new index for the assessment of hydromorphological alterations of the Mediterranean rocky shore. Ecological Indicators, 12: 26-36.
69. Orlando-Bonaca, M., Mannont, P. A. et Falace A. (2013). Assessment of Fucus virsoides distribution in the Gulf of Trieste (Adriatic Sea) and its relation to environmental variables.
70. Parsons TR, Maita Y, Lalli CM, editors (1984) A manual of chemical and biological methods for seawater analysis. Toronto: Pergamon Press.
71. Parsons, T.R., Maita, Y., Lalli, C.M., 1985. A manual of chemical and biological 434 methods of seawater analysis. New York, Pergamon press, 173 pp.
72. Pérès, J. M. & J. Picard. 1964. Nouveau manuel de bionomie benthique. Recueil des Travaux de la Station marine d'Endoume, 31 (47), 5-137.
73. Pielou E.C. 1969 An Introduction to Mathematical Ecology Wiley-Interscience, New York (286 pp.).
74. Pignatti S. (cur.), 1995, Ecologia Vegetale, UTET, Torino.
75. Pignatti S. (cur.), 1995, Ecologia Vegetale, UTET, Torino.
76. Pignatti S., 1982, Flora d'Italia, Edagricole, Bologna.
77. Pignatti S., 1982, Flora d'Italia, Edagricole, Bologna.
78. Pignatti, S., P. Menegoni, and S. Pietrosanti. 2005. "Bioindicazione attraverso le piante

vascolari. Valori Di Indicazione Secondo Ellenberg (Zeigerwerte) per le specie della Flora d'Italia [Biondication through vascular plants. Indication values according to Ellenberg (Zeigerwerte) for the flora species of Italy]." Braun-Blanquetia 39: 1–97.
79. Pitacco V., Mavric B., Orlando-Bonaca M. & Lipej L. (2013). Rocky macrozoobenthos mediolittoral community in the Gulf of Trieste (North Adriatic) along a gradient of hydromorphological modifications. Acta Adriatica, 54(1): 67-86.
80. Podani J., 2007, Analisi ed esplorazione multivariata dei dati in ecologia e biologia, Liguori Editore, Napoli, p. 515.
81. Ponti Massimo, Fava Federica, Abbiati Marco (2011). Spatial-temporal variability of epibenthic assemblages on subtidal biogenic reefs in the northern Adriatic Sea. Mar Biol (2011) 158:1447–1459
82. Ponti, and Mastrotaro. 2006. "DISTRIBUZIONE DEI POPOLAMENTI AD ASCIDIE Sui Fondali Rocciosi (Tegnùe) Al Largo Di Chioggia (Venezia)." Riassunti del 36° Congresso nazionale della Società Italiana di Biologia Marina. 13(Trieste. SIBM): 324.
83. Prisco, I.; Acosta, A.T.R.; Stanisci, A. 2021, A bridge between tourism and nature conservation: Boardwalks effects on coastal dune vegetation. J. Coast. Conserv. 25, 1–12.
84. R Core Team, 2020, R. A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria. Available online: http://www.r-project.org/index.html (accessed on 7 February 2021).
85. Raunkiær, C.C. 1934, The Life Forms of Plants and Statistical Plant Geography; Oxford University Press: Oxford, UK.
86. Raunkiær, C.C. 1934, The Life Forms of Plants and Statistical Plant Geography; Oxford University Press: Oxford, UK.
87. Ret, M. (2006). Bilancio idrologico e circolazione idrica della Laguna di Marano e Grado. Master's thesis, Faculty of Engineering, University of Udine, Italy (in Italian).
88. Riegl B. (1995) Effects of sand deposition on scleractinian and alcyonacean corals. Marine Biology, 121, 517– 526.
89. Santoro R, Carboni M, Carranza ML, Acosta ATR 2012. Focal species diversity patterns can provide diagnostic information on plant invasions. Journal for Nature Conservation 20:85-91.
90. Santoro R., Carboni M, Carranza M.L., Acosta A. T. R. 2012. Focal species diversity patterns can provide diagnostic information on plant invasions. Journal for Nature Conservation. 20 (2): 85-91. doi:10.1016/j.jnc.2011.08.003.
91. Šarić, T., I. Župan, S. Aceto, G. Villari, D. Palić, Dušan; G. De Vico & F. Carella. 2020. Epidemiology of Noble Pen Shell (Pinna nobilis L. 1758) Mass Mortality Events in Adriatic

