Assessment of Dusky grouper (*Epinephelus marginatus*) and related species population status in the area of potential MPA Katič (Montenegro)

- Phase 1 report -

Project: "Support local community's involvement in protection and promotion of the potential marine protected area – Katič"

December 2019

study done in partnership with

Background

This document has been produced within the framework of the project entitled "Support local community's involvement in protection and promotion of the potential marine protected area – Katič" funded by Critical Ecosystem Partnership Fund (CEPF). Beyond its various activities and goals, one specific aim of the project is to conduct field research about dusky groupers (*E. marginatus*) and build on it as a flagship species of the potential MPA.

Outline:

Specific goals and first results of activities related to this aim for phase1 (= 2019) are presented in the present document as follows:

Phase 2 will take place in 2020 and will notably include the Part C.

Contributions: this assessment was coordinated by the NGO "Montenegrin Ecologists Society" (MES, Podgorica, Montenegro), in collaboration with experts from the NGO "Septentrion Environnement" (SE, Marseille, France); it has been done in the framework of the project "Support local community's involvement in protection and promotion of the potential marine protected area – Katič", lead by the NGO "Green Home" (GH) and funded by the Critical Ecosystem partnership Fund.

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General introduction

One of the specific goals of the project is to ensure data for Dusky Grouper ecological valorization, management and protection within the future MPA Katic. The present study aims at assessing the state of populations of Dusky groupers (*Epinephelus marginatus*) as well as other species possibly found in the wider Katic MPA area (notably the Golden grouper *E. costae*). This assessment contains three parts: A) locating juvenile grouper nursery habitats and assessing the juvenile populations found in those nurseries (done in October 2019); B) mapping potential micro localities, i.e. potentially suitable habitats for adult groupers (done in July 2019); C) preparing methodology for describing the adult grouper populations found in the various zones of the potential MPA (field work planned for June 2020).

Part A: assessing groupers juvenile nursery habitats and juvenile populations

Introduction

Context

Due to an ever growing global human population and a general migration to coastal areas, oceans and seas are experiencing an unprecedented variety and magnitude of anthropogenic pressures. The Mediterranean, a semi-enclosed basin surrounded by inhabited land, and even more the Adriatic sea, is particularly susceptible to the effects of human induced pressures (Coll et al., 2010). This translates into a greater use of resources and increased modification of natural coastal habitats, just to name a couple of the many consequences. In this context, coastal areas represent great stakes: they contain habitats essential for species life cycles as well as concentrating a maximum of anthropogenic disturbances. Various fish species of both ecological and economical importance use shallow coastal habitats notably as nursery grounds for their juveniles, essential for the renewal of their population (Cuadros et al., 2017; Dulčić et al., 2002; Harmelin-Vivien et al., 1995; Matic-Skoko et al., 2007). It is notably the case for grouper species, which juveniles settle and grow in coastal heterogeneous rocky bottoms located in shallow (less than 2 meters depth) stretches of coastlines (Fig. 1). Coastal development programs threaten their integrity and thus its key function. As a consequence, even if adult populations become protected from fishing inside MPAs, nursery habitat degradation may impair population renewal by disrupting the species life cycle. Coastal managers therefore need detailed cartography and data about nursery to better focus their conservation effort (Cheminée et al., 2014a, 2017a; Cuadros et al., 2017).

Goals

Along the coast of the study area, among the various management zones of the potential MPA Katic, our study aimed at 1) locating potential nursery sites for juvenile groupers through aerial picture analysis and 2) assessing the density patterns of juvenile groupers and associated species present in these nursery habitats. This aimed at gathering data useful for decision makers in order to optimize the design of management measures and conservation efforts and therefore favor population renewal for these species of both ecologic and economic importance. Moreover, our aim was to compare it with other study zones abroad, to assess the spatial variability of juvenile populations at a larger scale.

Methods

Nursery habitat location, study sites selection, sampling design

A preliminary study using Geographic Information System was done: along the shoreline, we located sites displaying the juvenile habitat used by groupers and others economically and ecologically important species (notably *Epinephelus marginatus, E. costae,* the sparids *Diplodus* spp. and associated species). Juveniles of these species are known to use shallow (< 2 m depth) and gently sloping heterogeneous rocky bottoms as nurseries (Bodilis et al., 2003a, 2003b; Cheminée et al., 2017b) (Fig. 1). As in Cheminée et al. (2014a), a nursery site was identified as a stretch of coastline displaying a continuous portion of suitable habitat. These so-called nursery sites were identified and measured on ortho-photographs at a scale of 1:7000. Aerial picture analysis was performed using QGIS 2.14 (QGIS Development Team, 2016). In each management type planned in potential Katic MPA management plan (3 levels: A, B, and C), we selected various studied nursery sites (Fig. 2), and sites were grouped by study zones (6 zones) according to the following sampling design (Table. 1). In total, we studied 16 nursery sites located along the studied shoreline. Among those nursery sites, a total of 3.4 km were surveyed by belt transect. A total of 45 transect were realized.

Table 1. Sampling design according to the studied factors: number of replicates and length of underwater visual census (UVC) of fish juveniles done per sites, zones and management levels in the potential MPA Katic (Fig. 2)

Management type	Study zones	Studied nursery sites	Number of UVC transects per site	Total shoreline length surveyed per site (meters)
A	A	A1	4	309
A	A	A2	4	220
B	B Centre	BC1	4	215
B	B Centre	BC2	4	224
B	B Centre	BC3	2	108
B	B North	BN1	2	269
B	B North	BN2	2	303
B	B North	BN3	2	308
B	B South	BS1	3	183
B	B South	BS2	3	193
B	B South	BS3	3	142
C	C North	CN1	2	155
C	C North	CN2	2	186
C	C North	CN3	2	214
C	C South	CS1	3	152
C	C South	CS2	3	213
TOTAL		**16 sites**	**45 transects**	**3394 meters**

Figure 1. Example of various nursery sites from the area (see map in Fig. 2) – a: east coast of Katic island (site A2); b: a nursery site in the B north zone (site BN3); c: site BC1 in front of Buljarica; d: site BC3 in Petrovac harbor; e: Pecin cove with site BS2 (f)

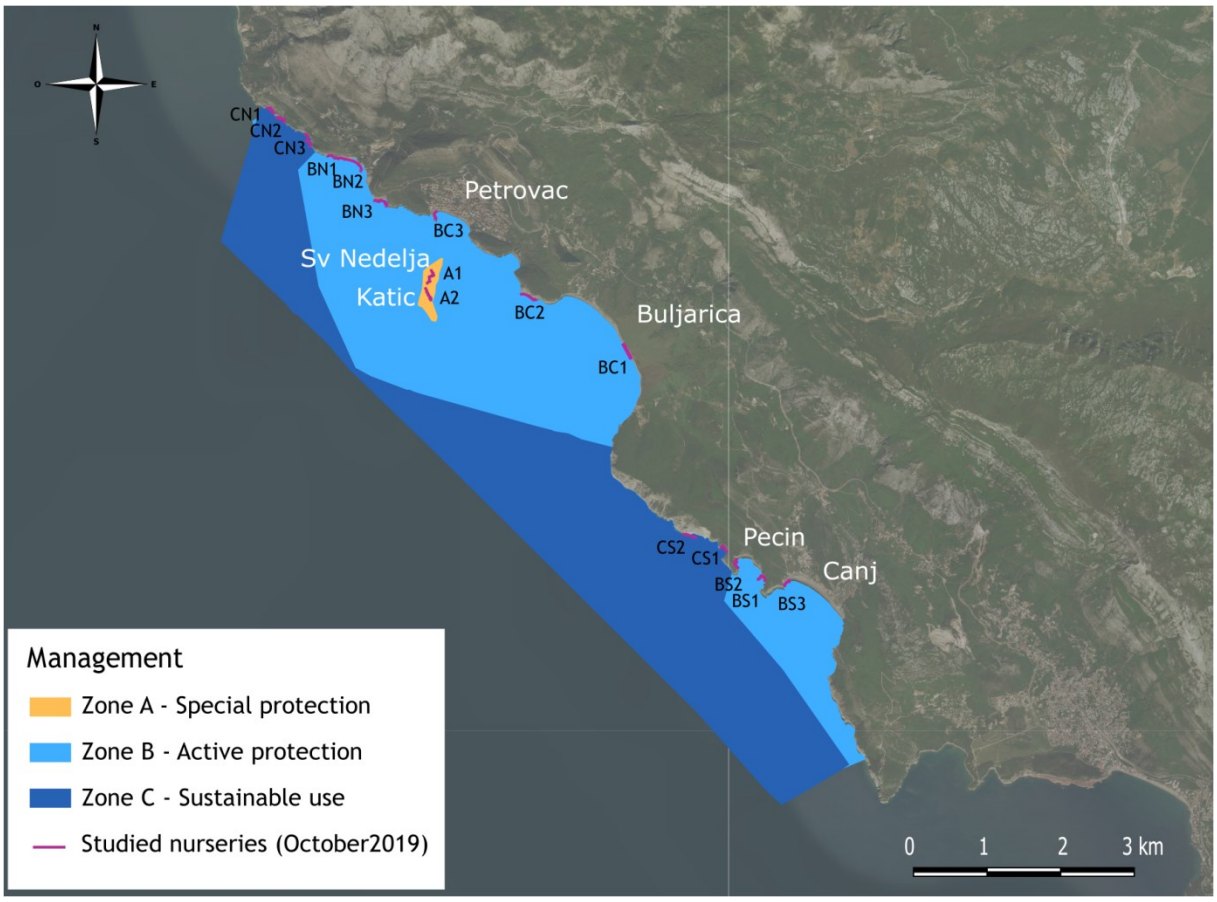


Figure 2. Studied nursery sites (in black) among the 3 levels of management types of the potential MPA Katic; the 16 sites are grouped by zones, from north to south: CN, BN, BC, A, CS, BS - Main localities mentioned in the text are written in white

Juvenile fish census

In each site (Table 1, Fig. 2), juvenile fish assemblages were described using taxa-specific abundances and individual sizes (TL, mm) during 3 consecutive days from October 26th to 28th, 2019.

