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Allegato 2 allo Studio per la Valutazione di incidenza ecologica
Marine Ecological Survey, Gela

the collection of biota and its subsequent analysis to determine the bioaccumulation of contaminants in the collected samples

Method Statement

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DISCLAIMER

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1.0 Preface

The Italian competent authorities (LIPU – Lega Italiana Protezione Uccelli) have requested a number of environmental studies (split into three phases) in relation to the proposed gas pipeline connection between Delimara (Malta) and Gela (Sicily) – Prot. U 2547 01/08/2019. The first two phases have already been carried out as part of the Preliminary Marine Route Surveys and EIA studies. These two phases which fall outside of the scope of this method statement, are summarized in the list below:

- Characterization of marine waters (TRIX, CAM, pH, Temp., Cond., Eh, Al, Fe, Cd, Crtot., Cr VI, Hg, Ni, Pb, Cu, Zn, As, V), Secchi disks, polycyclic aromatic hydrocarbons, tributyltin.
- Characterization of marine sediments (Al, Fe, Cd, Crtot., CrVI, Hg, Ni, Pb, Cu, Zn, As, V, radionuclides (uranium), total IPA and benzopyrene. Organochlorine compounds (pesticides and their metabolites), PCBs (polychlorophenyls), tributyltin), methylmercury and other possible organic mercury compounds, radioactivity levels.

This Sampling Plan will be carried out at the Gela offshore site with the scope of describing the methods of execution of the ***third and final phase*** of Prot. U 2547 envisaged for the characterization of the site:

- Characterization of the tissues of bivalve molluscs, stationary fish species and marine phanerogams to detect the presence of contaminants

The collection of samples of the fish fauna (molluscs, crustaceans and fish) and of the flora component for bioaccumulation analyzes will be carried out specifically in order to respond and satisfy LIPU's request (Lega Italiana Protezione Uccelli). The activities will be carried out within the project area, within the 20 m bathymetric zone for fish fauna and within the 10 m bathymetric zone for the aquatic flora component, thus obtaining representative results of the entire survey site.

The samples will also be taken in an area adjacent to the SIN, characterized by the same particle size and bathymetric conditions, but subject to lower anthropogenic pressure. This area, which will act as control, is located in the offshore zone located west of Gela.

The collection of:

- Molluscs by sorbona, operated by Underwater Technical Operators (OTS) to be carried out within the 10 m bathymetric zone.
- Ichthyofauna through gillnets to be lowered within a bathymetry of 20 m.
- Marine phanerogam *Cymodocea nodosa* by means of OTS within a bathymetry of 10 m.

Overall the sampling strategy will entail:

- Four (4) sampling stations for collecting bivalve molluscs with sorbona operated by Underwater Technical Operators (OTS) to be carried out within the 10 m bathymetric

zone. In total the survey will include two (2) impact area stations and two (2) control area stations, at depths of -3 m and -10 m.

- Four (4) stations for the collection of fish fauna (fish and crustaceans) with gillnets (tremaglio). Two (2) stations will be located in the impact area, and two (2) stations will be positioned in the control area, at depths of -5 m and -15 m.

- Two (2) stations for the collection of marine phanerogams (e.g. *Cymodocea nodosa*). One (1) station will be located in the impact area, whilst another (1) station will be positioned in the control area, located within the 10 m bathymetric zone.

The sampling activities and subsequent analyses will be carried out on individuals identified and listed in the list of reference organisms indicated in subsequent sections of this report, in accordance with the ISPRA-CNR-ISS 2015 procedure.

The use of Underwater Technical Operators and the provisions relating to the safety of diving operations and related equipment (composition of the OTS team, safety equipment and availability of a hyperbaric chamber) will be governed by the directives of the competent Harbor Master's Office (Gela).

