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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 2

PP1 – CMCC

Final version

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Project acronym	CASCADE
Project ID number	10255941
Project title	CoAStal and marine waters integrated monitoring systems for ecosystems protection AnD managemEnt
Priority axis	3 - Environment and cultural heritage
Specific objective	3.2 - Contribute to protect and restore biodiversity
Strategic theme	3.2.1 - Marine environment
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Activity number	Activity 4.1
Activity title	Set up and testing of the observing system
Partner in charge	PP1 – CMCC
Partners involved	All PPs

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2.7 Coastal area in Molise (IT)

In situ observing campaigns (e.g., chemical, biological, visual census) were set up and carried out. UNIMOL implemented biomonitoring in Coastal area in Molise (vegetation zonation sampling) along with beach litter pollution. This monitoring campaigns were essential for developing models of accumulation depicting the main factors affecting litter accumulation, the most vulnerable habitats to be pollutes as well as to predict the coastal tracts with higher litter pollution probabilities.
The presence of macro litter on sandy beaches and dunes represents a widespread and very serious environmental stress impinging coastal areas of the Mediterranean basin. Macro beach litter, composed by accumulated debris, includes fragments between 2.5 cm and 100 cm long, of non-biodegradable and highly frangible waste, accidentally or intentionally abandoned. The origin of marine and beach litter is heterogeneous coming from several sources from both: the emerged land and marine realms.
Land wastes on coastal areas may derive from recreational activities (e.g., bathing, sunbathing, picnics), as well as from polluted rivers, municipal drainage systems, ephemeral streams, and sewage, from domestic, agricultural, or industrial activities, and from refuse dumps located near beaches. On the other hand, marine sources of debris include commercial and recreational fisheries, as well as navigation activities (merchant ships, passenger ferries, and recreational boats). Concerning the material that constitutes coastal accumulated waste, plastic is the most abundant. A recent review based on litter data extracted existing reports (Regional Sea Conventions) and an ad hoc analysis of a beach litter data set from the year 2016 underline that plastic waste represents over 80% of marine litter items accumulated on European beaches.
Specifically, single-use plastic waste represents a large part of marine beach litter, and for this reason, they have been recently targeted in the EU Strategy for Plastics in the Circular Economy promulgated in the year 2018.
Great efforts are still needed to fulfil the Marine Strategy Framework Directive (MSFD) (2008/56/EC; European Commission, 2008) that commits the European states to take actions aimed at improving or maintaining the good environmental status (GES) of the marine environment by 2020. GES assessment is based on 11 qualitative descriptors (listed in Annex I of the MSFD), and descriptor 10 (D 10) is particularly focused on marine litter. To assure the correct implementation of the MSFD, the EC Directorate-General for the Environment established a Technical
Subgroup on Marine Litter (TSG ML) with a role of supporting Member States by providing scientific and technical background for the fulfilment of the requirements stated by the D 10. The work of
TSG ML focused on summarizing the information on monitoring protocols and evaluated the relation among GES assessment and environmental targets to identify strategies for preventing

further inputs of litter and reducing its total amount. From the beginning of the TSG ML, much has been achieved, and new efforts should be addresses to customize the monitoring methods to the different marine and coastal compartments and biota, to increase the knowledge and information by extending studies to new areas, and to better understand the sources of marine/beach litter.

In this activity and deliverable, UNIMOL sets out to explore how the different coastal dune ecosystems in the CASCADE pilot area 7 (Molise coast) are affected by the accumulation of macro litter.

2.7.1 Study area (Pilot 7)

As previously described Pilot area 7 comprises two Special Areas of Conservation (SACs; Habitats Directive 92/43/EEC: Biferno river mouth-Campomarino Coast -IT7222216, and Saccione mouth Bonifica Ramitelli -IT7222217) included in the municipalities of Campomarino and Termoli, as well as a 15 km long sandy seashore (Figure 64).

Figure 66. P7 Pilot Area is part of the Long Term Ecological Research network (LTER - https://deims.org/088fe3af-c5bb-4cc8-b479-fe1ea6d5be80) and includes two N2k sites: Biferno river mouth-Campomarino Coast (IT7222216), and Saccione mouth Bonifica Ramitelli (IT7222217).

2.7.2 Vegetation sampling

A set of 30 vegetation plots were done and georeferenced in a GIS environment. Plots are 16 m^2 wide (4m x 4m) and are carried out during the vegetative period (April – June). In each plot, the complete list of vascular plants is reported, along with their cover percentage, using the Braun-Blanquet scale of abundance/dominance. Vascular plants taxonomy conforms Conti et al. (2005) for natives.

To explore the effects of litter pollution we sampled litter accumulation across dune zonation.

2.7.3 Litter sampling

The sampling was carried out following the OSPAR Guidelines (for the complete list of material types and relative codes see OSPAR Commission 2010).

Data were collected and categorized during a field campaign performed in spring as follows:

• Small–medium litter with a diameter between 2 and 50 cm were sampled along two transects perpendicular to the coastline in four plots of about 200 m2 (Fig. 67).

• Large litter with a diameter more than 50 cm was sampled along three transects parallel to the coastline from the shoreline to the dune habitats of 1 km in length.

For each sampled area, the following information was also registered:

• The abundance of the litter, that is the number of elements collected in each plot;
• The material (paper, plastic, polystyrene, glass, mixed, not available);
• The source (recreational, health, fishing and food business, not available);
• The floating capacity of each kind of litter (classified in three levels: high, medium, and low).

Before characterizing the material, source, and floating capacity of the collected debris, the spatial trends of litter accumulation along the coastal zonation were also explored.

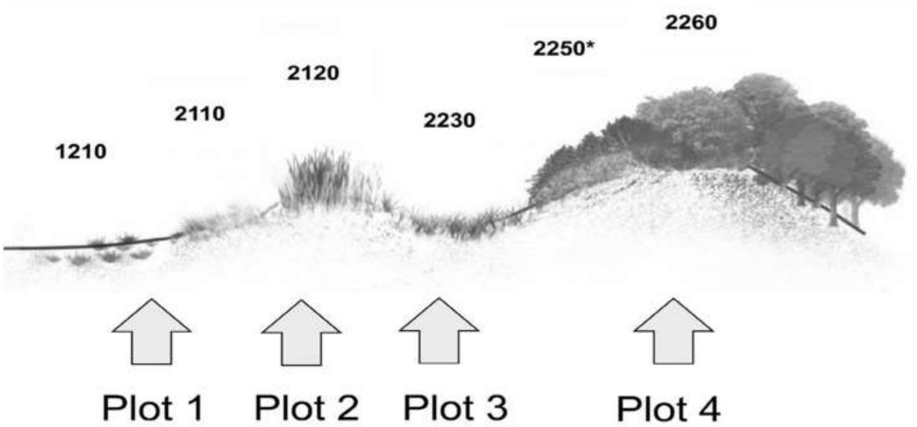

Figure 67. Schematic profile of distribution of litter sampling plots across the morpho-ecological dune zonation along with the EC habitats (sensu 92/43/EEC Habitat Directive). 1210: vegetation of the drift lines; 2110: embryonic shifting dunes; 2120: shifting dunes along the shoreline with Ammophila arenaria; 2230: Malcomietalia dune grasslands; 2250*: coastal dunes with Juniperus spp.; 2260: Cisto-Lavanduletalia dune sclerophyllous scrubs (profile form Bazzichetto et al. 2016 modified)

2.7.4 Preliminary results

During the litter sampling campaign, at least one litter item was found in all the plots. We collected more than 2500 elements with diameter between 2 and 50 cm, most of which was plastic followed by polystyrene and these debris mainly derive from food, fishing, and recreational activities. Almost all small–medium-sized litter (97%) presents high–medium buoyancy and therefore they are transported by waves and wind on the coast even at considerable distances from the seashore.

Moreover, the results showed different accumulation trends across the dune eco-morphological zonation. We observed higher accumulation near to the seashore on the upper beach and embryodunes impinging the vegetation of the drift lines (EC: 1210) and embryonic shifting dunes (EC: 2110) (43% of the litter elements). Litter accumulation slightly decreases towards the mobile and transition dune sectors with Ammophila arenaria (EC: 2120) and Malcomietalia dune grasslands (EC: 2230) (35% of the litter) and affects the fixed dunes with Juniperus spp. (EC: 2250*) and Cisto-Lavanduletalia dune sclerophyllous scrubs (EC: 2260) (18% of the litter) and here the polystyrene prevails due to its high volatility.

2.8 Northern-eastern Adriatic in Croatia (HR)

2.8.1 Short description of the pilot area

The North – Eastern Adriatic Sea is a shallow (up to 40 m depth), semi-enclosed coastal area with steep ecological gradients, which are mainly noticeable through changes in nutrient composition, salinity, temperature, and oxygen saturation. The area is under significant influence of freshwater inputs (Po River as the largest freshwater input into the Adriatic) and the established Adriatic Sea circulation. In addition to river dilution and nutrient enrichment, the area is characterised with periodic inflows of high salinity waters transferred by the Eastern Adriatic Current (EAC) from the southern to the northern part of the Adriatic Sea. Given the ecological characteristics, the North – Eastern Adriatic Sea is a highly productive and dynamic marine ecosystem. It is an ideal basin to study the influence of freshwater inflows and fast oceanographic changes on marine ecosystem.

Observing campaigns in the Northern – Eastern Adriatic Sea were organised at two stations (Figure 68, Table 25) were included and monthly sampling conducted in the period from December 2020 to December 2021. Variables that were measured include: temperature, conductivity (salinity), dissolved oxygen, Chlorophyll a and nutrients (N, P, Si). Samplings were conducted with the CMR – RBI research vessel Burin using CTD probe (temperature, conductivity, and dissolved oxygen) from the surface to the bottom and collecting sea water with Niskin bottles (Chlorophyll A and nutrients) on depths of 5, 10 and 20 m (Figure 69). All the samples were immediately laboratory processed and analysed in the CMR – RBI. Observing system of the Nothern – Eastern Adriatic Sea was supported with the real-time data collecting of the AdriaClim project installed observing oceanographic buoy (Figure xx) on the Rovinj – Po transect, position 5 nm from the coast of Croatia (Lat: 45.075492, Lon: 13.598978) during a period from January 2023 – June 2023. Oceanographic buoy enabled measuring of diverse set of meteorological (wind direction and speed, air temperature, relative humidity, atmospheric pressure, solar irradiation, precipitation and air visibility) and oceanographic variables (surface current, wave height, PCO2, temperature, conductivity (salinity), dissolved oxygen, light transmission, pH, phytoplankton pigments; phycocyanin, phycoerythrin and chlorophyll-a).

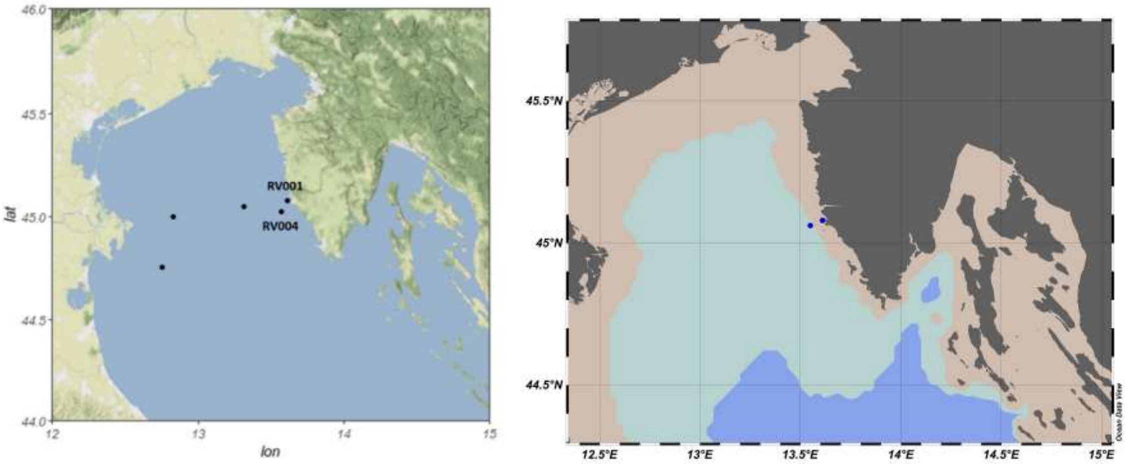

Figure 68. Selected sites within the Natura 2000 Map of the Rovinj – Po transect and samplin stations in the Northern – Eastern Adriatic Sea pilot area

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

Table 25. Description of the sampling stations included in the observing campaigns

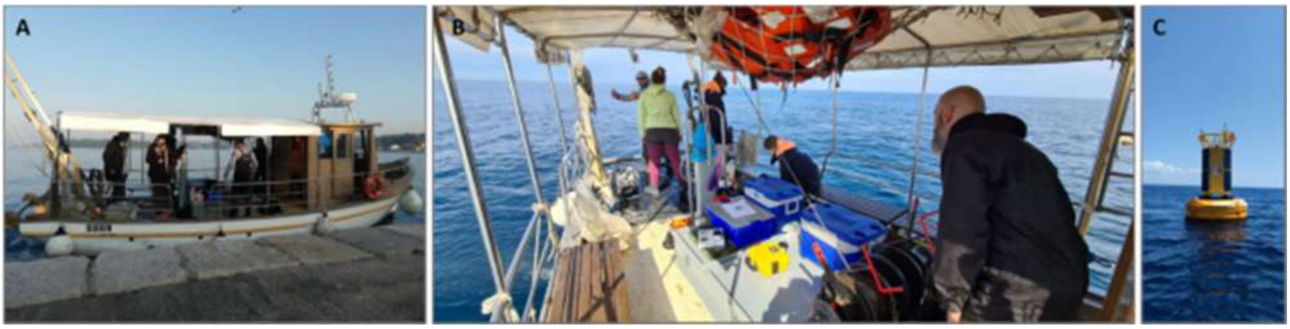

Figure 69. Research vessel (A), sampling procedure (B), and oceanographic buoy (C) in the Northern – Eastern Adriatic Sea pilot area

Figure 70. Research vessel Vila velebita, sampling with niskin samplers , phytoplankton nets and photos on board in the Northern –Adriatic Sea pilot area 8

2.8.2 Report on observing campaigns

Overall number of 24 observing campaigns (December 2020 – December 2022) were conducted in the Northern – Eastern Adriatic Sea. CTD probe (Sea Bird, USA) measurements of bottom – surface water column oceanographic parameters (temperature, conductivity, and dissolved oxygen) were collected for each campaign on two sampling stations (RV001 and RV004). In each campaign, Niskin bottles water column collections were conducted and 6 samples (2 stations x 3 depths) of 500 mL per parameter (chlorophyll-a and nutrients) were prepared for laboratory measurements. After prefiltration (200 µm mesh size), chlorophyll-a Niskin samples were filtered on Whatman GF/C filters and immediately frozen at -20°C until analysis (within a week). Total chlorophyll a concentrations were determined on a Turner TD-700 fluorometer after three hours extraction in 90% acetone (in the dark, with grinding). Nutrients analyses were performed using methods described in Strickland and Parsons (1972). All measuring instruments are calibrated by the manufacturer once per year, and CMR scientific team always checks the quality of the data collected during monitoring to avoid any issue.

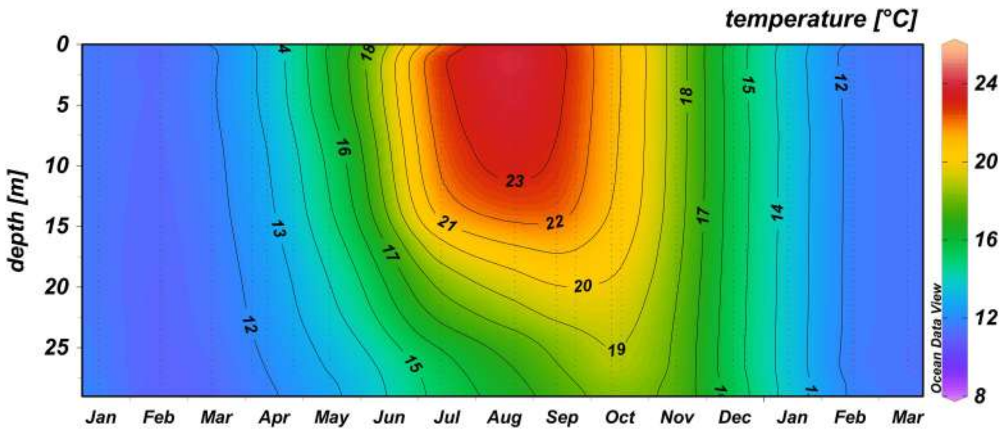

Figure 71. Yearly temperature distribution in water column on station RV001

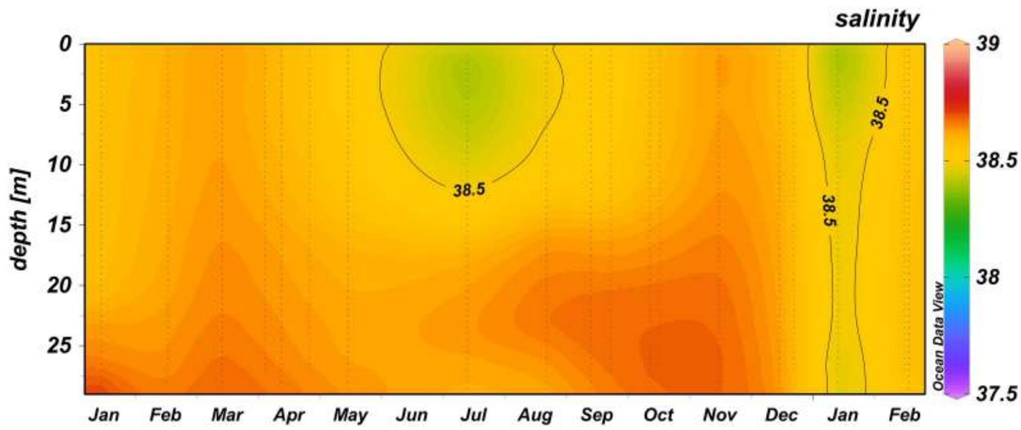

Figure 72. Yearly salinity distribution in water column on station RV001

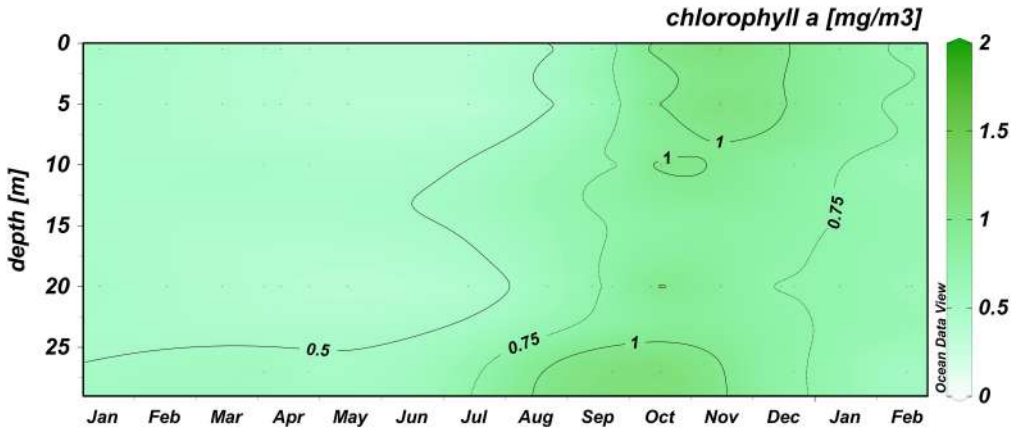

Figure 73. Yearly chlorophyll a distribution in water column on station RV001

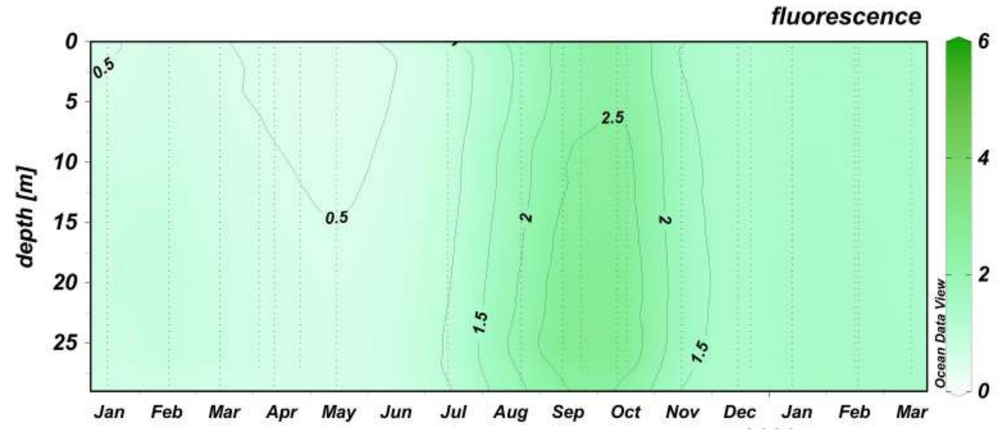

Figure 74. Yearly fluorescence distribution in water column on station RV001

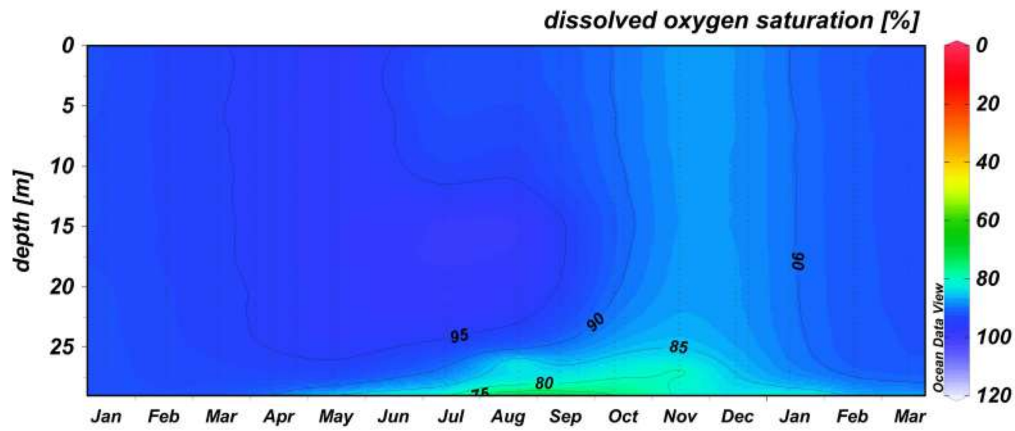

Figure 75. Dissolved oxygen saturation in water column on station RV001

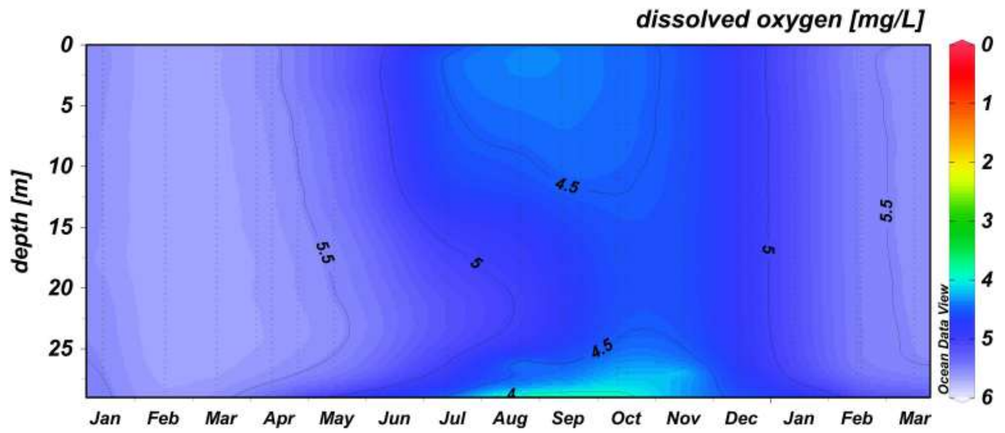

Figure 76. Dissolved oxygen concentration in water column on station RV001

2.8.2.1 Materials and methods for phytoplankton analysis:

Samples were collected in period 2020. – 2021. from two stations in front of Rovinj, Croatia: RV001 and RV004, 1 and 4 nautical miles offshore from Croatian coast. Sampling was done monthly (with exception in April, 2020. when sampling was not organized due to COVID-19 restrictions. Niskin bottles were used to sample the seawater from 0, 5, and 20 m depth (from January 2020 to May 2020 only 5 and 20 m depths were sampled). Samples for phytoplankton analysis (200 ml) were prefiltered through 300 µm mesh net and fixed with neutralized formaldehyde (2% final concentration). Subsamples of 50 ml were settled for 40 hours in sedimentation chambers and analysed following the Utermöhl's method. In total 130 samples were analysed on inverted zeiss axiovert microscope. All data analysis was made using R software (package vegan for ecological analysis and ggplot2 for graphical representation of results).

