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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.

D4.1.2

In situ observing campaigns set up and carried out by relevant partners.

Part 1

PP1 – CMCC

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Chapter 1 Aims and content of the document.

The purpose of this document is to collect information on the status of the in situ observing campaigns (e.g., chemical, biological, visual census) set up and carried out by relevant partners and to understand how the equipment described in D.4.1.1 has been used efficiently in relation to the objectives that each partner has set within its pilot area.

Chapter 2 In situ observing campaigns at the Pilot Scale

2.1 Grado and Marano Lagoon, and Gulf of Trieste (IT)

ARPA FVG has implemented two different activities simultaneously on its marine and transitional waters; therefore, they will be treated in two separate chapters to make the description of the activities more understandable.

2.1.1 Grado and Marano Lagoon observing campaign

ARPA FVG has installed three multiparametric probes in the Grado and Marano lagoon. Those probes provide material for the validation of the numerical models that has been developed in the lagoon area. Furthermore, the activity allows to enrich the ARPA database and to deepen the general knowledge of regional transitional waters.

Figure 1. A probe installed on board the buoy.

The choice of sites for the installation of the probes was made keeping in mind several needs:

- Investigate areas of interest for model validation (exchange areas between marine and fresh waters);
- ease reaching of the areas for maintenance;
- enough water depth to avoid periods of time out of the water;
- remain within the coverage areas of the normal GPRS network;
- no harm to navigation or fishing/shellfish farming activities.

Three sites with high dynamic hydrological characteristics were therefore chosen to highlight changes in physical-chemical characteristics as the currents vary, controlled by tidal flows and river flows.

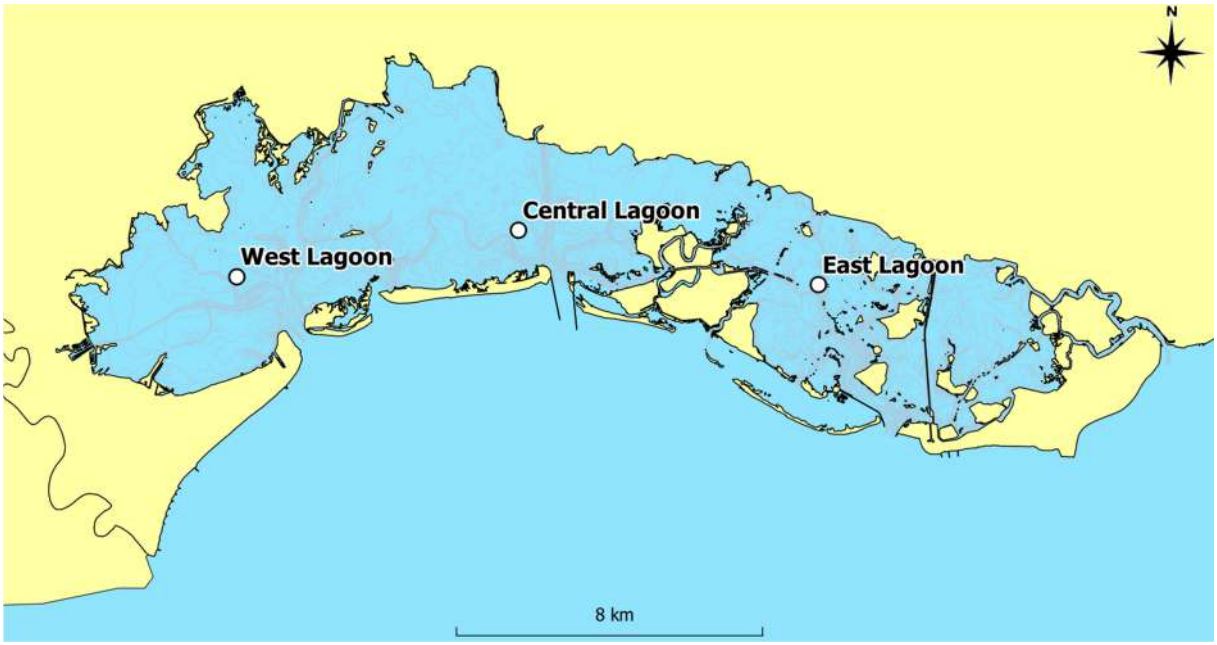


Figure 2. Instruments' position inside the lagoon

Furthermore, before mooring the instruments, it was verified that the tidal plain had average depths greater than 1.20 m (msl) to ensure that the instrument remained correctly immersed and that the buoy floated even during low tides.

The frequency of acquisition of the instruments was set at 15 minutes, a reasonable compromise to allow long periods of displacement at sea while obtaining a high number of daily recordings. In this configuration the probe batteries were able to last for over 6 months without any need for maintenance.

The GPS/GPRS modem, connected to the probe, is powered by an autonomous battery pack. In this case, the frequency for acquiring the position and sending the data recorded by the probe to the FTP server has been set to 12h. During the first tests it emerged that with this configuration the modem's batteries can withstand about 4 months, but it was observed that low temperature drastically reduces the voltage and can trigger fast discharge. Therefore, during 2022 the configuration was set to check position and send data only once every 24h, allowing to better preserve the batteries in the long term.

The installation required the use of a ponton boat capable of moving on shallow water and having a large working deck to accommodate the dismantled buoys that were loaded on board.

Figure 3. Some pictures taken during the installation: a) configuration of the instruments; b) anchoring; c) the site at the end of installation.

Once the selected points were reached, the buoys were assembled and launched. After that, a technician went down into water to ensure anchorage (10 m steel chain with 10kg brittany type anchor). The probes were configured as previously described and positioned in their housing inside the buoys.

Even if the probe has an antifouling system, the instrumentation was checked periodically and if necessary, an extraordinary cleaning of the sensors was carried out. Also, the buoy and the anchoring require maintenance as they are subject to the growth of algae and molluscs which tend to immediately colonize hard substrates, especially during spring and summer. Excessive accumulation could lead to unbalance buoyancy as well as potentially affecting the data recorded by the instrument and it was removed whenever necessary.

Figure 4. Maintenance activities on the flotation system

Other maintenance activities can be carried out to replace the batteries or to reboot the system in case of errors or disconnections. Furthermore, the configuration of the probe also allows to remove a defective sensor and take it away for repairs, while keeping the rest of the instrumentation perfectly operational.

The instrumentation was withdrawn in November, it was washed and dismantled, and the sensors were sent back to the manufacturer for maintenance and recalibration. On this occasion, an evaluation of the instrumental drift due to the long period of immersion was also requested to perform an estimation of the error on the recorded data (see Figure 12).

The recorded data were made available to the ARPA FVG modelling development équipe, according to the objectives of the pilot activity. However, even without the need for elaboration, the visualization of the data allows some interesting observations.

For demonstration purposes Figure 5, Figure 6 and Figure 7, show the graphs created by calculating daily averages from the data (approximately 96 data per day for each parameter) which can give a hint regarding type and quantity of data collected by using the equipment during the project activities.

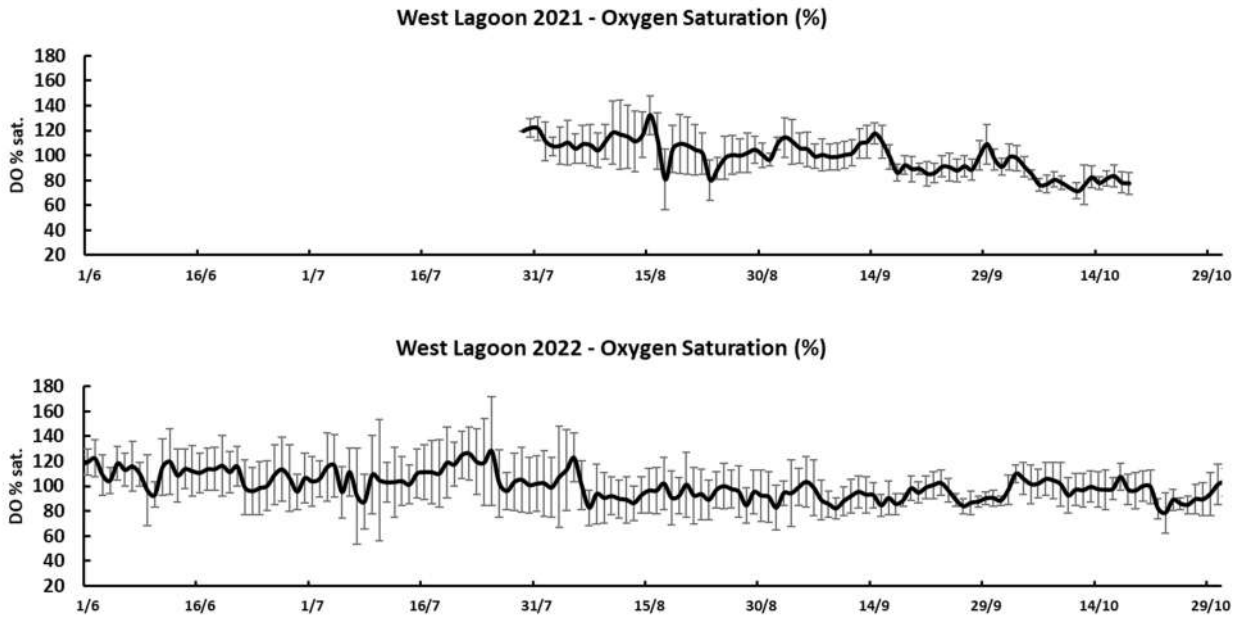


Figure 5. Seasonal trends of dissolved oxygen during 2021 and 2022

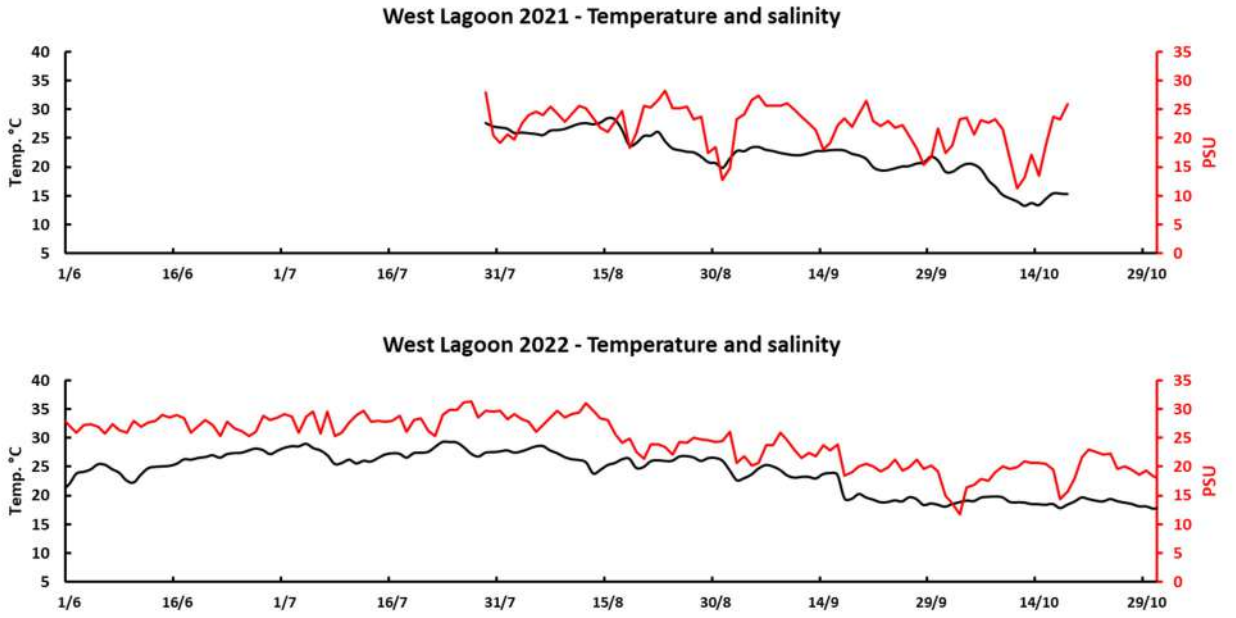


Figure 6. Seasonal trend of temperature and salinity during 2021 and 2022

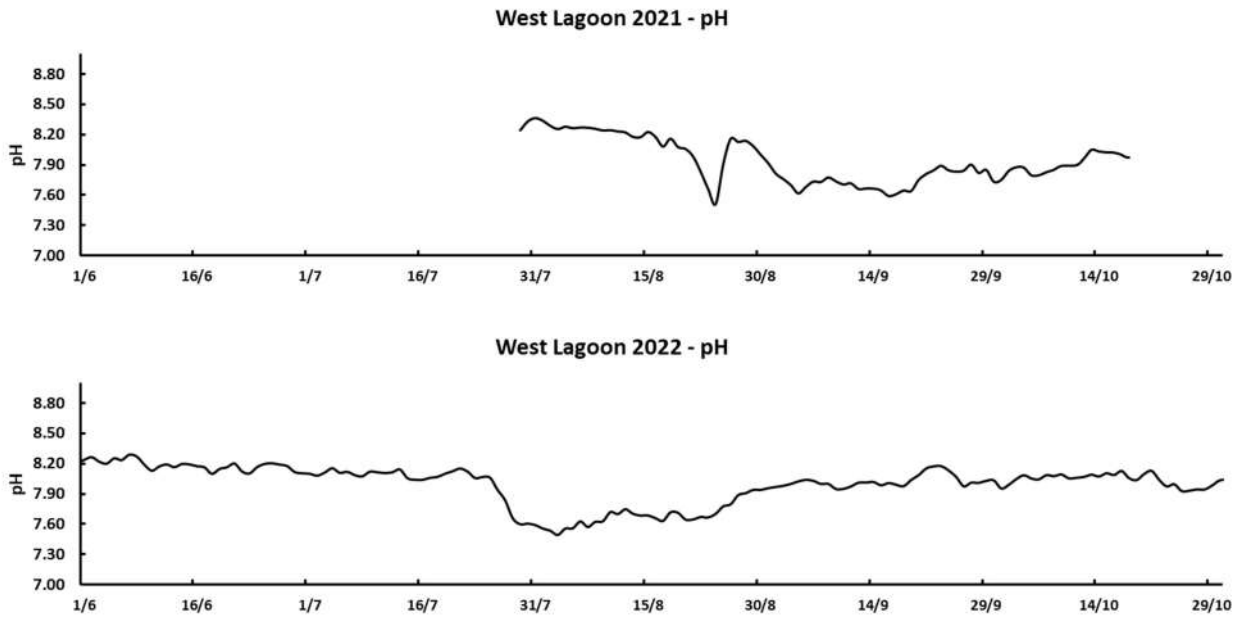


Figure 7. Seasonal trends of pH during 2021-2022

While data such as temperature follow quite similar patterns between the three sites, salinity allows to divide the lagoon basin in two main areas. The western lagoon shows more brackish characteristics and is strongly influenced by the presence of numerous rivers and dewatering pumps serving the near agricultural areas, while the central-eastern portion has a greater marine influence which is reflected in higher and decidedly more stable average values (Figure 8).

Moreover, because of the strong influence of rivers in the dynamics of the lagoon basin, it was possible to highlight the effect related to the drought that hit the region during most of 2022. Indeed, in Figure 9 the average salinity showed a sharped difference comparing summers 2021 and 2022, due to the lack of thunderstorms that, usually, hit the area during summer period and counterbalance the influence of sea water by means of river inputs. The anomaly in the salinity values is clearer in the central-western portion of the lagoon, while in the eastern portion, given the scarce presence of tributary watercourses, the variation was less appreciable (see Figure 10 b).

The drought of 2022 was also accompanied by higher water temperature values which can be observed by comparing 2021 and 2022 data, showing a reduced variability (see standard deviation bar in Figure 10a) throughout the lagoon basin.

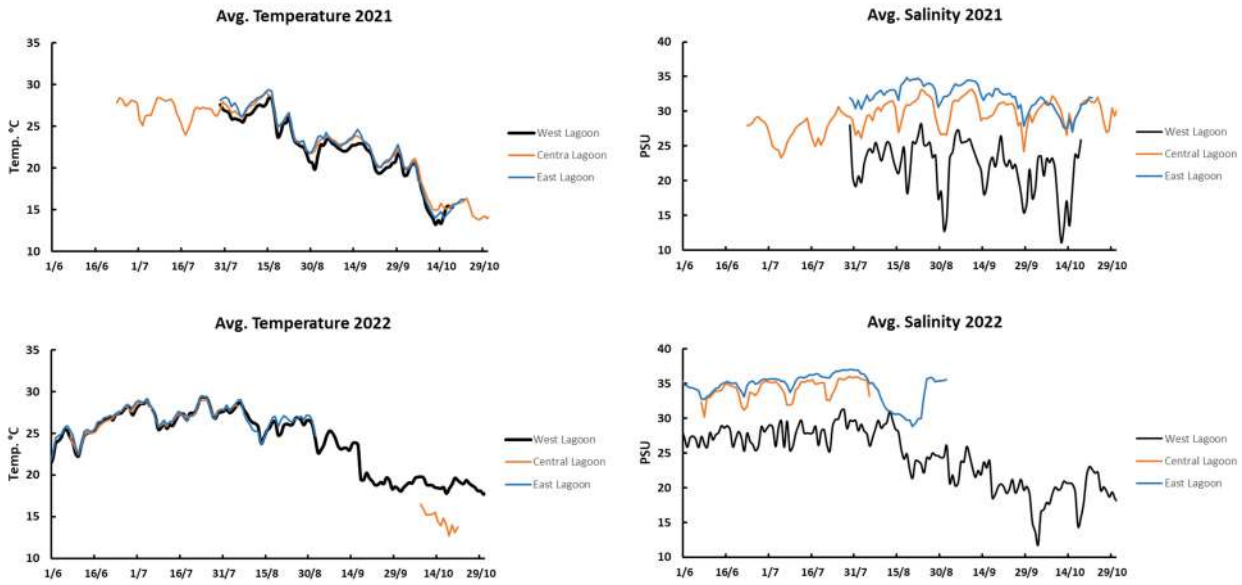


Figure 8. Comparison between different Lagoon areas 2021-2022

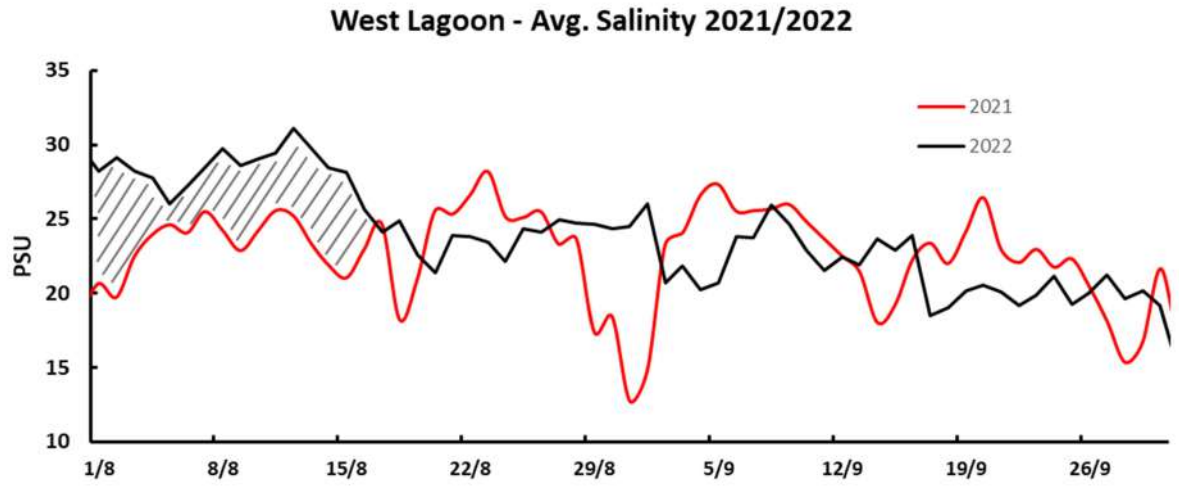


Figure 9. The effects related to 2022 drought on salinity trend observed in the same site.

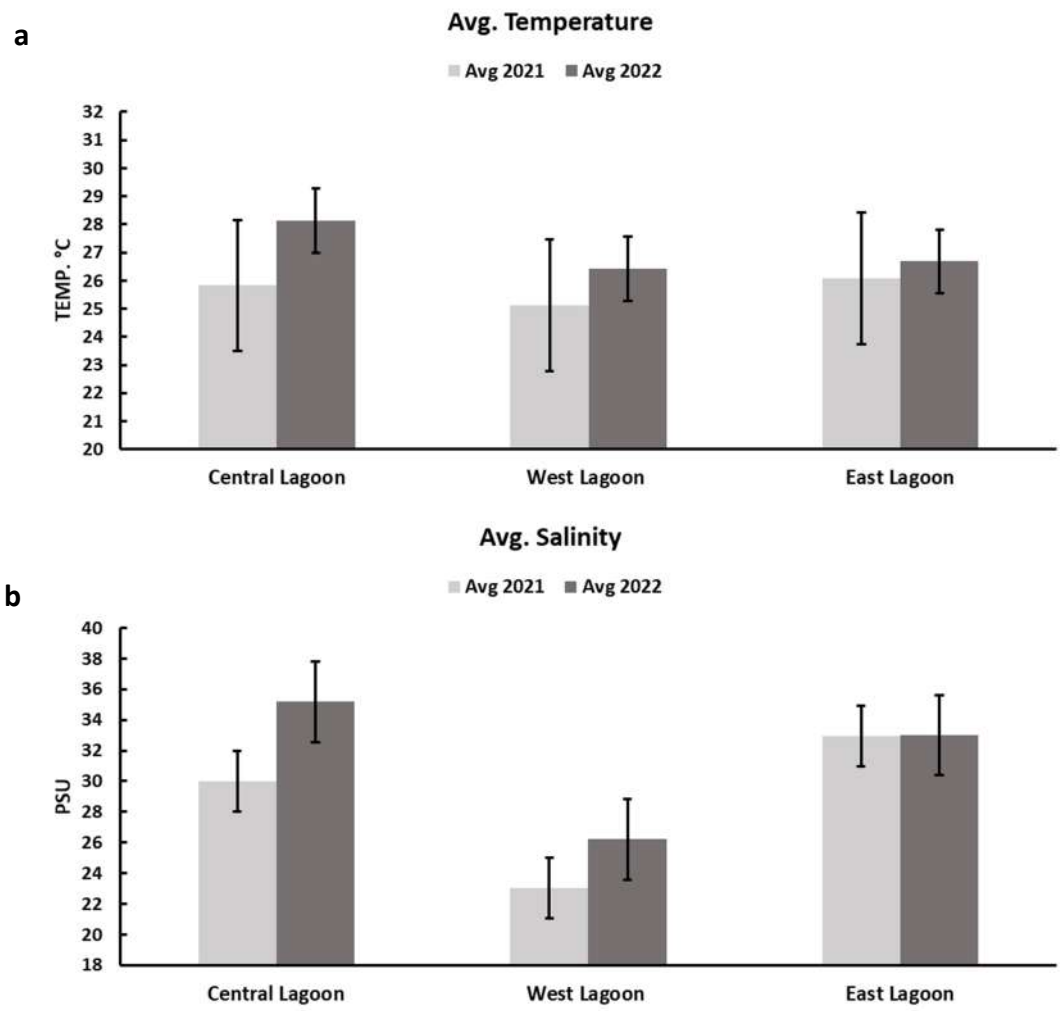


Figure 10. The high temperatures (a) and salinity (b) values registered during 2022 compare with 2021 data.

