Deliverable C1.5
Monitoring report on the field work of Sub Action C1.2

Reliable and innovative technology for the realization of a sustainable MARINe And coastal seabed management PLAN

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1. INTRODUCTION

On sandy coasts, sediment is transported along the shoreline by waves, tide and currents, and potentially accumulates in port areas. This accumulation of sediment results in reduced water depth in the approach channel and constitutes a risk for navigation and restricts the vessel size that ports can accommodate. Traditionally, in most of the ports periodic dredging is performed in order to keep the depth of channel for safe navigation. However, dredging activities have broad economic and environmental implications. Dredging costs vary according to sediment volume and dredging method, but they are usually very high and strongly impact local economy (Ezzeldin *et al*., 2019). Moreover, dredging activities have severe negative impacts on marine ecosystems, both in the dredged area as well as in the area where dredged sediment is dumped. Most studies show that dredging is usually accompanied by a significant fall in species diversity, population density and biomass of benthic organisms. The rate of recovery is highly variable depending (among other factors) on the type of assemblages that inhabits the sediments in the dredged area and surrounding, the latitude and the extent to which the assemblages are naturally adapted to high levels of sediment disturbance and suspended particulate load (Newell *et al*., 1998). Sediment deposition in navigation channel is considered a crucial issue according to economic and environmental point of view. Minimizing sediment volume in navigation channel to reduce maintenance costs has become urgent need. Several sediment bypassing solutions can be used to reduce sediment volume, but the proper methodology depends on volume and sources of sediment which differ from one place to another (Ezzeldin *et al*., 2019). Technologies involved in sediment handling could have various impacts on surrounding marine environment, calling for appropriate monitoring activities.

In MARINAPLAN PLUS LIFE project novel technology based on an open jet pump called “ejector” is used to minimize sediment deposition in front of the Cervia channel port. Detailed environmental impact assessment (EIA) of ejector bypassing system has never been done previously (Bianchini *et al.*, 2019). Thus, EIA in this project has to follow rigorous scientific criteria, using appropriate sampling designs, adequate replication in space and time, essential to certificate the validity of the proposed technology. In particular monitoring activities will include analyses of sediment characteristics (sediment grain size and content of organic matter), composition and structure of benthic assemblages and composition of fish assemblages.

The report describes the methodologies applied for organic and non-organic sampling, sample pre-processing and storage. The results of the samples analysis is presented in Deliverable C1.7.

2. STUDY AREA, SAMPLING PROCEDURE AND ANALYSES OF SAMPLES AND DATA

2.1 Study area and sampling design

Possible impacts of the ejectors demo plant were addressed simultaneously at a variety of spatial scales in the wider area where demo plant will be deployed, in order to take into account the variability of environmental conditions and benthic assemblages. Sampling areas were located in one putatively impacted location in front of the port of Cervia (location I; 44° 16.162′ N, 12° 21.667′ E) and in four control locations, placed 600 m (location N1; 44° 16.484’ N, 12° 21.512′ E) and 1200 m (location N2; 44° 16.718’ N, 12° 21.390′ E) north and 600 m (location S1; 44° 15.857’ N, 12° 21.822′ E) and 1200 m (location S2; 44° 15.573′ N, 12° 21.976′ E) south of the impact location (Figure 1). Two sampling areas (about 800 m^2 each), 20–30 m apart, were defined within every location. The two areas within the putatively impacted location are represented by the sediment removal area (where ejectors are positioned; previously dredged area) and sediment discharge area (where plant discharges sediment). In each area 6 sample replicates were taken, out of which four (a number that is sufficient for reliable statistical analyses and robust results) were aimed for analysis, while two were aimed for stock in case of sample loss.

Three sampling campaigns were planned: 6 months before the deployment of the demo plant, and 6 and 18 months after the deployment. This sampling timing is intended to incorporate the natural temporal variability of assemblages. The first sampling campaign was tentatively planned in May - June 2017, but it was done only one year later, because of the delays in other phases of the project. Therefore the first sampling campaign was done in May 2018, before the deployment of the ejectors (Table 1). Due to the problem of fishing boat access to the harbour, an unscheduled dredging campaign was imposed by the Coast Guard and was performed few weeks before the field campaign, on 16–19 April 2018, resulting in a potential impact on the area. Unfortunately, due to further obstacles, plant instalment was delayed too and it was realised in July 2019. Consequently, the second sampling campaign took place in the beginning of February 2020 and the third one at the end of June and the beginning of July 2020 (Table 1).