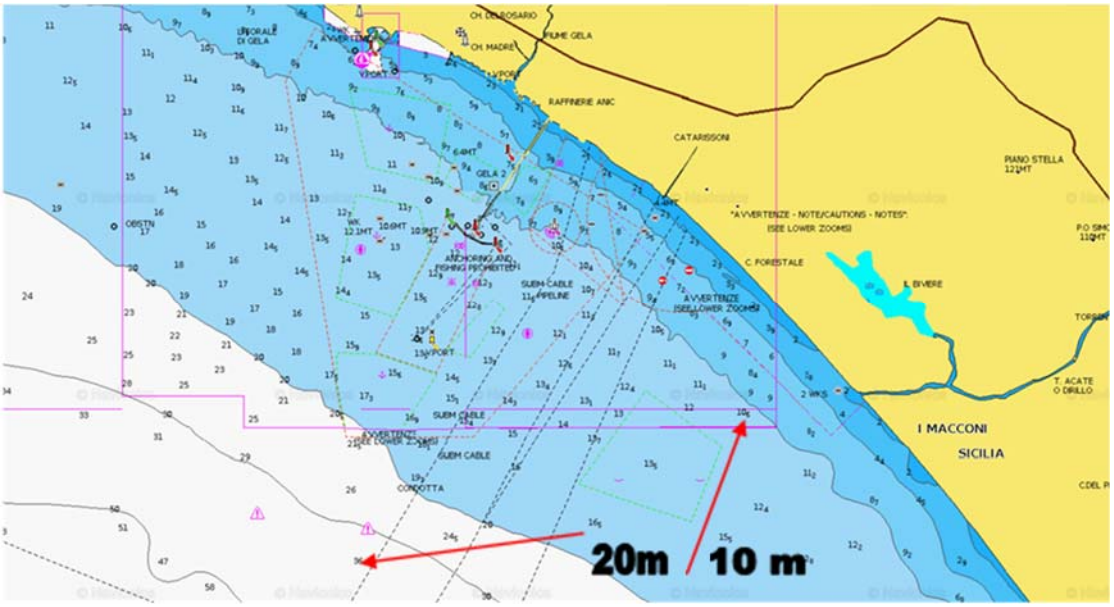

Figure 1: Nautical chart with reference bathymetry

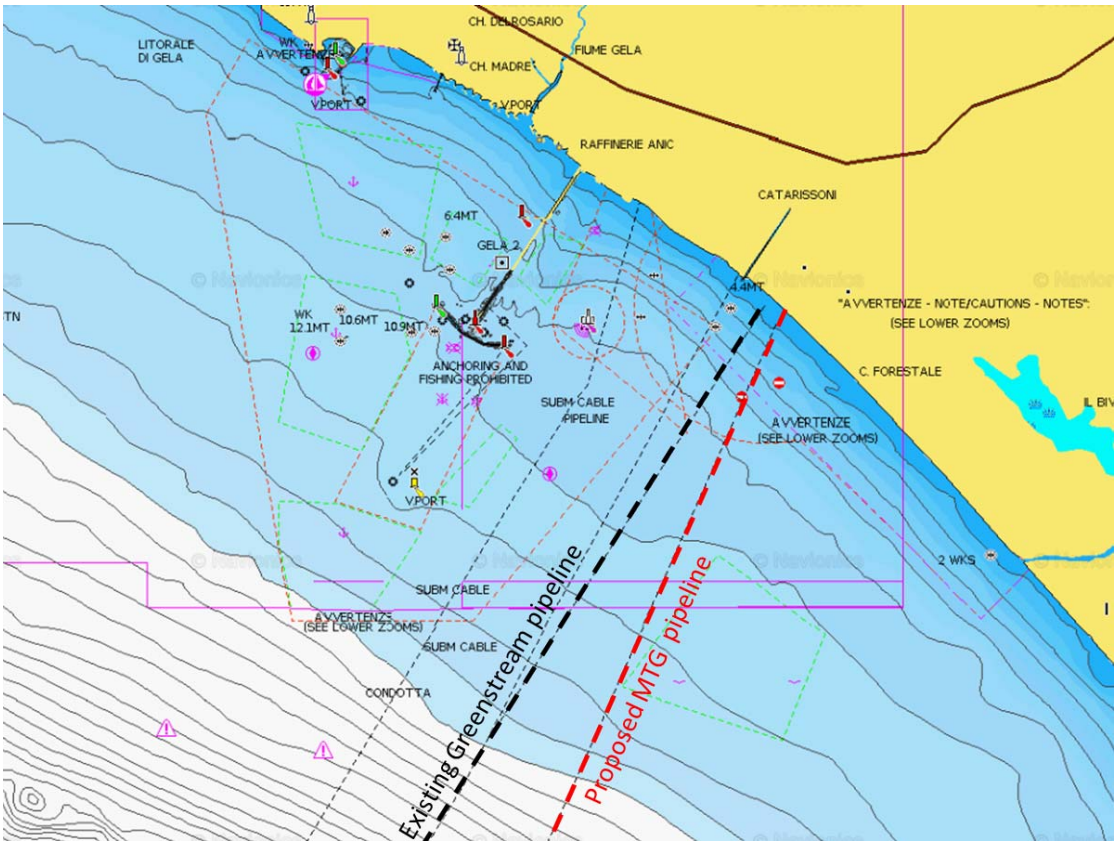

Figure 2: Nautical chart showing the existing Greenstream pipeline (on the left) and the proposed MTG pipeline (on the right)

2.0 Bivalve Molluscs

2.1 Bivalve molluscs: organisms and sampling method

The presence of communities of the Fine Calibrated Sands (SFBC) has been documented in the study area (Environmental Impact Study: Sealine Gela). The following bivalve molluscs are expected since these species are normally present in such biocoenoses:

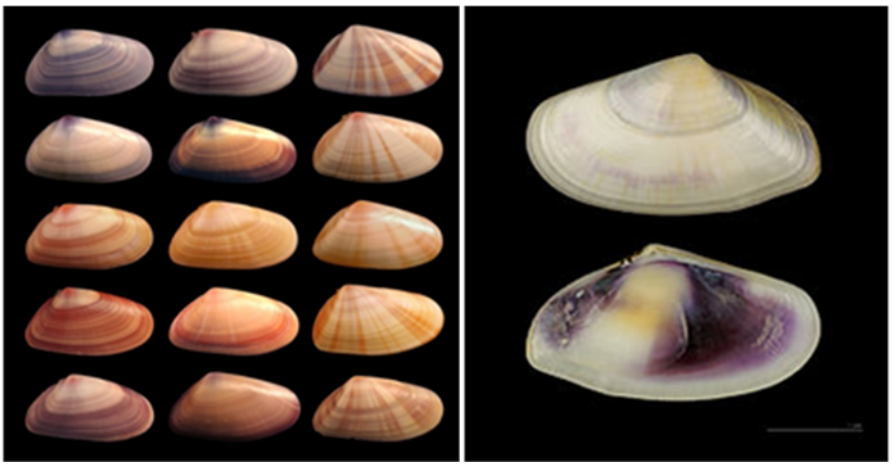

Figure 3: Photographs of Donax spp.

Figure 4: Photographs of Tellina spp.

Figure 5: Photographs and illustrations of Tapes spp.

Only one bivalve species from *Donax* spp., *Tellina* spp. & *Tapes* spp. will be analysed, depending on the most abundant or suitable species. The collection of bivalves will be carried out by means of an air sorbona (vacuum) maneuvered by a diver as indicated in Figure 6.

