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BIOLOGIA MARINA

DRIVER AMBIENTALI DELLE VARIAZIONI TEMPORALI NEGLI ASSEMBLAGGI ITTICI ASSOCIATI AD UNA PIATTAFORMA OFF-SHORE MEDIANTE MONITORAGGIO MULTIPARAMETRICO

ENVIRONMENTAL DRIVERS OF TEMPORAL VARIATIONS IN FISH ASSEMBLAGES ASSOCIATED TO A GAS PLATFORM BY MULTIPARAMETRIC OBSERVATORY MONITORING

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Summary

1. INTRODUCTION

The northern-central Adriatic Sea comprises the widest continental shelf in the Mediterranean Sea and it is of great economic value in the Italian and European contexts, contributing to global marine biodiversity while providing valuable ecosystem services to people (Liquete et al., 2016). This makes the Adriatic a highly exploited ecosystem for the extraction of natural resources as well as commercial and touristic activities. In terms of catches, this area is the most productive in the Mediterranean Sea (FAO, 2017) and, following the North Sea, together with the Ionian Sea, represents the second area for offshore installations devoted to hydrocarbons extraction in Europe. Since the1960s, an increasing offshore gas production has occurred in this region, leading to the construction of more than 120 gas platforms in the northern and central Adriatic Sea (Fabi et al., 2002).

Drilling operations are known to generate fluids of waste and debris that could cause strong environmental changes (Terlizzi et al., 2008). Furthermore, the physical structure of the platform can modify the local water flow and alter the composition of the surrounding benthic community (Wilson-Ormond et al., 2000). At the level of fish biodiversity, the platform act as a Fish Aggregating Device (FAD, Fig, 1.1), it can furnish shelter for protection from predation and trawling, additional food supply, and spawning substrate, and can act as a visual attractant for organisms not strictly dependent on hard bottoms (Fabi et al., 1998).

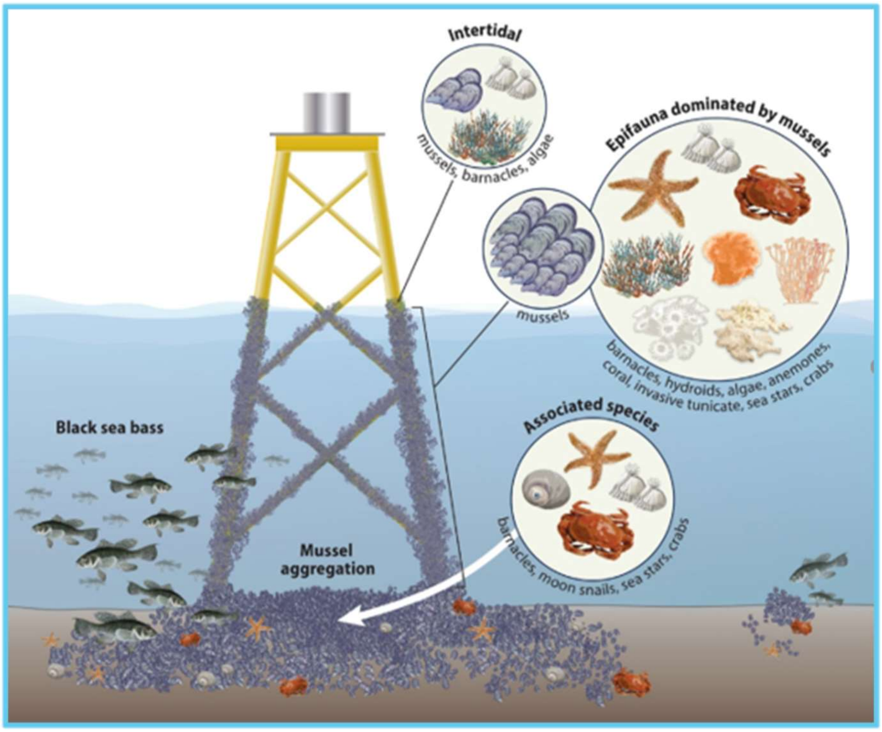


Figure 1.1 Reproduction of the FAD effect reproduced by an offshore platform.

Monitoring studies carried out in the Adriatic highlighted higher level of species richness and abundance near the platform rather than at a short distance from it (Consoli et al., 2013). When the platform reaches end-of-life it should be removed, a process known as decommissioning. This process represents a serious problem for the health and conservation of the marine environment and biota. Indeed, the partial dismantling or total removal of the structure from the seafloor requires the use of cutting tools or even explosives that impose profound transformations in the natural environment and, sometimes, irreversibly damage the latter (Jeremy et al., 2015). Furthermore, since the structure can provide connectivity in areas where natural reefs are scarce (i.e. the northern-central Adriatic Sea), acting as “stepping stones” to allow species to move from one area of the habitat to another, their loss can also affect a wider ecosystem area (Sommer et al, 2019).

It is necessary to develop an eco-sustainable model to address these issues, through monitoring of the impact conditions and a conversion of these model structures. An ecosystemic approach to the decommissioning of offshore platforms that evaluate where it is possible to completely or partially remove the structure, or where to leave it and adopt methods of reconversion of the latter (Sommer et al., 2019).

The assessment of fish assemblages or fish species, associated with Mediterranean off-shore platforms, has been until now carried out mostly by fishing gears (Scarcella et al., 2011a, b) and underwater visual census techniques (UVCs) (Andaloro et al., 2011). Unfortunately, these types of survey methods are often logistically challenging and not temporally representative, because of seasonal or sporadic sampling. In contrast, video monitoring can deliver observations at high frequencies, continuously and over long time periods, but with a rather limited spatial coverage (e.g. Aguzzi et al., 2020; Francescangeli et al., 2022). A video camera has in fact a field of view (hereafter FOV) limited to few cubic meters (depending on intrinsic and/or environmental conditions).

Nowadays, video-observatories are emerging tools that offer new opportunities without interfering with the individuals through remote monitoring of the marine environment (Aguzzi et al., 2012; 2015; 2019; Rountree et al., 2020; Lantieri et al., 2022). Even though observatories are limited in space, the main advantage they offer is a long-term and a low invasive technology. *In situ* video monitoring can provide a record of temporal changes in the number of fish and assemblages structures, providing insights on the daily cycles, seasonal and long-term temporal changes never obtained before (Aguzzi et al., 2013; Marini et al., 2018**)**. The seasonal timing of recurring biological processes is essential for organisms living in temperate regions such the Mediterranean, and yet is relatively understudied (Sbragaglia et al., 2018). This technology allows to study species in their natural ecosystems without interfere and to explore the temporal modulation of biological processes in the presence of environmental variables (Aguzzi et al., 2021).

1.1 PON plaCE project

This thesis is based on the results from a seabed platform developed within the framework of the PON Research and innovation project, in the Blue Growth specialization area, entitled "*Conversion of offshore Platforms for multiple eco-sustainable uses*" (PlaCE). The PlaCE project aims to test, for the first time at a national level, cutting-edge technologies, and solutions for the eco-sustainable reuse of offshore platforms located in front of the Abruzzo coast at the end of their production phase. In particular, miner growth technology through low voltage electrolysis of seawater to precipitate calcium carbonate on a cathode material to protect the platforms from corrosion, allowing the potential extension of their "life". In recent years this technology has been used in tropical areas for the recovery of coral reefs and field tests are also underway in the Baltic Sea. However, its efficiency in protecting offshore structures from corrosion is still largely unknown, especially in temperate systems such as the Adriatic, where temperature change can affect performance. The second purpose of the project aims to find innovative integrated multitrophic aquaculture solutions, that involve the use of sea cucumbers (*Holothuria tubulosa*), sea oysters (*Ostrea edulis*) and mussels (*Mytilus galloprovincialis*) as a component for the recycling of waste produced by fishing farming. The last goal of this project, elaborated within this thesis, aims to develop methodologies for the acquisition of biological/ecological parameters through continuous recording of environmental parameters and images, and their remote transmission by identifying the fish community present, its variability over time (i.e. seasonal, day/night) and finally the environmental drivers of such changes. In line with the sustainability requirements of Blue Growth, the use of renewable energy from different sources, such as photovoltaics and waves, with integrated management, will be evaluate to ensure self-consumption.

1.2 Offshore gas platform in the Adriatic Sea

In the last 50 years, the exploitation of non-renewable resources has greatly increased to meet the growing global demand for energy. The extraction of fossil fuels from offshore fields has largely increased and is becoming one of the most important activities for the exploitation of marine mineral resources (Terlizzi et al., 2008). In the Mediterranean, most countries have decided to grant new licenses for open oil exploitation throughout the whole basin.

In Italy, about 80% of the total energy comes from this type of activity (Brighenti et a., 2003), and, to date, around 120 offshore platforms have been installed in the northern and central Adriatic Sea (Fig 1.2), a production of about 86% (5,239 million standard cubic meters) of the total natural gas derived from Italy (Ministero dello Sviluppo Economico, 2013). The highest concentration of fossil fuel extraction platforms in the Mediterranean area.

These platforms are installed in a wide variety of environments, with different depths (from 20 to 80 meters) and types of sediments (from sandy to muddy), and are connected to each other / and or to the mainland through about 300 pipelines, which extend for a total of about 2300 km, of different lengths (from a few tens of meters to about 70 kilometres) in relation to the distance between the platforms or between the ground terminal platforms.

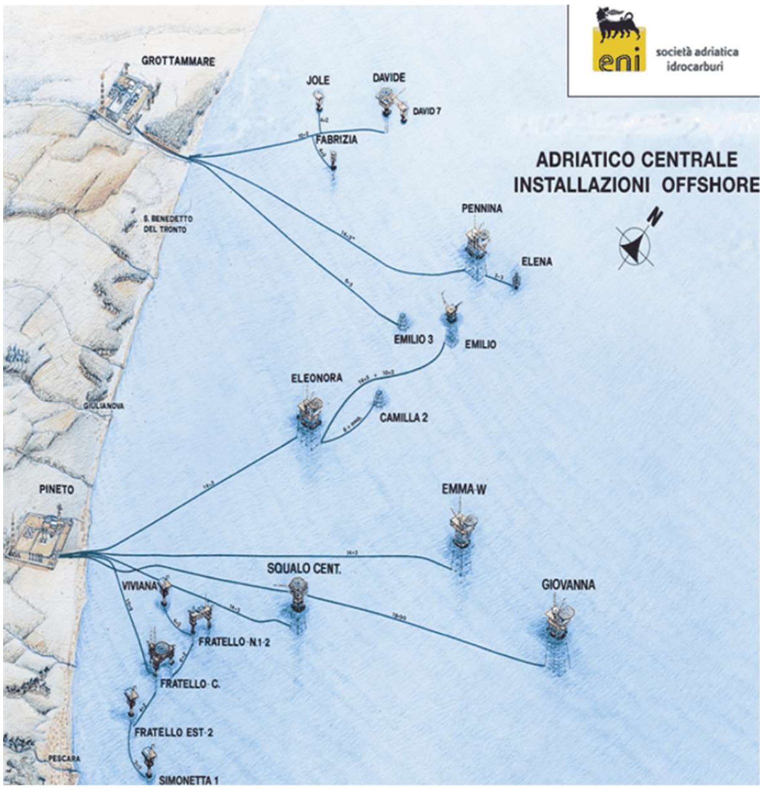


Fig. 1.2. Offshore platforms installations in the central Adriatic (ENI)

These pipes are laid or sunk into the sediment and their implementation requires different times, depending on their length and method of deployment. Over the years, numerous studies have been conducted to evaluate the effects of installing offshore oil and gas platforms on soft-bottoms benthic communities (i.e.; Manoukian et al., 2010; Spagnolo et al., 2014; Punzo et al., 2017) and fish assemblages around them (Andaloro et al., 2011; Fabi et al., 2002,2004; Scarcella et al., 2011a, b). Platforms extend throughout the entire water column, from the bottom to the upper layers, and their aggregating effect seems to be not limited exclusively to species inhabiting lower layers of the water column (Stanley and Wilson, 1998). The presence of these structures also affects pelagic species that are either attracted by their solid, reef-like nature (Gallaway and Lewbel, 1982), or by fouler, i.e., the smaller organisms attached on artificial structures like those usually observed in aquaculture facilities (Sarà et al., 2007). Basically, the role on fish biodiversity is well known, offshore platforms promote the aggregation of fishes that would otherwise be dispersed across larger areas in the ocean, as different studies carried out through the world says (Moreno et al., 2016). Monitoring studies carried out in the Adriatic Sea highlighted diverse fish species composition and densities at platforms placed at different depths, higher abundance at the rings in respect to the natural sandy-mud habitat, and temporal changes of the rings fish assemblages (Fabi et al., 2002, 2004). The installation of structures, drilling operations, and ship transport can cause an increase in metals, hydrocarbons, and other chemical agents in the environment causing potential impacts for both the abiotic and biotic components. The impact radius may vary depending on the number of platforms positioned in an area, the size of the structures, and biogeographical/climatic factors. Studies have highlighted the effects of platforms on pelagic communities over time, however, there is still a lot of research to be undertaken to understand the potential impacts of offshore platforms on the surrounding environment in the Adriatic area. These platforms are installed in a wide variety of environments, with different types of sediments and oceanographic settings, which can be influenced by rivers, such as the Po, which is the main source of fresh water in the northern Adriatic Sea. This situation, associated with the high drilling activity in the Adriatic basin, makes it difficult to develop general models for predicting the impact of offshore platforms (Cattaneo et al., 2003; Marini et al., 2008). The present

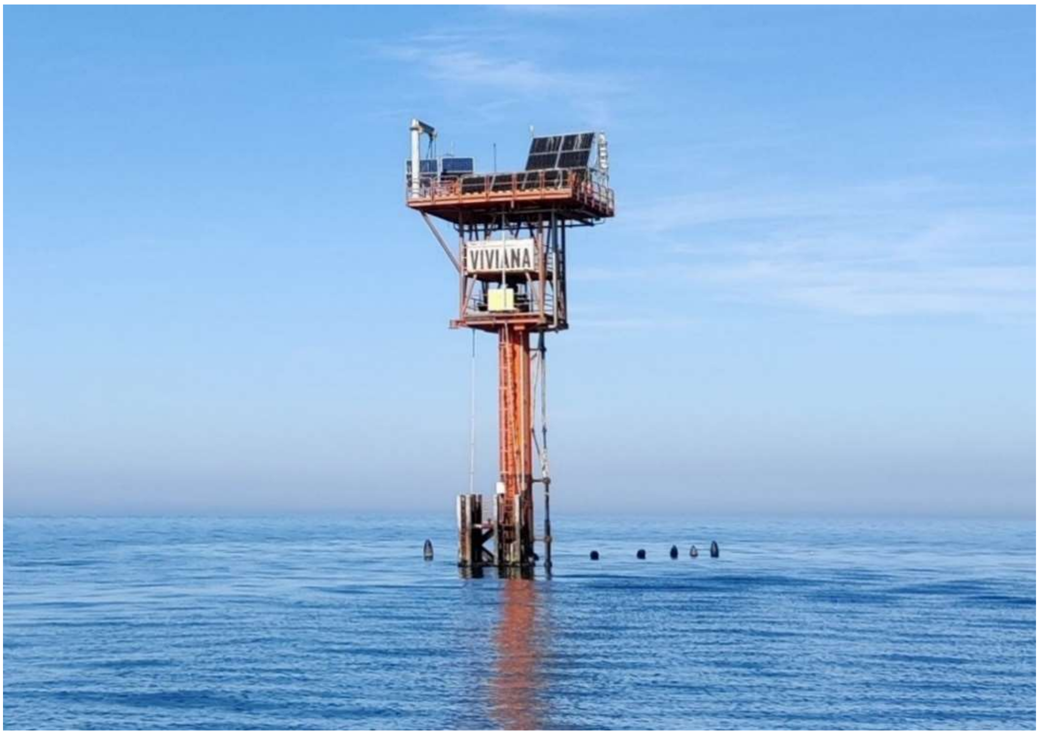


Fig.1.3. Study area, Viviana platform.

thesis, specifically, takes into consideration the sampling site around the "Viviana" platform (Fig. 1.3) located off the Abruzzo coast. The study area will be described in detail in the following chapters.

