UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea Magistrale

Biologia marina

METODOLOGIE SUBACQUE STANDARD PER IL MONITORAGGIO DEGLI EFFETTI DEL CAMBIAMENTO CLIMATICO LUNGO LE COSTE CROATE

STANDARDIZED UNDERWATER METHODOLOGIES TO MONITOR CLIMATE CHANGE EFFECTS ALONG THE CROATIAN COAST

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Sessione Febbraio 2022

Anno Accademico A.A 2020/2021

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ACKNOWLEDGEMENTS

This thesis would not have been possible without the support of my family and friends. I would like to pay my special regards to my father for being my "buddy" in almost all of the dives that were performed to collect data and for accompanying me throughout the entire journey. I would also like to thank my mother and sister Mirena for always being there for me. I would like to recognize the invaluable assistace of my boyfriend Mario that helped me every time I had issues when analysing data in Excel or writing this thesis. Thank you for being always being there to help me. I'm extremely grateful to my mentor Martina for providing guidance, teaching me how to perform the protocols but also for enduring my slow PC during our calls on Teams. I should also appreciate my italian friends Greta, Valentina, Federica, Tatiana, Chiara, Andrea and Viola for keeping me company but mostly for all the fun times that we spent together. I will cherish these memories forever. A special thanks goes to my friend Filippo for helping me during some of the toughest periods. I would also like to thank my croatian friends Dzara Juraj, Nikola, Matej, Marko, Marija Edita, Filip and Lovro for their constant encouragement.

Especially I would like to thank Anton Prekalj from Ronilački klub "Ugor", Vrsar for providing all the information we ever needed, Ronilački klub "Roniti se mora" especially Katarina Sprljan for performing the FVC protocol like a professional and everyone who was interested in learning about my project and the entire staff of DC "Marco Polo" for their great hospitality.

SUMMARY (ITALIAN)

Il cambiamento climatico continua ad essere uno dei principali problemi a scala globale ed il Mar Mediterraneo è particolarmente sensibile alle sue conseguenze a causa della sua geomorfologia e un alto tasso d'evaporazione che causano un aumento della temperatura ad una velocità del 20% maggiore della media mondiale. L'aumento della temperatura dei mari favorisce organismi non autoctoni e meglio adattati a temperature più calde e di conseguenza si osserva un cambiamento della struttura delle comunità con una potenziale perdita di biodiversità.

Per valutare gli effetti del cambiamento climatico e creare dei piani di mitigazione e adattamento, è stato sviluppato il progetto MPA Engage ("Engaging Mediterranean key actors in Ecosystems Approach to manage Marine Protected Areas to face Climate Change"), un progetto diretto dal CSIC-Spagna che coinvolge sei diversi partner europei e una rete di MPA (Marine Protected Areas) che insieme ai ricercatori cercano di trovare soluzioni per mitigare gli effetti del cambiamento climatico.

Grazie a questo progetto si sono creati 11 protocolli per il monitoraggio di diverse specie d'interesse che rappresentano degli ottimi indicatori dello stato di salute dei mari. Tre di questi protocolli sono stati implementati nell'ambito

di questa tesi e sono: il censimento visivo dei pesci (Fish Visual Census-FVC), il monitoraggio delle popolazioni di ricci marini (Sea Urchins Populations - URCH) e rapida valutazione dello stato di conservazione di *Pinna nobilis* (Fast Assessment *Pinna nobilis* Conservation Status-FAP).

I risultati ottenuti dalla realizzazione del protocollo FVC hanno dimostrato che finora non ci sono effetti della "meridionalizzazione" in atto nell'area di studio poiché sono state rilevate solo specie autoctone della zona.

Il protocollo URCH ha rilevato un numero ridotto di *Paracentrotus lividus* e *Arbacia lixula* mentre la maggioranza di individui trovati e misurati erano *Sphaerechinus granularis.* È importante notare che non sono stati trovati individui con la taglia minore di 4 cm.

L'implementazione del protocollo FAP ha rilevato solo diversi esemplari di *Pinna nobilis* morte. Tutte erano ancora ancorate nel substrato con la conchiglia integra o minimamente danneggiata e per questa ragione si ritiene che la morte di questi esemplari sia avvenuta relativamente di recente. *Pinna nobilis* è uno dei più grandi bivalvi del Mediterraneo che rischia l'estinzione se il trend di mortalità continua così velocemente.