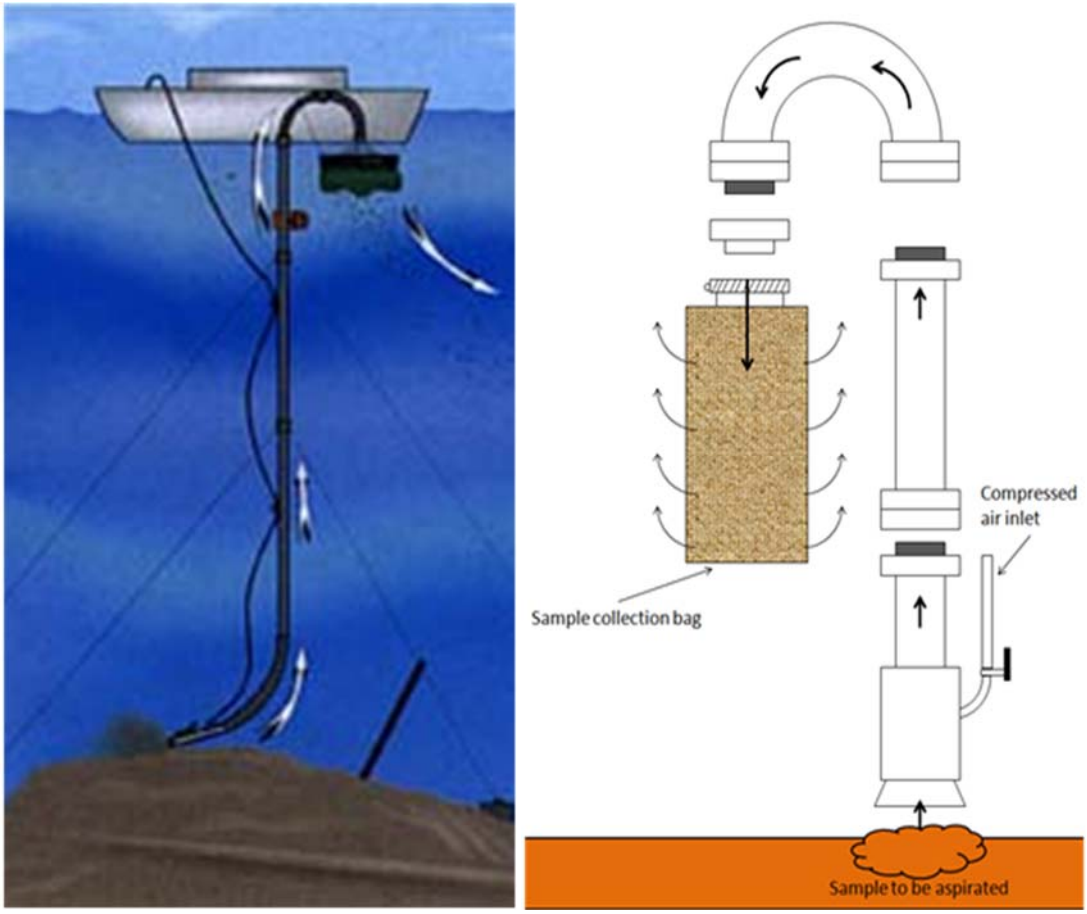

Figure 6: Example diagram of an air Sorbona

The Sorbona is a sampling equipment whose general operation can be summarized as a sample aspiration system thanks to the induced vacuum. The simplest system used to produce vacuum in underwater operations is generally the air one, through the use of a compressed air cylinder.

The Sorbona used for this study consists of a 10 cm diameter and 100 cm long PVC tube connected to a whip which is attached to a first stage regulator. The dispenser is fixed to a compressed air cylinder. Once the cylinder tap is opened, a handle allows the passage of air inside the PVC pipe; the depression that is generated causes a flow of air towards the surface that drags the sediments and the associated fauna. In the terminal part of the tube there is a nylon net with mesh sizes suitable for the types of organisms sampled. The terminal part can be composed of several overlapping nets with decreasing mesh sizes to capture all the aspirated organisms.

Figure 7: Diver making use of an air sorbona on sand

Figure 8. Diver making use of an air sorbona on rocky assemeblages

2.2 Bivalve molluscs: Samples number

For each station, a combined sample comprising of two sub-samples collected at the respective bathymetric depths of -3m and -10m will be obtained. The sampling scheme and the labeling methods of the samples are shown in Figure 9.

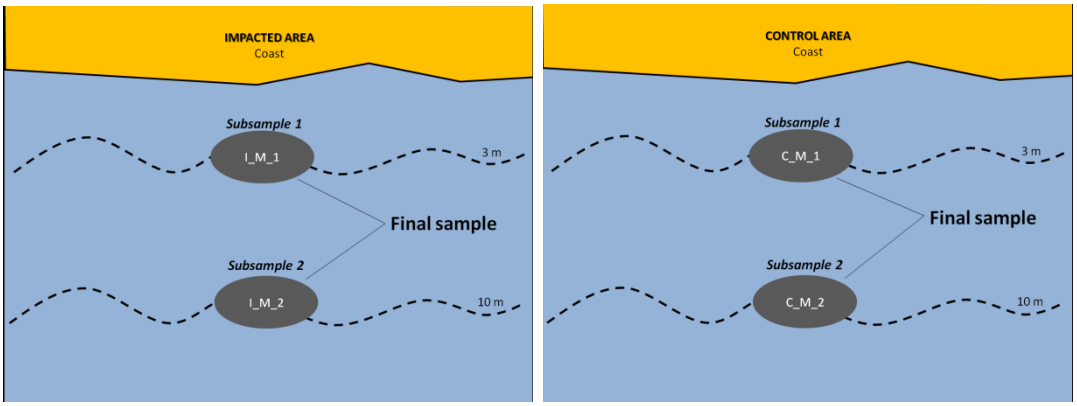

Figure 9: Mollusc sampling example scheme

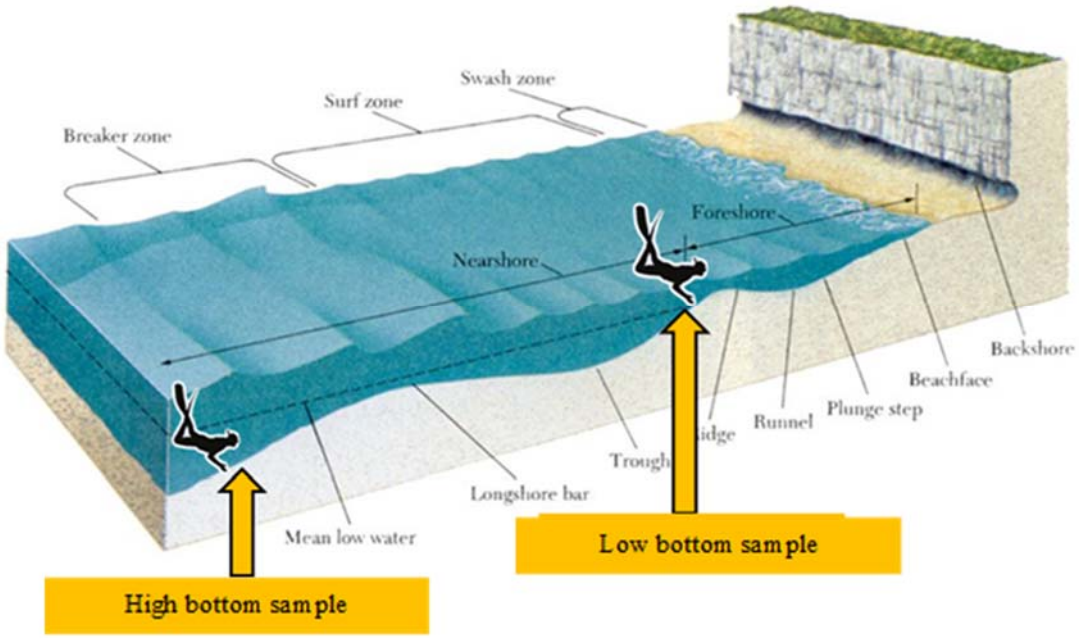

Figure 10: Diagram showing OTS operations for sorbona sampling

Table 1. Total number of samples to be analysed (bivalve molluscs)

Total number of samples
4 stations (two depths merged in one sample)
3 replicates for each station
1 species to process
Total: 12 samples

In order to determine the composition and abundance of the benthic macroinvertebrates of interest, the sediment samples collected will be sieved on the field with a 0.5 mm mesh.

The sample will be considered sufficient once a quantity of 2 kg of organisms of interest has been reached.

After collection, the samples destined for the study of bioaccumulation can be kept refrigerated at about 4 ° C, in a humid environment but not immersed in water, up to a maximum of 24 hours from the time of collection. The selected organisms will be homogeneous in size to represent approximately between 70 and 90% of the maximum population size. Analyses will be carried out on the soft tissues of at least 30 organisms (divided into at least 3 replicates), each replicate containing the tissues of 10 animals.

Utmost care will be taken to avoid contamination of the sample during the handling phase of the material to be analyzed; therefore, the animals will be handled with all the necessary precautions in order to reduce the risks of cross-contamination.