Studied species

Our study gathered data for juveniles of fourteen species, including groupers as well as other species that are known to use the same shallow heterogeneous rocky habitats as nurseries at that time of the year. They were grouped into the following taxa (Fig. 3 and 4, Table 2): dusky grouper (*Epinephelus marginatus*, Lowe 1834), golden grouper (*E. costae*, Steindachner 1878), dogtooth grouper (*E. caninus*, Valenciennes 1843*),* yellowmouth barracuda (*Sphyraena viridensis*, Cuvier 1829), ornate wrasse (*Thalassoma pavo*, Linnaeus 1758), Mediterranean rainbow wrasse (*Coris julis*, Linnaeus 1758*),* white seabream (*Diplodus sargus*, Linnaeus, 1758), sharpsnout seabream (*Diplodus puntazzo*, Walbaum 1792), red porgy (*Pagrus pagrus*, Linnaeus 1758), and common dentex *(Dentex dentex*, Linnaeus 1758). Opportunistic species, which have previously been documented as more ubiquitous in terms of juvenile habitat choice, were excluded from the presented data. This includes *Sarpa salpa*, *Diplodus vulgaris* and *Oblada melanura* (Cheminée et al., 2011; Garcia-Rubies and Macpherson, 1995; Harmelin-Vivien et al., 1995; Vigliola, 1998).

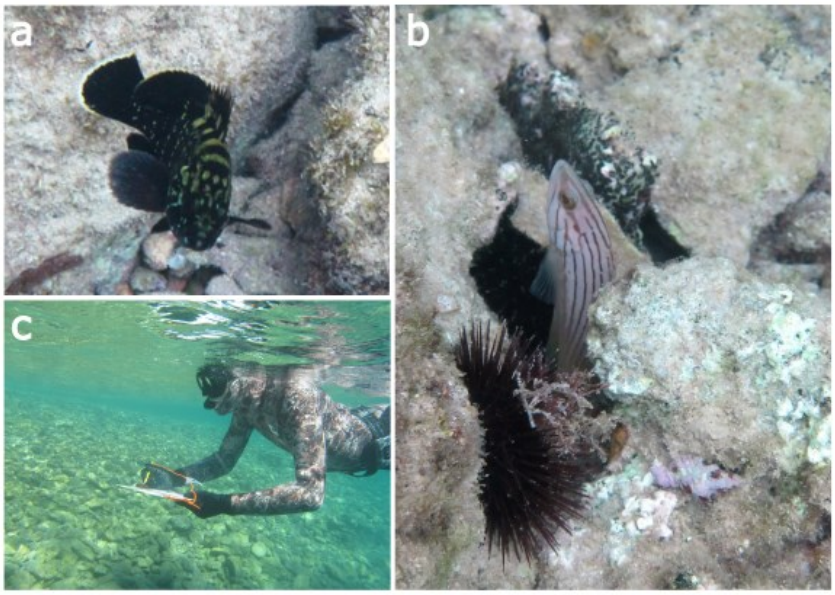


Figure 3. Juvenile groupers of *E. marginatus* (a) and *E. costae* (b), monitored by underwater visual census (c)

Figure 4. Examples of studied taxa: illustration of juvenile stages (total length in mm)

Fish sampling procedure

Method

In each of the surveyed sites, for each studied species, juvenile abundances and sizes were monitored during underwater visual censuses (UVC) done by snorkeling along belt-transects (i.e.

replicates) parallel to the shoreline, among the previously described habitat. Belt-transects were 1-meter-wide and in average 77 m length (Table 1, Fig. 3, 5 & 6). Two to four transects were sampled in each site, from October 26th to 28th. This allowed us to take into account not only settlers (yo individuals) but juveniles from a longer period of time as well (y+1 individuals, i.e. settlers from the previous year) (Crec'hriou et al., 2015; Froese and Pauly, 2011; Garcia-Rubies and Macpherson, 1995; Lejeune, 1985; Raventos and Macpherson, 2001). By doing so, we did not aim to consider the settlement peak of a given single species, but rather we performed an estimate of the recruitment level for a variety of species, i.e. quantifying the remaining juveniles after an arbitrary period of time following settlement (*sensu* Macpherson & Zika (1999)). The same divers, previously inter-calibrated, performed underwater visual censuses of juvenile fishes as described in previous works (Cheminée, 2012; Cheminée et al., 2017a, 2013, 2011; Cuadros, 2015; Cuadros et al., 2017; MacPherson, 1998; Pastor et al., 2013; Vigliola et al., 1998): the diver recorded the abundance and size per taxa of necto-benthic juveniles. The total length (TL) of individuals (±0.5 cm) was estimated with the help of fish silhouettes of different sizes on a submersible slate (Fig. 3). TL estimates were used *a posteriori* in order to study only juvenile individuals. Rough sea and poor visibility days were avoided.

For most rocky reef fishes in the Mediterranean, size at settlement is around 10 mm TL (Cheminée et al., 2013; Crec'hriou et al., 2015; Garcia-Rubies and Macpherson, 1995). The smallest specimens of the taxa studied were considered newly settled individuals. Our visual censuses took into account the young of the year (y0 individuals) and size-classes that may correspond to young of the previous year (y+1) (Table 2). At this stage most of the mortality has already occurred so the densities observed are a proxy of what should recruit into the adult population (Macpherson and Zika, 1999). *Thalassoma pavo* is a stenotherm species affiliated to warmer waters from southern parts of the Mediterranean; however, data from the last 20 years suggest a shift in its geographical range concomitant with global change and the warming of the Mediterranean (Guidetti et al., 2002; Milazzo et al., 2016). In the northern Mediterranean coasts, juveniles are still very rare and the detection of adult specimens is important data, as it indicates the shift of its expansion range towards the north. The ratio of *T. pavo* and *C. julis,* is used as an indicator to monitor changes due to global warming (Milazzo et al., 2016). For this reason, we recorded all sizes of these two species.

Table 2. Code and maximum juvenile Total Length (TL) considered for each of the fourteen surveyed taxa.			
Genus	**Species**	**Code**	**TL (mm)**
Epinephelus	*marginatus*	em	260
Epinephelus	*costae*	eco	225
Epinephelus	*caninus*	eca	260
Diplodus	*sargus*	ds	80
Diplodus	*puntazzo*	dp	90
Sphyraena	*viridensis*	sv	150
Dentex	*dentex*	dd	90
Pagrus	*pagrus*	pp	90
Dicentrarchus	*labrax*	dl	300
Sparus	*aurata*	sa	80
Sparisoma	*cretense*	scr	120
Sciaena	*umbra*	su	250
Thalassoma	*pavo*	tp	80
Coris	*julis*	cj	80

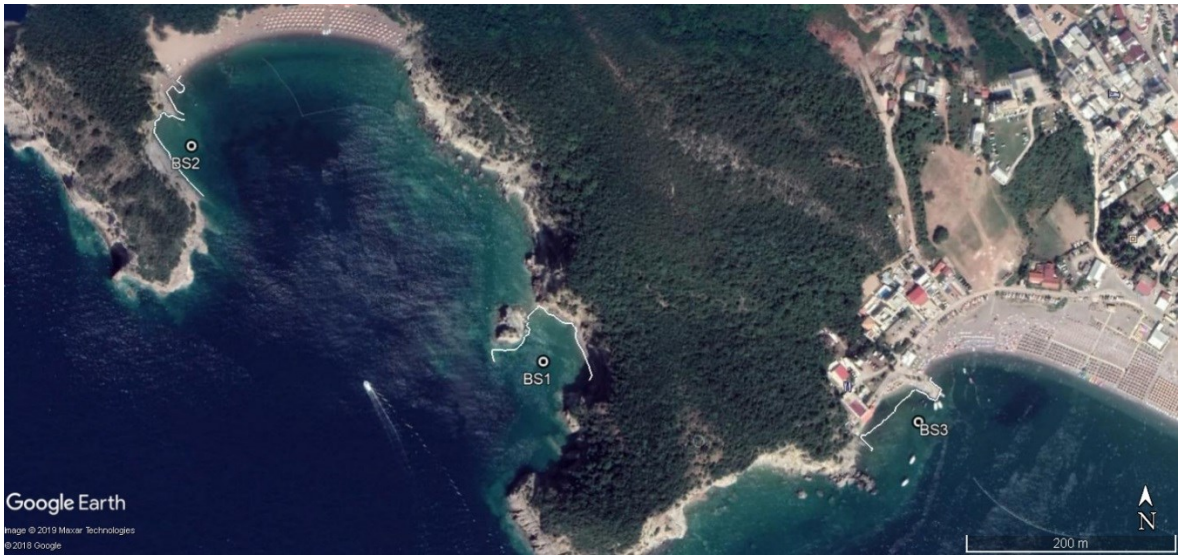


Figure 5. Studied nursery sites and localization of belt-transects (white stripes) used for juvenile fish visual censuses in Canj and Pecin cove (B south zone)

Figure 6. Groupers and other fish juveniles monitoring campaign: exploring the study area including Katic and Sv Nedelja islands (a) and adjacent costal zones; finalizing the selection of study sites (b); preparing material (c) for underwater visual census (e, f) using submersible plastic plates (c) in each studied nursery sites (d: illustration of the site BN2)

Results and discussion

Nursery sites

During the field work, we validated the preliminary localization of nursery habitats in the 16 surveyed sites, thus obtaining a ground trothing of the cartography of these essential habitats, althouth it is not exhaustive: more nursery habitats are potentially available in this area, and have not been mapped nor visited (Fig. 2).

Juvenile fish full assemblages

Of the fourteen studied taxa, five were not observed at the juvenile stage (*Dentex dentex*, *Pagrus pagrus*, *Sciaena umbra*, *Sparus aurata*, *Sphyraena viridensis*). Among the 9 other taxa, a total of 675 juvenile individuals were observed. The most frequently observed taxa at the juvenile stage were, in

order of decreasing proportion: *Coris julis*, *Diplodus sargus*, *Thalassoma pavo*, and *Diplodus puntazzo* (Fig. 7). They summed up to 90% of observed individuals. We counted 47 individuals of juvenile groupers (*E. costae*, *E. marginatus* and a single individual of *E. caninus*) accounting for 7% of all taxa juveniles.