2.8.2.2 Nanophytoplankton dynamics

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

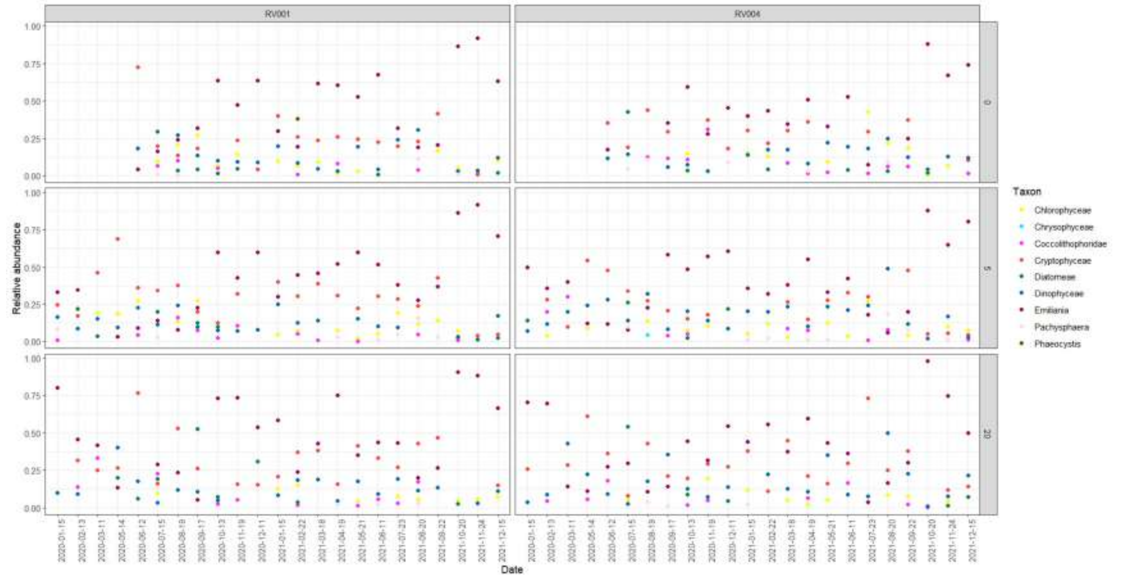

Figure 77. Relative abundances of nanophytoplankton taxa

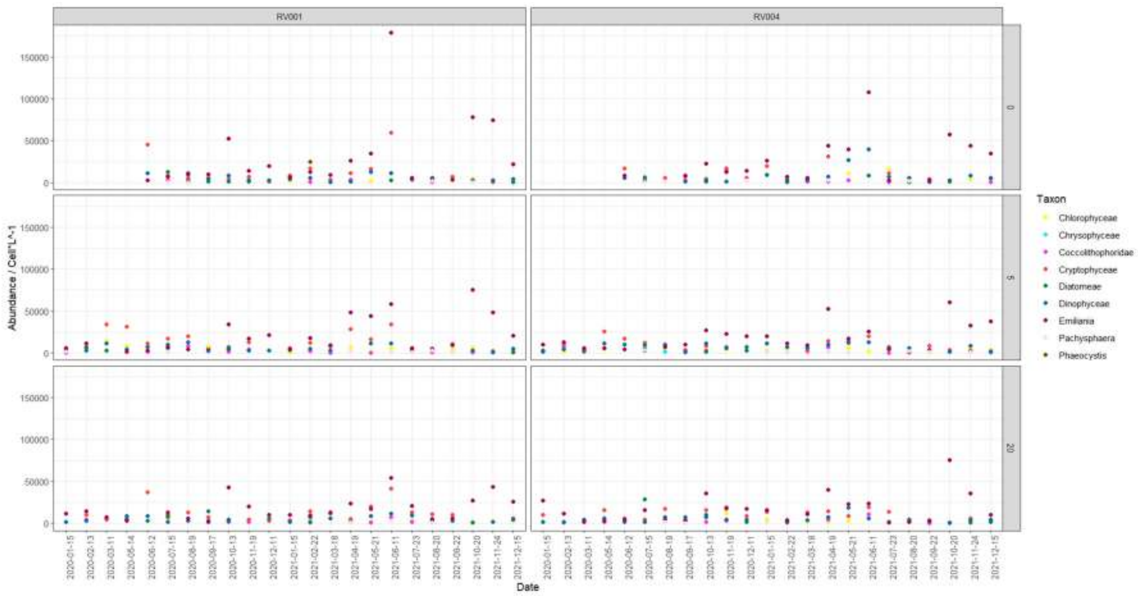

Figure 78. Total abundances of nanophytoplankton taxa

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

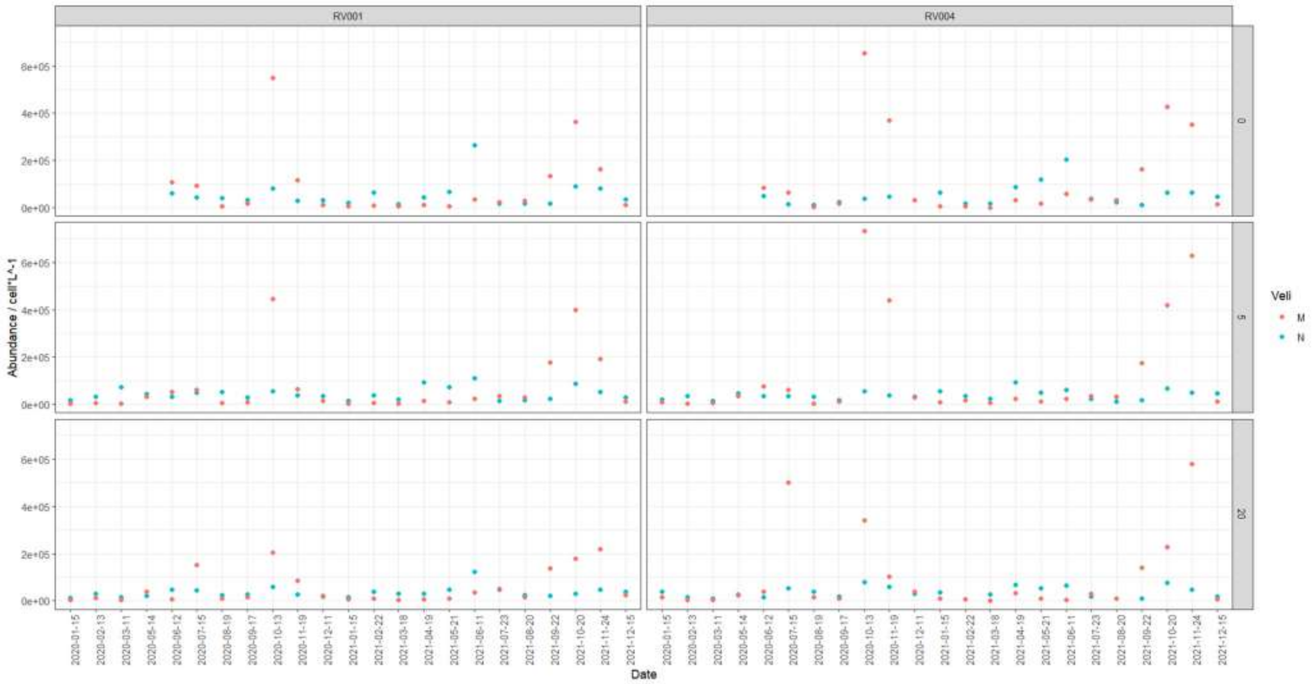

Figure 79. Total abundances of nanophytoplankotn (N, pink) and microphytoplankton (M, blue)

2.8.3 Microphytoplankton dynamics

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

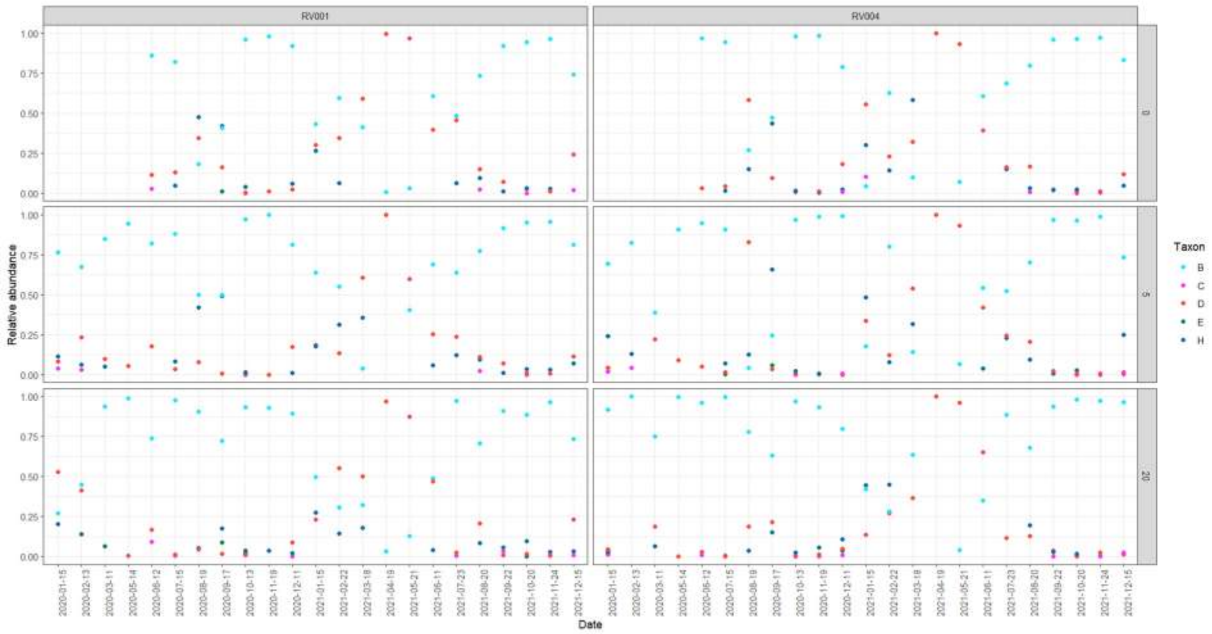

Figure 80. Relative abundances of microphytoplankton taxa: B - Bacillariophceae, C- Chrysophyceae, D- Dinophyceae, E - Euglenophyceae, H - Haptophyta

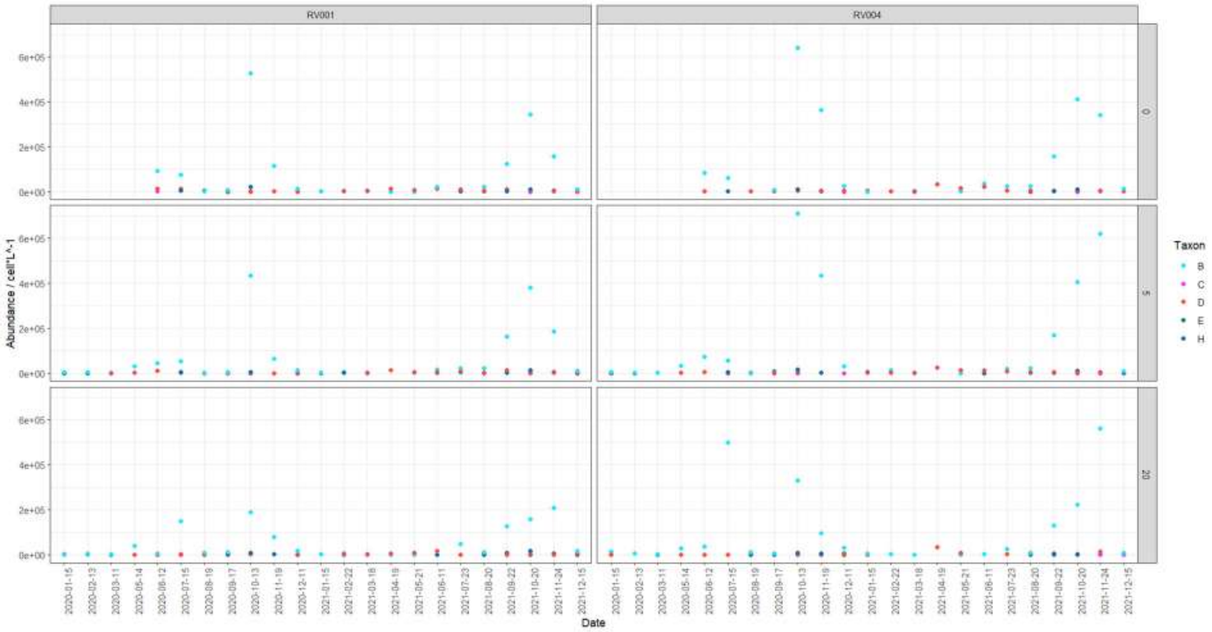

Figure 81. Total abundances of microphytoplankton taxa: B - Bacillariophceae, C- Chrysophyceae, D- Dinophyceae, E - Euglenophyceae, H - Haptophyta

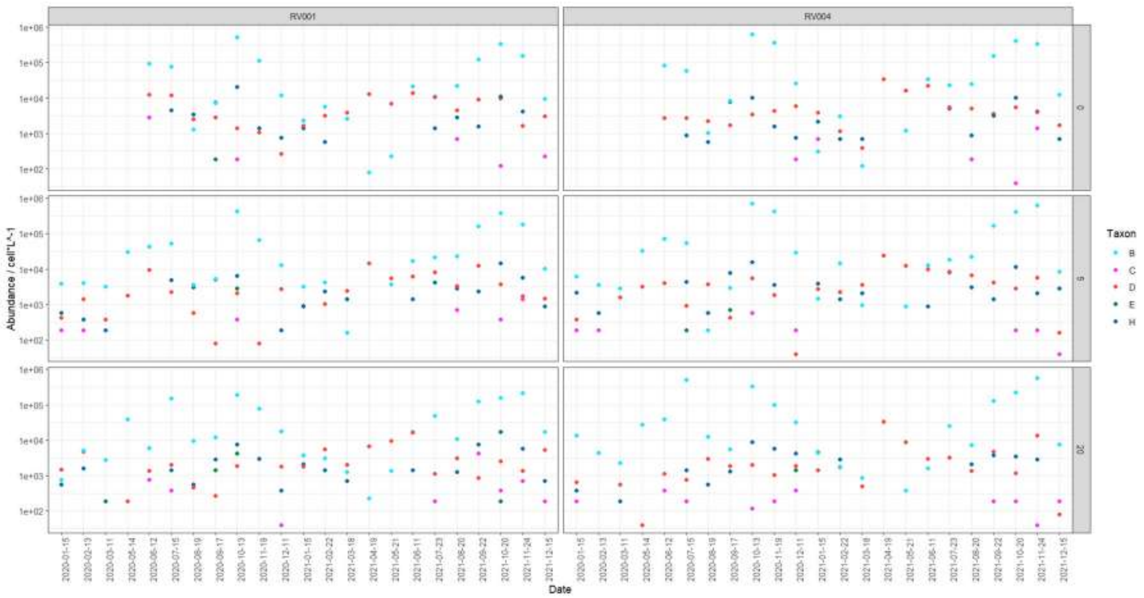

Figure 82. Total abundances of microphytoplankton taxa: B - Bacillariophceae, C- Chrysophyceae, D- Dinophyceae, E - Euglenophyceae, H - Haptophyta (log scale)

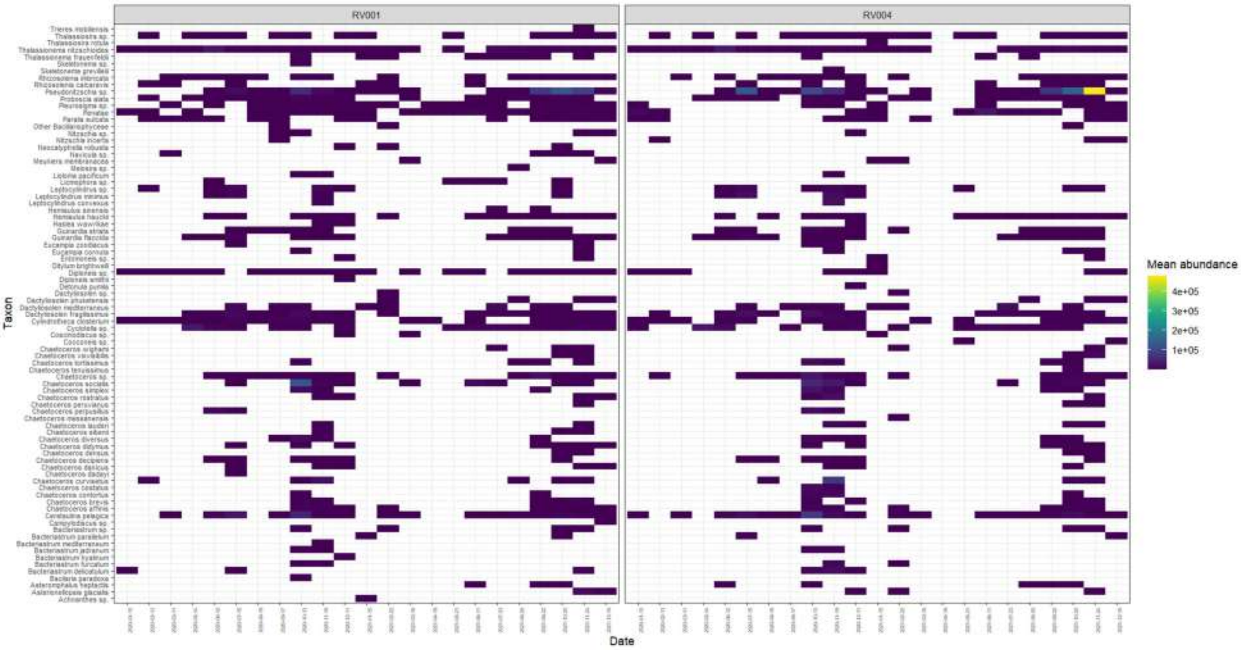

Figure 83. Monthly mean abundance dynamics of diatom species

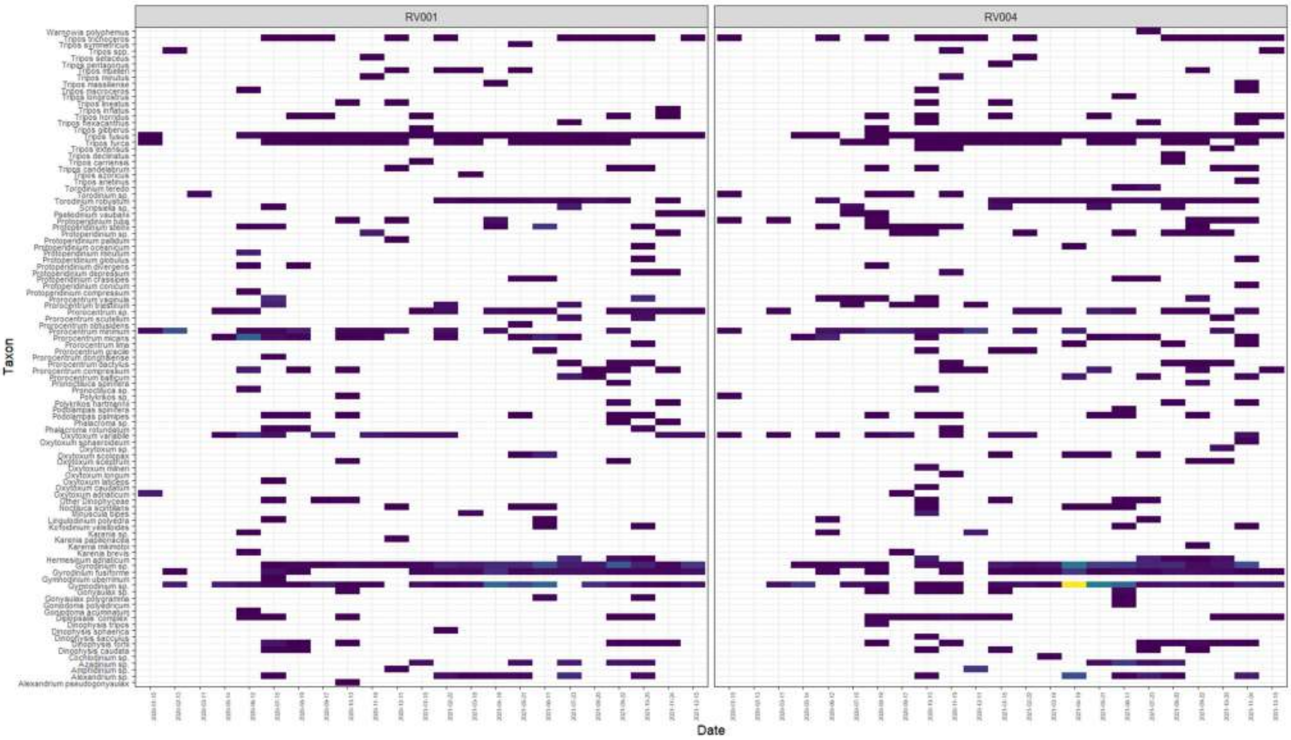

Figure 84. Monthly mean abundance dynamics of dinoflagellate species

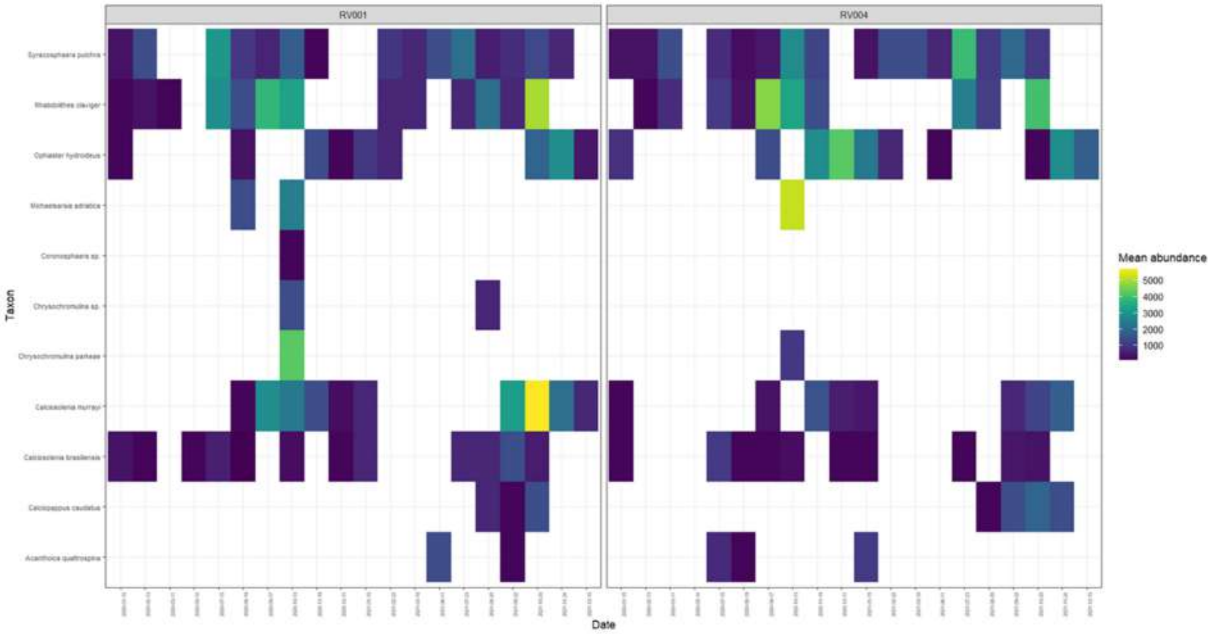

Figure 85. Species monthly mean abundance dynamics of coccolithophorid species

2.8.3.1 Metabarcoding analysis

In this metabarcoding study, a total of 7 785 ASVs, 3 229 99%-OTUs and 420 97%-OTUs were detected on genus or species level, of which 364, 453 and 172 were present with five of more reads per cluster (ASVs*, 97%-OTUs* or 99%-OTUs*). Most present phytoplankton groups were Dinophyceae and Bacillariophyceae no matter of clustering method used (Table 26).

