Interreg
Italy - Croatia
ADRIREEF
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Work Package 3 – Mapping of Adriatic Reefs from different perspectives

Activity 4 – Identification of technologies for underwater monitoring of reefs

FINAL REPORT

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1. EXECUTIVE SUMMARY

The crucial aspect when investigating a given area is the possibility to obtain the most possible information. This is particularly important in the case of reefs (both natural and artificial) that are very complex ecosystems. A lot of instruments and techniques are available for environmental studies, each with advantages and disadvantages for example in terms of costs, working time, efficiency etc. Fundamental in the choice of a sampling strategy is to evaluate possible environmental impacts and to found a compromise between the expected results and the safety of the environment.

An overview of the most used techniques and the newer technologies in environmental studies was conducted subdividing them in destructive and non-destructive for the environment. For each instrument/technology we described its appropriateness, advantages and disadvantages, also analysing the applicability of low impact technologies to reef monitoring purposes.

The above constituted a basis to select the most suitable monitoring technologies to be applied to each Case Study during the next phase of the ADRIREEF project (WP4 Activities 4.1 and 4.2) taking into account their feasibility on the basis of the peculiarities of each location and the available budget within the project, but even the potential sustainable activities that could be implemented in line with the Blue Economy.

2. CRITICAL OVERVIEW OF DIFFERENT METHODOLOGIES USED TO MONITOR REEFS, WITH EMPHASIS ON THEIR IMPACT ON THE NATURAL ENVIRONMENT AND TECHNOLOGIES WITH LOW ENVIRONMENTAL IMPACT

2.1. Introduction

Several methods have been used to monitor both natural (NRs) and artificial reefs (ARs) around the world. The aim of this overview is to provide an excursus of the different methodologies and evaluate either their suitability to be employed for reef monitoring and their impacts on the marine environment.

The document focuses on the main abiotic and biotic components (geomorphological features, water column, sediments, benthic and finfish communities) which are usually taken into account when monitoring a reef and to make the reading easier, we have debated the various environmental components separately, emphasizing both advantages and disadvantages of each technology in terms of results which can be obtained and possible environmental impacts due to its application (Table 1).

2.2. Geomorphological, geophysical and geochemical characterization of the seabed

Seabed characterization is fundamental to evaluate extension, morphological features (e.g., slopes, picks, etc.) and integrity of a reef, producing a valuable base for geomorphological and environmental analysis (Otto & Smith, 2013). In the case of reefs this monitoring method is also useful to verify eventual environmental changes on the adjacent seafloor and on the hard substrates (e.g. subsidence, sediment accumulation, texture, bio-constructors) and, as for ARs, some of those changes which can also affect the physical performance of the reef structures (Manoukian *et al*., 2004; Lo Iacono *et al*., 2018).

2.2.1. Sampling methods

2.2.1.1 Non-destructive methods

Acoustic systems: Acoustic systems (single- and multibeam echosounder, side-scan sonar) are efficient tools which allow to evaluate the physical performance of reefs without creating any disturbance to the environment.

The single-beam echosounder (SBS) has spatial limitations as it provides 3D measurements along track lines that have to be interpolated to get a 100% coverage of the seabed.

The side-scan sonar (SSS) technique can provide a 100% high resolution backscatter mosaic of the seabed, allowing to obtain detailed maps of habitats and sediment features (e.g. texture, occurrence of algae and/or seagrass meadows), but it cannot provide bathymetric maps. Due to the use of a towed fish, covering wide areas could be time consuming.

Conversely, high-frequency multibeam echosounder (MBES) has the potential of providing a 100% coverage of seabed morphology and water column offering a high-resolution picture of the overall investigated site (Fig. 1; Manoukian, 2011; Manoukian *et al*., 2011; Fabi *et al*., 2015; Tassetti *et al*., 2015). In respect to SSS it is less time consuming and, through the backscatter acquired data, can provide information on the habitats and sediment features although with a lower resolution. Therefore, MBES currently represents the most appropriate equipment to get a complete picture of a reef and monitor it over time. However, to obtain detailed information on the sediment texture, both SSS and MBES surveys need to be associated to collection of sediment samples.

The above instruments can be installed on a vessel, a Remote Operated Vehicle (ROV; see Box 1) or an Autonomous Underwater Vehicle (AUV; see Box 2).

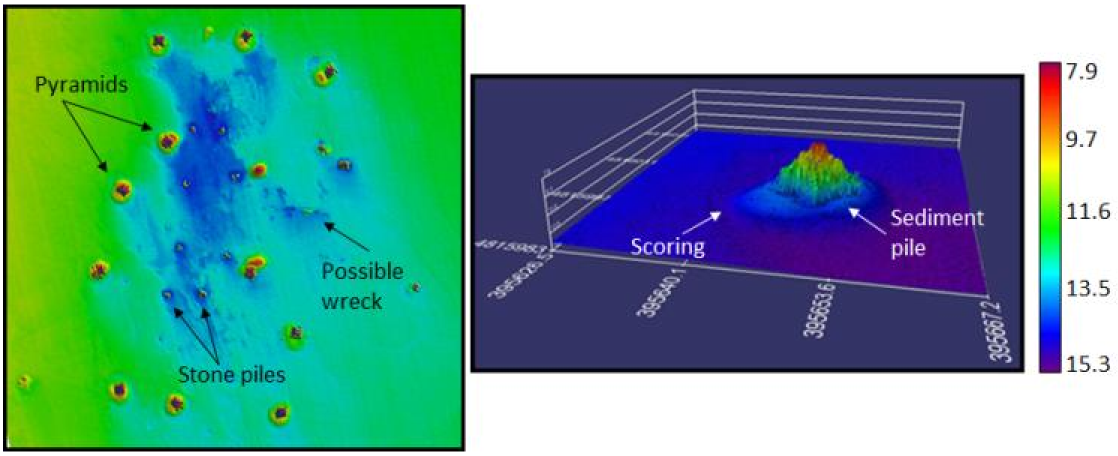


Figure 1. Multibeam echosounder images of on artificial reef in the Adriatic Sea, Italy. (from Fabi et al., 2015).

2.2.1.2 Destructive methods

Grabs, box-corer, multi-corer and hand corer samplers: these instruments (Fig. 2) are usually employed to take samples of sediments in soft seabed outside a reef and between the structures constituting an AR. These samples can be used for wide variety of analysis concerning the physical and chemical characteristics of the sediments.

Hand corers are inexpensive, easy to construct, and can be used in shallow water by a person wading, from a boat at depth of about 2 m, or by a diver up to 50 m depth (Pusceddu *et al*., 2003). An advantage of this device is the possibility to sample at a precise point; moreover, the sediment sample comes on board compact and it is possible to take sections at specific levels. Nevertheless, the dive operator must be careful to avoid the mixture of superficial sediments (Pusceddu *et al*., 2003).

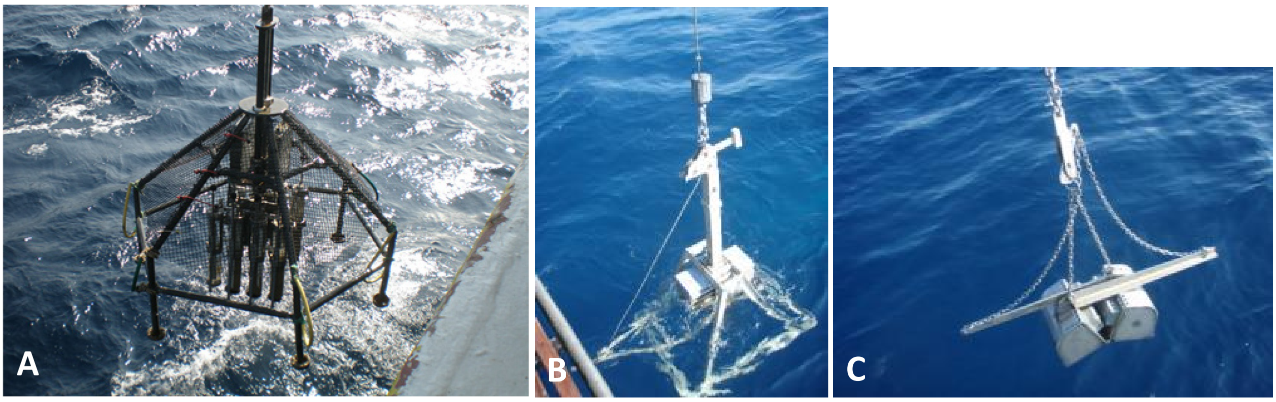


Figure 2: Instruments employed to take samples of soft-bottom: (A) multi-corer, (B) box-corer and (C) grab (courtesy of CNR-IRBIM Ancona).

Grabs, box-corers and multi-corers are surface operated and do not require underwater work. For this reason, their positioning on the seabed is not precise; in addition, the penetration of these instruments inside sandy bottoms may be difficult due to the substrate density.

Box-corers and multi-corers are usually preferred to take sediment samples for physical chemical, biochemical and ecotoxicological analysis because, even though they have a smaller capacity than grabs, they prevent mixing of the sample. Like hand-corers, sediment samples taken by box-corers and multi-corers come on board compact and it is possible to take sections at specific levels.

BOX 1 – Remotely Operated Vehicles - ROVs

ROVs are tethered, tele-operated, power-supplied underwater vehicles, highly manoeuvrable and operated by a crew either aboard a vessel/floating platform or on proximate land. They are common in deep water industries such as oil and gas exploration, telecommunications, geotechnical investigations and mineral exploration, but they are also used to study lakes, the ocean, and the ocean floor. ROV may sometimes be called Remotely Operated Underwater Vehicle (ROUV) to distinguish it from remote control vehicles operating on land or in the air (Azis *et al*., 2012).

The ROV system comprises an underwater vehicle, which is connected to the control platform and the operators on the surface by an umbilical cable, a handling system to control the cable dynamics, a launch system and an associated power supply. ROVs can vary in size from small vehicles with video's for simple observation up to complex work systems, which can have several dexterous manipulators, video cameras, tools and other equipment (Christ & Wernli, 2014). For example, survey and inspection ROVs are frequently fitted with SSS and MBES. Effective control schemes require relevant signals in order to accomplish the desired positions and velocities for the vehicle. A suitable controlling method of underwater vehicles is very challenging due to the nature of underwater dynamics.

There are four broad categories of ROV based upon vehicle size and capabilities (Christ & Wernli, 2014):

Observation class ROVs (OCROV): these vehicles range from the smaller micro-ROVs to a vehicle weight of 100 kg. They are generally small, DC-powered, inexpensive electrical vehicles used as either backup to divers or as a diver substitution for general shallow water inspection tasks. Vehicles in this classification are generally limited to depth ratings of less than 300 m of seawater. They are typically hand launched and are free flown from the surface with hand tending of the tether.

Mid-sized ROVs (MSROV): these vehicles weigh from 100 to up 1000 kg. They are generally a deeper-rated version of the OCROV with sufficient AC power delivery components and pressure housings capable of achieving deeper depths over longer tether/umbilical lengths. There also are generally all-electric vehicles with some hydraulic power for the operation of manipulators and small tooling package options. The vehicle electrical power is stepped down to a manageable voltage for operation of the various components and can be either AC or DC power. Vehicles in this classification are sometimes called "light work class". Due to their weight, a launch and recovery system as well as a tether management system is often needed.

Work-Class ROVs (WCROV): Work-class systems are generally heavy electromechanical vehicles running on high-voltage (>3000 V) AC circuits from the surface to the vehicle. The power delivered to the vehicle generally is changed immediately to mechanical (hydraulic) power at the vehicle for locomotion as well as all manipulation and tooling functions.

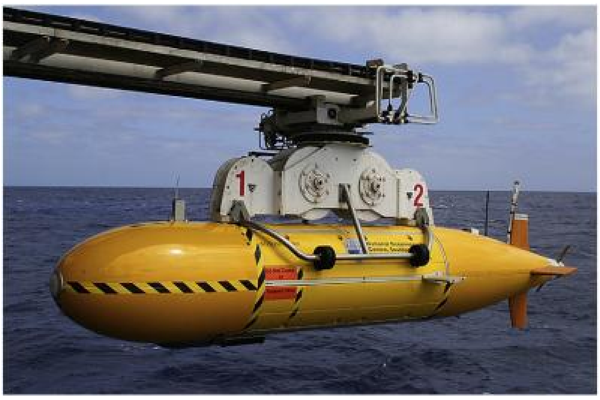
Special-use vehicles: vehicles not falling under the main categories of ROVs due to their non-swimming nature such as crawling underwater vehicles, towed vehicles, or structurally compliant vehicles (i.e., non-free-swimming).

Typologies of ROVs: Observation ROV, Work-Class ROV, Special-use ROV (from left to right) (from https://www.lerus.com.sa/blog/offshore-operations).

BOX 2 – Autonomous Underwater Vehicles (AUVs)

AUVs are unmanned, self-propelled vehicles that are typically immersed from a surface vessel and can operate independently of that vessel for periods varying from a few hours to several days. The wholly autonomous nature of AUVs means that the deploying vessel can be used for other tasks during the survey.