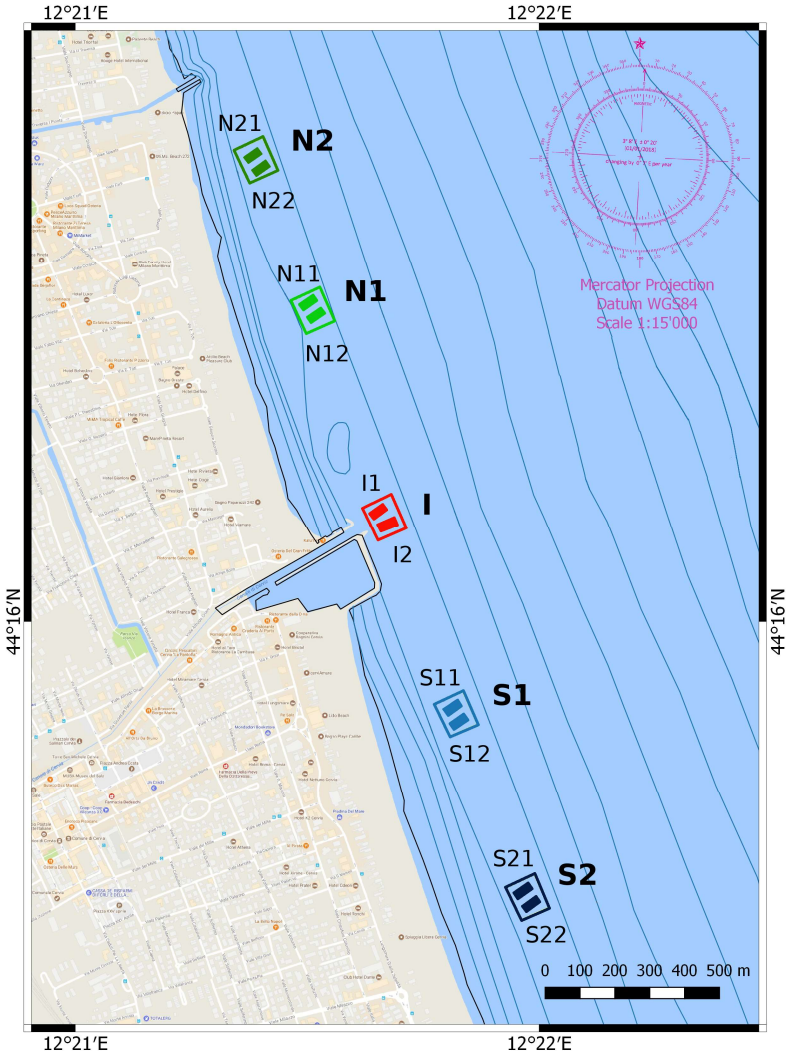


***Fig. 1**: Map of research area. N1 = control location 600 m north with related areas (N11 and N12), N2 = control location 1200 m north with related areas (N21 and N22), S1 = control location 600 m south with related areas (S11 and S12), S2 = control location 1200 m south with related areas (S21 and S22), I = impacted location with related areas (I1 and I2).*

Table 1. Summary of monitoring activities.

Date	**Activity**
11-05-2018	Site inspection
21-05-2018	1. Benthic sampling (suspended)
23-05-2018 – 25-05-2018	1. Benthic sampling
28-05-2018 – 30-05-2018	2. Fish video-sampling
07-06-2018	Site inspection
19-06-2018	Site inspection
01-09-2018 – 10-01-2019	Taxonomic identification of the benthic fauna from the first sampling campaign
11-01-2019 – 10-02-2019	Analyses of organic matter and sediment grain size from the first sampling campaign
11-02-2019 – 28-02-2019	Analyses of videos for the evaluation of fish abundances
01-03-2019 – 31-03-2019	Collection and analyses of literature, analyses of results and