Table 26. Proportions of differences for each phytoplankton group and clustering method used for sequences assigned to genus or species level

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

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

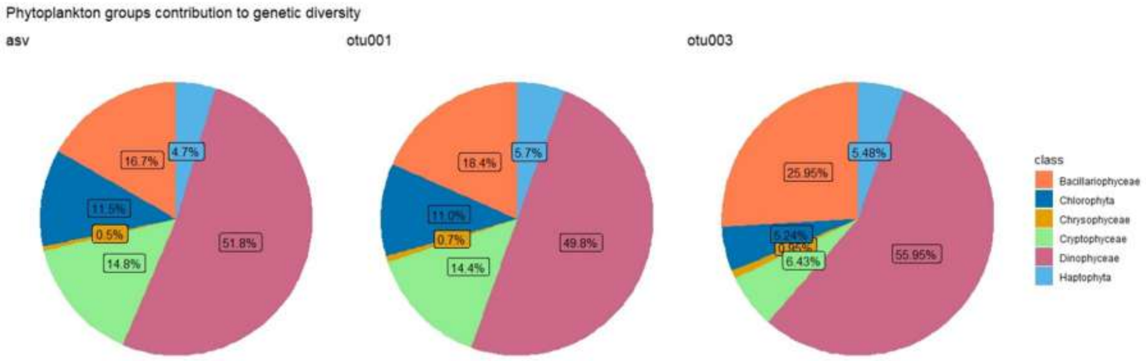

Figure 86. Relative contribution of phytoplankton groups. Changes in relative contribution in genetic diversity of different phytoplankton groups depending on clustering method used.

Five diatoms' genera were revealed with molecular methods that have not been reported in mentioned checklist. Four of them were assigned to species level containing one or more species per genus (Cymbella, Meuniera, Mediolabrus, Minidiscus), while Fragilariopsis was assigned up to genus level. Dinoflagellates group showed higher number of non-reported genera (9), and higher number assigned to species not mentioned in earlier complete checklist of this area of interest (Table 27).

Table 27. Diatoms and dinoflagellates species revealed by metabarcoding, assigning taxonomy based on ASVs as a clustering method with no reads cutoff, not present in checklist

Class	Order	Genus	Species
Bacillariophyceae	Chaetocerotales	Bacteriastrum	Bacteriastrum jadranum
Bacillariophyceae	Chaetocerotales	Bacteriastrum	Bacteriastrum parallelum
Bacillariophyceae	Cymbellales	Cymbella	Cymbella prostrata
Bacillariophyceae	Chaetocerotales	Chaetoceros	Chaetoceros eibenii
Bacillariophyceae	Leptocylindrales	Leptocylindrus	Leptocylindrus aporus

Bacillariophyceae	Thalassiosirales	Mediolabrus	Mediolabrus comicus
Bacillariophyceae	Naviculales	Meuniera	Meuniera membranacea
Bacillariophyceae	Thalassiosirales	Minidiscus	Minidiscus trioculatus
Bacillariophyceae	Thalassiosirales	Minidiscus	Minidiscus variabilis
Bacillariophyceae	Bacillariales	Pseudo-nitzschia	Pseudo-nitzschia galaxiae
Bacillariophyceae	Bacillariales	Pseudo-nitzschia	Pseudo-nitzschia pseudodelicatissima
Bacillariophyceae	Rhizosoleniales	Rhizosolenia	Rhizosolenia formosa
Bacillariophyceae	Thalassiosirales	Skeletonema	Skeletonema pseudocostatum
Bacillariophyceae	Thalassiosirales	Thalassiosira	Thalassiosira mala
Bacillariophyceae	Thalassiosirales	Thalassiosira	Thalassiosira profunda
Dinophyceae	Noctilucales	Abedinium	Abedinium dasypus
Dinophyceae	Gonyaulacales	Alexandrium	Alexandrium affine
Dinophyceae	Gonyaulacales	Alexandrium	Alexandrium andersonii
Dinophyceae	Gonyaulacales	Alexandrium	Alexandrium margalefii
Dinophyceae	Gonyaulacales	Alexandrium	Alexandrium ostenfeldii
Dinophyceae	Gonyaulacales	Alexandrium	Alexandrium tamarense
Dinophyceae	Amphidiniales	Amphidinium	Amphidinium massartii
Dinophyceae	Gonyaulacales	Amylax	Amylax triacantha
Dinophyceae	Blastodiniales	Blastodinium	Blastodinium contortum
Dinophyceae	Gymnodiniales	Balechina	Balechina pachidermata
Dinophyceae	Blastodiniales	Blastodinium	Blastodinium mangini
Dinophyceae	Blastodiniales	Blastodinium	Blastodinium navicula
Dinophyceae	Blastodiniales	Blastodinium	Blastodinium spinulosum
Dinophyceae	Gonyaulacales	Cucumeridinium	Cucumeridinium coeruleum
Dinophyceae	Pyrocystales	Dissodinium	Dissodinium pseudolunula
Dinophyceae	Gymnodiniales	Erythropsidinium	Erythropsidinium agile
Dinophyceae	Gonyaulacales	Fragilidium	Fragilidium mexicanum
Dinophyceae	Gonyaulacales	Fragilidium	Fragilidium subglobosum
Dinophyceae	Gonyaulacales	Gonyaulax	Gonyaulax cochlea
Dinophyceae	Gonyaulacales	Gonyaulax	Gonyaulax ellegaardiae
Dinophyceae	Gymnodiniales	Karenia	Karenia brevis
Dinophyceae	Gymnodiniales	Karlodinium	Karlodinium veneficum
Dinophyceae	Noctilucales	Kofoidinium	Kofoidinium pavillardii
Dinophyceae	Gymnodiniales	Lepidodinium	Lepidodinium chlorophorum
Dinophyceae	Dinophysiales	Ornithocercus	Ornithocercus heteroporus
Dinophyceae	Gymnodiniales	Paragymnodinium	Paragymnodinium shiwhaense
Dinophyceae	Gymnodiniales	Pelagodinium	Pelagodinium bei
Dinophyceae	Dinophysiales	Phalacroma	Phalacroma porodictyum

Dinophyceae	Prorocentrales	Prorocentrum	Prorocentrum cordatum
Dinophyceae	Gonyaulacales	Protoceratium	Protoceratium reticulatum
Dinophyceae	Gymnodiniales	Torodinium	Torodinium robustum

Table 28. Species belonging to phytoplankton groups Cryptophyceae, Chrysophyceae, Haptophyta and Chlorophyta, revealed by metabarcoding, assigning taxonomy based on ASVs as a clustering method with no reads cutoff, not present in checklist

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

Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonas parkeae

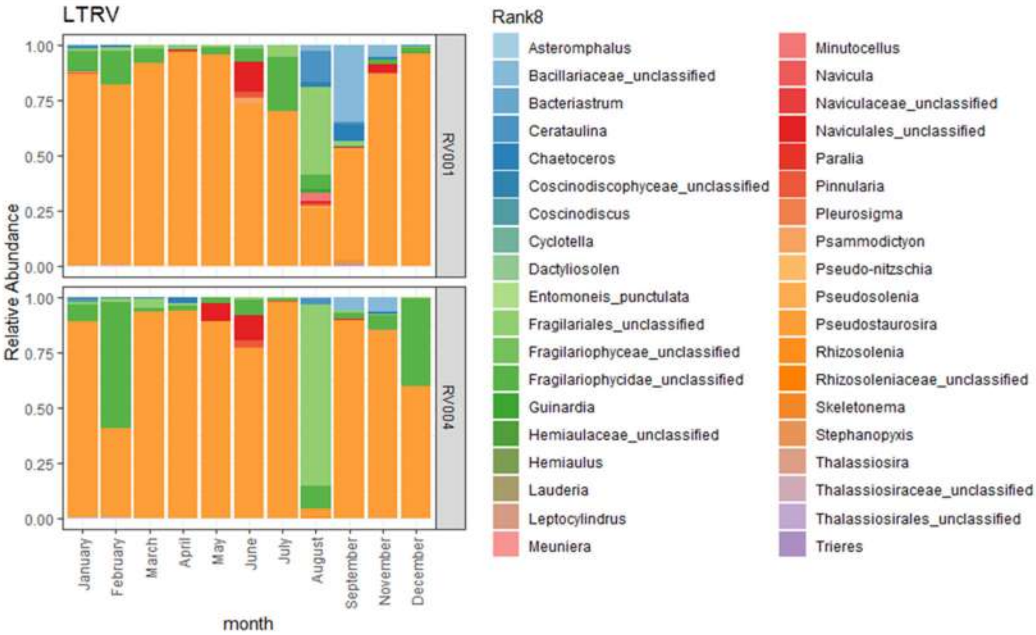

Figure 87. Comparison of the 2 sampling stations (RV001 and RV004) diatom community composition at the genus level

2.9 Cetina River mouth (HR)

The main aim of the present document is to report on the activities and results related to the establishment of a long-term monitoring (observation) system for the Natura 2000 site HR3000126 Cetina Estuary (Fig. 88) within the Interreg Italy-Croatia project CASCADE. Monitoring system involved innovative techniques (e.g., satellite images, photogrammetry) and tested it for benthic habitats (e.g., *Cymodocea nodosa* meadows, *Cladocora caespitosa* bank). It also included monitoring activities on the critically endangered noble pen shell (*Pinna nobilis*) and setting-up of in-situ continuous high-resolution sea water environmental parameters monitoring.

Figure 88. The extent of the Natura 2000 site Cetina Estuary.

Source: http://natura2000.dzzp.hr/reportpublish/reportproxy.aspx?paramSITECODE=HR3000126

In the scope of the CASCADE project we:

- Assessed the condition of the main benthic habitat-forming, structuring species in the pilot site, representative of the Natura 2000 key habitat type 1110 - Sandbanks which are slightly covered by the sea water all the time, i.e., seagrass *Cymodocea nodosa* and its meadows;
- Characterized and assessed the conservation status of the reef of the Mediterranean endemic coral *Cladocora caespitosa* that was discovered adjacent to the current southern border of the pilot Natura 2000 site Cetina Estuary;
- Monitored recruitment of once omnipresent and now critically endangered noble pen shell (*Pinna nobilis*);
- Initiated in-situ continuous high-resolution sea water temperature monitoring along depth gradient at the border of Cetina estuary Natura 2000 site next to endemic coral *Cladocora caespitosa*;
- Initiated in-situ continuous high-resolution sea water temperature, salinity, and oxygen monitoring in the centre of Cetina estuary Natura 2000 site near Cetina River freshwater inflow and *Cymodocea nodosa* meadow.

Monitoring (observation) system is based on technical reports produced by external experts in the framework of EU project CASCADE, namely *Conservation status assessment of representative marine habitats and species in the Natura 2000 site Cetina Estuary (HR3000126) with the proposal of conservation measures and monitoring activities* (Kipson 2023) and *Study of the Cymodocea nodosa meadows distributional range and cover in the Natura 2000 site HR 3000126 Cetina Estuary using remote sensing methods* (OIKON 2023) available at Sea and Karst premises, complemented with monitoring performed by Sea and Karst staff.

2.9.1 Assessment and monitoring of the *Cymodocea nodosa* meadows.

2.9.1.1 Habitat distributional range/habitat extent of Cymodocea nodosa meadows

The methodology applied for this study relied on the processing Remote Sensing data that is multi-sourced and multi-resolution, mainly satellite based Earth Observation (EO) data high-resolution aerial photogrammetric product, the official digital orthophoto maps of the Republic of Croatia. The main source of satellite-based Earth Observation data were the twin Sentinel-2 satellites from the Copernicus program and their MSI multispectral sensor.

The mentioned data sources were integrated for the purposes of the study, where the high-resolution orthophoto maps were used to estimate and map the spatial distribution of the *Cymodocea nodosa* meadows, and the multispectral EO imagery was used to detect and confirm the presence of *Cymodocea nodosa.*

Throughout the project Sentinel-2 multispectral data was used for several purposes:

- Estimation and validation of the *Cymodocea nodosa* meadows cover;
- Validation of mapped human and natural impact on the meadows cover;

For validation purposes, in specific minor cases several other EO data sources were also used.

In accordance with the project specifications, estimation of the coverage of *Cymodocea nodosa* meadows was conducted for the period of 2011-2021. The versioning of the meadows extent maps was based on the iterations of the official digital orthophoto maps. Additional data on *Cymodocea nodosa* meadows extent were available from SCUBA diving field work carried out in the framework of the EU project CASCADE (Kipson 2023)

Based on the meadows cover estimation mapped for each reference year, the dynamics of the meadows cover were analysed by calculating and comparing the coverage area between the reference years.

Table 29. Meadows cover area dynamics between 2011 and 2021

Year	Area [ha]	Increase/Decrease comparison to the previous year
2011	43.65	x
2014	46.37	+6.2%
2017	49.59	+6.9%
2019	51.00	+2.8%
2021	54.04	+5.9%

The results of the analysis show a constant increase of the area covered by *Cymodocea nodosa.* of approximately 6-7% every two to three years in comparison to the previous period, on the entire area of the Cetina Estuary Natura 2000 site. This trend was only hindered in the period 2017-2019, which however does coincide with the increase in sand extraction activities in the period from 2016 to 2017. The overall decadal increase of the coverage of *Cymodocea nodosa* habitat between 2011 and 2021 was approximately 10.39 ha (Fig. 89).

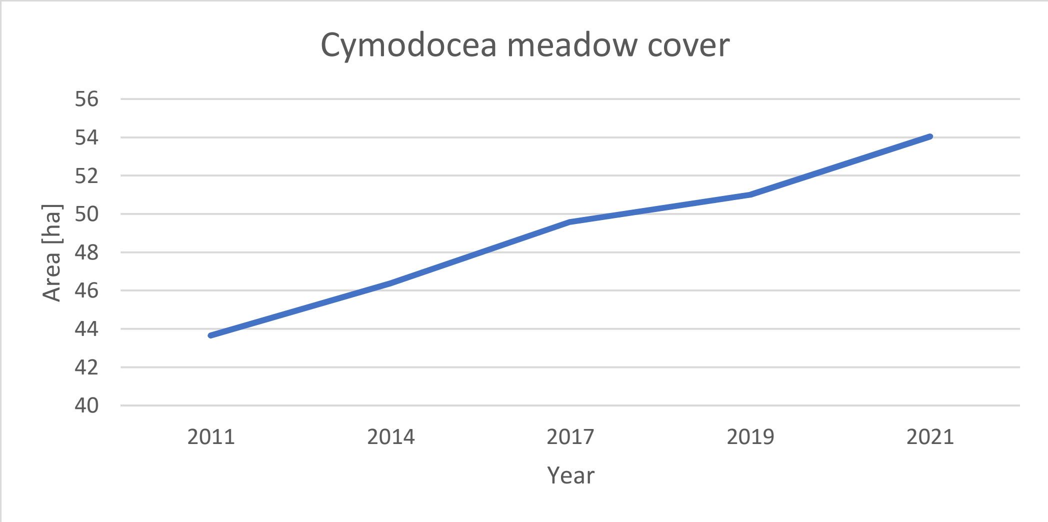

Figure 89. Area dynamics of Cymodocea nodosa meadows in the period 2011-2021

Analysis of the impact of human activities on the *Cymodocea nodosa* meadows, with a focus on changes observed between 2016 and 2017 was one of the key aspects of the Study. Due to the availability of high-resolution imagery, the changes were analysed for the period between reference years 2014 and 2017. The natural impacts on the CN meadows were proven to be hard to detect using this methodology so the analysis focused on human activities.

Two main types of impact on the *Cymodocea nodosa* meadows' structure that were the focus of this task are:

- Impact of sand extraction activities;
- Impact caused by coastline construction (dikes construction, beach replenishment, beach construction…).

It must be noted that the analysis conducted for this task confirms changes in the meadows' extent and pinpoints the morphology of those changes. The cause however of detected changes in the meadows' extent and structure cannot be always confirmed as humanly induced without expert assessment and *in situ* confirmation.

Changes which were estimated as being caused by sand extraction are located further from the shoreline and are circular in nature. On the location shown on Figure 90, approximately 2.500 m^2 of the CN meadows were affected. On the Omiš city beach location, shown on Figure 91, approximately 5.060 m^2 of CN meadow structure was affected.

Figure 90. Example of changes in the meadows structure between 2014 and 2017 which were estimated as being caused by sand extraction activities.

Another example of human-induced changes to the shoreline are changes of meadows structure and cover whose main cause was two dikes construction (significant lengthening and widening of existing dams) along the shoreline (Figure 92). The CN meadow area affected in this example is approximately 1.375 m^2.

Figure 91. Example of changes in the meadows structure near the Omiš City beach between 2014 and 2017 which were estimated as being caused by sand extraction activities.

Figure 92. Example of changes in the meadows area and structure between 2014 and 2017 which were estimated as being caused by dikes construction on the shoreline.

Figure 93. Changes of the meadows structure caused by possible sand extraction and coastal construction activities between 2014 and 2017.

Areas that were assessed to have been subjected to sand extraction activities were analysed with the goal of estimating the regeneration of *Cymodocea nodosa* after human activities with negative impact on the habitat. For this purpose, coverage maps for 2017 and 2021 were compared.

After 2017, there are indications that sand extraction activities happened with reduced frequency within the Cetina Estuary, with only a small number of additional sites subjected to extraction. On a majority of sites that were affected by activities that happened before 2017 the conditions for the regeneration of *Cymodocea nodosa* habitat were created, with limited degree of regeneration detectable on some of the sites (Figure 94).

Table 30. Area of Cymodocea nodosa meadows affected by human activities

Type of activity	CN meadows area affected [ha]
Sand extraction	1.81
Coastline construction	0.55

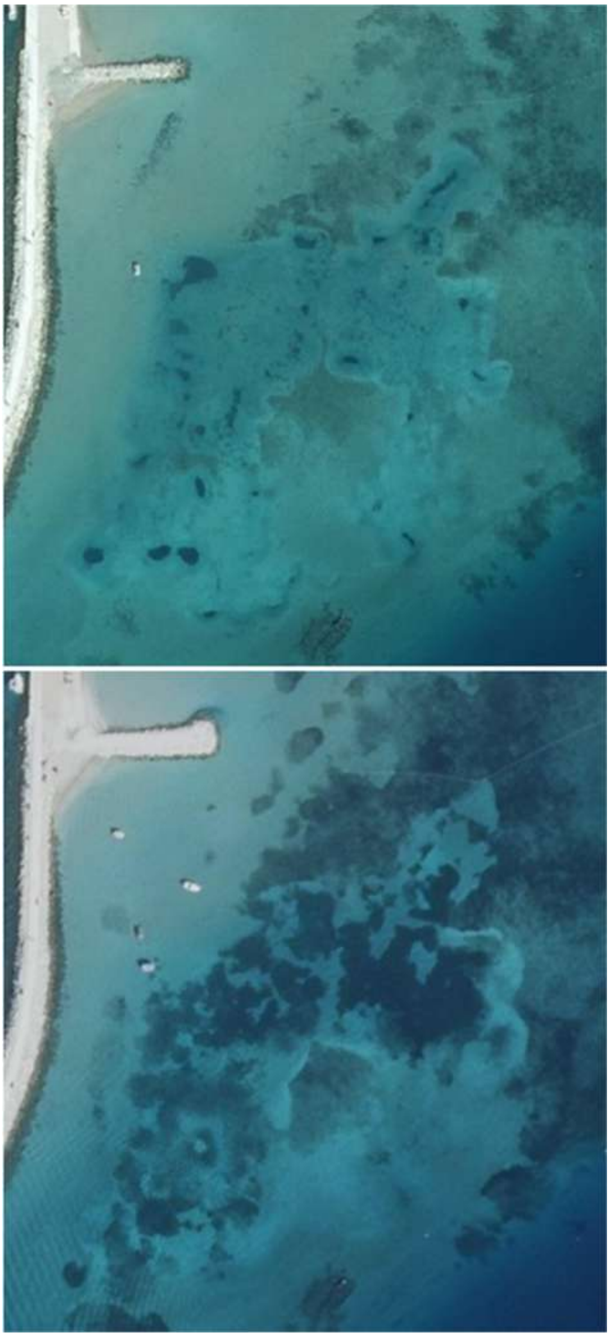

Figure 94. Example of meadows regeneration between 2017 and 2021

The results indicate a consistent increase in the area covered by *Cymodocea nodosa* meadows in the Cetina Estuary Natura 2000 site from 2011 to 2021, with an overall decadal increase of approximately 10.39 hectares. The growth trend was only slightly hindered in the period of 2017-

2019, potentially due to increased sand extraction activities in the area. Mapping for the last analysed reference year (2021) indicates the total area of *Cymodocea nodosa* meadows coverage on the habitat type "1110 – sandbanks which are slightly covered by sea water all the time" to be 54,04 ha which means a decadal increase of are covered by CN meadows of approximately 24% form 2011.

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

2.9.1.2.1 Assessment at the population level

To assess the current condition of *Cymodocea nodosa* habitat, the method proposed by Orfanidis et al. (2020) was used, that will enable calculation of the recently updated CymoSkew index, as outlined in the work plan (Kipson 2021). As we were dealing with an endangered and strictly protected species (with the permit issued by the Ministry of Economy and Sustainable Development , KLASA: UP/I-612-07/21-48/169; URBROJ: 517-10-1-1-21-4 from July 9 2021), destructive sampling was kept to a minimum – at each of 3 sites situated along the isobath of 3-4 m (close to the lower distributional depth limit of the meadow, chosen in consultation with the conservation manager-oceanographer of the Public Institution "More i krš" Jelena Kurtović Mrčelić: 1 – Omiš, Velika plaža; 2 – Omiš, kamp; 3 – Duće) and approximately 500 m apart from each other (Fig. 95), 5 replicates were collected within a well-developed meadow (as illustrated in Figs. 96 and 97b). A replicate consisted of all *Cymodocea nodosa* shoots thriving within 25 x 25 cm quadrat (Fig. 97a, c). They were collected in previously marked individual plastic bags and stored at -20 °C until further analysis.

Figure 95. Selected sites within the Natura 2000 Cetina Estuary for monitoring of *Cymodocea nodosa* meadows: 1 – Omiš, Velika plaža; 2 – Omiš, kamp; 3 – Duće. Numbered points indicate locations where shoot sampling was carried out (5 replicates collected at each site). Moreover, at each site video was recorded along three 50 m long transects, positioned perpendicularly to the coast (indicated at each site in different colours, with each colour corresponding to an individual transect, consult Table 1. and Fig.6 for further details). Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu. Figure preparation: J. Kurtović Mrčelić.

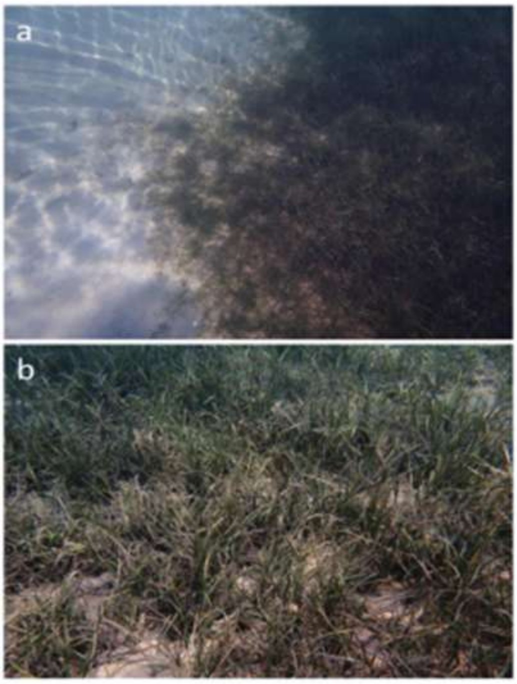

Figure 96. *Cymodocea nodosa* meadow within the Natura 2000 site Cetina Estuary at the site 1 – Omiš, Velika plaža: a) the edge of the meadow bordering with bare sand, b) detail of the *C. nodosa* meadow at 3 m depth. Photo credit: S. Kipson.