The following methodology will be adopted in the following stages:

- Recording the biometric parameters (weight of tissue, length and weight of shells) of the organisms intended for analysis;
- Cleaning the organisms from any deposits on the valves, removing the foreign material with a clean knife;
- Washing each specimen with distilled water or clean sea water;
- Removing the byssus from the closed valves;
- Using a second clean knife and inserting it between the valves where the fine byssus extrudes and open them gently by cutting the posterior adductor muscle (see Figure 11); making sure that the byssus has been completely removed.
- Washing of the soft tissue with distilled water; the sample will be picked up with clean tweezers and drained;
- Collection of the soft part; the composite weight of the soft parts considered will be noted and recorded as the weight of the sample pool;

- The soft parts collected must be closed in decontaminated aluminum sheets (in the case of the analysis of organic compounds) or in suitable containers - plastic bags - (in the case of trace metal analysis) and analyzed. If the samples are not analyzed immediately, it is possible to freeze them at -20°C, after having placed them in labeled containers.
- For the analysis of trace metals, dried samples that are not immediately subjected to the mineralization cycle must be stored in a dryer.

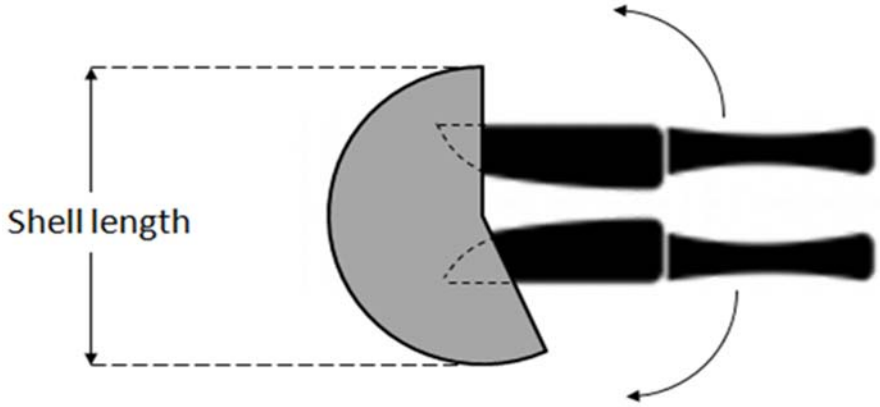

Figure 11: Illustration of how bivalve shells can be opened

For each pool-sample (replicate) a datasheet will be prepared with the specifications of the sampling and the recording of parameters, such as the number of organisms considered, the average length of the valves and the weight of the sample pool.

With the precise objective of obtaining a detailed description of the populations of molluscs present in the investigation area, it is of fundamental importance to know and study the main biometric measurements of the individuals collected.

The following figure shows which are the main measures to be determined and how these are to be measured

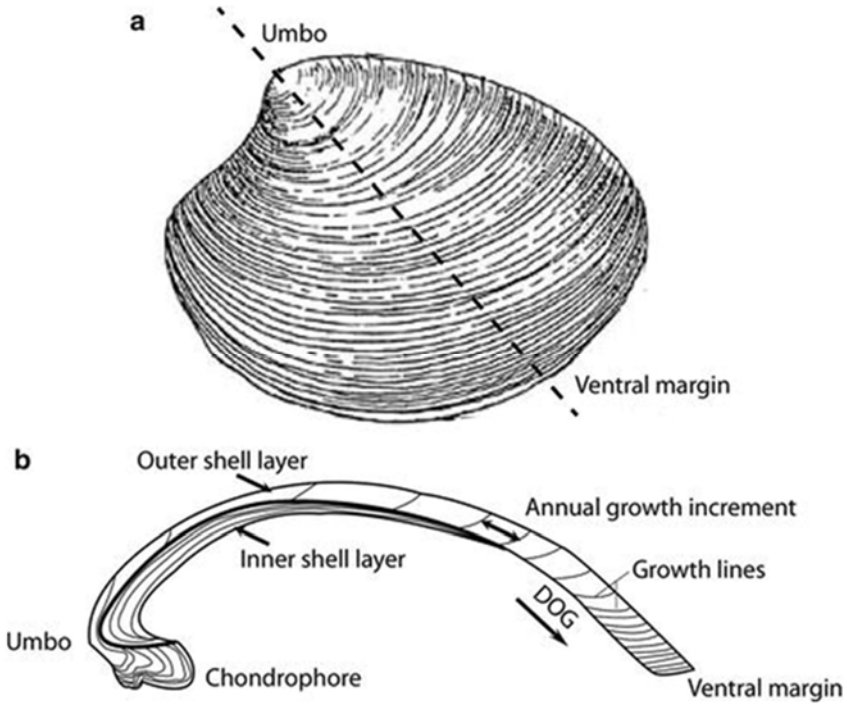

Figure 12: Calculation of the size (biometry) of molluscs

2.3 Bivalve molluscs: Sampling Stations

The individual sampling stations are identified by means of a data sheet as shown below:

- Two (2) impact area stations (two pick-up points at -3m and -10m)
- Two (2) control area stations (two pick-up points at -3m and -10m)

RECORD N° 001

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

RECORD N° 002

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

RECORD N° 003

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

RECORD N° 004

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

3.0 Fish Fauna

3.1 Fish Fauna: organisms and sampling method

Fish specimens will be caught using trembling gillnets. Immediately after the recovery of the fishing gear, the fish species having greater affinity for sediments will be selected from the catches, with the addition of *Paracentrotus lividus* and *Squilla mantis* potentially present in the sediments of the investigation area:

Figure 13: Photographs of Mullus barbatus

Figure 14: Photographs of Lithognathus mormyrus

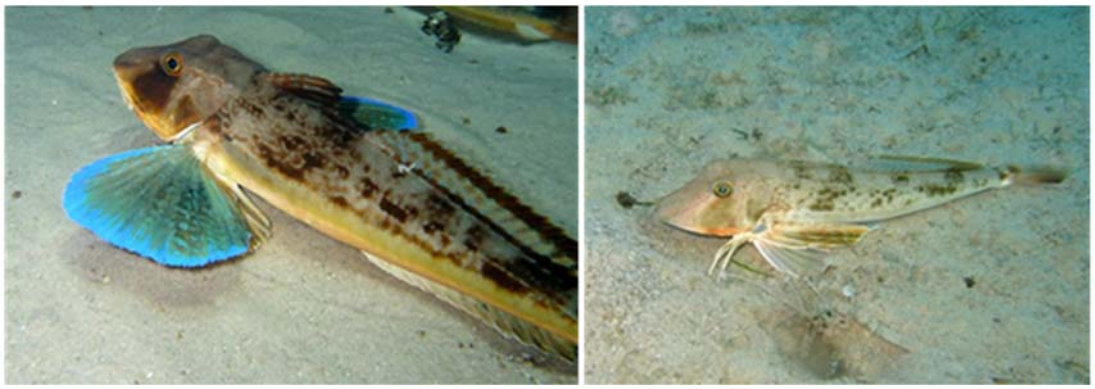

Figure 15: Photographs of Trigla lucerna

Figure 16: Photographs of Raja miraletus

Figure 17: Photographs of Dicentrarchus labrax

Figure 18: Photographs of Sparus aurata

Figure 19: Photographs of Paracentrotus lividus

Figure 20: Photographs of Squilla mantis

Gillnets are passive nets, intended to enclose or block water spaces in order to catch fish, molluscs and crustaceans that run into them. The trembling nets (Figure 21 to Figure 23) are the best-known capturing mechanism among gillnets and is made up of three overlapping and reinforced pieces of netting with different reinforcement ratio on the same two cork and lead-ropes.