Another interesting aspect observed during 2022 is the effect of an extensive bloom of *Alexandrium sp.* near the mouth of the Stella and Cormor rivers in the western basin. The unusually high values of dissolved oxygen (Figure 11) could be linked to the abundant presence of this microalgae in an area not far from where the probe was positioned.

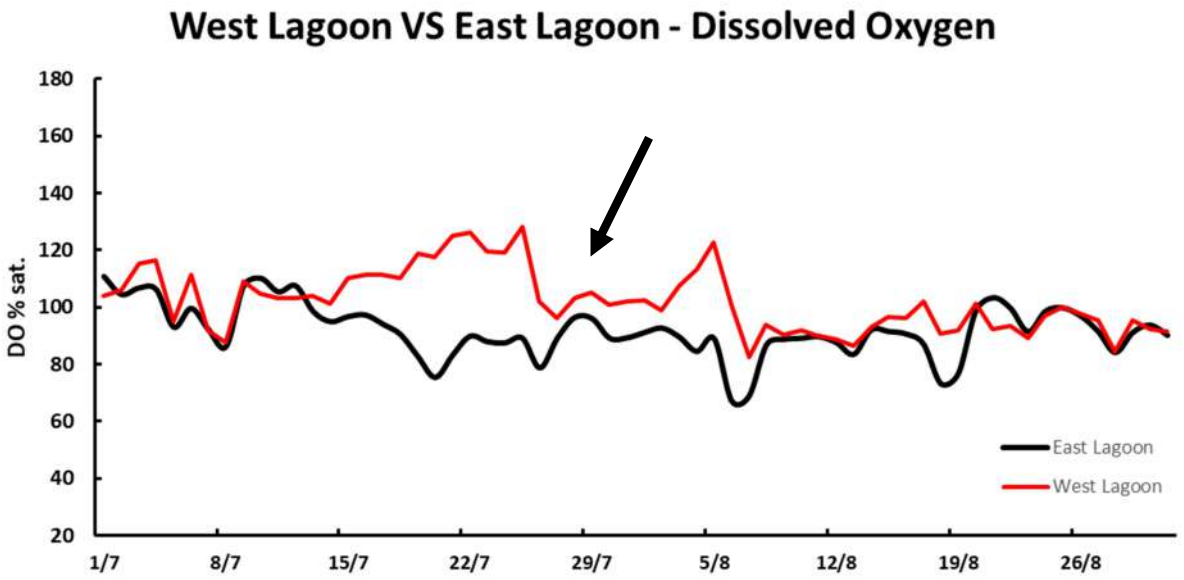


Figure 11. Dissolve oxygen anomaly during 2022, probably related to Alexandrium sp. bloom

The campaign encountered some minor issues that led to loss of part of the data. Mainly, during 2021, a problem affected the GPS/GPRS communication system of two of the three probes, creating both corrupted and/or overwritten data file. In the worst case, corrosion damage also occurred inside one of the modules due to excessive battery discharging (which caused a spill of battery salts). During 2022, however, a problem with two CT sensors (conductivity and temperature) forced the sensors to be disassembled and sent to the manufacturer with the relative loss of data during the time required for repairs.

To avoid similar problems in the future, we are evaluating the possibility of acquiring spare sensors or, to avoid new expenditures, installing only two of the available probes in 2023 and keeping the third in standby as a replacement in case any problem arises on one of those installed.

The sensors used during 2021 were tested in the manufacturer's laboratory to verify the stability of the measurements and the results were very satisfying. The sensor verification parameters are very stringent and therefore a recalibration was anyhow necessary for the conductivity and ph sensors. However, their drift showed a relatively low residual and such as not to affect the quality of the data collected despite the long period of exposure to the aggressive lagoon environment (see Figure 12). The temperature sensor, on the other hand, even passed the verification tests and a recalibration would not even been necessary (anyhow it was done for safety). The verification tests will also be carried out again during the winter of 2022-2023 and once recalibrated the sensors will be reinstalled on the probes for a new data acquisition season.

Température / temperature

Référence / reference : **SBE 37 SMP 15/002**
Date : **15/02/2022**

Points de vérification (°C) Verification points (°C)	Valeur lue (°C) Instrument output (°C)	Ecart (°C) Residual (°C)	Ecart maximum toléré: +/- 10m°C Maximum permissible error: +/- 10m°C
15,163	15,167	0,004	Conforme / Conform

Conductivité / conductivity

Référence / reference : **Reagecon standard lot(s) CSKC10M CSKC50M**
Date : **18/02/2022**
Température / Temperature (°C) **17,132**

Points de vérification (mS/cm) Verification points (mS/cm)	Valeur lue (mS/cm) Instrument output (mS/cm)	Ecart (mS/cm) Residual (mS/cm)	Ecart maximum toléré: +/- 0,5% Maximum permissible error: +/- 0,5%
0,000	0,001	0,001	Conforme / Conform
8,541	8,716	0,175	Non conforme / Not conform
42,426	43,670	1,244	Non conforme / Not conform

pH

Solution de référence / Reference solution: **pH Buffer Solution HANNA instruments**
Date: **16/02/2022**
Température d'ajustage / Calibration temperature (°C) **19,5**

Points de vérification (pH) Verification points (pH)	Valeur lue (pH) Instrument output (pH)	Ecart (pH) Residual (pH)	Ecart maximum toléré: +/- 0,1 pH Maximum permissible error: +/- 0,1 pH
4,00	3,656	0,344	Non conforme / Not conform
7,03	6,722	0,309	Non conforme / Not conform
10,07	9,774	0,292	Non conforme / Not conform

Figure 12. Verification tests on the sensor after the 2021 season

2.1.2 Gulf of Trieste observing campaign

The fieldwork was carried out *una tantum* within April and September 2021, in 15 stations between Villaggio del Pescatore and Lazzaretto. To reach the sampling site and to transport all the equipment, a vessel and a small support boat with oars were used (see D 4.1.1 for details).

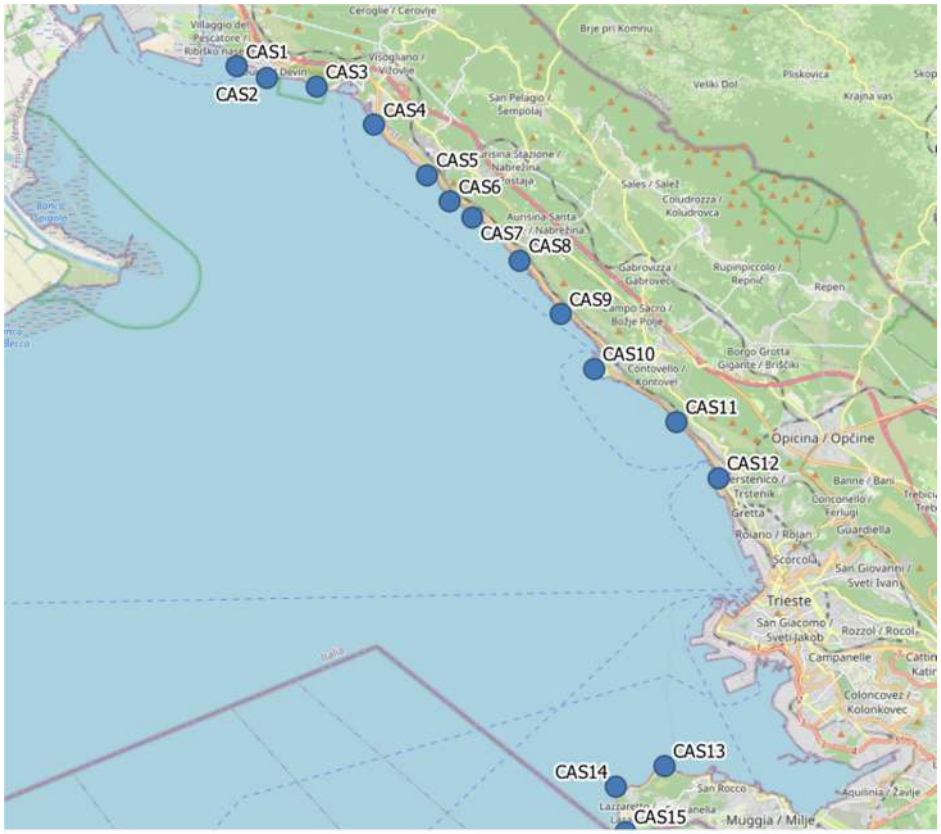


Figure 13. Sites of the observing campaign in the Gulf of Trieste

In situ observing campaign was divided into two sub-campaigns:

1. assessment of the macrofaunal community living in the medio- and upper-infralittoral rocky shores;
2. visual census of the fish fauna living in the medio- and upper-infralittoral rocky shores.

The first sub-campaign started by assigning an assessment, a value between 0 and 2 for each site, regarding materials, textures, structures, sedimentation, water retention and artificially induced mobility. These values will be used to calculate the BIRS index.

Secondly, for each station 3 subsamples of the upper mediolittoral, the lower mediolittoral and the upper infralittoral were collected. 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 as Chtamalida and *Mytilus galloprovincialis*.

The collection of samples in the two mediolittoral belts was done by snorkelling, while in the upper-infralittoral was used scuba gears to remain for the time needed on the sea bottom at 1.5 m. 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 funnelled 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 underwater vacuum called sorbona.

Photos of the surface of each square were taken with a digital camera, before (non-invasive method) and after (invasive method) organism's collection. During the main monitoring activities, physical-chemical parameters, and the presence/absence of the species *Fucus virsoides* (not found) and *Gobius cobitis* (found) were noted.

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. Overall, each station was sampled in about 1,5/2 hours and the campaign's activities were concluded in September 2021.

After the sampling phase, the BIRS (acronym for Benthic Index for Rocky Shore) has been evaluated. BIRS calculates the probability of presence of each species or taxon for each identified hydro morphological 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.

CLASS	HM stressor score range	HM site status
Class 1	0–2.8	Pristine
Class 2	2.9–5.6	Slightly altered
Class 3	5.7–8.4	Moderately altered
Class 4	8.5–11.2	Substantially altered
Class 5	11.3–14	Heavily altered

Table 1. HM Classes

CAS6				CAS7				CAS8				CAS9				CAS10			
UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean
1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00
2,00	2,00	1,00	1,67	2,00	2,00	1,00	1,67	2,00	2,00	1,00	1,67	2,00	2,00	2,00	2,00	0,00	0,00	0,00	0,00
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	1,00	0,00	0,67	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00
			3,67				3,67				5,33				6,00				0,00
			2				2				2				3				1

CAS11				CAS12				CAS13				CAS14				CAS15			
UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean
1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
1,00	1,00	1,00	1,00	2,00	2,00	1,00	1,67	2,00	2,00	2,00	2,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
1,00	1,00	1,00	1,00	0,00	0,00	1,00	0,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
0,00	0,00	0,00	0,00	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
1,00	1,00	1,00	1,00	2,00	2,00	2,00	2,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,00	1,00	0,00	0,33	0,00	1,00	0,00	0,33
			6,00				8,00				6,00				4,33				4,33
			3				3				3				2				2

Table 2. HM stressor classes in each site

Samples	CAS1				CAS2				CAS3				CAS4				CAS5			
Facies	UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean	UML	LML	UIL	Mean
Material	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Texture	1,00	1,00	1,00	1,00	1,00	1,00	0,00	0,67	0,00	0,00	1,00	0,33	1,00	1,00	1,00	1,00	0,00	1,00	1,00	0,67
Structures	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Sediment.	0,00	0,00	0,00	0,00	1,00	1,00	1,00	1,00	0,00	0,00	1,00	0,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
W. retention	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
AIM	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
Belt length	0,00	0,00	0,00	0,00	1,00	1,00	1,00	1,00	1,00	1,00	0,00	0,67	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Total HM				2,00				2,67				1,33				2,00				1,67
Class				1				1				1				1				1

The sampled organisms have been counted and identified to the higher taxonomic level under stereomicroscopes (Nikon SMZ18 and Leica M205) and an optic microscope. The species counted must have been alive at the time of collection. Identification of the taxa occurred thanks to a faunal list used to calculate all the univariate indexes for studying benthic communities.

The other sub-campaign, visual census of the fish fauna living in the medio- and upper-infralittoral rocky shores, consists in the identification of the main fish species of littoral by the means of non-invasive methods.

A 60 m long reel was laid on the sea bottom at a depth of 0.5-1 m. Following a rope an underwater operator identified and counted the fish fauna through visual census observing a buffer area of 2 meters on both side of the rope. To identify the undisturbed fish fauna, other two/three operators set four underwater action cameras on the bottom and inside the ravines. The identification was concluded by analysing all the information, excluding possible biases, and the results are shown in Table 2.

2.2 Transitional and coastal areas in Emilia Romagna (IT)

Monitoring activities performed by ARPAE within the CASCADE project consists of bathymetric surveys of the Sacca di Goro. A boat equipped with an echo sounder and GPS was used for the bathymetric measurements (see Figure 14) which concerned the northern part of the Sacca di Goro, because the available bathymetric data of this area date back to 2003. The aim of the survey is to update the existing data acquisition.

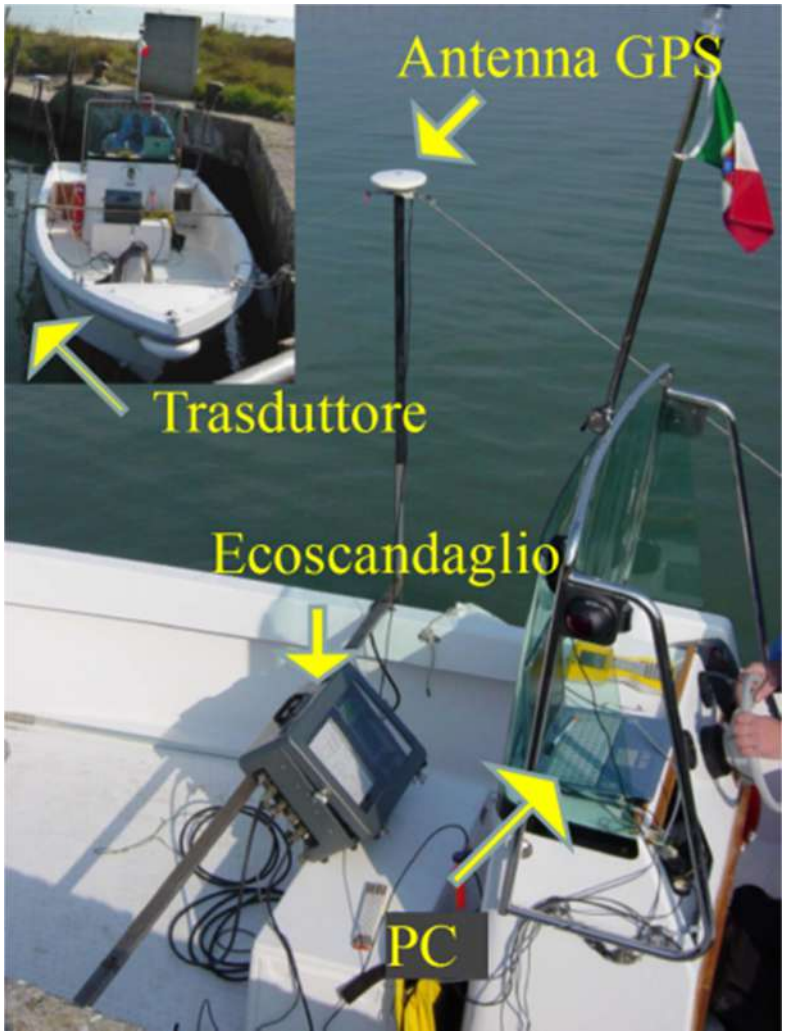


Figure 14. Boat used for the survey, equipped with the necessary instruments.

The GPS instrument used for the old survey does not provide the ellipsoidal height and consequently the depth measurement could not be corrected in real-time with the tide measure but required that this measure be entered a posteriori. This methodology could cause errors in the post-processing correction phase. The presence of waves due or oscillation due for example to the transit of other boats could affect the accuracy of the seabed depth measure.

The current RTK technology (the GPS Rover of the boat also receives data from the satellites as well as (via radio link) data from the Master station positioned on land on a point of known coordinates) and the GPS antenna positioned vertically to the transducer, allows the user to automatically compensate the GPS altitude with the depth detected by the echo sounder, completely avoiding level oscillations due to waves as well as tide.

The survey paths were drawn before the field survey, by maintaining the transects perpendicular to each other and at about 50 meters from each other (equidistant). The samples were collected with an interval of about 2.5 meters.

Figure 15. Survey design

Bathymetric surveys were carried out on the following dates:

- 19 April 2022
- 4, 11, 12 and 17 May 2022
- 7, 8, 16 June 2022
- 23 August 2022

Subsequently, measured field data has been reprocessed to eliminate errors due the interference detected by the echo sounder. These oscillations are caused by boats that pass in the bow of the boat equipped for the surveys. Once the error correction had been completed, the dataset was provided to colleagues who used these data to update the bathymetry input for the numerical model of the Sacca di Goro.

The updated bathymetric map of the Sacca di Goro was also produced by mixing all the bathymetric data collected up to now, taking care to use the most recent data where the area is covered by two overlapping surveys.

The updated bathymetric map of the Sacca di Goro is presented in Figure 16.

Figure 16. Updated bathymetric survey of the Sacca di Goro, Ferrara.

CIRSA-UNIBO performed monitoring activities in Sacca di Goro and Lago delle Nazioni that were related to phytoplankton and phytobenthos diversity and abundance and to the water column parameters. These activities are not currently performed by the University, as ARPAE performs routine activities every three months, but were aimed at a better knowledge of the environmental status of these transitional waters and at obtaining data to be used in the biogeochemical BFM model.

CIRSA implemented its instrumentation for samplings with a multiparameter probe useful to measure temperature and oxygen HQ30d Portable Meter (Hach-Lange GmbH). Oxygen is an important parameter to understand the presence of decaying organic matter due to algal blooms and condition of hypoxia (below 2-3 mg L-1) or anoxia (0,5 mg L-1) can have dramatic impacts on

marine life conditions and affected the ecosystems, including direct loss of habitat or habitat compression, altered trophic (food web) relationships, changes in migration patterns, and changes in biodiversity.

Results obtained in Sacca di Goro and Lago delle Nazioni were fully reported in D3.2.1 and partly in D5.1.1, the following graphs are an example of annual values measured by means of the multiparametric probe in the four sampling stations located into Goro Lagoon:

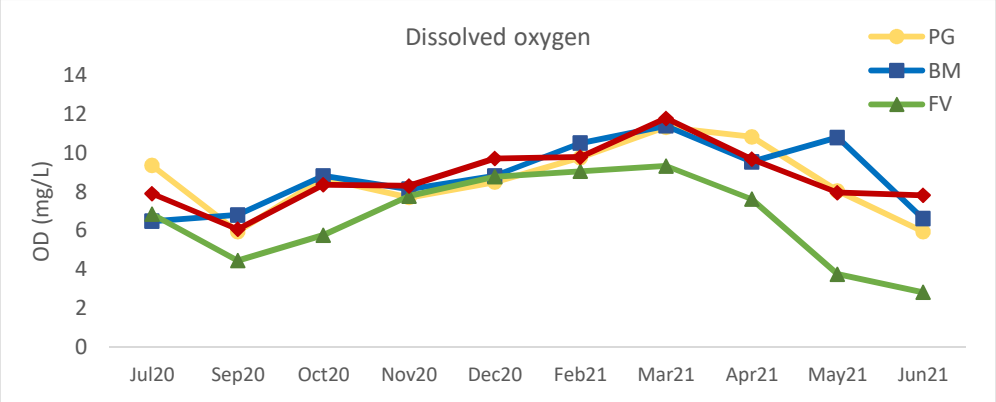


Figure 17. Dissolved oxygen (mg/L) measured by the multiparameter probe in the Goro Lagoon

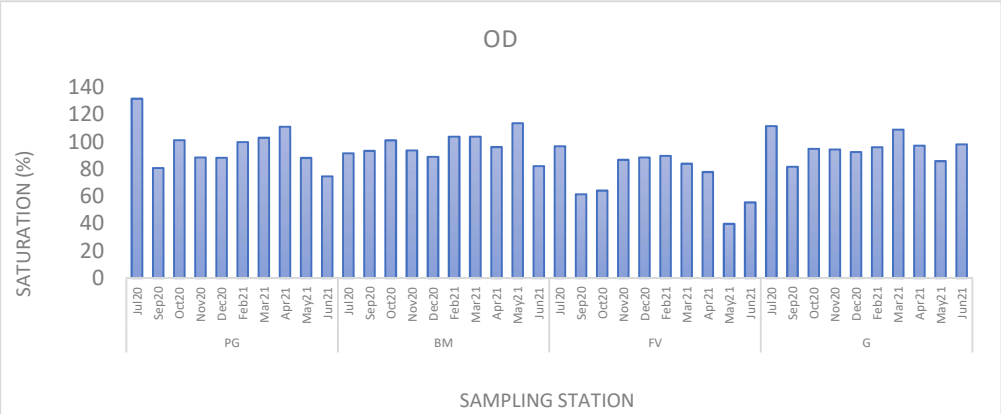


Figure 18. Dissolved oxygen saturation (%) measured by the multiparameter probe in the Goro Lagoon

The results obtained helped in clarifying some dynamics occurring in the Sacca di Goro: a critical oxygen concentration (2-3 mg L-1) was found only at the station FV in May 2021, while a border intermediate condition (3-5 mg L-1) was found during the autumn season (September-October 2020) in the same station; this site was also characterized by a higher chlorophyll concentration respect to the other three stations due to phytoplankton abundance that, after decaying of the biomass, is degraded by heterotrophic bacteria occurring in the sediments.