AUV is potentially capable of hosting a variety of sensor payloads including geophysical instruments (MBES, SBP, SSS, magnetometer), geochemical instruments (electrochemical redox sensors), seafloor-imaging tools (high-definition monochrome or colour cameras) and oceanographic instruments (CTD, Acoustic Doppler Current Profilers – ADCP). The sensors deployed determine the vehicle altitude, as well as its speed and endurance. Higher power sensors, e.g. SSS and SBP, reduce endurance due to their increased energy requirements, while high-resolution seafloor imaging with a colour camera system will require the AUV to fly slower and closer to the seabed than if it was undertaking a MBES survey using a vessel (Yoerger *et al*., 2007a, b). Depending on their pressure resistance, existing AUVs for scientific research can operate in water depths (WD) of up to 6000 m. The ability of deep-water AUVs to fly relatively close to the seabed means they are potentially capable of collecting seafloor mapping, profiling and imaging data of far higher spatial resolution (up to two orders of magnitude) and navigational accuracy than surface vessels equipped with towed instruments (e.g., SSS), and camera systems (Murton *et al*., 1992; Scheirer *et al*., 2000; Jones *et al*., 2009). AUVs therefore effectively bridge the spatial resolution gap between vessel-mounted or towed systems, e.g. multibeam echosounders (MBESs), side scan sonars (SSSs) and sub-bottom profilers (SBPs), and ROV-mounted systems. In many cases, AUVs are actually used in conjunction with these systems as part of a 'nested' multi-resolution survey of the seafloor (e.g. Ferrini *et al*., 2007; Yoerger *et al*., 2007a, b; German *et al*., 2008; Haase *et al*., 2009; Larroque *et al*., 2011; Römer *et al*., 2012; Paull *et al*., 2013).



The UK Natural Environment Council (NERC) Autosub6000 AUV, depth-rated to 6000 m, can be equipped with multiple payloads for marine geoscience research, including a high-resolution multibeam echosounder, sub-bottom profiler, and side-scan sonar, a color camera system, and Conductivity, Temperature, Depth (CTD) and electrochemical redox (Eh) sensors. The vehicle is 5.5 m long and has a dry weight of 1800 kg; it is capable of precision navigation and terrain following and has a sophisticated collision avoidance system (from Wynn et al., 2014).

2.3. Water column

Study of the water column includes physical (e.g., temperature, salinity, light penetration) and chemical (e.g., pH, dissolved oxygen, nutrient salts, chlorophylls) features of seawater at different depths of defined geographical points.

2.3.1. Sampling methods

2.3.1.1 Non-destructive methods

Sensors: a variety of instruments are capable of measuring water column variables These instruments can be used for a single measurement or for a continuous collection of data, installed at a fixed station or mounted on a vessels. Among the most used sensing instruments we can find current meters for the measurement of current direction and intensity, wave meters to record height, direction and intensity of waves, CTD (Conductivity/Temperature/Depth) systems to measure conductivity (fundamental for the calculation of salinity, density and sound speed), water pressure (for referring each parameters measurements to the depth) and temperature. Additional sensors can be included to measure other water column parameters such as dissolved oxygen, pH, irradiance, turbidity, oxidation-reduction potential, chlorophyll a, etc.

While the old current meter models were able to only record at the depth layer where they had been deployed, the new generation of Acoustic Doppler Current Profilers (ADCP) allow to investigate the entire water column both at a fixed point or from a sailing vessel as well as from AUVs (see Box 2).

New *in situ* technologies, which provide high-frequency (continuous or semi-continuous) observations, are also represented by instruments developed to monitor marine hydrological and physico-chemical variables. A few sensors currently in use can monitor concentration of CH4, CO2, IPA, heavy metals, organic pollutants and algal toxins (Danovaro *et al*., 2016).

However, as for any instrument deployed at sea for a long period, especially in nutrient-rich environments, sensors are subjected by rapid biological colonization (biofouling) which can limit overall deployment times (Mills & Fones, 2012). The use of wiper brushes, antifouling tablets, pumped flow and copper covers can limit the fouling phenomenon.

Wave meters are usually mounted at a fixed station (pole or buoy), while ADCP and sensors can be operated either at a fixed point, or from a sailing vessel as well as from AUVs (see Box 2).

Bottle-samplers: these samplers (Niskin, Van Dorn and Kemmerer) are commonly employed to withdraw a representative sample of water from a discrete depth at an established sampling point (Fig. 3). Collected samples can be used for different laboratory analysis like search of contaminants (e.g., heavy metals, PAHs, AHs), microbiological parameters, etc.

Bottle samplers can be operated by hand, from a vessels as well as from an AUV (see Box 2).

Figure 3: A Rosette equipped with Niskin bottles and CTD sensors (courtesy of CNR-IRBIM Ancona).

Sediment traps: these instruments are used to directly collect setting particles in a variety of marine and limnetic environments over long periods, either continuously or in time-fraction sequences. The collected samples allow the determination of downward mass fluxes of particles and associated elements, such as organic and inorganic compounds (e.g., organic C, carbonates), or trace elements and pollutants (e.g. selected trace metals, radionuclides).

This instrument can be deployed at any, except extreme, depths from any ordinary vessel as well as by scuba divers.

However, sediment trap measurements are not always accurate, especially in some settings and, in particular, at shallower depths, that is from the surface euphotic zone and down to roughly 1000 m where most sinking particles are remineralized, and trap accuracy is most likely to be an issue. This is because the processes that can bias the collection of sinking particles by traps are more likely to be significant at these depths. For example, currents are generally faster in shallower waters, thus increasing the chance of hydrodynamic biases for moored and drifting traps in the upper layers of water co column. Zooplankton is more abundant in surface waters, while decreasing numbers are found to migrate daily or live permanently down to 800-1200 m. Thus it is at the shallower depths zooplankton swimmers are more likely to actively enter traps and compromise both particle load and remineralized components. Finally, flux decreases with depth presumably due to the rapid remineralization (Buesseler *et al*., 2007)

A further possible problem encountered in this sampling methodology is the high possibility of contamination of the sample by boat exhaust or surface water (Grasshoff *et al*., 2009).

Remote sensing: this method consists in optical, thermal and radar images from airborne and satellite sensors for a direct monitoring of biodiversity (species assemblages and ecological communities) or for provision of environmental data layers (primary productivity, climate and habitat structure) which are needed to build predictive models of species and habitats (Turner *et al*., 2003; Pettorelli *et al*., 2014).

One of the major advantages of the remote sensing is to provide valuable data bridging the spatial and temporal gaps in observation complementing the in situ measurements.

Remote sensing presents however a few limitations: for example, the phytoplankton biomass contents evaluated by satellite-derivate Chl-a concentration are based on conversion factors; the weather condition (clouds) can interfere with information about the surface parameters, limiting spatial and temporal coverage. Moreover, satellite ocean colour imagery is generally underutilized or improperly interpreted in coastal and estuarine systems largely because standard approaches for retrieving water column properties from space cannot accommodate the optical complexity deriving from riverine delivery of dissolved and sedimentary material. Both the sensors and algorithms used for routine ocean remote sensing are optimized for open ocean conditions (Dierssen, 2010), reason why the estimated values have to be validated with in situ observations, which are essential to ensure the optimal quality of the data (Aurin & Dierssen, 2012). Moreover, the remote sensing cannot assess the benthic compartment (Danovaro *et al.*, 2016).

2.4. Benthic communities

Researches on soft- and hard-bottom communities can be carried out utilizing many monitoring techniques depending on the objective and questions to be answered. Sampling methods depend on the kind of research you are investigating, that can be microbiological analyses, description of meio- and macro-fauna, and algal communities. For example, the study of soft-bottom communities is important in the case of ARs, considering that deployment of new hard substrates may induce changes in the communities of the natural habitat as well as the development of new benthic communities. Otherwise, research on the animal component and macroalgae on hard substrates is important to assess the ecological role of a reef (Falace & Bressan, 1996). Moreover, studies on functional diversity can be used to investigate the effects of different anthropogenic disturbances on the functioning of marine systems (Diaz & Cabido, 2001). Studies can be also linked to some ecological and biological characteristics of the species such as feeding mechanisms, longevity, body size, and mobility to verify eventual changes that could affect communities exposed to stressors such as sewage pollution (Poore & Kudenov, 1978; Grizzle, 1984), anoxia (Beukema *et al.*, 1999) and fishing (Brown & Wilson, 1997; Ramsay *et al.*, 1998; Spencer *et al.*, 1998; Hall-Spencer *et al.*, 1999; Bremner *et al.*, 2003).

2.4.1. Sampling methods

2.4.1.1 Non-destructive methods

Underwater observations: underwater observations include both qualitative and quantitative methods to establish lists and zonation patterns; they can be performed directly through visual observation (Fig. 4a) of standard surfaces by divers or taking pictures or video (Fig. 4b), also in this case of standard surfaces through underwater photo- or video cameras operated by either divers or ROV or AUVs (see Boxes 1 and 2).

Videos can be also recorded by using video camera systems mounted on remote frames that are suspended above the seabed or on towed sleds in contact with the seabed moved by the motion of surface vessel. Transmission to the surface is by umbilical cable which will contain various wires to cover both power and control, as well as data transmission. Data transmission can be either analogue through a simple coaxial cable, or for maximum quality, digital through a fibre optic cable (Smith & Rumohr, 2005).

Photography and video survey may be used to estimate fauna and flora species composition, the number of organisms, coverage percentage and relative density and biomass of the sessile community (Beuchel *et al*., 2006). Moreover, it allows for an objective evaluation and the creation of a reference collection. Such methods can be used for soft- and hard-bottom communities and are useful for dominant and large organisms while they likely underestimate small or cryptic components of the community. Moreover, the records obtained through these techniques can be affected by low taxonomic precision, especially for small-sized organisms and algae.

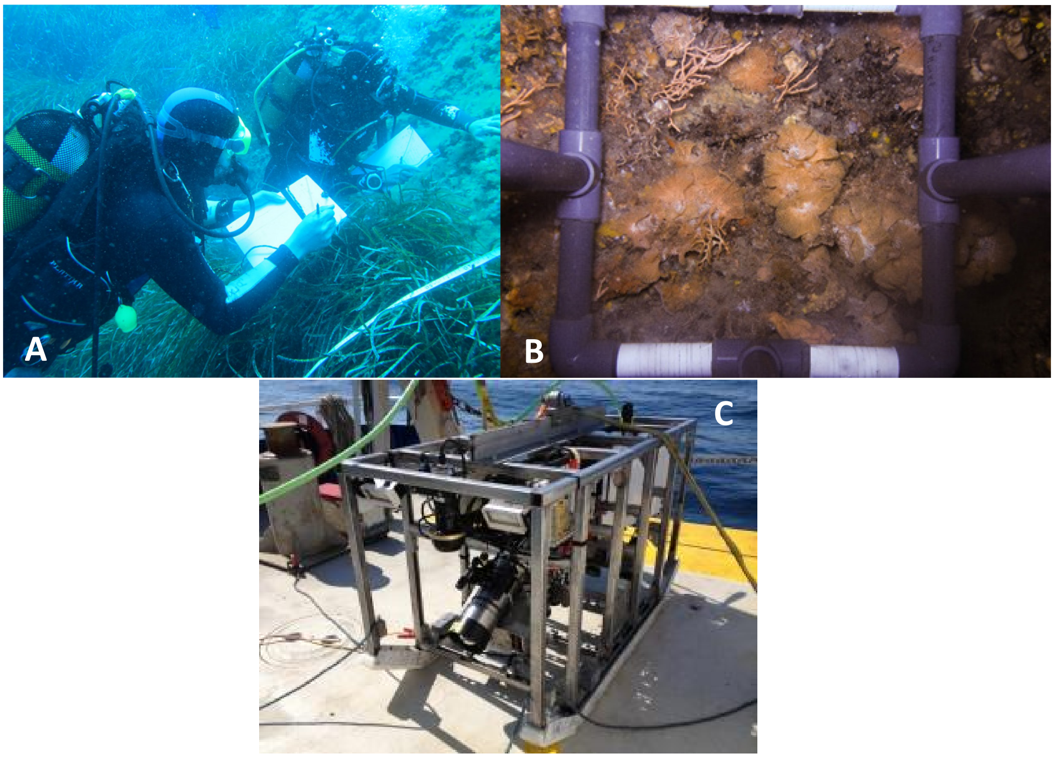


Figure 4: Examples of underwater observations techniques on reefs: visual census (A), photo acquisition (B), and towed video sled (C) (courtesy of CNR-IRBIM Ancona and from https://www.nurtec.uconn.edu/isis2-towed-system/).

2D mosaicing reconstructions: the first mosaic reconstruction methodology, developed by Lirman *et al*. (2007), consists of a two-dimensional (2D), spatially accurate, high-resolution construction of the benthic habitat starting from underwater pictures. However, 2D images are insufficient for species-level identification of many benthic taxa (Gintert *et al*., 2012). To overcame this limitation a second and a third generation were developed (Gintert *et al*., 2008, 2012).

However, despite the potential of the 2D mosaics, in some cases the information are still inadequate for characterization of reef structural complexity due to the ignorance of the 3D structure (He *et al*., 2012) and the strong distortions in the presence of steep slope and cliffs (Johnson-Roberson *et al*., 2010), typical of coral reefs.