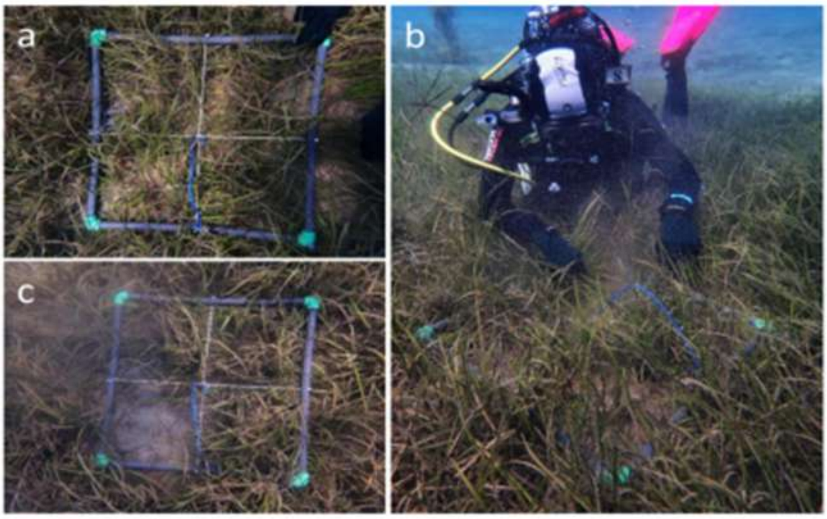

Figure 97. Illustration of the sampling to obtain data for calculation of Cymoskew index: a) a quadrat 50 x 50 cm divided in four 25 x 25 subquadrats positioned over the meadow, b) a SCUBA diver collecting all *Cymodocea nodosa* shoots within a subquadrat of 25 x 25 cm, c) unvegetated surface after the sampling of *C. nodosa* shoots. Photo credit: S. Kipson.

Prior to analysis, frozen samples were thawed 10 min then transferred into plastic washbasins filled with water (Fig. 98 a-b). Firstly, all shoots from each quadrat were counted to estimate shoot density (expressed as shoots/ m^2). The leaves (total leaf = sheath + photosynthetic part) of 20 shoots from each quadrat (i.e., 100 shoots per site) were randomly sorted, characterized as adult, intermediate or juvenile according to the sheaths differentiation degree and measured to the nearest mm (both the total length and sheath length, Fig. 98 c-e). Leaves with a well-developed sheath, that encompassed other leaves in its interior, were classified as adults, while those with no visible sheath were classified as juveniles. If a sheath was present but not yet fully developed and separated from the rest of the leaf, the leaf was classified as intermediate. The length and width of photosynthetic leaf part were used to calculate leaf surface area. Leaf area index (LAI) was determined by multiplying mean surface area of shoots (only one face considered) by a meadow shoot density.

Figure 98. Illustration of morphometric analysis of *Cymodocea nodosa* shoots: a) a sample retrieved from the freezer and left to thaw; b) all shoots collected within 25 x 25 cm quadrat, comprising 1 sample – 4 seeds are also visible; c) a shoot of *C. nodosa* with a seed attached to the base; d) separated leaves belonging to 1 shoot, consisting of photosynthetic leaf part (PLL) and a sheath; e) shoot with the longest observed leaf – however, not considered for further analysis as it was damaged (no apical part present). Photo credits: S. Kipson.

All quadrats consisted of > 20 *Cymodocea nodosa* shoots, hence all of them were included in the CymoSkew analysis. Photosynthetic leaf length (PLL) of differentiated leaves (adult and intermediate) per shoot were used as metrics to calculate the Cymoskew^m ecological index.

In general, the CymoSkew index may be estimated following the formula (Orfanidis et al. 2010):

$$\text{Skewness index} = N * M3/ [(N - 1) * (N - 2) * s^3]$$

Furthermore, the value of CymoSkew index can be transformed to Ecological Quality Ratio (EQR) under the Water Framework Directive's (2000/60/EC) standards and be used to classify a site to one of the five Ecological Status Classes i.e., High, Good, Moderate, Poor, or Bad (Table 31).

Table 31. Ecological class boundaries expressed for all known ecological indices based on a seagrass *Cymodocea nodosa*.

Ecological index	HIGH	GOOD	MODERATE	POOR	BAD	Ref.
Cymoskew^m (EQR)	0.75 to 1	0.5 to 0.75	0.25 to 0.5	0 to 0.25	0	1
Cymoskew^m	0 to 0.75	0.75 to 1.5	1.5 to 2.25	2.25 to 3	3	1
Cymoskew^{m}MAX	0 to 0.8	0.8 to 1.6	1.6 to 2.4	2.4 to 3.2	>3.2	1
Cymoskew^{m}MAX (EQR)	0.75 to 1	0.5 to 0.75	0.25 to 0.5	0 to 0.25	0	1
MediSkew	0 to 0.2	0.2 to 0.4	0.4 to 0.6	0.6 to 0.8	0.8 to 1	2
CYMOX	0.8 to 1	0.6 to 0.8	0.4 to 0.6	0.2 to 0.4	0 to 0.2	3

References: 1) Orfanidis et al. 2020; 2) Orlando Bonaca et al. 2015; 3) Oliva et al. 2012.

To assess potential differences within a *C. nodosa* meadow inside the Natura 2000 site Cetina Estuary, all metrics is expressed at the site level (Dućе, Kamp and Velika plaža). In addition, to accommodate the general sampling design proposed by Orfanidis et al. (2010, 2020) i.e., to sample seagrass at two sites approx. 500-800 m apart within the same meadow, values are also expressed for a combination of sites which are located next to each other, i.e., 800 m apart, such as Velika plaža and Kamp as well as Kamp and Dućе (10 samples in both cases). Lastly, the seagrass metrics and Cymoskew values are presented at the meadow scale (MEADOW) considering all available sites (3) and samples (15).

In total, 300 shoots were analysed (20 per replicate sample, 5 replicates per site, 3 sites) within the Natura 2000 site Cetina Estuary and 1337 leaves were counted. Out of these, 1236 were not damaged (421 juvenile, 118 intermediate and 724 adult leaves) and were measured for general morphometry (sheaths without any photosynthetic part were excluded), whereas 1 juvenile, 2 intermediate and 98 adult leaves had damaged apexes and hence their total length could not be measured.

Results of all analyses are presented in detail in technical report of Kipson (2023), while in the following tables a brief overview of them is presented (Table 32 – 34).

Table 32. *Cymodocea nodosa* abundance, leaf morphometric measurements and other observations (percentage of damaged leaves, number of seeds) within the Natura 2000 site Cetina Estuary. The standard deviation of the mean (within parentheses) is provided when possible.

Sites / Parameter	Duće	Kamp	Velika plaža	Duće + Kamp	Kamp + Velika plaža	Meadow
Shoot density (shoots/m2, n = 5 per site)	634	608	864	621	736	702
Total number of leaves per shoot	4.67 (0.68)	4.39 (0.74)	4.31 (0.71)	4.53 (0.72)	4.35 (0.72)	4.46 (0.72)
Max number of leaves per shoot	6	6	6	6	6	6
Leaf length (all ages, cm, n = 5 per site)	17.58 (8.93)	13.76 (7.3)	21.73 (11.28)	15.7 (8.44)	17.63 (10.27)	17.59 (9.84)
Leaf width (all ages, cm, n = 5 per site)				0.32 (0.07)	0.33 (0.08)	0.33 (0.08)
Maximum total leaf length (cm)	37	29.1	43.1	37	43.1	43.1
Adult Leaf length (cm, n = 5 per site)	23.10 (5.0)	18.66 (3.34)	29.39 (5.01)	20.91 (4.8)	23.64 (6.81)	23.45 (6.23)
Adult Leaf width (cm, n = 5 per site)	0.36 (0.04)	0.35 (0.05)	0.37 (0.04)	0.35 (0.04)	0.36 (0.05)	0.36 (0.04)
Intermediate Leaf length (cm, n = 5 per site)	19.93 (3.90)	14.01 (2.69)	25.48 (3.61)	17.41 (4.51)	19.83 (6.59)	19.87 (5.65)
Intermediate Leaf width (all ages, cm, n = 5 per site)	0.34 (0.04)	0.32 (0.05)	0.36 (0.04)	0.33 (0.05)	0.34 (0.05)	0.34 (0.05)
Juvenile Leaf length (cm, n = 5 per site)	6.44 (4.96)	4.56 (3.97)	9.45 (8.02)	5.51 (4.59)	7.10 (6.85)	6.88 (6.29)
Juvenile Leaf width (all ages, cm, n = 5 per site)	0.27 (0.08)	0.25 (0.09)	0.28 (0.09)	0.26 (0.08)	0.27 (0.09)	0.27 (0.09)
Percentage of damaged leaves (no apex)	4.71	3.64	7.42	4.19	5.52	5.24
Percentage of grazed leaves (visible bites)	1.07	0.91	3.71	0.99	2.30	1.87
Percentage of leaves with some epiphytes	5.14	7.74	4.87	6.40	6.32	5.91
Number of seeds (n = 5 per site)	0.8 (1.79)	2.8 (2.39)	0.6 (1.34)	1.8 (2.25)	1.7 (2.16)	1.4 (2.03)
Leaf area (cm2/shoot, n = 5 per site)	23.05 (7.51)	16.44 (5.84)	28.48 (8.3)	19.75 (7.5)	22.46 (9.36)	22.66 (8.78)
Leaf Area Index (m2/m2, n = 5 per site)	1.46	0.99	2.46	1.23	1.65	1.6

Table 33. Morphometry of differentiated leaves (i.e., adult and intermediate, excluding juvenile) of *Cymodocea nodosa*. PLL = photosynthetic leaf length; Total leaf length = sheath + PLL.

	TOTAL LEAF LENGTH (cm)		PLL (cm)	
	MEAN	SD	MEAN	SD
L1-Duće	22.50	4.90	17.20	4.11
L2-Kamp	18.09	3.60	13.58	2.75
L3-Velika plaža	28.93	4.98	22.69	4.04

Table 34. Ecological Quality Ratios (EQR) derived from Cymoskew^{m} values considering all 3 sites (MEADOW) and combination of 2 adjacent sites, based on iteration of different subsample's sizes (i.e. different number of leaves).

	Cymoskew^{m} EQR		
N of leaves	Duće + Kamp	Kamp + Velika plaža	MEADOW
100	0.90	0.88	0.82
125	0.90	0.89	0.83
150	0.91	0.90	0.85
200	0.94	0.91	0.88

Based on the high Ecological Quality Ratio resulting from the Cymoskew^{m} index assessed here as well as the evidenced increase of *C. nodosa* habitat extent within the Natura 2000 site Cetina Estuary in the past decade (OIKON 2023), the status of the seagrass meadow may be evaluated as favourable and thus contribute to the overall assessment of the habitat type 1110 Sandbanks which are slightly covered by the sea water all the time - where *Cymodocea nodosa* represents a typical, habitat-forming species (over 40% of the 1110 Habitat type extent), for the purpose of the EU Habitat Directive reporting. The morphometric characteristics of *C. nodosa* meadow within the Cetina Estuary fall within values previously recorded in other Mediterranean meadows.

2.9.1.2.2 Assessment of *Cymodocea nodosa* habitat structure

To assess *Cymodocea nodosa* habitat structure in the Natura 2000 site Cetina Estuary based on the habitat structure index (HIS; Irving et al. 2013), *in situ* video transects 50 m long and 1 m wide (3 per site) were recorded. One diver from a diving team was in charge of laying down the transect on the sea floor while also towing a surface buoy equipped with GPS in order to acquire georeferenced data, Fig. 99a). This diver would position the buoy directly above the starting and the end point of the transect to obtain the most precise georeferenced data (hence, any putative deviation corresponds to the deviation of the GPS itself during the satellite positioning). Moreover, he/she would note the exact time of the positioning by using the diving computer. Subsequently, the second diver would record a video along the transect (Fig. 99b). Paths of recorded transects are visualized in Fig. 100 and coordinates of the transects' start and end points are provided in Table 35. The GoPro camera was positioned perpendicular to the substrate during the video recording (Fig. 99b).

During the subsequent desktop video analysis all necessary data (e.g., percentage cover estimations, species identities of present vegetation) will be extracted for each 1 m^2 of the transect and HSI will be calculated by quantifying five habitat metrics in each transect (Irving et al. 2013).

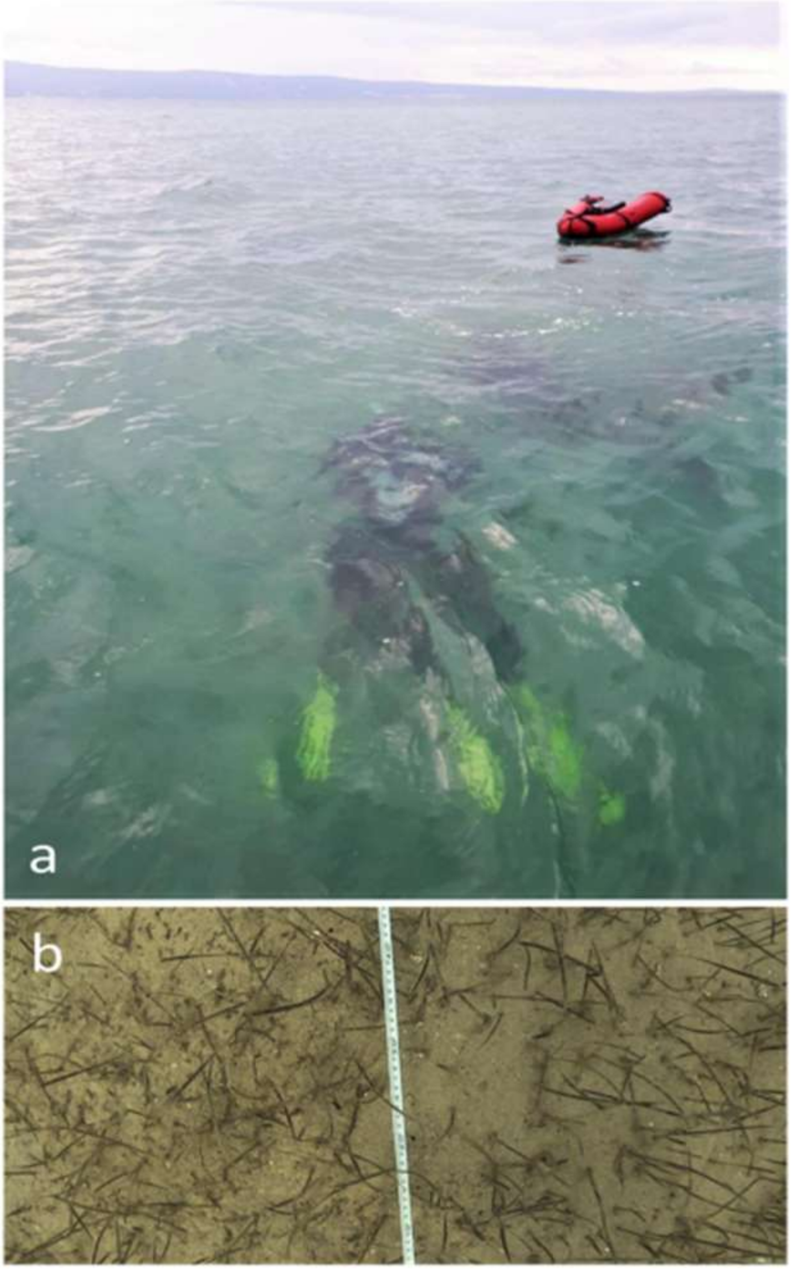

Figure 99. Acquisition of a video along the transect within the *Cymodocea nodosa* habitat: a) SCUBA diver towing a surface buoy equipped with the GPS, b) excerpt from the video of the transect 50 m long and 1 m wide. Photo credit: a) M. Belošević, b) S. Kipson.

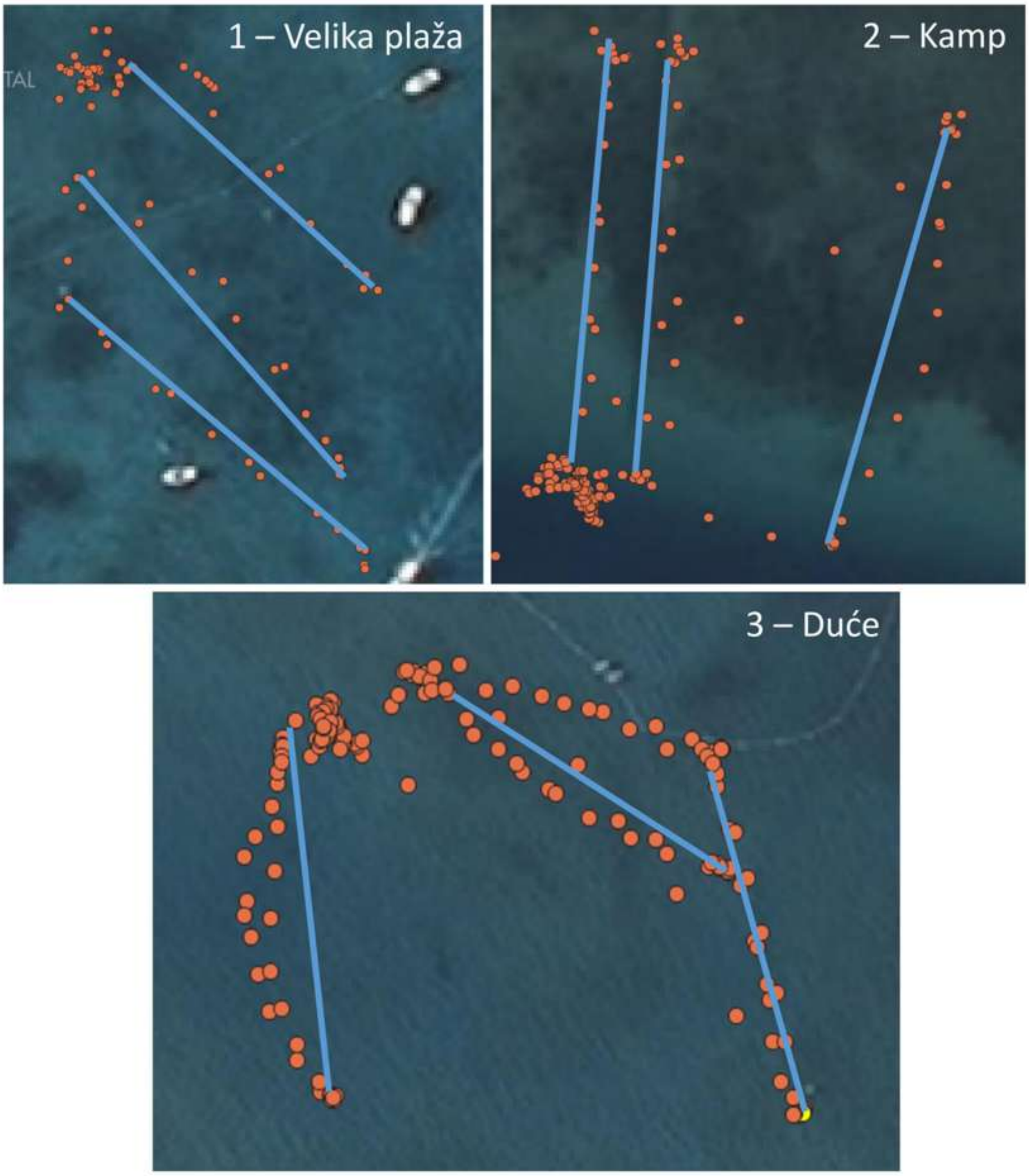

Figure 100. Details of the video transects within *Cymodocea nodosa* meadows with marked GPS positions at each of 3 sites. Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu. Figure preparation: J. Kurtović Mrčelić.

Site	Transect N	Date	Depth (m)	Coordinates initial	
			Initial	Y	X
Velika plaža	1	11/11/2021	2	4810976.55	515343.43
Velika plaža	2	11/11/2021	2	4810991.87	515306.08
Velika plaža	3	11/11/2021	2	4810942.56	515341.71
Kamp	1	12/11/2021	3.3	4810900.14	514648.37
Kamp	2	12/11/2021	3.1	4810898.67	514657.53
Kamp	3	12/11/2021	3.9	4810890.71	514679.15
Duće	1	13/11/2021	2.3	4811031.60	513002.89
Duće	2	13/11/2021	1.7	4811039.37	513025.83
Duće	3	13/11/2021	2	4811030.09	513063.12
Site	Transect N	Date	Depth (m)	Coordinates final	
			Final	Y	X
Velika plaža	1	11/11/2021	2.3	4811010.53	515308.21
Velika plaža	2	11/11/2021	2.3	4810953.32	515338.40
Velika plaža	3	11/11/2021	2.3	4810974.24	515301.86
Kamp	1	12/11/2021	1.3	4810949.30	514655.96
Kamp	2	12/11/2021	1.1	4810951.54	514661.70
Kamp	3	12/11/2021	1.1	4810940.48	514692.16
Duće	1	13/11/2021	1.9	4810990.42	513004.77
Duće	2	13/11/2021	2	4811015.02	513064.92
Duće	3	13/11/2021	1.8	4810981.06	513076.77

Table 35. Depths and GPS positions of the initial and end points of each video transect recorded at each of 3 sites within the *Cymodocea nodosa* habitat in the Natura 2000 site Cetina Estuary. Note: coordinates are provided in HTRS96 projected coordinate system.

The HSI was calculated by quantifying five habitat metrics in each transect (Irving et al. 2013):

(i) Area (A) – the total area of seagrass sampled within the transect, which provides data on overall seagrass abundance.

(ii) Continuity (C) – the number of patches of seagrass within the transect, which provides data on the degree of habitat patchiness. A patch is defined at the scale of the quadrat used, and is delineated by quadrats where seagrass is absent (e.g., sand)

(iii) Proximity (P) – the distance(s) between patches of seagrass within the transect, which provides data on the scale of habitat connectivity. The distances between patches of seagrass are quantified for this metric.

(iv) Percentage cover (K) – the average value of the observed percentage cover of seagrass within the transect. This metric essentially ranks the within-quadrat abundance of seagrass based on its percentage cover, assuming that quadrats with a greater percentage cover of seagrass represent better habitat

(v) Species identity (S) – the average value assigned to species present in the transect. Similar to K, this metric ranks the seagrass present in the transect based on its identity, assuming that slower growing but competitively dominant ''climax'' taxa create more valuable habitat

Once all of five habitat metrics are known, a preliminary HSI (termed HSI') is calculated using the Euclidean distance of the five integrated data points to define the sample's position in multivariate space relative to a common origin. This is done using the equation:

$$HSI' = \sqrt{(A^2 + C^2 + P^2 + K^2 + S^2)}$$

The final step is to apply a scalar so that the HSI scores each transect from 0 to 100 (100 = 100% cover of a 'climax' species, while structure worsens as the HSI approaches 0). HSI can then be directly compared among all samples. The value of this scalar depends on the length of the transect, the minimum sampling unit size (i.e. the 1 m2 quadrat), and on the number of metrics used in the calculation of HSI'.

Therefore,

HSI = HSI' x scalar (for a 50 m long transect the scalar is 0.4453; see Irving et al. 2013 for further explanation)

This value can be readily compared to other calculations of HSI, which allows direct spatiotemporal analyses of the overall structure of seagrass meadows and a quantitative assessment of their relative condition (Irving et al. 2013).

Results of all analyses are presented in detail in technical report of Kipson (2023), while in the following table a brief overview of HIS calculated for each transect is presented (Table 36).

Table 36. Habitat structure index (HSI) calculated for each transect at each of 3 investigated sites, together with values of 5 main metrics used for index calculation: A = area, C = continuity, P = proximity, K = percentage cover and S = species identity. The value of scalar used for a 50 m transect was 0.4453. Calculations are made for summer and autumn aspect (implying different percentage cover, and hence the observed Area). The summer aspect is estimated based on results of destructive sampling, images and literature data and presented both assuming the maximum species value (S_{max}) to be 3 and 2, respectively, given that *Cymodocea nodosa* was assigned 2 as the observed species value.