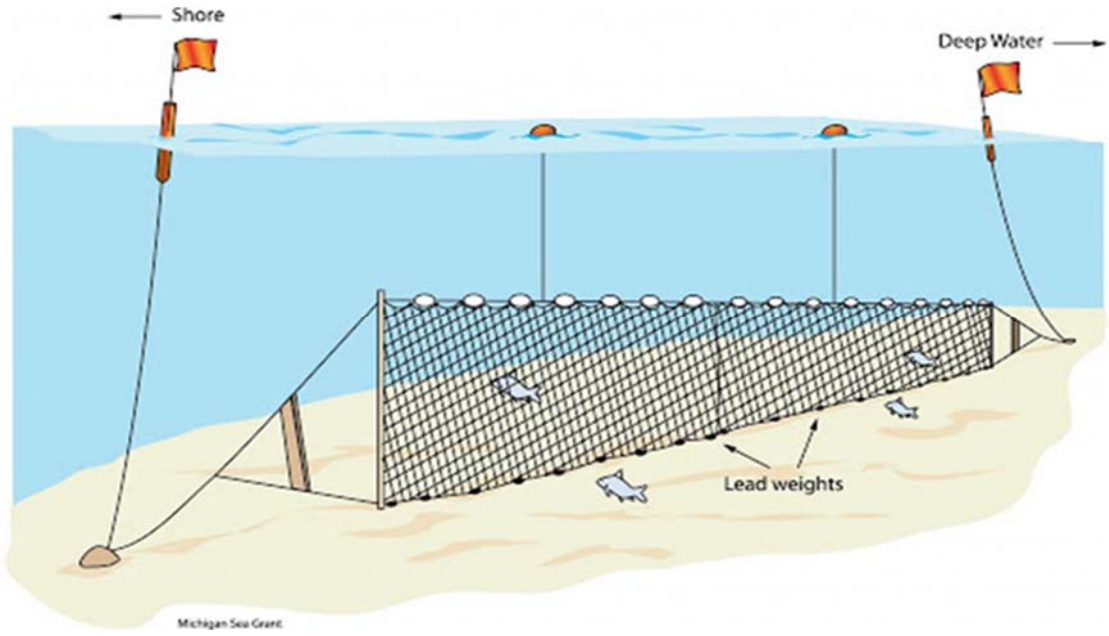

Figure 21: Illustration of a passive net

The trembling nets will have a height of about 3 m, internal mesh sized 72 mm and external mesh sized 400 mm. Six nets will be placed, each 500 m long. The nets will be positioned on the seabed parallel to the coast, at the 5 m and 15 m bathymetric depths, in order to cover as much of the range as possible. The fishing gear will be lowered at sunset and set sail at dawn, for an average stay in the sea of about 12 hours.

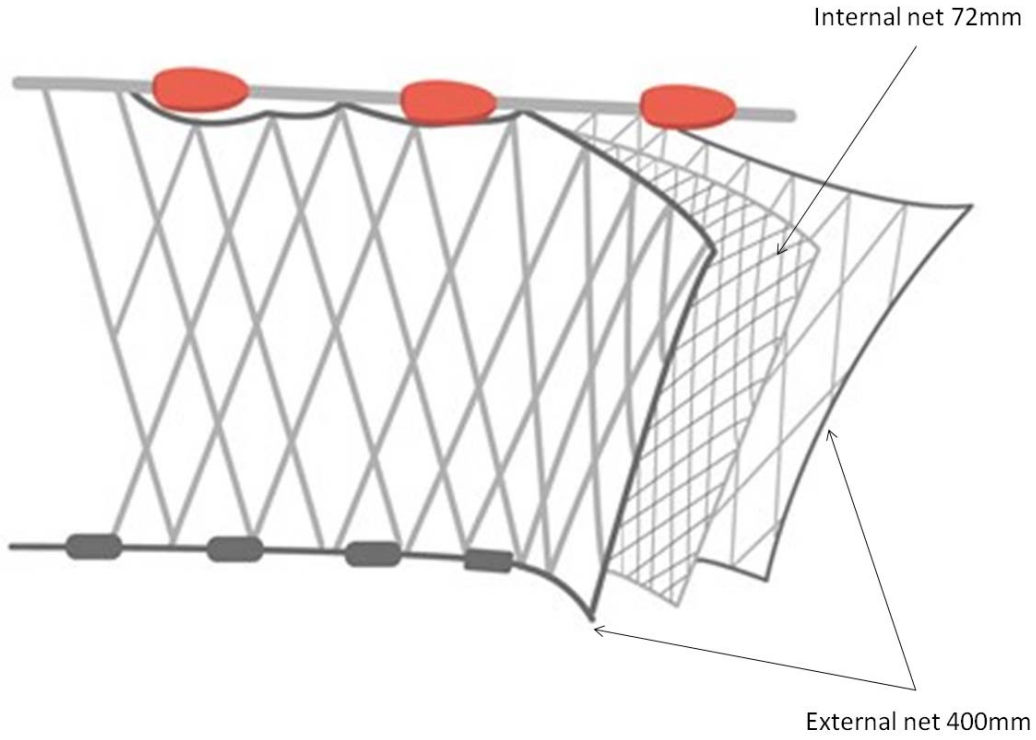

Figure 22: Trembling nets

Figure 23: Fishnet installation on the seabed

After capture, the samples will be stored at 4°C until the biological samples for bioaccumulation analysis are prepared, which in any case will be carried out as soon as possible (within a few hours of sampling).

Each specimen will be weighed and measured individually. In the case of echinoderms and crustaceans, the edible part will be taken for analysis (i.e. soft part for the curls and muscle of the appendages and abdomen for the crustaceans). In particular, pools of tissues from

different individuals of the same species will be prepared, on which the concentrations of the investigation parameters will be determined.

3.2 Fish fauna: Samples number

For each station, one collective (1) sample consisting of two (2) samples at the respective bathymetric depths of -5m and -15m will be obtained as shown in Figure 24.