Reconstructing 3D virtual environments: it is a powerful modern technology that allows reconstructing a 3D scene from a set of photos acquired by a single-camera or by a stereo-camera (Fig. 5).

This methodology, that allows the use of the photogrammetry (estimating real measurement from photographs), also provides information on the rugosity of hard surfaces and the structural complexity of reef habitats, hence it can be suitable for studies on biodiversity and ecological processes (e.g. nutrient cycling; Friedman *et al*., 2010, 2012; Burns *et. al*., 2015, 2016; Palma *et al*., 2018). 3D-measurement allows for accurate and repeatable measurements of size, shape, and biomass without the destruction of target organisms; additionally, stereo-photogrammetric techniques are not limited by size or orientation of surfaces and organisms (Shortis *et al*., 2000).

However, this technique presents some problems: most applications require specialized and expensive hardware (e.g., graphics card, multi-core processor, etc.) and software as well as clear images; moreover, it is time-consuming for processing images and measurements (Shortis *et al*., 2000).

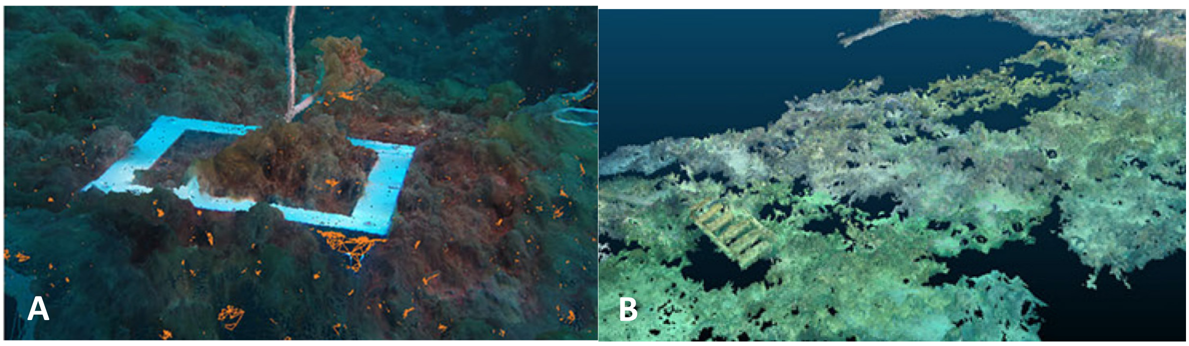


Figure 5: Overview of the 3D reconstructions of (A) colony and (B) reef (from Coro et al., 2018).

Autonomous Reef Monitoring Structure (ARMS): ARMS is a standardized sampling device for marine biodiversity assessment and an application of homogeneous protocols for morphological and molecular identification.

The device consists of nine 23 × 23 cm gray, Type I PVC plate stacked in an alternating series of layers that are either open to the current or obstructed, which are intended to mimic the three-dimensional structure of the reef environment (Fig. 6a). They should be deployed for 1–3 years and colonized by bacteria, algae and sessile and mobile fauna, including cryptic species, of different size ranges.

After recovery, both sides of each plate are photographed and then scraped, the organisms homogenized and analyzed using barcoding and metabarcoding techniques. The ARMS processing protocol applies a combination of morphology (for organisms >2000 µm) and molecular-based (all components) identification approaches to assess species richness (Leray & Knowlton, 2016).

However, the characterization of the surrounding environment where ARMS units are deployed should be carried out for a comparison with natural assemblages. Another main problem for use of ARMS as a routine sampler is that, although the costs of sequencing are dropping, they are still higher than morphological-based approach (Hayes *et al*., 2005).

Artificial Substrate Units (ASUs): ASUs, like ARMS, are another standardized sampling devices to assess benthic marine biodiversity. They consist of nylon pot scrubbers and have been used to study recruitment and taxonomic composition for over 20 years (Fig. 6b; Menge *et al*., 1994, 2002, 2009; Gobin & Warwick, 2006; Underwood & Chapman, 2006; Hale *et al*., 2011). They are used to mimic a habitat for species recruitment, like filamentous algae or kelp holdfasts (Menge *et al*., 1994).

After their recovery, ASUs are analyzed to identify species using morphological characters or molecular methods.

The advantages and disadvantages of ASUs are like those of the ARMS, which are detailed above. Comparing the two devices, ASUs are easier to deploy and the materials are less expensive; moreover, processing of an ASUs sample is faster than ARMS.

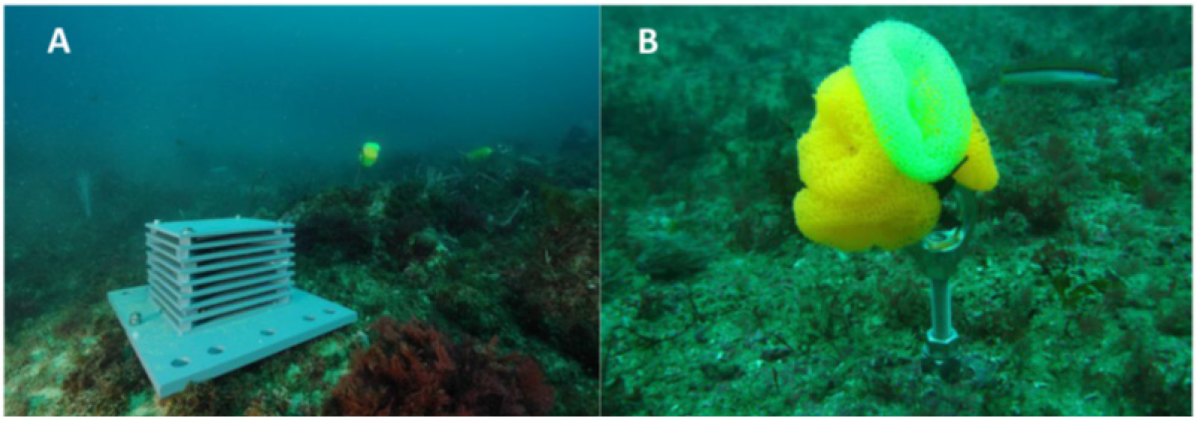


Figure 6: Standardized sampling devices to monitor hard-bottom benthic biodiversity: (A) Autonomous Reef Monitor Structure (ARMS) and (B) Artificial Substrate Unit (ASU) (from Danovaro et al., 2016).

2.4.1.2 Destructive methods

Grabs, box-corer, and multi-corer samplers: these instruments, described above (see Chapter 2), are also employed to take samples of sediments in soft seabed and study the associated benthic communities. Due to their small sizes, corers are less suitable to adequately sample macrofauna than grabs.

Advantages and disadvantages of these instruments are already described in Cap. 2.2.1.2; specifically for benthic communities, these samplers allow to carry out quantitative studies being characterized by known volumes.

Dredges: dredges are gears provided with a heavy metal frame and a net usually towed by a vessel. They are designed for breaking off pieces of rock, scarping organisms off hard surfaces, or for limited penetration and collection of sediments and fauna (Fig. 7) (Eleftheriou & Moore, 2005). Nevertheless, these instruments should not be used inside or close a NR or AR to preserve hard substrates.

This device is not useful in the case of quantitative studies because it is not able to adequately sample pre-defined quantities of sediment (Castelli *et al.*, 2003). In fact, it is not easy to estimate the exact time during which the gear is on the bottom. Moreover, dredges sample only a fraction of the fauna living on the surface of the seabed and very few burrowing animals, hence results merely represent minimum densities on the ground (Eleftheriou & Moore, 2005).

Suction samplers: these samplers are used to investigate soft-bottom benthic communities but may be useful for mobile fauna living on the horizontal walls of hard substrates (Fig. 8; Spagnolo *et al.*, 2004; Fabi *et al.*, 2006; Santelli *et al.*, 2013). Being operated by scuba divers these instruments allow sampling at precise points, but they may require a great effort to collect samples of adequate size and/or a sufficient number of replicates.

Scraping technique: this technique is commonly employed to sample hard-bottom communities (animals and algae). Similarly to the suction sampling, it requires the involvement of scuba divers and allows to sample specific areas with high precision.

In order to prevent losses of organisms due to underwater currents organisms should be gently removed by divers and placed in net bags with appropriate mesh size (Fig. 9; Spagnolo *et al.*, 2014).

Figure 7: Example of dredge to sample soft-benthic fauna (courtesy of CNR-IRBIM Ancona).

Figure 8: Example of zoobenthic sampling using a suction sampler (from Sciuto et al., 2017).

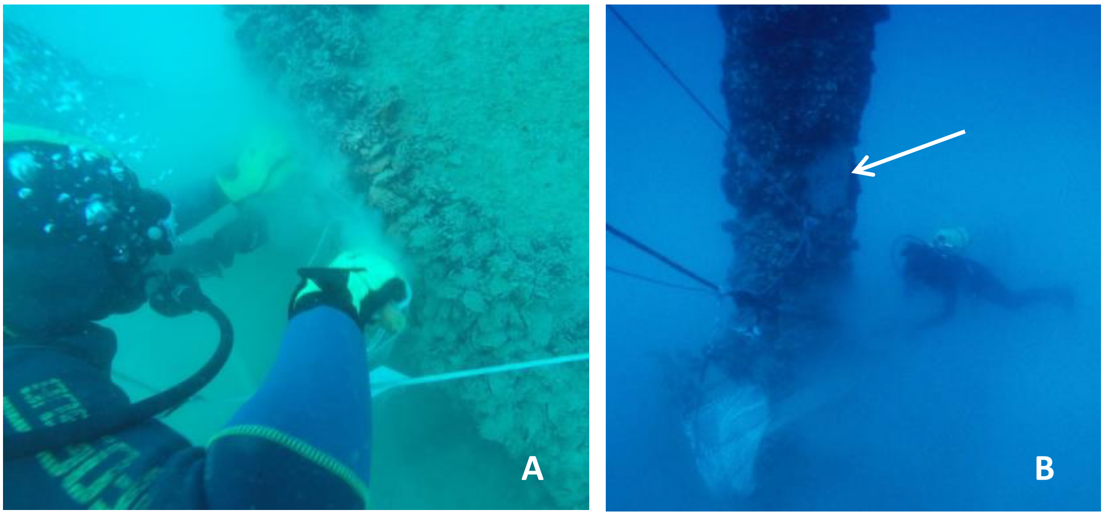


Figure 9: Sampling of a vertical wall using the scraping technique (A) and track of a sample on an artificial structure (white arrow) (B) (courtesy of CNR-IRBIM Ancona).

2.5. Fish assemblage

2.5.1. Sampling methods

2.5.1.1 Non-destructive methods

Underwater visual census (UVC): UVC by divers is historically the most common non-destructive method to evaluate the fish assemblage associated to a reef (Fig. 10), and a range of techniques in a variety of shallow marine habitats has been developed (Bortone & Kimmel, 1991). The most common are listed below:

- ***Strip transect***: the diver swims along a transect of pre-established length in a pre-established time interval listing the species encountered and recording number and size of specimens.
- ***Point count (fixed or mobile)***: the diver stands at a fixed point or swims around a predefined reef unit recording species an enumerating the organisms observed within a prescribed area or volume in a pre-established time interval.
- ***Species-time random count***: this method assumes that abundant species are likely encountered before then the rarer ones. The observer swims randomly over the survey area for a predefined time period either simply recording the species encountered or listing them in the order in which they were initially seen.
- ***Combinations of two or more the above-described methods***. Depending on the features of the reef, it could be more suitable to combine two methodologies rather than adopt only one. This can be the case of patch reefs and most artificial reefs, where open spaces alternate to boulders or manmade structures respectively.

In situ visual methods are relatively rapid, provide adequate levels of replication and can record a broad suite of variables such as relative abundance, density, size structure, species composition, and habitat characteristics (Bortone *et al*., 2000; Samoilys & Carlos, 2000). However, the limitations of diver-based methodologies have been well documented (Thresher & Gunn, 1986; Lincoln-Smith, 1988, 1989; Bombace *et al*., 1997; Thompson & Mapstone, 1997; Kulbicki, 1998) and related to physical limitations (e.g. water depth and visibility) as well as species-specific sources of "detection heterogeneity" (Kulbicki, 1998; Macneil *et al*., 2008), which can be summarized as the ability of the diver to see fish accurately and record their presence under variable conditions (Sale, 1997). A further limitation of UVC is that the fish species react in different ways to the presence of divers: some escape whereas others come closer, making it difficult to maintain the same level of accuracy during a survey.

On the other hand, UVC can be particularly suitable for recording obligate or partially-obligate reef-dwelling species, such as cryptic species living inside the holes of the hard substrates and nekton-benthic and pelagic fish swimming close to the substrates (Bombace *et al*., 1997).

Figure 10: Underwater visual census on a NR (from http://www.theislandergirl.com/category/academia/).

Baited remote underwater video (BRUV): innovations in the development of video technology have resulted in the widespread use of BRUV to monitor fish populations in a variety of habitats (Cappo *et al*., 2004) (Fig. 11). However, BRUV has some differences in rates of detection than the classic UVC, due to the ecological niche and behaviour of the target species (Lowry *et al*., 2011).

Figure 11: Deployment of a BRUV (from https://indooceanproject.org/2017/06/22/b-r-u-v/).