2.3 Torre Guaceto-Canale Reale, Punta della Contessa and Melendugno in Puglia (IT)

CMCC implemented two different activities to study the main characteristics of Torre Guaceto Marine Protected Area marine waters. The type of equipment with the main specific technical details and characteristics are reported in D4.1.1.

2.3.1 Torre Guaceto MPA fixed station data

As mentioned in the 4.1.1. Deliverable 'Equipment implemented/installed by relevant partners', CMCC installed a fixed monitoring system; indeed, one of the delimitation buoys moored in the Torre Guaceto Marine (see Fig. 19) Protected Area was made available for the integration of low-cost measurement instrumentations. The station is based on low-cost and open access technologies and allows to acquire different types of parameters.

Figure 19 Monitoring System installed on the Torre Guaceto MPA buoy.

Some examples of outputs are shown in Figure 20 for conductivity and temperature from 01/08/2021 to 16/10/2021, and Figure 21 for turbidity from 03/08/21 to 16/08/2021. Furthermore, in Figure 22, an example of intercomparison between different low-cost temperature sensors is highlighted.

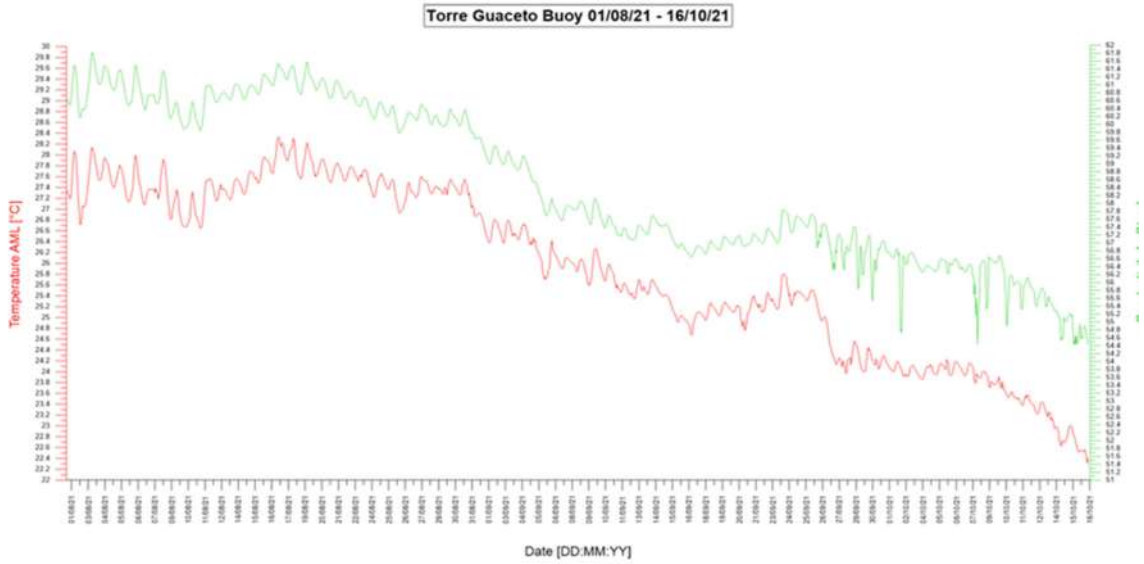


Figure 20. An example of Conductivity and Temperature data collected during the first period of installation.

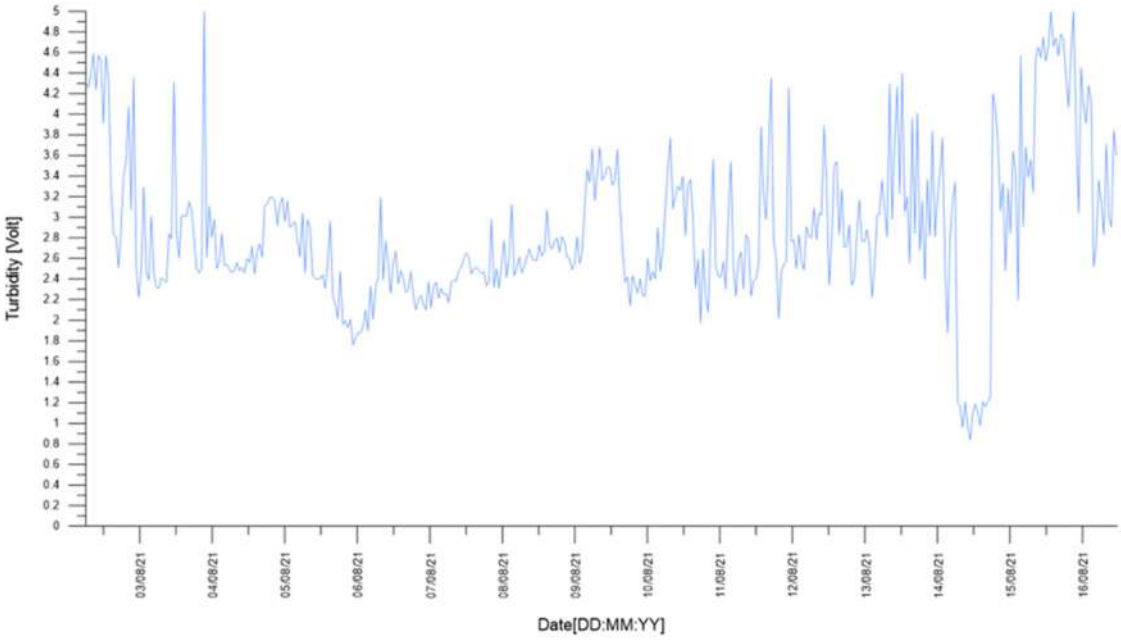


Figure 21. An example of Turbidity data collected during the first period of installation.

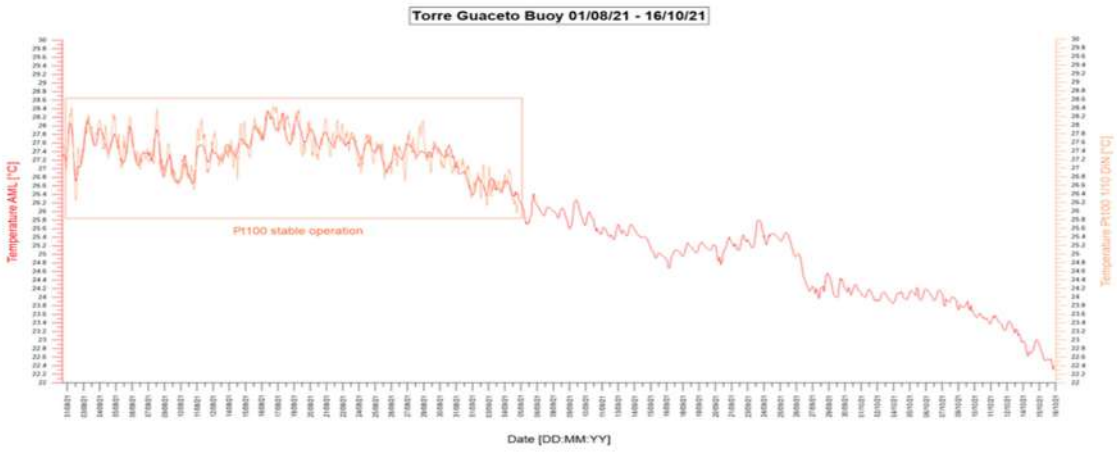


Figure 22. An example of comparison between different low-cost Temperature sensors data

During October 2021 the sensors installed in the underwater part of the buoy were pulled out by external actions, so all the system was removed and CMCC is working on a new deployment.

2.3.2 Torre Guaceto MPA monitoring survey data.

As mentioned in the 4.1.1. Deliverable 'Equipment implemented/installed by relevant partners', CMCC performed in July 2021 an extended survey in the Torre Guaceto MPA marine waters.

The campaign consists of n.20 stations, in each station a multiparametric probe was used allowing to perform a series of vertical profiles and to acquire along the water column the following parameters: depth, temperature, conductivity (salinity, density), dissolved oxygen, pH, chlorophyll a, turbidity. The spatial distribution of the profiles is shown in Figure 23.

Figure 23. Survey stations and spatial distribution

The data acquired by the multiparametric probe were processed to have the vertical distribution of the measured variables in all the stations. All the data will be used for numerical model validation. We report some preliminary results processed by Ocean Data View software. In particular, Figure 24 shows the iso-surfaces at 5m of temperature, salinity, dissolved oxygen, chlorophyll and suspended solids, while vertical profiles of temperature, salinity and chlorophyll-a are displayed in Figure 25 and 26 respectively.

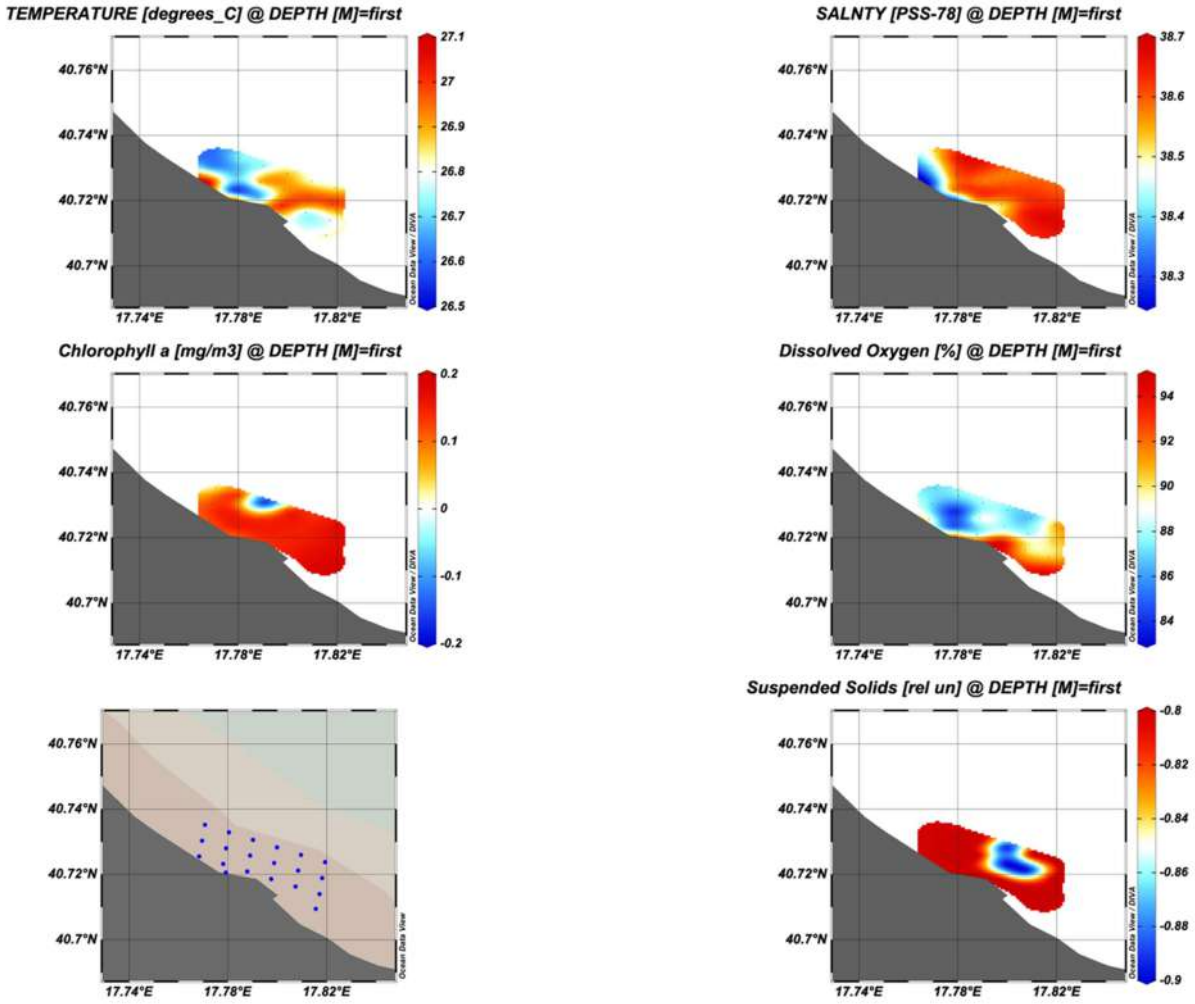


Figure 24. Isosurfaces at 5m of temperature, salinity, dissolved oxygen, chlorophyll, and suspended solids.

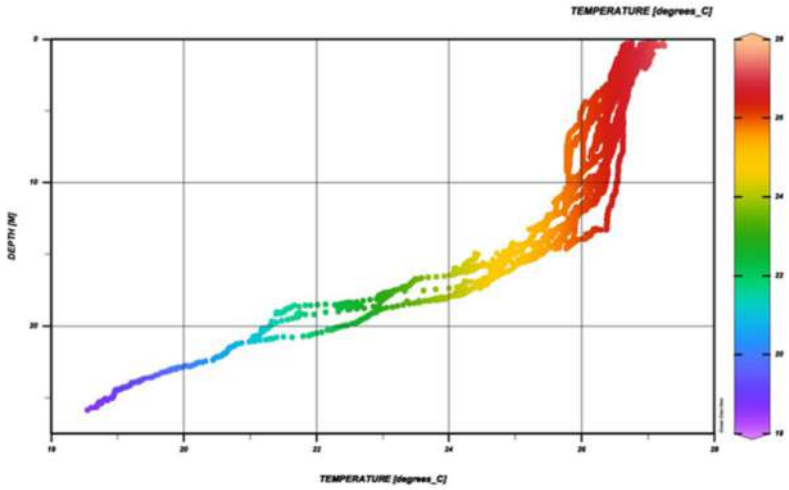


Figure 25. Temperature vertical profiles.

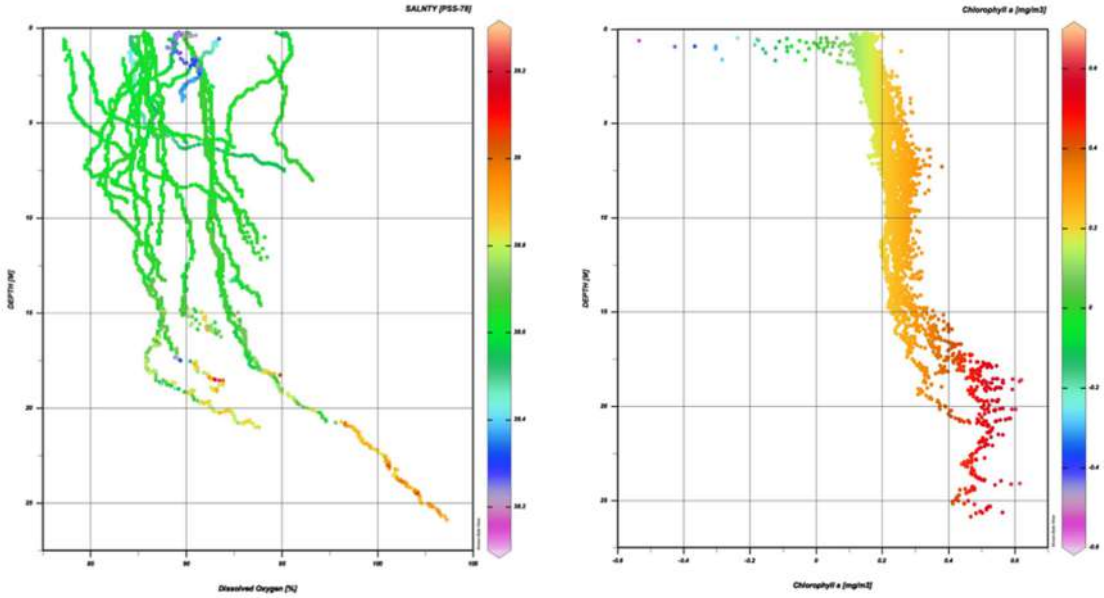


Figure 26. Salinity (left) and Chlorophyll-a (right) vertical profiles.

2.3.3 Pilot 3 chemical analysis

PP9 (UniSalento, analytical chemistry group) collected water and sediment samples in P3: the sampling points are listed in Table 3.

Table 3. sampling points in Pilot 3 area

eventDate	Matrix	EcosystemType	Locality	decimallatitude	decimallongitude	stationname
28/07/21	sediment	marinecoastal waters	AMP Torre Guaceto	40,7050	17,8071	TG1
28/07/21	sediment	marinecoastal waters	AMP Torre Guaceto	40,7099	17,8065	TG2
28/07/21	sediment	marinecoastal waters	AMP Torre Guaceto	40,7166	17,8049	TG2B
28/07/21	sediment	marinecoastal waters	AMP Torre Guaceto	40,7153	17,8032	TG3
28/07/21	sediment	marinecoastal waters	AMP Torre Guaceto	40,7075	17,8029	BAY2
22/12/21	sediment	marinecoastal waters	Melendugno	40,3223	18,3824	SF1
22/12/21	sediment	marinecoastal waters	Melendugno	40,3207	18,3835	SF2
22/12/21	sediment	marinecoastal waters	Melendugno	40,3191	18,3847	SF3
14/02/22	sediment	marinecoastal waters	Punta dellaContessa	40,6050	18,0358	PC1
14/02/22	sediment	marinecoastal waters	Punta dellaContessa	40,6081	18,0377	PC2
14/02/22	sediment	marinecoastal waters	Punta dellaContessa	40,6138	18,0376	PC2B
14/02/22	sediment	marinecoastal waters	Punta dellaContessa	40,6051	18,0417	PC3
14/02/22	sediment	marinecoastal waters	Punta dellaContessa	40,6045	18,0479	PC3B
28/07/21	water	marinecoastal waters	AMP Torre Guaceto	40,7050	17,8071	TG1
28/07/21	water	marinecoastal waters	AMP Torre Guaceto	40,7099	17,8065	TG2
28/07/21	water	marinecoastal waters	AMP Torre	40,7166	17,8049	TG2B

			Guaceto			
28/07/21	water	marinecoastal waters	AMP Torre Guaceto	40,7153	17,8032	TG3
28/07/21	water	marinecoastal waters	AMP Torre Guaceto	40,7075	17,8029	BAY2
22/12/21	water	marinecoastal waters	Melendugno	40,3223	18,3824	SF1
22/12/21	water	marinecoastal waters	Melendugno	40,3207	18,3835	SF2
22/12/21	water	marinecoastal waters	Melendugno	40,3191	18,3847	SF3
14/02/22	water	marinecoastal waters	Punta dellaContessa	40,6050	18,0358	PC1
14/02/22	water	marinecoastal waters	Punta dellaContessa	40,6081	18,0377	PC2
14/02/22	water	marinecoastal waters	Punta dellaContessa	40,6138	18,0376	PC2B
14/02/22	water	marinecoastal waters	Punta dellaContessa	40,6051	18,0417	PC3
14/02/22	water	marinecoastal waters	Punta dellaContessa	40,6045	18,0479	PC3B
15/02/22	water	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	A1 MEA
15/02/22	water	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	A1 MEB
15/02/22	water	transitional waters	Punta dellaContessa	40°36'33.69"N	18°01'42.23"E	A2 MEB
15/02/22	water	transitional waters	Punta dellaContessa	40°36'42.81"N	18°01'27.48"E	B1 MEA
15/02/22	water	transitional waters	Punta dellaContessa	40°36'49.62"N	18°01'30.10"E	B4 MEA
15/02/22	water	transitional waters	Punta dellaContessa	40°36'49.62"N	18°01'30.10"E	B4 MEB
15/02/22	water	transitional	Punta	40°36'52.59"N	18°01'25.15"E	B5A

		waters	dellaContessa			
15/02/22	water	transitional waters	Punta dellaContessa	40°36'52.59"N	18°01'25.15"E	B5 MEB
15/02/22	water	transitional waters	Punta dellaContessa	40°36'46.49"N	18°01'13.14"E	B8 MEA
15/02/22	water	transitional waters	Punta dellaContessa	40°36'46.49"N	18°01'13.14"E	B8 MEB
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	DET A1 MEA
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	DET A1 MEB
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'33.69"N	18°01'42.23"E	DET A2 MEA
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'33.69"N	18°01'42.23"E	DET A2 MEB
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'42.81"N	18°01'27.48"E	DET B1 A
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'42.81"N	18°01'27.48"E	DET B1 MEB
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'49.62"N	18°01'30.10"E	DET B4
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'52.59"N	18°01'25.15"E	DET B5 MEA
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'52.59"N	18°01'25.15"E	DET B5 MEB
15/02/22	Detrito	transitional waters	Punta dellaContessa	40°36'46.49"N	18°01'13.14"E	B8 DET MEA
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	EPI A1 MEA
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	EPI A1 MEB

15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'33.69"N	18°01'42.23"E	EPI A2 MEA
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'33.69"N	18°01'42.23"E	EPI A2 MEB
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'42.81"N	18°01'27.48"E	EPI B1 MEA
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'42.81"N	18°01'27.48"E	EPI B1 MEB
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'49.62"N	18°01'30.10"E	EPI B4 MEA
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'49.62"N	18°01'30.10"E	EPI B4 MEAB
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'52.59"N	18°01'25.15"E	EPI B5 MEA
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'52.59"N	18°01'25.15"E	EPI B5 MEB
15/02/22	Epilithon	transitional waters	Punta dellaContessa	40°36'46.49"N	18°01'13.14"E	B8 MEA
28/02/22	biota	transitional waters	Punta dellaContessa	40°36'33.69"N	18°01'42.23"E	*Gammarusaequicauda (10) A1 MEA*
28/02/22	biota	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	isopodi (20)A1 MEA
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	SED A1 MEA
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'37.11"N	18°01'43.65"E	SED A1 MEB
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'33.69"N	18°01'42.23"E	SED A2 MEA
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'33.69"N	18°01'42.23"E	SED A2 MEB
15/02/22	sediment	transitional waters	Punta dellaCont	40°36'42.81"N	18°01'27.48"E	SED B1 MEA

			essa			
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'42.81"N	18°01'27.48"E	SED B1 MEB
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'49.62"N	18°01'30.10"E	SED B4A
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'52.59"N	18°01'25.15"E	SED B5 MEA
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'52.59"N	18°01'25.15"E	SED B5 MEB
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'46.49"N	18°01'13.14"E	B8 MEA
15/02/22	sediment	transitional waters	Punta dellaContessa	40°36'46.49"N	18°01'13.14"E	SED B8 MEB

These samples have been analysed for heavy metals, chlorinated pesticides and microplastics. For metal analysis in sediments the protocol UNI EN 54321: 2021 was followed. In particular sediment samples were freeze-dried and sieved. Fractions < 2 mm were used for further metal analysis by ICPMS after wet digestion of the samples in closed vessel. The microwave digestion with nitric acid and hydrochloric acid was performed at 175 °C for 15 min.