1.2.1. Decommissioning problems

Knowing the basic structure of offshore platforms is useful for understanding decommissioning options. Each platform consists of five main sections, as shown in Fig. 1.3: (1) The upper structures of the bridge above the water which include the equipment and piping for oil and gas treatment, which must be treated separately to address potential contamination problems. (2) Conductors for wells, which are pipes that go from the upper deck to the well (at the bottom

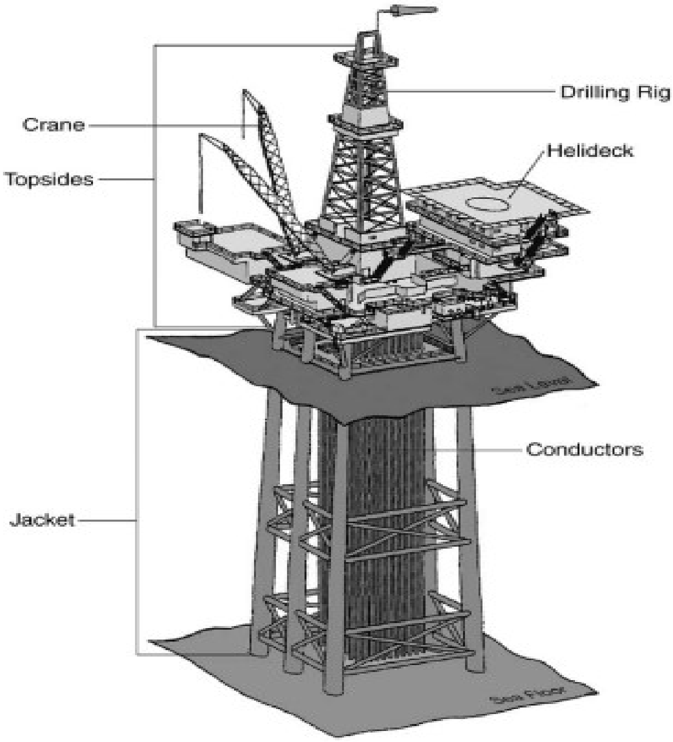


Fig. 1.4 main components of one of the most common offshore platforms (Manago and Williamson,1998, workshop notes p.223)

of the sea) for drilling, drilling and collecting oil and gas. (3) “The jacket”, a steel reticular structure that supports the deck and secures it to the seafloor and mounds of shells, drilling cuttings and debris on the seafloor around the platform. These structures include fallen remains of molluscs and other marine organisms that have grown on it, mixed with rock fragments and mud residues from drilling operations.

After the offshore platform has concluded its extraction phase due to resource exhaustion, the decommissioning can include one of four alternatives (Fig. 1.5):

a.1 Complete removal: explosives are detonated to sever the shaft conductors, pillars and support legs 5 meters below the seafloor and the structure is towed ashore and demolished;

a.2 Tow-And-Place: the severed structure is towed to a designated location and placed on the seabed;

b. Partial removal: the well conductors, poles and support legs are mechanically cut, usually 26 meters deep, and then optionally repositioned to the seafloor as an additional habitat;

c. Toppling: Explosives are detonated to sever conductors in the middle and support legs on three sides of the platforms, the whole structure is folded to stay in horizontal orientation on the seabed.

It is evident that all these practices have a disastrous effect on the integrity of the seabed with profound impacts on benthic communities and ecosystem functionality in general (Claisse et al., 2015**)**.

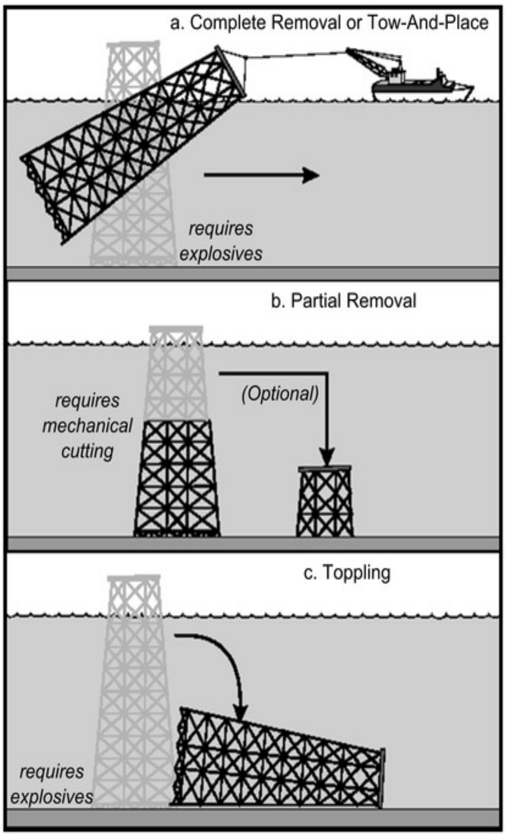


Fig.1.5 (Source: Jeremy et al., 2015)

For this reason, we are moving towards ecologically sustainable choices and procedures. Decommissioning offshore oil and gas infrastructure brings environmental challenges, but also opportunities. After decades at sea, these structures support marine life by attracting surprising concentrations of fish and providing a site for mussels, barnacles and other invertebrates, many of commercial interest. In the North Sea, for example, marine creatures attached to offshore platforms can increase the weight of these structures by 30% (Sommer et al., 2019). From a human perspective, removing the drill rings makes things seem pristine. But seen from below the surface of the water the situation can be different. Leaving structures in place can actually have benefits, and removing them can cause damage. When the platform to extract oil and gas are taken away, the biota that colonized them goes away with them and, since the structures can provide connectivity in areas where natural reefs are scarce, they act as "stepping stones" to allow species to move from one habitat area to another, their loss may also have effects on a larger marine ecosystem (Sommer et al., 2019). Paradoxically, active oil and gas platforms actually become a sort of marine protected area because safety regulations exclude fishing boats from the area around them. Disuse structures left fully or partially in place may retain some of these benefits by preventing trawling. For example, removing all oil and gas platforms from the North Sea would free approximately 400 km^2 for trawling (Sommer et al., 2019).

No dismantling method is the best for all situations. There is a need for all ecosystem approach to decommissioning that take into account the entire spectrum of environmental benefits and risks associated with the different operations (Fig. 5). This, in some regions, such as Southeast Asia and West Africa, will require more research on the habitat value of offshore platforms and also think about decommissioning in a more flexible way. In some regions, such as the North Sea, complete removal of end-of-life platforms is the default approach, but by limiting options upfront, planners may fail to identify the best approach for the environment. For example, a large offshore rig, such as the "Murchison" platform in the northern part of the North Sea, had attracted a large concentration of fish and formed a habitat for cold-water corals. However, the designers decided that since those species would not have been there if it was not for the platform, they did not have to take into account the effects of the removal of the platform on the marine community that had established itself (Sommer et al., 2019).

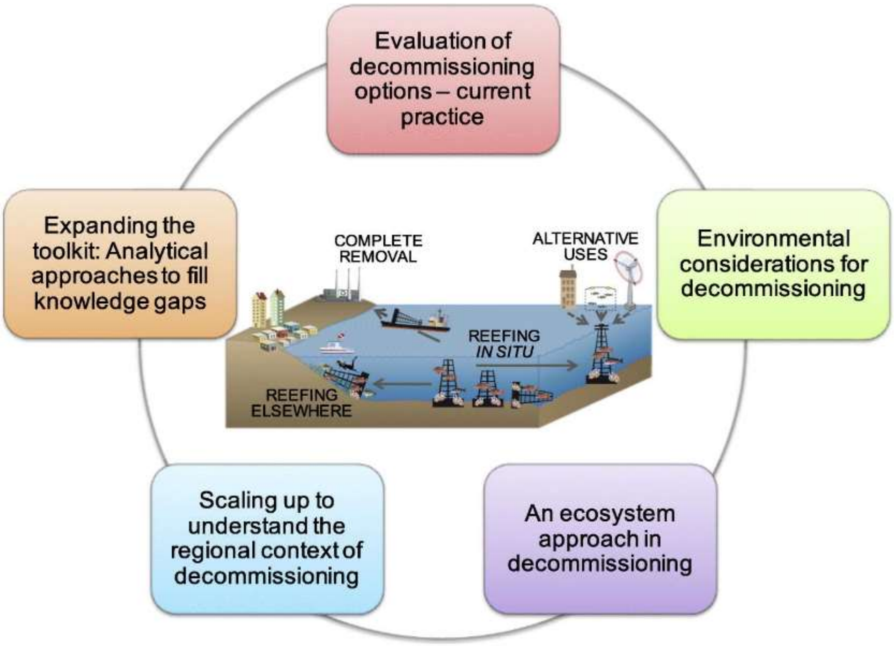


Fig.1.6. Decommissioning offshore oil and gas structures. Environemntal opportunities and challenges (Source: Sommer

Ecosystem functions and services increase with the age of the structure and vary according to geographic location so decommissioning decisions must adopt an ecosystem approach that takes into account their wider habitat and biodiversity levels. Aligning decommissioning assessment priorities between regulators and how they are assessed will reduce the likelihood of making variable and sub-optimal decommissioning decisions.

Ultimately, the range of allowed decommissioning options must be expanded to optimize the environmental results of decommissioning across the wide range of ecosystems in which the platforms are located (Sommer et al., 2019). There is a growing interest in developing alternative uses for old oil and gas infrastructures. The disused platforms could become sport fishing sites, diving spots, host wind or wave power plants, carbon capture and storage facilities, research stations or mariculture facilities. This is the fulcrum of the PON PlaCE project which has as the main objective the development of eco-sustainable methodologies for the conversion of offshore platforms.

1.2.2 Oil and gas offshore platforms as FADs

Associations of pelagic fishes with natural floating objects such as logs, mats of algae, debris etc. in the open ocean have been widely reported in the literature (Moreno et al., 2016), such structures are called Fish Aggregating Devices (FADs). Fish associated with drifting floating structures probably feed on invertebrates associated with the structure. Adult fish of some migratory species (tuna, dolphin, etc.) have also developed similar associative behaviour around drifting objects for other reasons (e.g., resting pace, presence of baitfish, geographical reference, and school reconstitution) (Castro et al., 2002). However, the main reasons for the attraction of fishes can be partially explained through the availability of food, shelter from predators, and orientation advantages. FADs are widely used as a fishing method due to their high efficiency (Fig. 6): ropes and lines encourage the settlement of marine plants and small crustaceans and molluscs, which in turn attract small fish. Echo-sounders such as a “Fishfinder” may be attached to a FAD allowing fishermen to electronically “connect” to the FAD and see how many and at what depth fish are located.

Because this is important for tuna fisheries, nearly 100,000 FADs are deployed by fishers every year in the world’s tropical oceans (Moreno et al., 2016). The principal factors in common between oil and gas platforms and FADs are (Franks, 2000):

- The creation of artificial habitats at or near the surface in midwaters;
- The high taxonomic diversity and abundance of pelagic fishes relative to the surrounding waters;
- The attraction effect towards similar families of fishes; resident and transient pelagic fishes;

- The influence they exert on regional pelagic fisheries;
- The significant increase in fisheries catch;
- the opportunity they offer to study the natural history and biology of pelagic fishes.

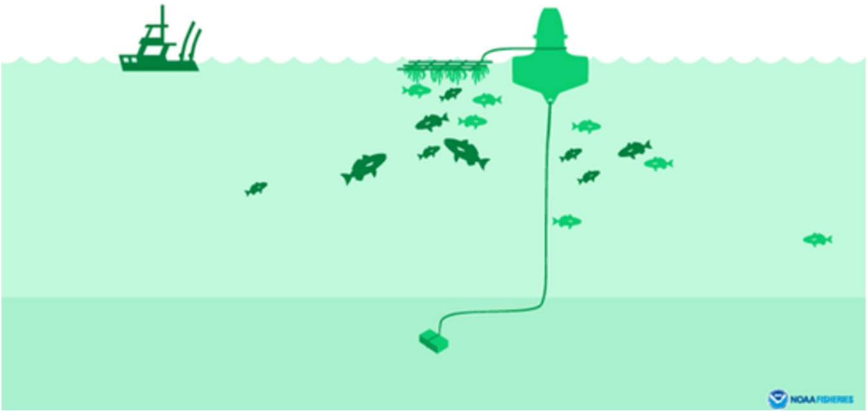


Fig.1.7 Fish aggregating device (Source: NOAA)

Moreover, oil and gas platforms also served as major “aggregation points” for a large number of fishes. These platforms, extending from the ocean bottom to above the water’s surface, provide vertical relief and new attachment sites attractive for different species inducing the development of new organisms’ assemblages (Terlizzi et al., 2008). The “platform effects” are not confined to benthic and demersal fishes alone, but also extend to pelagic fish which often exhibit high species diversity and typically represent the greatest fish biomass (Gallaway and Lewbel, 1982). The majority of these structures have been in place for decades and they may have functioned as artificial reefs, potentially acting as a network of marine protected areas (Fuji, 2015). Although the effects of platforms on biodiversity have been investigated in different areas of the world, such as the Gulf of Mexico, the southern California, and the west coast of Africa, their role on species trophic relationships has so far received little attention.

1.3 Underwater video monitoring system

Biological processes influence our population and biodiversity assessments if the frequency of sampling is too low and irregular through time (Aguzzi et al., 2012). Video monitoring, coupled with sensors (i.e. oceanographic and geochemical) that acquire profiles of water properties and oceanographic properties, allows the analysis of different biodiversity indicators such as the composition of communities (i.e. richness) and relative abundance of species (i.e. evenness), as well as ecosystem functions. These technologies together allow providing sufficient background measurements to characterize the dynamic relationships between changing environmental conditions and biological activities in association with offshore oil/gas platforms at fine temporal resolution. The result is a highly integrated biological and environmental monitoring that can, in principle, provide useful information in assisting decision-makers in case of decommissioning platforms and could offer information for the management of the areas beneath and surrounding the platforms. The most common techniques used to study and monitory biodiversity until now are mostly extractive (e.g., fishing, dredging), or alternatively based on acoustic (echosounders), or on UVCs. Technological progress regarding video cameras, sensors, battery life, and data storage, make these new technologies now accessible to a majority of users. As a consequence, underwater video monitoring systems are increasingly used in marine ecology studies (Mallet & Pelletier, 2014).

Fishing-based surveys (e.g. Petitgas et al., 2009) focus on catchable species without providing information on other species, nor on habitat, furthermore, catchability varies across species and as a function of weather conditions (Trenkel and Cotter, 2009) and vessels. Sampling effort by fisheries is considerable, but data interpretation may be difficult due to the uncontrolled sampling design. Scientific catch surveys instead provide small sample sizes compared to fisheries catch (Trenkel and Cotter, 2009). In addition, extractive techniques have an impact on biodiversity, which may not be desirable in the context of monitoring conservation strategies.

Underwater acoustics is currently effective for pelagic and semi-demersal species, and zooplankton (Trenkel et al., 2011). However, species present in the acoustic data have to be identified through complementary techniques, and benthic species are not well-observed.

UVC techniques have been used for over sixty years to monitor fish, macrobenthic organisms, and habitats (Brock, 1954). Advantages and disadvantages of UVC for estimating fish abundance and diversity have been reported in different papers (e.g. Dickens et al., 2011). The main limitation of UVC lies in the need for divers' presence underwater, which influences the observation of vagile macrofauna, restricts the number of observations that can be carried out, and constrains depth observation.

The first published work reporting the use of underwater video systems in the coastal environment dates to the 1950s and remote underwater video (RUV) has become to be used more frequently in marine sciences since the 1960s. It provided the first data on fish movement and behaviour in daytime and at night, which had not been previously studied without human disturbance (Mallet & Pelletier, 2014). RUV systems can include additional sensors and can be distinguished in terms of their autonomy (linked or autonomous). The latest linked systems are permanent observatories using cables for energy supply, data transfer, and instrument control (Aguzzi et al., 2012). In the western Mediterranean, set the OBSEA (20 meters depth) system linked to an internet video server (Aguzzi et al., 2011; Del Río et al., 2021), making the videos viewable in real-time on the World Wide Web (Fig. 1.8) (www.obsea.es).

Figure 1.8 OBSEA's underwater observatory (www.obsea.es)

The first autonomous RUV was placed for a week in the North Adriatic Sea, in order to study the behaviour and distribution of benthic and demersal species, their feeding activities and movement patterns, along with species interactions and the influence of environmental conditions (Fedra and Machan, 1979). Financial costs of this technology are difficult to evaluate because of the various types of cameras, manufacturing systems, and the differences in the characteristics of the sensors used.

The main RUV's problem, after some time underwater, is the fouling, i.e. the accumulation of organisms, impairing the quality of images. In some cases, the problem has been resolved by cleaning the lens surface using automatic windshield wipers. Underwater visibility is a limitation for all visual techniques, whether UVC, video, and photo. The second issue address to the time needed for image analysis, but the post-treatment of image balance the time gained in the field through other techniques (Pelletier et al., 2012). The required time is mostly directly dependent on the amount of data obtained, which should not be seen as a con. Last, but not least, correctly managing the data obtained is another obstacle, particularly because of long-term monitoring. developing shared protocols for collecting and utilizing the data available should be a priority.

1.4 Aims of this study

Within this context and considering a temporal window of one year, from February 2021 to February 2022, the aim of the present study was:

1. To define the fish pelagic community;
2. To analyse fish day/night and seasonal variability, defining fluctuations of mot occurring species;
3. To define the environmental drivers responsible for these changes

Discussing findings and considering the potential use of this technology within the context of EU monitoring strategy (i.e. MSFD 2008/56/EC), to analyse and define the trophic state and the hydrographic conditions around the “Viviana” platform’ area. This study proposes itself as a pioneer in the possible use of dismissed offshore platforms as points of observation and study of fish fauna and abiotic factors in an uncontrolled environment, thanks to their high distribution and abundance along the entire Adriatic Sea.

