

INTERREG MED PROGRAMME

FISHMPABLUE2 PROJECT

WP3 "Testing"

Deliverable 3.3.2 Monitoring Reports

Results of the "ex post" environmental-socio-economic monitoring activities

Updated with eDNA analysis

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1. INTRODUCTION

In the context of FishMPABlue2 project, one monitoring report has to be produced after each of the two monitoring campaigns planned for 2017 ("ex ante", i.e. before the implementation of the governance tools in each MPA, see Del. 3.3.2 - 'ex-ante' monitoring report) and 2018 ("ex post", i.e. after the implementation of the monitoring tools).

The current document represents the "ex post" monitoring report. Specifically, it describe the activities carried out and provides the main results, based on the data available at the moment of drafting the document, from monitoring activities carried out in each of the 11 pilot MPAs (Fig.1) between January and October 2018. These activities include:

- 1) Ecological monitoring,
- 2) Economic monitoring,
- 3) Social monitoring.



Figure 1. Map of the 6 countries and 11 pilot MPAs involved in FishMPABlue 2 project.

2. ACTIVITIES CARRIED OUT

As planned in the Terms of Reference of the Pilot Project implementation contracts (Deliverable 3.2.1), sampling activities were performed by University of Nice (UNS) and Conisma for what concerns the ecological monitoring and by the staff of each MPA (with the supervision of University of Nice, Conisma and responsible project partners) for what concerns the economic and social monitoring. A summary of the activities carried out can be found in Table 1.



Monitoring Activity	PERFORMED BY	TIME PERIOD
Ecological	UNS + Consima	June - July (2018)
Economic	MPA staff	January-October 2018
Social	MPA staff	July-October 2018

Table 1. Subjects in charge of each monitoring activity and time period of execution.

2.1 Ecological monitoring

The ecological monitoring aimed to assess MPAs reserve effect on fish assemblage (i.e. if MPAs have any effect on fish density, size distribution and biomass). This goal can be achieved by comparing data about descriptors of fish species and assemblage between each MPA and some unprotected control locations. To do so, we combined two techniques, already implemented during the first monitoring campaign (see Del. 3.3.2 - 'ex-ante monitoring report'), in order to collect information about a large spectrum of fish species:

- 1. underwater visual census (UVC) based on strip transects and
- 2. Baited Underwater Video systems (BUV)

These techniques allowed us to estimate species richness, along with fish density, size distribution and biomass per each species recorded for each level of protection present in the 11 MPAs considered. For a detailed description of sampling methodologies, see Deliverable 3.1.2 ('Common methodology for design and execution of sound scientific monitoring of small scale fishery within and around an MPA').

For the 'ex-post' sampling campaign a novel methodology called 'environmental DNA' (eDNA) was also implemented in each MPA. This next-generation technique allows to potentially determining the presence of all aquatic organisms, with a specific focus on fishes that are present in a certain area at a given time. The method is based on the fact that organisms produce an abundance of genetic material (in the form of sloughed cells, feces, or other exogenous processes) that can persist in aquatic environments as environmental DNA. The collection, concentration, and analysis of eDNA from water samples is an effective method for

monitoring aquatic organisms. In particular, this method can 'record' the presence of a set of species that are generally not targetable with standard UVC or BUV (e.g. cryptic and shy species). From this perspective, the combination of the three methodologies foreseen in this 2nd sampling campaign will allow to have a comprehensive picture of the boidoversity levels of the coastal fish assemblages in the selected MPAs.

The sampling campaign for the ecological monitoring started in June 2018 in the Pilot MPAs and was finalized in July 2018 (Table 2).

MPA	Start-end of sampling
Egadi	4-8 June 2018
Torre Guaceto	10-13 June 2018
Zakynthos	16-20 June 2018
Cabo de Palos	24-27 June 2018
Es Freus	29-31 June 2018
Bonifacio	4-7 July 2018
Cote Bleue	9-12 July 2018
Cap Roux	13-15 July 2018
Portofino	17-20 July 2018
Telascica	22-24 July 2018
Strunjan	25-27 July 2018

Table 2. Starting sampling date of the ecological monitoring in the 11 pilot MPAs.

As for the first campaign, the sampling effort of UNS and Conisma was huge, with more than 300 l of water analysed, about 500 h of videos recorded, 200 hours diving and about 10,000 km travelling for displacing among MPAs by car and ship (Fig. 2).





Figure 2. Sampling campaign for the ecological monitoring carried out in 2018. The yellow line indicates the paths travelled for displacing among the 11 MPAs (red dots).

In the following sections the main results concerning the ecological monitoring, based on the data available at the moment of drafting this report, are presented, dividing them on the base of three methodology used: UVC, BUV and Environmental DNA. An update of the results on additional analyses of Environmental DNA data are also presented.

For all the techniques implemented, samplings were performed in all the levels of protection present in each MPA (no-take, partially protected and unprotected) (Fig. 3a). In two MPAs (Cote Bleue and Cap Roux) only the no-take zone is present, with no partial protection zones (buffer) between the no-take and the unprotected (external) areas around. In these 2 MPAs external sites at different distance from MPA borders have been sampled (2 sites close to the MPAs and 2 sites far from the MPA borders) (Fig. 3b). For UVC and BUVs for each level of protection 2 sites were randomly selected and in each site 12 UVC (only in few sites we carried out a lower



number of replicates depending on site spatial extent) and 6 BUV replicates were performed (Fig. 3a and 3b).



Figure 3. Scheme of MPA zoning for: a) multi-use "standard" MPA, b) no-take only MPA (also called marine reserve); and schematic site distribution in each protection level (red dots) for UVC and BUV during the sampling campaign.

2.1.1 Underwater visual census

Underwater Visual Censuses, based on strip transects of 25x5 m (Fig. 4), were used to assess species richness, abundance, density and biomass. Overall 760 UVC transects were carried out in 66 sites. Actual number of fish encountered were recorded up to 10 individuals, whereas larger groups were recorded using categories of abundance (i.e. 11–30, 31–50, 51–200, 201–500, >500 ind.). Fish size (total length, TL) was recorded within 2 cm size classes for most of the species, and within 5 cm size classes for large-sized species (maximum size >50 cm) such as the dusky grouper *Epinephelus marginatus* and the brown meagre *Sciaena umbra*. Apart from the fish belonging to the family Mugilidae, for which conclusive species identification is not possible during UVC, for all the other fishes it was possible to get to the species level (or genus in very few cases) during the monitoring. Data about cephalopods and macro-crustaceans were recorded following the same methodology (carapace and mantel length were estimated respectively for crustacean and cephalopod).