Site	Transect	A	C	P	K	S	HSI
Summer aspect							
Duće	1	41.4	100.0	102.1	58.7	27.6	72.3
	2	44.1	100.0	102.1	64.7	29.4	73.7
	3	26.8	97.8	91.7	51.3	17.9	65.5
Kamp	1	53.9	100.0	102.1	66.0	35.9	75.8
	2	54.1	100.0	102.1	66.7	36.1	76.0
	3	54.4	100.0	102.1	66.7	36.3	76.0
Velika plaža	1	69.2	100.0	102.1	66.7	46.1	79.4
	2	70.0	100.0	102.1	66.7	46.7	79.6
	3	68.6	100.0	102.1	66.0	45.7	79.1
Autumn aspect							
Duće	1	20.7	100.0	102.1	33.3	27.6	67.1
	2	22.1	100.0	102.1	33.3	29.4	67.4
	3	13.4	97.8	91.7	33.3	17.9	62.3
Kamp	1	27.0	100.0	102.1	33.3	35.9	68.3
	2	27.1	100.0	102.1	33.3	36.1	68.4
	3	27.2	100.0	102.1	33.3	36.3	68.4
Velika plaža	1	34.6	100.0	102.1	33.3	46.1	70.2
	2	35.0	100.0	102.1	33.3	46.7	70.3
	3	34.3	100.0	102.1	33.3	45.7	70.1
Smax = 2 (summer aspect)							
Duće	1	41.4	100.0	102.1	58.7	41.4	73.6
	2	44.1	100.0	102.1	64.7	44.1	75.2
	3	26.8	97.8	91.7	51.3	26.8	66.1
Kamp	1	53.9	100.0	102.1	66.0	53.9	77.9
	2	54.1	100.0	102.1	66.7	54.1	78.0
	3	54.4	100.0	102.1	66.7	54.4	78.1
Velika plaža	1	69.2	100.0	102.1	66.7	69.2	82.6
	2	70.0	100.0	102.1	66.7	70.0	82.9
	3	68.6	100.0	102.1	66.0	68.6	82.3

Being a seasonal species, a decrease in *C. nodosa* biomass/coverage from summer to autumn is considerable (e.g., shoot density and mean leaf length decrease by 40-70% and 60-70%, respectively, from summer to winter; Peduzzi & Vuković 1990, Rismondo et al. 1997, Guidetti et al. 2001). Hence, concerning the percentage cover as one of the metrics to calculate HSI, also affecting calculation of Area and thus calculation of observed species identity, conducting the structure assessment in November was not ideal. Luckily the cover categories defined by Irving et al. (2013) are rather broad, and having also a notion of shoot density per site stemming from fully quantitative measurements and images of summer (July) aspect, it allowed an estimation that could serve as a baseline for future monitoring. While it would be more ideal to carry out future monitoring of habitat structure at the peak of vegetation season in July/August, the most important is to select a period within a year and to stick to it, to avoid seasonal bias and to enable comparisons over time. Hence, the baseline for autumn aspect (November, when the actual assessment was carried out within this study) is also provided here. Overall, based on Habitat structure Index, the individual sites, and the *C. nodosa* meadow in the Cetina Estuary showcase higher structural integrity in summer, however it is generally good disregarding the season. At the site level, the structural complexity is the highest at the Velika plaža site.

2.9.2 Assessment and monitoring of the *Cladocora caespitosa* bank.

To characterize and map yet undescribed *C. caespitosa* population forming a remarkable, relatively deep bank (from 24.6 down to 28 m) just outside of the southern border of the Natura 2000 site Cetina Estuary (Fig. 101,102; coordinates 43°24’53.8'' N, 16°43’03.5'' E) and to assess its current conservation status, we have carried out field activities in November 2021 and July 2022. Assessment and monitoring of the bank are described in detail in technical report of Kipson (2023), while in the following text a brief overview of them is presented.

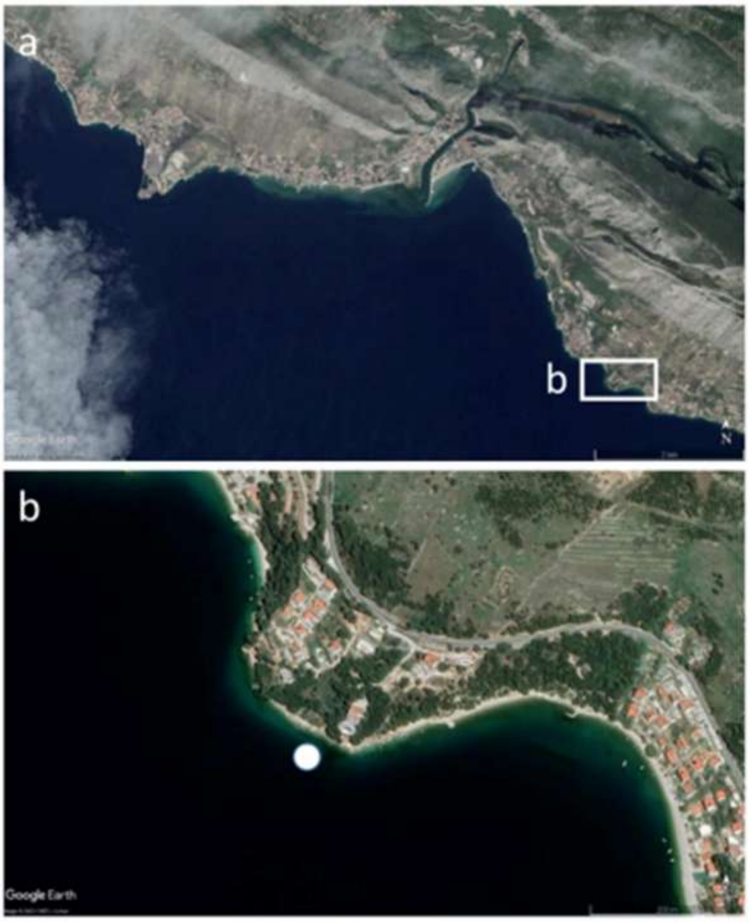

Figure 101. Location of the yet undescribed Cladocora caespitosa bank, next to the southern border of the Natura 2000 site Cetina Estuary and the site selected for the establishment of continuous, high-resolution monitoring of seawater temperature down to 30 m depth: the site Mala Luka (Balića rat). Map source: Google Earth ©2022

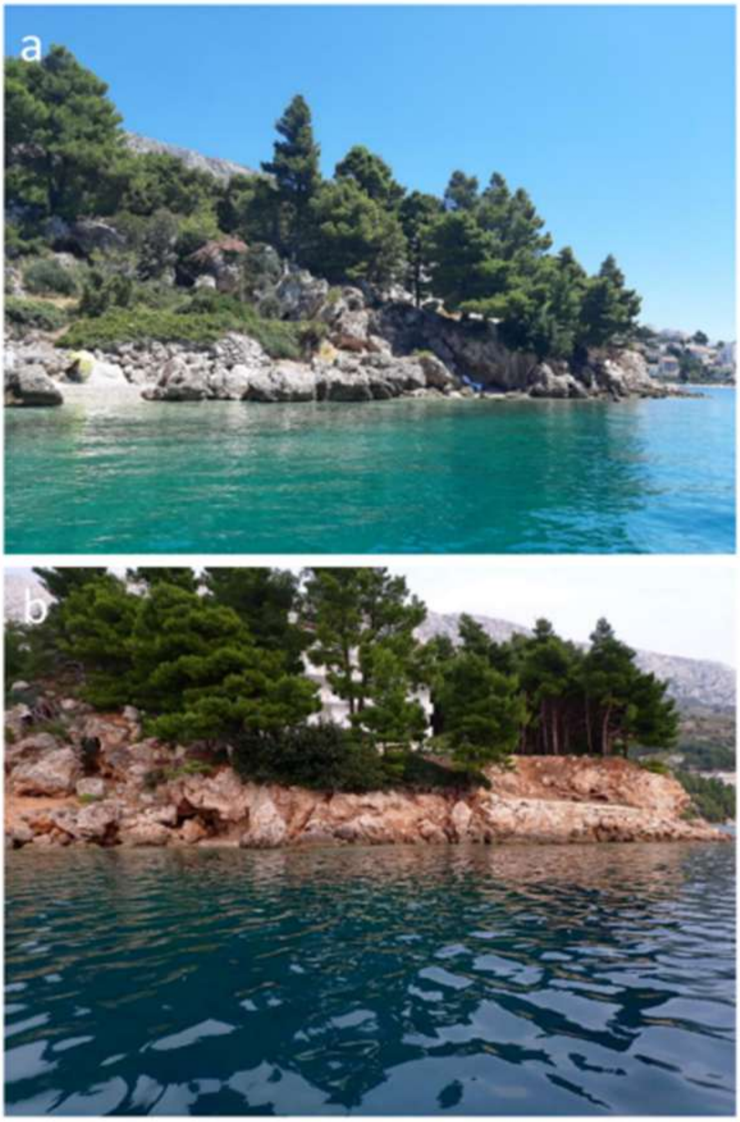

Fig. 15. The site Mala Luka (Balića rat): a) the settlement Velika Luka (Stanići) seen in the background, on the right, b) view of the coast from the immersion point, where patches of rocky bottom may be found on otherwise pebbly substrate at 5 m depth. Photo credit: S. Kipson.

2.9.2.1 Mapping of the Cladocora caespitosa bank

The *C. caespitosa* bank was mapped by the underwater photogrammetry in November 2021, enabling highly accurate measures of the surface area and volume of coral colonies and skeletons. Images required for 3 D reconstruction were acquired by a SCUBA diver, using a GoPro 8 camera mounted on a tray and equipped with 2 video lights and a wide-angle dome. Prior to photographing, 2 scales (rulers) were placed on the *C. caespitosa* bank (Fig. 102). The bank and scale references were then photographed in a way to capture overlapping photographs (at least 70% overlap) at a rate of ~1 photo every 1–2 seconds from different viewpoints using a standardized pattern. The pattern consisted of several consecutive arched passes at approximately 1 m from the coral bank, each pass capturing a series of overlapping images from top to bottom covering the entire surface of the coral bank. The camera was then moved ~45° to start the next pass, again following the several arched passes and lastly 1-2 360° spiral passes around the bank were made. In total, 1181 images of *C. caespitosa* bank were acquired for the purpose of photogrammetric reconstruction. Additionally, several measures were made *in situ* of the reef height, in the lower, middle, and upper part of the bioconstruction (Fig. 103).

Since good quality of images for photogrammetry was attained right from the first attempt, and due to logistic challenges related to the underwater work at depths of the *C. cladocora* bank (from 24.6 to 28 m) operational decision was made not to proceed also with the "classic" *in situ* mapping technique following Kružić & Benković (2008) that would imply counting and outlining all coral colonies inside the 25 m^2 frames which should be moved around to cover the whole coral bank.

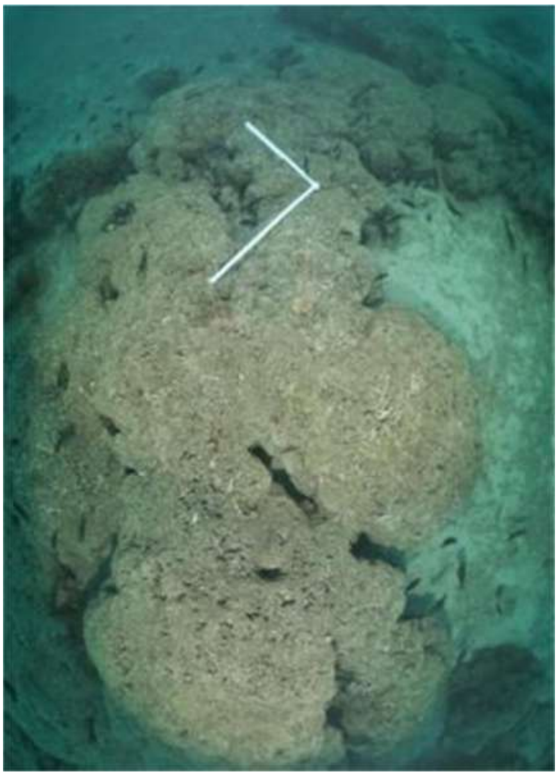

Figure 102. Ruler used as a scale bar during the acquisition of images for photogrammetry. Photo credit: M. Belošević.

Figure 103. Measuring the height of *Cladocora caespitosa* bank in different parts of bioconstruction. Photo credit: J. Kurtović Mrčelić.

Prior to producing 3D reconstructions by Photoscan (Agisoft LLC), images were processed. Once a 3D reconstruction was processed, it was scaled by using the ruler. After scaling, reconstruction was exported, and further measurements and analyses were undertaken. To assess the size of the *C. caespitosa* bank (and separate larger colonies nearby, including broken bank portions), its ortho-photo was imported in Photoquad, calibrated and its surface area was outlined using a freehand Region of Interest (ROI) selection tool.

The cushion coral *Cladocora caespitosa* bank stretches from 24.6 m to 28 m depth just outside of the southern border of the Natura 2000 site Cetina Estuary. It occupies an area of 36.1 m^2 (Fig. 104) with the maximum length of 15.8 m and the maximum width of 3.8 m. The bioconstruction is thicker in its lower part (from 27-28 m depth; with mean thickness of 50.17 ± 4.67 cm) than in the upper one (at approx. 25 m depth; 42.88 ± 6.24 cm).

Figure 104. The orthophoto mosaic of the *Cladocora caespitosa* reef and nearby colonies at the Mala Luka site. Two white L-shaped rulers positioned over the reef served as scale bars, with shorter end measuring 40 cm. The upper part of the image depicts the deepest part of the reef, whereas the lower part of the image depicts the shallowest part of the reef.

2.9.2.2 Population structure and conservation status of Cladocora caespitosa

To further assess the condition of the bank, during the field mission in November 2021 around 30 photographs of 50 x 50 cm quadrats were taken along 3 random transects, each 10 m in length (Fig. 105, 106). In addition, 5 video transects were recorded (each 20 m long and 1 m wide) of the nearby sea bottom hosting separate *C. caespitosa* colonies, not fused into a bank (Fig. 107). In each case one diver laid down the transect on the substrate, and after the video was recorded by the second diver, he/she recovered the transect. Based on acquired photographs and video the size of individual colonies will be determined, as well as areas affected by necrosis and the ratio of alive vs. dead % cover.

Figure 105. SCUBA diver laying down transects as a preparatory action to acquire images of 50 x 50 cm quadrats on the *Cladocora caespitosa* bank and to record video on the nearby sea bottom hosting individual *C. caespitosa* colonies not fused into a bank. Photo credit: M. Belošević.

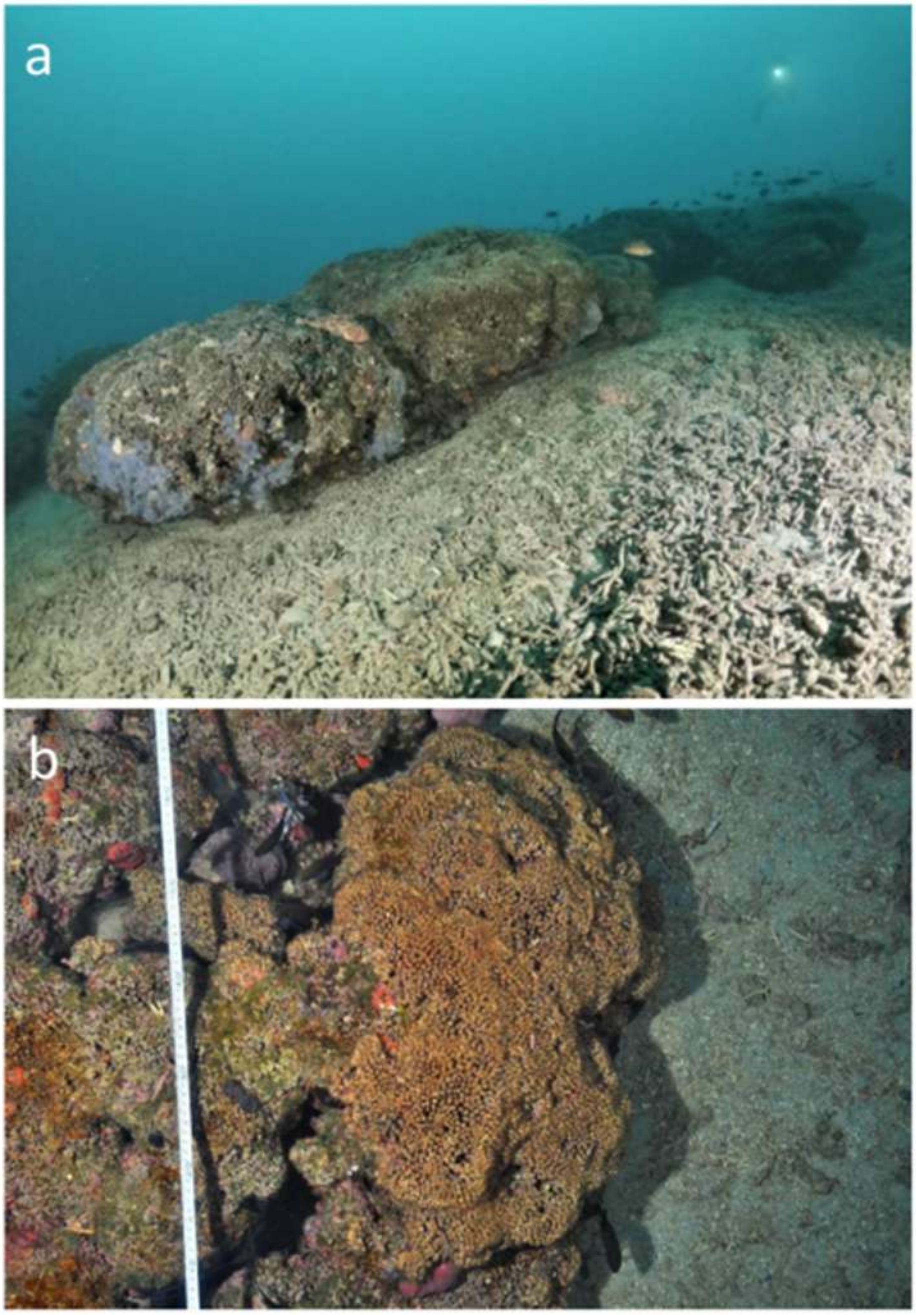

Figure 106. The *Cladocora caespitosa* bank stretching from 24.6 m down to 28 m depth at the site Mala Luka (Balića rat) near Omiš: a) view of the lower part of the bank, b) illustration of the transect laid down over a bank to acquire images that will be used for the assessment of its conservation status. Photo credit: M. Belošević.

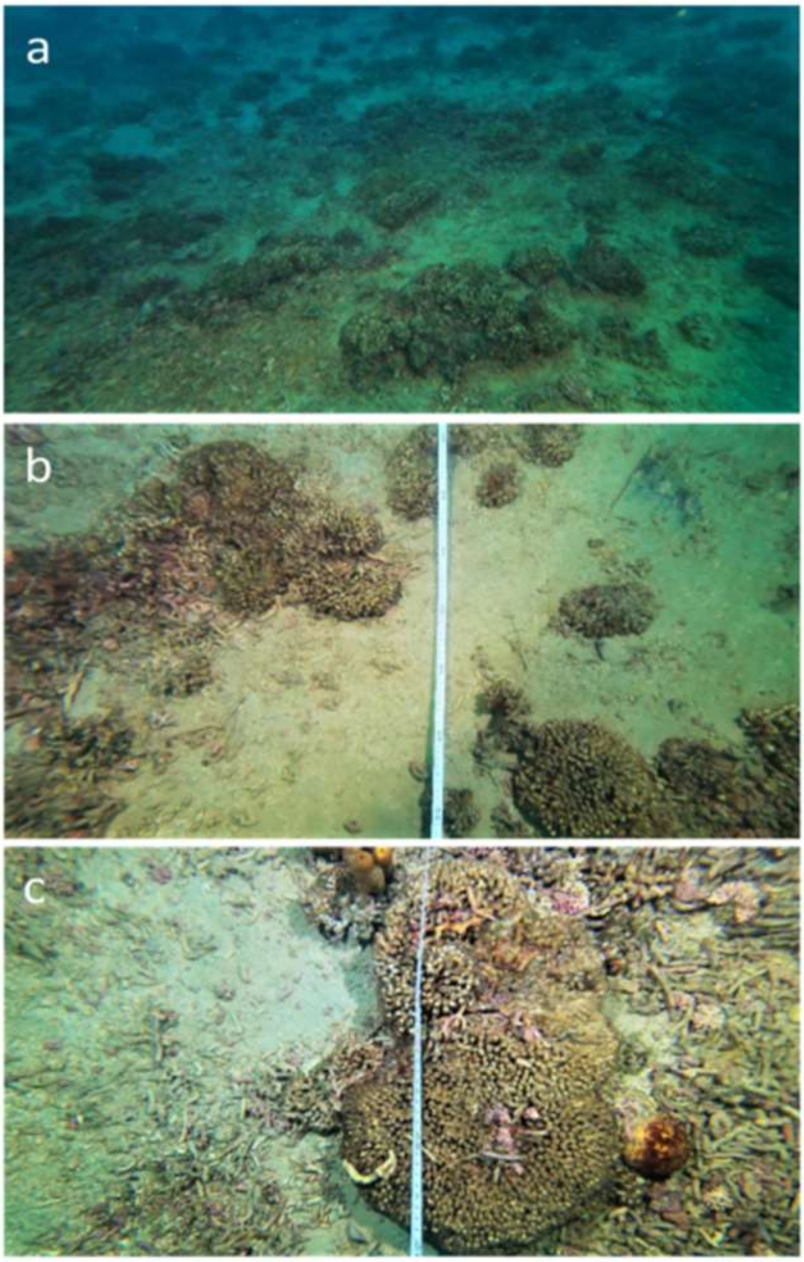

Figure 107. The sea bottom nearby the *Cladocora caespitosa* bank: a) numerous smaller *C. caespitosa* colonies scattered around, along with rubble of dead corallites, b) and c) excerpts of the video recorded along five 20 m transects. Photo credit: J. Kurtović Mrčelić.

The reef appeared to be the healthiest in its lower part (from 27-28 m depth), with mean live coral cover of 86.78 ± 19.63%, followed by considerably lower mean live coral cover in its central (29.52 ± 33.85%) and upper part (24.6-25 m depth) which hosted mere 6.96 ± 16.90% (mean ± SD) of live coral cover (Table 37). When assessing the condition on the scale of the whole reef, the mean percentage of live coral cover amounted to 45.03 ± 42.09% (n = 52). As notable from stated result, the percentage of live coral cover varied significantly at the level of 50x50 cm replicate quadrat, reaching values from 0 to 100%.

Dead portions of the *Cladocora* reef were still mainly uncovered by other organisms. The mean percentage cover of visible dead corallites in the reef's upper part amounted to 48.04 ± 18.16%, followed by 44.92 ± 26.77% in its middle and 8.34 ± 16.63% in its lower part (all values expressed as mean ± SD, Table 9). At the scale of the reef, dead coral portions still uncovered by epibionts reached mean % cover of 31.75 ± 27.73. Out of epibionts which have overgrown remaining dead portions of the reef, 4 algae, 6 sponges, 1 polychaeta and cyanobacteria were noted (Table 37). Among encrusting algae *Peyssonnelia* spp. and Corallinales were present, as well as green turf algae *Pseudochlorodesmis furcellata* and the unidentified red turf algae. Among sponge taxa, specimens of *Ircinia* sp., *Petrosia ficiformis*, *Chondorsia reniformis*, *Phorbas tenacior*, unidentified black massive keratose sponge and red encrusting sponge were recorded as epibionts on the investigated, horizontal surface of the *C. caespitosa* reef.

Table 37. Mean percent cover of alive coral, non-overgrown dead coral and different epibionts shown for each portion of the *Cladocora caespitosa* reef (deepest = 27 - 28 m, middle = 25.5 – 27 m and shallowest = 24.5 – 25.5 m depth). SD = standard deviation.