1. INTRODUCTION

Climate change (CC) is becoming one of the most important issues at a global scale. Human activities and the high input of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) caused an increase of the water temperature ultimately affecting the ocean dynamics at a global scale. Winters are becoming progressively warmer determining the ice melting with the consequent increase of the sea levels rise estimated in with an estimated 0.5-1.5 m in a span of 100 years. Moreover, more frequent heatwaves are becoming more frequent influencing the health status of living organisms experiencing mass mortality events or bleaching. On the other hand, high concentrations of atmospheric CO_2 are the main cause of surface water acidification, and a decrease in the capability of the oceans to store CO_2 and and ultimate effect on the global biogeochemical cycle of carbon (IPCC, 2021). One of the most affected and vulnerable regions in the world is the Mediterranean basin, which is rapidly getting warmer at a rate 20% faster than the world's average. This temperature rise is further favored by the basin's geomorphology of a semi-enclosed sea with negative water balance due to an excess of evaporation over precipitation and river runoff barely compensated by the inflow of Atlantic water (Pastor et al., 2020).

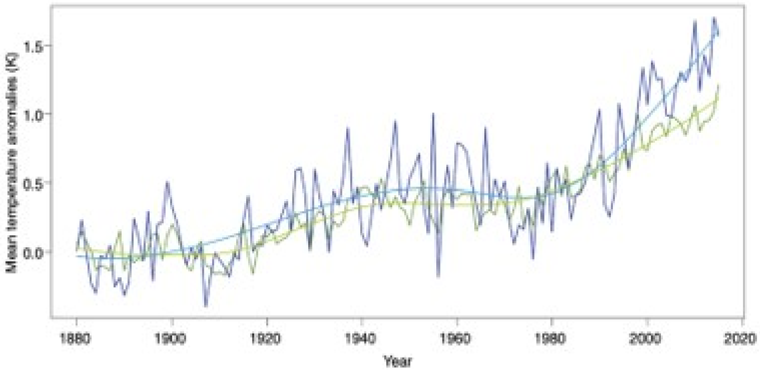


Fig. 1 Depiction of historic warming of the atmosphere on a global scale (green) and in the Mediterranean basin (blue). (Cramer et al. 2018)

Higher temperatures were recorded during summer seasons with the highest peaks occurred during the summer of 2003 and 2010 (Kim et al., 2019). We are witnessing a sort of "tropicalization" of the Mediterranean i.e. the increased occurrence of warm-water biota. due to a combination of four possible causes: lessepsian migrations, Atlantic influx, climate warming and anthropogenic action (Bianchi et al., 2003). Along with the tropicalization the Adriatic Sea is affected by “meridionalization”, an event where many thermophilic species such as *Sparisoma cretense, Sphyraena viridensis* and many others are expanding northwards (Azzurro 2008). Climate change is redistributing species across the Mediterranean basin and across the globe including marine invaders with warm-adapted fishes that are expanding across the basin (Azzurro et al., 2019). These types of organisms benefit immensely from these new climate conditions and adapt very quickly (D'Amen et al., 2019). Climate warming might favor warm-adapted species over cold-adapted species at the same site (Parmesan & Yohe 2003). Higher temperatures and the increase of a warm-water species may act synergistically causing a cold-water species to relocate to a less preferred habitat within the same thermal environment and alter its behavioral activity (Millazzo et al., 2013). The Adriatic Sea is a peculiar area showing a strong latitudinal gradient with low winter temperatures in the north and hot summers in the south (Civitarese et al., 2010) and local information on the dynamic of thermophilic species is scarce. The North Adriatic Sea is one of the coldest sectors of the Mediterranean Sea with biological characteristics resembling a boreal area, nevertheless this area is experiencing the abovementioned meridionalization process (Azzurro 2008).

Under current conditions of unfavorable thermal conditions several species will experience a decline in abundance, while at the same time, alien species will continue to propagate, as many of them have higher thermal tolerances (Corrales et al., 2018). This warm species migration was already noted in the southern Croatian waters (Dragičević et al., 2021).