Figure 4. Operator performing UVC in Cabo de Palos MPA (photocredit: Javier Ferrer).

A total number of 79 fish *taxa* was recorded overall (all the MPAs and unprotected locations) (Fig. 5). Table 3 reports all the fish taxa encountered during UVC, their commercial value (based on Claudet et al. 2008) and their trophic group (based on Guidetti et al. 2014). The fish families mainly represented were Labridae (16 species) and Sparidae (14 species). Also 2 species of Cephalopods and 3 species of macro-crustaceans were recorded (Tab. 3).

Table 3. List of fish *taxa* recorded in the 11 MPAs during UVC campaign in 2018. Commercial value: NC= no commercial value, LC= low commercial value, C= high commercial value. Trophic group: PL= planktivore, DE= detritivore, CA= carnivorous, AP= apex predator, HE= herbivorous.

SPECIES	COMMERCIAL VALUE	TROPHIC LEVEL	SPECIES	COMMERCIAL VALUE	TROPHIC LEVEL
Anthias anthias	NC	PL	Pagrus pagrus	С	CA
Apogon imberbis	NC	PL	Palinurus elephas	С	CA
Atherina spp	LC	PL	Parablennius gattorugine	NC	CA
Boops boops	С	PL	Parablennius rouxi	NC	CA
Chromis chromis	NC	PL	Parablennius zvonimiri	NC	CA
Conger conger	С	AP	Phycis phycis	С	CA



Coris julis	NC	CA	Pomatomus saltatrix	С	AP
Ctenolabrus rupestris	LC	CA	Pomatoschistus sp	NC	PL
Dasyatis pastinaca	LC	CA	Pseudocaranx dentex	LC	CA
Dentex dentex	С	AP	Sarpa salpa	LC	HE
Dicentrarchus labrax	С	AP	Sciaena umbra	С	CA
Diplodus annularis	С	CA	Scorpaena maderensis	С	CA
Diplodus cervinus	С	CA	Scorpaena notata	С	CA
Diplodus puntazzo	С	CA	Scorpaena porcus	С	CA
Diplodus sargus	С	CA	Scorpaena scrofa	С	CA
Diplodus vulgaris	С	CA	Sepia officinalis	С	CA
Dromia personata	NC	CA	Seriola dumerili	С	AP
Epinephelus costae	С	AP	Serranus cabrilla	С	CA
Epinephelus marginatus	С	AP	Serranus hepatus	NC	CA
Euriphia verrucosa	С	CA	Serranus scriba	LC	CA
Euthynnus alletteratus	С	AP	Siganus luridus	LC	HE
Gobius auratus	NC	CA	Siganus rivulatus	NC	HE
Gobius bucchichi	NC	CA	Sparisoma cretense	LC	HE
Gobius cobitis	NC	CA	Sparus aurata	С	CA
Gobius cruentatus	NC	CA	Sphyraena viridensis	С	AP
Gobius geniporus	NC	CA	Spicara maena	LC	PL
Gobius vittatus	NC	CA	Spicara smaris	LC	PL
Gymnotorax unicolor	NC	AP	Spondyliosoma cantharus	С	CA
Labrus merula	С	CA	Symphodus cinereus	NC	CA
Labrus mixtus	LC	CA	Symphodus doderleini	NC	CA
Labrus viridis	С	CA	Symphodus mediterraneus	NC	CA
Lichia amia	С	AP	Symphodus melanocercus	NC	CA
Lithognathus mormyrus	С	CA	Symphodus melops	NC	CA
Mugilidae	С	DE	Symphodus ocellatus	NC	CA
Mullus surmuletus	С	CA	Symphodus roissali	NC	CA
Muraena helena	С	AP	Symphodus rostratus	NC	CA
Mycteroperca rubra	С	AP	Symphodus tinca	LC	CA
Myliobatis aquila	NC	AP	Syngnathus acus	NC	CA
Oblada melanura	С	PL	Thalassoma pavo	NC	CA
Octopus vulgaris	С	CA	Trachinotus ovatus	С	CA
Pagellus erythrinus	С	CA	Tripterigion delaysi	NC	CA
Pagrus auriga	С	CA	Tripterigion tripteronotus	NC	CA

FISH

CEPHALOPOD

CRUSTACEAN





Figure 5. Pictures of some species encountered during UVC: a dusky grouper with a group of barracudas (upper panel); a couple of red scorpionfish (down-left panel); a big individual of dusky grouper (bottom-right panel) (photocredit: Javier Ferrer).



Overall, the number of species recorded was slightly different among the three levels of protection considering all the species (Fig.6 left), the species with high commercial value (Fig.6 center) and the apex predators (Fig.6 right) (refer to table 3 for the commercial value and the trophic level of the species encountered during UVC). The choice to consider both species with high commercial value and apex predators was made in order to highlight potential MPA effects on those species that are primarily benefited by protection, being the species mainly targeted by commercial and recreational fishing.



Figure 6. Total number of fish species recorded, pooling all the 11 MPAs, for each level of protection: considering all the species (left), considering only the species with high commercial value (center) and considering only the apex predators (right). Refer to Table 3 for species commercial value and trophic level.



The total density of fish per site, considering all the species, greatly ranged from \sim 7.5 individuals per transect (i.e. 125 m²) in an external site of Strunjan MPA to \sim 1,252 individuals per transect recorded in a no-take site in Cabo de Palos MPA.

Overall, the number of fish individuals appeared to be higher inside MPAs (both in the no-take zone and buffer) than outside (external), considering: all the species (Fig.7 left) and the species with high commercial value (Fig.7 center). Concerning apex predators the highest mean number of individuals was observed in buffer sites (Fig.7 right).