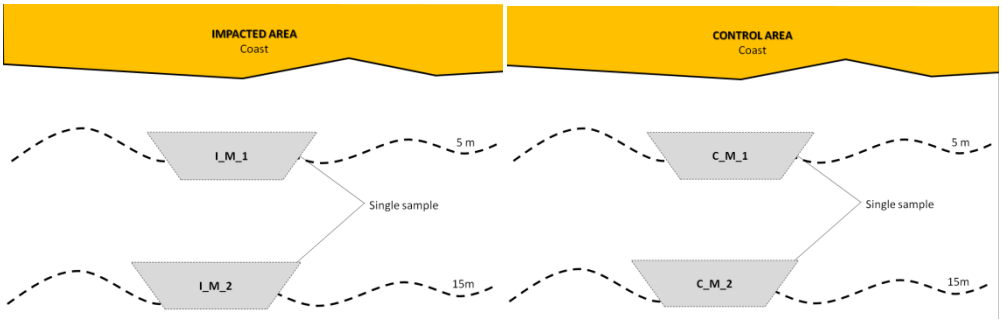

Figure 24: Sample scheme for fish sampling

For each area (study and control), and for each biota category, 3 pools (replicates) of tissues to be analyzed will be prepared, in order to allow an adequate statistical treatment of the data.

Once the sample is prepared for bioaccumulation analyses on the various categories of biota (always in triplicate in order to optimize the statistical treatment of the data), the sample will either be immediately analyzed or else stored at -20°C in suitable containers (e.g.: falcon or glass jars).

Table 2. Total number of samples to be analysed for fish fauna (fish, echinoderms and crustaceans)

Total number of samples
4 stations (two depths combined into one sample)
3 replicates for each station
8 species to process
Total: 96 samples

3.3 Fish Fauna: Sampling Stations

The individual sampling stations are identified by means of a data sheet as shown below:

- Two (2) impact area stations (-5m and -15m)
- Two (2) control area stations (-5m and -15m)

RECORD N° 001

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

RECORD N° 002

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

RECORD N° 003

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

RECORD N° 004

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

4.0 Marine Phanerogam

4.1 Marine Phanerogam: organisms and sampling method

The marine phanerogam species selected for bioaccumulation investigations is C*ymodocea nodosa,* a species of seagrass sometimes known as Neptune grass. As a seagrass, it is restricted to growing underwater and is found in shallow parts of the Mediterranean Sea and certain adjoining areas of the Atlantic Ocean.

Figure 25. Cymodocea nodosa (Neptune grass)

C. nodosa has light green or greyish-green leaves. Specimens are very narrow but may be up to forty centimeters long. Each leaf has seven to nine veins running along its length. The plant produces rhizomes which are only 1 mm in diameter and have leaf scars at intervals. Inconspicuous grass-like flowers are sometimes produced at the end of long stems in the spring when water temperatures begin to rise after their winter minimum. The pollen is liberated into the sea and the seeds remain dormant until the following spring.

If possible, the samples taken in the two areas (study area and control area) will be collected at similar depths and within the 10 m bathymetric zone.

The *Cymodocea* samples taken by immersion will be stored at 4°C in plastic bags until transported to the accredited laboratory. Chemical analyzes will be carried out on root samples, since they represent the most suitable tissue for the evaluation of the bioaccumulation potential. For each area, three different pools (replicates) will be prepared (one for each portion of the rhizome sampled), which will be immediately analyzed or can be stored at -20°C until the moment of analysis.

4.2 Marine Phanerogam: Samples number

For each station one (1) sample will be obtained. This sample is composed of a single rhizome at least one meter long with its roots, taken at a bathymetric depth of -10m. the sampling scheme is the one shown in the figure 26. Figures 27 and 28 shown below show the methods for taking the samples.