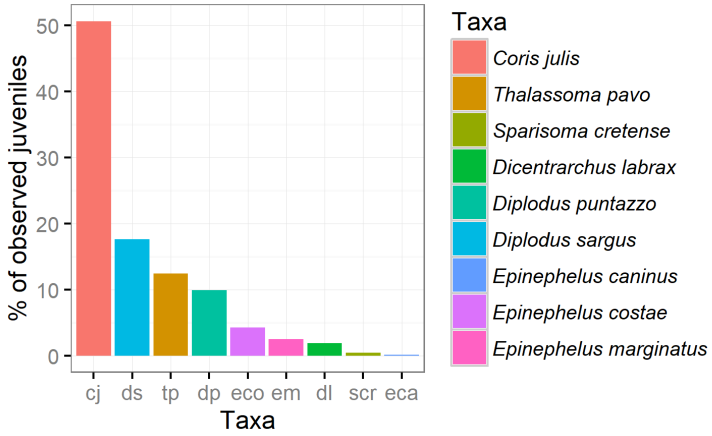


Fig. 7. Proportion of observed juvenile fish during the entire study

When looking at juvenile assemblage composition, juvenile assemblages differed between the various zones, while management type and site had no significant effect. In other word, samples from different zones tended to show various compositions in terms of juvenile relative abundance among taxa (Fig. 8 ; PERMANOVA, $p<0.05$).

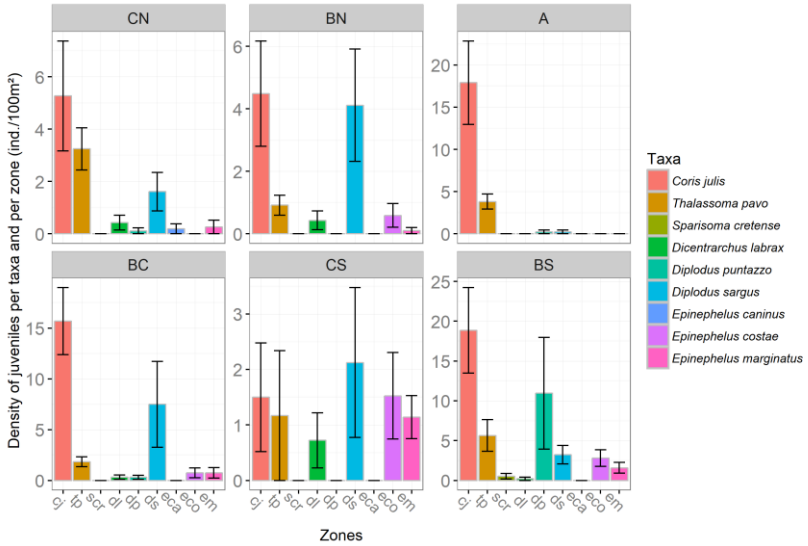


Fig. 8. Barplots of mean juvenile densities assemblages per zones – error bares = standard errors

Total juvenile densities, all taxa together, differed significantly between zones: zones BS, BC and A displayed significantly higher mean total densities. Similarly, mean species richness (i.e. the number of different taxa) differed significantly between zones: in average, samples from zone B south (BS) displayed significantly richer assemblages than all those from other zones (Fig. 9; PERMANOVAs, $p < 0.05$).

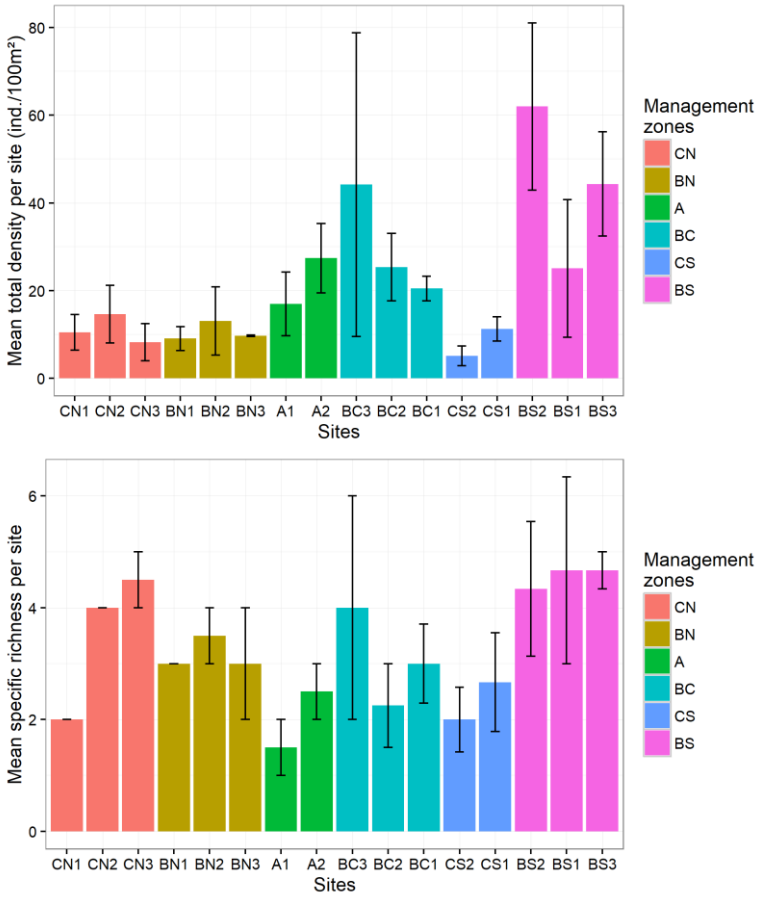


Fig. 9. Mean total densities and mean richness of juvenile fish assemblages per sites and zones – error bares = standard errors

Taxa specific patterns

As regards grouper juveniles (y0 and y+1 individuals), we observed a total of 29 *Epinephelus costae* juveniles and 17 *E. marginatus* juvenile individuals, sizing in average respectively 144.3 mm TL (min: 60 mm, max: 220 mm) and 132.9 mm TL (min: 50 mm, max: 90 mm) (Fig. 10, 11a and 11b). For *Epinephelus caninus* only a single juvenile was observed, located in site CN2, and sized 250 mm (TL).

Mean densities differed between sites and between zones: for both *E. marginatus* and *E. costae*, juvenile individuals were significantly more abundant in the BS and CS zones, while totally absent from the A zone (Katic and Sv. Nedelja Islands). In the south, the most southern studied site (BS3), at the small harbor of Canj, displayed notably high densities of small *E. marginatus* juvenile individuals (Fig. 12). BC zone displayed intermediate mean densities of groupers: it is to note that *E. costae* were particularly present in the BC1 site, in front of the beach of Buljarica, while *E. marginatus* individuals were notably observed in the small harbor of Petrovac, among blocks and pebbles located few tens of centimeter depth right at the feet of the main dock.

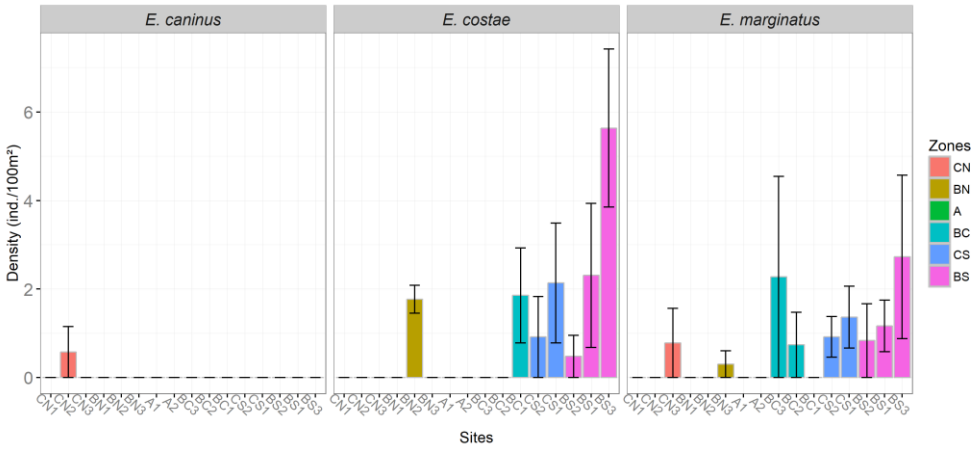


Fig.10. Mean densities of juvenile groupers per zones, sites and taxa

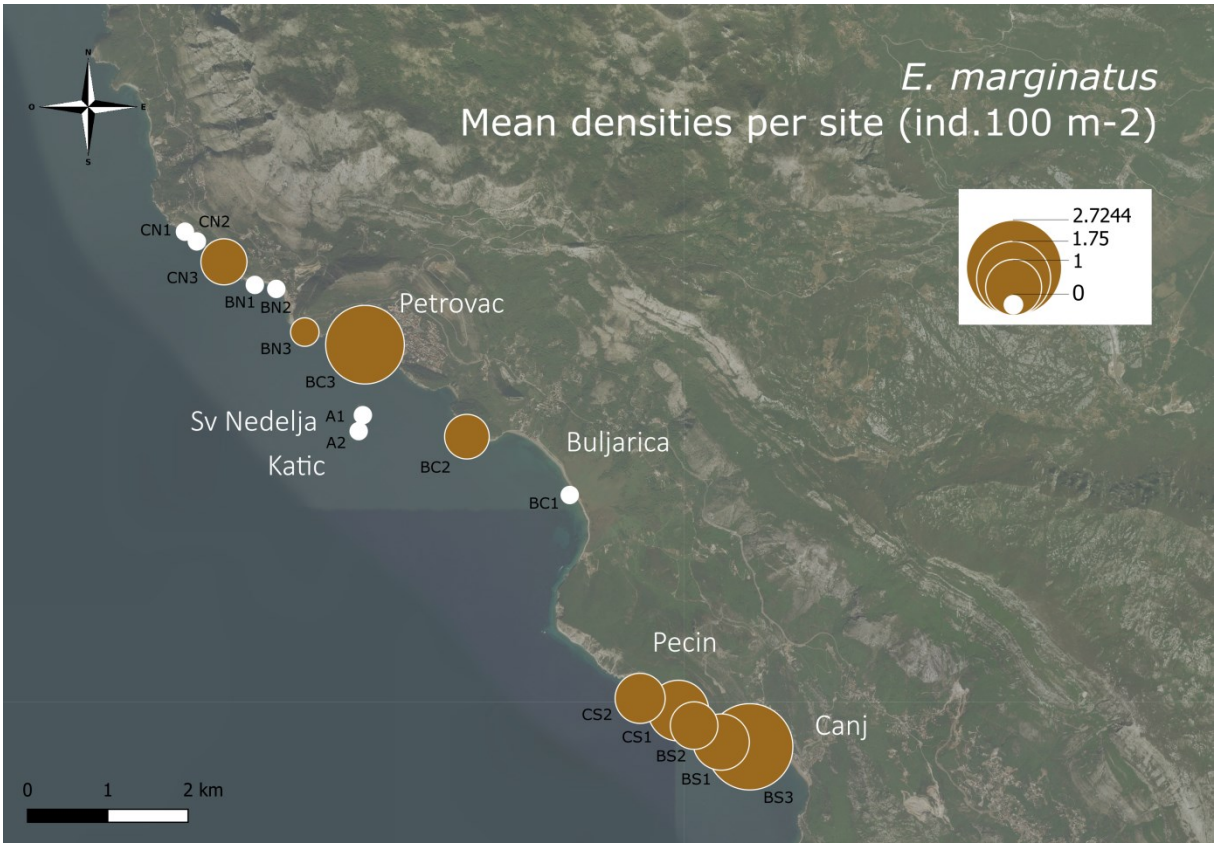


Fig.11a. Maps of juvenile groupers (*E. marginatus*) densities per sites and taxa