The water samples were suitably diluted in double-distilled water (referring to their conductivity) and acidified with aquaregia 1:10 (9 ml sample and 1 ml aquaregia water) using the protocol UNI EN 17294-2:2016.

Heavy metal determination on both solutions (from seawater and sediments) were carried out by ICPMS and relevant results are collected in Table 4.

Table 4. heavy metals in samples from Pilot 3 area(mlq=minore del limite di quantificazione)

station name	unit	sampling position	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
TG1	mg/kg p.s.		1,5	mlq	5,4	1,0	mlq	1,4	1,8	15,1

TG2	mg/kg p.s.		6,3	mlq	5,7	0,8	mlq	1,5	9,5	9,5
TG2B	mg/kg p.s.		9,4	mlq	14,8	2,0	mlq	3,0	3,4	25,9
TG3	mg/kg p.s.		13,2	mlq	7,9	0,9	mlq	2,1	1,9	6,0
BAY2	mg/kg p.s.		12,3	0,29	24,6	3,1	mlq	5,9	3,7	16,6
SF1	mg/kg p.s.		1,8	mlq	4,9	0,5	mlq	1,2	1,2	5,7
SF2	mg/kg p.s.		1,9	mlq	5,0	0,4	mlq	1,0	1,3	7,7
SF3	mg/kg p.s.		1,7	mlq	5,0	0,5	mlq	1,1	1,5	5,9
PC1	mg/kg p.s.		4,2	mlq	3,4	0,8	0,2	1,3	1,6	4,6
PC2	mg/kg p.s.		6,1	mlq	7,1	1,6	0,4	2,7	2,9	28,7
PC2B	mg/kg p.s.		6,2	mlq	4,4	1,1	0,1	1,4	2,3	9,0
PC3	mg/kg p.s.		14,1	mlq	6,0	1,0	0,3	1,8	2,6	7,4
PC3B	mg/kg p.s.		41,8	mlq	22,0	3,1	0,6	5,3	5,7	99,2
TG1	µg/l	bottom	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG2	µg/l	bottom	0,3	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG2B	µg/l	bottom	0,5	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG3	µg/l	bottom	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
BAY2	µg/l	bottom	0,4	mlq	mlq	mlq	mlq	mlq	mlq	mlq
SF1	µg/l	bottom	1,2	mlq	mlq	mlq	0,4	mlq	mlq	mlq
SF2	µg/l	bottom	0,7	mlq	mlq	mlq	mlq	mlq	mlq	mlq
SF3	µg/l	bottom	0,6	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC1	µg/l	bottom	0,4	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC2	µg/l	bottom	0,4	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC2B	µg/l	bottom	0,4	mlq	mlq	mlq	mlq	mlq	mlq	mlq

PC3	µg/l	bottom	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC3B	µg/l	bottom	mlq	mlq	1,3	mlq	mlq	mlq	mlq	mlq
A1 MEA	µg/l	surface	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
A1 MEB	µg/l	surface	42,6	mlq	mlq	mlq	mlq	mlq	mlq	mlq
A2 MEB	µg/l	surface	mlq	mlq	mlq	mlq	mlq	mlq	mlq	2076,0
B1 MEA	µg/l	surface	mlq	mlq	mlq	mlq	mlq	mlq	mlq	4916,8
B4 MEA	µg/l	surface	38,2	mlq	mlq	mlq	mlq	mlq	mlq	mlq
B4 MEB	µg/l	surface	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
B5A	µg/l	surface	44,3	mlq	mlq	84,6	mlq	62,3	mlq	mlq
B5 MEB	µg/l	surface	mlq	mlq	mlq	mlq	mlq	58,6	mlq	mlq
B8 MEA	µg/l	surface	39,2	mlq	mlq	mlq	mlq	69,5	mlq	mlq
B8 MEB	µg/l	surface	mlq	mlq	64,4	277,7	mlq	70,2	mlq	5916,7
DET A1 MEA	µg/kg		347,1	mlq	188,4	1352,3	mlq	3386,2	376,3	3748,1
DET A1 MEB	µg/kg		401,4	mlq	597,2	1016,0	mlq	543,5	315,5	6448,6
DET A2 MEA	µg/kg		mlq	mlq	211,3	915,9	mlq	259,7	mlq	26263,3
DET A2 MEB	µg/kg		mlq	mlq	247,0	166,9	mlq	204,8	mlq	50595,4
DET B1 A	µg/kg		619,5	mlq	785,0	488,8	mlq	747,8	901,1	1567,9
DET B1 MEB	µg/kg		mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
DET B4	µg/kg		6699,8	143,8	4330,3	9060,5	119,9	9695,9	13599,1	10277,9
DET B5 MEA	µg/kg		1072,0	mlq	554,0	2272,5	mlq	2858,9	2146,0	5218,1
DET B5 MEB	µg/kg		908,6	mlq	812,8	2489,8	mlq	2503,4	1103,4	8891,2
B8 DET MEA	µg/kg		796,0	mlq	1182,2	3114,0	mlq	1503,0	963,1	6398,8
EPI A1 MEA	µg/kg		1602,7	mlq	2785,5	3678,5	mlq	2149,2	1915,8	7408,1
EPI A1 MEB	µg/kg		1247,4	mlq	1069,0	2868,8	mlq	1265,5	1051,3	6385,4
EPI A2 MEA	µg/kg		679,7	mlq	512,6	768,8	mlq	481,5	571,5	3188,2
EPI A2 MEB	µg/kg		mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
EPI B1 MEA	µg/kg		1141,2	mlq	2681,9	1597,5	mlq	2170,6	2863,9	8985,3
EPI B1 MEB	µg/kg		317,7	mlq	655,6	339,6	mlq	662,4	653,4	2349,8
EPI B4 MEA	µg/kg		5463,1	371,2	7167,9	5944,5	mlq	6193,8	11090,5	12941,3
EPI B4 MEAB	µg/kg		3459,6	235,4	6056,4	6179,6	97,5	6064,5	8952,2	9978,4
EPI B5 MEA	µg/kg		2304,4	mlq	1368,4	1242,6	mlq	1504,9	2730,6	4733,9

EPI B5 MEB	µg/kg		3234,9	mlq	1395,6	1574,5	29,4	1933,4	2834,0	4611,0
B8 MEA	µg/kg		3143,7	mlq	5664,0	4295,0	mlq	6060,3	8965,6	13616,8
Gammarus a. (10) A1 MEA	µg/kg		987,4	mlq	712,3	14646,1	mlq	1638,2	684,6	21344,2
Isopodi (20) A1 MEA	µg/kg		mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
SED A1 MEA	µg/kg		3945,8	mlq	3283,1	1315,1	241,5	1497,4	1991,4	5687,9
SED A1 MEB	µg/kg		3021,9	mlq	2403,7	644,4	172,8	992,6	1449,8	3571,8
SED A2 MEA	µg/kg		7250,5	119,9	33806,9	27181,5	112,8	20363,7	13487,3	41458,9
SED A2 MEB	µg/kg		9138,4	182,0	39453,4	31774,9	142,6	24262,0	15624,8	49328,0
SED B1 MEA	µg/kg		7922,1	207,1	19461,3	13121,7	mlq	14754,5	17275,0	26613,3
SED B1 MEB	µg/kg		17128,4	220,2	37736,2	19105,9	mlq	25388,8	19352,6	40842,7
SED B4A	µg/kg		4219,6	185,6	17888,2	21292,4	mlq	17601,0	12728,3	71282,1
SED B5 MEA	µg/kg		12977,6	1788,1	63524,9	28131,9	251,3	37783,1	25969,7	58718,0
SED B5 MEB	µg/kg		8632,9	272,7	39321,2	20401,8	235,1	24066,6	18178,1	42756,7
B8 MEA	µg/kg		9780,4	191,3	37646,4	25037,6	mlq	26510,2	17117,0	44451,7
SED B8 MEB	µg/kg		10292,8	253,1	37664,9	20947,4	182,8	24296,6	23800,3	39298,4

Chlorinated pesticides in water were determined according to method EPA 3510C whereas method EPA 3550 was used to analyse these pollutants in sediments. Results are reported in table 3.

Table 5. chlorinated pesticides in seawater and sediments sampled in Pilot 3 (mlq=minore del limite di quantificazione)

stationname	4,4'-DDT	2,4'-DDT	4,4'-DDE	2,4'-DDE	4,4'-DDD	2,4'-DDD	Aldrin	Dieldrin	Endrin	alfa-Endosulfan
TG1	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG2	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG2B	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG3	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
BAY2	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
SF1	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq

SF2	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
SF3	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC1	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC2	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC2B	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC3	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC3B	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG1	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG2	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG2B	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
TG3	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
BAY2	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
SF1	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
SF2	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
SF3	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC1	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC2	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC2B	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC3	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq
PC3B	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq	mlq

Microplastics (MPs) in seawater were recovered by filtration on quartz filters afterdigestionoforganicsubstanceswith3%hydrogenperoxide. Filters were washed with prefiltered ultrapure water, dried and MPs analysed both microscopically and spectroscopically. Marine sediments were thawed, oven-dried at 50°C for 1 week, and manually sieved on a 2 mm metal sieve to remove coarse particles. An aliquot (30g) was weighed in a 400 mL beaker and 100 mL of a saturated NaCl solution containing 3% H_2O_2 was added. The resulting mixture was stirred overnight. Then the beaker was tilted and allowed to decant for 1hour. The solution was carefully transferred to two 50 mL via l sand centrifugated at 3000 rpm for 5 minutes to precipitate any sand residue. The supernatant was filtered on 0.7µm What man glass fiber filter store cover MPs. The filtrate was added to the sediment in the beaker and the extraction procedure was repeated, filtering the supernatant on the same 0.7 µm filter. After filtration, the filter was washed with 0.1 µm filtered

Milli Qwater to remove salt residues, dried for 24h then MPs were determined. The recovery of the analytical procedure was ensured by analysing samples spiked with known amount of PS spheres (6 µm diameter).

Results of microplastics analysis are in tables 6-8. The morphological characterization was carried out on a Nikon Eclipse microscope (Tables 6 and 7) whereas the chemical characterization of polymers was carried out by Raman spectroscopy (Table 8).

Table 6. number of microplastics in seawater and sediments sampled in Pilot 3

TG2B	item/30 g dry sediment	2	10	16	8	15	20
TG3	item/30 g dry sediment	0	2	4	9	8	18
BAY2	item/30 g dry sediment	0	3	8	17	12	16
SF1	item/30 g dry sediment	0	10	5	12	12	17
SF2	item/30 g dry sediment	1	11	4	14	14	20
SF3	item/30 g dry sediment	0	8	6	9	14	24
PC1	item/30 g dry sediment	3	13	13	26	24	20
PC2	item/30 g dry sediment	0	4	5	6	5	15
PC2B	item/30 g dry sediment	0	2	4	5	6	21
PC3	item/30 g dry sediment	1	2	5	18	15	31
PC3B	item/30 g dry sediment	0	1	1	2	2	8
TG1	item/L seawater	5	8	5	3	5	6
TG2	item/L seawater	0	2	6	11	16	20
TG2B	item/L seawater	0	5	2	7	8	14
TG3	item/L seawater	3	5	4	10	21	20
BAY2	item/L seawater	4	11	5	8	6	19
SF1	item/L seawater	1	1	2	2	4	10
SF2	item/L seawater	1	6	9	14	20	15
SF3	item/L seawater	0	9	6	7	9	14
PC1	item/L seawater	0	6	12	17	22	50
PC2	item/L seawater	1	3	4	4	2	10
PC2B	item/L seawater	1	6	5	12	7	11
PC3	item/L seawater	1	1	2	0	2	8
PC3B	item/L seawater	1	8	9	30	6	33

Table 7. morphology and colour of microplastics in seawater and sediments sampled in Pilot 3

stationname	Bead green	Bead black	Fiber black	Fiberblue	Fiberred	fiber green	Fiber orange	Fibertrasparent	Fiberpink	Fibergrey	Fiberbrown	Fiberyellow	fiberpurple	fragment black	Fragment blue	Fragment red	Fragment yellow	Fragment orange	Fragmentrasparent	Fragment purple	fragmentpink	Fragment green	Fragment brown	Fragment grey	spheretrasparent
TG 1	0	0	4	4	4	0	0	18	0	1	2	1	0	0	0	0	0	5	0	0	0	0	0	0	0
TG 2	0	0	10	10	3	0	0	8	0	1	0	0	0	1	0	0	0	0	0	3	0	0	0	0	0
TG 2B	0	0	12	14	1	1	0	17	0	2	0	5	0	0	7	4	1	0	0	0	0	6	0	1	0
TG 3	0	0	4	6	1	0	0	23	0	2	2	1	0	0	1	0	0	0	0	1	0	0	0	0	0
BA Y2	0	1	7	13	3	3	0	15	3	1	0	1	0	0	5	2	0	0	0	0	2	0	0	0	0
SF 1	0	0	8	7	1	3	1	17	1	1	3	1	1	3	3	0	2	1	0	2	1	0	0	0	0
SF 2	0	0	23	17	0	1	0	19	1	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0
SF 3	1	0	4	8	1	1	1	25	1	0	3	4	2	0	6	0	1	3	0	0	0	0	0	0	0
PC 1	0	0	40	7	2	1	1	18	0	2	1	0	0	4	10	10	0	0	0	1	0	2	0	0	0
PC 2	0	0	5	13	7	0	3	1	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
PC 2B	0	0	6	7	2	1	0	7	0	0	0	1	0	6	1	0	0	0	0	7	0	0	0	0	0
PC 3	0	0	5	7	2	1	9	18	2	0	0	8	0	11	2	0	0	0	0	1	4	2	0	0	0
PC 3B	0	0	1	1	2	0	0	7	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0
TG 1	0	0	4	6	3	0	5	0	0	0	0	0	0	10	0	0	0	3	0	1	0	0	0	0	0
TG 2	0	0	18	4	5	0	2	18	0	0	0	0	0	6	1	0	0	1	0	0	0	0	0	0	0
TG 2B	0	0	5	1	3	0	0	7	0	1	0	1	0	9	9	0	0	0	0	0	0	0	0	0	0
TG 3	0	0	10	5	2	0	0	14	0	1	0	4	0	5	21	0	0	0	0	0	0	1	0	0	0
BA Y2	0	0	6	8	1	0	0	2	0	1	0	1	0	14	17	0	0	0	3	0	0	0	0	0	0
SF 1	0	0	4	2	0	0	0	7	0	1	0	2	0	1	2	0	0	0	0	0	0	1	0	0	0
SF 2	0	0	6	27	3	0	0	15	0	0	0	0	0	4	7	0	0	0	0	0	0	1	0	0	2
SF 3	0	0	5	17	7	1	0	3	0	3	3	0	0	1	4	0	1	0	0	0	0	0	0	0	0
PC 1	0	0	17	40	13	1	2	30	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0
PC	0	0	5	3	2	0	0	7	0	2	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0

2																									
PC 2B	0	0	12	10	3	0	0	7	0	0	0	1	0	0	7	2	0	0	0	0	0	0	0	0	0
PC 3	0	0	1	4	1	0	0	7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
PC 3B	0	0	16	13	5	1	0	35	0	4	0	3	0	0	10	0	0	0	0	0	0	0	0	0	0

Table 8. chemical composition of microplastics in seawater and sediments sampled in Pilot 3

station name	unit	alkyd resin	PA	PE	PET	textile	PP	PUR	Polysulphone	PTFE
TG1	%	0%	15%	12%	6%	30%	0%	30%	0%	6%
TG2	%	0%	32%	4%	0%	32%	0%	29%	4%	0%
TG2B	%	0%	23%	3%	3%	26%	0%	42%	0%	3%
TG3	%	0%	20%	8%	0%	36%	0%	36%	0%	0%
BAY2	%	3%	33%	9%	0%	18%	0%	36%	0%	0%
SF1	%	4%	20%	0%	0%	52%	0%	24%	0%	0%
SF2	%	0%	4%	0%	4%	69%	0%	23%	0%	0%
SF3	%	8%	12%	4%	0%	44%	0%	24%	0%	8%
PC1	%	0%	22%	3%	8%	33%	0%	17%	0%	17%
PC2	%	0%	10%	5%	20%	35%	0%	30%	0%	0%
PC2B	%	0%	0%	0%	0%	65%	0%	30%	0%	4%
PC3	%	4%	12%	23%	4%	23%	0%	31%	0%	4%
PC3B	%	0%	6%	0%	0%	72%	0%	22%	0%	0%
TG1	%	0%	9%	0%	4%	26%	0%	57%	0%	4%
TG2	%	3%	31%	9%	0%	46%	0%	11%	0%	0%
TG2B	%	23%	23%	6%	6%	19%	0%	16%	0%	6%
TG3	%	29%	29%	3%	14%	20%	0%	6%	0%	0%
BAY2	%	26%	14%	2%	10%	29%	0%	14%	0%	5%
SF1	%	5%	16%	5%	5%	37%	0%	26%	0%	5%
SF2	%	9%	23%	3%	11%	26%	0%	29%	0%	0%
SF3	%	9%	30%	0%	5%	23%	0%	28%	5%	0%

PC1	%	3%	8%	10%	5%	41%	0%	31%	2%	0%
PC2	%	14%	7%	0%	0%	41%	0%	34%	3%	0%
PC2B	%	23%	2%	0%	0%	42%	0%	33%	0%	0%
PC3	%	0%	0%	13%	0%	73%	0%	7%	0%	7%
PC3B	%	15%	2%	4%	2%	59%	4%	13%	0%	2%

On considering data in the survey campaign carried out during Cascade project metal and organic pollutant in all Pilot 3 area sea water samples were all below the maximum allowable concentration. Arsenic and mercury in some sediment samples showed to be higher than Italian legal thresholds. In fact sediments from the sampling points TG3 and Bay2 in Torre Guaceto and PC3 and PC3B in Punta della Contessa had arsenic concentration higher than 12 mg/kg p.s.(d.w.) whereas PC2, PC3 and PC3B in Punta della Contessa had mercury levels equal or higher than 0,3 mg/kg p.s..

Overall arsenic and mercury threat remain still real in the sediments of the north area of pilot 3 (Torre Guaceto and punta della Contessa), whereas both cadmium and chlorinated pesticides deserve less attention as thresholds were not exceeded in any sample.

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

2.3.4 Pilot 3 status monitoring by using amphipods as bioindicators

Unisalento Human Anatomy group monitored Pilot 3 status by using amphipods as bioindicators: samples belonging to the Gammarus insensibilis species were collected in spring from populations located in small lagoons. Measurements of the enzymatic activity of the proteins involved in both metabolism and antioxidant response systems were carried out. Antioxidant defence biomarkers like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and metabolism enzymatic activities like cytochrome c oxidase (COX) and lactate dehydrogenase (LDH) were measured using the whole body of amphipods. The results obtained are collected in Table 9 and in histograms in Fig. 27-28.

Table 9. Data of enzymatic activities in PILOT 3 samples.

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

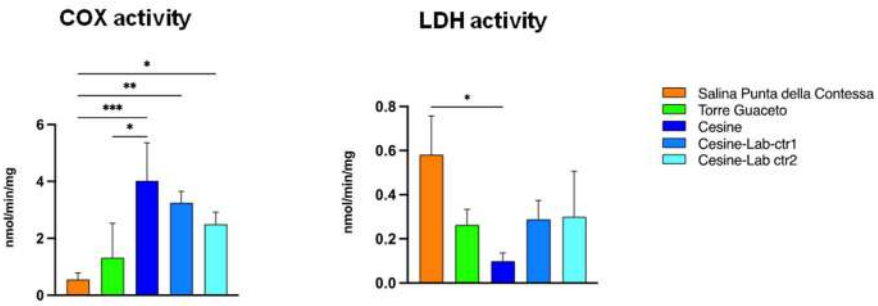


Figure 27. Quantification of metabolism enzymatic activity (COX and LDH).

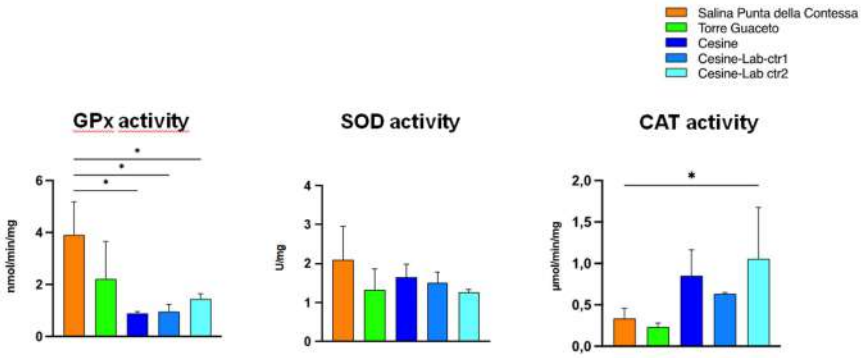


Figure 28. Quantification of antioxidant enzymatic activity (CAT, SOD and GPx).

To assess the protein amount of these enzymes in gammaridae, we analysed their protein expression by western blotting technique: the protein expression profiles showed no significant changes (Fig.29).