Figure 7. Fish density per transect (125 m^2) recorded during UVC (mean ± se), including all the 11 MPAs, for each level of protection: considering all the species (left), considering only the species with high commercial value (center) and considering only the apex predators (right). Bars represent the standard error. Refer to Table 3 for species commercial value and trophic level.



Also the recorded biomass per transect considerably varied among the sites considered, ranging from 0.06 kg/125 m² to more than 150 kg/125 m².

Considering the three levels of protection (no-take, buffer and unprotected), the results including all 11 pilot MPAs are shown in Fig. 8. Overall fish biomass, for the 3 groups considered, appeared to be higher inside the MPAs (with mean values in buffer sites higher than no-take ones) than outside (Fig. 8).



Figure 8. Fish biomass per transect (125 m²) recorded during UVC (mean \pm se), including all the 11 MPAs, for each level of protection: considering all the species (left), considering only the species with high commercial value (centre) and considering only the apex predators (right). Refer to Table 3 for species commercial value and trophic level.



Focusing on the single MPAs, the proportion of fish density in the three levels of protection highly differs among the 11 pilot MPAs, with the total fish density recorded also hugely varying among the MPAs (see size of the circles in Fig. 9). The total fish biomass recorded was very different among the 11 MPAs, with the minimum value recoded in Strunjan MPA and the maximum recorded in Cabo de Palos MPA, regardless the protection level. Considering the proportion of fish biomass within each level of protection, although a certain variability found among the MPAs, in the most of the cases, biomasses were higher in the no-take zone or buffer than in the unprotected sites around the MPAs (Fig. 10).



Figure 9. Proportion of fish density recorded in each level of protection in the 11 Pilot MPAs. The size of the pie charts is proportional to the total density of species recorded in each MPA, respectively.





Figure 10. Proportion of fish biomass recorded in each level of protection for each MPA. The size of the pie charts is proportional to the total biomass recorded in each MPA respectively.



2.1.2 Baited underwater video

In addition to UVC surveys, Baited Underwater Video systems (BUVs) were deployed in the 11 MPAs to assess species richness, abundance and biomass of fish. BUVs technique consists on the deployment of a steel structure equipped with two video cameras and a basket containing a bait allowing the attraction of fish species, such as large predators and more mobile species (see Deliverable 3.1.2 for further details), which usually are not recorded by other sampling methods.

To evaluate how fish assemblages composition varies among MPAs and among sites with different fishing pressure/protection levels (i.e. no-take zone, buffer zone and external zone), BUVs were deployed in 2 random sites for each of the 3 levels in the 11 MPAs and surrounding areas (see paragraph 2.1). A total number of 384 BUVs replicates were carried out in 66 sites. Each BUV was deployed for ~65 min on rocky bottom between 8:00 a.m. and 3:00 p.m. within a depth range of 5–15 m (Fig. 11). To avoid the repeated sampling of the same individuals, BUVs were deployed at a distance >150 meters from each other.



Figure 11. BUV sampling operations in Egadi Islands MPA.



The bait consisted of 400g of crushed sardines placed inside a net fixed in front of the cameras. From each BUVs deployment, we obtained a 60min video, which was successively analyzed to record species richness (S) and MaxN, a conservative measure of abundance calculated as the maximum number of fish of the same species seen over the observation period. To evaluate S, we recorded all the fish observed in the field of view, whilst the MaxN was calculated considering only the species observed within 2 meters around the bait. Further analyses aimed to estimate the biomass for each fish species are still under way.

Here we present the results from 354 BUVs replicates deployed in the 11 MPAs (for 30 replicates the video analysis was not possible due to low visibility). All the fishes were identified at the species level, except for Mugilidae, Clupeidae and Belonidae, whose accurate identification was not possible in many cases.

In total, 14607 fish individuals were recorded in the 11 MPAs. Total fish abundance was highest in the no take areas (5461 individuals) than in the buffer (5003 individuals) and external (4143) zones. Overall, BUVs deployments allowed the identification of 71 fish *taxa* (listed in Table 4) belonging to 31 families. Sparidae and Labridae were the most represented families (16 and 15 species, respectively; Table 4). The most frequent species were *Coris julis* (91% of the replicates), *Chromis chromis* (81%) and *Serranus scriba* (74%; Table 4). In addition, two species of Cephalopod (*Sepia officinalis* and *Octopus vulgaris*), one species of macrocrustacean (*Maja squinado*) and one species of Reptilia (*Caretta caretta*) were recorded.

Table 4. List of fish *taxa* recorded through BUVs deployments (n=354) in 11 MPAs during the monitoring campaign in 2018. For each *taxon*, the percent frequency (percent of replicates in which the taxon was observed), its commercial value (CV) and trophic group (TG) are reported. Refer to Table 3 for the abbreviations of species commercial value and trophic groups.

Species	CV	ΤG	%	Species	CV	TG	%
Coris julis	NC	CA	91.0	Blennidae	NC	CA	3.1
Chromis chromis	NC	PL	81.1	Diplodus cervinus	С	CA	3.1
Serranus scriba	LC	CA	74.0	Epinephelus costae	С	AP	2.8
Diplodus vulgaris	С	CA	72.9	Parablennius rouxi	NC	CA	2.8
Symphodus tinca	LC	CA	63.0	Apogon imberbis	NC	PL	2.5
Diplodus sargus	С	CA	60.2	Dasyatis pastinaca	LC	CA	2.5
Diplodus annularis	С	CA	46.9	Gobidae	NC	CA	2.3
Thalassoma pavo	NC	CA	41.8	Sciaena umbra	С	CA	2.3
Symphodus ocellatus	NC	CA	37.9	Symphodus doderleini	NC	CA	2.0