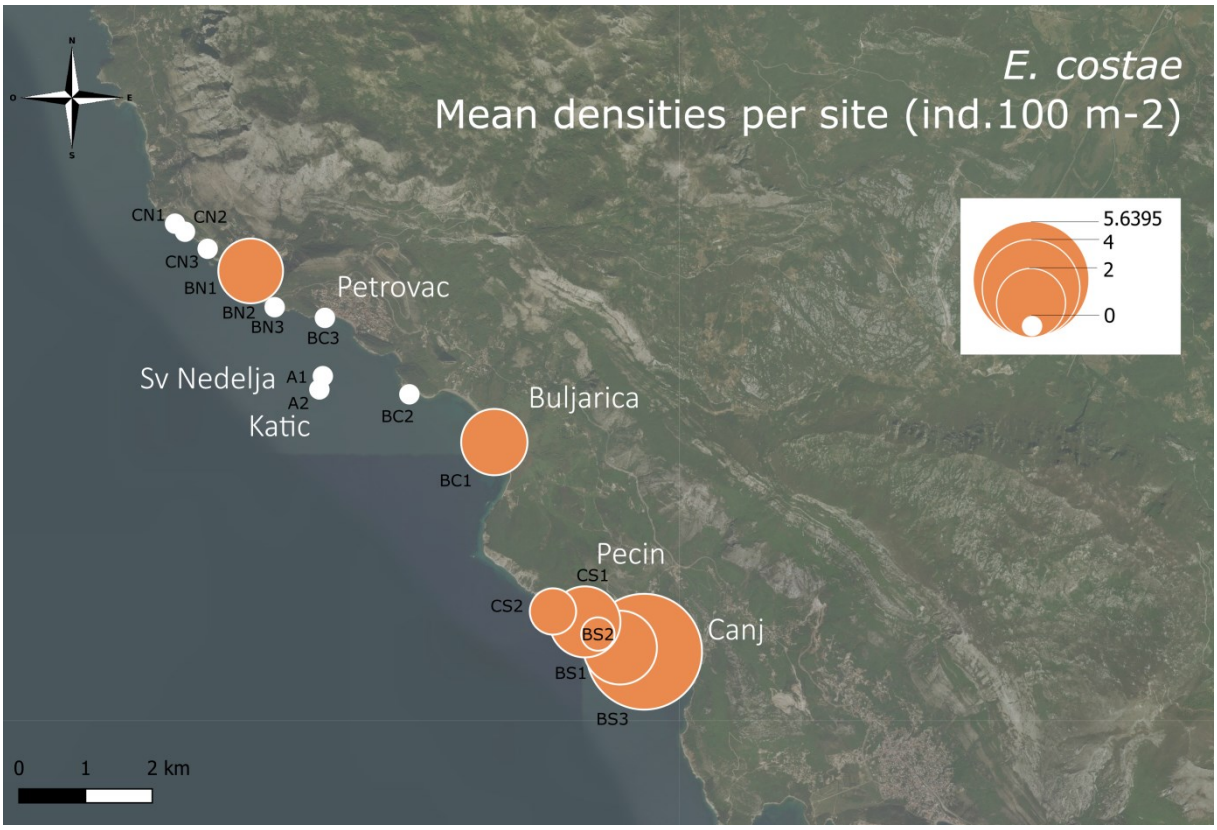


Fig.11b. Maps of juvenile groupers (*E. costae*) densities per sites and taxa

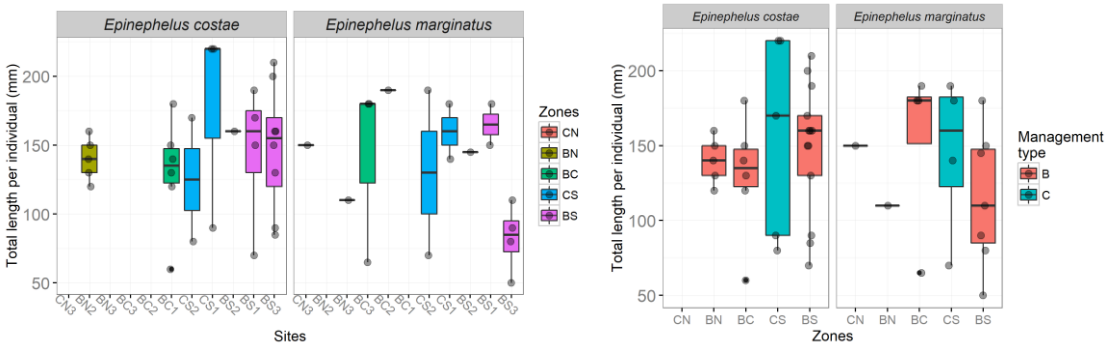


Fig.12. Boxplot of individual total length (mm) per taxa, sites, and zones (left panel) or grouped per zones and management type (right panel)

As regards, *T. pavo* and *C. julis* juveniles ratio of densities, *C. julis* was more abundant than *T. pavo* in almost every sites (Fig. 13).

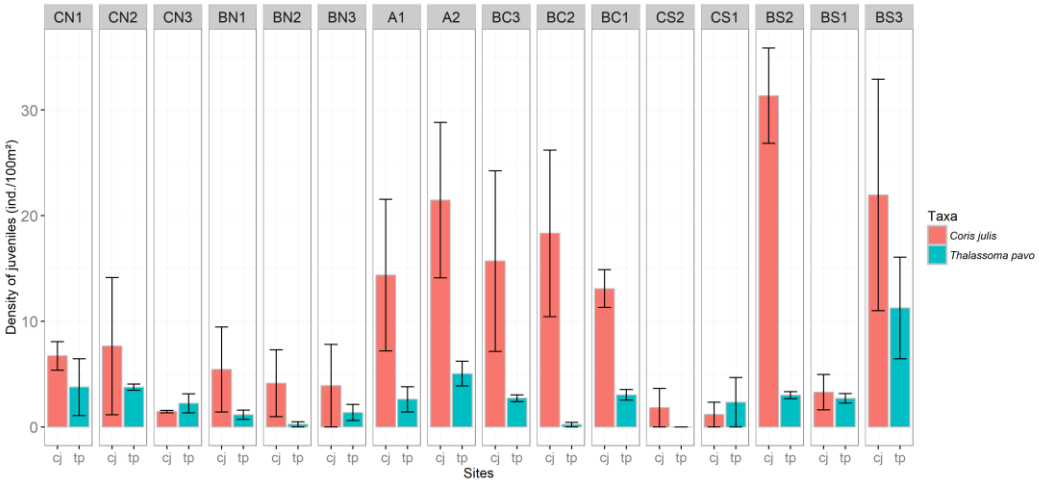


Fig.13. Barplots of mean densities of *C. julis* and *T. pavo* juveniles per sites – error bares = standard errors

Discussion and perspectives

Grouper juveniles were scarce (7% of observed juvenile individual all taxa together). Moreover they were located only on coastal sites, and absent from the potential "A" core management zone, although nursery habitat were present there. Such pattern, displaying contrasted densities between nurseries from mainland *versus* island, is observed for other species in other MPAs: in Port-Cros MPA (France), juvenile of *Diplodus sargus* are scarce in the island sites, although they display suitable nursery habitat, in comparison to coastal mainland sites (Harmelin and Vigliola, 1998). The same has been observed in the Calanques National Park (Riou Island) (Cheminée et al., 2011; Leboulleux, 1992). These patterns have been interpreted as a consequence of currents running parallel to the shoreline and that export the produce of adult reproduction (eggs, larvae) away from the island nurseries, while coastal nursery sites are more favorable for larvae retention. Consequently, for grouper populations' renewal and conservation, island sites rely on the migration of sub-adults and adults that first settled and grew up in coastal nursery sites, illustrating the concept of ecological solidarity between various units of the seascape (Cheminée et al., 2014b). In the case of Katic potential MPA, this outcome is highly important because it stresses out the need to protect not only the island zone (A zone in current management plan) but as well sites located along the shoreline, such as in Buljarica beach or Canj.

From a manager point of view, conservation of these nursery habitats is not only a matter of designating no-take zones: the key condition to preserve the nursery function of these habitats is to conserve the three-dimensional structure and characteristics of bottoms. For example, in order to keep fully functional nurseries, sites such as those displaying high densities of grouper juveniles (BC3 in Petrovac harbor, BC1 in Buljarica beach, sites B South 1, 2 and 3 near Canj, see Fig. 2) should be sufficiently protected to prevent strong modifications and transformations of habitat characteristics.

For *Thalassoma pavo* juveniles, during a previous study in the nearby region of Split (Croatia) it was observed an increasing gradients of densities form north-western towards south-eastern sites: *T. pavo* was present and more abundant than *Coris julis* in the southern sites (Vis, Hvar) while absent in the northern sites from Kornati archipelago (Cheminée, 2016). Such pattern is in agreement with previous studies: this stenotherm species is usually found on the southern Mediterranean coasts, in warmer waters. According to the maps presented in Guidetti *et al*. (2002) and Milazzo et al. (2016), the Gulf of Lion remain one of the last areas of the Mediterranean not populated by *T. pavo*.