BRUV system has inherent biases such as difficulties in determining the area sampled due to variables associated with the dispersion of bait (Priede & Merrett, 1996, 1998; Bailey & Priede, 2002), conservative relative abundance estimation (Farnsworth *et al*., 2007), and reliance on acceptable visibility (Watson *et al*., 2005).

Moreover, the kind of bait can affect the species and individuals (i.e., adults and/or juveniles) which are attracted depending on their feeding behaviour (Watson *et al*., 2005; Harvey *et al*., 2007). Therefore, BRUV is likely to sample a small portion of the fish population inhabiting a reef (Lowry *et al*., 2011).

Automated estimate of fish abundance: it is an imaging device provided with an autonomous sensor to quantify biotic components at a global scale for an extended period. This experimental imaging device has been specifically designed for acquiring images, recognising and extracting the relevant image content (Marini *et al*., 2013; Corgnati *et al*., 2016). The information extracted from the acquired images can be either stored on board the system or transmitted through a dedicated communication device.

A number of experiments have been carried out to assess the automated recognition performance (Marini *et al*., 2018a, b): the fishes contained in several test images were manually counted and the resulting time-series was compared with the time-series automatically generated founding a good correlation between them.

Hydroacoustic techniques: hydroacoustic techniques, represented by stationary or mobile systems such as single-, dual-, split- and multi-beam echosounders for fish, can provide valuable estimates of fish biomass and spatial distribution over reef-specific natural/artificial sites and relative areas of influence.

Hydroacoustic fish survey methods have also the advantage that they offer a non-destructive means of conducting rapid surveys, and are not hampered by issues such as water clarity, strong currents or diver depth limits. Having minimal impact on fish behaviour, they result in a “snapshot” of fish biomass.

Hydroacoustic methods, however, require for calibration and ground truthing (e.g., underwater observations, fishing sampling) to gain species-specific information, especially in mixed-species assemblages such as those typically inhabiting reefs (Fabi *et al*., 2015), and for the most accurate calculations of fish lengths and weight. This makes it possible determine not only the overall fish biomass around the reef, but also species and relative abundances (Barker, 2016).

Echosounders for fish have been successfully employed in surveying fish assemblages at hydroelectric facilities in riverine environments and around oil and gas platforms (Thorne *et al*., 1990; Stanley *et al*., 1994; Thorne, 1994; Stanley & Wilson, 1998; Soldal *et al*., 2002; Gaetani *et al*., 2016). However, applying this technique to reefs has been very limited so far (e.g., Thorne *et al*., 1989; Fabi & Sala, 2002; Sala *et al*., 2007; Kang *et al*., 2011).

Stationary hydroacoustic methods, when strategically placed and combined with computerized data records, have the advantage of collecting long-term time-series data along the entire water column or at specific depths and providing for the evaluation of fish biomass and spatial distribution and its daily behaviour. The weak point, on the other hand, is the spatial limitation of the sampling area.

Mobile hydroacoustic systems can cover a much greater area per unit of time, allowing large spatial scales to be studied which may be necessary to sample highly mobile species. The relatively fast water column data acquisition of these hydroacoustic methods also adds the time-saving (and therefore often cost-saving) benefits when compared to alternative fish survey methods, and data are immediately digitally recorded and ready to be processed. 3D school detection algorithms can be later used to automatically extract targets and related metric and acoustic features allowing to create 3D virtual scenes of the artificial habitat (Fig. 12).

Although very powerful to estimate the biomass of nekto-benthic and pelagic fish, the hydroacoustic techniques tend to underestimate benthic fish because these are often seen by the system as spikes of the seabed.

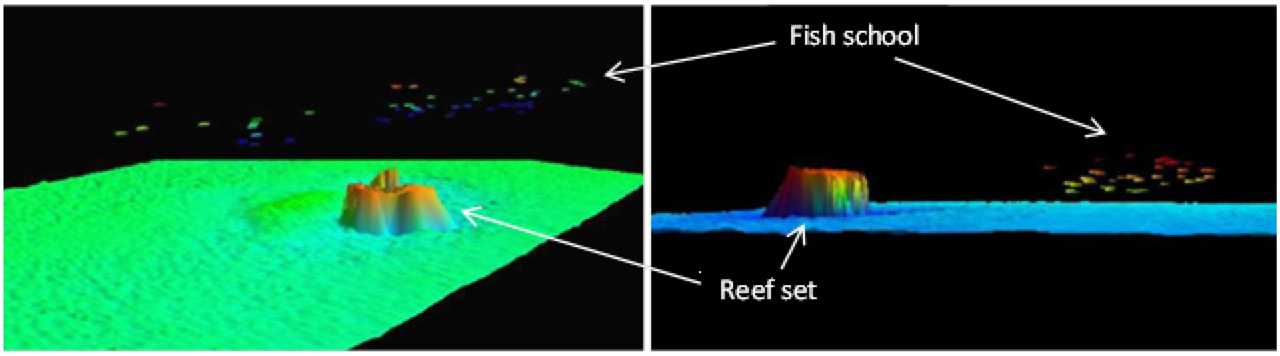


Figure 12: Multibeam Echosounder images of fish schools around artificial reef structures in the Adriatic Sea, Italy: (from Fabi et al., 2015).

2.5.1.2 Destructive methods

These monitoring methods include adaptations of commercial fishing techniques such as traps, longlines, set nets (Gannon *et al*., 1985; Kelch *et al*., 1999) as well as bottom trawling, even though the last one is the least suitable method to be used for monitoring a reef site due to the physical presence of the hard substrates.

The advantages related to the use of fishing techniques include the availability of specimens for biological studies (e.g., gonadic stage, growth, etc.) which are relevant to understand the ecological role of the reef towards different species. Feeding behaviour investigations can be also undertaken if the applied gear has not to be baited. Moreover, the possibility to sample at day and night and in each season over the year, independently from water transparency, allows studying the species assemblages as well as the seasonal changes of the reef fish community.

Oppositely, the potential habitat degradation due to the use of some fishing techniques, the impossibility to observe the behavioural aspects of the species associated with the reef, and the possible underestimation in terms of both size and species due to the selectivity of the gear employed, are clear weaknesses of such approaches. Moreover, these methodologies are often prohibited in sensitive areas such as marine parks (Willis *et al*., 2000; Lipej *et al*., 2003; Cappo *et al*., 2004).

Traps: they are baited, set devices of various shapes depending on the target species (Fig. 13). They can be deployed inside an AR and close to crevices of a NR allowing to sample cryptic and reef-dwelling species. A weakness of traps is their selectivity due to both the kind of used bait and the trap shape.

Longlines: they consist of a main line with baited hooks attached at intervals by means of branch lines. They can be used to sample either pelagic or nekto-benthic species depending on their positioning along the water column (Fig. 14); indeed, longlines can be set as bottom lines (including very rough bottom and/or coral reefs), in midwater or even not far from the surface (http://www.fao.org/). The kind of bait and the hooks' dimension make this device highly selective.

Set nets: set nets can be made of a single wall of netting (gill nets) or three walls (trammel nets), the two outer panels being of a larger mesh size than the loosely hung inner netting one. A third typology is represented by the "combined gill net – trammel net", that is a bottom-set gear made with a gill net with the lower part replaced by a trammel net.

Ste nets can be made of different filaments, mesh size, etc. Due to its features (Fig. 15), trammel net is less selective than gill net so being more suitable to investigate the fish assemblage associated to a specific habitat, catching benthic, nekto-benthic and pelagic species.

Nevertheless, as set nets cannot be used strictly close to the hard substrates, this approach tends to underestimate cryptic or sedentary fish.

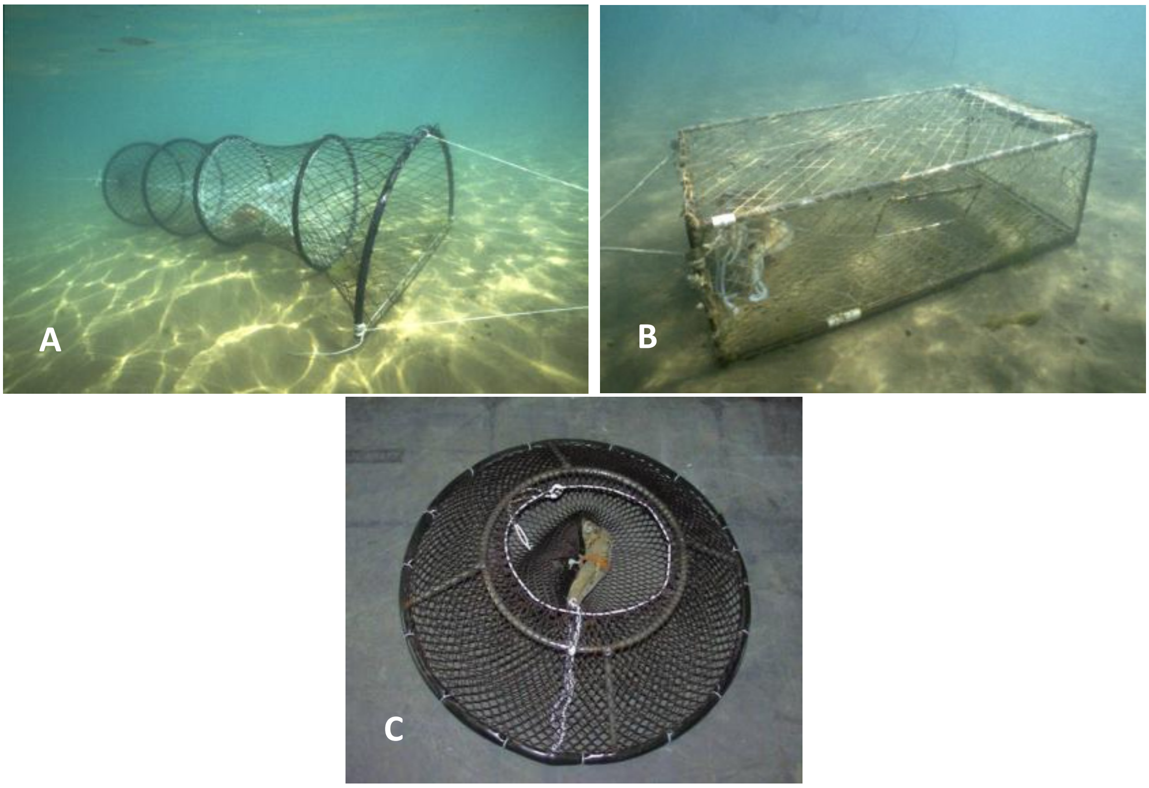


Figure 13: Examples of trap. A and B: fyke net and trap commonly used to catch cuttlefish; C: basket trap for gastropods (courtesy of CNR-IRBIM Ancona).

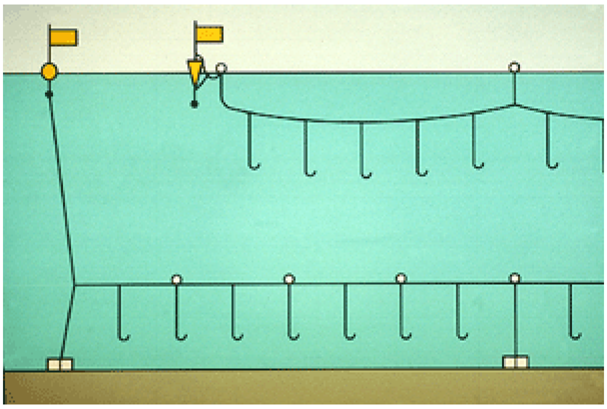


Figure 14: Set longlines at two different depths (from http://www.fao.org/).

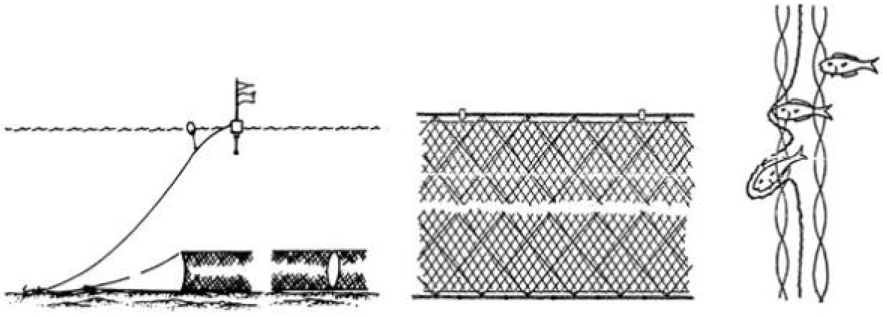


Figure 15: Trammel net features and catching modality (from http://www.fao.org/).

Bottom trawling: the bottom otter trawl net is a cone-shaped net that ends in a bag or coded and it is towed by a vessel, on the seabed.

Bottom trawling is the least selective fishing technique, but even the most destructive for the environment. Moreover, it cannot catch reef-dwelling species due to the impossibility to be carried out inside or strictly close to a reef. Hence, regardless to its scarce selectivity, this methodology does not provide a satisfactory picture of a reef fish assemblage.