2. MATERIAL and METHODS

2.1 Site and platform description

The Adriatic Sea is an elongated basin with a NW-SE orientation (Fig 2.1a). The northern area is shallow, it rarely exceeds a depth of 46 meters, while in the central part the depth reaches 270 meters. The continental slope is located about 500 kilometres from the northern border and separates the central basin from the southern one, where the depth reaches 1200 meters. The central Adriatic is characterized by three pits collectively called Mid-Adriatic-Deep (MAD). In this area, Palagruža Sill (170 m deep) acts as a spill area between the three pits and the Southern Adriatic Basin (Marini et al., 2016). The main circulation is dominated by the Eastern Adriatic Current (EAC), which flows counter-clockwise from SE to NW along the easter side, and the Western Adriatic Current (WAC), which flows from NW to SE along the western side. The offshore area is characterized by more stable conditions compared to the coastal area, being less affected by the Adriatic cyclonic circulation and by the terrigenous contribution of rivers (Djakovac et al., 2015). The western side is characterized by soft bottoms that gradually change from coastal mud to offshore relict sands (Fig. 2.1b).

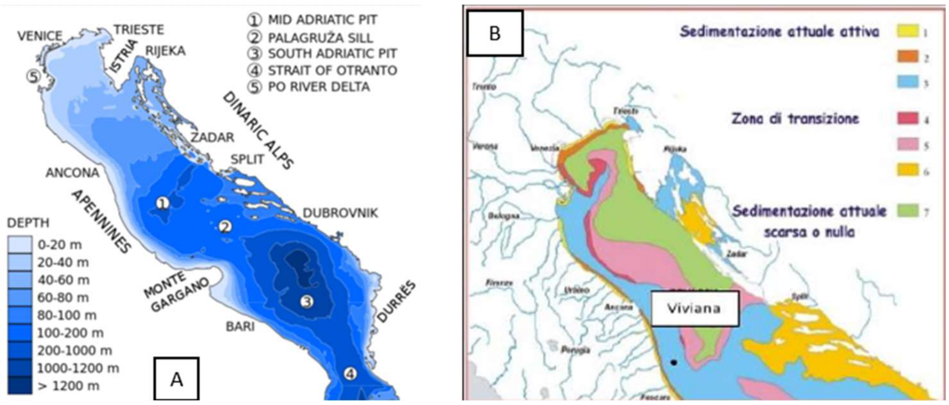


Figure 2.1 a) bathymetric diagram of the Adriatic Sea. 1: Mid Adriatic Pit (Pomo-Jabuka Pit); 2: Plagruža Sill; 3: South Adriatic Pit, b) Scheme of the distribution of superficial sediments in the Adriatic. 1: coastal sands; 2: silty sands and sandy silt; 3: clayey silts and silty clays; 4: loam; 5: clayey sands; 7: platform sands.

The study area is located in the central Adriatic Sea, which extends from the Ancona-Zara junction to the Gargano. The "Viviana" offshore platform (ENI operator) is a mono-tubular platform, formerly a gas extractor, installed in 1998 and located about 10 kilometres offshore Giulianova coast, Abruzzo, Italy (longitude: 14.15423203W, Latitude: 42.65742097N) (Fig. 2.1b). It is connected to the Pineto power station and nearby Fratello Nord offshore platform and is deployed at the depth of 20 meters. The platform' associated observatory is located at 18 meters depths.

2.2 Instruments description

The observatory consists of a Seabed Platform instrumented with a CTD probe (Fig. 2.2a) for measuring conductivity (and thus salinity), temperature, and depth, an oxygen probe (recording both dissolved oxygen concentration and oxygen saturation), a multi-channel fluorimeter (to have indication of pigments concentration, Fig. 2.1b)) and, an Acoustic Doppler Current Profiler (ADCP) for the recording of current data and waves (Fig. 2.1c). This allows also to record the significant height of the wave, the period of the significant, wave and the direction of the significant wave.

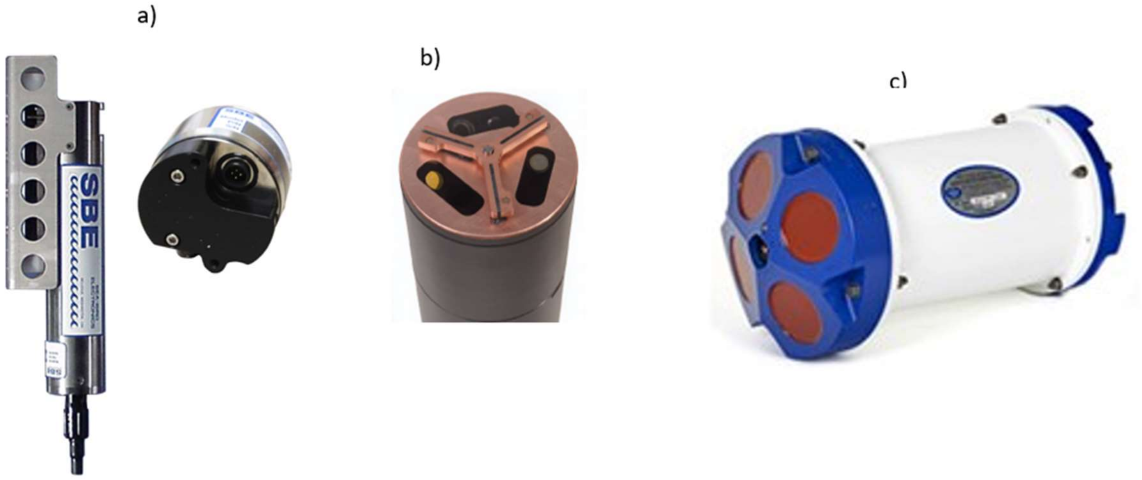


Figure 2.2 Instrument installed in the Viviana's' observatory for recording environmental variables, (a) Conductivity, Temperature, Depth-Optical Dissolved Oxygen sensor, (b) multichannel fluorimeter (c) Acoustic Dropper Current Profilers.

The whole system has been set up to allow low maintenance of the instrumentations, using optical technologies, antifouling systems, and when necessary, an automatic mechanical cleaning system.

The integrated image (photo and video) acquisition system was installed using suitable brackets, equipped with an IP full HD underwater camera (Fig. 2.3a) with 1920x1080-pixel resolution H264/MJPEG compression, 1 / 2.9''CMOS sensor, streaming and remote recording function. The structure includes an integrated device for mechanical cleaning of the optical window (Zebra Tech Hydro-Wiper) (Fig. 2.3b) and underwater LED light (OceanTools OceanLED Subsea LED lighting) remotely controllable by the LISC Datalogger (Fig. 2.3c). It is installed in an underwater housing, always pointing to the same Field Of View (FOV): a portion of the structure used for the electrification experiment and the surrounding water column (Fig. 2.4).

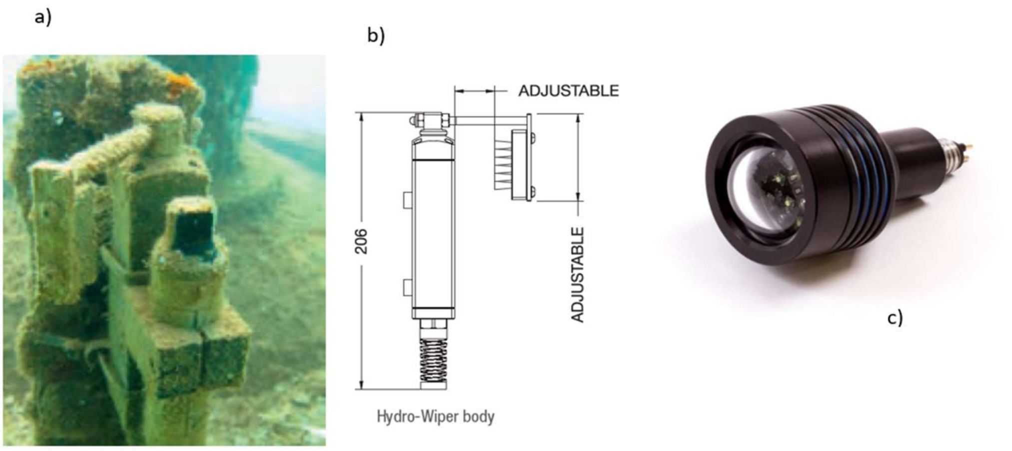


Figure 2.3. The integrated image (photo and video) acquisition system's components: (a) camera and underwater housing, (b) wiper, (c) led light.

Figure 2.4 The underwater camera ‘Field of View’.

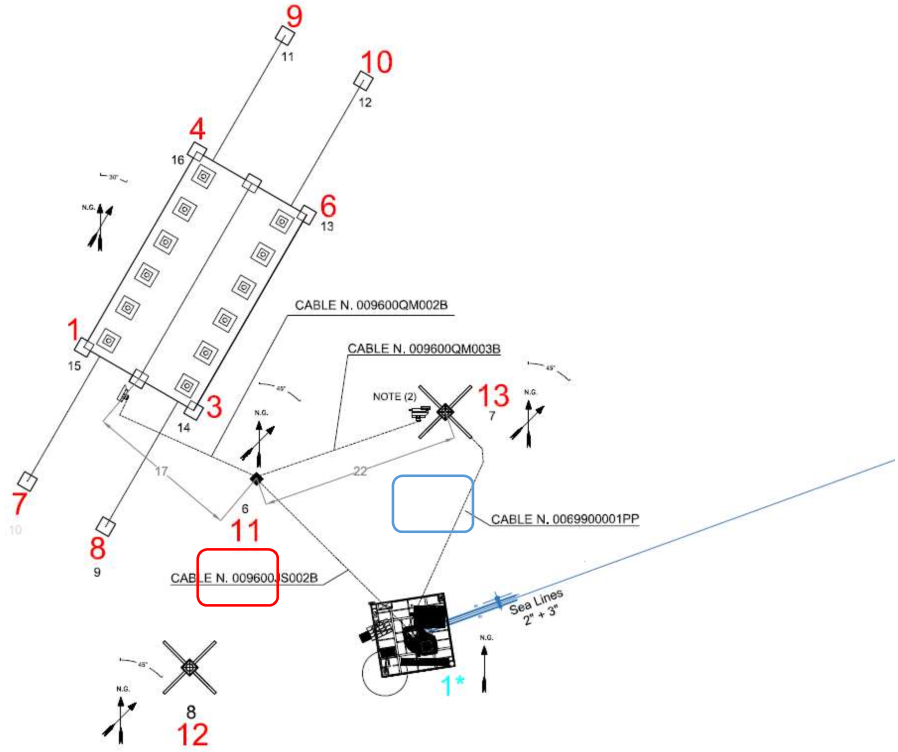


Figure 2.5. ‘Viviana’ observatory structure: 1=Viviana platform, 1-10=Integrated Multi-Trophic Aquaculture (IMTA), 11=seabed platform (circled in red). From the Seabed platform two cameras are connected, one pointing to the IMTA and the other one (circled in blue) to the electrified mineral accretion system (circled in blue and indicated by the number 13), our FOV (Fig. 2.4). The structure indicated with the number 12 is the non-electrified mineral accretion structure.*

Both systems were connected to a Junction Box positioned in the Seabed Platform located on the bottom close to the VIVIANA platform, through cables terminated with Underwater Pluggable connectors, which are explicitly declared for connections and disconnections at depth (fig. 2.5).

A single cable terminated with a connector on the connection terminal with the Junction Box, connects the Seabed Platform to the LISC system positioned on the VIVIANA deck. The use of an Underwater Pluggable connector allows the Seabed Platform to be recovered for ordinary or extraordinary maintenance operations, after a technical diver has disconnected them, thus facilitating all the recovery and repositioning operations of the Seabed Platform alone, which is therefore independent of the cameras and the CTD-ODO. The LISC control and management system of the instrumentation is located in a protected area on the VIVIANA platform, for data transmission to SZN through a GSM-4G system and where the images are stored. Furthermore, a weather station is connected to the LISC which is also positioned on the VIVIANA deck.

2.3 Protocol for data acquisition

In order to study the temporal variations in fish assemblages, as a product of the behavioural response of individuals to environmental changes, a time-lapse footage of 60 seconds every hour was continuously acquired.

The data acquisition occurred every hour from 23.02.2021 to 23.02.2022. The LED light allowed the analysis of the night shoots, turning on at the same time as the camera starts recording the videos.

With the video imaging, the environmental data was acquired concomitantly, in order to link behavioural activity to environmental changes, every hour.

2.3.1 Faunal data extraction from videos

Per each video, individuals that appeared in the FOV were visually classified and counted one by one (see below). Individuals were summed up for all (motion-detected) videos and the same (still) individuals that appeared in consecutive images were not recounted. However, recounting errors may have been introduced (i.e. considering as a new entry each fish re-entering the FOV). The potential effect of artificial light at night on the fish behaviour may exist (e.g. Doya et al., 2014) but that factor was impossible to control. To obtain a comprehensive richness list each video was screened for the presence of all visible individuals, which were subsequently classified by species, deriving the time sequence of the count, according to the protocol described in Aguzzi et al. (2020). All individuals (isolated or in schools) were classified to the lowest possible taxonomic level, using the latest scientific nomenclature. Classification of taxa to the species level occurred only when the morphological features of the individuals were sufficiently visible, in other cases, individuals were assigned to a greater taxonomical category (i.e., order, genus) or indicated as “unidentified”. At the end of each video, all the counts for each species and unidentified ones were summed up to obtain the total numbers for every hour. The presence of sessile organisms was not reported.

During one year of filming the observatory has captured all the most critical acquisition conditions that could have affected the quality of the data videos, including changes in water transparency, the biofouling on the camera, crowded scenes (i.e., presence of large fish schools), and light variation between day and night (Fig. 2.6).

The number of individuals per each species was compiled into a time series within a 1-hour interval. Relative percentages of species in video-counts were obtained for the entire observation period and for each month separately.

All statistical analyses were conducted using univariate and multivariate analyses using the softwares PAST 4.10 (Hammer et al., 2001) and PRIMER6&PERMANOVA+ (Clark and Gorley, 2008; Anderson et al., 2008).

Figure 2.6. Examples of the most relevant condition of images acquisition occurring at the Viviana observatory during the daylight. From the top to the bottom clockwise: clear and turbid water, heavy biofouling on the camera, and crowded scenes.

2.4 Univariate analysis

Before the analysis, abundance data were averaged into two temporal blocks accordingly to the irradiance data: irradiance values greater than 0 W/m^2 were classified as day, if values were null, those samples were ascribed to night. Averaging was used to reduce variance in species count data (Aguzzi et al., 2020).

The experimental design used for both univariate and subsequent multivariate analyses was based on two crossed factors: ‘month’, fixed with 13 levels (from

February 2021 to February 2022) and ‘day/night’, fixed with two levels (day and night). A univariate Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson et al., 2008) was performed on the Euclidean resemblance matrix of square root-transformed abundance data. Significance was set at $p < 0.05$, and p-values were obtained using 9999 permutations, with permutation of residual under a reduced model as permutation method. Box-plots graphs were also used to visualize temporal changes in total abundance and also considered day and night counting, separately.

2.5 Multivariate analysis

To analyse changes in community structure over time, multivariate statistical techniques that consider several variables at the same time have been used. These make a comparison between all the samples and allow to highlight the similarities in terms of composition and structure.

First, data ordination was visualized through non-metric Multi-Dimensional Scaling (nMDS) which provides an unconstrained graphical two-dimensional ordination. Then differences among months, between day and night assemblages, and for the interaction term were tested by two-way PERMANOVA on the same experimental design described above. PERMANOVA tests were run on the Binomial deviance resemblance matrix of square-root transformed abundance data, and PERMANOVA pairwise tests allowed to identify the source of variations. Binomial deviance measure compared to Bray-Curtis measure has the added advantage of being based on the likelihood theory, it also should be able to handle different sample size without a fixed upper limit but can vary among sites with no shared species. ASIMPER (Similarity Percentage) analysis were carried out, to identify the main fish taxa contributing to similarities within each month in both diurnal and nocturnal assemblages and the average dissimilarities between pair of months. SIMPER analyses were run without unidentified species which prevented from highlighting the effective contribution of the identified species to each month/period of the day.

The Shannon-Weaver diversity index (H') was then calculated to show temporal changes in fish diversity. Changes in diversity were also tested by univariate PERMANOVA, run on the Euclidean resemblance matrix of untransformed H' values.

2.7 Waveform analysis

A waveform analysis to assess the phase of visual count rhythms in terms of the peak timing for the 4 most abundant species counted (the bogue *Boops boops,* the annular seabream *Diplodus annularis,* the white seabream *D. sargus,* and the two-banded seabream *D. vulgaris*) was done. All the time series were subdivided into 24 h segments (at 1-hour sampling frequency) and a fluctuation was obtained by averaging all values of the different segments at the corresponding 1-hour time intervals (Francescangeli et al., 2022). The resulting mean (± SD) values were plotted to determine the waveform' peaks and troughs. The peak temporal amplitude was then computed according to the Midline Estimating Statistic of Rhythm (MESOR) method (Aguzzi et al., 2006). The MESOR value was estimated by re-averaging all waveform averages and the result presented as a threshold horizontal line superimposed onto the waveform plot. All mean values above the line defined a significant increment in the visual counts. The Onset and Offset of activity were estimated by considering the first and the last value above MESOR, respectively. Because of the substantial variability, the activity was considered continuous if no more than 3 values occurred below the MESOR (Aguzzi et al., 2020d). Fish count fluctuations were considered as a proxy for average levels of swimming rates in the population (being the chance of animal spotting into the FOV proportional to the overall motility rates within the overall population (Francescangeli et al., 2022). The waveform output plots' y axis was not standardized to the same extent in order to make visible the fluctuations of the less abundant months, which would otherwise have been flattened.