Serranus cabrilla	С	CA	36.2	Dactylopterus volitans	NA	CA	1.7
Symphodus mediterraneus	NC	CA	33.3	Dicentrarchus labrax	С	AP	1.7
Muraena helena	С	AP	33.1	Lithognathus mormyrus	С	CA	1.4
Oblada melanura	С	PL	28.8	Spicara smaris	LC	ΡL	1.4
Symphodus roissali	NC	CA	27.7	Conger conger	С	AP	1.1
Sarpa salpa	LC	HE	26.0	Myliobatis aquila	NC	AP	1.1
Spondyliosoma cantharus	С	CA	22.3	Raja sp.	С	CA	0.8
Symphodus melanocercus	NC	CA	17.5	Tripterygion delaisi	NC	CA	0.8
Diplodus puntazzo	С	CA	15.0	Caranx crysos	С	AP	0.8
Sparus aurata	С	CA	14.1	Clupeidae	С	ΡL	0.6
Mullus surmuletus	С	CA	12.7	Lichia amia	С	AP	0.6
Mugilidae	С	DE	11.6	Mullus barbatus	С	CA	0.6
Labrus merula	С	CA	9.6	Pagellus erythrinus	С	CA	0.6
Dentex dentex	С	AP	9.0	Pagrus pagrus	С	CA	0.6
Siganus luridus	LC	HE	8.8	Parablennius gattoruggine	NC	CA	0.6
Boops boops	С	PL	8.2	Siganus rivulatus	NC	HE	0.6
Sphyraena sp.	С	AP	7.6	Spicara maena	LC	ΡL	0.6
Symphodus rostratus	NC	CA	7.6	Labrus bergylta	С	CA	0.3
Sparisoma cretense	LC	HE	7.1	Labrus mixtus	С	CA	0.3
Labrus viridis	С	CA	6.8	Mola mola	С	AP	0.3
Seriola dumerili	С	AP	5.9	Scorpaena sp.	С	CA	0.3
Symphodus cinereus	NC	CA	5.9	Symphodus bailloni	NC	CA	0.3
Pagellus sp.	С	CA	4.5	Dentex gibbosus	С	AP	0.3
Atherina sp	LC	PL	3.7	Belonidae	С	CA	0.3
Epinephelus marginatus	С	AP	3.7	Solea spp	С	CA	0.3
Mycteroperca rubra	С	AP	3.7	Euthynnus allitteratus	С	AP	0.3
Serranus hepatus	NC	CA	3.4				

When considering all the 11 MPAs together, the average species richness was 9.8 (±0.2 se). Overall, the number of species recorded in the no-take, buffer and external zones were 62, 59 and 52, respectively (Fig. 12a). The highest mean species richness value was found in Bonifacio and Es Freus MPAs (11.7 species), whilst the lowest value was recorded in Strunjan MPA (4.6 species). Es Freus and Zakynthos resulted the MPAs with the highest number of identified species (36 *taxa* each).

As for UVC data, we assigned a commercial value to the identified *taxa*, following the categorization of Claudet et al. (2008). Overall, we recorded 3033 individuals from 41 species with high commercial value in the 11 MPAs. When considering the different levels of protection we identified 33 species with high commercial value in both the no-take and buffer zones, and 28 species in the external ones (Fig. 12b). The highest abundance of commercial fish was recorded in the buffer zones (1198 individuals). *Diplodus sargus* was the most frequent among these species, as it was observed in 73% of the BUVs deployed in the 11 MPAs (Table 4). The highest



number of *taxa* with high commercial value was recorded in Cabo de Palos AMP (23 species).

We identified a total of 334 fish from 15 apex predator species in the 11 MPAs. When considering the three levels of protection, we recorded 12 species in both the no-take and buffer zones and 8 species in the external ones (see Fig. 12c). *Muraena helena* was the most frequent predator species (33% of the replicates; see Table 4).



Figure 12. Total number of species recorded through BUVs deployments in the three levels of protection of 11 MPAs during the monitoring campaign of 2018. a) Number of all fish species recorded; b) number of species with high commercial value; c) number of apex predator species. Refer to Table 4 for species commercial value and trophic groups.

Overall, the mean relative fish abundance (MaxN) was similar among the three levels of protection. On average, we recorded 46.3 (\pm 4.3 se) individuals in the notake zones, 41.7 (\pm 3.4 se) individuals in the buffer zones and 35.7 (\pm 2.9 se) individuals in the external zones (Fig. 13a). When considering every single MPA, the highest mean fish abundance was recorded in Cabo de Palos MPA (83.7 \pm 9.0 se individuals).

The species with high commercial value were more abundant in the buffer zones (MaxN: 10.0 \pm 1.0 se individuals) than in the no-take (MaxN: 7.8 \pm 0.6 se individuals) and external zones (MaxN: 7.8 \pm 0.9 se individuals; Fig. 13b). The highest abundance of fish with high commercial value was found in Telascica MPA (MaxN: 13.3 \pm 2.2 se



individuals). The apex predators (Fig. 14) resulted on average more abundant in the buffer zones (mean MaxN: 1.2 \pm 0.3 se individuals) than in the no-take (0.9 \pm 0.2 se) and external zones (0.6 \pm 0.1 se; Fig. 13c).



Figure 13. MaxN (mean ± se) relative to all the species recorded through BUVs deployments in the three levels of protection of 11 MPAs during the monitoring campaign of 2018. MaxN values are reported for a) all fish species; b) species with high commercial value; c) apex predator species. Refer to Table 4 for species commercial value and trophic groups.



Figure 14. Frames extracted from BUVs deployments carried out during the monitoring campaign of 2018, showing a common stingray (left panel) and a common dentex (apex predator, right panel).



2.1.3 Environmental DNA

Environmental DNA (eDNA) metabarcoding was applied in the framework of the 2018 sampling campaign with the aim of providing additional meaningful information about species richness in the 11 Mediterranean MPAs. This novel methodology is currently considered one of the most promising non-invasive tools for the assessment of biodiversity in both terrestrial and aquatic environments. It consists on the amplification and subsequent sequencing of particular genetic markers using the DNA present in specific samples of water, sediment, air, etc. as template. The obtained sequences are then used as barcodes against a reference database containing genetic information of known specimens.