Table 1 - Advantages, disadvantages and environmental impact of the different methodologies

GEOMORPHOLOGICAL, GEOPHYSICAL and GEOCHEMICAL CHARACTERIZATION OF THE SEABED			
METHODOLOGY	**ADVANTAGES**	**DISAVANTAGES**	**ENVIRONMENTAL IMPACT**
Acoustic systems	Detailed maps of habitats and sediment features	Some instruments (i.e., single-beam echosounder and side scan sonar) have spatial limitations and/or navigation uncertainties.	none
Grabs, box-corer, multi-corer samplers	No underwater work; possibility to carry out a wide variety of physical, chemical and microbiological analysis	Positioning on the seabed not precise	low
Hand corer	Precise positioning on the seabed; possibility to carry out a wide variety of physical, chemical and microbiological analysis	Underwater work; sampling depth limitation	low
WATER COLUMN			
METHODOLOGY	**ADVANTAGES**	**DISAVANTAGES**	**ENVIRONMENTAL IMPACT**
Sensors	Possibility to monitor in continuous a wide variety of physical, chemical and biological parameters	Subjected to biofouling	none
Bottle-samplers	Possibility to sample at discrete depths and at established sampling points		none
Sediment traps	Possibility to determine downward mass fluxes of particles and associated elements		none
Remote sensing	Possibility to monitor wide areas in continuous	Weather conditions; some parameters based on conversion factors; benthic compartment cannot be assessed	none
BENTHIC COMMUNITIES			
METHODOLOGY	**ADVANTAGES**	**DISAVANTAGES**	**ENVIRONMENTAL IMPACT**
Underwater observations (diving samplings)	accurate detection of crypto-benthic species and taxonomic recognition; possibility to estimate fauna and flora species composition, number of organisms, coverage percentage and relative density and biomass of the sessile community	Influenced by the experience of the operator; limited operating depth; monitoring only possible in good conditions of water transparency;	none
Underwater observations (photos and videos)	Possibility to operate with divers or from on board using ROV or AUV; possibility to estimate fauna and flora species composition,	Underestimation of small or cryptic species; possible low taxonomic precision	none

	number of organisms, coverage percentage and relative density and biomass of the sessile community; possibility to create a reference collection		
2D mosaicking reconstructions	High-resolution construction of the reef; spatial accuracy	Information not always sufficient for a comprehensive understanding of the area	none
Reconstructing 3D virtual environments	Allows the use of the photogrammetry; accurate and repeatable measurements of size, shape and biomass of organisms; not limited by size and orientation of surfaces and organisms	Expensive and time-consuming technique; images with sharp definition are necessary	none
ARMS	Standardized sampling device	Highly expensive; long processing of samples; benthic organisms settled on ARMS could not represent the real benthic composition of the area due to the different materials with which they are made	none
ASUs	Standardized sampling device; processing of samples faster than ARMS	Benthic organisms settled on ASUs could not represent the real benthic composition of the area due to the different materials with which they are made	none
Grabs, box-corer, and multi-corer samplers	No underwater work; possibility of carrying out qualitative and quantitative studies	Positioning on the seabed not precise; only for soft-bottom samplings	low/medium
Dredges	Useful for preliminary surveys to discover the nature of the bottom and its fauna	Unable to adequately sample pre-defined quantities of sediment; it allows sampling only a fraction of the fauna living on the surface of the seabed and very few burrowing animals	very high
Suction samplers	Precision in the sampling points	Underwater work; depth limitation	low/medium
Scraping technique	Precision in the sampling points	Underwater work; depth limitation; possible loss of organisms during the sampling operations	high
FISH ASSEMBLAGE			
METHODOLOGY	**ADVANTAGES**	**DISAVANTAGES**	**ENVIRONMENTAL IMPACT**
UVC	Relatively rapid methods; possibility to record a broad suite of variables (e.g. relative abundance, density, size structure, species composition)	Underwater work; good visibility; depth limitation; Influenced by the experience of the operator and by fish behaviour	none

BRUV	Monitoring of fish population in a variety of habitats; possibility to monitor specific portions of the finfish community (e.g., carnivorous, herbivorous) depending on the used bait	Good visibility; bait tipology can affect the species with a loss of information in terms of species richness	none
Automated estimate of fish abundance	Automated image acquisition of macro and mega fauna; work in different environmental conditions	Biofouling; expensive	none
Hydroacoustic techniques	Valuable estimates of fish acoustic biomass, density, spatial distribution, and area of influence of reef on fish assemblage; independent from the visibility	Calibration needed; difficulty of identifying the species; underestimation of benthic species	none
Traps	Possibility to be deployed inside an AR and close to crevices of a NR with consequent sampling of cryptic and reef-dwelling species	High selective	medium
Longlines	Possibility to be deployed close to hard substrates at different depths	High selective	medium
Set nets	Depending on the net typology, they can be suitable to catch benthic, nekto-benthic and pelagic species. Studies on feeding behaviour of the species are possible	Cannot be used strictly close to the hard substrates; possible underestimation of cryptic and/or sedentary fish	medium
Trawling	Very low selective fishing technique	Cannot be used close to the hard substrates; not suitable to sample cryptic and/or sedentary fish	very high

3. ANALYSIS OF THE APPLICABILITY OF LOW IMPACT TECHNOLOGIES TO MONITORING PURPOSES

The crucial aspect of the investigation of a given area is the possibility to obtain the most possible information. It is particularly important in the case of reefs (both natural and artificial) that are very complex ecosystems. Many studies stated that the use of a single monitoring technique is reductive because each has advantages and disadvantages.

Seabed characterization represents a fundamental issue to evaluate extension, morphological features (e.g., slopes, picks, etc.) and integrity of a reef. Among the possible, non-destructive, instruments historically used to obtain information on geomorphological and geophysical features, MBES appears the most suitable providing a 100% coverage of seabed morphology and water column, offering a 3D high-resolution picture of the overall investigated site. Moreover, as SSS, MBES also provides a description of sediment features, although at a lower resolution. Regarding this last aspect, both these instruments can provide information on the presence of sand, mud or rock but they cannot discriminate grain size at a higher level and the percentage of the different components; hence, they should be associated to collection of a number of sediment samples. Instead, geochemical characterization needs of taking sediment samples to analyse in the lab. For this purpose corers appear more appropriate than grabs avoiding mixing of the sample and making it possible to take specific sections.

Study of water column (physical and chemical features) does not induce any impact on the environment. Various instruments can be used, depending on the aspects to be investigated. However, the use of sensors can provide complete information and continuous collection of data.

With regard to the benthic communities settled on hard substrates, photographic or video techniques, possibly associated with 3D reconstruction, appear to be the best low-impact technologies. Their limit is the inability to recognize many species (e.g., sponges, polychaetes, crustaceans) whose require a deep microscopial analysis for correct taxonomic classification. This limit may be overcome by using ARMS or ASUs associated with morphological analysis. However the benthic assemblage settled on these devices could not really represent the community living on the reef substrates because of the different material they are made of. So, the characterization of the surrounding environment where ARMS or ASUs units are deployed should be carried out for a comparison with natural assemblages.

In the case of soft-bottom benthic communities, photograph or video techniques appear suitable to detect the presence of macro-organisms living on the surface (e.g., crabs, some molluscs and echinoderms), but they are not useful to obtain an exhaustive picture because most of species live inside the bottom and their presence can be observed only analysing samples of sediment. For these reasons, it is necessary to adopt other sampling methods, preferably grabs, box-corer or suction samplers which have lower impact in respect to dredges and allow to obtain quantitative data.

Regarding the fish assemblage, UVC is suitable for recording cryptic species but it monitors a smaller fraction of the reef population than that sampled by fishing gears which give a broader picture of the fish community living in and around the reef (Bombace *et al*., 1997).

The most suitable UVC technique to be adopted depends on the habitat that has to be investigated. Strip transects and fixed/mobile point counts are mostly used in natural rocky habitats (Vacchi & Tunesi, 1993; Francour, 1994; Vacchi *et al*., 1998; García-Charton *et al*., 2004) and comparison between both have been carried out by Bortone *et al*. (1989) and Guidetti *et al*. (2005) in natural habitats and by Consoli *et al*. (2007) in artificial ones. Bortone *et al*. (1989), studying different UVC methods to assess the fish assemblage living at a natural coral reef, stated that transect and point methods appear to be most useful when evaluating a reef biotope as both methods permit divers to record numbers of species and individuals as values relative to area. Random method, while clearly allowing divers to record more information in terms of numbers of species, merely presents a score of relative abundance without regard to area. Thus, the use of different UVC methods allows obtaining complementary information (D'Anna *et al*., 1999; Guidetti *et al*., 2005).

Obviously, UVC has to be carried out by expert divers with a deep knowledge of the species living at the reef. Instead, the use of other methodologies such as foto- video cameras fixed on the substrates or mounted on ROV or AUV allows to record images that can be analyzed successively. Nevertheless, these are unsuitable in identifying crypto-benthic species due to their tendency to hide in hole and crevices that make these fish not visible to the camera (Andaloro *et al*., 2013).

In conclusion, it is a matter of fact that a reef, natural or artificial, is a very complex ecosystem and its assessment is a very hard task. As scientific objectives surrounding complex ecological questions are very broad and include a number of more specific goals, the type and quantity of data to be collected depend on the kind of questions to be answered.

Monitoring programs exist for Croatian natural reefs (Garrabou *et al*., 2014; RAC/SPA – UNEP/MAP, 2014), where monitoring techniques are described in detail; instead, artificial habitats are often criticized for a lack of planning in the development of monitoring programs.

Due to the great variety of rocky habitats in terms of reef arrangement or, in the case of artificial habitats, structures, materials, etc., it is difficult to identify a common methodology to monitor the various abiotic and biotic aspects; in fact, no single technique is capable of completely describing an aspect and a combination of techniques should be employed and adjusted according to the morphological and geographical characteristics of each reef site.

Independently from the techniques used, it is essential to standardize results from studies carried out using different methodologies.

In the context of the ADRIREEF project, a few low impacting methodologies were identified to investigate the different components of the marine ecosystems associated to NRs and ARs and evaluate their suitability for implementing already existing economic activities and/or developing new ones according to the blue economy (Table 2). These methodologies have been included into the monitoring protocols to be applied to the different CSs (see Chapter 4).

Table 2 – Possible low impacting methodologies for the monitoring of the CSs.

ADRIREEF MONITORING PROTOCOL FOR NATURAL AND ARTIFICIAL REEFS												
	Evaluation of environmental load			**Implementation of diving**			**Implementation of Recreational fishing**			**Extensive mussel culture**[1]		
	Scope	**Methodology**	**Timing**	**Scope**	**Methodology**	**Timing**	**Scope**	**Methodology**	**Timing**	**Scope**	**Methodology**	**Timing**
Geomorphological mapping	Description of morphological features of the reef; map for planning the other investigations	MBES and/or SSS[2]	1 survey	Description of morphological features of the reef; map for planning the other investigations	MBES and/or SSS	1 survey	Description of morphological features of the reef; map for planning the other investigations	MBES and/or SSS	1 survey	Description of morphological features of the reef; map for planning the other investigations	MBES and/or SSS	1 survey
Water column	Dispersion of pollutants	Current direction and speed	In continuous	Investigate the suitability of the site for diving	Current direction and speed	In continuous						
	Characterization of the water column	Physical parameters (T°, S, turbidity, O_2)	In continuous or spot sampling (minimum: once per month)	Investigate the suitability of the site for diving and identify the most suitable season/s over the year	Physical parameters (T°, turbidity)	In continuous				Characterization of the water column	Physical parameters (T°, S, turbidity, O_2)	In continuous or spot sampling (minimum: once per month)
	Evaluation of the environmental load due to tourism	PH, Redox, nutrients Chlorophyll, CDOM, PAH, AH, *Escherichia coli* (according to the local law)	Spot sampling (once per month)							To evaluate the water quality	PH, Redox, nutrients Chlorophyll, CDOM, PAH, AH, etc. (according to the local law)	Spot sampling: once per month or according to the local law
Shellfish										To evaluate shellfish quality	Heavy metals, organohalogenate substances, PAH, faecal coliforms, *E. coli*, phycotoxins, etc. (according to the local law)	Spot sampling: every 6 months or seasonally according to the local law

Benthic community settled on the reef	Investigate the status of the community in terms species and coverage	Photos equipped with macrophotography at fixed georeferenced points	Seasonally	Investigate the attractiveness of the reef for divers	Photos at fixed georeferenced points	Seasonally				To evaluate mussel biomass without collecting them	Photos equipped with macrophotography + 3D model	Seasonally
Fish assemblage				Qualitative and quantitative assessment of the fish assemblage	Visual census (UVC) with ROV or drone[3]; UVC with scuba divers	Monthly (to evaluate the best season for diving)	Qualitative and quantitative assessment and structure by size classes of the fish assemblage	UVC with ROV equipped with stereo-photocamera; UVC on fix points with stereo-photocameras[5]	monthly			
					UVC on fix points with videocameras or photocameras[4];	In continuous						
				To get complementary information and validate the remoted visual census	UVC with divers	At least once every 3 months (at least from spring to fall)	To get complementary information and validate the remoted visual census	UVC with divers	At least once every 3 months			

Additional methodologies for dissemination/communication	BRUV, 360° videocamera

[1] Extensive mussel culture: natural settlement of wild mussel populations on artificial substrates.