2.6 Correlation with environmental variables

To identify the environmental drivers of fish communities and their structure across the sampling period, biotic data were correlated to environmental variables. Environmental data were tested for collinearity among variables by using a Draftsman plot, with irradiance, turbidity, and direction of the wave

(Deg) data being Log (X+1)-transformed to fit a linear distribution in the Draftsman plot. Finally, a DistLM (Distance-based linear models, Anderson et al., 2008) was run with turbidity, salinity, temperature, dissolved oxygen, chlorophyll-*a*, irradiance, density, wind direction, height, period, and direction of origin of the wave. As environmental variables, using “step-wise” as selection procedure and “AIC (Akaike Information Criterion)” as selection criterion. Three DistLM models were run, one for the overall assemblages and then two separate models for the day and night assemblages, respectively.

3. RESULTS

3.1 Fish community composition

The total number of videos acquired was 8,672 for a total of 155.5 hours of video-monitoring. Out of these, 7,802 were viable for fish classification and counting being the rest discarded for camera errors at shooting (black footages), or high biofouling. A total of 25 fish taxa, belonging to 10 families and one order (Clupeiformes) were identified. Clupeiformes generally comprise small species swimming rapidly. These characteristics hampered their identification to lower taxonomic resolution during our observations, because diagnostic morphological features were not sufficiently visible, thus to avoid identification bias they were grouped to higher taxonomical level (i.e. the order). Out of the 83,138 individuals enumerated, 76,831 were counted during the day and 6,189 during the night. The most represented family was Sparidae (12 species), followed by Carangidae, Centracanthidae and Serranidae (all families represented by 2 species) (Annexes 1 and 2). The most abundant species identified, that together represent the 40% of the observations during the whole year, were the bogue *Boops boops,* the annular seabream *Diplodus annularis,* the white seabream *D. sargus,* the two-banded seabream *D. vulgaris* and the black seabream *Spondyliosoma cantharus* (Fig. 3.1). Unidentified individuals, targeted but not classified because of different constrains such as high turbidity, biofouling on the FOV, or other reasons, corresponded to the 66% of the total individuals (Annex 1). Excluding unidentified individuals, the percentage composition of the different classified species was dominated by a few dominant species (Fig. 3.2).

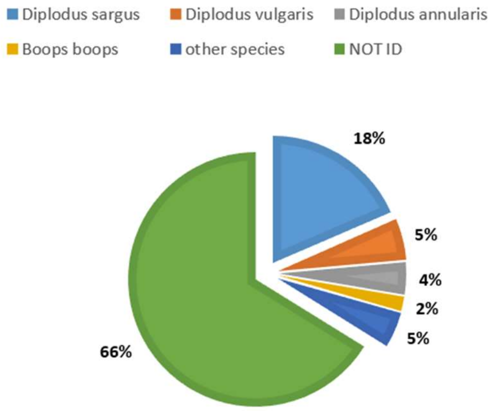


Figure 3.1. Taxonomic composition, % of the abundance of classified and unclassified species (NOT ID).

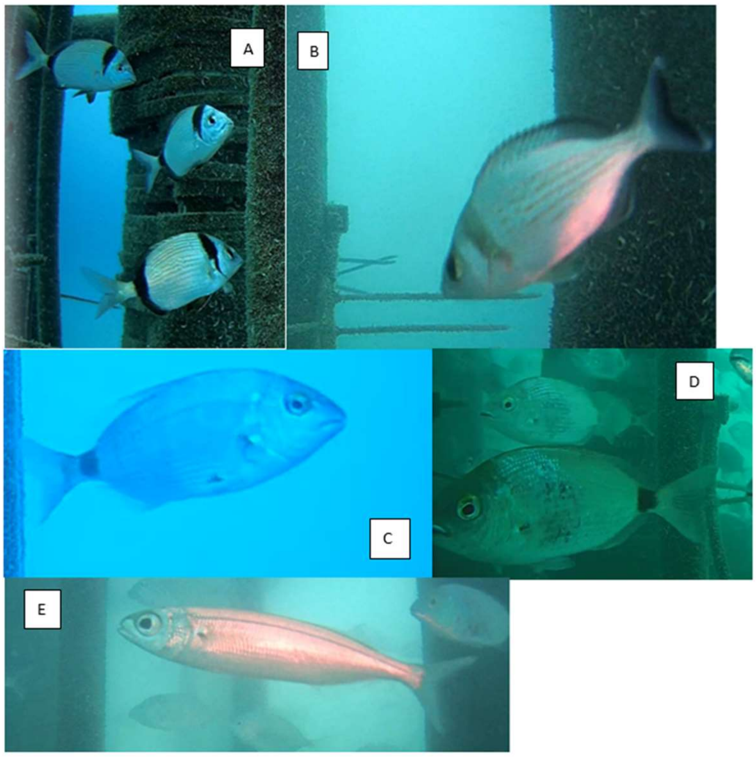


Figure 3.2 Images of the most abundant species classified at the Viviana observatory. A: Diplodus vulgaris; B: Spondyliosoma cantharus; C: Diplodus annularis; D: Diplodus sargus, E: Boops boops.

Among all, three benthic species have been regularly recognized: the scorpion fish *Scorpaena porcus*, the brown comber *Serranus hepatus,* and the comber *Serranus cabrilla* (Fig. 3.3).

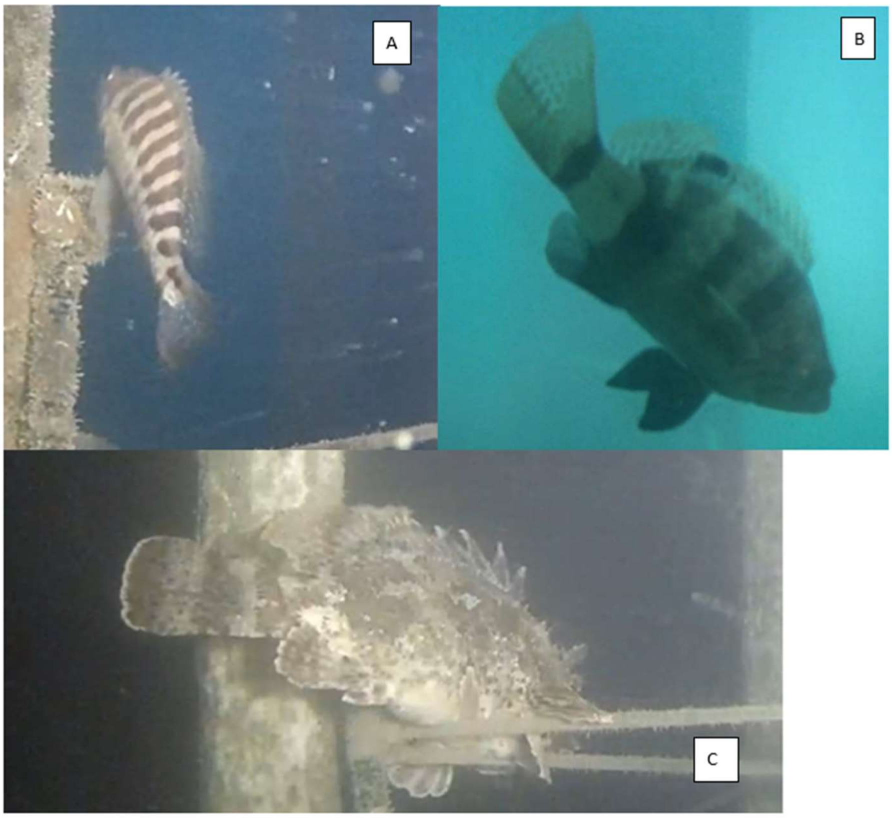


Figure 3.3. Images of benthic fish species classified in the Viviana observatory. A: Serranus cabrilla; B: Serranus hepatus; C: Scorpaena porcus.

3.2 Seasonal changes in fish abundance

The box plot of the total abundance showed a clear pattern of increasing abundance in warmer spring-summer periods (Fig. 3.4).

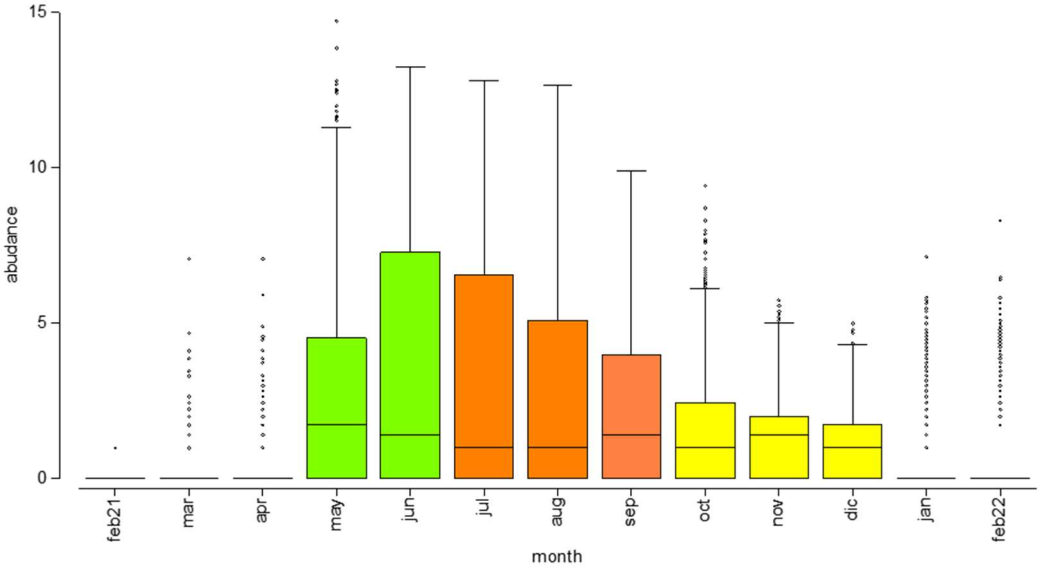


Figure 3.4. Box Plot of the average fish abundance across the year (square-root transformed data). Colours indicate the different seasons: GREEN: spring; ORANGE: summer; YELLOW: autumn; BLUE: winter.

When separating day observations from night (Figure 3.5), the abundance of fishes during the day followed the pattern described above for the whole dataset (Figure 3.5a), while that at night was greater in June and from October to January (Figure 3.5b). The univariate PERMANOVA test showed significant differences in the fish assemblage according to seasons, day/night variations and for the interaction factor (Table 3.1).

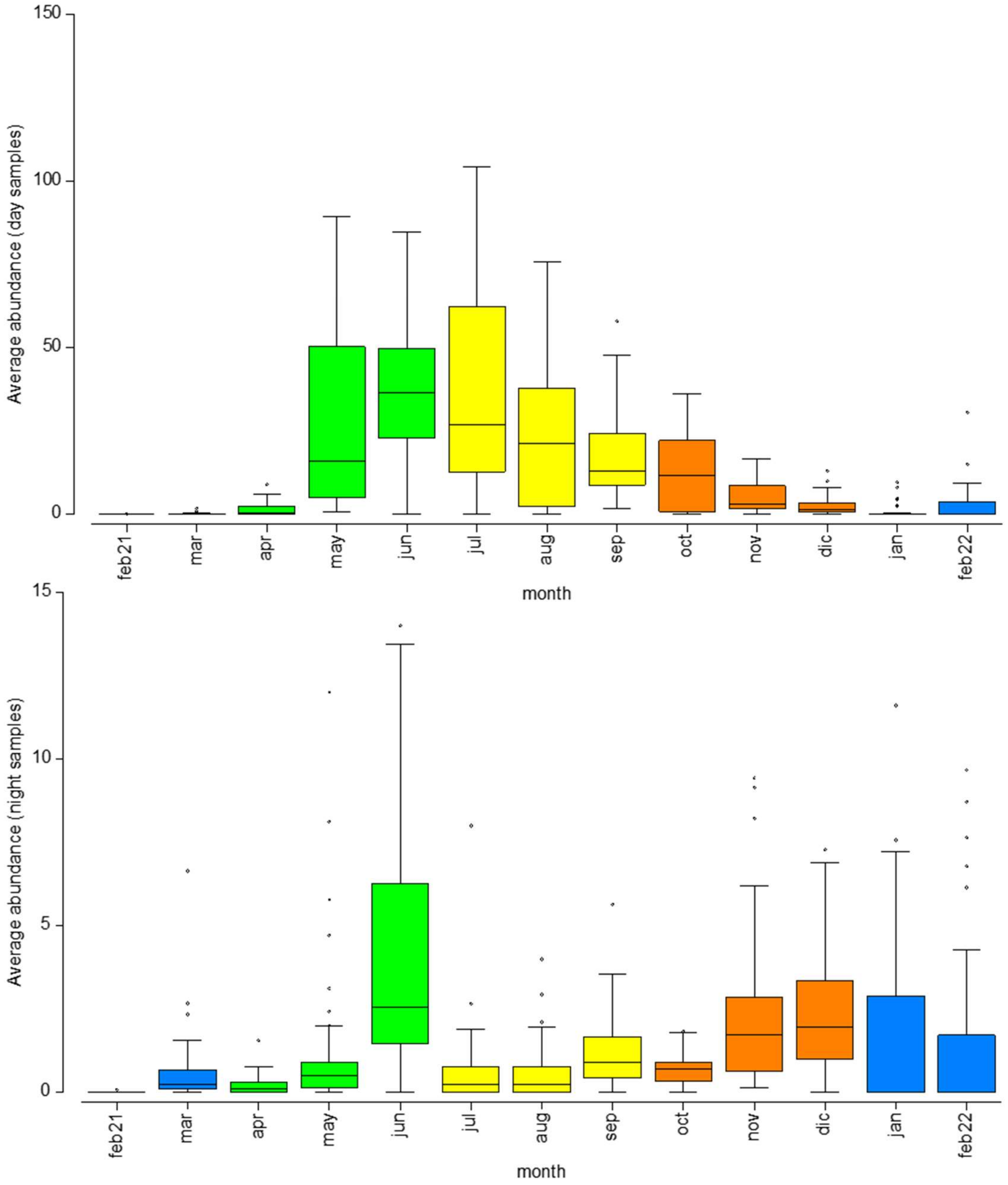


Figure 3.5. Box Plot of the average fish abundance at day (a) and night (b) along the year (untransformed data). Colours indicate the different seasons: GREEN: spring; ORANGE: summer; YELLOW: autumn; BLUE: winter.

The pair-wise comparisons among months per pair of levels of factor day and night, separately, allowed to highlight the source of month variations for day and night samples, respectively (Table 3.2a and b).

Table 3.1. Result of univariate PERMANOVA main test for abundance data. Df indicates degrees of freedom, MS is Mean Squares and Pseudo-F is the F-value calculated by the PERMANOVA.

Source	***df***	***MS***	***Pseudo-F***	***P(perm)***
Month	*12*	*2666.9*	*21.64*	*0.0001*
Day/Night	*1*	*18,456*	*149.76*	*0.0001*
Month x Day/Night	*12*	*2462.4*	*19.98*	*0.0001*
Residuals	*691*	*123.23*		
Total	*716*			

The pair-wise comparisons among months per pair of levels of factor day and night, separately, allowed to highlight the source of month variations for day and night samples, respectively (Table 3.2a and b). Since the null hypothesis (months' abundances are equal among them) is rejected when the p-value is less that our significance level $\alpha = 0.05$, during the daytime (Tab. 3.2a), contiguous months that demonstrated a significant level of difference were March and April, April and May, October and November and, November and December. The comparison between July and August provided a p-value very close to 0.05, thus the null hypothesis was accepted. Considering only night-time samples, differences were almost always significant except for autumn-winter months and July and August (Tab. 3.2b).

Table 3.2 Result of univariate PERMANOVA pair-wise for abundance data for day (a) and night (b) samples showing only differences among contiguous months. t is statistic t and P(perm) is P-value.

a)

Groups	***t***	***P(perm)***
February21, March	*1.07*	*0.26*
March, April	*3.26*	*0.0003*
April, May	*5.34*	*0.0001*
May, June	*1.53*	*0.12*
June, July	*0.13*	*0.89*
July, August	*2.00*	*0.049*
August, September	*1.16*	*0.24*
September, October	*1.65*	*0.10*
October, November	*3.08*	*0.003*
November, December	*2.26*	*0.02*
December, January	*1.74*	*0.08*
January, February22	*1.14*	*0.29*

b)

Groups	***t***	***P(perm)***
February21, March	*1.20*	*0.15*
March, April	*1.80*	*0.04*
April, May	*2.70*	*0.0004*
May, June	*2.96*	*0.003*
June, July	*4.43*	*0.0002*
July, August	*0.15*	*0.9*
August, September	*2.25*	*0.02*
September, October	*2.49*	*0.01*
October, November	*3.77*	*0.0002*
November, December	*0.43*	*0.66*
December, January	*1.15*	*0.25*
January, February22	*0.43*	*0.67*

3.3 Temporal changes in assemblage structure

The nMDS plot showed a clear separation between day and night samples (Fig. 3.6), with day samples being more dispersed than night ones.