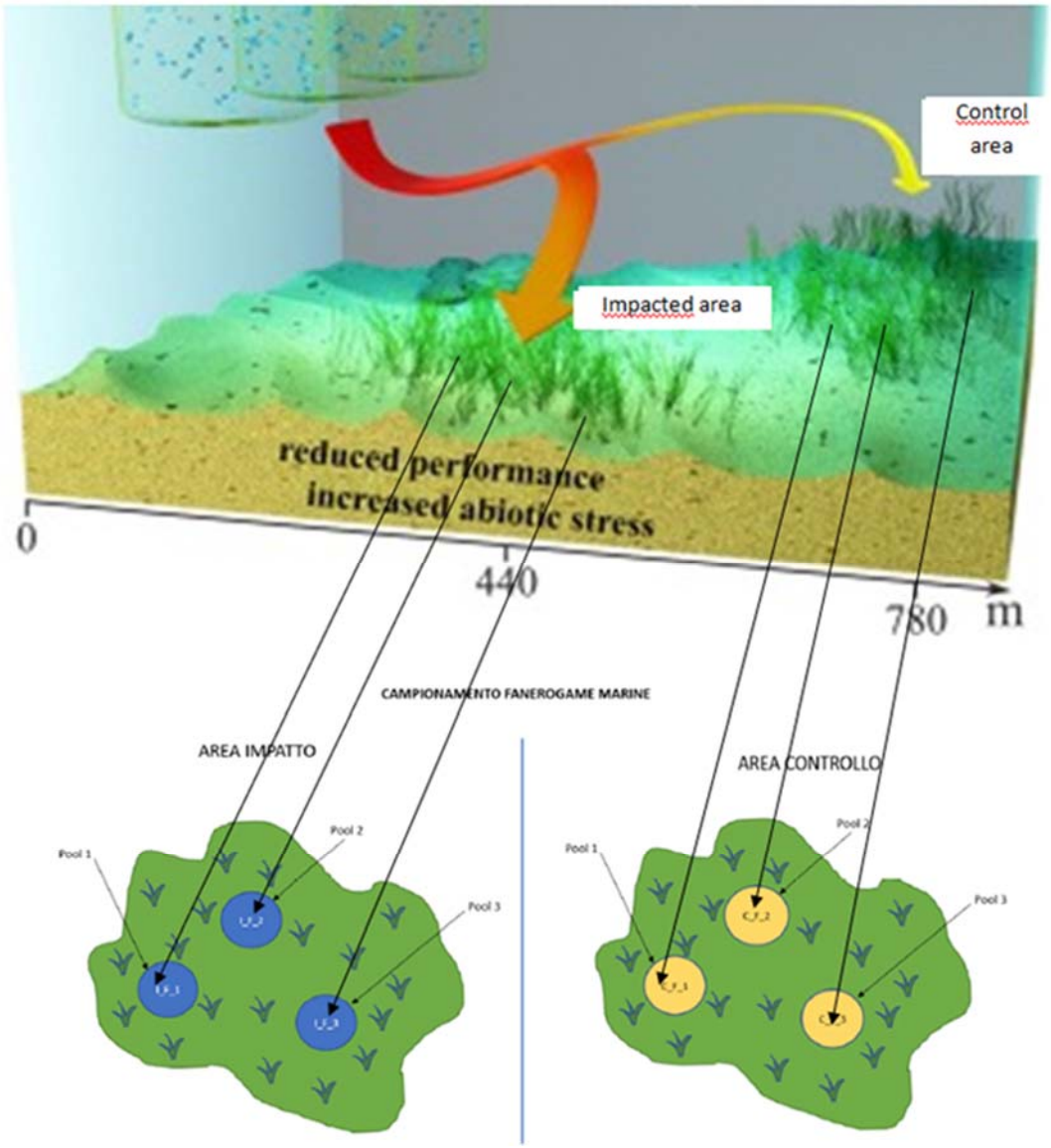

Figure 26. Marine phanerogam sampling sample scheme

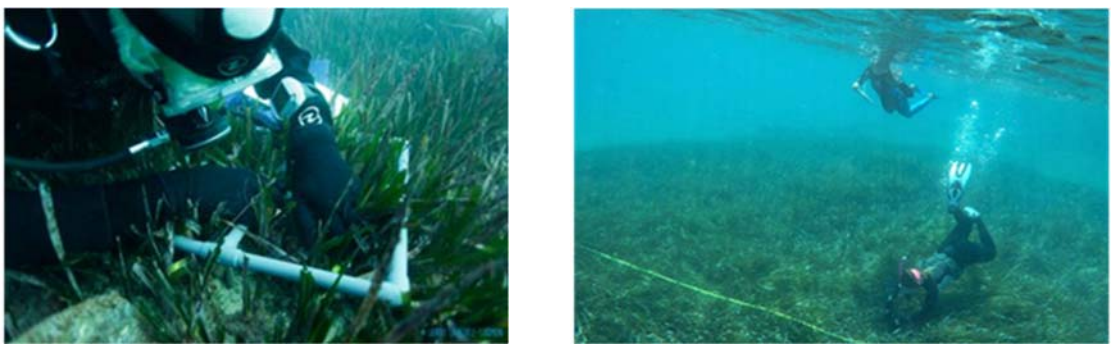

Figure 27: Quadrat sampling of seagrass species on the seabed carried out by divers

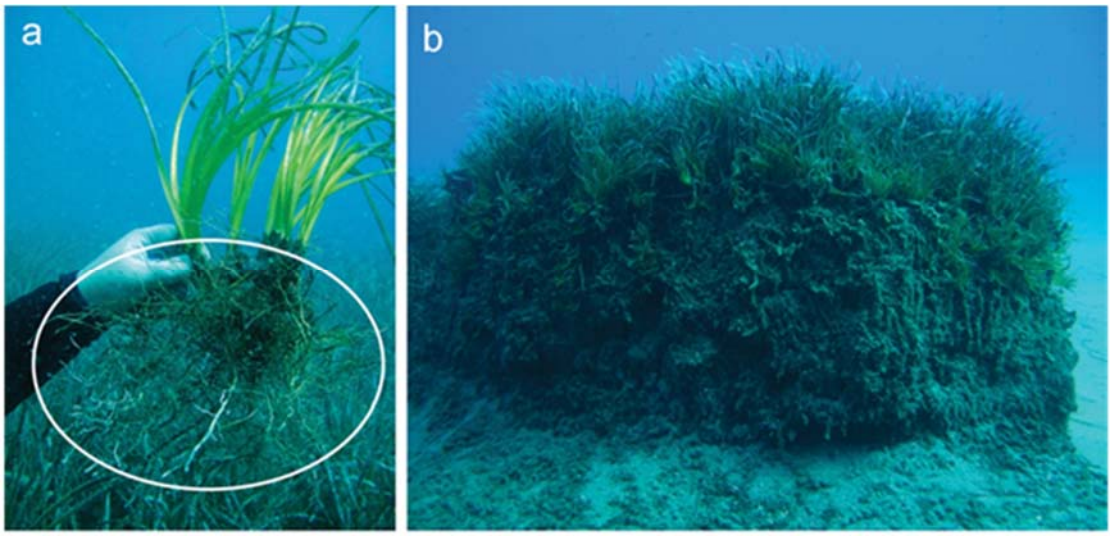

Figure 28. Underwater survey phases and sampling; a) Rhizomes, b) Matte

These rhizomes will be sampled for a total of 3 replicates for each station at a distance of at least 5 m from each other, proceeding orthogonally to the prevailing direction of the rhizomes to select different individuals for an appropriate statistical data processing.

Table 3. Total number of samples to be analysed for cymodocea nodosa

Total number of samples
2 stations
3 replicate for each station
1 species to process
Total: 6 samples

4.3 Marina Phanerogam: Sampling Stations

The individual sampling stations are identified by means of a data sheet as shown below:

- One (1) station impact area (-10m)
- One (1) control area station (-10m)

RECORD N° 001

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

RECORD N° 002

Client			
Station Code			
Coordinates WGS84			
Type of sampling			
Analitycal profile			

5.0 Analytical Set for Bioaccumulation Determination in Biota

The analytical chemical parameters to be assessed for bioaccumulation purposes are summarized in the sections below:

5.1 Bivalve Molluscs

Parameters	u.o.m.	Method	LOD	LOQ	Ref. Regulamentation 1881/06
Lead	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	1.5 mg/kg
Cadmium	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.03	1 mg/kg
Mercury	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	0.50 mg/kg
Chrome	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Nickel	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Vanadium	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Copper	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Arsenic	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.05	
Methylmercury			0.008	0.025	
Sum (upper bound) of Dioxins and furans	ng/kg	EPA 1613 B 1994	1	2	3.5 pg/g fresh weight
PCB Dioxin like (Reg.1881/2006)	ng/kg	EPA 1668 C 2010	1	2	8.0 pg/g fresh weight
Benzo (a) pyrene	µg/kg	UNI EN 16619:2015	0.3	0.6	10 µg/kg
Pesticides organochlorines: Cis-chlordane, Trans-chlordane, Aldrin, Dieldrin, Endrin, α-HCH, β-HCH, γ-HCH (Lindane), 2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, 2,4-DDD, 4,4-DDD, HCB, Heptachlor epoxide.	mg/kg	EPA 3541 1994 + EPA 3630C 1996+ EPA 8081B 2007	0.05	0.01	