	report writing
10-06-2019 – 21-06-2019	Additional taxonomic determination of molluscs
24-06-2019 – 24-07-2019	Work on invertebrate collection
12-07-2019	Meeting with project partners in Bologna
25-07-2019 – 26-07-2019	Underwater plant inspection in Cervia
29-07-2019 – 31-07-2019	Analyses of photos taken during inspection and upload on server
01-10-2019 – 31-01-2020	Collection and analyses of literature regarding soft bottom benthic communities and species life traits
03-02-2020 – 04-02-2020	2. Benthic sampling
07-02-2020 – 08-02-2020	2. Benthic sampling
10-02-2020 – 06-08-2020	Taxonomic identification of the benthic fauna from the second sampling campaign
10-04-2020 – 25-07-2020	Analyses of data and preparation of project report
01-06-2020 – 20-06-2020	Analyses of organic matter and sediment grain size from the second sampling campaign
30-06-2020 – 03-07-2020	3. Benthic sampling, fish video-sampling and site inspection with side scan sonar
31-08-2020 – 04-09-2020	Measurements of *Mytilus galloprovincialis* molluscs that colonized ejector's tubes and calculation of colonization period
07-09-2020 – 15-09-2020	Further analyses of data and preparation of project report
31-08-2020 – 06-11-2020	Taxonomic identification of the benthic fauna from the third sampling campaign
02-11-2020 – 11-11-2020	Analyses of organic matter and sediment grain size from the third sampling campaign
09-11-2020 – 20-11-2020	Work on invertebrate collection
16-11-2020 – 18-11-2020	Analyses of videos for the evaluation of fish abundances
16-11-2020 – 15-12-2020	Analyses of data and preparation of project report

2.2 Sampling operations

Sampling operations were carried out by an inflatable boat equipped with a WAAS/EGNOS enabled GPS ensuring a positioning estimated accuracy of 2-3 m (Figure 2a). The operations were carried out with calm sea, wind from absent to light breeze and clear or partly cloudy sky. In case of worsening of the weather-marine conditions the operations have been suspended. Before and after sampling, areas were inspected to check for depth and presence of obstacles, positioning and tide prediction accuracy, etc. The depth was ranging from 1.5 to 2.5 meters (below Mean Lower Low Water, MLLW) at all sampling areas.

At each sampling area six replicated samples of sediments were manually taken by scientific SCUBA divers (Figure 2b) for the analyses of: 1) sediment grain size, 2) percentage of organic material in the sediment, 3) benthic macrofauna. Equal sampling area was assured using an aluminium frame (23.5×13.5 cm). From each sediment sample, a 50 ml subsample was stored in labelled plastic container and frozen for subsequent grain size and organic matter analyses. The remaining sample was sieved through 0.5 mm mesh, fixed in 90% alcohol and stored in labelled plastic containers for subsequent macrofaunal sorting (Figure 2b, c). As already stated, out of the

six samples taken per area, four (a number that is sufficient for reliable statistical analyses and robust results) were analysed, while two replicates were aimed for stock in case of sample loss.

***Fig 2a**: Navigation operations*

***Fig 2b**: SCUBA diving operations and sample sieving*

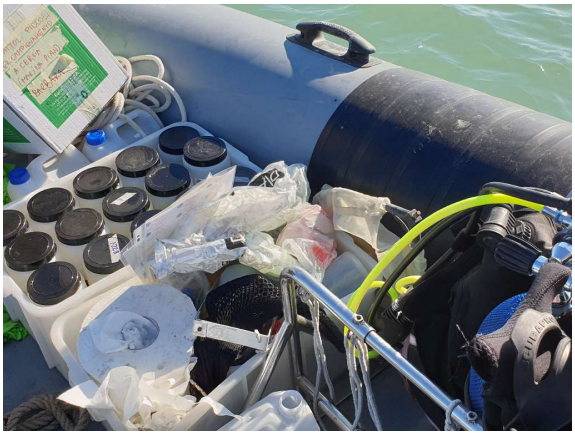


***Fig 2c**. Samples stored in plastic containers*

Fish assemblages were sampled in May 2018 and June/July 2020 by video cameras randomly placed within each study area. High definition (Full-HD) thirty minutes digital videos were recorded during each deployment (Figure 3). Videos were recorded using GoPro Hero 5 cameras. All digital videos were stored in a file server for subsequent image analysis. Unfortunately, due to extremely poor visibility, in February 2020 it was not possible to record videos for the fish visual census.