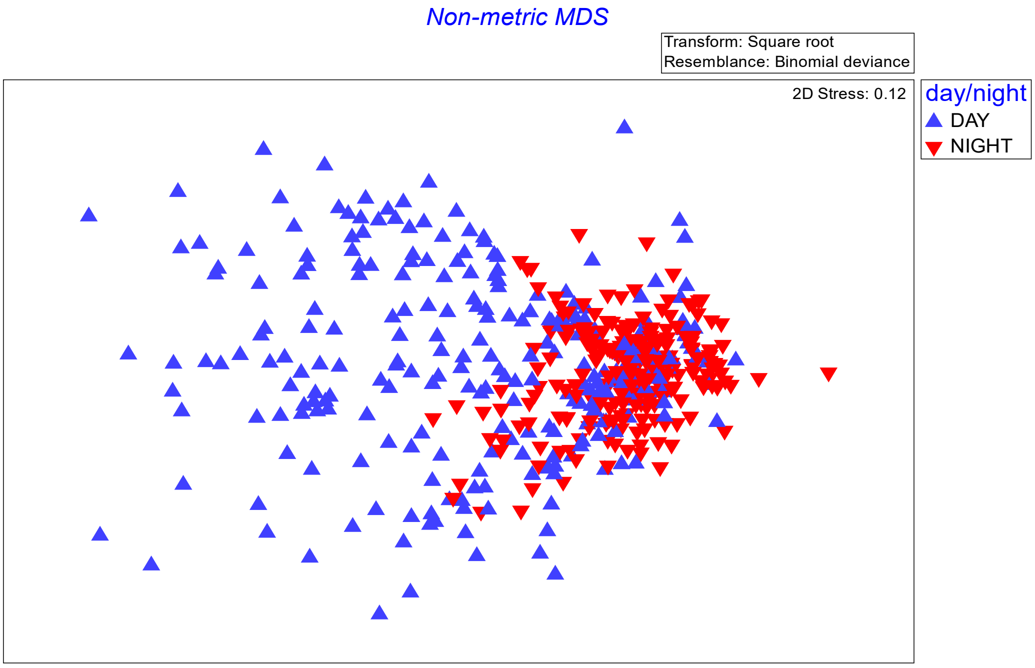


Figure 3.6. nMDS plot obtained through Binomial deviance resemblance index for the day-night fish assemblages recorded from February 2021 to February 2022.

The nMDS plot carried out separately for day and night samples showed different seasonal patterns of fish assemblage. For the fish assemblages observed during the day, there was a separation of samples from winter/spring from those from summer and from autumn and the second winter here observed (January-February 2022, Fig. 3.7a). The night assemblages were more homogeneous and two main group were evident, one encompassing samples from February 2021 to September, and the other including those from October to February 2022 (Fig. 3.7b).

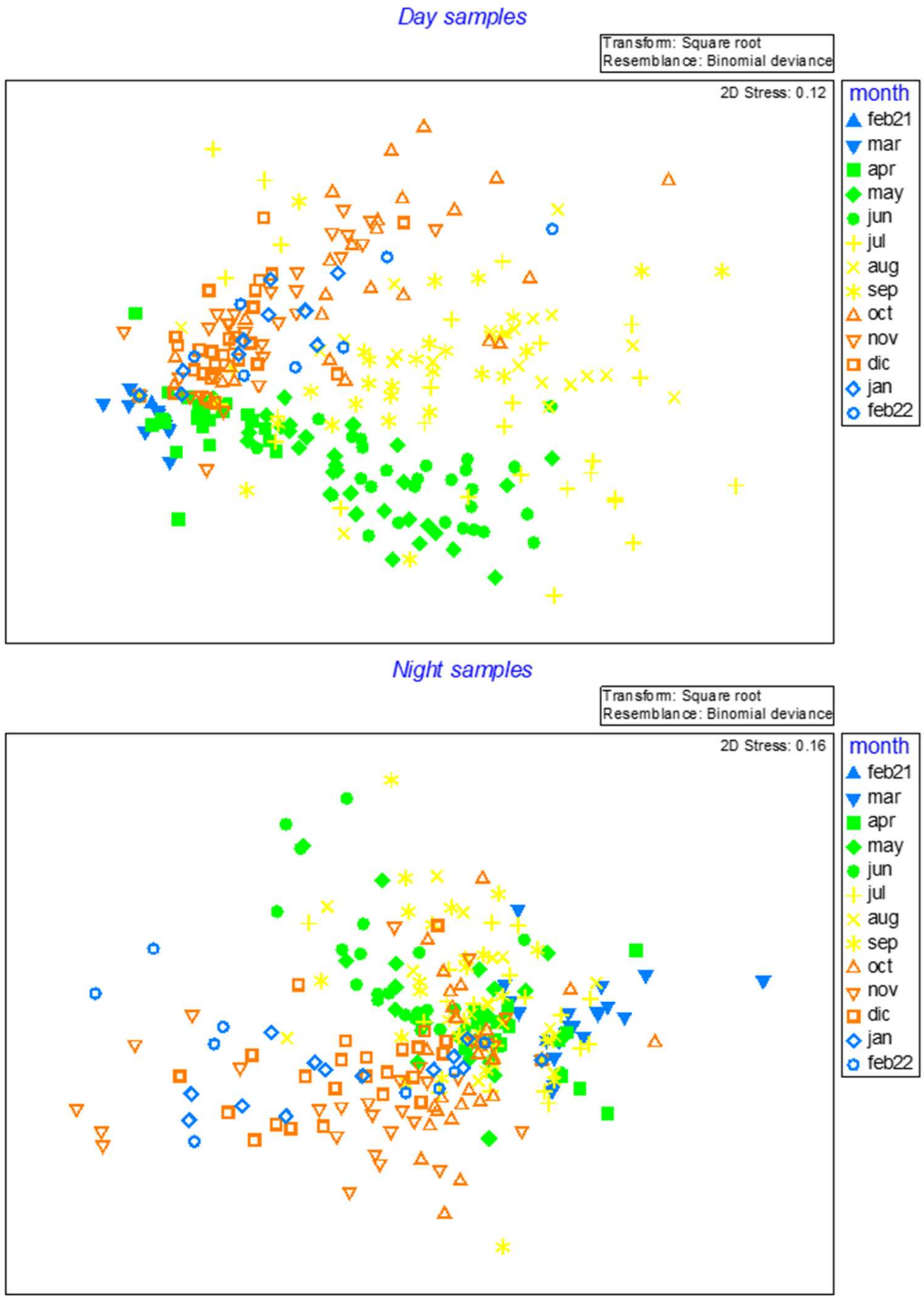


Figure 3.7 Two-dimensional ordination nMDS plot of day (top) and night (bottom) fish assemblages recorded from February 2021 to February 2022. Colours indicate different seasons (BLUE: winter; GREEN: spring; YELLOW: summer; ORANGE: autumn).

The PERMANOVA showed significant differences in fish assemblages' structure by month, time of day, and for the interaction term (table 3.3).

Table 3.3 Result of multivariate PERMANOVA test carried out on binomial deviance distance resemblance-matrix of square root transformed data. Df is degrees of freedom, MS is Mean Square and Pseudo-F is the F-value calculated by the PERMANOVA.

Source	**df**	**MS**	**Pseudo-F**	**P(perm)**
Month	12	125.02	38.73	0.0001
Day/Night	1	591.04	183.11	0.0001
Month x Day/Night	12	67.70	20.97	0.0001
Residuals	691	3.22		
Total	716			

The SIMPER analysis identified those species that contributed the most to assemblage changes by month and depending on the time of the day, excluding February 2021, where all similarities are zero. During the day (Tab 3.4), the white seabream *D. sargus* was the most typifying species of the assemblage from March to July, reaching more than 95% of contribution in March and April. In August and September, the bogue was the dominant species followed by *D. sargus*, while from October to February 2022 *D. vulgaris* dominated the fish assemblage together with *Spondyliosoma cantharus* and *Spicara maena*.

Table 3.4 Result of simper analysis carried out on factor month for the fish assemblages on day time.

Species	*Average Number*	*Av. Sim.*	*Contrib. %*	*Cum. %*
Group March	**Average similarity: 12.29**			
Diplodus sargus	0.18	11.78	95.85	95.85
Group April	**Average similarity: 29.30**			
Diplodus sargus	0.67	28.96	98.84	98.84
Group May	**Average similarity: 46.62**			
Diplodus sargus	1.19	28.01	60.09	60.09
Diplodus annularis	0.63	15.31	32.84	92.93
Group June	**Average similarity: 50.13**			
Diplodus sargus	1.51	30.41	60.66	60.66
Boops boops	0.94	13.85	27.63	88.28
Group July	**Average similarity: 33.19**			
Diplodus sargus	2.63	14.66	44.17	44.17
Boops boops	1.37	6.94	20.9	65.07
Diplodus annularis	1.06	6.06	18.26	83.33
Group August	**Average similarity: 39.45**			
Boops boops	2.27	17.54	44.46	44.46
Diplodus sargus	1.74	10.24	25.95	70.41
Group September	**Average similarity: 50.92**			
Boops boops	2.65	31.58	62.02	62.02
Diplodus sargus	0.98	9.69	19.03	81.05
Group October	**Average similarity: 31.60**			
Diplodus vulgaris	1.33	11.77	37.24	37.24
Diplodus sargus	0.98	8.82	27.91	65.16
Spondyliosoma cantharus	0.79	6.62	20.96	86.11
Group November	**Average similarity: 34.23**			
Diplodus vulgaris	0.87	19.84	57.96	57.96
Spondyliosoma cantharus	0.38	5.91	17.26	75.22

Group Dicember	**Average similarity: 18.03**			
Diplodus vulgaris	0.44	11.44	63.46	63.46
Spondyliosoma cantharus	0.19	3.62	20.09	83.55
Group January	**Average similarity: 6.37**			
Diplodus vulgaris	0.22	3.98	62.54	62.54
Spicara maena	0.11	1.29	20.25	82.8
Group February 2022	**Average similarity: 6.80**			
Diplodus vulgaris	0.36	5.06	74,42	74,42

During the night SIMPER results showed Clupeoid fishes as the most typifying taxon of the assemblages from March to May and July while the two-banded seabream *D. vulgaris* was the dominant species from November to February 2022 (Tab 3.5). In August and September, the bogue was the dominant species, similarly to the day time. A dissimilarity index between contiguous months (night and day sample separately) was also calculated using the SIMPER analysis (Tab. 3.6).

Table 3.5 Result of simper analysis carried out on factor month for the fish assemblages on night time.

Species	*Average Number*	*Av. Sim.*	*Contrib. %*	*Cum. %*
Group March	**Average similarity: 34.73**			
Clupeiformes	0.45	32.57	93.79	93.79
Group April	**Average similarity: 7.06**			
Clupeiformes	0.07	3.61	51.22	51.22
Diplodus sargus	0.12	3.12	44.23	95.45

Group May	**Average similarity: 11.65**			
Clupeiformes	0.13	6.78	58.25	58.25
Trachurus trachurus	0.13	3.47	29.84	88.09
Group June	**Average similarity: 6.65**			
Diplodus annularis	0.12	2.59	38.95	38.95
Trachurus trachurus	0.06	2.02	30.31	69.26
Clupeiformes	0.11	0.88	13.17	82.43
Group July	**Average similarity: 8.30**			
Clupeiformes	0.13	3.04	36.69	36.69
Spondyliosoma cantharus	0.08	2.31	27.86	64.56
Boops boops	0.06	1.38	16.65	81.21
Group August	**Average similarity: 16.62**			
Boops boops	0.24	11.15	67.1	67.1
Diplodus sargus	0.15	3.1	18.64	85.74
Group September	**Average similarity: 14.31**			
Boops boops	0.32	7.21	50.38	50.38
Clupeiformes	0.16	2.39	16.71	67.09
Diplodus sargus	0.11	2.08	14.54	81.63
Group October	**Average similarity: 16.82**			
Spondyliosoma cantharus	0.17	4.94	29.35	29.35
Diplodus vulgaris	0.18	4.22	25.11	54.45
Diplodus sargus	0.12	2.92	17.37	71.82
Group November	**Average similarity: 29.01**			
Diplodus vulgaris	0.54	13.71	47.25	47.25
Spondyliosoma cantharus	0.31	7.24	24.95	72.2
Group Dicember	**Average similarity: 23.76**			
Diplodus vulgaris	0.46	15.23	64.09	64.09
Spondyliosoma cantharus	0.14	2.24	9.44	73.53

Group January	**Average similarity: 10.71**			
Diplodus vulgaris	0.29	8.07	75.35	75.35
Group February 2022	**Average similarity: 8.39**			
Diplodus vulgaris	*0.36*	*5.78*	*68.9*	*68.9*
Boops boops	*0.2*	*0.96*	*11.47*	*80.37*

Table 3.6 Table of dissimilarity between assemblages from contiguous months during the day(a) and during the night(b). Feb21: February 2021: Mar: March, Apr: April; Jun: June; Jul: July, Aug: August, Sep: September; Oct: October; Nov: November; Dec: December; Jan: January, Feb21: February 2021.

	Mar	***Apr***	***May***	***Jun***	***Jul***	***Aug***	***Sep***	***Oct***	***Nov***	***Dec***	***Jan22***	***Feb22***
Feb21	*100*											
Mar		*82,74*										
Apr			*74,11*									
May				*57.17*								
Jun					*69.18*							
Jul						*65.47*						
Aug							*58.47*					
Sep								*72.20*				
Oct									*72.86*			
Nov										*76.59*		
Dec											*91.12*	
Jan22												*92.96*
Feb22												

	Mar	***Apr***	***May***	***Jun***	***Jul***	***Aug***	***Sep***	***Oct***	***Nov***	***Dec***	***Jan22***	***Feb22***
Feb21	*100*											
Mar		*87.71*										
Apr			*91.92*									
Ma				*92.59*								
Jun					*94.64*							
Jul						*89.91*						
Aug							*84.81*					
Sep								*87.82*				
Oct									*79.47*			
Nov										*74.52*		
Dec											*86.56*	
Jan 22												*89.93*
Feb22												

Fish diversity (as expressed by H' index), was greater in summer and autumn than in the other two seasons (Fig. 3.8), and especially from September to November, being on average 0.96 (± 0.47).

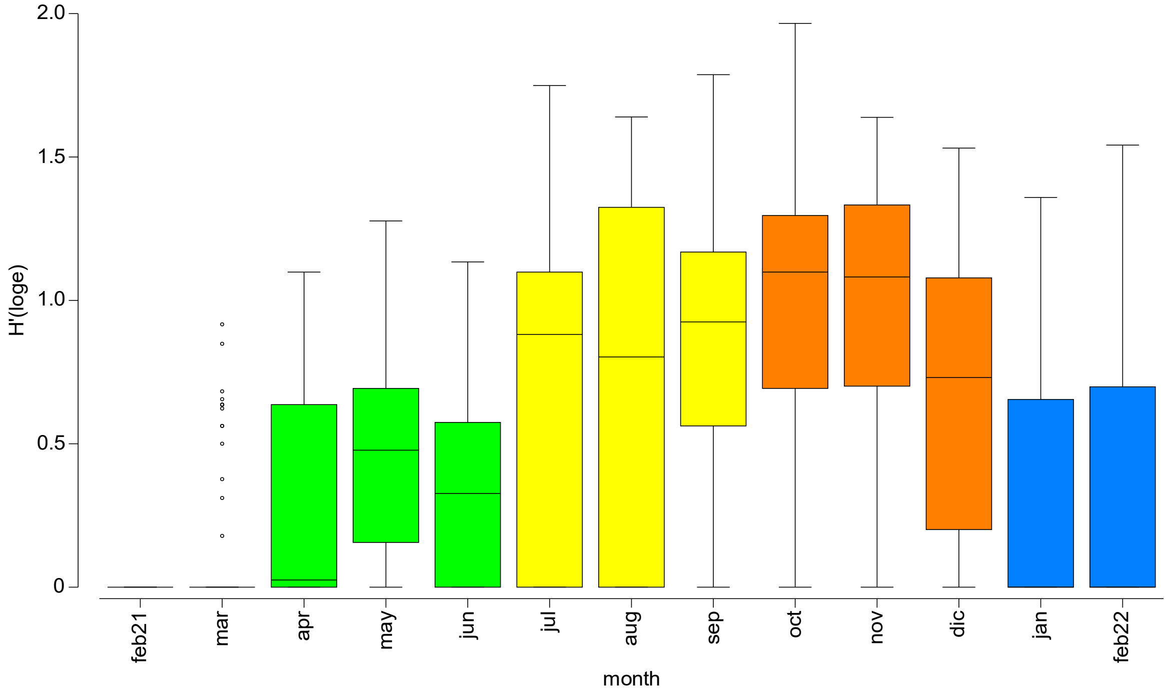


Figure 3.8 Box Plot of the fish diversity by month, based on Shannon index (H').