[2] MBES would be preferred as it provides a complete picture of the bathymetric and ecological features of the reef; moreover it allows to get 3D views of the reef (useful for example for dissemination/communication).

[3] UVC with ROV equipped with a single video camera can only provide data on fish species and number of fish but it does not allow to get data on the structure by size of the fish population. It is useful to explore depths where divers cannot arrive. UVC with ROV and with divers are complementary.

[4] Fixed video/photo cameras can only provide qualitative data on the species of the fish assemblage but they cannot properly record highly mobile species as well as cryptic species which stay out of their field of view, hence they should be integrated with UVC performed by divers and/or ROV in order to have a complete picture of the fish assemblage species composition.

[5] Stereo video/photo cameras have the capacity of accurately and precisely measure the size of both fish and survey area.

4. FEASIBILITY STUDY FOR THE FOLLOWING PHASE IN WP4 - ACTIVITIES 4.1 AND 4.2

The review of the monitoring methodologies, the identification of those which are less impacting on the environment and the resources (Chapter 2), and the evaluation of their applicability in different conditions (Chapter 3) constituted the basis to identify the most suitable and in a few cases even innovative techniques to be adopted to monitor the CSs in order to assess their potential for implementing sustainable economic activities in line with the Blue Economy. As described in the report of Activity 3.3, these activities have been potentially identified for each CS basing on its peculiarities and available knowledge (Scanu *et al.*, 2019).

Therefore, the present Chapter describes the methodological protocol chosen to be implemented at each CS, taking also into account the feasibility of the different methodologies in relation to the features of each location and the available budget within the ADRIREEF project.

Preference has been given to not impacting and, where possible, new techniques, while the employment of traditional, more impacting techniques (e.g., scraping of surfaces to collect sampling of sessile community) will be occasional and exclusively aimed to validate the most innovative methodologies.

4.1. Case Study "Paguro" wreck (Artificial reef)

Existing information on the "Paguro" wreck derives from studies and monitoring programs carried out by research institutes (e.g. University of Bologna, Centro Ricerche Marine – Cesenatico) since the mid-90s. These activities were aimed at describing the evolution of biotic communities inhabiting the wreck, thus estimating their potential ecological and economic value and posing the basis for the SCI management plan. More recently, ARPAE Emilia-Romagna chose the "Paguro" area as sampling site for the Marine Strategy Framework Directive (MSFD). This sampling forecasts: the collection of annual samples of sediments and biota in the period 2018-2020 to investigate the presence of environmental contaminants.

Within ADRIREEF project, ARPAE Emilia-Romagna aims at testing innovative monitoring techniques with low environmental impact, thus diversifying the data available for the SCI site and increasing its attractiveness for scuba divers.

A description of the geomorphological features of the reef will be obtained through a one-time multibeam echo sounder (MBES) survey, covering both the "Paguro" wreck and the additional iron structures sunk in 1990-1991 and 1999-2000.

Physical parameters (i.e. temperature, salinity, conductibility, pH, Optical Dissolved Oxygen, PAR, chl-a and turbidity) will be sampled continuously from the surface to the sea bottom by using a CTD multiprobe IDRONAUT 316 Plus. Water samples for nutrients will be collected at the sea surface and at sea bottom using a Niskin bottle. Multiprobe data will be published on social media using interactive web-interfaces. ADCP and data loggers will be placed at the sea bottom for collecting recording data on intensity and current direction.

Photogrammetric surveys will be performed on the study site at three different spatial scales (small, medium and large) according to local sea conditions and water quality:
(1) Small scale photogrammetric surveys will cover areas less than 20 m^2 each and will be performed with horizontal visibility between 1 m to 5 m. Additionally, traditional sampling will be carried out on standard area to be used as a reference to estimation of communities' biomass and diversity by photogrammetry.
(2) Medium scale photogrammetric surveys will cover areas between 20 m^2 and 50 m^2 each and will be performed with more than 5 m of horizontal visibility between 5 m and 10 m. The resulting point clouds will be classified to community facies and associations and used to generate 3D benthic maps.
(3) Large scale photogrammetric surveys will be performed on the study site at large scale cover areas greater than 50 m^2 and will be performed according to local sea conditions and water quality (i.e. horizontal visibility greater that> 10 m).

Moreover, Underwater Visual Census (UVC) for fish assemblage will be performed by scuba divers and will be aimed to: [1] add informative contents to the areas mapped with photogrammetric approaches; [2] validate visual census data obtained by using stereo video cameras positioned at fixed stations.
[1] standard approaches of UVC along transects or at fixed stations will be chosen accordingly to the topography and extension of the mapped area. Visual census at fixed stations will be preferred for locations already defined for the deployment of the stereo camera. Data on fish abundance and diversity for the most representative species will be added to the 3D model of the area. 3D models will be available on an interactive online web-interface. Before starting the surveys, scaled boards will be placed across the study areas to scale the 3D models and estimate their accuracy.
[2] UVC will be carried out by 2 scuba divers the same date and at the same location of stereo cameras surveys. The census will last 10 minutes and will be replicated twice at each sampling event before recording videos with the stereo cameras. Data on fish abundances, diversity, and size will be used to validate the stereo camera records.

Visual census on fish community will be performed with digital video cameras mounted on a stereo configuration. Cameras equipped with an illumination system will be positioned on fixed stations. The cameras will record videos at full HD over 1 hour and 15 minutes. Fixed stations will be selected considering the best functioning of the stereo video software and the automatic fish tracking and identification. At the beginning of each survey, the stereo camera will be calibrated by the mean of a control frame with dimensions of 60 cm x 40 cm. A self-calibrating photogrammetric network solution is used to calibrate both the internal and external parameters of the camera and to obtain length estimates of fishes. Images obtained will be analysed to identify and count fishes encountered, and then processed with the associated software (SEBASTES or similar) to get length estimations.

	Case study "Paguro" wreck		
Type of analysis	**Scope**	**Methodology**	**Timing**
Geomorphological mapping	Description of morphological features of the reef	The survey will consist of parallel transects by MBES to cover the artificial reef area within SIC-ZPS IT4070026 including the Paguro wreck and other iron debris sunk in 1990-1991 and 1999-2000.	One survey in autumn 2019
Water column parameters	Characterization of the water column (physical and chemical parameters), current direction and speed along the water column Give stakeholders (divers) updated information on oceanographic status	Physical parameters (i.e. temperature, salinity, conductibility, pH, Optical Dissolved Oxygen, PAR, chl-a and turbidity) will be sampled continuously from the surface to the sea bottom by using a CTD multiprobe IDRONAUT 316 Plus. Water samples for nutrients will be collected at the sea surface and at sea bottom using a Niskin bottle. Multiprobe data will be published on social media using interactive web-interfaces. ADCP and data loggers will be placed at the sea bottom for collecting data on intensity and current direction.	Water column physical and chemical parameters: fortnightly during the summer period and monthly from fall 2019 to spring 2020 Nutrients: seasonally ADCP data: acquisition frequency 10 min; data collected seasonally
Benthic community settled on the reef	Describe the status of the community in terms of species composition and coverage to provide information for divers	Photogrammetric surveys at three different spatial scales (small, and medium, and large) according to local sea conditions and water quality: (1) small scale photogrammetric surveys on areas less than 20 m^2 each and with horizontal visibility between 1 m to 5 m. Traditional sampling will be carried out on standard area to be used as a reference to estimation of communities' biomass and diversity by photogrammetry; (2) medium scale photogrammetric surveys on areas between 20 m^2 and 50 m^2 each and horizontal visibility between 5 m and 10 m; (3) large scale photogrammetric surveys at areas greater than 50 m^2 according to local sea conditions and horizontal visibility greater than 10 m. The resulting point clouds will be classified to community facies and associations and used to generate 3D benthic maps. Before starting the surveys, scaled boards will be placed across the study areas to scale the 3D models and estimate their accuracy.	Seasonally
Fish assemblage	Describe the fish population in terms of species composition and abundance to provide stakeholders (e.g. diving) with updated	Photogrammetric surveys will be performed on the study site at large scales (i.e. greater than 50 m^2) according to local sea conditions and water quality (i.e. horizontal visibility > 10 m). Fish visual census will be performed by scuba divers and will be aimed to: [1] add informative contents to the areas mapped with photogrammetric approaches, [2] validate visual census data obtained by using stereo video cameras positioned in fixed stations (see following cell).	Seasonally (at least from spring to fall), potentially with increased frequency during the summer season

	information on the fish assemblage	[1] within the areas selected for the photogrammetric mapping. Standard approaches of visual census along transects or at fixed stations will be chosen accordingly to the topography and extension of the mapped area. Visual census at fixed stations will be preferred for locations already defined for the deployment of the stereo camera. Data on fish abundance and diversity for the most representative species will be added to the 3D model of the area. 3D models will be available on an interactive online web-interface. Before starting the surveys, scaled boards will be placed across the study areas to scale the 3D models and estimate their accuracy. [2] Underwater Visual Census (UVC) will be carried out by 2 scuba divers the same date and at the same location of stereo cameras surveys. The census will last 10 minutes and replicated twice at each sampling event before recording videos with the stereo cameras. Data on fish abundances, diversity, and size will be used to validate the stereo camera records.	
	Evaluate the effectiveness of the visual census carried out by using digital video cameras mounted in a stereo configuration, to describe the fish community	The comparison of the data collected by scuba divers with those gathered by processing the stereo videos, will be used to estimate the effectiveness of the visual census carried out by using digital video cameras mounted in a stereo configuration, to describe the fish communities.	Seasonally (at least from spring to fall)

4.2. Case Study Plić Lagnjići (Natural reef with a ship wreck)

To date, the CS location is exploited only by fishing activity; the coastal area is accurately avoided by the maritime traffic because of the danger due to the pitfalls of some shoals, protruding from the bottom.

The knowledge on the features of the site is currently limited to the characterization of the underwater habitats of the western side of Pliči, carried out by Sunce Association in 2009. Since the CS location is included in the ecological network of Luka Soliščica (HR3000067), aimed to protect habitats of sandbanks, *Posidonia* beds, and bays, to implement investigations in the area could be particularly useful not only for conservation but even for sustainable development purposes.

Activities that are planned to be implemented through the monitoring phase include: geomorphological mapping of the reef location, measurement of water column parameters in order to assess the water quality and oceanographic status, investigations on the sediments surrounding the reef, algae and phanerogams (*P. oceanica* and *C. nodosa*) occurrence, benthic community settled on the soft and the hard substrates, and the associated fish assemblage.

Results will be used to characterize the entire site and detect the strengths of the location for the implementation of tourist activities in agreement with the Blue Economy, such as recreational scuba diving.

	Case study Plić Lagnjići		
Type of analysis	**Scope**	**Methodology**	**Timing**
Geomorphological mapping	Description of morphological features of the reef Production of maps useful for planning the other investigations	Parallel transects with HELIX 10 – SOLIX 10 (Humminbird). The survey will assure a 100% coverage of the reef area.	One survey in 2019
Water column parameters	Characterization of the water column and dispersion of pollutants Give stakeholders (divers) information on water quality and oceanographic status	All physical and chemical parameters will be monitored (temperature, oxygen, salinity, pH). In addition, the following chemical and biological parameters will be determined: nutrients (nitrate, nitrite, phosphate, silicate) ammonium, PAH, dissolved organic matter, sanitary quality (faecal coliforms, *E. coli).*	Monthly
Benthic community settled on the reef and sediments	Describe the community status in terms of species assemblages and coverage Provide information to divers	Photographic samplings of standard areas frames. The factors taken into account in the sampling strategy are: (1) two sides of the reef, the east ''inner'' side and west open water side (2) and proportional coverage of the reef in percentages (%) of all present basic habitat types, including: rock (R), unconsolidated sediment (U), algae on sediment (A), *P. oceanica* (P) and *C. nodosa* (C). The following compounds will be determined n sediments: oxygen, PAH, dissolved organic matter, sanitary quality (faecal coliforms, *Escherichia coli).*	At the beginning and end of the monitoring program
Fish assemblage	Describe the fish population in terms of species composition and abundance to provide stakeholders (e.g. diving; recreational and professional fisheries) with updated information on the fish assemblage	Visual census by a remotely operated vehicle (ROV) equipped with digital video camera. Visual census using a Baited Remote Underwater Video (BRUV) mounted in a stereo configuration. The factors taken into account in the sampling strategy are (1) two sides of the reef, the east ''inner'' side and west open water side, (2) two points on each side of the reef will be used for species composition and abundance estimation: a) N-MAX (maximum number of species per frame) and b) T1 (the time of first arrival) as proxy for encountering the fish by diver. For BRUV there will be 5 replicates per system and 6 systems will be used for a total of 30 replicates per each survey. Adoption of stereo BRUV system will allow to use the SEAGIS Event Measure software to estimate fish size. Each system will be calibrated before the survey.	Monthly (to evaluate the best season for diving)
	Evaluate the effectiveness of the remotely operated vehicle (ROV) and BRUV to describe the fish communities	Underwater Visual Census (UVC) with scuba divers and using a Lure Assisted Visual Census. The sampling strategy is the same adopted for the UVC conducted by the ROV and BRUV. One scuba diver will investigate each transect done with ROV. Data obtained will be used to validate the observations obtained with ROV. The Lure Assisted Visual Census will be used to validate the observation conducted with BRUV.	Seasonally (at least from spring to fall)

4.3. Case Study Porto Recanati – Porto Potenza Picena (Artificial reef)

The majority of information on the AR derives from a monitoring programme aimed to evaluate the environmental effects of the reef deployment, carried out in the period 2000-2005 by the CNR-IRBIM of Ancona. In this context, the reef structural conditions were assessed by periodic diving, the soft- and hard-bottom macrozoobenthic assemblages sampled by suction samplers and scraping, and the fish assemblages investigated using bottom trammel nets placed both the AR and at an open sea control site.