In order to assess the MPAs' biodiversity and highlight possible differences in fish taxa composition among protected and unprotected zones, water samples were collected randomly in three sites inside and outside each MPA (Fig. 15). Possible fish assemblage variabilities related to depth were explored replicating the sampling at -2 m and -20 m for each site. Moreover, to enhance the detection of resident fish species, a water sample was collected also in the fishing port closest or within each each MPA. The idea was that the genetic material carried by fishing nets and traps could be more concentrated near the port area where small scale fishermen usually rinse their tools. Finally, a field negative control (Field Blank) was included in the sampling design for each MPA to identify possible sources of contaminations. The Field blanks were collected following the same protocol applied to the experimental samples, but using filtered instead of marine water. Overall 154 water samples were collected (143 study samples, 11 field blanks). The sampling was performed by scuba diving and each sample consisted of two liters of water (Fig. 15). All the equipment used in each step of the sampling protocol was previously sterilized using 50% bleach to avoid cross-contaminations among samples. The collected water was vacuum filtered using 0.45 µM pore size nylon filter (Millipore) within two hours of collection and filters stored in sterile cryotubes at -20C until DNA extraction. The laboratory work consisted in the following steps: a) DNA extraction; b) DNA quality check; c) amplification by Polymerase Chain Reaction (PCR) of a mitochondrial 12S gene fraction (12S marker); PCR products quality check; libraries construction and quantification by qPCR; sequencing in Illumina MiSeq run for a total of 164 samples (143 study samples, 11 field blanks, 8 DNA extraction blanks and 2 PCR blanks). Bioinformatic analyses were performed using the Obitools pipeline and the output thoughtfully manually checked (Fig. 15).





Figure 15. Schematic representation of the eDNA analyses pipeline

Environmental DNA metabarcoding is a young methodology and several issues still need to be addressed to perform to its full potential. One of the most important is the lack of fully complete and reliable public databases to be used as reference for the taxonomic identification of marine species. Unfortunately, to date the available 12S rRNA reference sequences are far to cover the entire Mediterranean fish diversity. Consequently, many sequences cannot be recognized even if present in the collected samples, with a severe loss of information about biodiversity. In order to partially overcome this issue, during 2019 a set of fish species missing in the public databases was collected with the purpose of producing new voucher sequences. Starting from pieces of tissues of 33 specimens of 20 different species, genomic DNA was extracted and the 12S fragment marker amplified and sequenced. The new sequences were added to the ones already available in the public databases to improve the taxonomic resolution of the present study. The selected species were:

Table 5. List of fish *taxa* for which new reference sequences were generated to improve the available database.



Diplodus vulgaris **Boops** boops Oblada melanura Belone belone Gobius paganellus Parablennius gattorugine Scorpaena porcus Scorpaena scrofa Labrus viridis Diplodus annularis Sarpa salpa Symphodus tinca Serranus scriba Serranus cabrilla Diplodus sargus Helicolenus dactiloperus Spicara maena Symphodus mediterraneus Spondyliosoma cantharus Gobius bucchichi

During 2019, a selection of the samples analyzed using the 12S metabarcoding marker was also chosen for further exploration with a Cytochrome Oxidase subunit 1 (COI) marker using the same protocol. We did not include all the samples to contain the costs, choosing only samples that previously showed the best yield with 12S. All the MPAs were represented in the samples subset, with both the protection levels (inside and outside the protected area). All the blanks were included to check for contaminations.

Most of the laboratory work and part of the bioinformatics concerning the 12S and COI metabarcoding fish biodiversity survey were carried out at Salford University (UK), which provided specialized facilities and expertise essential for the execution of the eDNA metabarcoding activities of the FishMPABlue2 project.

Overall, 95 different taxa were recognized in all the AMP by eDNA metabarcoding. The taxonomic resolution of the 12S and COI mitochondrial markers in a few cases was not high enough to distinguish among congeneric or confamiliar species, but the taxonomic resolution was considerably improved after the production of new reference sequences. Along with teleost fishes, some cartilaginous fishes were identified, such as *Raja* spp. and *Torpedo marmorata*.



eDNA metabarcoding provided a substantial contribute to the global species richness estimates, proving itself as complementary to the traditional monitoring methods. Indeed, eDNA, UVC and BUV found together 131 different fish *taxa*, 43 of which found exclusively by eDNA and 39 exclusively by UVC and BUV. Moreover, for some *taxa* particularly difficult to identify in the field due to homogeneous morphological traits, eDNA provided a fundamental help. For instance, eDNA registered six different mugilids, nearly morphologically indistinguishable without an accurate inspection and consequently reported as Mugilidae by UVC and BUV.

Table 6. List of fish *taxa* recorded through eDNA metabarcoding (n=143) in 11 MPAs during the monitoring campaign in 2018. In green all the taxa recorded exclusively by eDNA, in black *taxa* recorded also by the other applied monitoring methods.

ΤΑΧΟΝ	ΤΑΧΟΝ	ΤΑΧΟΝ
Ammodytes tobianus	Gadus morhua	Sardinella aurita
Anguilla anguilla	Gobius bucchichi	Sarpa salpa
Aphia minuta	Gobius cobitis	Scomber colias
Apogon imberbis	Gobius niger	Scomber scombrus
Argyrosomus regius	Gobius paganellus	Scorpaena porcus
Atherina boyeri	Gymnammodytes cicerelus	Scorpaena scrofa
Atherina hepsetus	Labrus viridis	Seriola dumerili
Auxis rochei	Lipohrys trigloides	Serranus cabrilla
Belone belone	<i>Lutjanus</i> sp.	Serranus scriba
Blennidae	Merlangius merlangus	Siganus luridus
Boops boops	Merluccius merluccius	<i>Siganus</i> sp.
Chelidonichthys sp.	Millerigobius macrocephalus	Solea solea
Chelon auratus	Mugil capurrii	Sparus aurata
Chelon saliens	Mugil cephalus	Sphyraena viridensis
Chelon sp. (C. labrosus or C. ramada)	Mullus barbatus	Spicara maena
Chromis chromis	Mullus surmuletus	Spicara smaris
Clinitrachus argentatus	Muraena helena	Spondyliosoma cantharus
Conger conger	Oblada melanura	Sprattus sprattus
Coris julis	Oedalechilus labeo	Symphodus mediterraneus
Deltentosteus quadrimaculatus	Pagellus acarne	Symphodus ocellatus
Dentex dentex	Pagellus bogaraveo	Symphodus sp.
Dentex gibbosus	Pagellus erythrinus	Symphodus tinca
Dentex sp.	Pagrus pagrus	Taurulus bubalis
Dicentrarchus labrax	Parablennius incognitus	Thalassoma pavo
Diplodus sargus	Platichtys flesus	Thunnus sp.
Diplodus vulgaris	Pomatomus saltatrix	Torpedo marmorata
Echelus myrus	Pomatoschistus sp.	Trachinus draco
Enchelycore anatina	Pseudaphya ferreri	Trachurus mediterraneus