Univariate PERMANOVA showed significant differences in fish diversity among months, between day and night, and for the interaction factor (Table 3.7). Pair-wise comparison within level 'day' of factor 'day and night' for pair of contiguous months (Tab. 3.8) showed significant differences ($p<0.05$) between March and April, April and May, June and July, November and December, and December and January.

Table 3.7 Result of univariate PERMANOVA main test for diversity of data between months. Df indicates degrees of freedom, MS is Mean Squares and Pseudo-F is the F-value calculated by the PERMANOVA.

Source	***df***	***MS***	***Pseudo-F***	***P(perm)***
Month	*12*	*5.35*	*28.72*	*0.001*
Day/Night	*1*	*1.39*	*7.47*	*0.007*
MonthxDay/Night	*12*	*0.65*	*3.51*	*0.001*
Residuals	*691*	*0.19*		
Total	*716*			

Table 3.8 Results of the Univariate PERMANOVA, pair-wise comparison carried out on the Shannon index within level 'day' of factor 'day and night' for pair of contiguous months.

Groups	***t***	***P(perm)***
February21, March	*0.43*	*1*
March, April	*5.24*	*0.0001*
April, May	*3.46*	*0.0008*
May, June	*0.52*	*0.59*
June, July	*4.22*	*0.0002*
July, August	*0.17*	*0.86*
August, September	*0.40*	*0.68*
September, October	*1.08*	*0.28*
October, November	*0.95*	*0.34*
November, December	*3.28*	*0.001*
December, January	*3.73*	*0.0005*
January, February22	*0.62*	*0.53*

Pair-wise comparison within level 'night' of factor 'day and night' for pair of contiguous months (Table 3.9) showed significant variations between May and June, June and July, November and December, and December and January.

Table 3.9 Results of the Univariate PERMANOVA pair-wise comparison carried out on the Shannon index, within level 'night' of factor 'day and night' for pair of contiguous months.

Groups	*t*	*P(perm)*
February21, March	*1.87*	*0.10*
March, April	*0.46*	*0.64*
April, May	*1.39*	*0.17*
May, June	*2.19*	*0.03*
June, July	*2.03*	*0.046*
July, August	*0.55*	*0.57*
August, September	*1.24*	*0.21*
September, October	*1.66*	*0.10*
October, November	*1.08*	*0.28*
November, December	*2.69*	*0.008*
December, January	*3.55*	*0.001*
January, February22	*0.66*	*0.51*

3.4 Activity rhythms of fishes

A waveform analysis was conducted to determine the swimming activity phase of the four most abundant species count rhythms over the 24h timing.

The results showed the presence of defined diurnal phase for all the four species. At night, between 04:00 and 18:00 or 05:00 and 17:00 with variations linked to seasons, the visual count dropped near zero with some exceptions (i.e. *Boops boops* in April). In particular, count peak temporal limits were: *B. boops* showed a diurnal activity, on average between 04:00 and 16:00 with exclusion for the months of March and April (Figure 3.9).

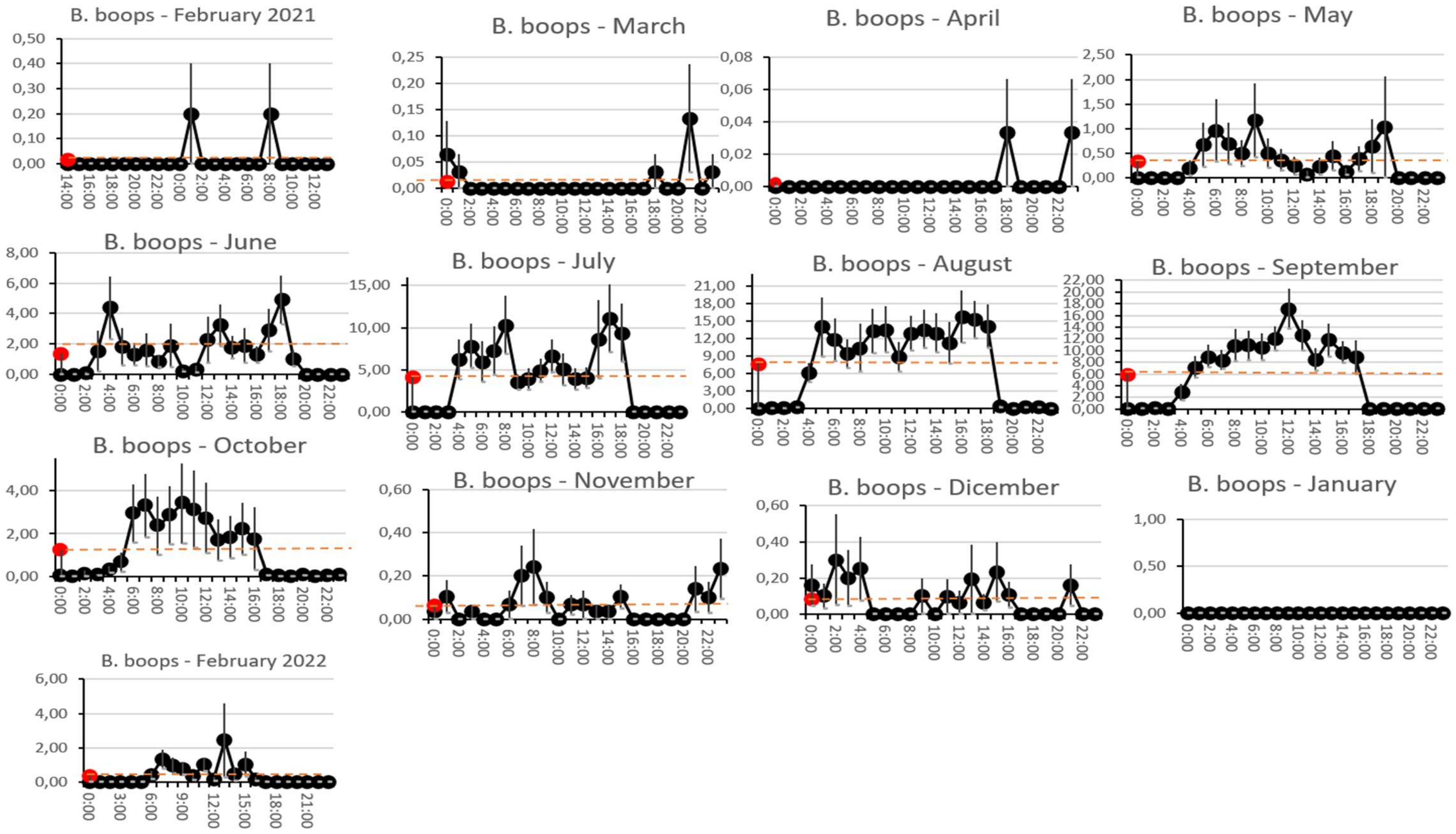
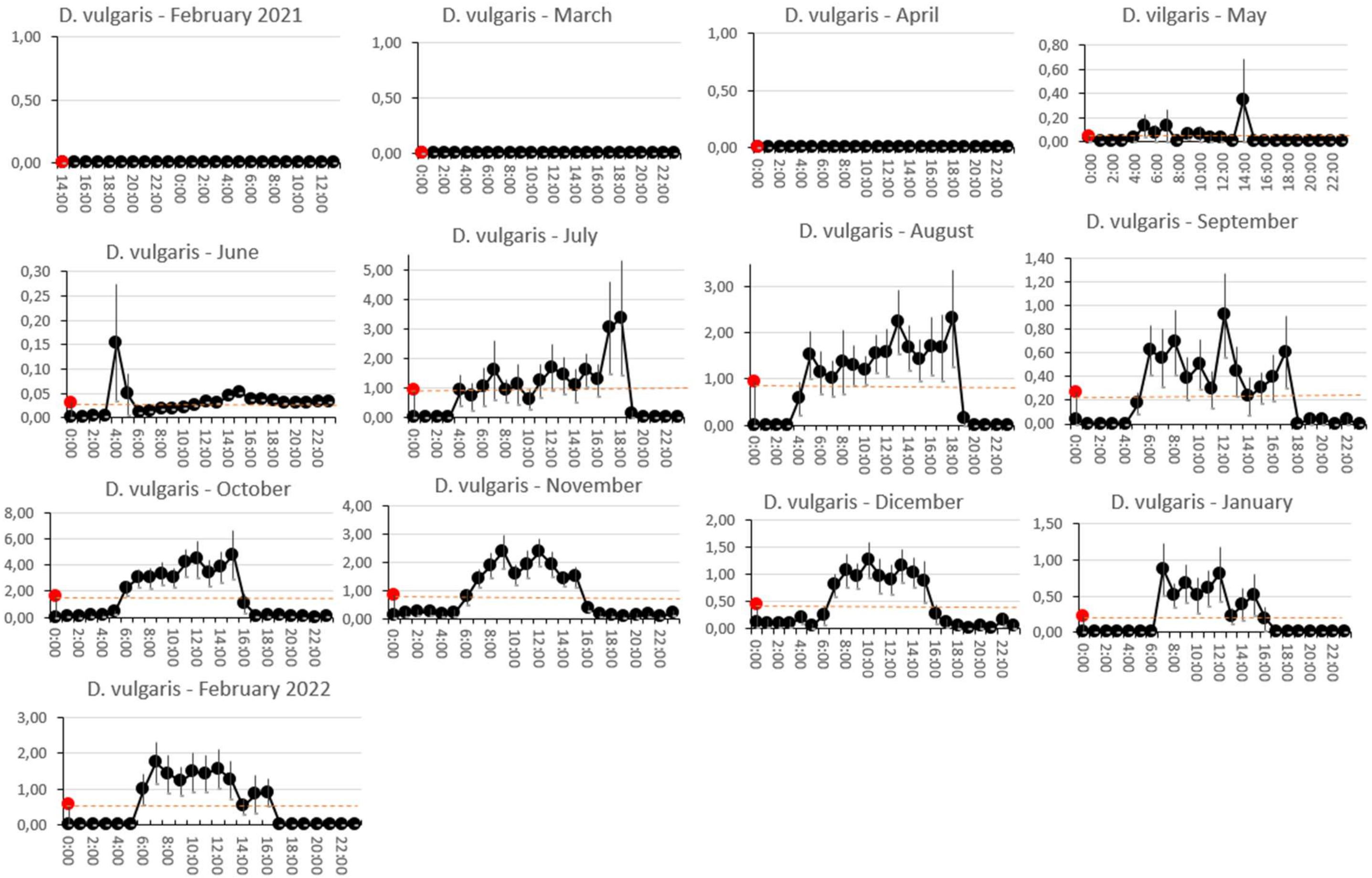


Figure 3.9 Waveform analysis output (mean ± SD) for time series of D. annularis count (note different scale) obtained during 1 year of continuous video monitoring by the "Viviana" observatory. The phase of rhythms of visual counts is identified by values above the MESOR.

D. vulgaris was not detected in the first three months of observation, it presented an increasing presence from April to the maximum peak of September. Peak time limits were from 06:00 to 18:00, and only rarely present before and after (Fig. 3.10).

D. sargus diurnal activity was determined to be between 6:00 and 18:00 during the winter-spring period and between 4:00 and 18:00 during summer-autumn. Also, presented an increasing pattern until the peak in July with and a subsequent and gradual decrease of individuals (Fig. 3.11).

D. annularis also showed a pattern similar to *D. sargus* with a diurnal activity on average between 4:00 and 18:00and the maximum peack in July (Fig. 3.12).


D. vulgaris - February 2021

D. vulgaris - March

D. vulgaris - April

D. vilgaris - May

D. vulgaris - June

D. vulgaris - July

D. vulgaris - August

D. vulgaris - September

D. vulgaris - October

D. vulgaris - November

D. vulgaris - Dicember

D. vulgaris - January

D. vulgaris - February 2022

Figure 3.10 Waveform analysis output (mean ± SD) for time series of D. vulgaris count (note different scale) obtained during 1 year of continuous video monitoring by the "Viviana" observatory. The phase of rhythms of visual counts is identified by values above the MESOR.

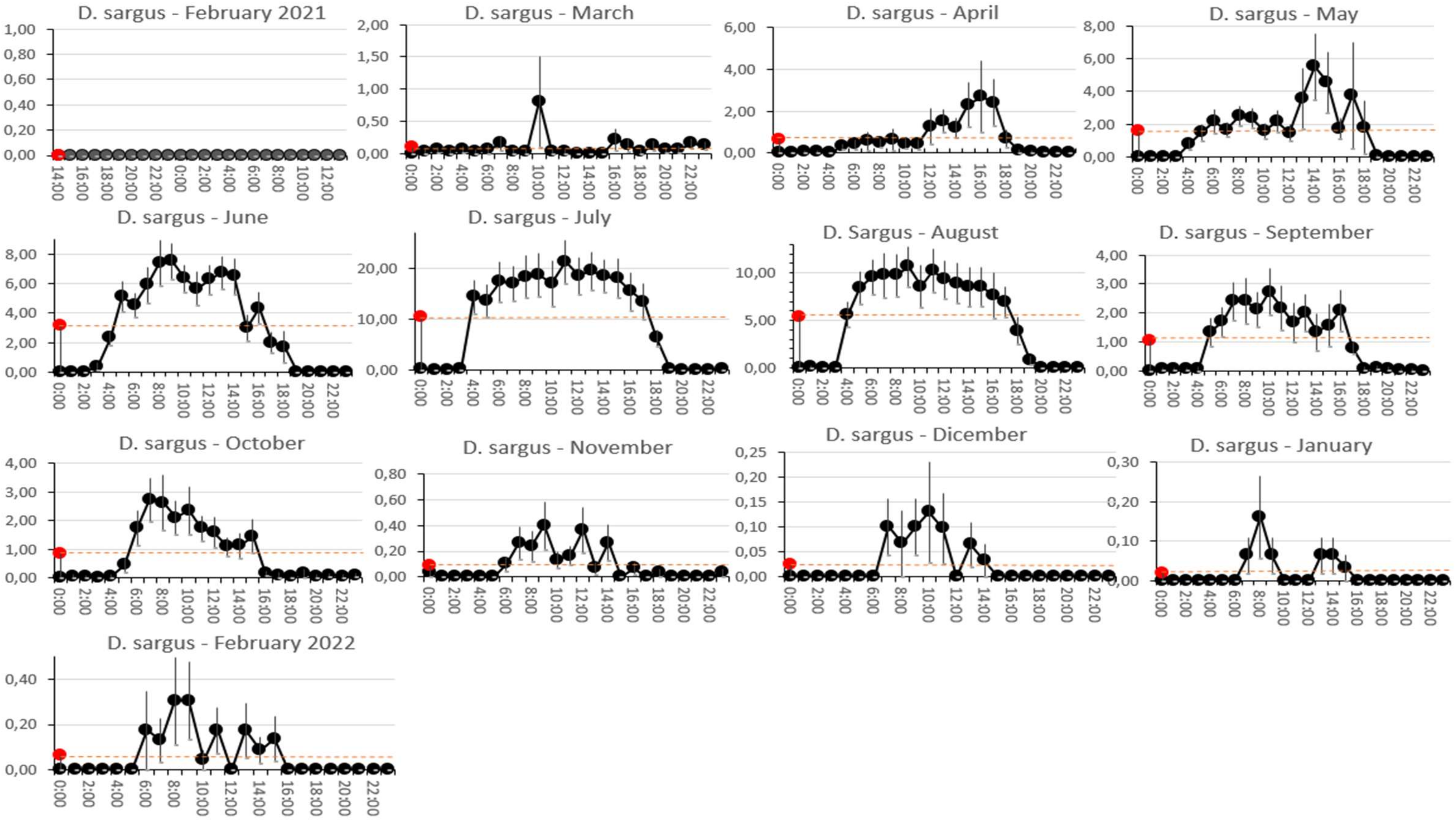


Figure 3.11 Waveform analysis output (mean ± SD) for time series of D. sargus count (note different scale) obtained during 1 year of continuous video monitoring by the "Viviana" observatory. The phase of rhythms of visual counts is identified by values above the MESOR.