***Fig 3**: Example of video frame for fish assemblages' analysis*

2.3 Processing of samples

Analyses of sediment, benthic macrofauna and fish assemblages were done in the laboratories of the Interdepartmental Research Centre for Environmental Sciences (CIRSA) of the University of Bologna in Ravenna. Samples taken in the first sampling campaign were processed from 1st September 2018 to 28th February 2019 (Table 1). Processing of samples from the second sampling campaign started on 10th February 2020 but was slowed down due to the lockdown imposed by the Covid-19 emergency, and was finished at 6th August 2020. Samples from the third sampling campaign were analysed from 31st August 2020 to 6th November 2020.
In the laboratory, samples for analyses of the benthic macrofauna were sieved under the running water through 3 sieves, 0.5 mm, 2 mm and 4 mm mesh size, embedded in each other, in order to separate sample fractions for easier later sorting. Each fraction was then placed in plastic containers or Petri dishes for further separation of organisms (Figure 4). Sediment from 0.5 mm sieve was coloured with few drops of Bengale Rose solution and rinsed after 30 minutes – a procedure that serves to colour organisms in order to facilitate their separation from sediment particles.

***Fig 4**: Sieving of sample*

Organisms were separated and identified to the lowest possible taxonomic level using stereoscope (Leica Wild M3B and Nikon SMZ1500), light microscope (Nikon ECLIPSE 50i), and with the help

of keys for identification (Figure 5a, b). Specimens belonging to each taxon were conserved separately in labelled plastic containers in 90% alcohol (Figure 5c).

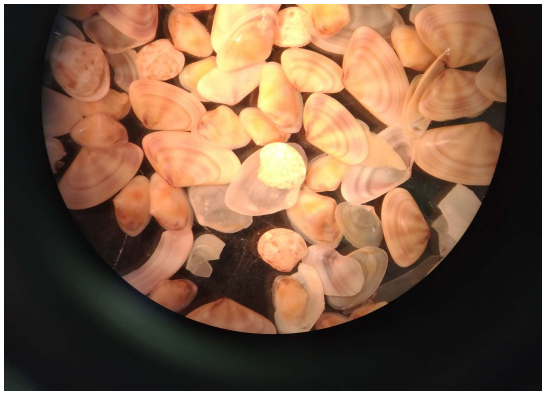
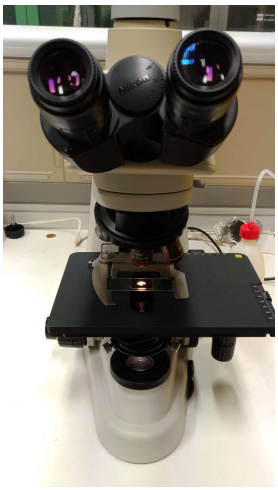


***Fig 5a**: Analyses of sample on stereomicroscope*



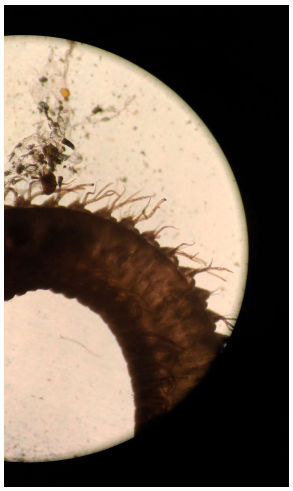


***Fig 5b**: Analyses of sample on light microscope*

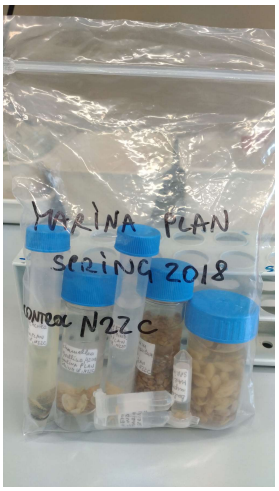


***Fig 5c**: Conservation of organisms*

The shell debris, after the separation of organisms, was placed in an aluminium container, left to dry overnight in stove on 70-80°C and weighted (Figure 6).