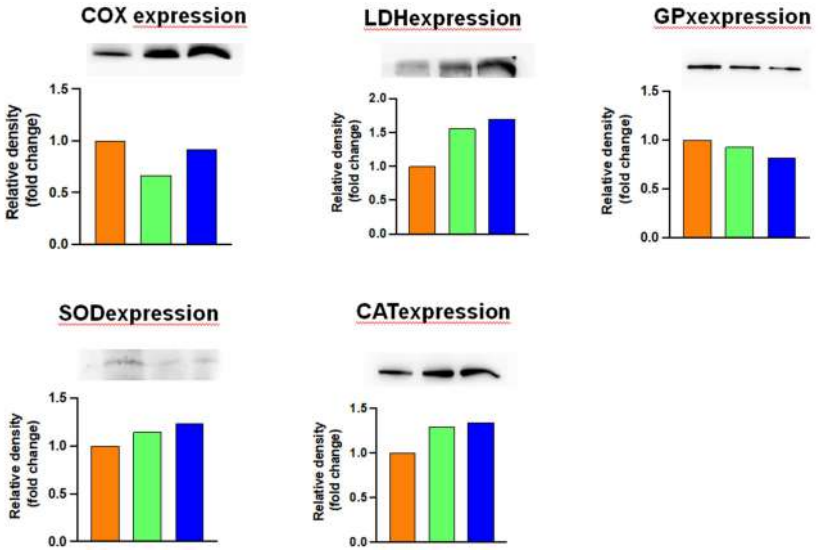


Figure 29. Protein expression analysis (COX, LDH, CAT, SOD and GPx).

Analysis of metabolism enzymatic activities in gammaridae showed a decrease in aerobic metabolism (COX reduction) and an increase in anaerobic one in specimens sampled in P3 sites compared to controls (LDH increase). The kinetic activation of LDH indicates an acclimatory response of G. Insensibilis to systemic hypoxia and may serve as a short-term ATP-generating system under critical conditions.

GPx and SOD activities increased in amphipods. Besides protecting against ROS, this enzyme has multiple functions, including xenobiotic detoxification. Thus, its higher activity may be related to the enhanced tolerance level to various environmental factors. The activity of these enzymes is reduced by negative feedback resulting from the excess of the substrate or the damage caused by the oxidative modification. Furthermore, CAT activity was reduced. This data seems to be in contrast with the other assessments, but CAT activity may increase or decrease in contaminated environments depending on the pollutant. Decreases in CAT activity has been reported in aquatic organisms exposed to various pollutants. Therefore, alterations in metabolism and antioxidant enzymes activities of Gammarus insensibilis may potentially be used as sensitive biomarkers for Pilot 3 monitoring.

2.4 Neretva River mouth (HR)

PP10 (IOF) collected mussel (*Mytilus galloprovincialis*) and sediment samples at the mouth of the Neretva River. The underwater drone of PP3 (DNC) was used to find the best locations for sampling considering micro conditions. The sites at the mouth of the Neretva River were chosen for their characteristics. It is the only river in this region that has a delta at its mouth. The delta itself,

the lagoons and the spaces filled with brackish water are areas where numerous fish and crayfish spawn. These areas are crucial for migratory movements. In addition, the Neretva Delta has a great diversity of habitats, especially water bodies and wetlands. In Croatia, the delta area is considered very important for the protection of nature and biodiversity. In addition, the entire project area, especially its fragile karst and wetland ecosystems, is threatened by various activities, such as anthropogenic impacts on freshwater ecosystems and global climate change.

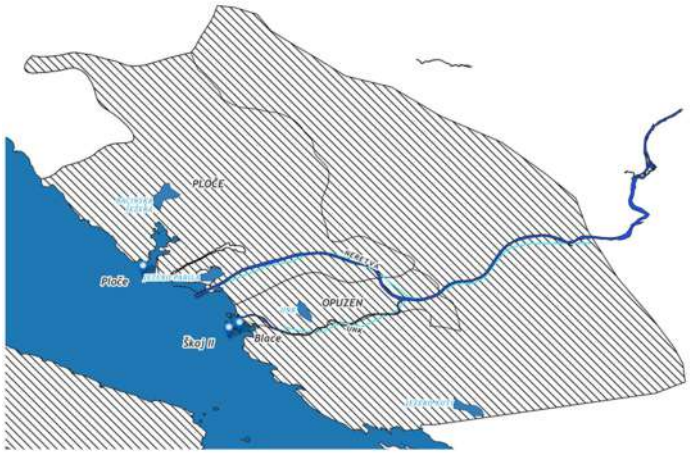


Figure 30. Sampling sites

DNC deployed the vessel and marine equipment to protect the coast from possible spills.

2.4.1 Sediment analysis

2.4.1.1 Metal analysis

Sediment samples were freeze-dried and sieved. Fractions < 2 mm were used for further trace metal analysis. Closed vessel microwave digestion with nitric acid and hydrofluoric acid was used for wet digestion of the samples. Analysis of trace metal concentrations in the digested samples was performed with an atomic absorption spectrometer, using graphite furnace (Cd, Cr, Pb and Ni) or flame (Cu, Fe, Mn, and Zn) atomic absorption techniques. The methods were validated by determining precision (reproducibility, repeatability), accuracy, and detection limits. The quality and accuracy of the analytical procedure was ensured by analysing certified reference materials (CRMs).

Station name	Cr (mg/kg)	Cu (mg/kg)	Fe (g/kg)	Mn (mg/kg)	Zn (mg/kg)	Ni (mg/kg)	Hg (mg/kg)	Cd (mg/l	Pb (mg/k
Blace 2021	31,07	7,04	16,07	399,20	46,16	19,53	0,02	0,19	13,66
Ploce 2021	21,87	3,40	11,37	252,70	36,98	12,15	0,02	0,16	9,29
Skoj 2021	112,11	13,38	28,57	546,70	103,00	25,09	0,04	0,26	26,68
Ploce 2022	30,09	6,77	15,26	450,80	38,15	11,81	0,02	0,22	9,57
Skoj 2022	51,08	9,17	17,39	379,30	61,45	70,72	0,03	0,25	14,19
Blace 2022	37,47	9,13	15,40	358,20	48,54	17,21	0,04	0,30	15,19

Table 10. Results of metal analysis at 3 sampling sites (Blace, Ploče and Škoj).

Heavy metal concentrations in sediment were analysed at all sampling sites and did not exceed the proposed ERLs (cadmium 1.2 mg/kg body weight; copper 34 mg/kg body weight; mercury 0.15 mg/kg body weight; lead 47 mg/kg body weight; zinc 150 mg/kg body weight; concentrations above the ERL may have adverse effects on marine organisms).

2.4.1.2 Analysis of organic matter, carbonate, and grain size

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

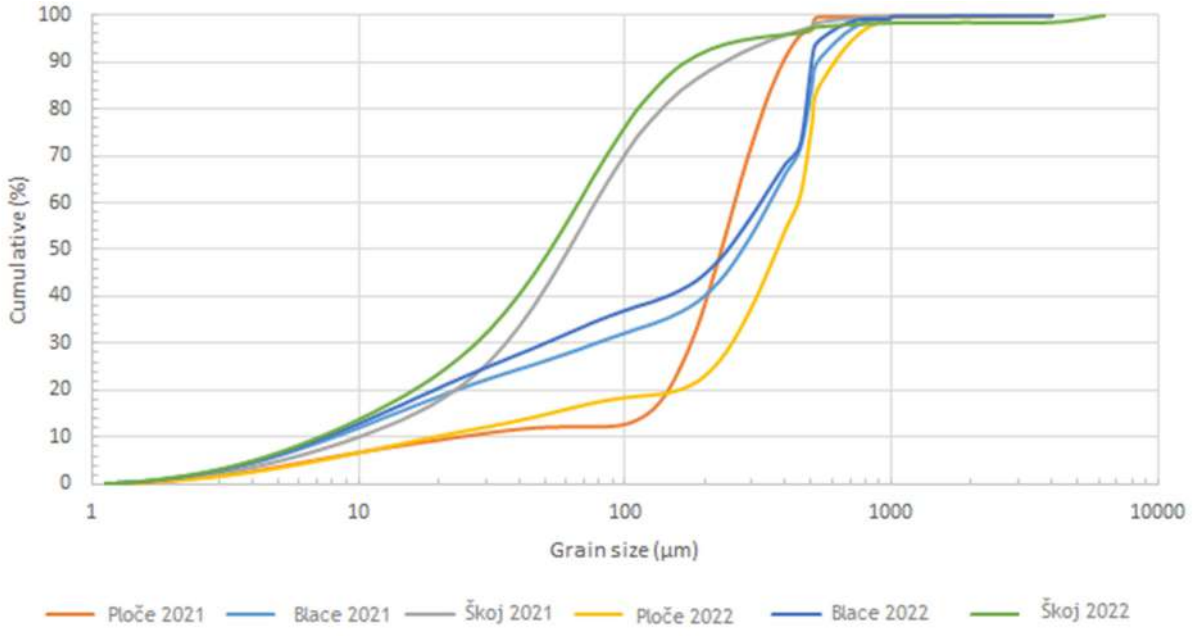


Figure 31. Results of grain size analysis at sampling sites Blace, Ploče and Škoj.

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

2.4.2 Mussel analysis

Samples were collected in plastic sampling bags and stored at -20 °C until the further analysis. Each sample was homogenized with a blender.

2.4.2.1 Metal analysis

Whole body tissue samples were freeze-dried. Closed vessel microwave digestion with nitric acid were used for sample digestion.

Analyses of trace metal concentrations in digested samples were carried out with an atomic absorption spectrometer using graphite furnace (Cd, Cr, Pb and Ni) or flame (Cu, Fe, Mn, and Zn) atomic absorption techniques. Methods were validated by determination of precision (reproducibility, repeatability), accuracy and detection limits. Quality and accuracy of the analytical procedure were ensured by analysis of Certified Reference Materials (CRMs).

Station name	Cr (mg/kg d.w.)	Cu (mg/kg d.w.)	Fe (mg/kg d.w.)	Mn (mg/kg d.w.)	Zn (mg/kg d.w.)	Ni (mg/kg d.w.)	Hg (mg/kg d.w.)	Cd (mg/kg d.w.	Pb (mg/kg d.w.)
Blace 2021	0,99	27,66	136,20	11,71	94,67	0,96	0,06	0,85	0,36
Ploce 2021	1,74	7,35	194,10	6,59	112,93	2,79	0,05	0,90	0,53
Skoj 2021	1,06	6,29	180,90	4,92	150,22	2,50	0,07	0,81	0,65
Ploce 2022	6,10	11,57	766,30	15,21	122,51	4,22	0,10	0,85	1,42
Skoj 2022	6,71	10,14	1130,00	25,97	89,14	8,34	0,11	0,80	1,89
Blace 2022	2,74	10,00	395,60	21,29	72,31	2,56	0,07	0,92	1,17

Table 11. Results of metal analysis at sampling sites Blace, Ploče and Škoj.

For biota, the moisture content of mussels was calculated by recording the difference between fresh weight and dry weight. The average moisture content in soft tissue of mussels is 80-85%. The data sets were evaluated and the concentrations of all analysed metals in mussels were below the permitted levels (according to the EU Regulation 1881/2006, the maximum permitted level for Cd is

1.0 mg/kg and for Pb 1.5 mg/kg wet weight); moreover, no concentrations unsuitable for human consumption were detected at any station.

2.4.2.2 PAH analysis

The QuEChERS method was modified, for the sample extraction step, a 5.0 g sample of mussel homogenate was placed into a 50-mL centrifuge tube containing 2.0 g of anhydrous MgSO4 and 0.5 g of NaCl. Then, 9 mL of acetonitrile was added to the tube, and the sample was vortexed for 2 min to achieve a homogeneous sample. After vortexing the samples, were centrifuged to produce a clear supernatant layer. For the sample purification step, 1.5 mL of the extract was transferred to a centrifuge tube containing 50 mg of PSA sorbents and 150 mg of anhydrous MgSO4. The sample tube was shaken vigorously for 1 min and centrifuged. A 1-mL aliquot of the extract was filtered using a 0.2 µm nonsterile syringe filter, and then, 1000 µL of the cleaned extract was placed in an autosampler vial for the UHPLC/FLD analysis.

2.4.2.3 ASP and lipophilic toxin analysis

Two grams of mussel homogenate was vortex mixed with 9 ml of methanol for 3 minutes and centrifuged for 10 minutes. The supernatant was transferred to a volumetric flask and the residue was extracted again with 9 ml methanol. After the second extraction, the volume of the collected supernatant was adjusted to 20 ml with methanol. An aliquot of 1.5 ml of the extract was filtered using a 0.2 µm or 0.45 µm non-sterile syringe filter, and then 1000 µl of the extract was added to an autosampler vial for LC-MS/MS analysis. Then, 2.5 ml of basic methanol extract was transferred to a tube and hydrolysed with HCl and NaOH. The purified extract was again added to an autosampler vial for LC-MS /MS analysis.

Hydrophilic biotoxins, namely ASP, and lipophilic toxins, including okadaic acid (OA) and its derivatives, dinophysistoxins (DTX), pectenotoxins (PTX), azaspiracids (AZA), yessotoxins (YTX), and cyclic imines (CI), were not detected.

(LOD – limit of detection)

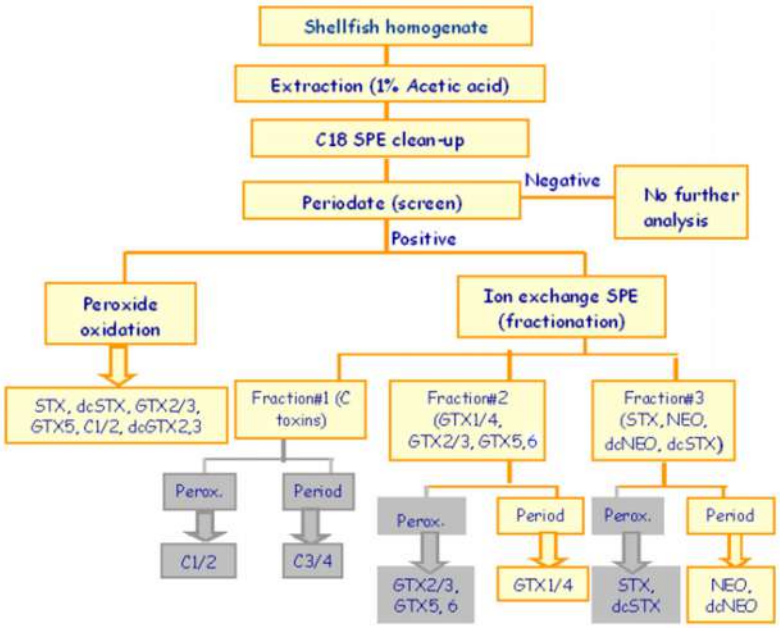
Station name	YTX (mg/kg)	hYTX (mg/kg)	GYM (mg/kg)	SPX (mg/kg)	AZA 1 (mg/kg)	AZA 2 (mg/kg)	AZA 3 (mg/kg)	DTX 1 (mg/kg)	DTX 2 (mg/kg)	PTX 1 (mg/kg)	PTX 2 (mg/kg)	DA (mg/kg)	OA (mg/kg)
Blace 2021	0,03	< LOD	0,01	0,01	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Ploce 2021	0,13	< LOD	0,02	0,01	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Skoj 2021	0,12	< LOD	0,01	0,01	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Ploce 2022	0,02	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Skoj 2022	0,01	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Blace 2022	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

Table 12. Results of ASP and lipophilic toxin analysis at 3 sampling sites (Blace, Ploče and Škoj).

2.4.2.4 PSP toxins analysis

The content of hydrophilic marine biotoxins causing Paralytic Shellfish Poisoning (PSP) was determined by UHPLC with fluorescence detection.

The extraction procedure was carried out for 5 g of the homogenate (see scheme below).



Station name	PSP (mg/kg)
Blace 2021	< LOD
Ploce 2021	< LOD
Skoj 2021	< LOD
Ploce 2022	< LOD
Skoj 2022	< LOD
Blace 2022	< LOD

Figure 32. PSP extraction procedure

Hydrophilic biotoxins, namely PSP were not detected. The low toxin levels in the bivalve molluscs analysed in this report demonstrate the excellent status of the areas studied and the safety of the molluscs for public health.

Table 13. Results of PSP toxins analysis at 3 sampling sites (Blace, Ploče and Škoj).

2.4.3 Seawater analysis

2.4.3.1 Temperature, salinity, oxygen saturation and nutrient salts

Excessive inputs of nutrient salts of phosphorus, nitrogen, and silicates into the water column result in an ecosystem response in the form of eutrophication. Eutrophication is defined as a change in the ecosystem caused by an increase in the rate of generation and external input of organic material. Eutrophication can occur naturally, but also because of human intervention in the environment. In the marine environment, the state of eutrophication can be assessed by the

Station name	% Oxygen saturation 0m	% Oxygen saturation 5m	% Oxygen saturation 7m	Temperature 0m	Temperature 2m	Temperature 5m	Temperature 7m	Salinity 0m	Salinity 2m	Salinity 5m	Salinity 7m
Blace 2021	123,0	131,0	-	25,1	24,1	24,0	-	34,1	36,6	36,8	-
Ploce 2021	130	112	-	25,3	24,0	23,0	-	36,0	36,3	37,8	-
Skoj 2021	129,0	-	112,0	25,6	24,2	23,5	23,0	35,0	36,3	35,5	36,9
Ploce 2022	97	101		13	13,3	13,7	-	36,8	37,2	37,1	-
Skoj 2022	99	-	-	13,5	13,5	13,4	13,4	37,9	37,9	37,9	-
Blace 2022	100	108	-	12,9	13,7	13,9	-	32,3	37,7	37,9	37,9

Station name	NH_4^+ µmol dm-3	NO_2^- µmol dm-3	NO_3^- µmol dm-3	TIN µmol dm-3	Ntot µmol dm-3	Norg µmol dm-3	PO_4^{3-} µmol dm-3	Ptot µmol dm-3	Porg µmol dm-3	SiO_4^{4-} µmol dm-3
Blace 2021	0,383	0,000	0,655	1,039	20,3	19,2	0,092	0,154	0,062	3,78
Ploce 2021	1,152	0,028	0,305	1,486	16,88	15,39	0,047	0,352	0,304	3,65
Skoj 2021	0,056	0,018	0,326	0,400	16,77	16,37	0,072	0,159	0,087	2,48
Ploce 2022	0,192	0,096	0,290	0,579	20,26	19,68	0,033	0,128	0,096	2,35
Skoj 2022	0,623	0,085	0,381	1,088	16,27	15,18	0,018	0,184	0,166	2,09
Blace 2022	0,296	0,083	0,409	0,788	18,40	17,62	0,045	0,948	0,903	2,87

Table 14. Results of oxygen saturation, temperature, and salinity at sampling sites Blace, Ploče and Škoj.

following indicators: transparency of the sea, oxygen saturation, concentration of nutrient salts of phosphorus, nitrogen and orthosilicates in the water column.

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

The analysis of the results of all studied parameters allows us to conclude that the ecological condition of the mouth of the Neretva River is very good.

2.5 Coastal area in Veneto (IT)

As described in deliverable 3.3.1, monitoring activities performed within CASCADE at

Natura 2000 site "le Tegnùe di Chioggia" (IT3250047) 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.

To achieve these goals, a comprehensive experimental plan was carried out between June 2021 and December 2022. This included 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) 2 campaigns with gillnets targeting fish fauna (November and March 2021, and December 2022);

D) 3 campaigns implementing an active bioacoustics survey, targeting fish community present in the area, and carried out from fall 2021 until March 2022.

Main results of these activities are summarized in the following paragraphs.

2.5.1 Video recording survey with ROV

Investigation with ROV (Remotely Operated System) instrumentation for the acquisition of HD videos and images for the study of fish populations and macro-benthic communities was carried out in the survey area on June 23, 2021, along the sections represented in Figure 33. Activities were carried out in collaboration between CORILA and OGS and took into consideration the guidelines provided by the Italian Ministry of the Environment for coralligenous monitoring, with respect to the MSFD.

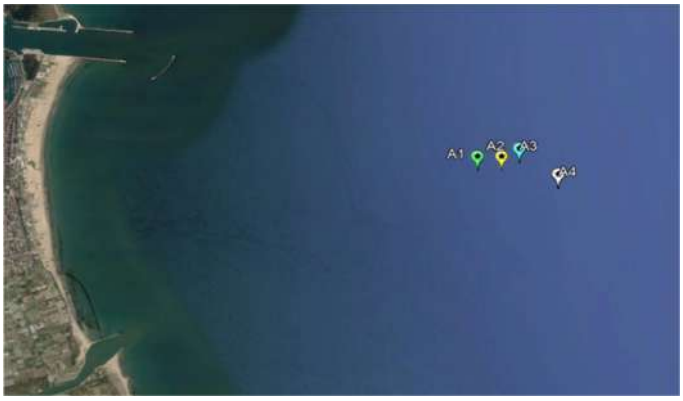


Figure 33. ROV monitoring activities at P5 Tegnùe di Chioggia. Points refer to the following coordinates: A1: 45°12,683'N; 12°22,790'E; A2: 45°12,684'N; 12°23,053'E; A3: 45°12,759'N; 12°23,251'E; A4: 45°12,522'N; 12°23,664'E.

From the videos and HD photographs analysed relating to the 4 sampled areas, it was possible to distinguish a total of 76 different taxa, 60 of which recognized at species level. Overall, the most represented taxon was that of porifera (18%), followed by crustaceans and fish (12%), molluscs and tunicates (11%), polychaetes and algae (9%) and echinoderms (8%). Bryozoans, cnidarians, and flatworms were the least represented taxa, with values of 5%, 4% and 1%, respectively (Figure 34).

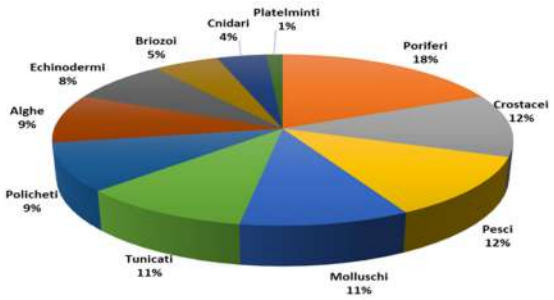


Figure 34. Relative abundance of the main observed taxa.

From an ecological habitus point of view, the organisms were mainly made-up of benthic-sessile species (60%), such as algae, porifera, cnidarians, tunicates, annelids, some molluscs, and bryozoans, followed by benthic-vagile species (28%), like most molluscs, crustaceans, echinoderms, and the flatworm *Pseudobiceros splendidus*. The remaining 12% of the assemblage was made up of fish, with mainly benthic (67%) and benthos-nektonic behavior (33%) (Figure 35).

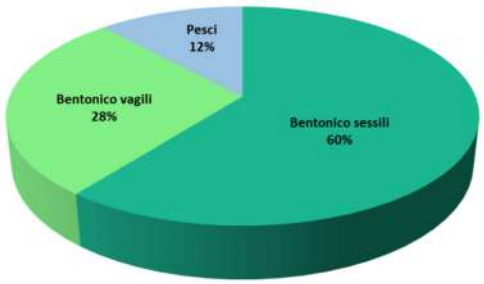


Figure 35. Relative abundance of observed taxa, by ecological habits.