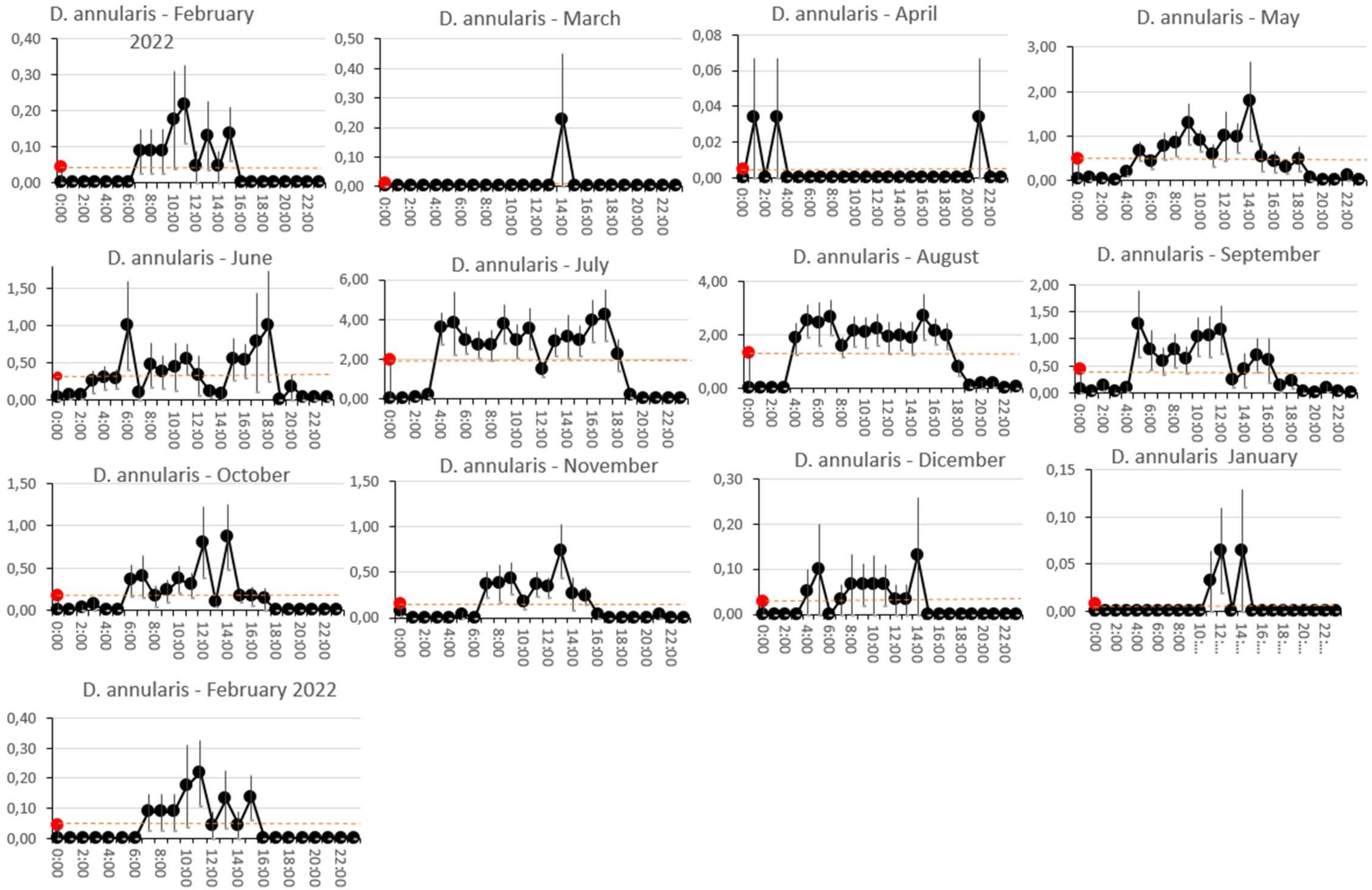


Figure 3.12 Waveform analysis output (mean ± SD) for time series of D. annularis count (note different scale) obtained during 1 year of continuous video monitoring by the "Viviana" observatory. The phase of rhythms of visual counts is identified by values above the MESOR.

3.5 Correlation with environmental variables

From the Draftman plot output, the resemblance matrix, all environmental variables with a high linearity (> ± 0.7) were selected to be sub sequentially eliminated: current speed, wind speed (both correlated with the height of the wave), direction of origin of the wind with respect to the sensor (correlated with the direction of origin of the wind with respect to the N) and the percentage of water saturation (correlated with the dissolved oxygen concentration).

The CCA plot evidenced a clear relationship between seasons and some of the environmental variables (Fig. 3.13a), specifically summer records were mostly linked to temperature and to the current direction, while spring ones seemed to be mostly related to irradiance. Winter samples were instead more correlated to water density and to dissolved oxygen concentration, while autumn ones to wind direction and turbidity. When species, instead of seasons, were shown (Fig. 3.13b), species as *D. annularis, P. erithrinus* and *P. acarne, B. boops, S. hepatus, S. dumerilii, M. surmuletus, P. pagrus* and *C. julis* seemed to be mostly linked to temperature, while species as *D. puntazzo, D. sargus, D. dentex* and *S. umbra* appeared to be more related to irradiance. *Spondilyosoma cantharus, S. porcus*, *S. cabrilla, T. trachurus, S. fluxuosa, M. cephalus, D. cervinus* and clupeiformes where instead more related to current direction and turbidity. Finally, *D. vulgaris*, *S.maena*, *C. chromis* and *O. melanura* are more correlated to density and dissolved oxygen concentration.

Finally, *D. vulgaris, S. maena, C. chromis* and *O. melanura* are more correlated to density and dissolved oxygen concentration.

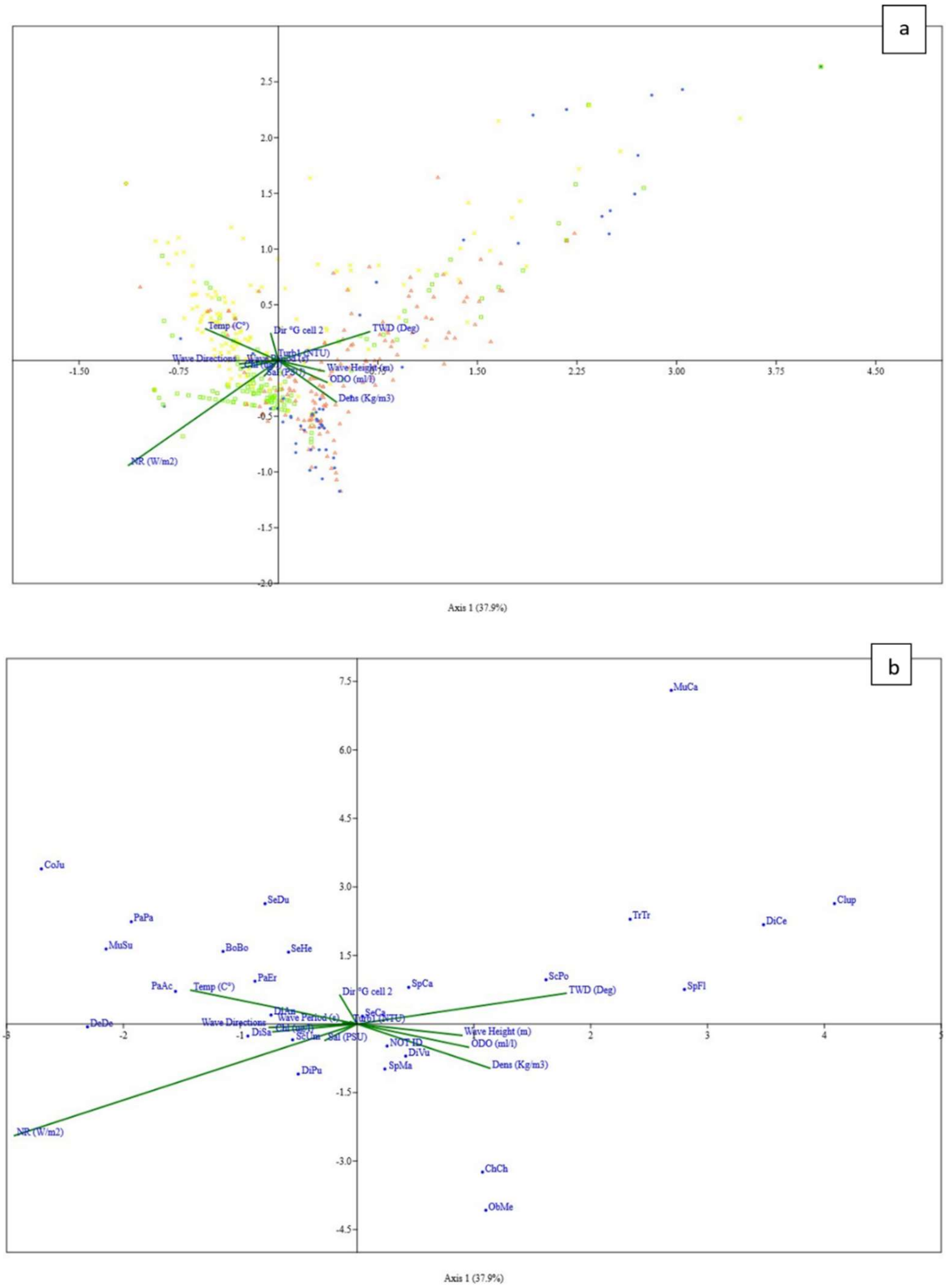


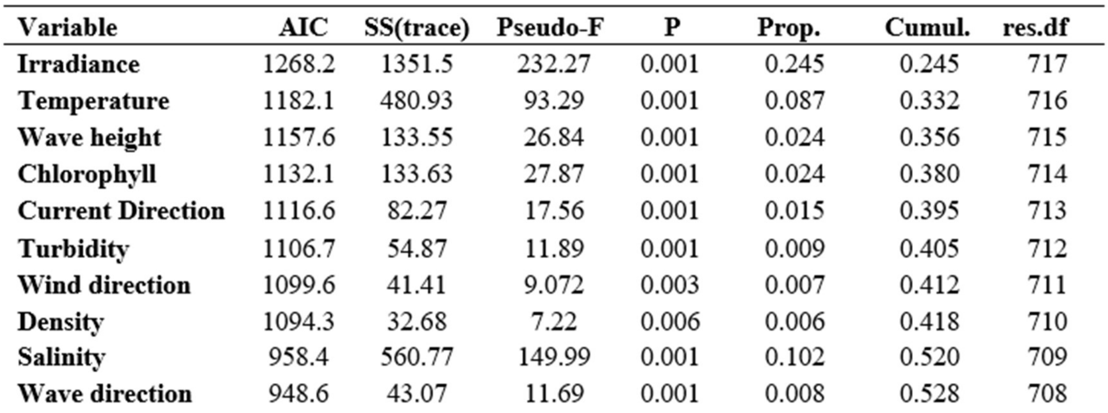
Figure 3.13 CCA output for the analysis of species counts temporal response upon some environmental variables (b) combined with the month factor (a). Environmental variabels are vectors.

Environmental data were tested for collinearity among variables by using a Draftsman plot The Distance-Based Linear Model allowed to determine the environmental variables that best explained the variance of the fish communities across seasons for all samples together (Tab. 3.8a) and for samples relating to daytime (Tab. 3.8b) and to night-time (Tab. 3.8c).

The best explanatory variables of the variance of the whole community (Tab. 3.8a) were irradiance, salinity, and temperature, together contributing to explain to 43.4%. The variance of the diurnal community was mainly explained by temperature, accounting alone to 17.1% and secondarily by wave height (ca. 6.7%) and chlorophyll-*a* concentration (4.8%) (Tab 3.8b), all the other variables, although significant, contributed less than 4% to the total variance. The best explanatory variables for the nocturnal community (Tab. 3.8c) were temperature, and dissolved oxygen, contributing for 10.7% and 4.2%, respectively, to the total variance. Similarly, for the daytime model, although several other variables were significant, they did not account for a large portion of variance, contributing each for less than 2%.

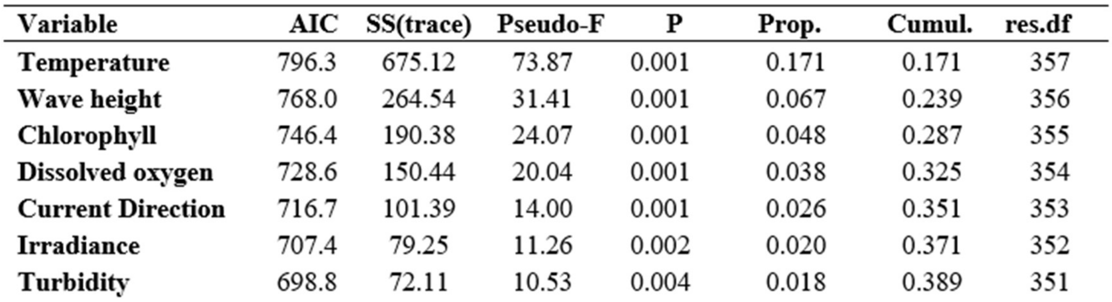
Table 3.8 Distance base Linear Mode (Dist LM) sequential test output for (a) all community sample; (B) the diurnal community sample portion; (c) the nocturnal community sample portion.

a



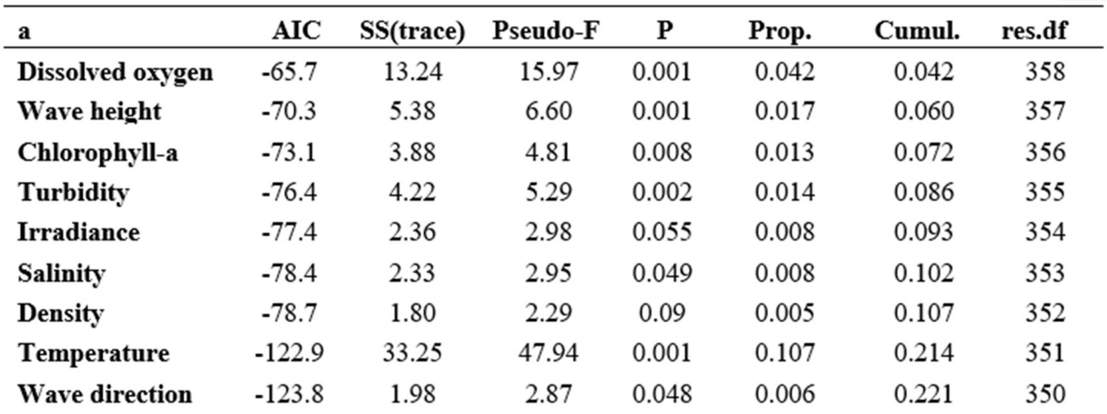
Variable	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Irradiance	1268.2	1351.5	232.27	0.001	0.245	0.245	717
Temperature	1182.1	480.93	93.29	0.001	0.087	0.332	716
Wave height	1157.6	133.55	26.84	0.001	0.024	0.356	715
Chlorophyll	1132.1	133.63	27.87	0.001	0.024	0.380	714
Current Direction	1116.6	82.27	17.56	0.001	0.015	0.395	713
Turbidity	1106.7	54.87	11.89	0.001	0.009	0.405	712
Wind direction	1099.6	41.41	9.072	0.003	0.007	0.412	711
Density	1094.3	32.68	7.22	0.006	0.006	0.418	710
Salinity	958.4	560.77	149.99	0.001	0.102	0.520	709
Wave direction	948.6	43.07	11.69	0.001	0.008	0.528	708

b



Variable	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Temperature	796.3	675.12	73.87	0.001	0.171	0.171	357
Wave height	768.0	264.54	31.41	0.001	0.067	0.239	356
Chlorophyll	746.4	190.38	24.07	0.001	0.048	0.287	355
Dissolved oxygen	728.6	150.44	20.04	0.001	0.038	0.325	354
Current Direction	716.7	101.39	14.00	0.001	0.026	0.351	353
Irradiance	707.4	79.25	11.26	0.002	0.020	0.371	352
Turbidity	698.8	72.11	10.53	0.004	0.018	0.389	351

c



a	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Dissolved oxygen	-65.7	13.24	15.97	0.001	0.042	0.042	358
Wave height	-70.3	5.38	6.60	0.001	0.017	0.060	357
Chlorophyll-a	-73.1	3.88	4.81	0.008	0.013	0.072	356
Turbidity	-76.4	4.22	5.29	0.002	0.014	0.086	355
Irradiance	-77.4	2.36	2.98	0.055	0.008	0.093	354
Salinity	-78.4	2.33	2.95	0.049	0.008	0.102	353
Density	-78.7	1.80	2.29	0.09	0.005	0.107	352
Temperature	-122.9	33.25	47.94	0.001	0.107	0.214	351
Wave direction	-123.8	1.98	2.87	0.048	0.006	0.221	350

4. DISCUSSION

In this study, the fish community around the “Viviana” offshore platform was identified and its variability, characterized by high-frequency video and environmental multiparametric monitoring, analysed. We highlighted dominant species, their pattern of seasonal variations and key controlling environmental drivers.

Temporally-intensive video-monitoring revealed the presence of marked biodiversity changes at the diel and seasonal level of this fish community dwelling at shallow depths (ca. 20 m depths), close to the platform.

Although many studies exist dealing with fish community associated to gas platform in the Adriatic Sea (Fabi et al., 2004; Andaloro et al., 2011; Consoli et al., 2013; Tassetti et al 2020), this is the first attempt based on remote video-monitoring carried out at high temporal frequency (i.e. one image every hour within 24 hours for 13 months), encompassing day and night sampling and taking into account the environmental control.