The same institution is also responsible, within the ADRIREEF project, for the monitoring programme aimed at verifying the reef suitability for the implementation and/or development of potential economic activities. Methodologies applied in this context are thought to pursuit three main objectives: to create a data set useful to promote the AR usages, monitor the biological status of the reef, and develop and test low impact, automated sampling methodologies. Parameters of interest regard the structural conditions of the reef modules, the water column characteristics, benthic community status, shellfish coverage and the fish assemblage.

A survey with a multibeam echo sounder was already conducted in 2018-2019 to assess the bulk volume of the artificial reef and to produce an updated map of the site. Subsequently, a fixed buoy equipped with solar panel, rechargeable battery, a data logger and a router + 4g antenna will be moored within the reef perimeter. This setting enables continuous sampling, on a frequency of 15/20 minutes, and real-time data transmission. Parameters collected consist in the characterization of the water column and currents' tracking and would be disseminated to diving centers. The oceanographic information is helpful for planning diving activities, as it permits to evaluate a priori those parameters crucial for a safe and nice dive, such as currents and turbidity.

The samplings for mussel coverage and biomass estimation are scheduled on a biannual frequency, through underwater photogrammetry. Elaborations obtained will be compared with the results of scraping samplings to validate the estimation. The study of mussel biomass throughout the year and of the re-colonization patterns aims to identify the better areas and the harvest rates suitable to implement a long-term sustainable exploitation strategy. The final goal will be the enforcement of a system of pre-established quotas assigned to local fishermen associations.

Mussels samples obtained for validating SfM estimates will be also utilised to evaluate the suitability for human consuming. Analysis will be carried out to investigate the levels of heavy metals, organohalogenate substances, PAH, faecal coliforms, *E. coli*, phycotoxins, etc. according to the local law.

Photographic samplings of the benthic community settled on the reef will be also collected on a biannual basis, while the fish assemblage will be assessed on a seasonal basis using a combination of visual census methodologies: scuba divers and a remotely operated vehicle (ROV). Scuba divers data serve to validate the ROV estimation, which will be the main sampling methodology especially during winter months. Pictures of benthic community and ROV videos will be used, at one hand, to describe the status of the community and to make a comparison with samples collected in the past. On the other hand, the information will be transmitted to diving centres to promote the AR as dive site and to decide the best period for diving. Assessment of the fish assemblage occurring at the reef will be also used to evaluate a possible exploitation of the site by recreational fishers and/or by the local small-scale fishermen using set gears.

	Case study Porto Recanati – Porto Potenza		
Type of analysis	**Scope**	**Methodology**	**Timing**
Geomorphological mapping	Description of morphological features of the reef Production of maps useful for planning the other investigations	Parallel transects with MBES EM 2040C-D (Kongsberg Simrad). The survey assured a 100% coverage of the reef area.	One survey in fall 2018 and winter 2019
Water column parameters	Characterization of the water column and dispersion of pollutants Give stakeholders (divers) real-time information on oceanographic status	A fixed buoy moored within the AR area provided with CTD sensor, Acoustic Doppler Current Profiler (ADCP), solar panel, rechargeable battery, a data logger and a router + 4g antenna would ensure the collection and transmission of water column parameters. The transmission would occur in real time to a land-based receiver, with a 15 minutes frequency. Water column parameters collected are Temperature, Salinity, Conductibility, Optical Dissolved Oxygen, and Turbidity. The ADCP will collect data on Intensity and direction of currents along the water column.	In continuous (15 minutes frequency)
Shellfish	Describe the patterns of biomass and density distribution of mussels for implementing a sustainable harvesting strategy Developing non-invasive methodologies	Underwater photogrammetry and scraping samplings for photogrammetry validation. The factors taken into account in the sampling strategy are: (1) north-south gradient, consisting of three level corresponding to three sampling areas located at the north-side, central and south-side of the reef; (2) seafloor elevation, which includes < 2m and >2m from the bottom; and (3) structure shape, considering two levels: (pyramids and poles). The number of replicates considered is one by each level of factors, meaning 3 * 2 *2 = 12 replicates to be collected on each survey. At each of the survey locations, an high-definition camera is used to collect imagery for Structure from Motion (SfM) analysis and samples (40 cm x40 cm frames) are scraped. The digital information collected within the photogrammetric surveys are processed to calculate 3D dense point clouds. Subsequently, scaled point clouds, 3D models and quasi-orthorectified projected images are used to estimate mussel colonies' structure, morphometries and biomass. SfM estimates are validated against the ground truth dataset constructed on the scraping samplings of standard areas	Biannual samplings
	Evaluate the shellfish quality	Analysis aimed to investigate the levels of heavy metals, organohalogenate substances, PAH, faecal coliforms, *E. coli*, phycotoxins, etc. according to the local law.	Biannual samplings
Benthic community settled	Describe the status of the community in terms of	Photographic samplings of standard areas frames. The factors taken into account in the sampling strategy are: (1) north-south gradient, consisting of three level corresponding to three sampling	Biannual samplings

on the reef	species assemblages and coverage To provide information for divers	areas allocated at the north-side, central and south-side of the reef; (2) inclination, including the two levels “Horizontal face” and “Vertical wall”. The number of replicates considered is 3 by each level of factor, meaning 3 * 2 * 3 = 18 replicates to be collected on each survey. The images will be analyzed with Image Analysis systems (PhotoQuad or similar), considering (1) coverage (cm^2) and (2) species number as variables to be registered.	
Fish assemblage	Describe the fish population in terms of species composition and abundance to provide stakeholders (e.g. diving; recreational and professional fisheries) with updated information on the fish assemblage	Visual census by the use of a remotely operated vehicle (ROV) equipped with digital video cameras mounted in a stereo configuration. The factors taken into account in the sampling strategy are (1) the north-south gradient, consisting of three level corresponding to three sampling areas allocated at the north side, central and south side of the reef; (2) the aggregation effect of AR modules, considering as levels “proximity to modules” and “open spaces” (spaces between the structures). The number of replicates considered is 3 by each level of factor, meaning 3 * 3 * 3 = 18 replicates to be collected on each survey. To account for different levels of the factor “aggregation”, the method adopted is based on a mixed methodology: strip transects + point count (modified from Samoilys & Carlos, 2000). At the beginning of each survey, the stereo camera will be calibrated by the mean of a control frame with dimensions of 1×1. A self-calibrating photogrammetric network solution is used to calibrate both the internal and external parameters of the camera and to obtain length estimates of fishes. Images obtained will be analysed to identify and count fishes encountered, and then processed with the associated software (SEBASTES or similar) to get length estimations.	Monthly (to evaluate the best season for diving)
	Evaluate the effectiveness of the remotely operated vehicle (ROV) to describe the fish community	Underwater Visual Census (UVC) with scuba divers. The sampling strategy is the same adopted for the UVC conducted by the ROV. Two scuba divers would carry out each replicate simultaneously, and the result would be a mean of the observation. Data obtained would be used to validate observation conducted with the ROV.	Seasonally (at least from spring to fall)

4.4. Case Study Torre Guaceto Marine Protected Area (Natural reef)

The investigations that will be implemented in the CS area will be aimed at improving the current knowledge on the NR, with reference to the habitat 1170 of the Habitat Directive "REEFS" defined as "*either biogenic concretions or of geogenic origin. They are hard compact substrata on solid and soft bottoms, which arise from the sea floor in the sublittoral and littoral zone. Reefs may support a zonation of benthic communities of algae and animal species as well as concretions and corallogenic concretions*".

Since a geomorphological mapping of the area is already available, the activities will have two different goals: monitoring with innovative methods and improving information for the sustainable exploitation, according to the rules of the Marine Protected Area, of the reefs. For the first goal, the following investigations are forecasted:

- characterization of the water column;
- evaluation of the population and spatial distribution of *Pinna nobilis*;
- description of the status of the benthic community settled on the reef in terms of species assemblages and coverage;
- description of the fish population in terms of species composition and abundance to provide stakeholders (e.g. diving; recreational and professional fisheries) with updated information;
- evaluation of the effectiveness of the remotely operated vehicle (ROV) to describe the fish assemblages.

With concern to the second goal, the information got through the monitoring activities will be used to improve the attractiveness of the reef towards the wide society. In particular, the main aim is to create appropriate underwater paths for divers, that will be used to collect sensitive data on the species of interest. In this way an increase in scientific information would be obtained through the involvement of citizens (citizen science).

Case study Marine Protected Area of Torre Guaceto			
Type of analysis	**Scope**	**Methodology**	**Timing**
Geomorphological mapping	Description of morphological features of the reef Production of maps useful for planning the other investigations	Parallel transects with MBES EM3002D (Kongsberg Simrad). The survey assured a 100% coverage of the reef area.	The geomorphological mapping is already available from the project BIOMAP (BIOcostruzioni MArine in Puglia), promoted by Puglia Region, as a part of the program "PO FESR 2007/2013 – AXIS IV – line 4.4: intervention for the ecological network"
Water column parameters	Characterization of the water column	A bimonthly water column measures of principal chemical-physical (salinity, oxygen, nutrients, etc.) parameters will be taken.	Bimonthly
		Data log of temperature and light	In continuous
***Pinna nobilis* Monitoring**	Evaluate the population and distribution of *P. nobilis* to provide information to divers	Protocol about the characterization of the state of the population: number of individuals for m^2, state of health of the individuals, depth, type of substrate, size of the shell (total length, maximum and at the base width) reporting of specific critical issues and / or impacts from human activities. Visual census and biometric data collection will be carried out by divers in three area of 100m*100m (three transepts of 100 m length for each area).	Once
Benthic community settled on the reef	Describe the status of the community in terms of species assemblages and coverage Provide information to divers and investigate the attractiveness of the reef for benthic organisms	Photographic samplings of standard areas frames. The factors taken into account in the sampling strategy are: (1) North-South gradient, consisting of three level corresponding to three sampling areas allocated at the north-side, central and south-side of the reef; ; (2) inclination, including the two levels "Horizontal face" and "Sloped face". The number of replicates considered is 3 by each level of factor, meaning 3 * 2 * 3 = 18 replicates to be collected on each survey. The images will be analyzed using Image Analysis systems (PhotoQuad or similar), considering (1) coverage (cm^2) and (2) species number as variables to be registered.	Biannual samplings (2 seasons)
	Identification of ASPIM species to create points of interest during the development of underwater routes	The use of routes by divers, appropriately trained, will be used to collect the presence on the species of interest. In this way an increase in scientific information would be obtained through the involvement of citizens (citizen science).	Biannual samplings (2 seasons)

Fish assemblage	Describe the fish population in terms of species composition and abundance to provide stakeholders (e.g. diving; recreational and professional fisheries) with updated information on the fish assemblage	Visual census carried out by divers in fixed monitoring stations. The monitoring strategy 3 stations taking into account (1) geographic exposition: north-south gradient consisting of three level corresponding to three sampling areas allocated at the north, central and south-side of the reef; (2) aggregation effect of natural reef, considering different levels of coralligenous patchiness and morphology. The stations will be positioned based on the identification of the points of interest (ASPIM) identified during the preliminary investigations. When the remotely operated vehicle (ROV) (approximately spring/summer 2020), equipped with digital video and cameras mounted in a stereo configuration will be available, the fish assemblages analysis will be operated through video analysis. The factors taken into account in the sampling strategy are (1) the north-south gradient, consisting of three level corresponding to three sampling areas allocated at the north side, central and south side of the reef; (2) aggregation effect of natural reef, considering different levels of coralligenous patchiness and morphology. The number of replicates considered is 3 by each level of factor, meaning 3 * 32 * 3 = 18 replicates to be collected on each survey.	Spring and summer, with 3 replicates for each season (to evaluate the best condition for diving)
	Evaluate the effectiveness of the remotely operated vehicle (ROV) to describe the fish community	Underwater Visual Census (UVC) carried out by scuba divers. The sampling strategy is the same adopted for the UVC conducted by the ROV. Two scuba divers would carry out each replicate simultaneously, and the result would be a mean of the observation. Data obtained would be used to validate observations conducted by using the ROV.	Spring and summer, with 3 replicates for each season

4.5. Case Study Trezza San Pietro (Natural reef)

The majority of information on the NR derives from two monitoring programmes aimed to evaluate the environmental value of the reefs locally called "trezze": the project "Le trezze dell'Adriatico: studio di alcune aree di particolare pregio ambientale ai fini della valorizzazione delle risorse alieutiche locali" in the period 2007-2009, and the Interreg ITA-SLO project "TRECORALA - Trezze e coralligeno dell'Alto Adriatico" in the period 2012-2015, both carried out by the OGS Trieste as lead partner. In this context, the reef structural conditions were assessed by periodic diving, the hard-bottom macrozoobenthic assemblages were sampled by photographic sampling with a fixed standard frame, and the fish assemblages investigated both by underwater visual census and experimental fishing with set nets.