Engraulis encrasicolus	Raja brachyura	Trachurus trachurus
Epinephelus costae	Raja clavata	Trisopterus sp.
Epinephelus sp.	Raja polystigma	Zeus faber
Euthynnus alletteratus	Sardina pilchardus	

Overall, according to eDNA metabarcoding, the species richness was slightly higher inside the MPAs than in the near unprotected zones, with some exceptions represented by Egadi, Es Freus, Portofino, Torre Guaceto and Zakynthos, where the number of taxa identified was slightly higher outside the MPAs (Fig. 16). In Telascica no differences where highlighted between areas with different protection levels (Fig. 16). Interestingly, with the exceptions of Cabo de Palos, Es Freus, Strunian, Telascica and Zakynthos, the number of fish taxa identified in the ports was comparable with those found in the surrounding areas, confirming the utility of small fishery ports as collectors of biodiversity signatures.



Figure 16. Species richness (absolute values) relative to all the species recorded by eDNA outside (EXT: red bars), and iside (MPA: green bars) the 11 MPAs during the monitoring campaign of 2018. Blue bars represent the global species richness registered in each area, including the *taxa* identified inside the ports.



2.2 Economic monitoring

The aim of economic monitoring campaign was to assess the economic status of SSF catches within and around each MPA 'after' the implementation of the pilot actions planned in the PPIPs. Specifically, the effect of MPAs on small scale fishers catches and revenues is assessed by comparing the "Catches per unit of effort" (CPUE) and the "Revenue per unit of effort" (RPUE), obtained by fishers within the MPA (in the areas where SSF is allowed), and those obtained in open fishing areas outside the MPA, using the same gear and approximately within the same bathymetric range and habitats.

A monitoring methodology was developed in order to obtain reliable data on small scale fisheries catches. In particular, small scale fishery landings (i.e. the amount of harvested fish brought to the land) were selected as source of data. In particular, 40 landings inside and 40 outside the MPA were planned to be monitored, i.e. photographed, in each MPA during 2018 (till the end of the pilot action). This number was chosen in order to have an exhaustive characterization of fish catch composition and quantities targeted by SSF. Further information on the monitoring methodology are available in Deliverable 3.1.2 ('Common methodology for design and execution of sound scientific monitoring of small scale fishery within and around an MPA').

For each landing monitored, we collected information about:

- 1) catches,
- 2) fishing effort and
- 3) fish first selling price for each species

The latter was assessed in order to calculate, together with information on fishing vessels, the variable cost (i.e. related to fuel consumption) that fishers had to sustain for each fishing operations. In addition we collected information about exvessel price of each species captured.

The methodology used was developed in a way in which sampling time in the field and fish manipulation were minimized. This was done in order to cause to fishers the least disturb possible during monitoring operations. Specifically, the operator placed the catch over a flat surface (e.g. a table or the fish box to minimize manipulation) and takes one/multiple pictures where a ruler (as length reference)



has to be visible and on the same plane as fishes (Fig. 17). Each picture was associated to an unique identifier of the fishing operation (i.e. a small piece of paper with a unique code) (Fig. 17 and 18). For species with a low commercial value, generally identified as 'soup', and for molluscs the operator directly weighted all the specimens at once and annotated the total weight, taking notes of species composition.



Figure 17. Operator carrying out photo-sampling of small-scale fisheries catch at landing in Torre Guaceto MPA.





Figure 18. Pictures of small scale fisheries landings taken at Cabo de Palos Marine Reserve (up-left), Torre Guaceto MPA (up-right), Strunjan MPA (bottom-left) and Zakynthos Marine Park (bottom-right) using photo-sampling technique. Note the ruler and the code present in each picture.

Once all the pictures relative to a specific MPA have been collected, in the laboratory an operator processed them by using the image-analysis software ImageJ (Fig. 19). This allowed to extract from each picture information on length, and then estimate the wet weight of each specimen using specific length-weight relationships. The total number of catches to monitor for the ex post campaign was planned to be 880, considering all the 11 MPAs (440 inside the MPA and 440 outside). The initial aim of the economic monitoring campaign was to assess the economic status of SSF in each MPA 'after' the implementation of the pilot actions planned in the PPIPs. However, in order to have a better picture of the economic status in each MPA, the experimental design and the relative data collection, in almost all the pilot MPAs, was focused on assessing differences between inside and outside the MPA, specifically considering potential temporal trends over the



monitored years, rather than a 'before' vs 'after' comparison. See further details on the 5th Monitoring of Pilot Project Implementation (deliverable 3.1.5). In this way, the economic monitoring took into account the temporal variability of fishing catch descriptors (e.g. catch per unit of effort and revenue per unit of effort) that are likely to be influenced by seasonal factors. Only in three MPAs (i.e. Cap Roux, Strunjan and Zakynthos) the 'ex ante' and 'ex post' monitoring campaigns were carried out actually before and after the implementation of the pilot action.



Figure 19. Example of fishery catches photo-analysis with the software ImageJ.

In this document, the catches monitored after the drafting of the 'ex ante' monitoring report (thus not included in it) are described (i.e. considering the data gathered over the period May-October 2018). For a complete analysis of the entire dataset of ssf catches collected in the two monitoring campaigns see Del. 3.4.1 'Scientific assessment of the effect of governance toolkit implementation'. During the abovementioned period, 691 fishing catches were monitored: 355 in the buffer zones and 236 outside. In the analyses carried out 152 taxa were identified. In the most of the cases photo analysis allowed to identify the species, but in some cases (e.g. for species of the family Mugilidae) species recognition was not possible using a photo and the individual was assigned to the a taxon at highest resolution of



taxonomic identification possible. The total list of species identified in each MPA is presented in table 5.

Table 7. List of taxa identified, in the 11 MPAs, during small scale fisheries catches photo analysis from May to October 2018.