Previous studies already highlighted the role of offshore platforms in producing qualitative and quantitative changes in the fish communities inhabit the surrounding natural habitat (Fabi et al., 2002). However, the establishment of a well-structured community requires more than two years and it is likely related to the different life cycle and responses to the occurrence of an artificial hard structure of individual species (Fabi et al., 1999, Scarcella et al., 2010).

Viviana platform was installed in 1998, while the production ended ca. 5 years ago, thus at the time of the beginning of our experiment there was a well-established fish community. The result obtained were considered as a proxy for diel (24-h based) and seasonal patterns of changes in local abundance of fish populations because the probability of animal spotting using video monitoring depends upon rhythmic behaviours such as spatiotemporal displacement through different environments or between sleeping and activation (e.g., Myers et al., 2016).

4.1 Temporal changes in abundance

In this study, the presence of seasonal changes in fish abundance was evaluated by averaging number of the total detected fishes per each month. The abundance increased in late spring and summer, more precisely between May and September, with a maximum peak in June, similarly to what observed in another study conducted in Spain where the abundance of the majority of the species was highest in the summer, and lowest in winter months (Fernando et al., 2012). Additionally, a study conducted at Šibenik (Croatia) (Aguzzi et al., 2020) for four months (from January to April) recorded an increasing abundance towards spring, with a small peak also in February. In some Mediterranean coastal fishes, an increase in population sizes can be observed during summer months as a result of successful recruitment of juvenile cohorts (Harmelin-Vivien et al, 1985). Fish abundance usually shows "intermediate" levels between October-December and March-April and they are considered to be transition periods for fish community composition in Mediterranean coastal area (Santos et al., 2005). The lowest abundances were observed between February and April. This is consistent with the pattern observed for the pelagic fish fauna in the central Adriatic Sea and was likely due to changes in temperature (Duli et al., 2005). Indeed, in winter, in the Northern and central Adriatic basin the water temperature drastically drops to ca. 8-10 ° C (Artegiani et al., 1997) causing most of the species to migrate towards deeper and warmer waters, with re-population occurring gradually in spring and summer (Bombace, 1992). To date, there is no comprehensive theory for patterns of diel variation in aquatic systems. To a certain extent, diel dynamics of marine fish reflect habitat use and activity patterns, that are species-specific (Arrington and Winnemiller, 2003), changes that have been mostly attributed to variations in feeding behaviour, spawning behaviour and to predator avoidance (Wolter and Freyhof, 2004). These changes have been clearly demonstrated by the high-frequency time-lapse video analysis conducted.

The diurnal trend was similar to the whole one as the daytime samples are characterised by the largest number of individuals recorded and thus drove the overall trend. So the considerations reported for the whole community can be extended to the daytime one.

The overall trend of nocturnal samples was more homogeneous, presenting a smaller number of species than the diurnal sample and a lower variability of the abundance during the year with two small peaks, one in June and a smaller one in in December. To date there are few studies with such a long-term monitoring of fish abundance during night to make an adequate comparison. It can be assumed (see below) that these two peaks in abundance are related to the periods of greatest abundance of the dominant nocturnal species.

4.2 Seasonal changes in fish community

A multivariate analysis of underwater faunistic surveys allows us to define which species were present in a long-term monitoring and determine the day to day and seasonal variations between different species.

A relationship between fish count and the solar irradiance have been detected (see paragraph 4.4): the fish assemblage composition and structure varied consistently between diurnal and nocturnal censuses with a marked decrease in both abundance and species richness during the night as observed in north-western Mediterranean coast at the OBSEA observatory (Santamaria et al., 2013) and other studies (i.e. Azzurro et al., 2007, Hawley et al., 2017). The daily sample is much more dispersive, the greater number of species which compose the daytime community (compared to the nocturnal sample) has a much more marked seasonal variation. Even if overall considered more diversified, it presented some species (precisely *D. sargus, B. boops* and *D.*

*vulgari*s) that for several months predominated over the others, contributing between 60% and 95% of the total composition of the species present, differently from the results obtain by Aguzzi et al., 2020 study conducted in Croatia were the most predominant species registered' (during the day) contribute was between 23% and 50%. A very different trend than the nocturnal one that showed a more homogeneous composition among species. The Shannon diversity index indicated a higher diversity in the fish population during autumn, an intra annual variation that is considered a reliable proxy for seasonal pattern of change in local fish abundance (Condal et al., 2012). The peaks of abundance registered during the night were caused in June by *D. annularis* and *T. trachurus*. *Diplodus annularis* had higher values of abundance during the entire period of observations and had a clear daily rhythm: this abnormal nocturnal aggregation can be explain by the fact that the spawning season, in the Adriatic Sea, begin in July (www.fishbase.org, version 02/2022). *Trachurus trachurus*, a benthopelagic (Colloca et al., 2004), is among the typical fish species affected by light/vision spectrum (Abaunza et al., 2003) that would form large schoals near sea bottoms during the day and dispersing during the night (Barange et al., 2005). Besides, Atlantic horse mackerel is believed to be a crepuscular predator, during both dawn and sunset (Helfman 1986). It is not clear why it is particularly abundant during the night of June. The other peak, weaker from the one just described, was register during the month of December by an increasing number of *D. vulgaris*' specimen. Probably it is linked to the mating period which ends, in the Adriatic Sea in the month of December (www.fishbase.org, version 02/2022, see below). The peak shows by the day sample, during the month of July was mostly due to the presence of individuals of *D. sargus,* which trend is explained below. The community structure presents a seasonal turnover showed by a sample separation between the winter/spring and the summer/autumn period. There was a clear change in the community composition and a shift from *S. cantharus* to *D. sargus* and *D. annularis* during the day and from *T. trachurus* and *B. Boops* to Clupeiformes in the night. Seasonal variability is expected to play a crucial role in the ecological succession of coastal communities, as background community composition and relative abundances changes substantially moving from summer to autumn (Condal et al., 2012). The continuous presence of reef-associated (i.e. belonging to the genus *Diplodus*) or reef-dwelling benthic species (belonging to the genera *Scorpaena* and *Serranus*), was characteristics of the platform-associated fauna, as platforms act as FADs (Scarcella et al., 2010) thus providing shelter, and/or food, as already observed from other authors (Fabi et al., 2002), increasing species richness and diversity.

4.3 Dominant species rhythms

Waveform analysis for visual count time series for the four different species identified the photic measures at which animal presence significantly increased or decreased at the "Viviana" platform study site. All the four species analysed presented seasonal fluctuations for all species considered and showed diurnal phase with the exclusion of the *B. boops*, the only one with a nocturnal behaviour established just during the winter season.

The sparid *B. boops* showed a single and compact seasonal increase lasting several months, probably explained by its gregarious behaviour and long spawning period (Dobroslavić et al., 2017) that for this species in the Adriatic Sea occurred from January to May. Differently, *D. sargus* and *D. annularis* showed a more definite increase during the month of July. *Diplodus sargus* counts were mainly correlated with daily photoperiod and peaked in July. This was in contrast to what observed at OBSEA observatory (Sbragaglia et al., 2018), where white sea bream rhythms were mainly correlated to water temperature and peaked in October. The white seabream shows a unique seasonal peak in spawning, which occurs in the western Mediterranean between March and June (www.fishbase.org, version 02/2022). Probably, the summer aggregation behaviour registered in this study was related to something other than reproduction, but probably a reflection of the fluctuations of the available prey in the environment (Pallaoro et al., 2006). Artificial reefs like off-shore platforms have been demonstrated to be important feeding sites for the omnivorous *D. sargus,* which mainly feed on bivalves, echinoderms, and algae (Sala et a., 1997). Thus, the artificial reef is likely used as a foraging ground, supporting individuals' dietary requirements, just before the onset of their spawning period.

Diplodus annularis summer aggregations can be instead associated to reproduction, in fact, the spawning period for this species in the Adriatic Sea is between June and August (www.fishbase.org, version 02/2022). Telemetry and tag-recapture experiments showed that the carnivorous annular seabream has a high site fidelity (March et al., 2011). This information can justify why the abundance of this species was on average lower than the other Sparidae in our study. In fact, the camera was activated immediately after the assembling of the observatory, that offers a more complex, three-dimensional shelter than the single-pole "Viviana" platform, and enhance fish abundance. However, this species would have need more time to be attracted by this change. According to Macpherson (1981), *D. annularis* is a generalist species, capable of adapting to environment variations and exploiting the food resources available.

The two-banded sea bream D. *vulgaris* showed a scattered pattern, it started to appear around April and then the abundance shows sparse peaks over the year, with the highest one in October. The late appearance can be justified by the new presence of the structure on which the individuals of *D. vulgaris* found refuge a few months later. The spawning season occurs in January and December (Sbragaglia et al., 2018). The increase of counts in October around the platform could be related to the dietary requirements for reproduction (Sbragaglia et al., 2018).

4.4 Environmental drivers of changes of fish assemblage at Viviana platform

Temporal variations in the fish community associated to Viviana platform and the artificial structure here deployed were correlated with different environmental variables, according to seasons. Species video-based lists and relative counts indicated the occurrence of marked swimming rhythms controlled by solar light intensity and photophase length cycles (respectively, for day-night and seasonal controlled rhythms). The importance of water temperature, as environmental driver has already been described for many fish species (Vinagre et al., 2016**;** Van Der Walt et al., 2021). Temperature deeply affects fish presence/absence, because it influences directly species physiological performance (Day et al., 2018)**.** Species video-based lists and relative counts indicated the occurrence of marked swimming rhythms controlled by solar light intensity and photophase length cycles (respectively, for day-night and seasonal controlled rhythms). In this last case, strong association with dissolved oxygen registered during the night with the firs axis is presumably combined with the effect of temperature, which to a great extent determines the oxygen solubility in the water column (Lipizer et al., 2014).

5. CONCLUSIONS

Taken together, these results evidenced the need of temporally replicated routines for fish species data collection to sustain biodiversity based conservation and management policies. At the same time, the enforced monitoring technological approach highlights the relevance of decommissioned oil and gas infrastructures to be used for the monitoring of live components of oceans. Our findings show the potential of underwater observatories to produce data for fish monitoring complementary to those obtained with other more classic sampling techniques (e.g. visual census, trammel nets and fishing). It allowed tracking fish assemblages at high-frequency and over a long period, opening new grounds for investigating biological rhythms at population level. Moreover, the possibility of installing an observatory on dismissed off-shore platforms opens the possibility to create a data collection replicated monitoring network. Advances along this technological path can provide novel and effective monitoring tools for environmental assessment of marine ecosystem in the context of European management policies like the Marine Strategy Framework Directive (EC 2008/56) that have already accepted high-definition cameras as a promising approach for monitoring the marine environment, specifically regarding the Descriptor 1 ‘biodiversity”. Also, remote underwater monitoring could cover other descriptors like the D3 ‘commercial fish and shellfish species’ and D4 ‘food webs’. The video-monitoring may also help detected alien species (Descriptor 2) or define levels of parasitism in the different populations. A standardised protocol for data acquisition and treatment may help for comparison of richness and biodiversity across areas with different monitoring platforms bearing similar sensor assets. The results from the “Viviana” platform will allow also gathering information to complement other ongoing monitoring initiatives using permanent observatories in the Mediterranean Sea (i.e., OBSEA in Spain: www.obsea.es; Šibenik Natura 2000 in Croatia) and the Atlantic (i.e., SmartBay in Ireland: https://www.smartbay.ie/; Molene in France: http://www.emso-fr.org/EMSO-Molene or Martha's Vineyard in USA at https://www.whoi.edu/mvco).

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Annex

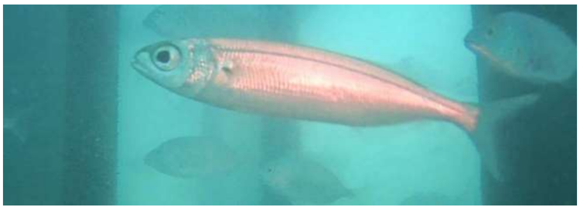
1. *List of vide-detected species and fish code are reported along with relative abundance and percentage.*

Species	**Common name**	**Codes**	**Total**	**%**
Boops boops (Linnaeus, 1758)	Bogue	BoBo	12179	14.7
Chromis Chromis (Linnaeus, 1758)	Damselfish	ChCh	256	0.3
Clupeiformes	/	Clup	295	0.4
Coris julis (Linnaeus, 1758)	Mediterranean rainbow wrasse	CoJu	1	0.001
Dentex dentex (Linnaeus, 1758)	Common dentex	DeDe	2	0.002
Diplodus annularis (Linnaeus, 1758)	Annular seabream	DiAn	2874	3.5
Diplodus cervinus (Lowe, 1838)	Zebra seabream	DiCe	59	0.07
Diplodus puntazzo (Cetti, 1777)	Sharpsnout seabream	DiPu	35	0.04
Diplodus sargus (Linnaeus, 1758)	White seabream	DiSa	13342	16.1
Diplodus vulgaris (Geoffroy Saint Hilaire, 1817)	Two-banded seabream	DiVu	3711	4.5
Mugil caphalus (Linnaeus, 1758)	Mullet	MuCe	4	0.004
Mullus surmuletus (Linnaeus, 1758)	Surmullet	MuSu	5	0.01
Oblada melanura (Linnaeus, 1758)	Saddled seabream	ObMe	26	0.03
Pagellus acarne (Risso, 1826)	Axillary seabream	PaAc	5	0.006
Pagellus erythrinus (Linnaeus, 1758)	Common pandora	PaEr	214	0.3
Pagrus pagrus (Linnaeus, 1758)	Red porgy	PaPa	13	0.02
Sciaena umbra (Linnaeus, 1758)	Brown meagre	ScUm	16	0.02
Scorpaena porcus (Linnaeus, 1758)	Black scorpionfish	ScPo	72	0.09
Seriola dumerili (Risso, 1810)	Greater amberjack	SeDu	79	0.1
Serranus cabrilla (Linnaeus, 1758)	Comber	SeCa	37	0.04
Serranus hepatus (Linnaeus, 1758)	Brown comber	SeHe	9	0.01
Spicara flexuosa (Rafininesque, 1810)	Pickerel	SpFl	220	0.3
Spicara maena (Linnaeus, 1758)	Blotched pickerel	SpMa	444	0.5
Spondyliosoma cantharus (Linnaeus, 1758)	Black seabream	SpCa	1222	1.5
Trachurus Trachurus (Linnaeus, 1758)	Common scad	TrTr	227	0.3
NOT ID	/	NOT ID	47791	57.5

2. List of video-detected species.

Species

Boops boops (Linnaeus, 1758)

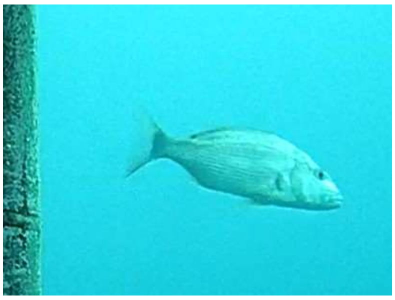


Chromis Chromis (Linnaeus, 17589)

Clupeiformes

Coris julis (Linnaeus, 1758)

Dentex dentex (Linnaeus, 1758)



Diplodus annularis (Linnaeus, 1758)

Diplodus cervinus (Lowe, 1838)

Diplodus puntazzo (Cetti, 1777)

Diplodus sargus (Linnaeus, 1758)

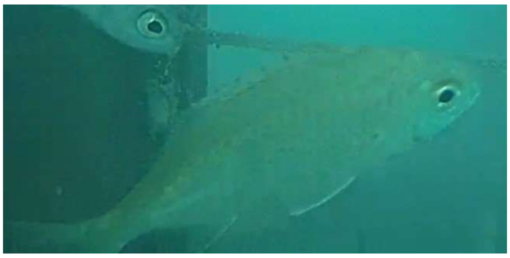
Diplodus vulgaris (Geoffroy Saint Hilaire, 1817)

Mugil caphalus (Linnaeus, 1758)

Mullus surmuletus (Linnaeus, 1758)

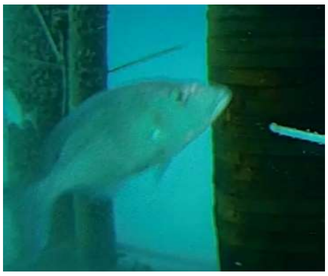
Oblada melanura (Linnaeus, 1758)

Pagellus acarne (Risso, 1826)



Pagellus erythrinus (Linnaeus, 1758)

Pagrus pagrus (Linnaeus, 1758)



Sciaena umbra (Linnaeus, 1758)

Scorpaena porcus (Linnaeus, 1758)

Seriola dumerili (Risso, 1810)

Serranus cabrilla (Linnaeus, 1758)

Serranus hepatus (Linnaeus, 1758)

Spicara flexuosa (Rafininesque, 1810)

Spicara maena (Linnaeus, 1758)

Spondyliosoma cantharus (Linnaeus, 1758)

Trachurus Trachurus (Linnaeus, 1758)