Methodologies applied in the context of the ADRIREEF project are thought to pursuit three main objectives:

(i) to create a data set useful to promote reef use;
(ii) to monitor the biological status of the reef;
(iii) to develop low impact, automated sampling methodologies.

The interest of the monitoring focuses on the structural conditions of the natural reef, the water column characteristics, the macrobenthic community status, and the fish assemblages.

A one-time survey with a multibeam echosounder and side scan sonar will be conducted to complete the assessment of the extension and the morphology of the natural reef and to produce an updated map of the site.

Subsequently, a fixed buoy equipped with solar panel, rechargeable battery, and a data logger with a Lora One system, or Modem, will be moored in the vicinity of the natural reef. This setting will enable continuous sampling, on a frequency of 15/20 minutes, and will ensure the collection of water parameters and of intensity and direction of currents through ADCP, as well as real-time data transmission. The oceanographic information will be helpful for the planning of diving activities, as it permits to a priori evaluate those parameters crucial for a safe and nice dive, such as currents and turbidity.

The photographic sampling of macrobenthic community by use of a standard frame settled on the reef will be performed during two different seasons, choosing the more suitable conditions for diving and checking the organisms' assemblages.

The fish assemblage will be assessed by underwater visual census on a seasonal basis, using the "strip transect" technique, taking into account different gradients and environmental conditions. Pictures and videos taken by scuba divers will be used to describe the status of the community, to identify the best conditions for diving, and to promote the trezze as diving sites.

	Case study Trezza San Pietro		
Type of analysis	**Scope**	**Methodology**	**Timing**
Geomorphological mapping	Description of morphological features of the reef. Production of maps useful for planning the other investigations	Multibeam bathymetric survey and Side Scan Sonar analysis will be performed on the area occupied by the natural rock outcrop.	One survey in 2019 or 2020 (evaluate the best condition to operate at sea)
Water column parameters	Characterization of the water column and dispersion of pollutants Investigate the suitability of the site for diving and identify the most suitable season/s over the year Give stakeholders (divers) real-time information on oceanographic status	A fixed buoy moored close to the natural reef provided with solar panel, rechargeable battery, a data logger and a Lora One system (or Modem) will ensure the collection and transmission of water column parameters. The transmission will occur in real time to a land-based receiver, whit a 15 minutes frequency. The collected water column parameters will be: Temperature at surface; Temperature and Turbidity close to the bottom (1-2 m from the bottom). The Acoustic Doppler Current Profiler (ADCP) will collect data on intensity and direction of currents along the water column.	In continuous (15 minutes frequency)
Benthic community settled on the reef	Describe the status of the community in terms of species assemblages and coverage Provide information for divers and investigate the attractiveness of benthic organisms of the reef	Photographic samplings of fixed frame areas at georeferenced points will be performed in each of the following conditions: (1) geographic exposition: north-south gradient consisting of three level corresponding to three sampling areas allocated at the north, central and south-side of the reef; (2) substrata inclination: two levels, “horizontal face” and “vertical wall”. The number of replicates considered will be 3 for each level of factor, meaning 3 * 2 * 3 = 18 replicates to be collected on each survey. The images will be analysed with Image Analysis systems (PhotoQuad or similar), considering (1) coverage (cm^2) and (2) species number as variables to be registered.	Biannual samplings (2 seasons)
Fish assemblage	Describe the fish population in terms of species composition and abundance to provide stakeholders with information on the attractiveness of fish fauna present on the reef	Underwater Visual Census (UVC) with scuba divers. Two scuba divers will independently operate using the “strip transect” technique, taking into account: (1) geographic exposition: north-south gradient consisting of three level corresponding to three sampling areas allocated at the north, central and south-side of the reef; (2) aggregation effect of natural reef, considering as levels “proximity to rock outcrop” and “open spaces” between the natural structures. The number of replicates considered will be 3 for each level of factor, meaning 3 * 2 * 3 = 18 replicates to be collected on each survey.	Seasonally, with 3 replicates for each season (evaluate the best condition for diving), at least from spring to fall

4.6. Case Study Plić Seget (Natural reef)

The flora and fauna of the reef hasn't been officially studied, but it could be really interesting to obtain scientific information regarding the whole area. In fact, the CS site is located in Plić Seget, a submarine volcano, included in the Geopark Vis archipelago, a member of UNESCO's Global Geoparks Network. It consists in a series of interconnected reefs built from eruptive rock, combined with rocks of old and young creation. Plić Seget is also included in the area HR3000469 Vis archipelago geopark of the ecological network Natura 2000, which is important for preserving Common Bottlenose Dolphin (*Tursiops truncates*).

To date, the CS site is located right next to important touristic locations and, being a rich fishing spot, professional and recreational fishery occur through the whole year. However, since the CS reef is located along the edge of the coastal zone, here the Spatial plan restricts the use of the gill-nets. The most important activity yet implemented in this CS area is recreational scuba-diving, mostly in the summer season because during winter it is extremely demanding for diving due to strong currents, constant sea traffic and rapid weather changes, which is why it is recommended only to well-trained and prepared dive teams.

Through ADRIREEF project it will be possible to set up investigations aimed to assess the possible impacts due to human activity. The one-year monitoring will also allow to identify peculiar characteristics of the environment to be protected otherwise to invest on in the view of the Blue economy. It could also be a location of an interest for geological and biological studies, as it is one of rare volcanic formations in the Adriatic sea and hosting different megafauna species could be fish watching, fish feeding, trips with submarine could be achieved.

Activities that are planned to be implemented through the monitoring phase include: geomorphological mapping of the reef location, measuring measurement of water column parameters in order to assess the water quality and oceanographic status, investigations on the sediments surrounding the reef, algae and phanerogams (*P. oceanica* and *C. nodosa*) occurrence, benthic community settled on the reef and sediments, and the associated fish assemblage. There are no permanent mooring systems at the location, therefore, a permanent mooring could be set up to facilitate the organization of site visits.

	Case study Plić Seget		
Type of analysis	**Scope**	**Methodology**	**Timing**
Geomorphological mapping	Description of morphological features of the reef Production of maps useful for planning the other investigations	Parallel transects with HELIX 10 – SOLIX 10 (Humminbird). The survey will assure a 100% coverage of the reef area.	One survey in 2019
Water column parameters	Characterization of the water column and dispersion of pollutants Give stakeholders (divers) information on oceanographic status	Water quality will be investigated by monthly sampling. All physical chemical parameters will be monitored (temperature, oxygen, salinity, pH). In addition, the following chemical and biological parameters will be determined: nutrients (nitrate, nitrite, phosphate, silicate) ammonium, PAH, dissolved organic matter, sanitary quality (faecal coliforms, *E. coli).*	Twice (beginning end of the monitoring)
Benthic community settled on the reef and sediments	Describe the community status in terms of species assemblages and coverage Provide information for divers	Photographic samplings of standard areas frames. The factors taken into account in the sampling strategy are: (1) two sides of the reef, the east ‘’inner’’ side and west open water side (2) and proportional coverage of the reef in percentages (%) of all present basic habitat types, including: rock, unconsolidated sediment, algae on sediment, *Posidonia oceanica* and *Cymodocea nodosa*. In sediments the following compound will be determined: oxygen, PAH, dissolved organic matter, sanitary quality (faecal coliforms, *E. coli).*	At the beginning and end of the monitoring program
Fish assemblage	Describe the fish population in terms of species composition and abundance to provide stakeholders (e.g. diving; recreational and professional fisheries) with updated information on the fish assemblage	Visual census by the use of a remotely operated vehicle (ROV) equipped with digital video camera. Visual census using a Baited Remote Underwater Video (BRUV) mounted in a stereo configuration. The factors taken into account in the sampling strategy are (1) two sides of the reef, the east ‘’inner’’ side and west open water side, (2) on each side of the reef will be used two points for abundance: a) N-MAX (maximum number of species per frame) and b) T1 (the time of first arrival) as proxy for encountering the fish with diver. For BRUV there will be 5 replicates per system and there will be 6 systems used so it is 30 replicates per each survey. Using the stereo BRUV system is important because it can be used with SEAGIS Event Measure software to estimate the fish sizes. Each system will be calibrated before the survey.	Twice (beginning end of the monitoring)
	Evaluate the effectiveness of the remotely operated vehicle (ROV) and BRUV to describe	Underwater Visual Census (UVC) with scuba divers and using a Lure Assisted Visual Census. The sampling strategy is the same adopted for the UVC conducted by the ROV and BRUV. One scuba diver would carry out each transect as the one done with ROV. Data obtained would be used to	Once

	the fish communities	validate the observation conducted with the ROV. The Lure Assisted Visual Census will be used to validate the observation conducted with BRUV.	

4.7. Case Study Plićina Konjsko (Natural reef)

To date, the reef has been not explored and the monitoring program that will be implemented within the ADREEREF project will be aimed at assessing the current status of the reef and the potential impacts due to possible economic activities that are in line with Blue Economy such as fishing tourism around the reef and researching activities.

Investigations that are planned to be implemented through the monitoring phase include: geomorphological mapping, measurement of water column parameters, assessment of the benthic community settled on the reef and of the fish assemblage, analysis of sediments, and quantification of environmental loads. Environmental loads include the evaluation of human load related to number of visitors, number of divers, number of fishing boats and associated activities, tourist pressure in the surrounding areas, cargo and passenger traffic in the neighboring areas. In addition, evaluation of physical garbage quantities and type on the seafloor will be examined.

	Case study Plićina Konjsko		
Type of analysis	**Scope**	**Methodology**	**Timing**
Geomorphological mapping	Description of morphological features of the reef Production of maps useful for planning the other investigations	Parallel transects with SOLIX 12 (Humminbird). The survey will assure a 100% coverage of the reef area. Video recording using a remotely operated vehicle (ROV) equipped with digital video camera.	Two surveys in 2019
Water column parameters	Characterization of the water column and dispersion of pollutants To provide information to divers on oceanographic status	Water quality will be analyzed by monthly sampling. All physical chemical parameters will be monitored (temperature, oxygen, salinity, pH). In addition, the following chemical and biological parameters will be determined: nutrients (nitrate, nitrite, phosphate, silicate) ammonium, PAH, dissolved organic matter, sanitary quality (faecal coliforms, *E. coli).*	Monthly
Benthic community settled on the reef and sediments	Describe the community status in terms of species assemblages and coverage Provide information to divers	Photographic samplings of standard areas. The factors taken into account in the sampling strategy are: (1) two sides of the reef, the east ''inner'' side and west open water side (2) and proportional coverage of the reef in percentages (%) of all present basic habitat types, including: rock (R), unconsolidated sediment (U), algae on sediment (A), *P. oceanica* (P) and *C. nodosa* (C). In sediments the following compounds will be determined: oxygen, PAH, dissolved organic matter, sanitary quality (faecal coliforms, *E. coli).*	At the beginning and end of the monitoring program
Fish assemblage	Describe the fish population in terms of species composition and abundance to provide stakeholders (e.g. diving; recreational and professional fisheries) with updated information on the fish assemblage	Visual census by the use of a remotely operated vehicle (ROV) equipped with digital video camera. Visual census using a Baited Remote Underwater Video (BRUV) mounted in a stereo configuration. The factors taken into account in the sampling strategy are (1) two sides of the reef, the east ''inner'' side and west open water side, (2) on each side of the reef will be used two points for abundance: a) N-MAX (maximum number of species per frame) and b) T1 (the time of first arrival) as proxy for encountering the fish with diver. For BRUV there will be 5 replicates per system and there will be 6 systems used so it is 30 replicates per each survey. Using the stereo BRUV system is important because it can be used with SEAGIS Event Measure software to estimate the fish sizes. Each system will be calibrated before the survey.	Quarterly (to evaluate the best season for diving)
	Evaluate the effectiveness of the remotely operated vehicle	Underwater Visual Census (UVC) with scuba divers and using a Lure Assisted Visual Census. The sampling strategy is the same adopted for the UVC conducted by the ROV and BRUV. One scuba	Seasonally (at least from spring to fall)

	(ROV) and BRUV to describe the fish communities	diver will inspect the same transects surveyed by ROV. Data obtained would be used to validate the observations conducted with ROV. The Lure Assisted Visual Census will be used to validate the observation conducted with BRUV.	
Environmental load	Evaluation of human load: number of visitors, number of divers, number of fishing boats and associated activities, tourist pressure in surrounding areas, cargo and passenger traffic in the neighbouring areas	Data acquisition and analysis from the harbour masters' offices in the area, state statistical databases, real time ship's Automatic Identification System records, and personal recordings on site during one week monitoring in the peak season from sunrise to sunset.	Personal monitoring: one week period during monitoring phase. Others include data collection on yearly basis
	Evaluation of physical garbage quantities and type on the seafloor	Presence of garbage and a build-up rate from human activities estimated by reef survey using a remotely operated vehicle (ROV) on the beginning and the end of the peak season.	Two surveys: 2019-2020

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