Generally *T. pavo* adults are the first to be observed colonizing a new area, followed by the juveniles (Figueiredo et al., 2005). Recent works highlighted the warming-related shifts of this species' distribution towards the north-western parts of the Mediterranean (Milazzo et al., 2016). In our case, along the coast of Katic potential MPA, our observations may be used as baseline data to monitor future possible signs of such distribution shift. Other stenotherm or thermophilic species, including non-native invasive ones such as *Fistularia sp*. or the puffer fish, may follow such trends, notably in the Adriatic. A yearly survey of these species would help to analyze and determine the extent and rapidity of this process.

PART B - Mapping potential micro localities where adult Dusky groupers may be present

Introduction

The Dusky grouper (*Epinephelus marginatus* Lowe, 1834) inhabits hard, rocky substrates which are rich in shelters distributed from the surface to the depth of 250 m (Heemstra and Randall, 1993). There are at least four subpopulations of the species in the world, affected by strong and long-lasting fishing pressure especially in the Mediterranean with landing decline of 86% at global level (Pollard et al., 2018). Herewith we are testing the methodological approach to be used in mapping out the area where Dusky grouper could be found by using the sonar feature mentioned above. The method should entail production of maps with micro localities and areas suitable for the target species. That will enable further data collection by underwater observations in the field research.

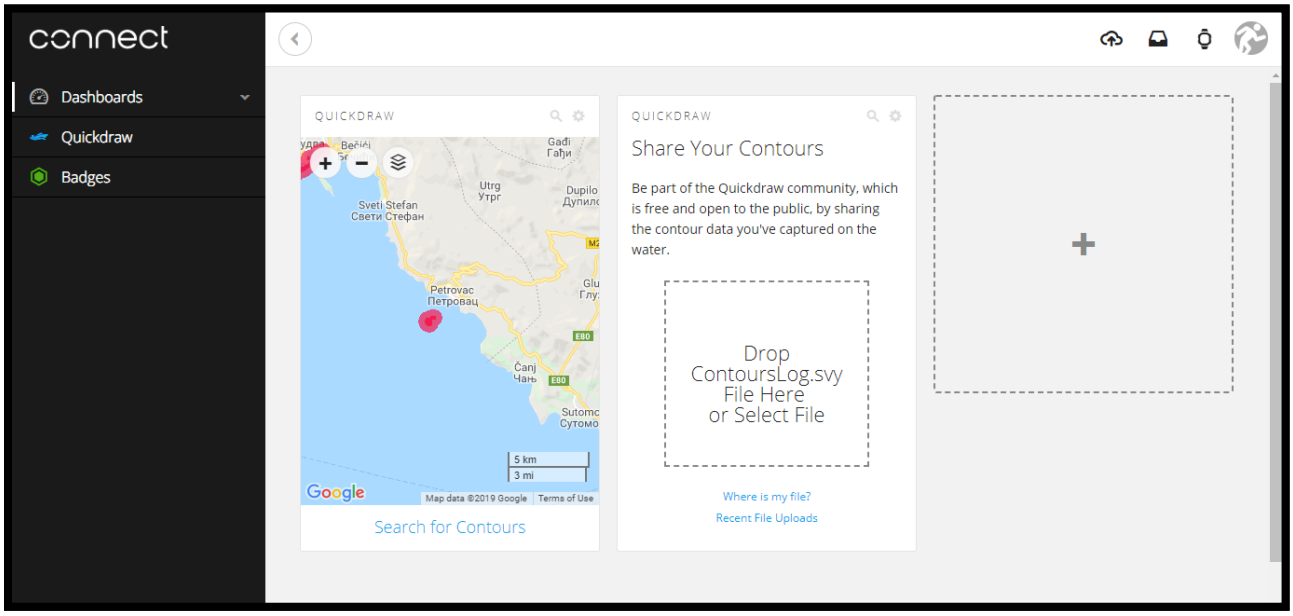
Materials and methods

In relation to the first research aim to make an inventory of appropriate micro localities for *E. marginatus* in the target area, Garmin Quickdraw™ Contours[1] tool was employed. This feature enables mapping of sea bottom contours with their depth related data and therefore creates an opportunity to find out unevenness at sea bottom, such as reefs, boat wrecks etc. These represent suitable habitats for the target species. An example of its functioning can be seen here https://www.youtube.com/watch?v=A3CKh0CIj00. Garmin has developed a website entitled Garmin Connect (https://connect.garmin.com/) which is an open access database with quickdraw mapping data from all around the world uploaded by different users (Picture 1). Every user can easily create an account and have access to the data, upload new one or download existing for a desired area. Each Garmin sonar device saves the produced quickdraw data as a file with .svy extension which is afterwards transferred to the computer and can be uploaded to the Quickdraw Community (Picture 2). Contours data for the area targeted by the research did not exist in the Quickdraw Community database currently.

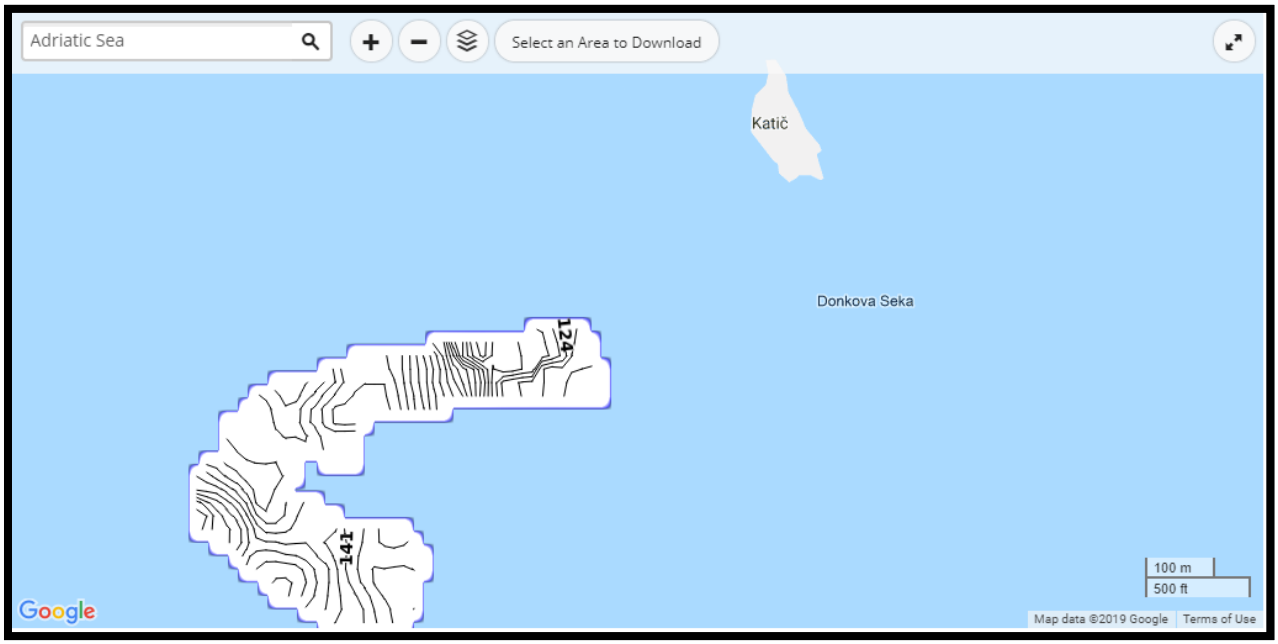
The chosen area for which it will be collected is the Zone B defined as a zone of active protection (Picture 3). Accordingly to the previous studies dealing with the zonation of the area, two localities fall under the Zone B. First part and the bigger one of the Zone B, expands from Perazića školjić reef on the north up to the southern end of the Buljarica beach to the south. The second part is smaller and covers area around the coastal settlement of Čanj which is relatively far away of the first part of Zone B. Bigger part of the Zone B has been chosen as the target area of the research and sonar mapping was undertaken there. This area was chosen as it will be the zone with the active protection, so it is assumed that it will be the most important part of the future MPA in the term of biodiversity protection. The majority of the areas rich in species habitats, such as Donkova seka and Katici islands, are included into this part of the future MPA. As well, the Buljarica cove, which is already a protected area, fall under this zone. The zone C is considered for sustainable use and likely be the area with fishing and other activities allowed at some point. Also, the surface of this zone is too large to be covered by this project and would need much more time and resources.