Overall, among sessile organisms, the largest number of species was observed for the phylum Porifera, with 14 different taxa present, followed by crustaceans and fish with 11 and tunicates with 8 (Table 15). Considering the vagile organisms, the most represented group was that of crustaceans with 9 different taxa, followed by echinoderms and molluscs, with 6 and 5 different taxa respectively (Table 16). As regards the fish fauna, it was possible to recognize a total of 9 species (Table 17), 5 of which were found in tegnùe A3 and A4. The tegnùa that reported the highest number of species was the one closest to the coast (A1), with 47 taxa, followed by A3 (45) and A4 (44). Tegnùa A2 was found to be the least diversified, although it still reported 40 different taxa. This partial and, in any case, modest difference could be connected to the greater quantity of remains of trawling nets (as many as 14; Table 18) which were found in correspondence with tegnùa A2 and in the immediately adjacent seabed. However, these remains of nets and peaks were highly colonized, a sign of the important impact that this anthropic activity probably had in recent times (probably before the creation of protected Area 1, in August 2002).

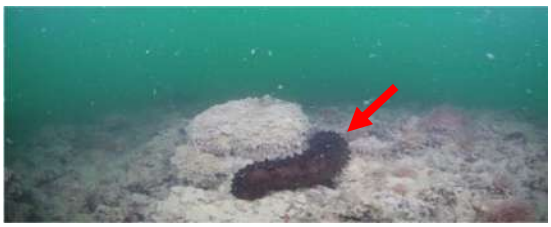


Figure 36. Holothuria tubulosa *in tegnùa A1 (red arrow).*

Figure 37. *The sponge* Cliona viridis*, in tegnùa A1.*

Phylum	Specie	Presenza			
		A1	A2	A3	A4
Algae (Rhodophyta)	*Botryocladia botryoides* (Wulfen) (Feldmann, 1941)	*	*	*	*
	Ceramiales nd (Oltmanns, 1904)	*	*	*	*
	Colpomenia sinuosa (Mertens ex Roth) (Derbès & Solier, 1851)		*		
	Cryptonemia lomation (Bertoloni) (J. Agardh, 1851)	*	*		*
	Halymenia floresii (Clemente) (C. Agardh, 1817)	*	*		*
	Lithophyllum stictiforme (J.E. Areschoug) (Hauck, 1877)	*			*
	Lithothamnion sp. (Heydrich, 1897)		*	*	
Porifera	*Antho (Antho) inconstans* (Topsent, 1925)	*		*	
	Aplysina aerophoba (Nardo, 1833)	*	*	*	*
	Chondrilla nucula (Schmidt, 1862)	*			*
	Cliona viridis (Schmidt, 1862)	*	*	*	*
	Dictyonella incisa (Schmidt, 1880)	*	*	*	*
	Dysidea avara (Schmidt, 1862)	*			
	Dysidea fragilis (Montagu, 1814)	*	*	*	
	Geodia cydonium (Linnaeus, 1767)	*	*		*
	Haliclona (Soestella) mamillata (Griessinger, 1971)			*	*
	Irciniidae nd (Gray, 1867)	*	*		*
	Petrosia (Petrosia) ficiformis (Poiret, 1789)		*		
	Phorbas tenacior (Topsent, 1925)		*		
	Pleraplysilla spinifera (Schulze, 1879)	*	*	*	
	Terpios fugax (Duchassaing & Michelotti, 1864)	*			
Cnidaria	*Caryophyllia (Caryophyllia) smithii* (Stokes & Broderip, 1828)				*
	Cornularia cornucopiae (Pallas, 1766)	*	*	*	
	Epizoanthus arenaceus (Delle Chiaje, 1836)	*		*	*
Anellida (Polychaeta)	*Chaetopterus variopedatus* (Renier, 1804)	*			*
	Lanice conchilega (Pallas, 1766)	*			
	Protula sp. (Risso, 1826)	*		*	*
	Sabellidae nd (Latreille, 1825)		*	*	*
	Serpula vermicularis (Linnaeus, 1767)	*			*
	Serpulidae nd (Rafinesque, 1815)	*	*	*	*
	Terebellidae nd (Johnston, 1846)	*	*	*	*
Mollusca	*Chama gryphoides* (Linnaeus, 1758)		*		
	Ostrea sp. (Linnaeus, 1758)				*
	Rocellaria dubia (Pennant, 1777)	*	*	*	*
Bryozoa	*Pentapora fascialis* (Pallas, 1766)	*		*	
	Schizomavella (Schizomavella) mamillata (Hincks, 1880)		*	*	*
	Schizoporella errata (Waters, 1878)	*	*	*	*
	Smittina cervicornis (Pallas, 1766)			*	
Chordata (Ascidiacea)	*Aplidium cf conicum* (Olivi, 1792)				*
	Aplidium sp. (Savigny, 1816)	*		*	
	Aplidium tabarquensis (Ramos-Espla, 1991)	*			
	Botryllus sp. (Gaertner, 1774)			*	
	Microcosmus vulgaris (Heller, 1877)	*	*	*	*
	Phallusia fumigata (Grube, 1864)	*	*	*	
	Polycitor adriaticus (Drasche, 1883)	*	*	*	*
	Pyura microcosmus (Savigny, 1816)	*	*	*	*
Totale		33	27	27	28

Table 15. List of the sessile organisms observed, with an indication of their presence within the 4 sites (A1, A2, A3 e A4).

Phylum	Specie	Presenza			
		A1	A2	A3	A4
Plathyelminthes	*Pseudobiceros splendidus* (Lang, 1884)	*			
Mollusca	*Bolinus brandaris* (Linnaeus, 1758)	*			
	Calliostoma sp. (Swainson, 1840)	*	*	*	*
	Flabellina sp. (McMurtrie, 1831)	*		*	
	Mimachlamys varia (Linnaeus, 1758)			*	*
	Pecten jacobaeus (Linnaeus, 1758)				*
Arthropoda (Crustacea)	*Dromia personata* (Linnaeus, 1758)			*	*
	Galathea sp. (JC Fabricius, 1793)		*	*	
	Galathea strigosa (Linnaeus, 1761)		*	*	
	Maja crispata (Risso, 1827)		*		
	Munida cf rugosa (JC Fabricius, 1775)				*
	Paguristes eremita (Linnaeus, 1767)	*		*	*
	Paguroidea nd (Latreille, 1802)	*	*	*	*
	Pagurus anachoretus (Risso, 1827)		*	*	
	Pilumnus sp. (Leach, 1816)	*	*	*	
Echinodermata	*Amphipholis squamata* (Delle Chiaje, 1828)	*	*	*	*
	Holothuria (Holothuria) tubulosa (Gmelin, 1791)	*	*	*	*
	Ocnus planci (Brandt, 1835)	*			
	Ophiothrix fragilis (Abildgaard, in O.F. Müller, 1789)	*	*	*	*
	Ophiuroidea nd (Gray, 1840)				*
	Paracentrotus lividus (Lamarck, 1816)	*			
Totale		12	10	13	11

Table 16. List of the vagile organisms observed, with an indication of their presence within the 4 sites (A1, A2, A3 e A4).

Phylum	Specie	Ecologia	Presenza			
			A1	A2	A3	A4
Chordata (Pisces)	*Monochirus hispidus* (Rafinesque, 1814)	bentonica (B)	*			
	Pegusa lascaris (Risso, 1810)	bentonica (B)				*
	Parablennius tentacularis (Brünnich, 1768)	bentonica (B)			*	
	Scorpaena porcus (Linnaeus, 1758)	bentonica (B)				*
	Scorpaena scrofa (Linnaeus, 1758)	bentonica (B)			*	*
	Serranus hepatus (Linnaeus, 1758)	bentonica (B)	*	*	*	*
	Chromis chromis (Linnaeus, 1758)	bentonectonica (BN)		*	*	
	Spicara maena (Linnaeus, 1758)	bentonectonica (BN)		*		*
	Trisopterus minutus (Linnaeus, 1758)	bentonectonica (BN)			*	
Totale			2	3	5	5

Table 17. List of the fish observed, with an indication of their presence within the 4 sites, and their ecological habitus (A1, A2, A3 e A4).

During the sampling with the ROV it was possible to observe from the videos also numerous remains of nets and ropes (Table 18, Table 19) used for trawling, a sign of the intense exploitation, which occurred over the years, of these environments that have always been known for their abundance of fish.

The high degree of colonization of the observed fishing gear remains (Figure 38), such as for example their engulfment by large porifera (Figure 39), lead us to assume that, at least most of

them, may derive from fishing activities prior to the creation of the 4 protected areas (including area 1 with the tegnùe we sampled), which took place with a ministerial decree of 5 August 2002.

CIME	min. video	
cima	5.49	A1
cima	8.34	
cime	8.38	
cima	2	A2
cima	10.14	
cime	12.44	
cima	16.05	
cime	18.55-19.02	
cima grossa	21	
cima inglobata in Geodia cydonium	21.59	
cime	23.27	
cime	23.42	
cime	24.14	
cime	25.23-44	
cime	27.35	
cima	29.45	
cime	4.29-44	A3-1
cima	9.01	
cime	9.28-56	
cime	10.30-58	
cime	11.31	
cima	12.14	
cime	0.39	A3-2
cime	02.08-38	
cime	2.59	
cime	3.19	
cime	3.38	
cime	4.32	
cime	5.48	
cime	7.42-48	
cime	8.36	
cime	9.11-19	
cime	9.32-42	
cime	10.21	
cima	15.38	
cime	15.59	
cime	6.23-28	A4
cima	9.58-10.02	
cima	13.03-13-35	
cime	14.05	
cime su Geodia cydonium	22.04	
cime	22.2	
cime	22.29-23.14	
groviglio di cime	23.43-24.26(groviglio)	
cima	26.33	
cime	28.46	

Table 18. List of ropes detected during the monitoring within the 4 stations, with the respective time within the video. A3 video was partitioned in 2 different recordings (A3-1 e A3-2).

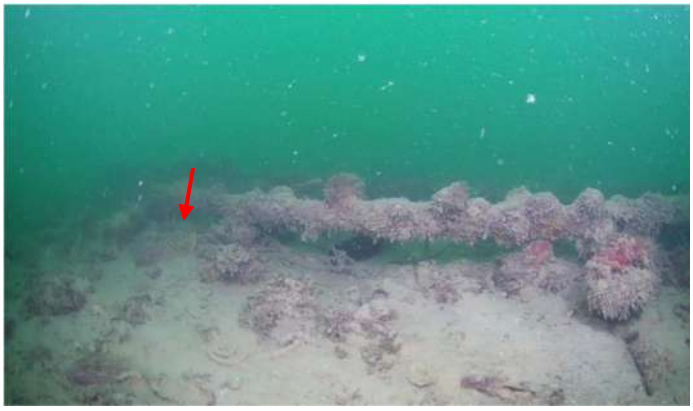


Figure 38. Highly colonized rope observed in Tegnùa A1 (min. 11.18). A fragment of trawling net can also be observed, partially covered by sediment (red arrow).

Figure 39. Large G. cydonium specimen covered by a trawling net fragment (tegnùa A4, min. 26.53).

Nets	Time video A1
Part of trawling net	5.07-5.47
Part of trawling net	7.36
Part of trawling net + large rope	11.18-11.23
Assemblage of trawling net + ropes	12.54-12.57
Part of trawling net	19.18-19.36
Part of trawling net + rope	22.22-22.23
Part of trawling net	34.39

Table 19. Trawling nets observed during the monitoring at tegnùa A1, with respective time within the video.

2.5.2 Photographic sampling survey by scuba diving

Species composition and abundances of the epibenthic assemblages on coralligenous biogenic reefs were investigated through in-situ monitoring on July 15, 2021, at 6 selected sites within the no-take zone off Chioggia. The survey was carried out in collaboration with PP6-University of Bologna, by the team of Prof Massimo Ponti. Locations and examples of the type images acquires are provided in Figure 40.

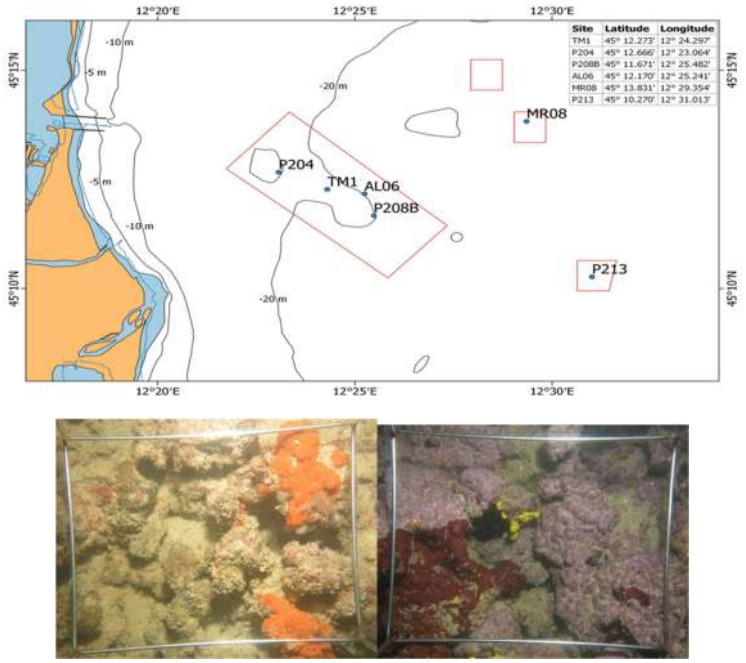


Figure 40. Photographic sampling survey by scuba diving. Stations covered by the survey (top map); examples of pictures acquired for organisms' identification and coverages estimations (bottom images).

The epibenthic assemblages found on the investigated northern Adriatic coralligenous reefs were very heterogeneous in terms of percent cover of the most abundant taxa.

The epibenthic assemblages were characterized by algal turf (percent cover between 1.46 and 36.63%), crustose coralline algae (17.15–91.17%), *Peyssonnelia* spp. (0.73-27.32%), encrusting sponges (2.64–53.32%), massive sponges (9.59–60.76%), boring sponges (6.14–25.11%), large, massive colonial ascidians (1.27–9.63%), erect sponges (0–11.88%) and boring mollusk (3.83–11.6%). The percent cover of "detritus + sediment" ranges from 43.68 to 89.16% (Fig. 41).

The dominant reef-forming organisms were crustose coralline algae that mostly consisted of *Lithophyllum incrustans* Philippi, 1837 and *Lithothamnion* sp., while other red algae belonging to the genus *Peyssonnellia* included *Peyssonnelia rosa-marina* Boudouresque & Denizot, 1973 and *Peyssonnelia rubra* (Greville) J.Agardh, 1851. Algal turf was mainly composed by the Rodophyta, *Antithamnion* sp. and *Nitophyllum punctatum* (Stackhouse) Greville, 1830. The main bioeroders were the boring sponges, *Cliona viridis* (Schmidt, 1862) and *Cliona rhodensis* Rützler & Bromley, 1981, and the endolithic bivalve *Rocellaria dubia* (Pennant, 1777). In terms of trophic guilds, epibenthic invertebrates included filter feeders, among which the most common were the sponges *Dictyonella incisa* (Schmidt, 1880), *Tedania (Tedania) anhelans* (Vio in Olivi, 1792), *Antho (Antho) inconstans* (Topsent, 1925), *Myxilla (Myxilla) rosacea* (Lieberkühn, 1859), *Sarcotragus spinosulus*

Schmidt, 1862, *Geodia cydonium* (Linnaeus, 1767), *Dysidea avara* (Schmidt, 1862), the zoantharian *Parazoanthus axinellae* (Schmidt, 1862) and the ascidian *Polycitor adriaticus* (Drasche, 1883).

The first two axes of the principal coordinate analysis (PCoA) explained 25.9 and 14.3 % of the variability of epibenthic assemblages, respectively. The scatter plot discriminated the assemblages inhabiting different sites; in particular, the MR08 site is characterized by the cover of CCA, *Peyssonnelia* spp. and large, massive colonial ascidians, while the P213 site by massive and erect sponges. The other four sites (i.e., AL06, P204, P208B and TM1) show high variability of the assemblages and are mainly described by algal turf, encrusting sponges and "detritus + sediment" (Fig. 42).

In photographic samples, species richness varies from 8 to 13 taxa at P204 and TM1, respectively (Fig. 43). The Hill's N1 index ranges from 3.5 at MR08 to 6.4 at TM1 (Fig. 44), while the Hill's N10 index from 0.4 at MR08 to 0.6 at P204 in photographic samples (Fig. 45).

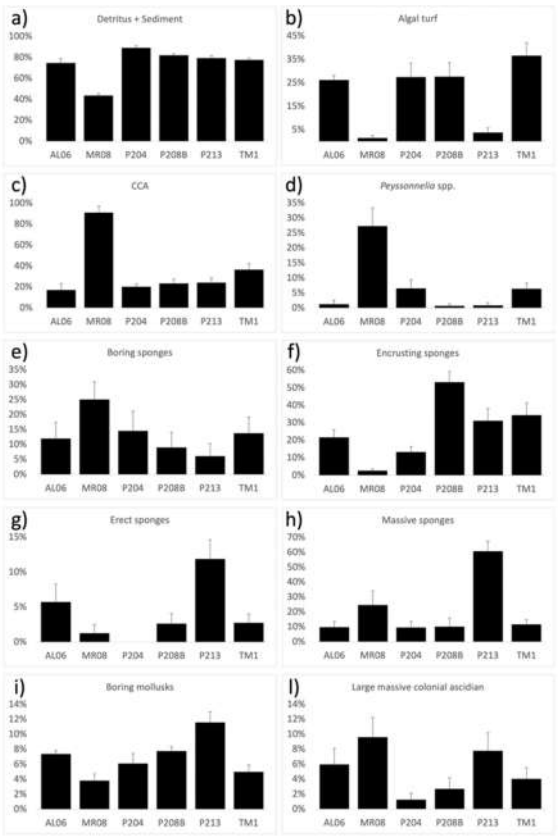


Figure 41. Mean (+SE, n = 10) percent cover values of following taxa/morphological groups: a) detritus and sediments, b) algal turf, c) crustose coralline algae (CCA), d) *Peyssonnelia* spp., e) boring sponges, f) encrusting sponges, g) erect sponges, h) massive sponges, i) boring mollusks and l) large, massive colonial ascidians

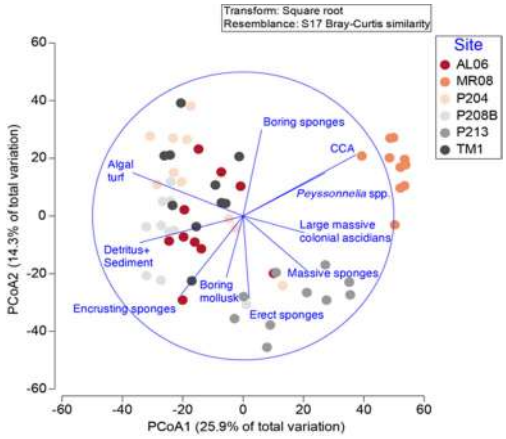


Figure 42. Unconstrained principal coordinate analysis (PCoA) ordination plots of benthic assemblage data in 2021at the 6 study sites, illustrated in different colors. Vectors superimposed to plot represent the correlations with the PCoA axes.

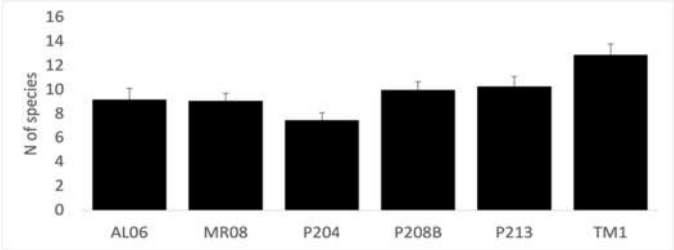


Figure 43. Mean (+SE, n = 10) species richness (S) in photographic samples (21 × 28 cm).

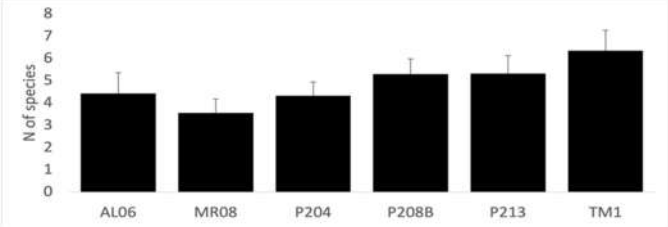


Figure 44. Mean (+SE, n = 10) species diversity in terms of effective number of taxa (Hill's N1) in photographic samples (21 × 28 cm).

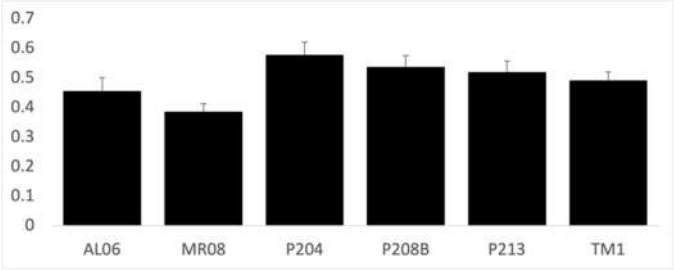


Figure 45. Mean (+SE, n = 10) evenness (Hill's N10) in photographic samples (21 × 28 cm).

2.5.3 Gillnets sampling

The samplings took place on 12/18/2021, 12/19/2021, 12/14/2022, 12/15/2022 (December samplings) and 03/26/2022 and 03/27/2022 (March samplings) with the fishing boat "Andrea G.", belonging to the Chioggia fleet, using monofilament gillnets (type "Barracuda"). People involved included the fishing vessel staff, and two researchers of the University of Padova (group of Prof Mazzoldi). For each point sampled, a set with a total length of 400 meters was used, made up of thirteen pieces with a mesh opening of 30 mm and one of 20 mm. Sampling was carried out at 3 sites during the first outing and 4 in the second, for a total of 7 hauls, with an average permanence of the nets in the water of 1 hour and 49 minutes. During each survey, which took place near Areas 1 and 3, the nets were lowered inside and outside the ZTB (Figure 46). Sampling took place both in areas with a sandy bottom and in correspondence with the rocky outcrops. For each sampling site, the following data were collected: date, coordinates of the start and end of set point, depth, start and end time of fishing and set inside or outside the ZTB. Furthermore, during the December 2022 samplings, the water temperature was recorded using temperature sensors for the points with a drop on a soft bottom. For each species caught, the total number of individuals, the size classes and the total weight of the catch were recorded.