Portion of the Reef	Deepest		Middle		Shallowest	
% Cover	Mean	SD	Mean	SD	Mean	SD
Cladocora alive	86.8	19.6	29.5	33.8	7.0	16.9
Cladocora dead	8.3	16.6	44.9	26.8	48.0	18.2
Scattered corallites	0.6	1.9	1.9	3.3	4.6	11.7
Green algal turf	1.8	4.9	12.8	11.7	19.4	9.0
Peyssonnelia sp.	0.9	1.8	3.6	2.3	4.1	2.1
Encrusting Coralinalles	1.0	2.1	1.7	1.8	3.2	2.8
Red algal turf	0.0	0.0	3.6	8.3	5.2	6.2
Cyanobacterial mat	0.1	0.3	1.3	2.1	7.6	8.4
Black keratose sponge	0.1	0.6	0.0	0.1	0.2	0.6
Ircinia sp.	0.1	0.5	0.5	1.9	0.2	0.7
Petrosia ficiformis	0.2	0.7	0.1	0.3	0.0	0.0
Chondorsia reniformis	0.0	0.0	0.0	0.1	0.2	0.5
Phorbas tenacior	0.0	0.0	0.1	0.2	0.1	0.2
Red encrusting sponge	0.0	0.1	0.0	0.1	0.1	0.1
Serpulidae	0.0	0.0	0.0	0.0	0.1	0.3

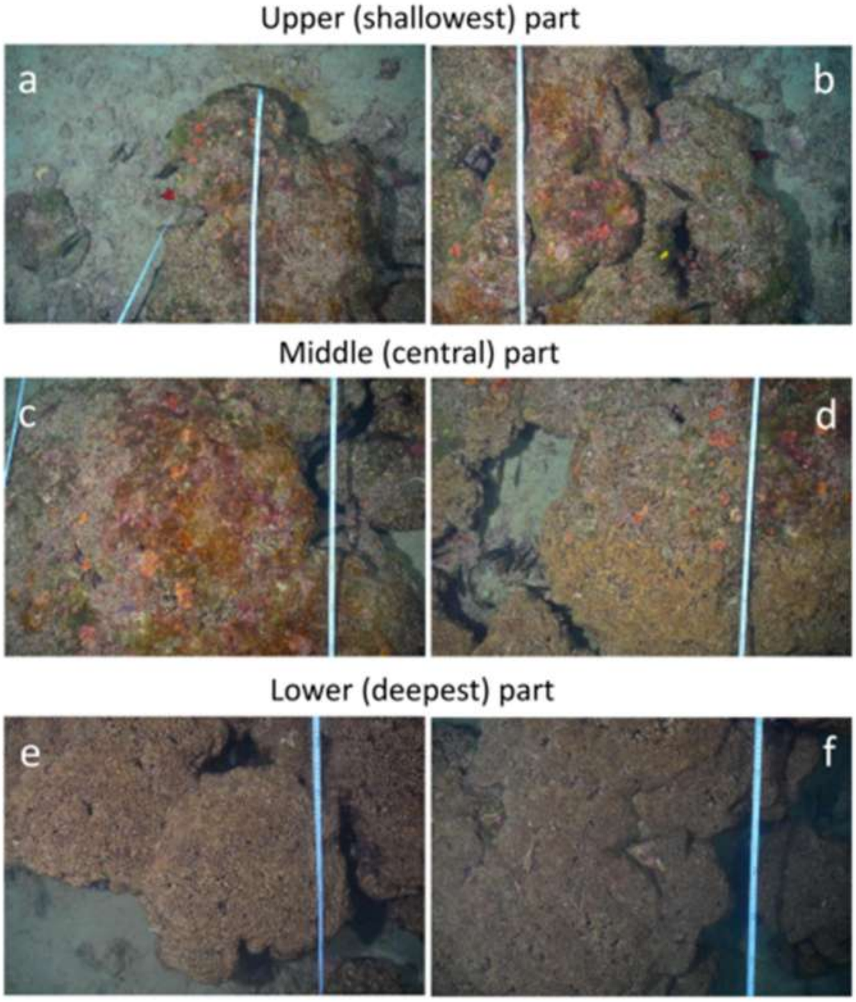

Figure 108. Illustration of different portions of *Cladocora caespitosa* reef: a and b) upper (shallowest) part (24.6-25.5 m); c and d) middle (central) part (25.5 – 27m); e and f) lower (deepest) part (27-28 m). Photo credits: M. Belošević.

Besides the main bioconstruction in form of colonies "fused" into a bank, several non-fused colonies are present next to the reef. Their total surface area amounts to additional 4.77 m^2, ranging in size from 0.01 to 2.1 m^2. Moreover, there are numerous smaller colonies present nearby, scattered over an area that stretches for at least 100 m (Fig. 109). While most of alive colonies were attached to the substrate, also unattached "spherical" colonies were observed, previously referred to as "coral nodules" and observed by Kersting et al. (2017) in a population from Formentera (Spain). Moreover, small individual colonies were observed also in the shallows of the same site, around 10-15 m depth. Details of all analyses of non-fused colonies are available in technical report of Kipson (2023).

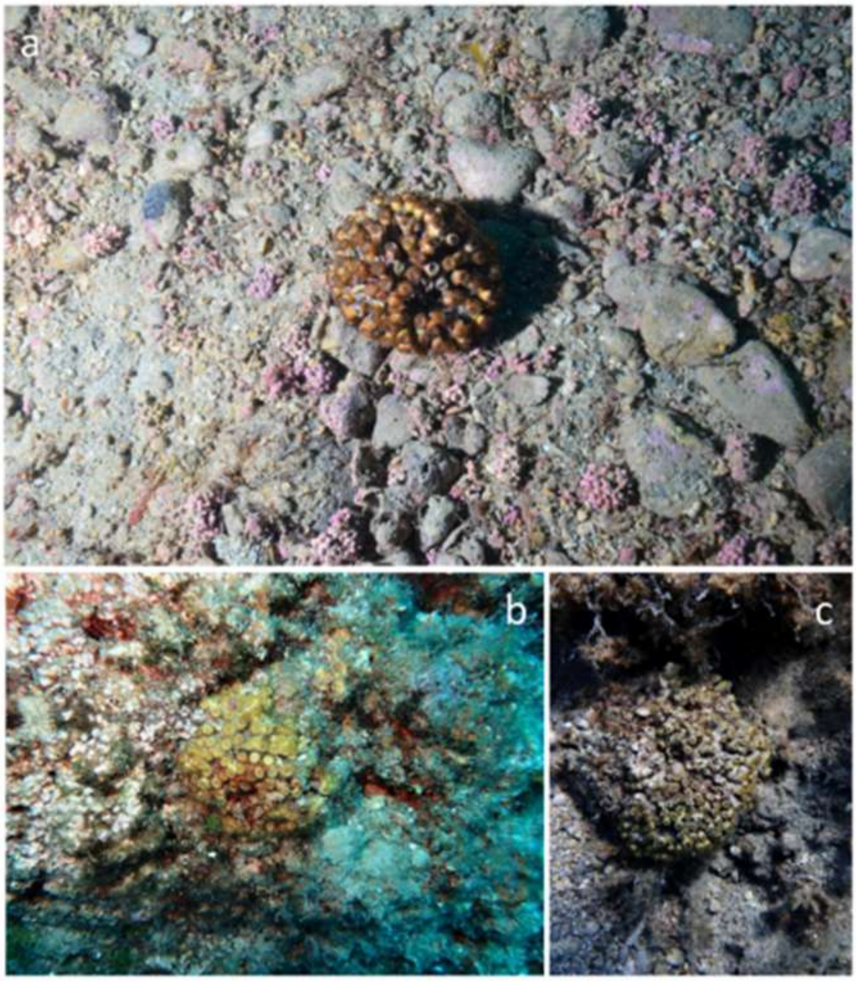

Figure 109. *Cladocora caespitosa* colonies at the Mala luka site: a) unattached, rounded colonies observed at 25 m depth; b and c) small individual colonies present on shallow rocky bottom, around 10 m depth. Photo credits: a) M. Belošević, b and c) S. Kipson.

The most adverse impact observed on the reef was evident physical damage. Around the main bioconstruction, there were quite a bit of scattered, detached reef parts (Fig. 110). Mainly, they still have alive corallites, while on dead parts (previously inner portions of the bioconstruction) the presence of epibionts is noticed. Damaged portions of colonies are also observed scattered across a wider area, among smaller alive colonies. Whereas elevated hydrodynamism may be partially responsible for some of mechanical damages observed, most likely the majority of them are caused by human-induced causes such as anchoring. Moreover, abandoned/lost fishing gear is present on the site, such as net ropes, longlines and monofilaments, sometimes covering or entangling *C. caespitosa* colonies (Fig. 111), with putative abrasive effect. Out of other potential stressors, mucilaginous algal aggregates were recorded on the reef at the end of June 2021 whereas cyanobacterial mats were noticed in November 2021, during assessment of the reef's condition (Fig. 112).

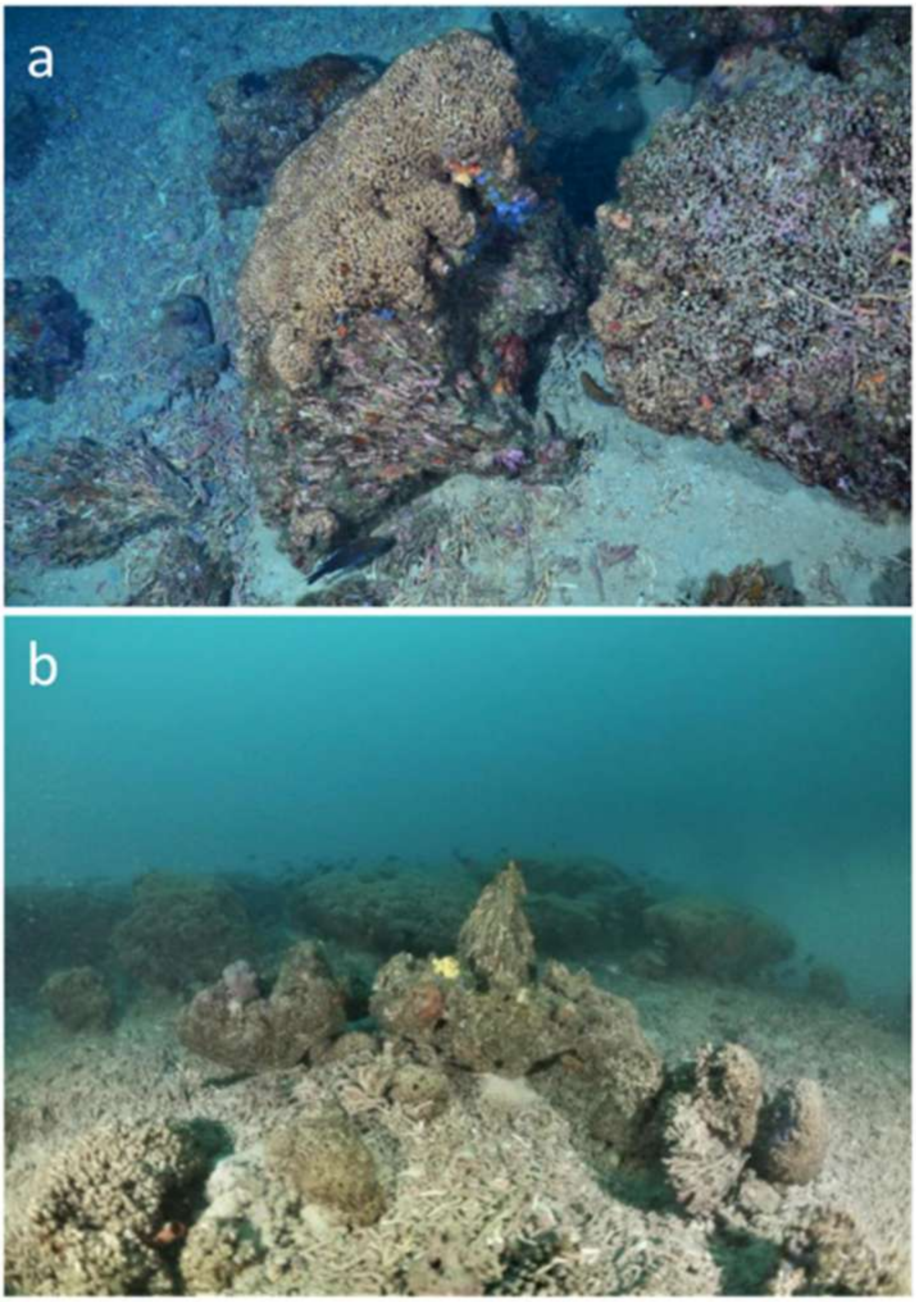

Figure 110. Illustration of mechanical damage on *Cladocora caespitosa* reef, resulting in detached reef parts scattered around the main bioconstruction. Photo credits: M. Belošević

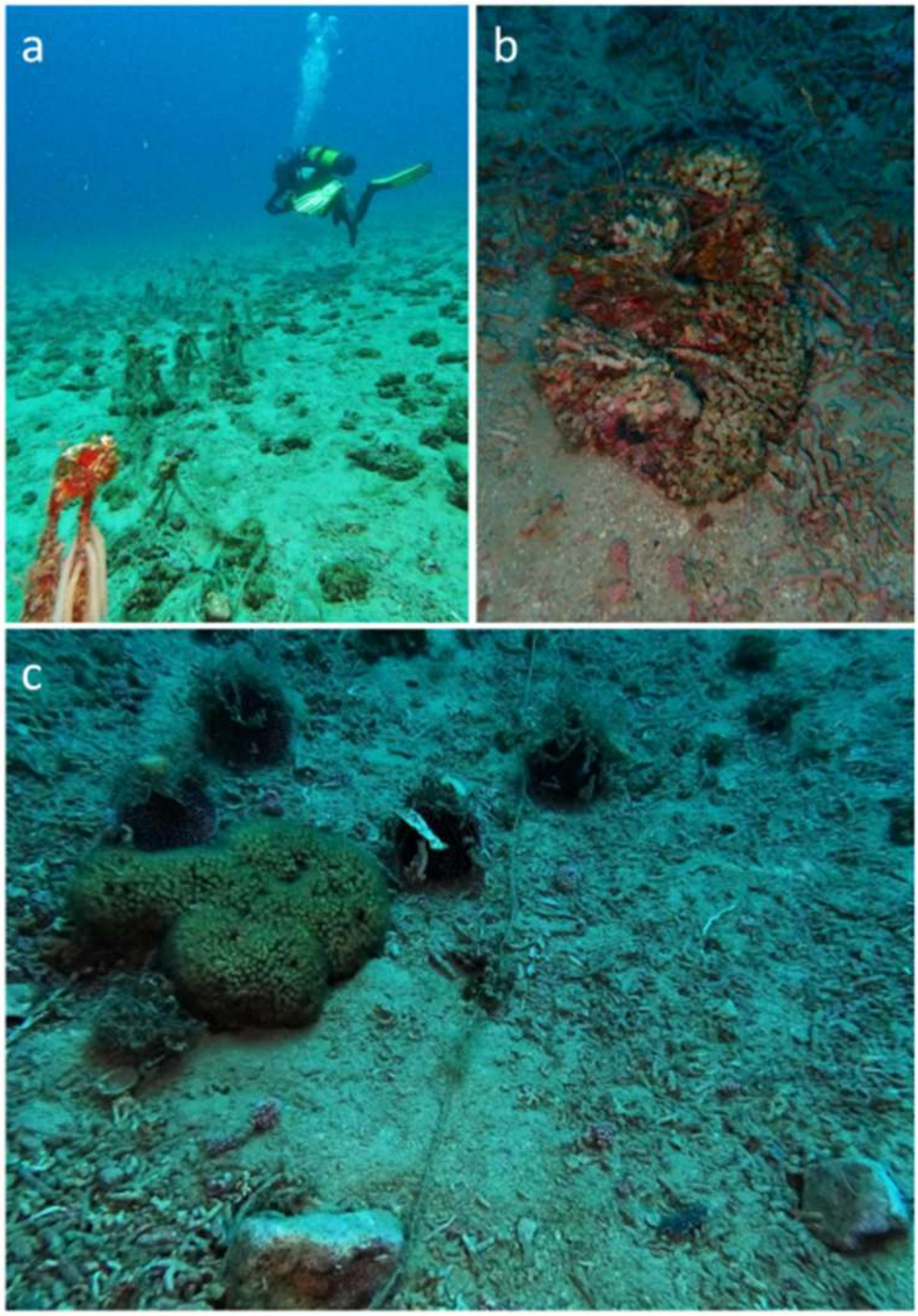

Figure 111. Abandoned/lost fishing gear observed at the *Cladocora caespitosa* site Mala luka: a) net rope, b) monofilaments, c) long-lines. Photo credits: a) J. Kurtović Mrčelić, b and c) S. Kipson.

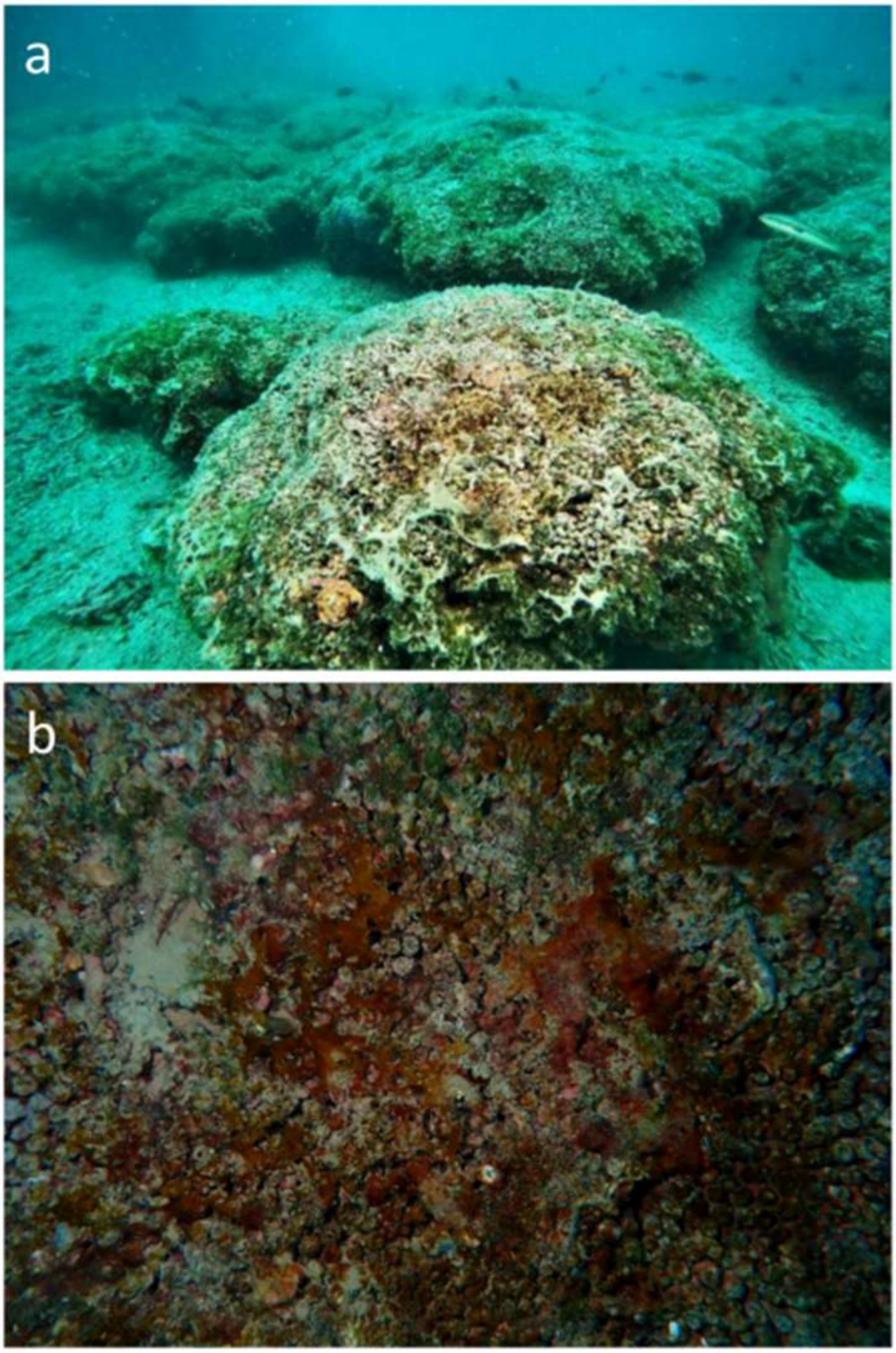

Figure 112. Biotic stressors observed on *Cladocora caespitosa* reef: a) mucilaginous algal aggregates deposited over corallites on June 30 2021 and b) cyanobacterial mat recorded on November 15 2021. Photo credit: a) J. Kurtović Mrčelić and b) S. Kipson.

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

To follow eventual recovery of impacted populations, *Pinna nobilis* larval collectors were installed at 3 sites of Cetina estuary, namely Water polo playground, Shallow mark, and Mala luka bay in 2020., 2021. and 2022. (Fig. 113).

Figure 113. Sites for monitoring of noble pen shell (*Pinna nobilis*) recruitment: 1) Water polo playground (HTRS96 515605.220; 4811327.260, 4m depth), 2) Shallow mark (HTRS96 515345.760; 4810867.340, 6m depth) and 3) Mala luka bay (HTRS96 517358.460; 4808803.840, 12m depth). Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu.

Water polo playground before mass mortality event that started in Croatia in 2019. (Čižmek H. et al, 2020) was location with high population density, 57 individuals/m^2 (Kurtović Mrčelić J., 2019). Shallow mark was location with mid density population while Mala luka bay had few scattered individuals. Mala luka is however characterized by sea currents from the south that transport water masses towards the first two locations. In 2020. permanent mooring lines were constructed for installation and removal of larval collectors each year. An anchoring screw is placed in the sea bottom holding all mooring line floating thanks to the floating buy, with CASCADE visibility elements, placed at the sea surface. Each location has 3 replicas spaced apart minimum 10m. Each year *Pinna nobilis* larval collectors were constructed according to Kersting D. et al. (2019) Short guidance for the construction, installation, and removal of *Pinna nobilis* larval collectors, IUCN and placed at different depths with a mutual distance of 2 m (Fig. 114, 115). The dimensions of each collector are 50x80 cm. In such a way, while floating up and down, collectors collect marine organisms carried out by sea currents from the whole water column.

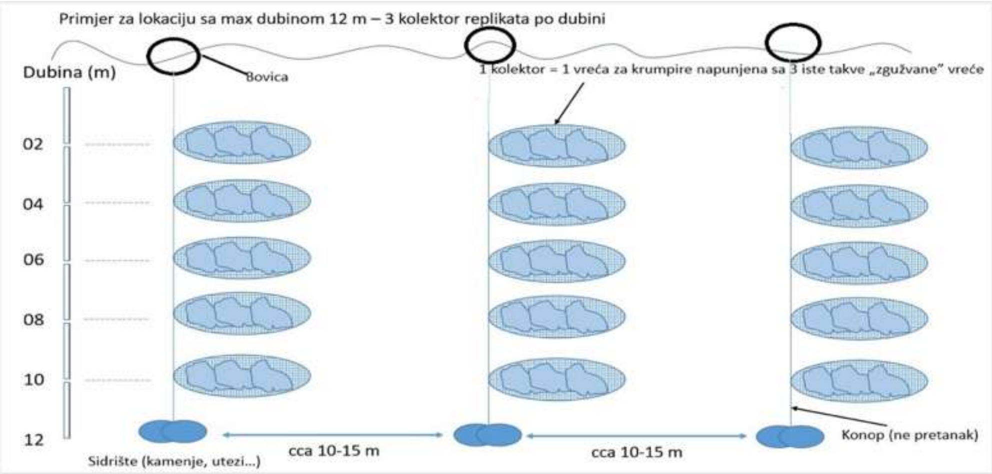

Figure 114. Schema of *Pinna nobilis* larval collectors with 3 replicas placed at one site.

Larval collectors are placed in the water column by SCUBA diving at latest in June and removed at earliest in October (Tab. 38) to cover the main reproduction period of *P. nobilis* (May – August) and the main settlement period (July – September).

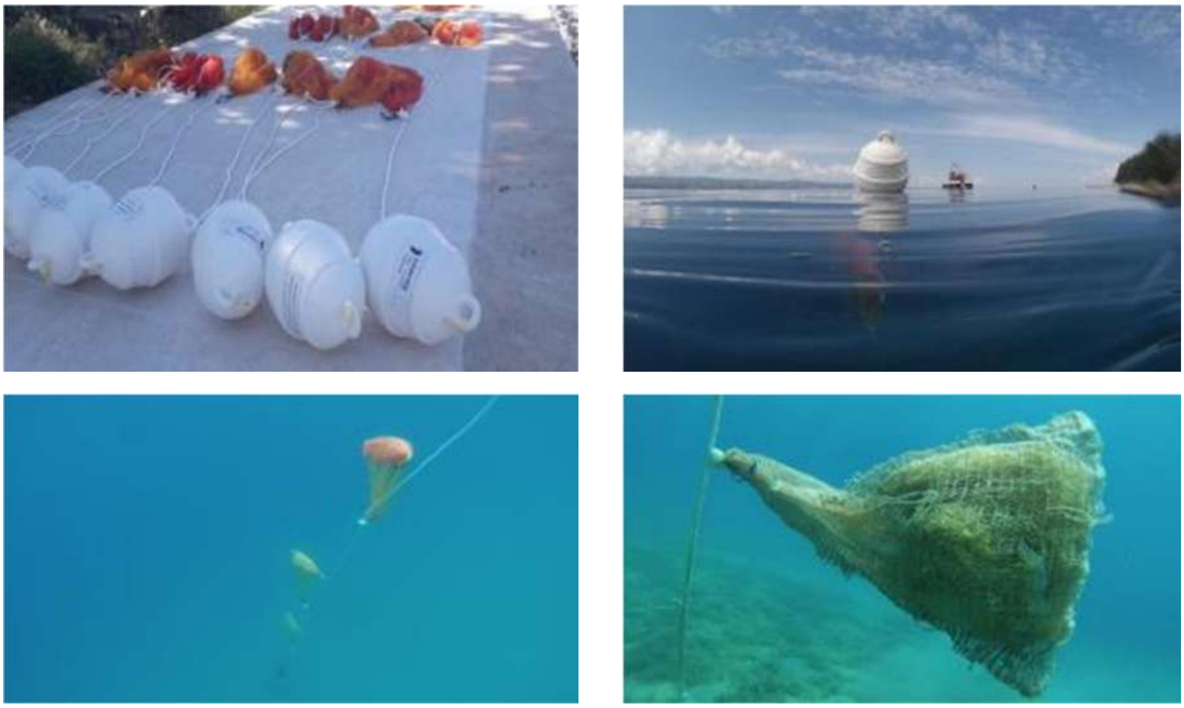

Figure 115. Installed *P. nobilis* larval collectors.