Taxon	Taxon	Taxon	Тахоп
Arnoglossus.sp	Labridae	Raja.brachyura	Solea.sp
Auxis.rochei	Labrus.merula	Raja.clavata	Sparisoma.cretense
Balistes.capriscus	Labrus.viridis	Raja.miraletus	Sparus.aurata
Belone.belone	Lagocephalus.sceleratus	Raja.montagui	Sphyraena.sphyraena
Boops.boops	Lichia.amia	Raja.polystigma	Sphyraena.viridensis
Bothus.podas	Liocarcinus.depurator	Raja.radula	Spicara.flexuosa
Chelidonichthys.cuculus	Lithognathus.mormyrus	Raja.sp	Spicara.maena
Chelidonichthys.lastoviza	Loligo.sp	Rajidae	Spicara.smaris
Chelidonichthys.lucerna	Loligo.vulgaris	Rhinobatos.rhinobatos	Spicara.sp
Chelon.auratus	Lophius.piscatorius	Rostroraja.alba	Spondyliosoma.cantharus
Chelon.labrosus	Lophius.sp	Sarda.sarda	Squilla.mantis
Chelon.ramada	Maja.squinado	Sardinella.aurita	Symphodus.bailloni
Citharus.linguatula	Melicertus.kerathurus	Sarpa.salpa	Symphodus.mediterraneus
Conger.conger	Merluccius.merluccius	Sciaena.umbra	Symphodus.melops
Coris.julis	Merluccius.sp	Scomber.colias	Symphodus.ocellatus
Coryphaena.hippurus	Microchirus.ocellatus	Scomber.japonicus	Symphodus.roissali
Dactylopterus.volitans	Mugil.cephalus	Scomber.scombrus	Symphodus.sp
Dasyatis.pastinaca	Mugilidae	Scomber.sp	Symphodus.tinca
Dasyatis.sp	Mullus.barbatus	Scophthalmus.maximus	Synapturichthys.kleinii
Dasyatis.tortonesei	Mullus.surmuletus	Scophthalmus.sp	Syngnathidae
Dentex.dentex	Muraena.helena	Scorpaena.elongata	Synodus.saurus
Dicentrarchus.labrax	Mustelus.mustelus	Scorpaena.maderensis	Thalassoma.pavo
Dicentrarchus.punctatus	Mustelus.punctulatus	Scorpaena.notata	Torpedo.marmorata
Diplodus.annularis	Mycteroperca.rubra	Scorpaena.porcus	Torpedo.sp
Diplodus.cervinus	Myliobatis.aquila	Scorpaena.scrofa	Trachinotus.ovatus
Diplodus.puntazzo	Oblada.melanura	Scorpaena.sp	Trachinus.araneus
Diplodus.sargus	Octopus.vulgaris	Scyliorhinus.canicula	Trachinus.draco
Diplodus.vulgaris	Pagellus.acarne	Scyliorhinus.sp	Trachinus.radiatus
Eledone.moschata	Pagellus.bogaraveo	Scyliorhinus.stellaris	Trachurus.mediterraneus
Epinephelus.caninus	Pagellus.erythrinus	Scyllarides.latus	Trachurus.sp
Epinephelus.costae	Pagrus.pagrus	Scyllarus.arctus	Trachurus.trachurus
Epinephelus.marginatus	Palinurus.elephas	Sepia.officinalis	Trigla.lyra
Epinephelus.sp	Palinurus.mauritanicus	Seriola.dumerili	Triglidae
Euthynnus.alletteratus	Pegusa.lascaris	Serranus.cabrilla	Trisopterus.capelanus
Gaidropsarus.mediterraneus	Phycis.phycis	Serranus.scriba	Umbrina.cirrosa
Gobiidae	Pomatomus.saltatrix	Siganus.luridus	Uranoscopus.scaber
Gobius.cruentatus	Pseudocaranx.dentex	Siganus.rivulatus	Xyrichtys.novacula
Homarus.gammarus	Raja.asterias	Solea.solea	Zeus.faber

More than 17,000 fish individuals were analyzed for this report. From the information on individual fish length the total wet-weight of each fish was calculated and then the total weight of the catch was extracted by summing up the weight of



all the individuals in the net. The catch per unit of effort (CPUE) was calculated by dividing the weight of the catch for the total length of the net.

Data were averaged by level of protection. For those MPA were the buffer is not present (Cote Bleue and Cap Roux), catches close to the no-take zone and those far from the no-take zone were considered as 'buffer' and 'external' catches, respectively (Fig. 20). Although CPUE was found to be higher in absolute values for catches carried out inside the MPA, no significant differences were observed between the 2 levels of protection considered.



Figure 20. CPUE (mean±SE) per level of protection considered in ssf catches analysis.

Data were then averaged by level of protection (buffer and external) and for each MPA. The preliminary results are shown in Fig. 21. Specifically, the map shows, for each MPA, the average relative contribution of each level of protection in which fish catches were recorded (buffer and external) to the total CPUE. Considering the single MPAs, a clear pattern does not emerge from these first results, with some MPAs characterized by higher CPUE inside the buffer and others in which an opposite pattern was highlighted. Please note that for those MPA were the buffer is not present (Cote Bleue and Cap Roux), catches close to the no-take zone and those



far from the no-take zone were considered as 'buffer' and 'external' catches, respectively.



Figure 21. Proportion of CPUE recorded for each level of protection considered in small scale fisheries catch analysis (buffer and external), over the total CPUE recorded in each MPA. In the case of Cote Bleue and Cap Roux, where no buffer is available, green colour represents external catches close to the no-take zone and red colour represents external catches far from the no-take zone. The size of the pie charts is proportional to the total CPUE recorded in each MPA respectively.

As for CPUE, the same preliminary analyses were carried out for fishers' revenue per unit of effort (RPUE). The gross revenue of each catch was calculated by multiplying the weight of each individual by the average price per kg of the relative species and then summing up the values from all the individuals from the same catch. The total value of the catch was then divided by the length of the net to obtain the RPUE. Overall, also for RPUE no differences were highlighted between the 2 levels of protection considered (buffer and external) (Fig. 22). As for CPUE, RPUE data were also averaged by level of protection (buffer and external) and for each MPA. The preliminary results are shown in Fig. 23.



Figure 22. RPUE (mean±SE) per level of protection considered in small scale fisheries catches analysis.