Sea Is Characterised with Rapid Spreading and Acute Disease Progression. Pathogens, 9 (2020), 776; 1-21
92. Shannon C., Weaver W. (1949). The Mathematical Theory of Communication, University of Illinois Press, Urbana, US.
93. Smagorinsky, J. (1963). General circulation experiments with the primitive equations. The basic experiment. Monthly Weather Review 91,99–152.
94. Solidoro, C., Bastianini, M., Bandelj, V., Codermatz, C., Cossarini, G., Melaku Canu, D., Ravagnan, E., Salon, S. et Trevisani, S. (2009). Current state, scales of variability, and trends of biogeochemical properties in the northern Adriatic Sea. J. Geophys. Res. 114: C07S91. doi:10.1029/2008JC004838.
95. Stanisci A., Acosta A.T.R., Carranza M.L., de Chiro M., Del Vecchio S., Di Martino L., Frattaroli A.R., Fusco S., Izzi C.F., Pirone G., Prisco I., 2014. EU habitats monitoring along the coastal dunes of the LTER sites of Abruzzo and Molise (Italy). Plant Sociology 51 (Suppl. 1): 51-56.DOI 10.7338/pls2014512S1/07
96. Stanisci A., Carranza M.L., 2008, Lo stato di conservazione del litorale molisano, in Marchetti M., Marino D., Cannata G. (cur.), Relazione sullo stato dell'ambiente della regione Molise, Università degli Studi del Molise Campobasso, pp. 95-96
97. Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. 167: 310.
98. Supić N, Kraus R, Kuzmić M, Paschini E, Precali R, et al. (2012) Predictability of northern Adriatic winter conditions. Journal of Marine Systems 90: 42–57.
99. Trygonis, V., Sini, M., 2012. photoQuad: a dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. Journal of Experimental Marine Biology and Ecology 424-425, 99-108. doi:10.1016/j.jembe.2012.04.018
100. Turk, T. 2011. Below the surface of the Mediterranean (in Croatian). Školska knjiga, Zagreb, 592pp.
101. Umgiesser G, Ferrarin C, Cucco A, De Pascalis F, Bellafiore D, Ghezzo M, Bajo M. (2014). Comparative hydrodynamics of 10 Mediterranean lagoons by means of numerical modeling. J Geophys Res Oceans. 119(4):2212–2226.
102. Umgiesser, G., Canu, D.M., Cucco, A. and Solidoro, C., 2004. A finite element model for the Venice Lagoon. Development, set up, calibration and validation. Journal of Marine Systems, 51(1-4), pp.123-145.
103. Umgiesser, G., Melaku Canu, D., Cucco, A., Solidoro, C., (2004). A finite element model for the Venice Lagoon. Development, set up, calibration and validation. J. Mar. Syst. 51, 123–145. doi:10.1016/j.jmarsys.2004.05.009.
104. UNEP/MAP (2000) River Cetina watershed and the adjacent coastal area: environmental

and socio-economic profile, Split
105. Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilungen der Internationale Vereinigung für theoretische und angewandte Limnologie 9: 1-38.
106. Valentini, A., Delli Passeri, L., Paccagnella, T., Patruno, P., Marsigli, C., Cesari, D., Deserti, M., Chiggiato, J. and Tibaldi, S., 2007. The sea state forecast system of ARPA-SIM. Bollettino di Geofisica Teorica e Applicata, 48(3), pp.333-350.
107. Vichi M., Cossarini G., Gutierrez Mlot E., Lazzari P., Lovato T., Mattia G., Masina S., McKiver W., Pinardi N., Solidoro C., Zavatarelli M. (2015): The Biogeochemical Flux Model (BFM): Equation Description and User Manual. BFM version 5.1. BFM Report series N. 1. March 2015, Bologna, Italy, pp. 89.
108. Westhoff, V. and van der Maarel, E. (1973) The Braun-Blanquet Approach. In: Whittaker, R.H., Ed., Ordination and Classification of Communities, Dr. W. Junk, Dordrecht, 617-626.