5.2 Fish fauna

5.2.1 Fish

Parameters	u.o.m.	Method	LOD	LOQ	Ref. Regulamentation 1881/06
Lead	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	0.30 mg/kg
Cadmium	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.004	0.015	0.05 mg/kg
Mercury*	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	0.50 mg/kg*
* with the exception of mullet and rays	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	1 mg/kg*
Chrome	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Nickel	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Vanadium	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Copper	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Arsenic	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.05	
Methylmercury	µg/kg	MP 2196 REV 0 2016	0.008	0.025	
Sum (upper bound) of Dioxins and furans	ng/kg	EPA 1613 B 1994	1	2	3.5 pg/g fresh weight
PCB Dioxin like (Reg.1881/2006)	ng/kg	EPA 1668 C 2010	1	2	8.0 pg/g fresh weight
Benzo (a) pyrene	µg/kg	UNI EN 16619:2015	0.3	0.6	2 µg/kg
Pesticides organochlorines: Cis-chlordane, Trans-chlordane, Aldrin, Dieldrin, Endrin, α-HCH, β-HCH, γ-HCH (Lindane), 2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, 2,4-DDD, 4,4-DDD, HCB, Heptachlor epoxide.	mg/kg	EPA 3541 1994 + EPA 3630C 1996+ EPA 8081B 2007	0.05	0.01	

5.2.2 Crustaceans

Parameters	u.o.m.	Method	LOD	LOQ	Ref. Regulamentation 1881/06
Lead	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	0.5 mg/kg
Cadmium	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.004	0.015	0.5 mg/kg
Mercury	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	0.50 mg/kg
Chrome	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Nickel	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Vanadium	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Copper	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Arsenic	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.05	
Methylmercury	µg/kg	MP 2196 REV 0 2016	0.008	0.025	
Sum (upper bound) of Dioxins and furans	ng/kg	EPA 1613 B 1994	1	2	3.5 pg/g fresh weight
PCB Dioxin like (Reg.1881/2006)	ng/kg	EPA 1668 C 2010	1	2	8.0 pg/g fresh weight
Benzo (a) pyrene	µg/kg	UNI EN 16619:2015	0.3	0.6	2 µg/kg
Pesticides organochlorines: Cis-chlordane, Trans-chlordane, Aldrin, Dieldrin, Endrin, α-HCH, β-HCH, γ-HCH (Lindane), 2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, 2,4-DDD, 4,4-DDD, HCB, Heptachlor epoxide.	mg/kg	EPA 3541 1994 + EPA 3630C 1996+ EPA 8081B 2007	0.05	0.01	

5.3 Marine Phanerogam

Parameters	u.o.m.	Method	LOD	LOQ	Ref. Regulamentation 1881/06
Lead	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	0.30 mg/kg
Cadmium	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.03	1 mg/kg
Mercury	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.04	0.50 mg/kg
Chrome	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Nickel	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Vanadium	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Copper	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.05	0.1	
Arsenic	mg/kg	UNI EN 13805:2014 + UNI EN 15763:2010	0.01	0.05	
Methylmercury	µg/kg	MP 2196 REV 0 2016	0.008	0.025	
Sum (upper bound) of Dioxins and furans	ng/kg	EPA 1613 B 1994	1	2	3,5 pg/g fresh weight
PCB Dioxin like	ng/kg	EPA 1668 C 2010	1	2	8.0 pg/g fresh weight
Benzo (a) pyrene	µg/kg	UNI EN 16619:2015	0.3	0.6	10 µg/kg
Pesticides organochlorines: Cis-chlordane, Trans-chlordane, Aldrin, Dieldrin, Endrin, α-HCH, β-HCH, γ-HCH (Lindane), 2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, 2,4-DDD, 4,4-DDD, HCB, Heptachlor epoxide.	mg/kg	EPA 3541 1994 + EPA 3630C 1996+ EPA 8081B 2007	0.05	0.01	

6.0 Reporting

At the end of the analytical activities, the analytical data will be submitted through Test Reports dated and signed by the functions in accordance with the provisions of the UNI CEI EN ISO / IEC 17025 Standard, on whose document they will be uniquely identified:

1. Description of the sample;
2. Date of receipt;
3. Test start date;
4. End date of tests;
5. Sampling date;
6. List of parameters with relative analytical result;
7. Test method;
8. Unit of measurement;
9. Measurement uncertainty;
10. Limit of quantification;
11. Recovery;
12. Sampling method;

The analytical results can be returned by means of a report in excel format and in PDF format. All analytical tests developed by the laboratory will be available for any audits by the client. The results will then be analysed by MT-IT JV and included in the Italian EIA documentation.

7.0 Impact Assessment

Considering the areas subject to potential impact on nearby Natura 2000 sites SPAs ITA050012 - "Torre Manfria, Biviere and Piana di Gela", SPA ITA050011 - "Torre Manfria" and ZSC ITA050001 - "Biviere and Macconi di Gela", the authoris have carried out an impact assessment to assess whether the proposed sampling activities are envisaged to have any negative and/or direct impact on the environment. Based on the assessments made with regard to possible incidence factors, any type of incidence with the mentioned Sites is excluded.

With regard to the assessment of the significance of the effects of the expected interventions on habitats and species within Natura 2000 sites, for which there is a potential ecological connection, the fact that the site is located outside of Natura 2000 habitats is fundamental.

Further considerations for the impact assessment include:

- extreme temporary nature of the actions (duration in the order of a few days);
- timely and limited interventions in the area;
- reversibility of environmental conditions;
- complete absence of any type of emissions after the works;
- sampling procedures which involve the use of divers and manual instruments (sorbona, underwater camera, etc);
- vessels used (fishing boat and motorboat);
- type of fishing net used for catching fish.

Cross-analysis of potential impact factors and conservation objectives of the site and in the adjacent areas of intervention, conclude that the possible incidence of impacts is remote and insignificant. All activities will be conducted with full respect and consideration of the environmental context by technically competent and experienced personnel and in compliance with all the limitations imposed by the local authorities.

8.0 Schedule of Activities

This section provides a brief schedule of activities which are envisaged throughout the execution of the sampling exercise. Kindly note that the commencement of the sampling activities is subject to the acquirement of permits and concessions from the competent Italian authorities which are operating under severe limitations due to the ongoing COVID-19 pandemic.

Table 4: Schedule of activities

Item	Estimated time	Note
Request for concessions and permits for carrying out the activities	3 weeks	Authorizations will be requested from the Gela harbor master's office. On 12 March 2020, MT-IT JV representatives visited the offices of the port authority of Gela to seek the necessary authorizations. Due to the ongoing COVID-19 emergency, the port authority was unable to present any documentation.
Monitoring of Fish Fauna	4 days	One day has been estimated for each fishing area
Bivalve Molluscs	6 days	One day has been estimated for each underwater sampling. another 2 days were added for sampling *Paracentrotus lividus*
Marine Phanerogam	4 days	Two days have been estimated for the control area and one day for the impact area.
Analytical activities	4 weeks	All the analytical activities foreseen by the study and the determinations provided by the method (ecological index) were considered
Final report processing	2 weeks	Technical report, spatial elaborations, definition of thematic maps & statistical processing

Table 5: Schedule of activities

Shedule of activities												
Items	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11**	**Week 12**
Request concessions and permits												
Monitoring of Fish Fauna												
Bivalve Molluscs												
Marine Phanerogam												
Analytical activities												
Final report processing												