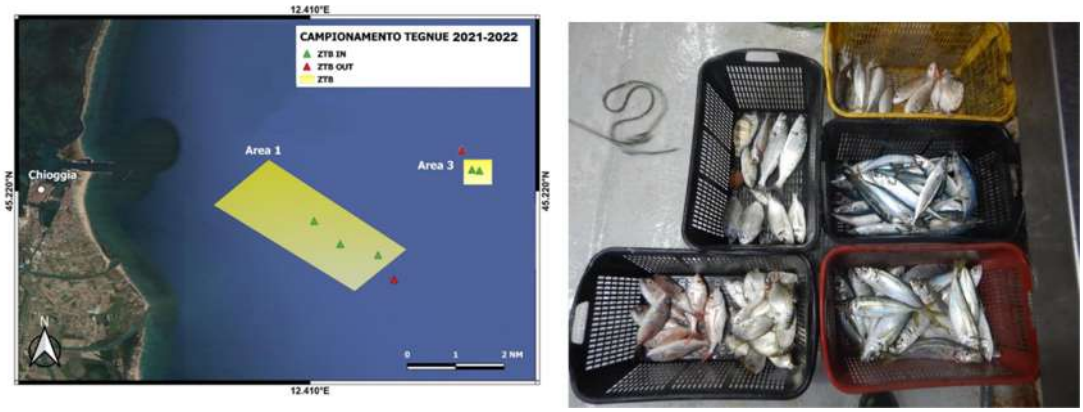


Figure 46. Gillnet sampling stations for fish fauna (left); example of fish caught at area 3 in dec 2021.

During the two seasons, 30 species were identified, with a total of 406 individuals. In the December 2021 sampling, 23 species were identified during the two sampling days (Table 20), the species with the highest number of individuals was *D. annularis* (n= 76).

Species	N dec 2021	N march 2022	N dec 2022	N tot
Aequipecten opercularis	2	0	0	2
Alosa fallax	1	0	0	1
Boops boops	11	12	1	24
Chelidonichthys lucerna	0	1	0	1
Diplodus annularis	24	11	41	76
Diplodus vulgaris	3	1	5	9
Dromia personata	2	0	3	5
Maja crispata	0	0	1	1
Merlangius merlangus	9	0	0	9
Mullus barbatus	0	0	4	4
Oblada melanura	2	2	0	4
Pagellus acarne	1	3	1	5
Pagellus erythrinus	7	14	33	54
Pagrus pagrus	3	0	0	3
Pomatomus saltatrix	2	0	0	1
Sciaena umbra	0	1	1	2
Scomber colias	0	0	2	2
Scomber scombrus	39	4	2	45
Scorpaena notata	2	1	2	5
Scorpaena scrofa	0	1	0	1
Solea solea	2	1	13	16
Sparus aurata	2	0	28	30
Spicara flexuosa	7	0	1	8
Spondyliosoma cantharus	1	0	0	1
Squilla mantis	1	0	0	1
Trachurus mediterraneus	40	15	2	57
Trisopterus capelanus	16	19	2	37

Table 20. Total number of individuals (N) for each sampled species during the three campaigns.

In the December samplings, the cove with the greatest quantity of fish and species diversity was the one carried out on 12/18/2021 on a hard bed, at a depth of 21.5 metres, within the ZTB of Area 3, with a total of 21.3 kg of catch and 16 species: *B. boops*, *D. annularis*, *D. vulgaris, D. personata, O. melanura, P. acarne, P. erythrinus, P. pagrus, P. saltatrix, S. scombrus , S. notata, S. aurata, S. smaris, S. cantharus, T. mediterraneus* and *T. capelanus*. Another set with high quantities of catch was the one carried out on 12/15/2022 on a hard bottom, at a depth of 21.9 meters, in the same site, with a total result of 13.4 kg of catch and 12 species, including: *B boops, D. annularis, D. vulgaris, P. acarne, P. erythrinus, P. pagrus, S. umbra, S. colias, S. scombrus, S. notata, S. aurata, S.*

flexuosa and *T. mediterraneus* . All the other sampled sites, however, showed low total catch values (1 kg or less in total), with few individuals caught and few species detected.

During the March sampling, 15 species were identified during the two sampling days, the species with the highest number of individuals being *P. erythrinus* (n=17). Laboratory analyses also highlighted an advanced stage of gonadal maturity in *B. boops* and *T. capelanus*. The presence of highly developed ovaries and testicles in the specimens indicate full reproductive activity in the March sampling period. As regards the month of March, the haul that showed the greatest quantity of fish and species diversity was the one carried out on 03/26/2022 on a hard bottom, at a depth of 21.2 metres, inside the ZTB of the 'Area 3, with a total result of 7.6 kg of fish and 12 species: *B. boops, D. annularis, D. vulgaris, O. melanura, P. acarne, P. erythrinus, Sciaena umbra, S. scombrus, S. notata , S. scrofa, T. mediterraneus* and *T. capelanus*. All the other sampled sites, on the other hand, showed low total catch values (less than 1 kg), with few individuals caught and few species detected.

The mean total length was calculated for each teleost species (Table 21). Invertebrates were excluded, due to their low number detected during sampling.

	Total length (cm, media±dev.st)	Sum of individual N
2021		**171**
December		**171**
Alosa fallax	34.5	1
Boops boops	23.5 ± 2.6	10
Diplodus annularis	17.2 ± 1.2	27
Diplodus vulgaris	18.5 ± 4.8	3
Merlangius merlangus	21.3 ± 2.7	9
Oblada melanura	21.8 ± 0.4	2
Pagellus acarne	15.5	1
Pagellus erythrinus	21.5 ± 0.9	7
Pagrus pagrus	18.0 ± 0.5	3
Pomatomus saltatrix	29.8 ± 0.4	2
Scomber scombrus	28.0 ± 2.2	37
Scorpaena notata	15.5 ± 0.7	2
Solea solea	22.0 ± 0.7	2
Sparus aurata	16.5 ± 8.5	2
Spicara flexuosa	17.2 ± 1.6	7
Spondyliosoma cantharus	20.0	1
Trachurus mediterraneus	25.7 ± 4.6	39
Trisopterus capelanus	18.9 ± 2.9	16
2022		**220**
March		**79**

Boops boops	23.6 ± 2.7	13
Chelidonichthys lucerna	21.0	1
Diplodus annularis	16.4 ± 1.0	11
Diplodus vulgaris	16.0	1
Oblada melanura	25.5 ± 1.4	2
Pagellus acarne	16.8 ± 1.2	3
Pagellus erythrinus	21.8 ± 2.0	17
Sciaena umbra	28.5	1
Scomber scombrus	25.3 ± 1.3	4
Scorpaena notata	14.5	1
Scorpaena scrofa	22.5	1
Trachurus mediterraneus	21.0 ± 2.8	15
Trisopterus capelanus	18.3 ± 2.3	9
December		141
Boops boops	23	1
Diplodus annularis	15.9±2.92	46
Diplodus vulgaris	17.5±1.08	4
Mullus barbatus	21.8±5.07	4
Pagellus acarne	23	1
Pagellus erythrinus	22±2.55	33
Sciaena umbra	20	1
Scomber colias	33.5	1
Scomber scombrus	30.5±0.71	2
Scorpaena notata	16.7±0.35	2
Solea solea	22.5±1.11	13
Sparus aurata	20±1.37	28
Spicara flexuosa	17.5	1
Trachurus mediterraneus	22.5±6.37	2
Trisopterus capelanus	17.5±4.24	2
Total		391

Table 21. Size and number (N) of specimens sampled within the different fishing campaigns.

For the statistical analyses, Primer software was used, and the following analyses were carried out: PCA (Figure 47), cluster analysis (Figure 48) and SIMPER analysis (with a maximum of 10 species), to evaluate the contribution of each species in determining the dissimilarity pattern (Table 22). Finally, the PERMANOVA analysis was applied (Table 23), using the R.studio statistical analysis software. The following factors were considered to analyse the catch: net mesh size (20 and 30 mm), month (December and March), inside/outside ZTB, area (1 and 3) and bottom type (hard/soft). The results of the analyses showed that the factor that most influences the composition of the catch was the type of bottom, in particular the hard bottom was the one with the greatest number of species and

the largest quantities of catch. In fact, from the results of the PCA, the most numerous catches, with *D. annularis*, *S. scombrus* and *T. mediterraneus* as the most abundant species both in terms of weight and individuals, were those with set of "spiral" nets on the bottom hard during the December campaigns. Furthermore, from the cluster analysis it is evident that the greatest similarity in the catches in terms of species and total weight is present in sets on soft bottoms.

SIMPER analysis also shows that *B. boops*, *Diplodus* spp., *P. erythrinus*, *S. scombrus* and *T. mediterraneus* are more associated with a hard bottom than other species such as *T. capelanus* and *M. merlangus*, which they prefer a mobile backdrop. As regards *S. scombrus* and T. mediterraneus, on the other hand, despite being mainly pelagic species, they are probably attracted by the greater availability of resources of a hard bottom compared to a sandy one, thus explaining the high number of specimens of the two species caught in the areas hard bottom in December 2021.

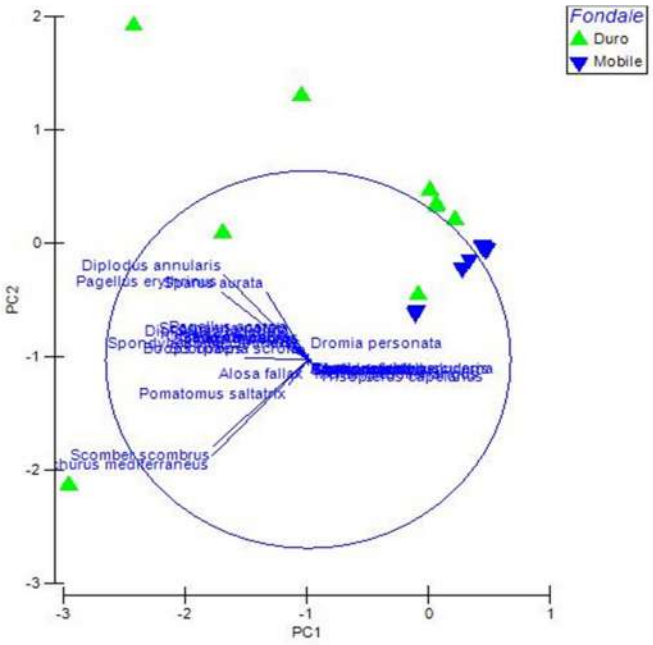


Figure 47. PCA with factor type of bottom substrate.

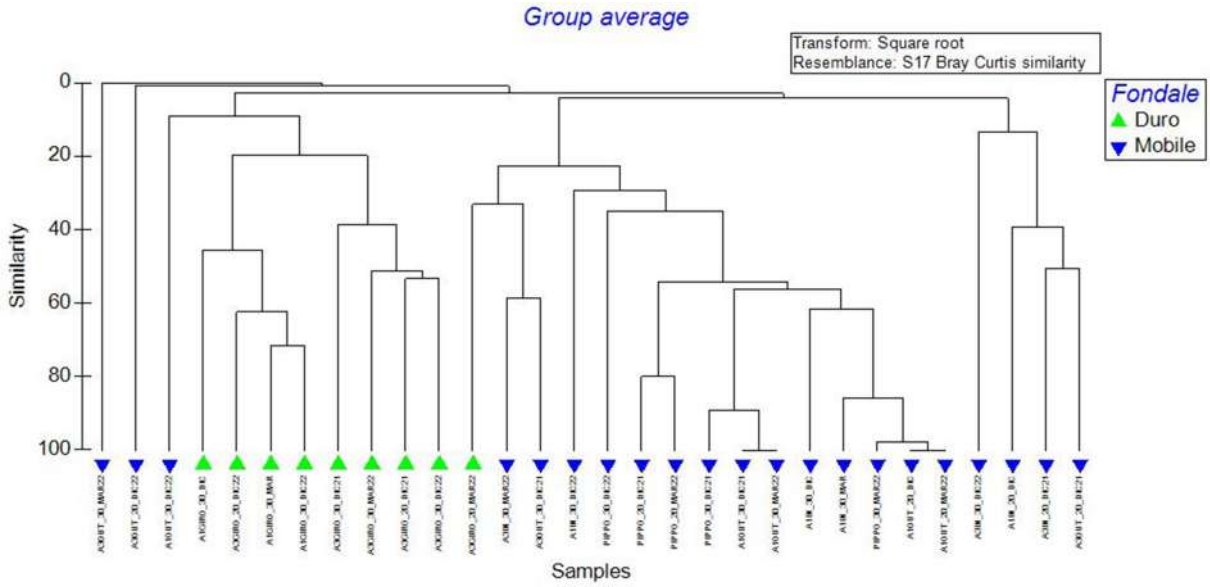


Figure 48. Cluster analysis with factor type of bottom substrate. Higher similarity is visible between sampling efforts performed on soft bottom.

Groups Duro & Mobile

Average dissimilarity = 95.81

	Group Duro	Group Mobile				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Diplodus annularis	0.72	0.00	16.97	1.74	17.71	17.71
Pagellus erythrinus	0.66	0.00	15.01	1.14	15.67	33.37
Trachurus mediterraneus	0.56	0.07	10.29	0.76	10.74	44.11
Trisopterus capelanus	0.10	0.26	8.27	0.82	8.63	52.74
Scomber scombrus	0.42	0.07	5.73	0.60	5.98	58.72
Sparus aurata	0.29	0.00	5.38	0.59	5.61	64.33
Boops boops	0.40	0.00	4.75	0.76	4.96	69.29
Dromia personata	0.09	0.01	4.48	0.57	4.68	73.96
Diplodus vulgaris	0.21	0.00	4.00	0.71	4.17	78.13
Pagellus acarne	0.20	0.00	3.68	0.65	3.85	81.98
Merlangius merlangus	0.00	0.08	2.45	0.38	2.55	84.53
Scorpaena notata	0.13	0.00	2.31	0.62	2.41	86.95
Oblada melanura	0.13	0.00	1.65	0.53	1.71	88.67
Solea solea	0.00	0.04	1.45	0.34	1.51	90.18

Table 22. Dissimilarity indexes in the species composition of catches between samplings belonging to the two bottom types.

The result of the PERMANOVA test gave the seabed, the area and the month as the most significant factors, the interaction between seabed and area was also significant (Table 18). The ZTB factor was not significant. Furthermore, by graphically representing the MDS (Figure 49) it is evident, once again, a greater dispersion in coves with soft bottom compared to those on hard bottom.

	Df	SumOfSqs	R2	F	Pr(>F)
Month	**1**	**0.5347**	**0.04977**	**2.2861**	**0.037 ***
ZTB	1	0.1370	0.01275	0.5857	0.793
Area	**1**	**1.7185**	**0.15995**	**7.3468**	**0.001 *** **
Bottom type	**1**	**2.0328**	**0.18920**	**8.6905**	**0.001 *** **
Month:ZTB	1	0.2190	0.02038	0.9361	0.477
Month:Area	1	0.1817	0.01691	0.7767	0.606
ZTB:Area	1	0.2539	0.02363	1.0853	0.336
Month:Bottom type	1	0.2219	0.02065	0.9486	0.481
ZTB:Bottom type	1	0.2054	0.01912	0.8782	0.512
Area:Bottom type	**1**	**0.6851**	**0.06376**	**2.9288**	**0.012 ***
Month:ZTB:Area	1	0.4751	0.04422	2.0313	0.054
Month:Area:Bottom type	1	0.1025	0.00954	0.4384	0.908
Residual	17	3.9765	0.37011		
Total	29	10.7442	1.00000		

Table 23. Results of the PERMANOVA test. The type of bottom resulted as the significant factor, along with the area.

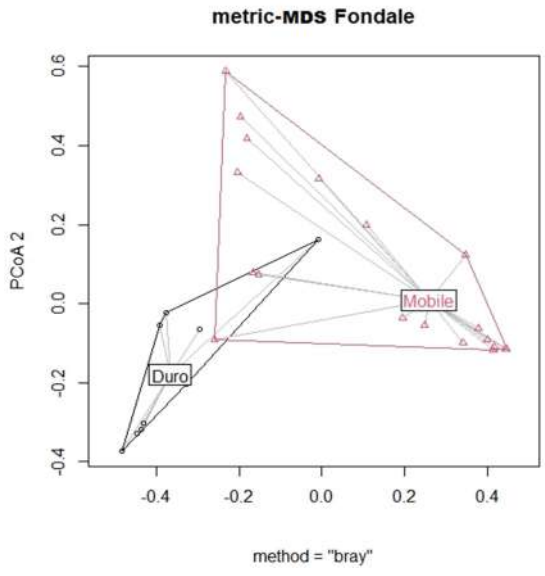


Figure 49. MDS representation. A lower dispersion of data points within soft bottom with respect to hard bottom can be observed.

2.5.4 Bioacoustics survey

The 3 acoustic sampling campaigns were performed in parallel to activity C (gillnet sampling), focusing on the winter period. Samplings took place on 14 December 2021, 26 January 2022, and March 2022 (Figure 50). Active acoustic data for nekton monitoring were collected by means of a *Scientific Echosounder* Simrad EK8, wide band, equipped with *split-beam* technology and frequency modulation. To highlight the distribution of the targets in the water column, the study area was ideally divided into three layers: from the water surface to 10 meters depth, between 10- and 15-meters depth, and beyond 20 meters. Furthermore, to investigate a possible spatial variability in the distribution of organisms, the targets detected in the four zones into which the SCI is divided were considered separately. Figure 48 shows some examples of ultrasound recorded during the three campaigns, while Figure 52, Figure 53 and Figure 54 show the distribution, in terms of density, of the detected targets.

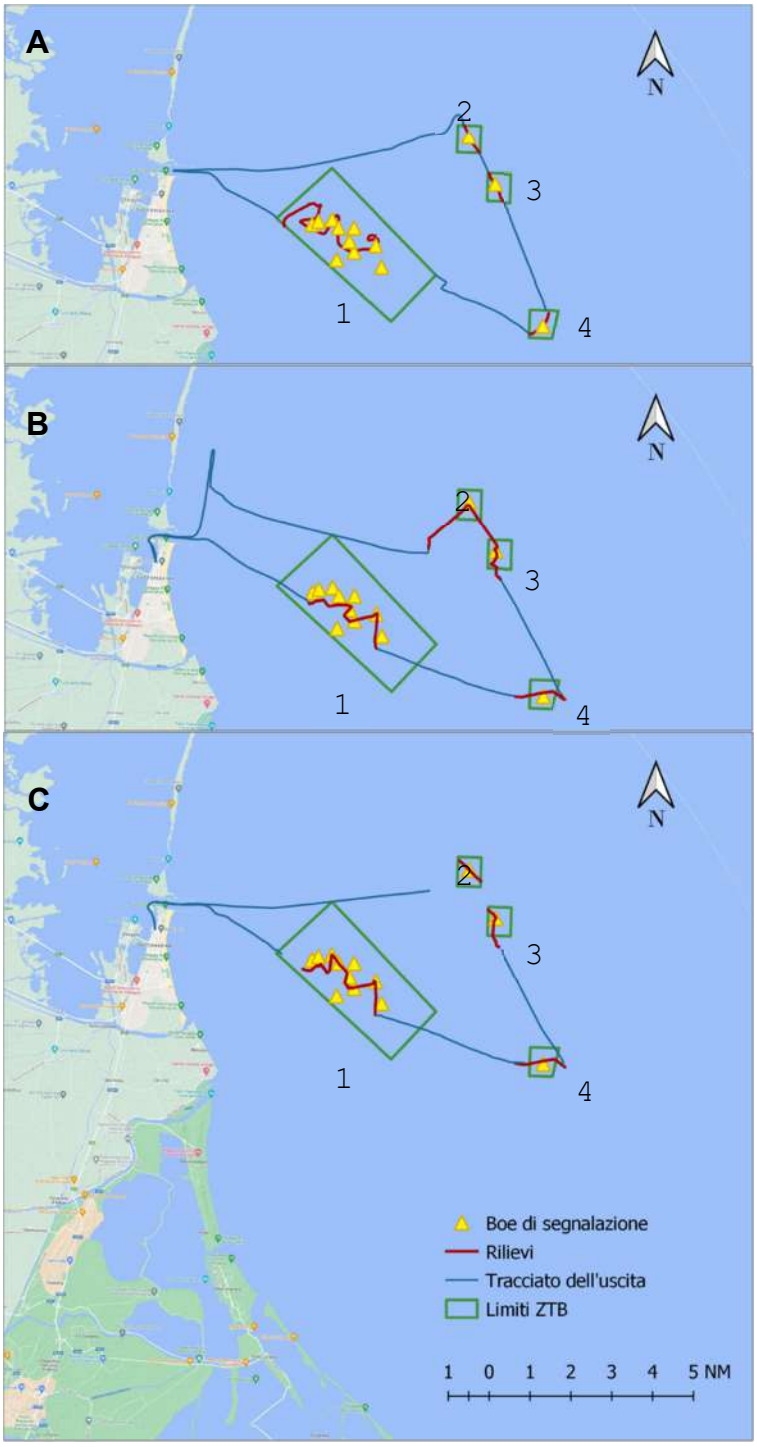


Figure 50. Scheme of the monitoring campaigns: A) December 2021; B) January 2022; C) March 2022. In red are evidenced the recording transects.