Fig. 6 *Shell debris weighting*

For the analyses of organic matter, about two teaspoons of unfrozen sediment were put in a previously weighted ceramic container, left to dry overnight in stove on 60-70°C and subsequently dry weight was measured. Afterwards, samples were left for 8 hours in a muffle furnace on 450 °C and the percentage of organic matter in a sample was calculated as a weight loss (Figure 7).

Fig 7*: Analyses of organic matter percentage*

For grain size analyses about two teaspoons of unfrozen sediment were carefully rinsed with wash bottle through 250 µm and 63 µm sieves embedded in each other and placed above a plastic container. Each fraction (> 250 µm, 63-250 µm and <63 µm) was carefully rinsed and filtered through previously weighted Whatman qualitative filter paper (Grade 1, pore size 11 µm), using

vacuum flask system (Figure 8a). Filter papers with sediment were left to dry overnight in stove on 70-80°C and then weighted (Figure 8b). The percentage of each fraction in total sediment was then calculated corresponding to the percentage of mud (silt and clay; <63 µm), fine sand (63-250 µm) and medium sand (> 250 µm).

***Fig 8a**: Analyses of grain size*

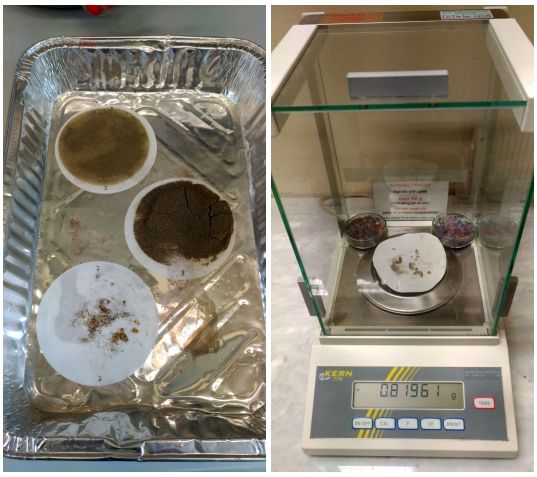


***Fig 8b**: Analyses of grain size – filter paper weighting*

Videos for the analyses of fish assemblages were carefully observed and data about species composition and abundance were registered in an Excel file. Each video, lasting approximately 30 minutes, was divided in four 7-8 minutes sections, considered as four replicates. Mean number of fishes per minute was calculated for each replicate.

2.4 Data analyses

Data on sediment variables (mass of shell debris, percentage of grain size fractions and percentage of organic matter) and abundances of taxa found in each sample were compiled in an Excel table. As for macrobenthic communities, for each replicate sample univariate indices of diversity, namely species richness (*S*), total abundance (*N*), Hill's species diversity index (*N1*; N1 = Exp H', where H' is the Shannon's index based on natural logarithm) and Hill's evenness index (*N10*; N10 = N1/S), were calculated. Hill's diversity index gives the number of species that would have been found in

the sample if all the species have been equally abundant (Hill, 1973). Evenness indicates the distribution of the individuals among species, and ranges in value from 0 to 1 (equally distributed). Multivariate analyses were applied to environmental and biotic data, to estimate and test similarity of both environmental data and structure of faunal assemblages between samples, within and among control and impacted locations. Non-metric multidimensional scaling ordination (nMDS) (Clarke, 1993), based on Bray-Curtis similarity matrix of square root transformed data, was produced to enable visualization of differences in structure of faunal assemblages among samples. A greater distance between points in an nMDS plot indicates a greater dissimilarity between samples. The match between the similarity matrix and the nMDS plot is ensured by the stress coefficient value (stress < 0.05 provides an excellent representation, < 0.1 good, < 0.2 fair, and > 0.2 poor representation).