Figure 23. Proportion of RPUE recorded for each level of protection considered in small scale fisheries catch analysis (buffer and external), over the total RPUE recorded in each MPA. In the case of Cote Bleue and Cap Roux, where no buffer is available, green colour represents external catches close to the no-take zone and red colour represents external catches far from the no-take zone. The size of the pie charts is proportional to the total RPUE recorded in each MPA respectively.

2.3 Social Monitoring

The social monitoring aimed to describe the human dimension in small scale fisheries and more precisely the human well-being of small scale fishers communities in the 11 MPAs. Specifically, the second monitoring campaign was aimed to analyse a series of social descriptors associated to the implementation of the governance measures selected by local governance clusters (LGC, i.e. MPA management board and fishers) in each MPA, with the aim to assess potential social effects of the governance measures adopted in the pilot MPAs. Thus, this analysis represents the core part of the social effects of toolkit implementation in each MPA. From this perspective, the complete report of the second social monitoring campaign is presented in Del. 3.4.1 'Scientific assessment of the effect of governance toolkit implementation' being the data collected with the second monitoring campaign a direct evaluation of social effect of governance measures implementation. In the present report, the demographic characterization of the fishers interviewed is described. The questionnaire has been administered to a relevant proportion of small scale fishers within each pilot MPAs (Fig. 24). As for the first questionnaire, given the huge variability in the number of fishers within each community, a target number of interviews to be carried out was identified for each MPA. We considered a minimum percentage of each community (i.e. not below the 30% of the total number of fishers in the community) that allowed to properly characterize the social status of SSF in each MPA. It is important to remark that participation to the social monitoring was totally voluntary, thus the percentage of fishers interviewed strongly depended on their willingness and availability to fill in the questionnaire. For the small communities (i.e. composed by less than 10 fishers) we chose to interview all the fishers willing to participate to the social monitoring. A total of 121 questionnaires were administered in the 10 out of the 11 MPAs (Table 6). In Bonifacio MPA, in 2018, it was not possible for MPA manager to administer the questionnaires to the fishers (see Del. 3.1.5, 6th Monitoring Report of Pilot Project Implementation).

	# of interviews
MPA	done
Egadi	24
Torre Guaceto	4
Portofino	14

 Table 8. Total number of interviews carried out in each MPA.



Zakynthos	17
Es Freus	11
Cabo de Palos	11
Cap Roux	8
Cote Bleue	14
Strunjan	8
Telascica	10



Figure 24. An operator interviewing fishermen in Egadi MPA.

2.3.1 General characteristics of the fisheries

All the fishers interviewed were men who have been living in same village most of their lives, as so often in SSF communities of the Mediterranean Sea. The age distribution of interviewees was skewed toward older age classes, with the class 50-



59 years old being the most represented and the youngest class (20-29 years old) the least represented (Fig. 25).



Figure 25. Frequency of fishers age classes in the 10 MPAs where social monitoring was carried out



Most of fishers had a medium level of education, with less than 1% of the interviewees holding no education titles and about the 5% holding a University or higher degree (Fig. 26).



Figure 26. Frequency distribution of the level of education completed by the fishers interviewed.

Fishers generally have families formed by 2 or 4 members (Fig. 27) and, in most of the case, 2 or 3 of the members are employed, with fishing representing the only or main source of household incomes. Most of the interviewees declared to eat fish 3 or 4 days per week and about 5% of them eat fish almost every day of the week (Fig. 28).

Mediterranean FishMPABlue 2



Number of persons leaving in the same noueshold

Figure 27. Frequency distribution of the number of people leaving in fishers' household





Figure 28. Frequency distribution of the number of people leaving in fishers' household

2.3.2 General characteristics of the small scale fishery fleet

Most of fishers uses a variety of traditional fishing gears (Fig. 29). The most used gears are fixed nets (including trammel nets and gill nets), used by almost 100% of the fishers. About 50% uses either traps for cephalopods (generally mainly targeting cuttlefish) or traps for lobsters.



Figure 29. Fishing gears used by fishers in the 11 MPAs

The great majority of fishers own one boat, while less than 20% owned 2 or 3 boats (Fig. 30) with an average dimension of 7.3 meters.





Figure 30. Distribution of number of boats owned by fishers

Regarding the fishing effort, in many cases interviewees declared to fish approximately the same number of days within and outside their MPAs, in the cases where they are allowed to fish inside (Fig. 31). In 2 MPAs (Cabo de Palos and Torre Guaceto) fishers generally fish 4 times more outside the MPA than inside (Fig. 31). Although a certain variability among MPAs, fishers generally deploy each day nets that range in length between 1000m and 3000m (Fig. 32)



Figure 31. Proportion of days fishermen fish inside and outside each of the 10 MPAs monitored in the second social campaign. Note that in Cote Bleue and Cap Roux it is not possible to fish inside the MPA, so fishers always fish outside.





Figure 32. Frequency distribution of net lengths



3. CONCLUSION

This document reports the results of the "ex post" monitoring campaign carried out after the implementation of the governance tools in the 11 MPAs.

Results from ecological monitoring showed that in most of the MPAs selected, although a certain variability, both no-take zones and buffer zones, were associated on average to higher values of fish density and biomass, compared to external unprotected zones. eDNA metabarcoding provided a substantial contribute to the global species richness estimate, proving itself as complementary to the traditional monitoring methods. eDNA, UVC and BUV found together 131 different fish *taxa*, 43 of which found exclusively by eDNA and 39 exclusively by UVC and BUV. Remarkably, whatever the monitoring technique used, the diversity of fish species was slightly higher in protected than unprotected zones.

Concerning the economic monitoring, as for the 'ex ante' report, a clear pattern was not highlighted both for CPUE and RPUE, with inconsistency between MPAs.

For what concerns the social monitoring, the analyses highlight the high heterogeneity characterising each community with age structure and education level highly diverse within each community. This heterogeneity was also remarked analysing the fishing-related features of fisher communities, identifying a wide range of fishing tools and techniques and fishing effort (i.e. net length) implemented in the 10 MPAs considered for the second social campaign.

For a complementary analysis on ecological, economic and social effects of governance toolkit implementation in each MPA, see Del. 3.4.1 'Scientific assessment of the effect of governance toolkit implementation'.