[1] https://connect.garmin.com/start/quickdraw/

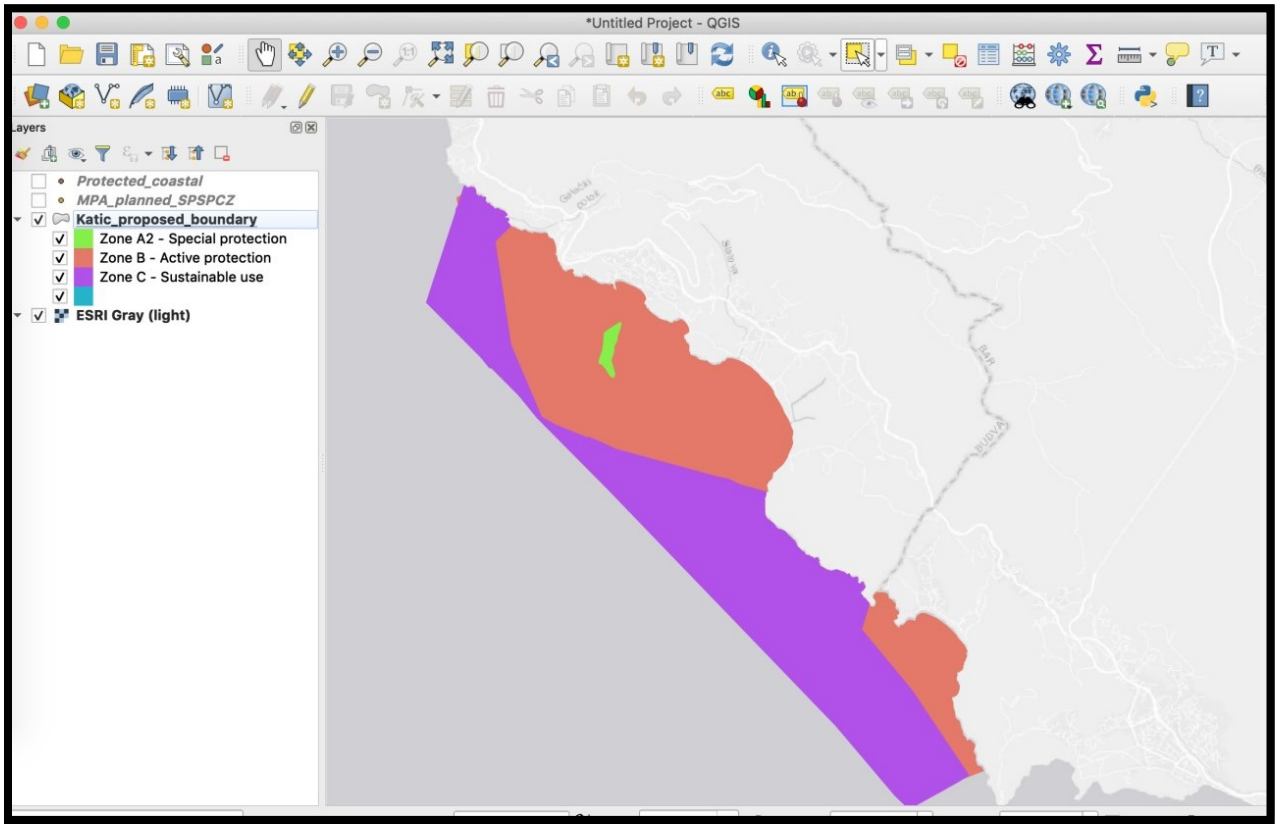
The used Garmin device for mapping the seabed contours was Garmin GPSMAP 721xs (https://buy.garmin.com/en-SG/digital/p/119865). As well, in order to achieve better synchronization among the used maps and other data, other Garmin programs were used instead of those from other manufacturers. For setting up the survey transects and their following in the field, Garmin HomePort software and Garmin ActiveCaptain app were used.



Picture 1. Garmin Connect website for quickdraw data.



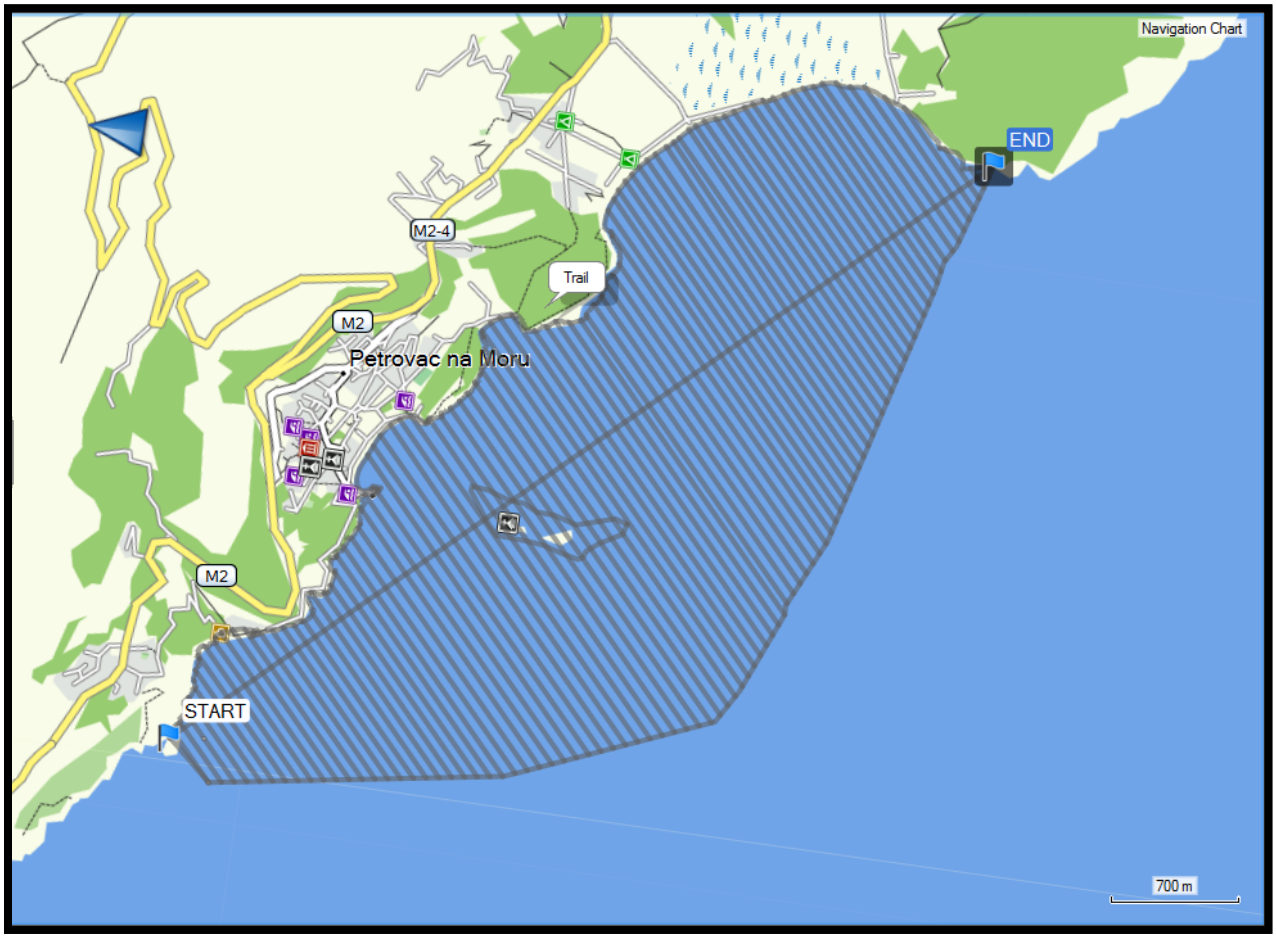
Picture 2. A small sample of the data uploaded to the Quickdraw Community in the target area.



Picture 3. Proposed boundaries of the future MPA Katič with zone B coloured in red.

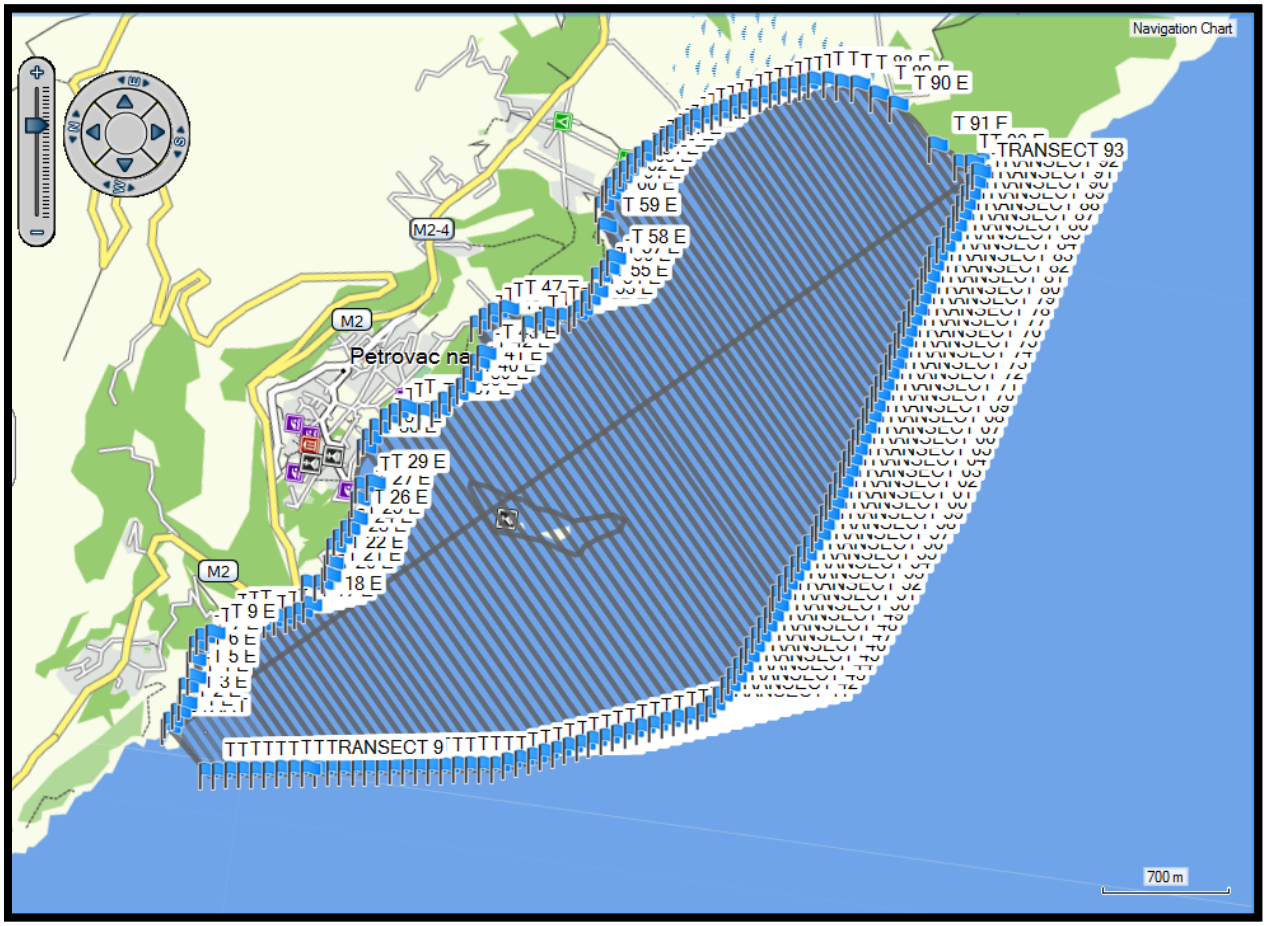
The boundaries of the target area are converted to the .gpx files and processed within another Garmin's software called HomePort which provides a variety of possibilities. The area subjected to the survey was firstly divided into transects as follows:

1) Boundaries of the area are placed as a .gpx file on the Garmin HomePort chart
2) An orientation axis has been created between the northernmost point of the target area and the southernmost one
3) Each transect has been made to cut the orientation axis as a perpendicular line with its beginning on the offshore border of the area and with its end on the coastline
4) Range between two transects was made to be 70 meters in correspondence with used sonar device characteristics and possibilities, as there are differences between characteristics of each sonar model, so this value should be a variable (Picture 4)
5) The used boat speed was defined to be 4 to 5 knots (nautical miles/hour) which is found to be enough slow to create contours correctly as it should be
6) The mapping was done in the morning hours, in calm days without wind and with sea surface being completely flat in order to avoid turning off the course caused by either wind or sea currents
7) Every micro locality with unevenness was marked with a waypoint on sonar. If the certain zone should be created, Garmin sonar enables entering a waypoint on the screen where needed, which can be later connected in HomePort and transferred to the shape files to be used in GIS and other systems.