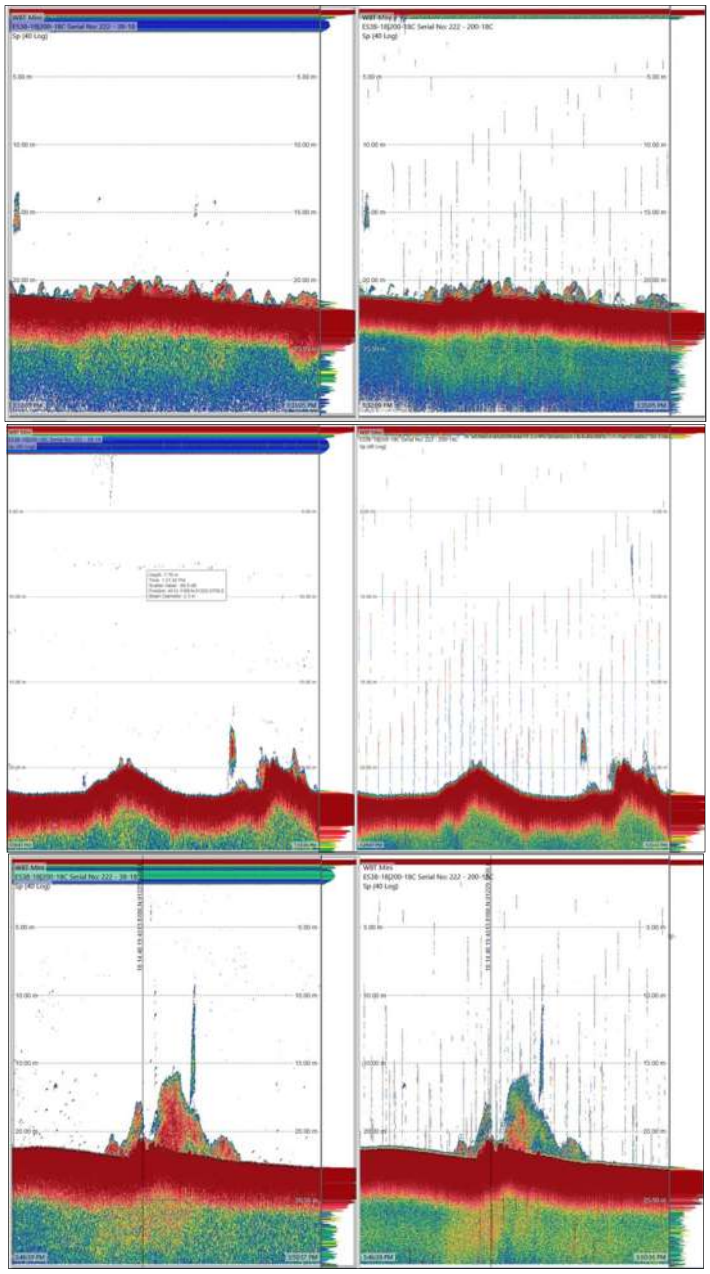


Figure 51. Examples of echograms detected during the campaigns, at the 38 kHz frequency. Top panel: December 2021, area 3, concentration of targets close to the bottom (possible demersal organisms); central panel: January 2022, area 1, possible demersal organisms close to the bottom; bottom panel: March 2022: concentration of targets above a Tegnùa.

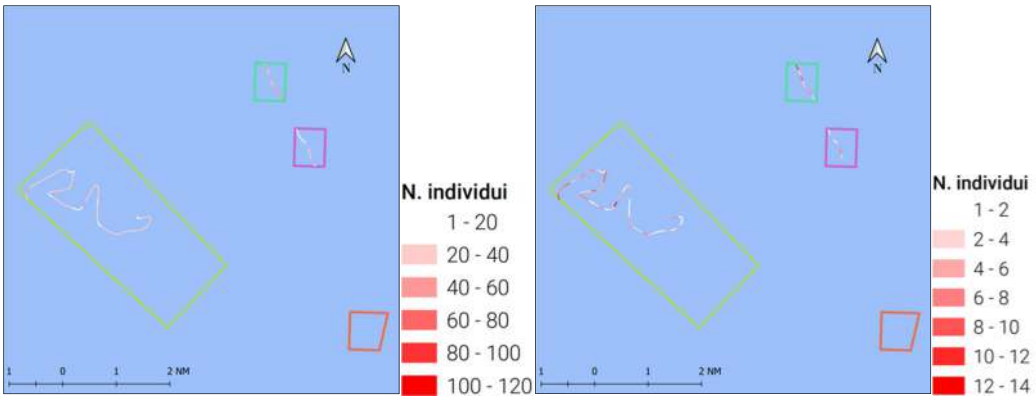


Figure 52. Map of the target density detected within the December campaign – darker colour is associated to a higher target density within the 200m segment. Left: entire dataset; right: TS > -65db.

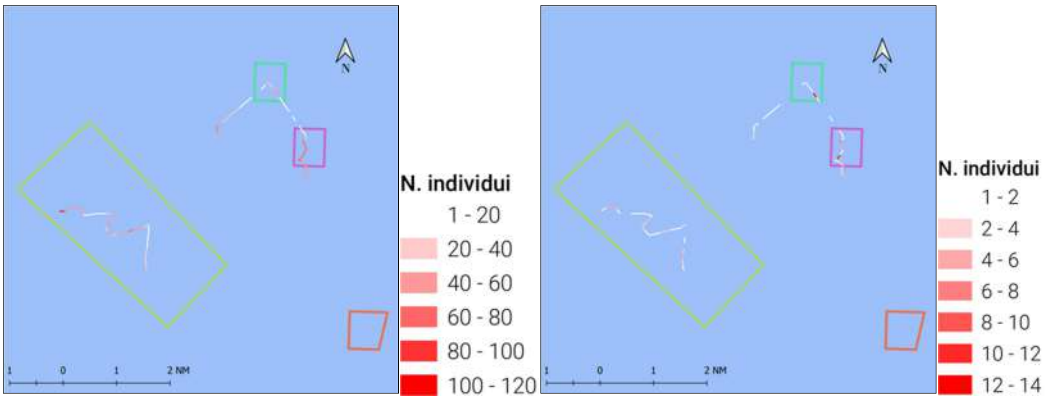


Figure 53. Map of the target density detected within the January campaign – darker colour is associated to a higher target density within the 200m segment. Left: entire dataset; right: TS > -65db.

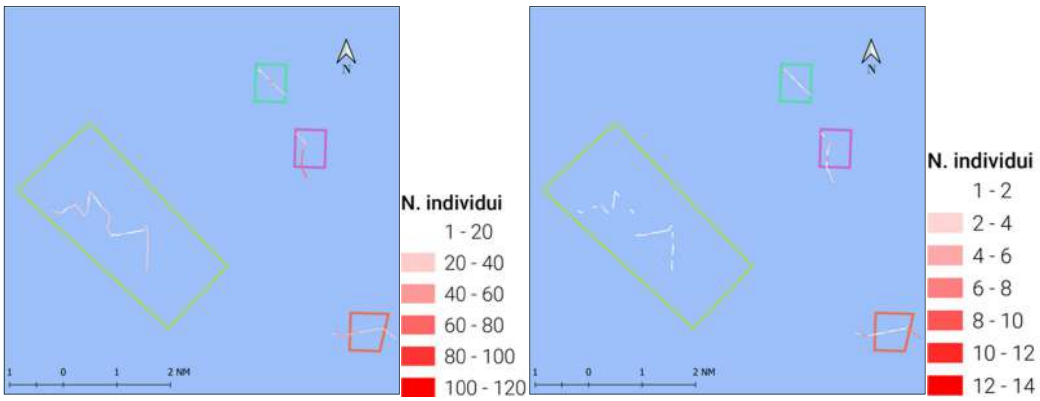


Figure 54. Map of the target density detected within the March campaign – darker colour is associated to a higher target density within the 200m segment. Left: entire dataset; right: TS > -65db.

Overall, 2237 targets were detected in the December campaign, 2217 in January and 2048 in February, with an average Target Strength (TS) of about -67 dB in all cases. The variation range is between -77 and -31 dB (corresponding to size classes of the order of 5 mm and 1 m respectively), but most of the observations fall within a much narrower range, between - 69 and -66 dB approximately. Indicatively, therefore, more frequently individuals were sampled who can be attributed a size in the range of 2 - 3 cm, if the "standard" conversion relationship TS - Total length is used (Simmonds & MacLennan, 2005). In this context, therefore, the indications in terms of total length of the nektonic fauna identified are not to be considered definitive as they are obtained from a generic empirical relationship. Precisely, for this reason it is preferred to report the measurements made in terms of decibels, i.e., the physical quantity directly measured by the instrumentation, as is also typically done in the scientific literature concerning monitoring and sampling with acoustic instrumentation. More reliable estimates of the size of the targets, and indications regarding their taxonomic classification, can be obtained by comparing the acquired acoustic data with the results of the scientific fishing campaigns conducted in other lines of the CASCADE project.

Observing the histogram of the TS distribution of the targets (Figure 55), a bimodal distribution is evident, with a first peak around -77 dB due to macroplanktonic organisms (absent in January), and a second, more pronounced peak around -68 dB, corresponding to size classes attributable to large macroplankton or small nektonic organisms. Finally, there is a limited number of major TS targets, corresponding to fish from about 4-5 cm up to 1 m in length. These larger targets are more represented in the December sample, while they tend to decrease in the subsequent ones. This decrease in the largest individuals from December to March is particularly evident in the bar graph in Figure 56. The reduction particularly affects zone 1, the largest and closest to the coast.

Going into the details of the four zones constituting the SCI, it is possible to highlight appreciable differences, not only in terms of time, but also based on depth.

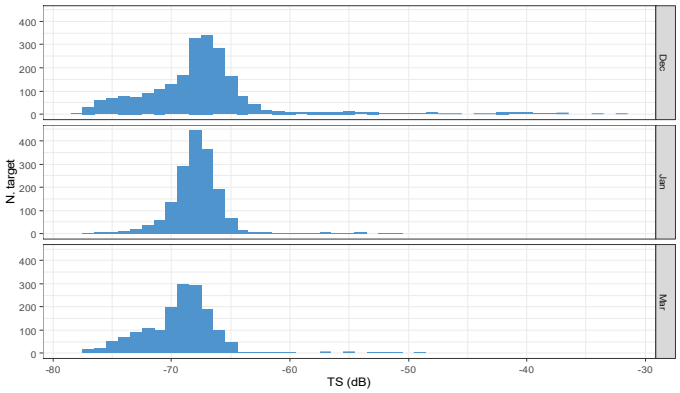


Figure 55. TS distribution of detected targets, within the three campaigns.

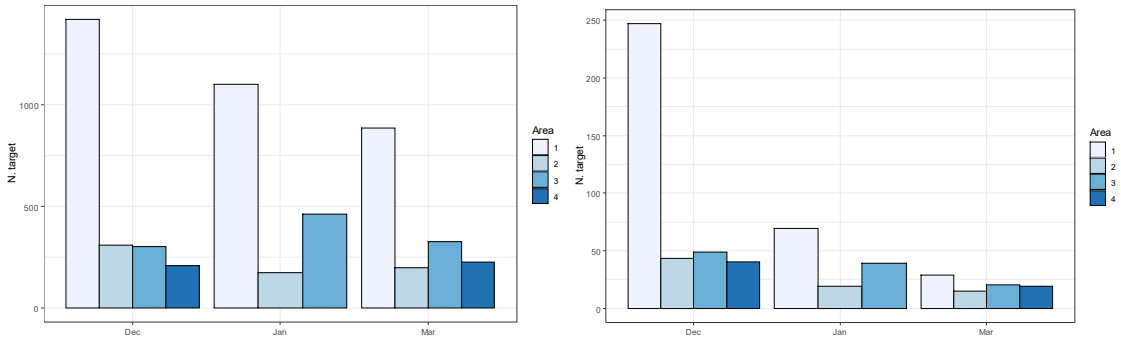


Figure 56. Number of targets detected within the 4 areas, during the three campaigns. Left panel: entire dataset; right panel: targets with TS > -65 dB.

The box plots shown in Figure 57 show that, with increasing depth, the TS values of the targets, and therefore the size of the organisms, generally tend to increase in all areas and for all campaigns. A partial exception is constituted by area 4, in which in December the TS of the targets showed rather wide ranges of variation, and overlapping for the three bands of depth; in the January campaign it was not possible to make significant observations in the area due to a momentary malfunction of the instrument, while in March the trend of the TS with respect to depth was in line with the general one.

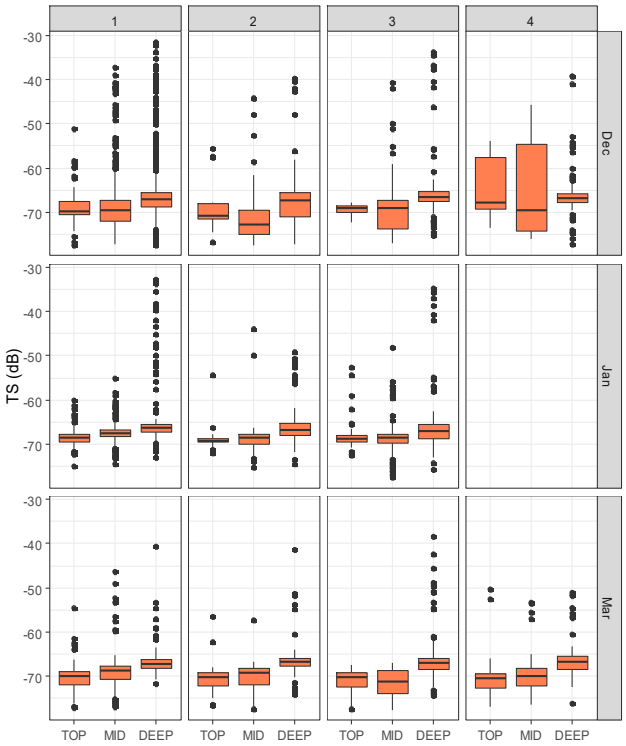


Figure 57. Range of TS variation in the observed targets, based on the depth interval, for each observing campaign.

2.6 Miljašić Jaruga river mouth, Nin Bay (HR)

The research was carried out on three occasions during 2022. The first survey was carried out in April, following in May and June, and lasted for two consecutive days in each month. The BRUV (Baited Remote Underwater Video) method was used for monitoring and permanent documentation of the condition of the reef as one of the most reliable methods for monitoring fish habitats in the coastal sea.

2.6.1 Baited Remote Underwater Video (BRUV)

The rapid development of technology has enabled the use of underwater video cameras to collect accurate and precise data and estimate the diversity of fish species and the abundance and length of fish in the shallow as well as in the deep sea (Watson et al., 2005). The two basic ways of recording are with the use of one camera and stereo video recording. In both modes, the camera can be positioned either perpendicular to the seabed or parallel to the seabed. Also, in both ways bait can be added to attract more fish. Cameras can also be equipped with infrared lights for night research or research at greater depths where there is no sunlight. The great advantage of such methods is the possibility of placing cameras at great depths and multiple daily repetition of recording.

2.6.2 Stereo system

A stereo video camera system has been developed to enable the collection of more precise and accurate data on fish, including the size of the organisms. Comparisons of studies in which bait was used for recording and recordings without bait showed that the addition of bait increases the abundance of carnivorous fish species and does not decrease the abundance of herbivorous fish species (Harvey et al., 2007). It can be used with the help of a diver (Figure 58) or mounted on a metal frame and placed on the seabed. Below is a description of the stereo BRUV system that was used for the purposes of this research (Figure 59).

Figure 58. Stereo BRUV used with the help of a diver.

The method of underwater recording with the use of bait is widespread because it is non-catchable and harmless and can be used in different types of habitats and at different depths, it is cost-effective and has high statistical power (Langlois et al., 2010, Cappo et al., 2007). The stereo video system consists of two cameras that are placed in a waterproof housing. The camera housings are mounted on a metal frame that is placed on the seabed, filming parallel to it. The cameras are 0.8 meters apart and rotated 8° towards each other to achieve an optimal field of view with visibility up to 7 meters away (Langlois et al., 2010). The bait bag is placed at 1 m from the cameras. The cameras used in this research are GoPro brands and Silver 4 and 3 models. These cameras were specifically chosen for their compatibility with the computer program for measuring the length of fish SeaGIS.

Figure 59. Stereo BRUV system used in the research.

Due to poor visibility, it was not possible to determine the length of the fish and this system was replaced by the mono system. When the conditions are better, the stereo system will be returned to the monitoring phase. The mono BRUV system is described below.

2.6.3 Mono system

Part of the research using this method is carried out in such a way that the camera is placed vertically on the seabed, where the fish that pass through the visible area of a small surface are counted. Data collected in this way are like data obtained by experimental fishing (Langlois et al., 2010, Willis et al., 2000). Langlois et al. (2006) recorded a lower number of species with a vertically mounted camera compared to a horizontally mounted camera.

Figure 60. Mono system

The camera is more often placed in a horizontal position (Figure 60), and it stands parallel to the seabed (Watson et al., 2005, Harvey et al., 2004, Cappo et al., 2004). The camera can be mounted on a support that is stationary and placed in the sea at a specific location or it can be mounted on a system that is carried by a diver while swimming a certain transect (Watson et al., 2005, Bortone and Martin, 1991). In the case of using a diver, there are limitations caused by the physical limitations of the diver.

Measuring the length of fish is possible, but it is much less accurate than using the stereo video recording method. The time BRUV stays in the sea is not standardized, but research has shown that most species are recorded in more than 36 minutes, and target (carnivorous) species are recorded in 60 minutes (Watson, et al. 2005). Research has shown that 20-30 minutes is enough to spot species that react quickly to the bait. But this time could be extended so that species with a slower reaction could be recorded (Lowry et al., 2012). Also, neither the type nor the amount of bait used is standardized. A different effect on the composition of observed fish species was observed depending on the type of bait. Greater abundance of fish was recorded using the small pelagic fish as bait compared to using white fish as bait (Wraith et al., 2013).

The collected images are analysed with a special program, e.g., SEAGIS EventMeasure (Figure 61), and the MaxN (the largest number of individuals) of a species at a certain moment is recorded, and at that moment the number of individuals and their length are determined. The measurement of the body length of the fish is done at the time of MaxN to avoid re-measuring the same individual. Using the data on the length of the fish, the biomass is calculated using length-mass ratios for a particular species. This method is also suitable for researching nocturnal species. For night research, BRUV is equipped with infrared lights. Night surveys showed a greater presence of olfactory species of fish that are attracted by the smell of the bait. The ability of a species to follow the scent of a bait affects the abundance of that species (Basset et al., 2011). Since the visibility on and around the ridge was extremely poor, the systems were placed around the ridge at 1m and 2m. The recording time for each system was 180 minutes.

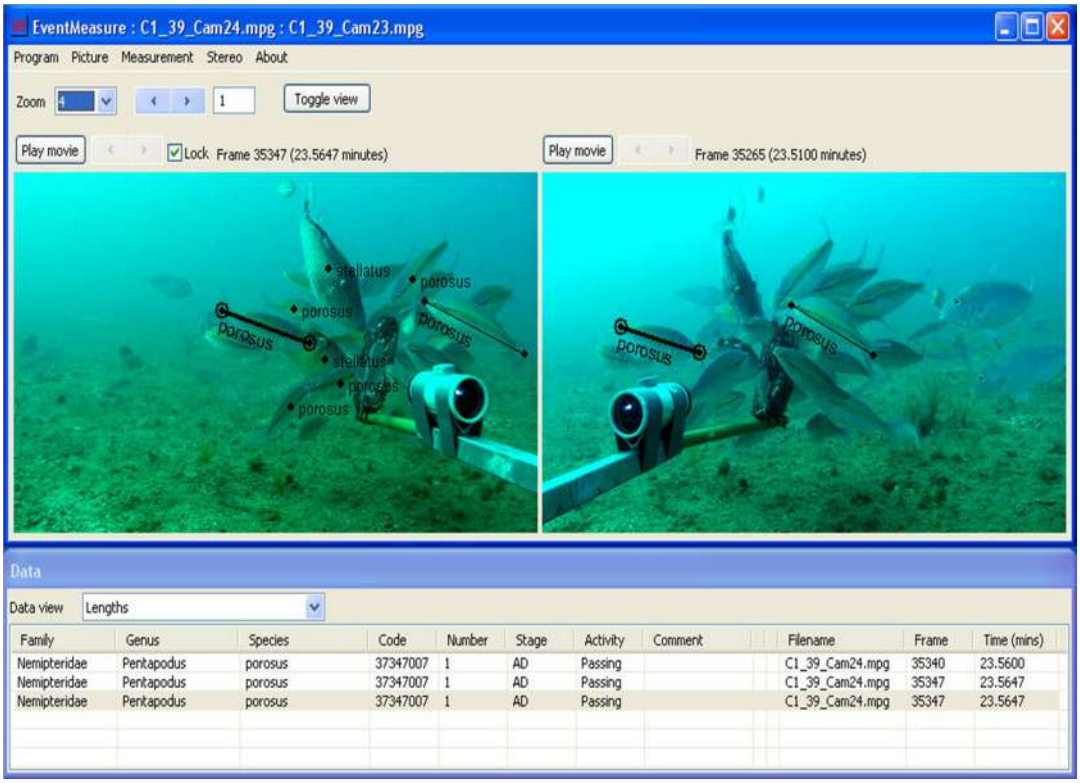


Figure 61. SeaGis EventMeasure programme

2.6.4 Biological features of the location and results

The bottom is mostly sandy with widely dispersed smaller rocks of irregular shape, which indicates little or no anthropogenic intervention. Observations were made at two control stations, one 5 meters from the mouth upstream (P1) and the other 20 meters from the mouth towards the Nin Bay (P2). In this way, we wanted to cover as much ground as possible and see if there are differences in the composition of fish communities within the locality.

2.6.4.1 Ichthyofauna

In the research, all species of fish that were observed and which could or could not be identified, but were included in the total number of species, were taken. A total of 13 species of fish were recorded and all of them were determined with 100% certainty, except for one that we know belongs to mullet, but we cannot be sure of the exact species. Table 24 shows the list of species and their occurrence at the locality or stations. At the beginning of the research in early April, the number of species as well as the abundance of individual species were smaller. With the increased temperature of the sea, that number increased, which is visible in the data for May.

Species	April	May	June	Location
Spicara flexuosa		X	X	P1
Symphodus tinca	X	X	X	P1/P2
Diplodus vulgaris		X	X	P2
Mullus barbatus	X	X	X	P1/P2
Lithognathus mormyrus		X	X	P2
Diplodus annularis	X	X	X	P1/P2
Sparus aurata		X	X	P2
Serranus scriba		X	X	P2
Dicentrarchus labrax		X	X	P2
Mugilidae spp.	X	X	X	P1
Symphodus cinereus	X	X	X	P1
Diplodus sargus		X	X	P1
Sarpa salpa		X	X	P1/P2

Table 24. Fish species and abundance

Figure 62. School of sea bass recorded with BRUV method.

Figure 63. Common two-banded sea bream recorded with BRUV method.

Figure 64. School of sand steenbras (striped seabream) and dreamfish recorded with BRUV method.

Figure 65. Sea bream recoreded in vicinity of the Miljašić channel