A distance-based permutational analysis of variance (PERMANOVA) (Anderson, 2001; McArdle & Anderson, 2001) was performed to test for: 1) differences in environmental variables, 2) differences in biodiversity indices and 3) differences in structure of faunal assemblages, between: control and impact; locations within control and impact; areas within locations. The experimental design included five factors: before/after (fixed, 2 levels), time (random, 3 levels, of which 1 nested in before and 2 in after), control/impact (fixed, 2 levels), location (random, 5 levels, of which 4 nested in control and 1 in impact) and area (random, 2 levels, nested within location) (Table 2). Because of the different impact present in two areas within impacted location (area 1 – ejectors, area 2 – ejectors discharge), analysis were done comparing separately impacted area 1 (I1) and impacted area 2 (I2) with all control areas. PERMANOVA was based on Euclidean distances of untransformed data for univariate analyses of single variables (mass of shell debris, percentage of organic matter and percentage of grain size fractions, and for diversity indices – number of taxa, number of species and Hill's indices N1 and N10) and on Bray-Curtis similarity matrix of square root transformed data to test multivariate differences in the structures of assemblages. Factor Area and its interactions have been pooled when alfa≥0.25, since in those cases they represented non-significant source of variation (Underwood, 1997). Posteriori pair-wise comparisons were performed in order to detect the source of significant variations. When number of permutations was low (less than 1000) Monte Carlo probability (P(MC)) was considered instead of permutational probability (P(perm)). To calculate *p* values for PERMANOVA, 9999 permutations were used. All analyses were performed using as permutation method, permutation under a reduced model.

A similarity percentage (SIMPER) routine (Clarke, 1993) (70% cut off), based on Bray-Curtis similarity matrix of square root transformed data, was done in order to detect taxa most responsible for faunal similarity within impact and control and dissimilarity between impact and control in different sampling periods.

The BIOENV procedure (Clarke & Ainsworth, 1993) was run to find the best match between the multivariate patterns of assemblages and patterns of environmental variables, which reflects the degree to which the abiotic data explain the biotic pattern. Analysis was based on Euclidian distance of normalised data of environmental variables. In BIOENV procedure the faunal similarity matrix is fixed, while subsets of environmental variables are used in the calculation of the environmental similarity matrix. The Spearman rank correlation coefficient is calculated between the two matrices and the BEST subset of environmental variables are identified and further subjected to a permutation to determine significance.

All analyses were done using the computer program PRIMER v6 (Clarke & Gorley, 2006), including the add-on package PERMANOVA+ (Anderson *et al.*, 2008).

Table 2. Cross table representing experimental design.

		Impact	**Control**							
	Location→	I	N2		N1		S1		S2	
	Areas→	I1 or I2	N21	N22	N11	N12	S11	S12	S21	S22
Before	May 2018	n=4								
After	Feb 2020									
	Jul 2020									

2.5 Analysis of biological traits

Assessment of functional diversity of benthic assemblages is important for bringing conclusions on state of marine environment. One of the approaches to measure functional diversity is the analysis of species biological traits. Biological traits are series of life history, morphological and behavioural features of species (e.g. are body-size, mobility, diet, reproduction etc.), which govern their ecological roles, important for regulating ecosystem processes (Bremner *et al.*, 2006). Variability in biological traits in benthic assemblages is governed by the environmental variables, thus changes in patterns of biological traits can indicate environmental stress (Bremner *et al.*, 2006; Oug *et al.*, 2012). Based on these premises it was decided to perform analyses of species biological traits. Analyses are still in progress. Series of biological traits will be chosen and associated to each species, based on those proposed by Costello *et al.* (2015). In the period from October 2019 to January 2020 literature was collected and analysed that will serve for further analyses of biological traits.

2.6 Creation of invertebrate collection

Representative specimens of all identified species were intended for the permanent collection of the Laboratory "Ecologia, Conservazione e Ripristino degli Ambienti Marini e Costieri (MARECOL)" of the University of Bologna Ravenna Campus. They will enrich already existing Laboratory collection, important to facilitate determination of specimens from the future projects and eventually resolving taxonomic position of marine invertebrates. The work on collection was performed during June and July 2019 and November 2020. Each species was photographed under stereoscope (Nikon SMZ1500) using appositely mounted DS-5M-U1 Digital Photomicrographic Camera System or Moticam by Motic Europe (both with 5Mpixels sensor) (Figure 9) and subsequently stored separately in appositely labelled plastic container in 90% alcohol. Photographs will make part of the virtual collection of the Laboratory for ecology hosted on the server of the University of Bologna.

Fig. 9 *Stereoscope with mounted Moticam*

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