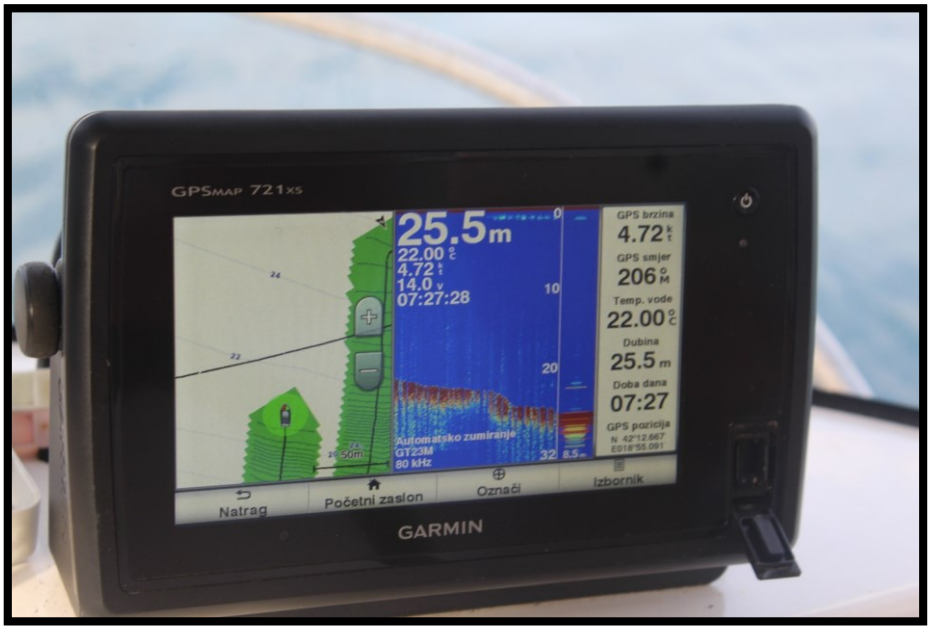
Picture 4. Target area divided by transects of different sizes in Garmin HomePort navigation chart.

The Quickdraw Contours software gives a great opportunity by observing the results of the contour mapping in the real time directly on sonar device's screen. This enables immediate recording of the coordinates of micro localities that are specifically interesting to the survey. As for big amount of data it provides, it's difficult to display contours themselves of such bigger area on a single map, but it can be divided into smaller maps of the locations which fall under the interest of the observer.

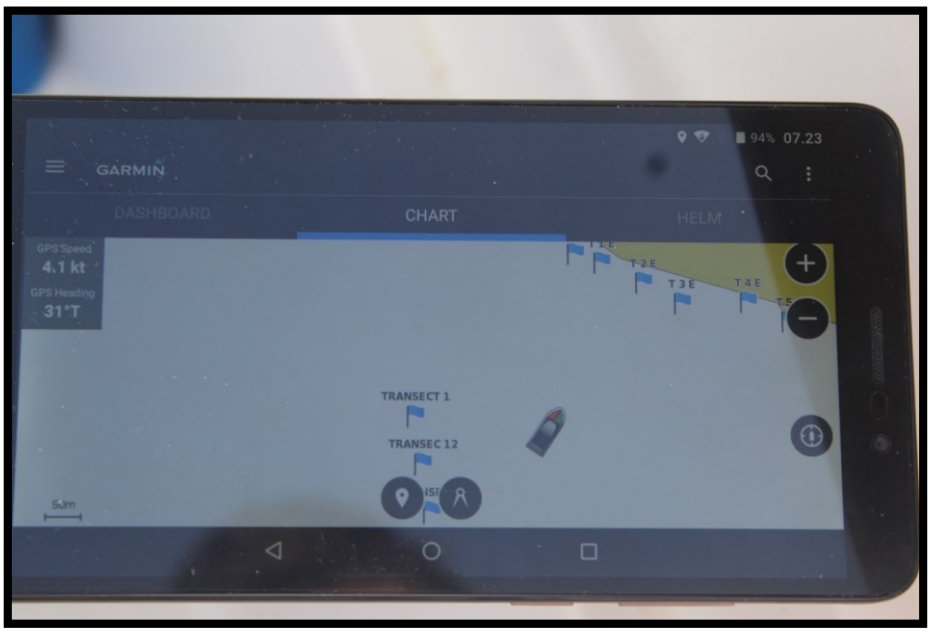


Picture 5. The target area has been divided into the 93 transects of different length with the beginning and end point of each one. This data are inputted into sonar device before the contour mapping, so the boat course can be set up for each transect during the survey.

Quickdraw Contours gives an opportunity to record the locations of interest immediately (Pictures 6 to 8), so one can obtain a map with results at the end of the survey. A shortage of the feature is reflected in the impossibility of imputing the contours data as a layer either in HomePort or another system as GIS, so one can't observe and mark another interesting localities and areas on computer. This is solved by a possibility to enter the new coordinates after the contour mapping directly on sonar device (Picture 9), which enables defining new localities by reviewing mapped contours. Some of the unevenness cannot be spotted immediately, such as dents, but afterwards when looking at created contours with depth data, they can be observed and marked.



Picture 6a. Contour mapping along a single transect in the target area and in real time.

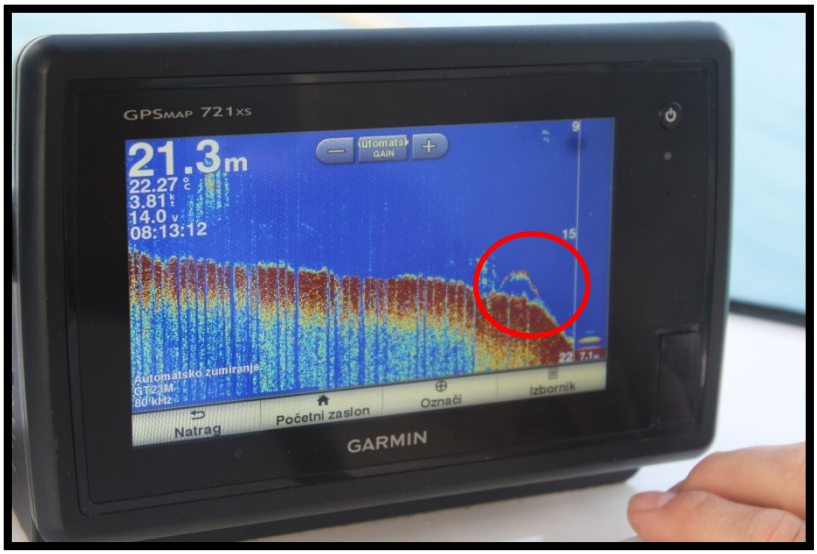


Picture 6b. The boat course is tracked in the Garmin ActiveCaptain app on the other device separately from the sonar.

Results

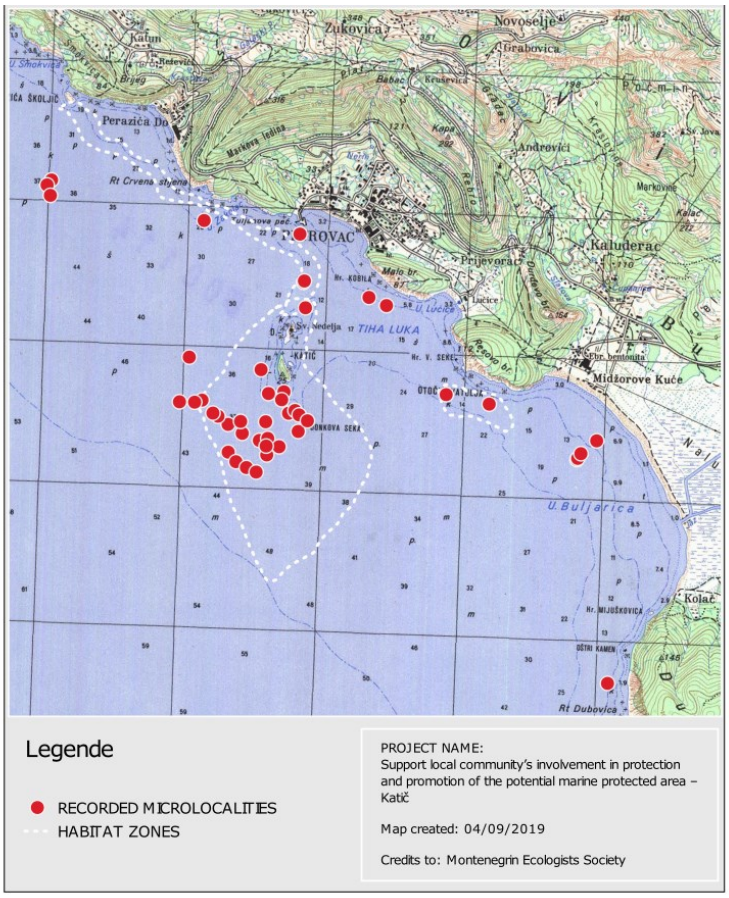
The area where the contour lines are tight (Picture 9) represents the slope in infralitoral zone where depth rapidly drops from approximately 20 to 30-35 meters. In this zone, communities of benthic fish species are found to be abundant. The area after it, in the offshore direction, represents the flat muddy/sandy bottom where depth falls slowly and is not suitable habitat for the Dusky grouper. In such area, smaller locations with either rocks or boat wrecks can be present and identified as micro localities suitable for the species. These suitable habitat zones and micro-localities are spotted on picture 10.