Each removed collector was processed by 1-2 persons in separate 70L container filled with fresh seawater, where all fouling organisms were manually separated. Organisms from Pinnidae family were identified first and if found removed in a separate smaller container and measured. Then all content of the collectors was filtered through a 30µm sieve and stored in 96% alcohol until further laboratory analysis (Fig. 106).

Figure 116. Processing of fouling community including individuals from Pinnidae family

In 3 years, period we succeeded to record recruitment only in year 2020. One individual from Pinnidae family of anterior-posterior length of 12 mm was found on the 2nd replica mooring line, in collector placed at 6m depth, at Mala luka bay site. The found individual was transported to the Pula Aquarium in a dedicated portable aquarium for breeding in ex-situ conditions (Fig. 117). Furthermore, the individual was identified as *Pinna nobilis* specie. In ex-situ conditions individual reached 32 mm of anterior-posterior length in half-year period (growth of 20 mm). However, individual died on June 04th 2021. (Aquarium Pula report, June 2021.). Further tastings on the presence of pathogens show that the individual was positive for *Mycobacterium* spp. (LIMIA report, December 2021).

Figure 117. Breeding of P. nobilis in ex-situ conditions in Aquarium Pula

site	coordinates_HTRS96	depth_m	date installed	date recovered	No. p. rudis	N. p. nobilis	No. Pinctada
Water polo playground	515605.220; 4811327.260	4	12.06.2020	10.11.2020	0	0	0
Shallow mark	515345.760; 4810867.340	6	12.06.2020	28.11.2020	0	0	0
Mala luka bay	517358.460; 4808803.840	12	17.06.2020	12.11.2020	0	1	1
Water polo playground	515605.220; 4811327.260	4	25.05.2021	01.10.2021	0	0	0
Shallow mark	515345.760; 4810867.340	6	25.05.2021	10.11.2021	0	0	0
Mala luka bay	517358.460; 4808803.840	12	25.05.2021	11.11.2021	0	0	0
Water polo playground	515605.220; 4811327.260	4	28.04.2022	28.10.2022	0	0	0
Shallow mark	515345.760; 4810867.340	6	10.05.2022	28.10.2022	0	0	0
Mala luka bay	517358.460; 4808803.840	12	10.05.2022	3.11.2022	0	0	0

Table 38. Results from monitoring of *P. nobilis* recruitment

In February 2022, thanks to citizens science approach developed through national project *Conservation of the noble pen shell in the Adriatic Sea* (haop.hr/hr/projekti; https://www.facebook.com/CROpinnanobilis), a live in-situ individual of Noble Pen shell was recorded at Cetina Estuary near local Aquapark (coordinates: 43.439110, 16.689698) at 2m depth inside *Cymodocea nodosa* meadow. After the start of the MME in the Croatian part of the Adriatic Sea in 2019, this is for now the only recorded living individual of the Noble Pen shell south of Zadar and has a symbolic name *New Hope*.

Dimensions of the individual shell were taken to estimate maximum antero-posterior shell length (Ht). According to Garcia-March (2007) three measurements are basic to estimate Ht, i.e., maximum and minimum width (W and w) and unburied length (UL). Gape orientation (Or) is also important from an ecological point of view (Fig. 118). Measurements were taken by a ruler (Fig. 119b).

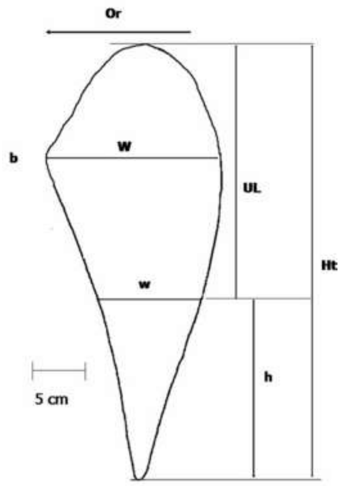

Figure 118. Measurements of interest to estimate maximum shell length (Ht) of Pinna nobilis. b, bend. h, length of the buried part. Or, orientation of the gape. UL, unburied length. W and w maximum and minimum width (Garcia-March et al., 2007).

Several equations have been proposed up to date, based on simple or multiple regression, for the estimation of Maximum shell length (Ht) of *Pinna nobilis*, although probably each population will have intrinsic parameters in the regression between Ht and unburied shell dimensions.

Equation by de Gaulejac and Vicente (1990) for the Population of Diana lagoon (Corsica)
$Ht = 2.186W + 1.6508$
$R = 0.98$

Equation By García -March and Ferrer (1995) for the population of Moraira (Spain)
$h = 1.79w + 0.5$
$R = 0.99$
$Ht = h + UL$

Equation By García -March (2006) for the population of Moraira (Spain)
$Ht = 1.29W^{1,24}$
$R = 0.98$

Based on these equations maximum shell length (Ht) of New Hope was estimated at the time of its discovery which was 22 to 25 cm (Tab. 39). Maximum shell length indicates that it is probably an adult individual that already survived developmental phase when there is a high risk of predation. Individual settled at very shallow depth, of 2m only, where the impact of waves from the south and southeast quadrants is considerable during wintertime. Fortunately, it settled in *Cymodocea nodosa* meadow that provides him a great anchoring position for its byssus threads. It also settled near Aquapark which an opportunity and a threat to the individual at the same time. Opportunity because boats do not anchor neither catch fish at that location and threat because there is a high concentration of Aquapark visitors that can damage the individual. However individual is far enough from the Aquapark so visitors cannot jump on it. Another benefit is floating beach fens. Aquapark and beach fens are installed during the summer season only, beginning of June, and retrieved beginning of October. Because of all these circumstances decision was taken to construct a cage which is not completely closed and has a shape of a tent (Fig. 119a). Because of shallow depths (not possible to put a massive concrete block) and *Cymodocea nodosa* meadow presence, cage was anchored with an eco-mooring system, a screw screwed 0,5m deep into the sand at 2 opposite sides of the cage. In such a way there is a lower waves impact on the cage, lower overgrowth of the cage, individual is accessible for taking measurements and at the same time is protected from eventual mechanical damage. In addition, warning panels were put at each side of the cage to inform visitor that the species is strictly protected and should not be disturbed. Its condition is being monitored each month from May to November, and once during the winter period (Figure 119c). During a period of 15 months, the individual has grown at least 10 cm and his maximum shell length (Ht) is estimated to be between 34 cm and 38 cm (Tab. 39).

Figure 119. Living specimen of the Noble Pen shell New Hope: installation of the cage over the individual (a), taking of the measurements with a ruler (b), individual taken in photo during wintertime.

Table 39. Estimation of New Hope maximum shell length (Ht).

Name	Date	w (cm)	W (cm)	UL (cm)	Ht1 (cm)	Ht2 (cm)	Ht3 (cm)
New Hope	24.02.2022.	5	10,6	12,6	24,1	22,1	24,8
New Hope	06.06.2023.	7,7	15,2	19,8	37,7	34,1	34,9
Difference	15 months	2,7	4,6	7,2	13,6	12,0	10,1

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

To acquire long-term, high-resolution information on temperature conditions along the depth gradient we have followed the protocol for seawater temperature monitoring of the T-MEDNet network (https://t-mednet.org/; Garrabou et al. 2018). According to this protocol sea water temperature conditions are characterized based on hourly data records by HOBO onset data loggers deployed and recovered over an annual or a semi-annual period. Data loggers are placed every 5 m from surface down to a chosen depth (usually 40 m but can be also placed shallower if such depth is not available in the selected coastal area) to acquire information about seasonal stratification dynamics and temperature conditions at depth. HOBO onset data logger's specifications are described in detail in CASCADE project deliverable D 4.1.1 *Equipment implemented/installed by relevant partners*. In the case of the Natura 2000 site Cetina Estuary dataloggers were placed down to the maximum depth of 30 m at the site Mala luka (Fig. 120, coordinates N43.4149470, E16.7176420). HOBO® Pendant temp/light data loggers were launched using the base station with the appropriate coupler connected to the computer and set up to record seawater temperature every hour, starting from July 16 2022 at 1 am. The underwater deployment at the site Mala Luka (Balića rat) took place on July 15, 2022.

Figure 120. Selected sites for the establishment of continuous high-resolution monitoring of ecological parameters. Seawater temperature, salinity and oxygen are monitored at Shallow mark site (1), while seawater temperature is monitored at Mala luka site (2). Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu.

The first dive was dedicated to the inspection of the terrain to detect the most suitable locations for data logger fixation on rocks at 5, 10 and 15 m depth. Due to the nature of the sea bottom, devoid of natural rock below 17 m depth, and with unsuitable depth of the sediment bottom to safely place a drill type of anchor, it was decided to place small concrete blocks (approx. 20 kg of weight) on selected spots at 20, 25 and 30 m depth (in the vicinity of the *Cladocora caespitosa* reef). Hence, 6 data loggers were placed from 5 down to 30 m depth (i.e. 2 m below the lower depth limit of the reef, Figs. 121, 122). Serial number of each data logger fixed at a specified depth is indicated in Table 40.

Depth (m)	Data logger's serial number
5	21237184
10	21237185
15	21237183
20	21237190
25	21237188
30	21237193

Table 40. Serial numbers of HOBO® Pendant temp/light data logger placed at 6 depths, from 5 to 30 m at the site Mala Luka (Balića rat) close to Omiš.

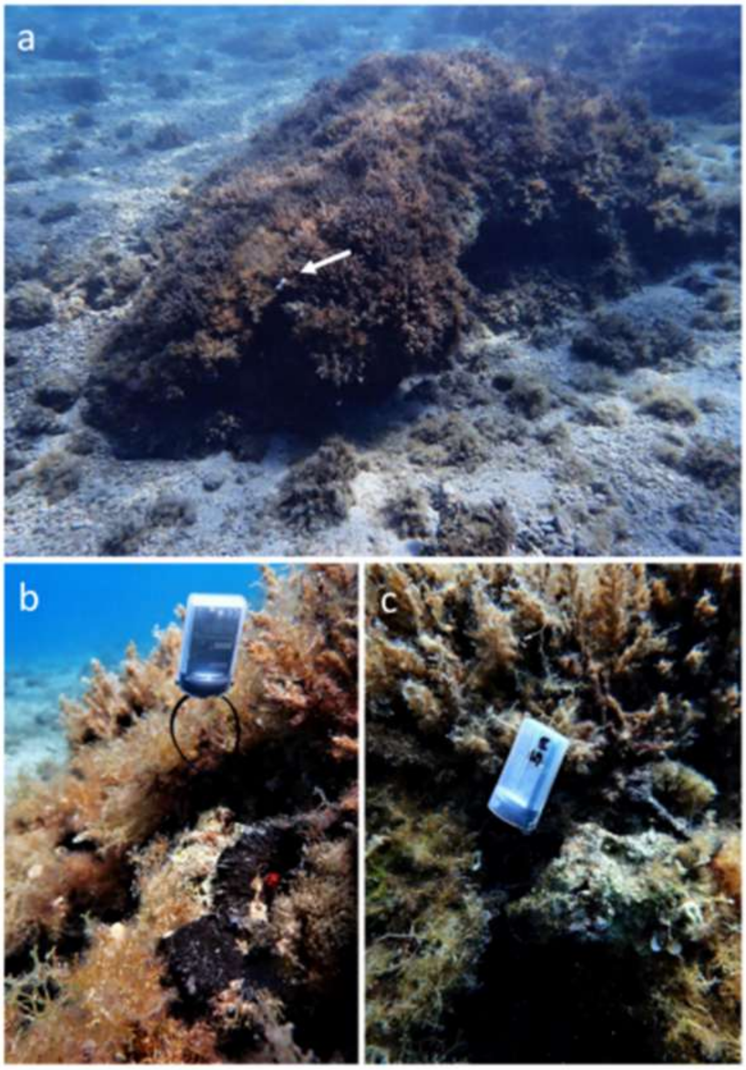

Figure 121. HOBO® Pendant temp/light data logger placed at 5 m depth: a) position of the data logger on the boulder (white arrow, image taken from approx. 6 m depth), b) black keratose sponge (lower right corner) thriving next to the data logger, c) detail of the data logger with a serial number 21237184. Photo credit: S. Kipson.

HOBO® Pendant temp/light data loggers will be retrieved in July 2023 after CASCADE project implementation period.

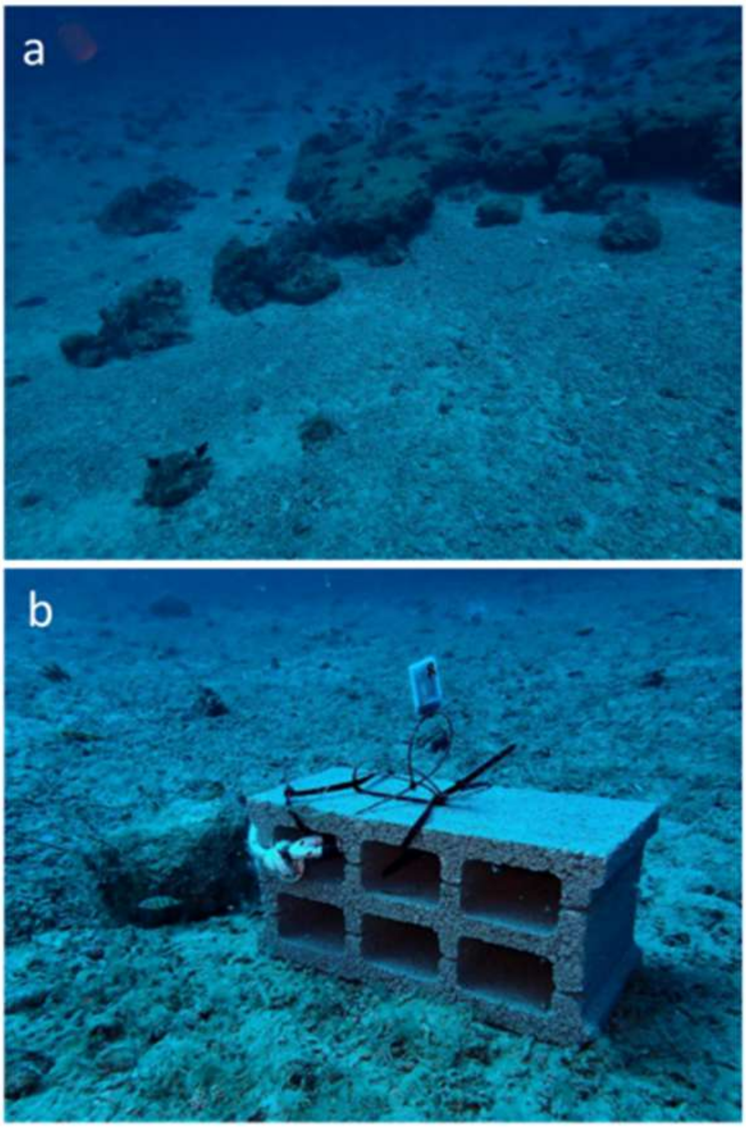

Figure 122. HOBO® Pendant temp/light data logger placed at 30 m depth: a) lower part of the Cladocora caespitosa reef – datalogger is placed several meters away from it, b) concrete block as a fixation point for the data logger with the serial number 21237193. Photo credit: S. Kipson.

2.9.5 Continuous, high-resolution seawater, salinity, and oxygen monitoring

To acquire information on the hydrodynamics between freshwater and coastal water an *in situ* multiparameter probe Aqua TROLL 500 was placed at the Shallow mark at 6 m depth (Figs. 122, 123, coordinates N43.4373300, E16.6894940). Aqua TROLL 500 specifications are described in detail in CASCADE project deliverable D 4.1.1 *Equipment implemented/installed by relevant partners*. As the Shallow mark is owned and maintained by state company Plovput ltd, the same was engaged for the preparation of technical documentation for the installation of the probe and for carrying out installation itself. Sea and Karst also signed an Agreement on the maintenance of the installation with Plovput ltd. while the last one obtains recoded data.

Figure 123. Shallow mark (in red) in front of town of Omiš and noble pen shell larval collectors (white buoys behind the shallow mark)

Prior to installation the probe software was updated and launched using VuSite application downloaded on the mobile phone (Fig. 124a-b). Through setting options time was set-up on the probe, then SIM card was inserted and VuLink log created by equipment provider Pileus ltd. on its FTP server called meteo (Fig. 124c). Meteo server is easily accessible through a web link https://meteo.pileus.si/, username and password. After testing, probe was ready for transmitting all data from the probe, via telemetric set of equipment, to meteo server.

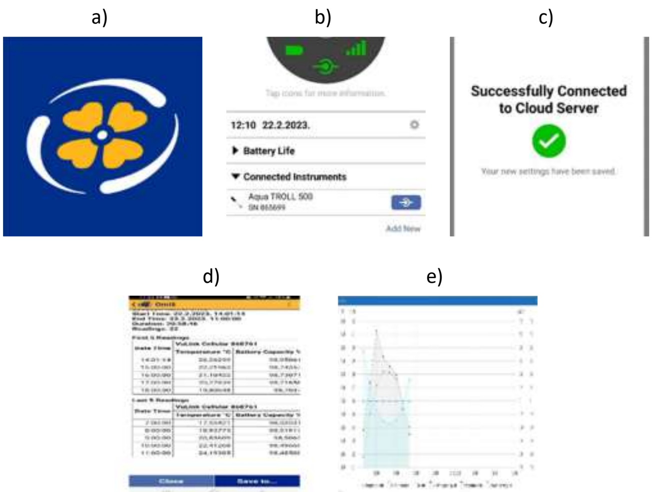

Figure 124. Setting-up of Aqua TROLL 500 multiparametric probe: a) VuSite mobile application, b) connection of the probe to the VuSite mobile application, (c) Vulink log created on meteo server, d) testing of data recording via VuSite mobile application, e) testing of data recording via meteo server

Then setting-up of all parameters was done through creation of a Log using VuSite mobile application. Probe was set-up to collect data each hour, and then upload interval was set-up to 20 hours. GPS location was not activated as the probe is mounted on a fixed shallow mark. A testing of probe functioning was done in the office conditions during 20:58:46 hours period of data collection (Fig 124d). Test was performed to confirm proper probe functioning before submerging it. Tomorrow data were visualised through VuSite mobile application which is working as long as the probe is next to the mobile phone (Fig 125d). Data were also visible on the meteo server (Fig 124e). Probe was ready for submersion. Before submersion new Log was created with data collection interval of 1 hour and data upload interval of 24 hours. Aqua TROLL 500 was installed on March 6th 2023 with the assistance of Plovput ltd which prepared mounting elements for the telemetric system placed at the upper part of the shallow mark (Fig.125c), for cable connecting telemetric Vu link system to the probe and for the probe which was placed at 6m depth on the submerged part of the shallow mark (Fig. 125d).

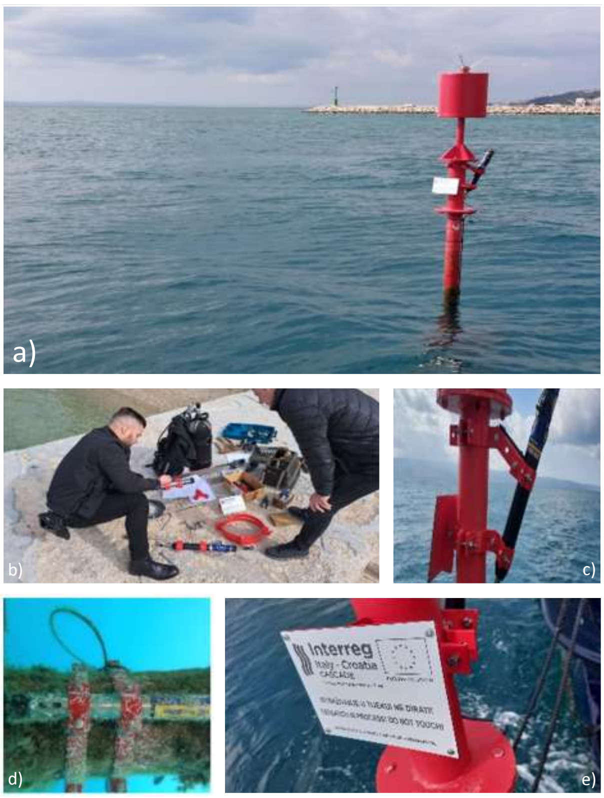

Figure 125. Aqua TROLL 500 installation on the shallow mark of Plovput ltd: probe mounted on the shallow mark (a), preparation of all the equipment and mounting elements (b), mounting elements for the telemetric system placed at the upper part of the shallow mark (c), probe at 6m depth on the submerged part of the shallow mark (d), visibility elements of the Interreg It-Cro CASCADE project.

Starting from March 7th 2023 it acquires data at hourly bases on temperature, salinity and dissolved oxygen. Data are continuously transmitted to Public Institution Sea and Karst through a set of elements for telemetric data transmission and download from an *in situ* multiparameter probe to a meteo FTP server.

In the long-term, these data series will build robust baselines and track hydrological and environmental changes to better understand potential impacts on marine coastal biodiversity. I*n situ* multiparameter probe is place next to Cetina River freshwater inflow to western part of marine area, 10 m from the border of *Cymodocea nodosa* meadow and 20 m from the noble pen shell (Pinna nobilis) larval collectors (Fig 123).

2.10 Torre del Cerrano, Pineto, Abruzzo (IT)

In situ observing campaigns (e.g. chemical, biological, visual census) were set up and carried out. UNIMOL implemented biomonitoring in Coastal dunes of the Torre Cerrano Marine Protected Area (vegetation zonation sampling) along with beach litter pollution. This monitoring campaigns were essential for developing models of accumulation depicting the main factors affecting litter accumulation, the most vulnerable habitats to be pollutes as well as to predict the coastal tracts with higher litter pollution probabilities.

Beach litter threatens coastal dunes integrity across the world and the European countries are committed to improve the environmental status of the marine and coastal environment by 2020. To do this further effort to reduce the gap of knowledge about litter accumulation patterns in coastal environments are needed.

On pilot 10 (and surrounding coasts) UNIMOL have analysed the distribution pattern of waste, differentiated by material and origin, in the coastal dune vegetation mosaic along the marine protected area placed in the Adriatic seashore (Central Italy).

2.10.1 Study area (Pilot 10)

The Pilot area 10 is the Torre Cerrano Marine Protected area (MPA). Among the coastal biodiversity facets Torre Cerrano is known for (https://www.torredelcerrano.it/), we focused on coastal dunes hosting valuable relicts of well-preserved natural ecosystems.

The Pilot area 10 includes well preserved coastal dune ecosystems representative of the Central Adriatic coast along a 6 km long seashore. It hosts several species and habitats of European conservation concern (Habitats Directive 92/43/EEC). Major dune habitats are Habitat 1210: Annual vegetation of drift lines; 2110: Embryonic shifting dunes; 2120: Shifting dunes along the shorelines with Ammophila arenaria, white dunes; 2230 Malcolmietalia dune grasslands; 1410: Mediterranean salt meadows - Juncetalia maritimi; 2270: Wooded dunes with Pinus pinea and/or Pinus pinaster.