Annexes

Forthcoming documents

1. Coastal studies 2021 _ Marche Region
2. PP 7 - 12 "Oceanographic characters of the central-northern Adriatic sea and the coast of Marche region" - Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Rocco De Marco 4,5, Federico Spagnoli1,2 , 4.5, Mario Tramontana 3.6
3. PP 13 - 28 “Historical and current evaluation of the coast of Marche region” - Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Federico Spagnoli 2,4, Mario Tramontana 2,3,5
4. PP 65 – 72 “Bathing of the coastal waters of the Marche - Gian Marco Luna 4, Elena Manini 4
5. PP 83-78 Marine-coastal biocoenosis in the Marche region“ - Elisa Punzo 4, Alessandra Spagnolo 4
6. PP 31-64 „Quality of sediments located at the back of breakwaters“ - Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Gian Marco Luna 4,5, Elena Manini 4,5, Emanuela Frapiccini 4,5, Federico Spagnoli 1,2,4,5, Mario Tramontana 3,6, Gianni Scalella 7, Stefano Parlani 7, Mauro Sinigaglia 7, Giordano Forchielli 7, Fabrizio Mazzoli 7, Diego Magnoni 7, Carmine Bellino 7, Daniele Pernini 7
7. Protection and enhancement of the residual coastal dunes in the Marche Region - Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Simona Casavecchia 4, Roberta Gasparri 4, Simone Pesaresi 4, Federico Spagnoli 2,5, Gianni Scalella 7, Mario Tramontana 3,8, Silvia Zitti 4,

Stefano Parlani 7, Mauro Sinigaglia 7, Giordano Forchielli 7, Fabrizio Mazzoli 7, Diego Magnoni 7, Carmine Bellino 7, Daniele Pernini 7

8. pp 143 - 148 “Final consideration” Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Federico Spagnoli 1,2,4,5, Mario Tramontana3,5

Websites

1. http://envixlab.unimol.it/
2. http://lifemaestrale.eu/
3. https://deims.org/088fe3af-c5bb-4cc8-b479-fe1ea6d5be80
4. https://www.consiglio.marche.it/banche_dati_e_documentazione/iter_degli_atti/paa/pdf/d_am67_10.pdf - Marche Region ICZM Plan
5. http://www.gnrac.it/ Gruppo Nazionale Studi Costieri
6. http://www.regione.molise.it/web/grm/ambiente.nsf/0/4A4D333C181C6E63C125757C003EFE54?OpenDocu ment
7. https://www.isprambiente.gov.it/it/pubblicazioni/manuali-e-linee-guida/manuali-per-il-monitoraggio-di- specie-e-habitat-di-interesse-comunitario-direttiva-92-43-cee-in-italia-habitat
8. https://www.voda.hr/sites/default/files/25_-
   a. _guidance_document_on_chemical_monitoring_of_sediment_and_biota_under_the_water_frame work_directive_-_eng.pdf
9. COPERNICUS Marine Service (2021). https://marine.copernicus.eu/access-data.
10. NAUSICA, Progetto NAUSICA – ARPA FVG – CRMA. (2018).
    a. http://www.arpa.fvg.it/export/sites/default/tema/aria/utilita/Documenti_e_presentazioni/tecnico_scientific he_docs/2018gen01_arpafvg_crma_goglio_nausica_rap2018_001.pdf.
11. NAUSICA, Progetto NAUSICA interim – ARPA FVG – CRMA. (2019)
    a. http://www.arpa.fvg.it/export/sites/default/tema/aria/utilita/Documenti_e_presentazioni/tecnico_scientific he_docs/2019gen05_arpafvg_crma_goglio_nausica_interim_rap2019_01.pdf.
12. SHYFEM model GitHub repository (2021) https://github.com/SHYFEM-model.
13. SHYFEM model home page (2021). official web page http://www.ismar.cnr.it/shyfem

List of figures

List of tables