Picture 7. The found and recorded micro locality consisted of a several rocks suitable for the Dusky grouper individuals in the surveyed area.



Picture 8. A larger fish observed over the edge of an infralitoral slope.

Picture 9. Created contour map for the northern end of the surveyed area.



Picture 10. Habitat zones and micro-localities suitable for adult grouper individuals

Discussion

The Quickdraw feature was designed by Garmin originally to improve fishing, but in this research it is found to be suitable for different kind of surveys in environmental projects and research. Its foremost good feature is that it is relatively cheap in comparison with other similar technologies as you need only Garmin device with its charts. It can be of significance in research in coastal areas of sea in relatively smaller depths (up to 60-70 m, maybe even more). Currently, the contour data can be inputted only in Quickdraw Community database which is good as users can easily share their and download others data. Garmin announces the plan to connect it with the HomePort software which would be of great significance and would easier the data analyses.

Part C: preparation of methodology for visual underwater survey of adult Dusky groupers and the ichthyofauna community in potential MPA Katic

Introduction

Once the suitable habitat and micro-localities mapping has been done (Part B), the next step of the study will be to perform underwater visual censuses of adult individuals of Dusky groupers, as well as other groupers and global fish assemblage. The aim of this part is to describe the methodology and study sampling design that will be used to assess the composition of assemblages and the population structure of specific taxa in the different zones of the potential MPA Katic. Ultimately, this fish assemblage description will give baseline data for further advising the management of the area and the development of sustainable activities.

Proposed material and methods

Studied species

The population censuses will focus on a list of species representative from the necto-benthic taxa that may be found in coastal Mediterranean habitats. The list should be constructed by sampling individuals from some of the main categories of necto-benthic taxa (Harmelin, 1987) (Fig. 1). Studied species will notably include some of those sensitive to human uses (fisheries) and management measures. As a minimum list, species used for the juvenile fish study (Part A) should be employed. It will be enriched with taxa under considerations for management purposes, as well as with taxa indicators of global change: thermophilic and Non Indigenous Species (NIS) (Table 1).

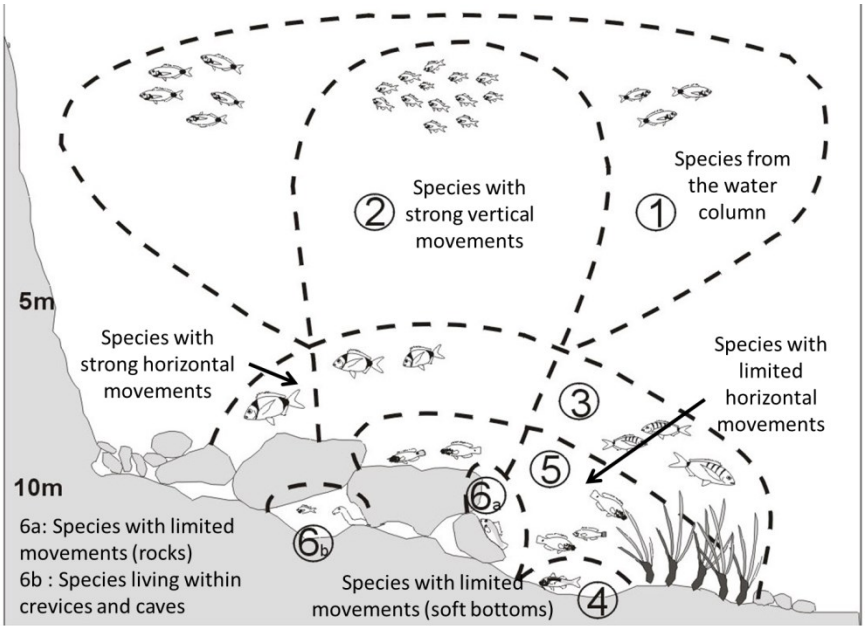


Figure 1. Main categories of necto-benthic taxa for coastal Teleosts found in the Mediterranean; Modified from Harmelin (1987)

Table 1. Proposed list of taxa considered for adult surveys			
Genus	**Species**	**Categories (Harmelin, 1987)**	**Thermophilic or Non-Indigenous Species (NIS)**
Epinephelus	*marginatus*	*3*	
Epinephelus	*costae*	*3*	
Epinephelus	*caninus*	*3*	
Diplodus	*sargus*	*3*	
Diplodus	*puntazzo*	*3*	
Sphyraena	*viridensis*	*1*	*Thermo*
Dentex	*dentex*	*3*	
Pagrus	*pagrus*	*3*	
Dicentrarchus	*labrax*	*1*	
Sparus	*aurata*	*3*	
Sparisoma	*cretense*	*5*	*Thermo*
Sciaena	*umbra*	*3*	
Thalassoma	*pavo*	*5*	*Thermo*
Coris	*julis*	*5*	
Fistularia	*commersonii*	*3*	*NIS*
Siganus	*spp.*	*3*	*NIS*
Lagocephalus	*spp.*	*3*	*NIS*

Sampling design and protocol

Fish assemblage will be described by underwater visual census (UVC). Since the objective is to obtain a comparison of fish assemblages between various zones and sites in order to advice management zoning, sampling should be done according to a stratified random sampling design. This means that censuses will be repeated various times, obtaining replicates, and that various replicates (i.e. samples) should be done in each configuration of the sampling design. Taking into account the objectives of the present study, classical and well recognized methodologies of Underwater Visual Censuses will be used (HARMELIN-VIVIEN and HARMELIN, 2013; Harmelin-Vivien et al., 1985), although others, more recent experimental protocol may also exist and may be considered for future research (Prato et al., 2017).

By crossing the management zoning of the potential MPA Katic (see Fig. 2, Part A) and the results from micro-localities cartography (Fig. 10, Part B), it is possible to propose a sampling design including eight studied sectors (Fig. 2, part C). In each sector, various random sites will be used to collect samples, summing an equivalent number of samples per sector. Habitat type is not considered as an explaining factor: all censuses, in every site, will be performed in the same single habitat type, i.e. rocky reefs, excluding for example sandy areas or meadows. Eight sectors (Fig. 2) are considered (white boxes), in each sector 3 sites (red dots) will be randomly selected and in each site 2 to 4 replicates (15' counts) will be

sampled. A sampling unit (= replicate = sample) will consist of 15 minutes count during which one diver will randomly swim at constant depth (+/-5 meters) and speed, searching for fishes. Divers will work by team, always staying within the visual field of each other's. Two divers will count fishes, while a third diver will accompany the two counters for security.

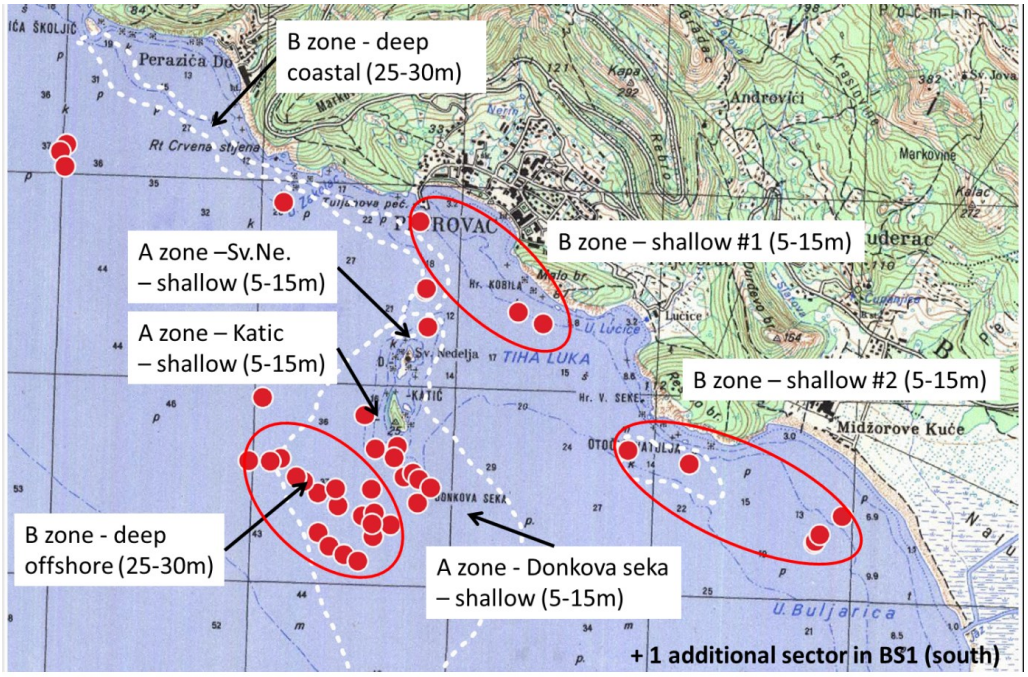


Figure 2. Sampling design for adult fish assemblages – 8 sectors are considered (white boxes), in each sector 3 sites (red dots) will be randomly selected and in each site 2 to 4 replicates (15' counts) will be sampled

Recorded response variables will include abundance and individual sizes for each taxa of the list of species. Further ecological data analysis will consist of exploratory (barplots, boxplot, ordinations) and inferential (uni- or multi-variate ANOVA) approaches, aiming at studying fish at the assemblage level (multivariate relative composition) and univariate taxa-specific metrics (population structure) (Anderson et al., 2008; Anderson and Millar, 2004).

Before the underwater visual censuses are done, previous *in situ* field training should be organized in order to guaranty an inter-calibration between observers for abundances and size estimation. This can easily be done during a few dives prior to field work, using for example plastic fish shapes enabling the training (Cuadros, 2015; Harmelin-Vivien et al., 1985). Underwater observations will be recorded using waterproof plastic sheets and slate (Cheminée, 2012; Harmelin-Vivien et al., 1985).

Sampling will be done during adequate weather conditions and seasons, ecologically representative. Winter should be avoided since fishes display a reduced biological activity, and may be hard to observe. Late spring (June) or Late summer (September) are good moments usually used for fish censuses (Grane-Feliu et al., 2019). Factors not included in the sampling design as well as stresses susceptible to disturb or interfere with censuses should be avoided: sampling should be always performed at the same day time (i.e. at least during

daylight), and low visibility and rough sea conditions (stormy weather, etc.) should be avoided.

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