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

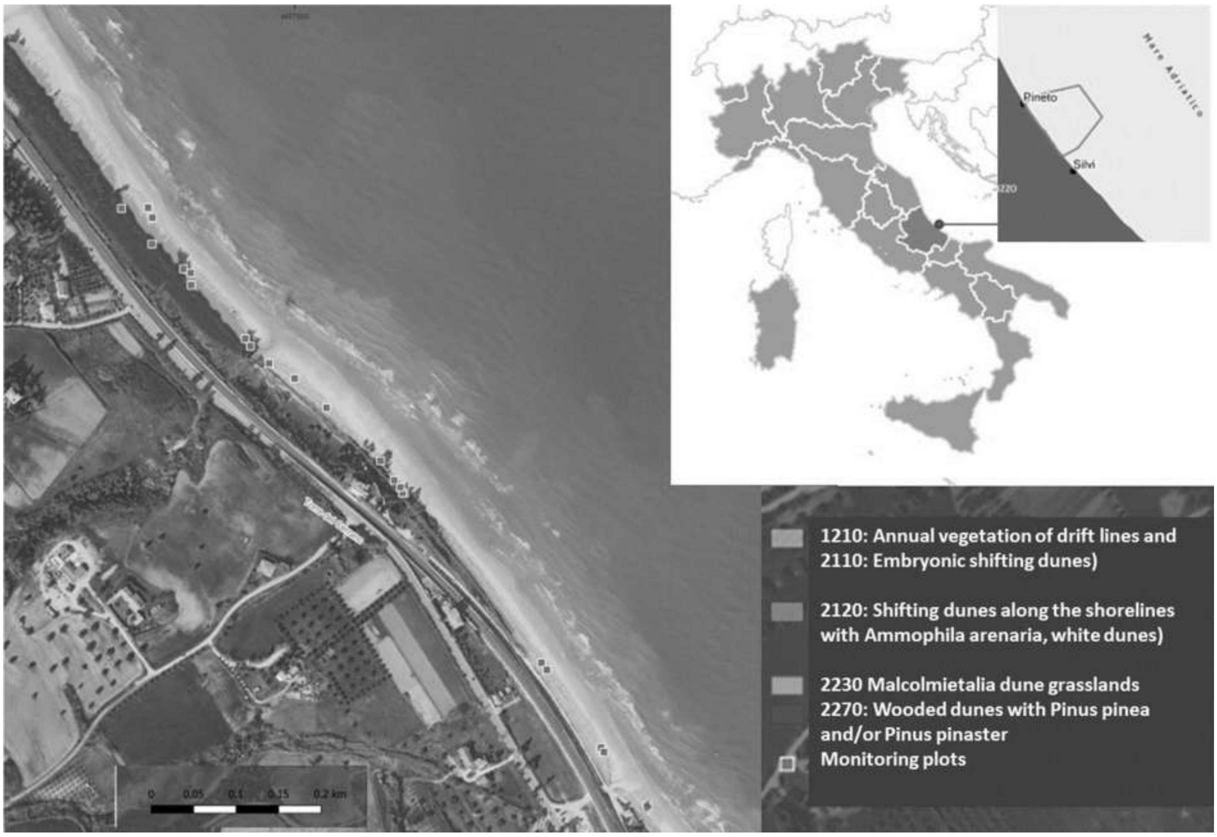

Figure 126. P10 Pilot area is a Marine Protected area (https://www.torredelcerrano.it/area-marina-protetta.html) along with coastal dune habitats (Habitats Directive 92/43/EEC). Some monitoring plots are also reported.

2.10.2 Beach Litter Data

Beach litter sampling was conducted through a stratified random protocol based on 2 × 2 m survey units. Specifically, we randomly placed 60 plots considering, as sampling strata, the EU habitat types, occurring in the study area. This sampling protocol and plot size are particularly effective for describing the highly heterogeneous eco-geomorphology and the fine-scale environmental gradients that characterize coastal dunes. Sampling was carried out in the vegetative period (April–May), making sure no mechanical cleaning had been carried out in the study area for several months. All the plots were visited only once by a single

	CATEGORY	ITEMS	% plot	% items
MATERIAL	PLASTIC	Bottles cups, pull tabs plastic, plastic bottles, plastic drums, fishing nets plastic, plastic plates, plastic forks, plastic bags, plastic sheets, soap containers, snack cards, straws, food trays, packaging of medicines, monofilament lines.	38.0	44.0
MATERIAL	POLYSTIRENE	Polystyrene boxes, polystyrene cups.	29.6	40.4
MATERIAL	PAPER	Paper and cardboard.	1.7	0.5
MATERIAL	GLASS	Glass bottles.	6.4	4.8
MATERIAL	ALUMINUM	Drink cans.	4.4	0.9
MATERIAL	MIXED MATERIALS	Cigarette butts, lighters, fluorescent light tubes, light globes, processed timber, rags, clothing, shoes, hats, tableware, toys, tires and inner tubes, rubber/chewing gum, wires, building materials, nappies, cotton buds, syringes, plasters, sanitary pads, foams, strapping bands, buoys, fishing nets not plastic, fishing related, ropes.	19.9	9.3

	CATEGORY	ITEMS	% plot	% items
ORIGIN	CONTAINERS	Bottle cups, pull tabs plastic, plastic bottles <= 2L, plastic bottles >2L, plastic drums>2L, glass bottles.	22.1	15.8
ORIGIN	FISHING AND BOATING	Buoys, fishing nets not plastic, fishing nets plastic, fishing related, monofilament lines, ropes, polystyrene boxes.	28.3	50.5
ORIGIN	FOOD AND BEVERAGE	Drink and food packages, cups, food trays, drink cans, ice c. sticks, chip forks, plastic plates, straws, snack cards, chips bags.	13.6	7.71
ORIGIN	PACKAGING	Foams, papers and cardboards, plastic bags, plastic sheets, strapping bends, soap containers.	14.2	8.98

OTHER	Fluorescent light tubes, light globes, processed timber, rags, clothing, shoes, hats, tableware, toys, tyres and inner tubes, rubber/chewing gums, wires, building materials. Cigarette packaging, cigarette butts, cigarettes lighters. Sanitary packaging, nappies, cotton buds, syringes, plasters, packaging of medicines, sanitary pads.	21.8	17.1

Table 41. Litter categories adopted for waste "material" and "origin" classification along with the list of items we have recorded for each class. The abundance of polluted plots in % per category of material and origin, and the % of items per class are also reported.

2.10.3 Preliminary results

Most of the plots included at least one waste element being plastic. Plastic was the most abundant material, and fishing and touristic the most polluting activities. Waste distribution varies across coastal dune vegetation types and involves the back dune zone too. Our results stress the need for (a) specific cleaning tasks able to preserve the ecological value of coastal dune habitats and (b) actions aimed at preventing litter production and accumulation.

2.11 Marche coastal area (IT)

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

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

2.11.1 Analysis of biocenosis emerged.

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

- Identification and updating of coastal habitats;

- The identification of specimens of Charadrius alexandrines;

- The eventual identification of the Charadrius alexandrinus nesting sites.

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

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

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

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

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

Four different habitats have been surveyed on the coast.

2.11.1.1 Habitat 1210: Annual vegetation of marine deposit lines

Annual herbaceous formations (therophytic-halonitrophilous vegetation) that colonize the sandy beaches and with thin pebbles, near the shoreline where the organic material carried by the waves accumulates and decomposes, creating a substrate rich in sea salts and decomposing organic matter. It is an exclusively coastal habitat and therefore linked to the dynamism of the shoreline but which, given the ecological characteristics of the present organisms, can maintain itself even in conditions of high anthropogenic pressure.

The indicator species of the habitat are represented by Cakile maritima, Salsola kali, Euphorbia peplis, Polygonum maritimum, Matthiola sinuata, Xanthium orientale subsp. italicum. The presence of young individuals of Elymus farctus and/or Sporobolus virginicus is frequent, due to the chain contact with the vegetation of the embryonic dunes.

The newly discovered phytocoenosis connected to this habitat is the Salsolo-Cakiletum one.

2.11.1.2 Habitat 2110: Mobile embryonic dunes

It refers to sandy micro-dunes, which are formed due to some specialized plants' soil retaining action. These plants, with their underground apparatus formed by long and intricate rhizomes, create a network which locks the sand in place thus forming small accumulations which form a dune cordon, with the capability of opposing the wind action from the sea.

The habitat indicator species are Elymus with: Sporobolus pungens (= S. arenarius; more recently referred to as S. virginicus), Euphorbia peplis, Otanthus maritimus, Medicago marina, Anthemis maritima, A. tomentosa, Eryngium maritimum, Echinophora spinosa, Calystegia soldanella, Cyperus capitatus, Polygonum maritimum, Silene corsica, Rouya polygama, Lotus creticus, Lotus cytisoides ssp. conradiae, Solidago litoralis, Centaurea subciliata, Spartina juncea.

The found phytocoenosis connected to this habitat is the Echinophoro-Agropyretum.

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

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

2.11.1.4 Habitat 2230: Dunes with meadows of the Malcolmietalia

Predominantly annual vegetation, with prevalent late-winter-spring phenology of sandy substrates, weakly to strongly nitrophilous, located in clearings of perennial vegetation belonging to the Ammophiletea and Helichryso-Crucianelletea classes; it is affected by the evolution of the dune system, in relation to the winds action and to the animals and people passage. The habitat is distributed on sandy coasts with both Mediterranean and temperate macrobioclimates. The phytocoenosis connected to this habitat is the Sileno-Vulpietum one.

In some cases, these habitats are found as mosaic associations since the structure does not allow a clear differentiation of the habitats (1210-2110 and 2110-2120).

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

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

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

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

- 1210: Annual vegetation of marine deposit lines

- 2110: Mobile embryonic dunes

- 2230: Dunes with meadows of the Malcolmietalia

Figure 127. Transept 01bis_fr_220 - The signs of mechanical means on the sand are visible

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

Figure 128. Transept 07_fr_260 - Charadrius alexandrinus

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

Figure 129. Transept 14_he_401 - Type of beach with gravel filling

No habitats of interest have been surveyed in these areas, as the conformation of the mountain slope overlooking the beach means that no dune and back-dune vegetation could be created.

There are no sea cliffs, and the action of the waves significantly erodes the mountain clayey side, causing frequent landslides.

Figure 130. Transept 15_he_404 - Landslides and landslides of the upstream slope

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

Figure 131. Transept 19_he_448 - Vegetation present

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

The area investigated in the Sentina Nature Reserve (Porto d'Ascoli coast) has common characteristics for all transepts, except 20_fr_772 which falls further north and is much poorer in vegetation. There are no sea cliffs and the beach is characterized by reduced width, between 15 and 25 meters, with very fine sand. Throughout the area you can see deposits of organic material and woody material, even of large dimensions, transported and deposited by the sea.

The area corresponding to the transept 21_fr_774 is heavily eroded in the northern part, while the other transects show no evident signs of erosion.

The present phytocenoses are those of Salsolo-Cakiletum for the annual deposit vegetation (habitat 1210) and Echinophoro-Agropyretum for the mobile embryonic dunes (habitat 2110).

The species that characterize the strip of vegetation in front of the dune, when present and not eroded by the sea, are those that are able to settle among the timber deposits of the beach, mainly Salsola kali and Cakile maritima; there are also some specimens of Raphanus raphanistrum on the transept 20_fr_772.

Figure 132. Transept 23_fr_777 - Vegetation in front of the dune

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

The rear part is characterized by the humid environment's vegetation, with the Arundo donax and Phragmites australis.

Figure 133. Transept 21_fr_774 - Vegetation behind the dune belt

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

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

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

Figure 134. Transept 20_fr_772 - Presence of strong erosion

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

2.11.2 Analysis of biocenosis submerged

The Analysis of submerged biocenosis will be carried out in the month of June, as in the period of May it was not possible to carry out the boat trips due to bad weather.

Instrumental detection surveys will be carried out using a state-of-the-art ROV generation equipped with Full HD camera, lighting system and positioning system Built-in USBL.

The investigation protocol normally used for "Marine Strategy" projects will be applied.

On board of the vessel that will be used for this activity, in addition to the ROV Video Ray Pro4 and yours integrated positioning system USBL Micronav the following instrumentation will be present:

- Multi Beam Echosounder R2 Sonic for morphobathymetric data acquisition

- IMU model Ixea Octans III for data acquisition of: Heading, Pitch, Roll, Heave and relative compensation of the dynamic effects induced with a frequency of 80Hz refresh;

- SVP On line Mini SVS Valeport for determining the speed of sound in real time;

- DGPS Trimble NetR9S for determining:

  - absolute position (NMEA CGA string)
  - synchronization parameters (NMEA ZDA string and PPS Output pulse)
  - real-time range of the tide.

All sensors are pre-installed on the vessel to reduce installation and calibration time. In any case, we will proceed, at the beginning of the surveys of each area, to calibrate the system through the procedure "Patch Test", as required in the Technical Regulations for the Standardization of Hydrographic Surveys drawn up by the Hydrographic Institute of the Navy, 2021 edition.

For navigation, control, and data recording operations it is proposed to use the Software Teledyne PDS 2000 release 4.3.8.0 2020.

Chapter 3 Conclusions

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

ARPA FVG has implemented two different activities in the pilot area of the CASCADE Project: while in the Grado and Marano lagoon the observing campaign was carried out through the probes continuously; in the Gulf of Trieste the fieldwork was carried out *una tantum* during spring and summer 2021. ARPA FVG took advantage of the continuously recording of the probes acquiring large amount of information on the lagoon environment. The system has demonstrated to provide quality data even after long periods in the field, increasing efficiency and reducing costs for the agency to collect data. Data acquired by the sensors were then supplied to the CRMA (Centro Regionale di Modellistica Ambientale) to support the validation of the SHYFEM model.

Data collected from the fieldwork in the Gulf of Trieste have been summarised in the AMBI index that seemed to be a good descriptor in term of water quality on the status of the meso/infralittoral macrozoobenthic community. Based on the AMBI index, the communities seemed to be in an undisturbed/slightly disturbed condition: 75% of the sampling sites were in pristine/slightly altered conditions, whereas 25% were moderately altered. The monitoring and the methodology applied on rocky shore invertebrates of the Gulf of Trieste proved that it could be adopted also in other Adriatic areas.

3.2 Transitional and coastal areas in Emilia Romagna (IT)

By updating the Sacca di Goro bathymetric dataset, a better geomorphological comprehension of the system's evolution and man-made impacts can be achieved. Additionally, the new bathymetric dataset leads to a better representation of the bathymetric setting that can be used for modelling purposes as well as an improved knowledge of the processes taking place inside Sacca di Goro.

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

The availability of distributed and continuous observations along coastal areas and in the MPAs is fundamental to acquire data to study the quality of marine ecosystems and to validate numerical models.

In Pilot 3 area the fieldwork was carried out una tantum during winter and summer 2021 and winter 2022. Thanks to the Cascade project, it has been possible to verify that organochlorines are a minor problem than in the past, while arsenic and mercury pollution continues to persist in some, albeit limited areas. Also, for the first time it was possible to map the presence of micro plastics in water and sediment throughout pilot 3.

Analysis of metabolism enzymatic activities in amphipods sampled in P3 sites showed a decrease in aerobic metabolism and an increase in anaerobic one, as well as a modification of oxidative stress marker enzymes activities, particularly in animals sampled in Punta della Contessa, allegedly because of the presence of environmental stresses.

3.4 Neretva River mouth (HR)

The main objective was to assess the potential pollution and ecological status of the Neretva estuary by characterizing the general water and sediment quality. The analysis of the results of all studied parameters leads to the conclusion that the ecological status of the Neretva River estuary is very good. The data obtained should contribute to the preservation of the Adriatic ecosystem and especially the estuaries.

3.5 Coastal area in Veneto (IT)

Activities carried out at P5 allowed to test and compare the potential for integrating different monitoring methodologies, deriving useful indications for supporting the future management of this site. Distribution of hard substrata assemblages shown consistency with previous records in the Tegnùe di Chioggia area, while fish communities shown a substantial decrease in abundance with respect to previous records. Acoustic campaigns performed show an interesting potential to spatially extend the results obtained by scientific fishing campaigns, suggesting the need for further cross-validating the results obtained with these two monitoring methodologies. ROV campaigns integrated in-situ monitoring by scuba diving and confirmed previous observations about the accumulation of marine litter within the rocky outcrops.

3.6 Miljašić Jaruga river mouth, Nin Bay (HR)

The use of the BRUV method proved to be very successful for use in monitoring the ichtyo population of the mouth of the Miljašić ravine, especially in combination with the visual survey that was used earlier. Although the same number of species was determined as with the visual examination by diving, a very large diversity of ichthyopopulations was observed in relatively small research area.

3.7 Coastal area in Molise (IT)

The analysis of washed-up litter on the Molise Coast as part of a network of natural protected areas in the central Adriatic coast provided an accurate description of the type of material and the origin categories of macro-litter accumulated in dune habitats of European conservation concern using an accurate and replicable procedure.

We registered a widespread presence of litter on coastal dunes with over the 80% of the inspected random plots polluted which place our coastal dunes on a comparable situation with other areas of the world as the Caribbean Sea or other Mediterranean coasts. As on other areas, most of the plots with washed up waste contains plastic elements and polystyrene, results which are in line with the recent Italian and European reports, but also with observations reported for other areas in the Mediterranean and for different study cases across the globe.

The abundance of plastic in the Adriatic coasts as well as on most of the coastal areas of the world are not only due to the diffuse utilization of plastic but also to the specific characteristics of hard and soft plastic items, such as flotation, high and increased use by modern society, and persistence in the environment.

As observed in Mediterranean Sea that most of the marine litter washed up on the analysed coasts comes from land-based rather than sea-based sources ad similarly to which was observed on other coasts, also in the Adriatic seashore, recreational activities (e.g., bathing, sunbathing, water, and beach sports) appeared to be the major source of litter, affecting ~50% of the surveyed plots. Activities connected with recreation were found to pollute the studied beaches with plastic tableware, bottles and bags, glass and aluminium containers and litter items.

On the other hand, as occurring in the seafloor of the Mediterranean Sea and in the Tyrrhenian coast an important marine source of litter washed up along the sand dunes is navigation and fishing (e.g. shipping, fishery and fish farming) with plots containing buoys, fishing nets, ropes and polystyrene boxes. Similar values were observed on remote oceanic areas and are higher to which is reported in other Mediterranean coasts. High proportions of litter originating from fishery should be due protection status and moderate touristic facilities that balance in some tracts the contributions from other sources.

The analysis of beach litter here carried out along with data collected on the network of protected areas in the central Adriatic coast, contribute to increase the knowledge necessary to implement the European regulation concerning sea litter and to define adequate strategies aimed at maintaining or improving their GES (Good Environmental Status) at different administrative levels (local, national and in the entire Mediterranean Region).

The data collected, which offers a starting point for comparisons and monitoring the litter accumulation on beaches, confirm the dominance of plastic and polystyrene material and depict that both marine and terrestrial proveniences of litter are very important. Litter tends to deposit heterogeneously across the sea inland gradient impinging dune ecomorphology in a variety of ways which suggests the need of dedicated cleaning procedures.

3.8 Northern-eastern Adriatic in Croatia (HR)

Activities carried out at pilot P8 Northern Adriatic Sea allowed to test and compare the potential for integrating different monitoring methodologies, deriving useful indications for supporting the future management of this Natura 2000 site. Traditional monitoring with sampling campaigns (Chemical, biological oceanography) with traditional microscopy against metabarcoding monitoring for marine phytoplankton. Phytoplankton distribution and composition revealed by microscopy and comparison with metabarcoding data show more species founded with molecular approach hence increased biodiversity in marine phytoplankton, but not all the species recorded with microscopy were revealed by metabarcoring. Suggesting the need for further cross-validating the results obtained with these two monitoring methodologies.

3.9 Cetina River mouth (HR)

The monitoring of the state of the Natura 2000 Cetina Estuary ecosystem in relation to EU biodiversity directives was until now not carried out. Accordingly, in the framework of Interreg It-Cro CASCADE project, Sea and Karst developed and tested monitoring (observing) system which improved knowledge on the state of Natura 2000 site for the purpose of carrying out conservation activities even beyond project implementation period.

In summary, a constant increase in the area covered by *Cymodocea nodosa* of approximately 6-7% every two to three years over the entire area of the Cetina Estuary Natura 2000 site is observed. Overall, in a decadal period (2011-2021) *C. nodosa* extent has increased from 43.65 to 54.04 ha within the Cetina Estuary Natura 2000 site, ultimately comprising 40% of the Annex I 1110 Habitat type present within the site (in total 135 ha reported in Natura 2000 Standard Data Form). *C. nodosa* and its meadows, as a representative habitat of 1110 *Sandbanks which are slightly covered by the sea water all the time* Habitat type remained in favourable status within the Cetina Estuary during the past 10 years, despite of sporadic mechanical damage caused by sand extraction and human activities along the coastline.

The results of the analysis of the Noble Pen shell (*Pinna nobilis*) larval collectors during the three years period (2020-2022), after the occurrence of the Mass Mortality Event at Croatian part of the Adriatic Sea in 2019, showed that only in 2020 a recruitment of the species occurred. However, discovery of a resistant individual and its growth of at least 10 cm over 15 months period gives a new hope to the recovery of the population at Cetina Estuary.

The implemented observing system show that cushion coral *Cladocora caespitosa* bioconstruction that was discovered adjacent to the current southern border of the pilot Natura 2000 site Cetina stretches from 24.6 m to 28 m depth. It occupies an area of 36.1 m^2 with the maximum length of 15.8 m and the maximum width of 3.8 m. Besides the main bioconstruction in form of colonies "fused" into a bank, several non-fused colonies are present next to the reef as well as numerous smaller colonies present nearby, scattered over an area that stretches for at least 100 m. The presence of such endemic species and the sole zooxanthellate scleractinian reefbuilder in the Mediterranean merits revision of the Natura 2000 Cetina Estuary borders and priority habitat types list to include on it reefs (1170) as well.

Results of high-resolution monitoring of environmental conditions in the sea (temperature, salinity, oxygen, etc.) will be visible at longer term and cannot be discussed during CASCADE project implementation period.

3.10 Torre del Cerrano, Pineto, Abruzzo (IT)

Similarly to previous observations that reported plastic as the most widespread marine litter, also along the central Adriatic coast plastic and polystyrene are the most common litter item. Moreover, higher densities of litter were recorded in the Adriatic Sea than in other basins as seen in the Mediterranean Sea, and the artificial polymer materials are the most abundant litter fraction recorded.

The distribution of litter along the sea-land eco-morphological gradient is heterogeneous and differences of litter spatial distribution were found between the upper beach and embryodunes sector, the mobile and transition dunes and the fixed dunes. In fact, several habitats are particularly affected by litter accumulation with higher pressure on the Vegetation of the drift lines and

Embryonic shifting dunes followed by the mobile and transition dune sectors with Ammophila arenaria and Malcomietalia dune grasslands, and by the fixed dunes with Juniperus spp. and Cisto-Lavanduletalia dune sclerophyllous scrubs. Given that all of these formations are habitats of European conservation concern (European Commission 1992), the necessary cleaning actions must be carefully planned in order to avoid both sand extraction and dune morphology simplification. Indeed, an experimental work on cleaning up procedures of coastal dunes have observed that with debris, a high amount of sand tends to be also removed. In view of such observations the management measures aimed at beach cleaning should be aware of the risk, when removing litter, of unintentional sand and natural dune herbaceous vegetation removal that should affect coastal dune habitats subsistence over time.

On the other hand, the lighter litter elements tend to remain trapped in the woody vegetation growing in internal woody dune sectors. The great volatility and trend to occur on tiny fragments of the polystyrene determine its accumulation in the fixed dunes that host one of the last relicts of fixed coastal dunes with Juniperus spp. and Cisto-Lavanduletalia dune sclerophyllous scrubs (EC: 2250 and EC: 2260) along the whole Adriatic coast and a refuge and reproductive site of Testudo hermanni.

The ecological effects of the large presence of plastics and polystyrene are still largely unknown. Surely, the presence of large percentages of plastic, whose negative impact on the environment is known, could cause degradation and loss of functionality of the habitats. Also important is the loss of landscape value and attractiveness for tourist activities, of fundamental socio-economic importance, due to the presence of beach litter along the beaches and in dune habitats.

The standardized data collection and analysis of beach litter in the central Adriatic coast represent an important contribution to the knowledge of the distribution of waste along coastal dunes and such information could help to the identification of integrated management strategies aimed at mitigating the negative impact on biodiversity and on the recreational and socio-economic capital of sandy coasts due to presence of beach litter.

3.11 Marche coastal area (IT)

The analysis of the emerged biocenoses highlighted a deterioration in the conservation of the areas along the entire coastal strip. The threats and pressures are mainly related to anthropization, humans' presence, trampling and the presence of pets not on a leash, which can cause damage to vegetation and nesting fauna.

At the moment, conclusions for submerged biocenoses are not available.

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