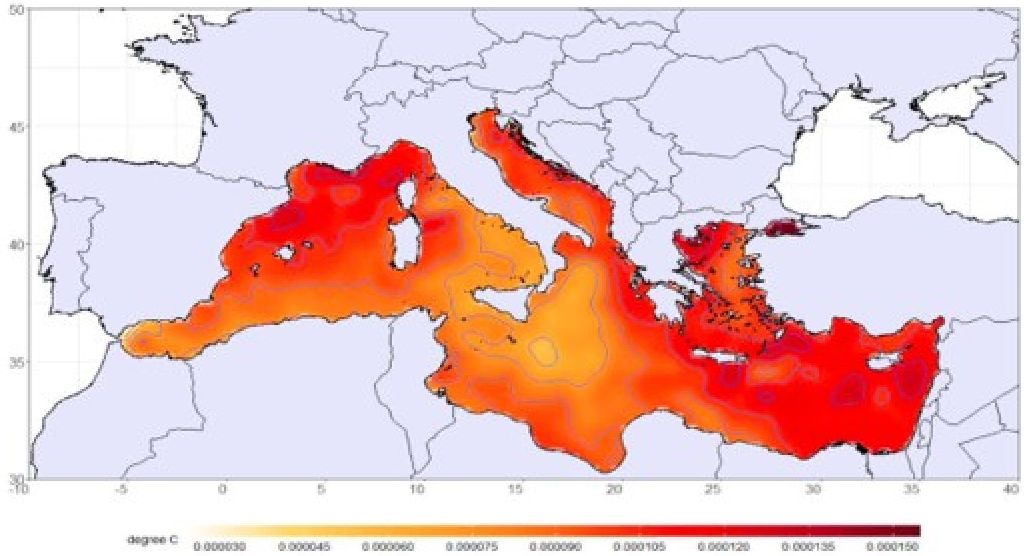


Fig. 2 Daily warming trend in the Mediterranean basin from 1982 to 2019. Lower warming values are found in the southern half of the Tyrrhenian sea and southern Italy, while higher values are located in the northern half of the western basin and large parts of the Levantine basin (Pastor et al., 2020).

Apart from the shift in native species distribution and the spread of alien species, climate change in the Mediterranean Sea is responsible for the following additional impacts: frequent jellyfish population and harmful algal blooms, reproductive events with a shifted phenology, the occurrence of unprecedented mass mortalities events, and a general detrimental impact on the food webs (Corrales et al., 2018, Cramer et al., 2018, Garrabou et al., 2019).

European union saw these climate changes as an important issue that needed immediate action and, in this sense, different European programs have been funded to monitor the risks related to climate change, to mitigate these risks and to develop specific adaptation plans. Marine Protected Areas (MPAs) play a crucial role in these programs being at the frontline for adaptation to climate change in the Mediterranean Sea through nature-based solutions (i.e. actions to protect, sustainably manage and restore natural or modified ecosystems providing economic, social and environmental benefits).

1.1. MPA ENGAGE

The Interreg MED project MPA Engage ("Engaging Mediterranean key actors in Ecosystems Approach to manage Marine Protected Areas to face Climate Change") (https://mpa-engage.interreg-med.eu/) brings together a network of MPAs (from seven European countries namely, Spain, France, Italy, Croatia, Greece and Albania), marine scientists, conservation and management practitioners promoting MPAs as nature-based solutions for the implementation of adaptation and mitigation actions to confront the effects of CC. This project capitalizes the results of a previous Interreg MED project MPA ADAPT that developed 5 monitoring protocols (See Box 1, in Bold) to early detect the effects of CC relying on a participatory approach and a direct involvement of the citizen science for the data collection. Scientists and researchers involved in the MPA Engage project developed other 6 new protocols to monitor and early detect the effects of CC (See Box 1).

BOX. 1

LIST OF PROTOCOLS OF THE MPA Engage PROJECT:

- **MONITORING TEMPERATURE CONDITIONS**
- **ASSESSMENT AND MONITORING OF MASS MORTALITY**
- **LEK 1: EXPLORING LOCAL ECOLOGICAL KNOWLEDGE TO RECONSTRUCT HISTORICAL CHANGES**
- **LEK 2: EXPLORING LOCAL ECOLOGICAL KNOWLEDGE FOR PERIODICAL MONITORING**
- **FVC: FISH VISUAL CENSUS OF CLIMATE CHANGE INDICATORS**
- LEK 3: MASS MORTALITIES
- BRD: BENTHIC SPECIES RAPID DETECTION
- URCH: SEA URCHINS POPULATIONS MONITORING
- POFA: FAST ASSESSMENT ON MEADOWS CONSERVATION
- FAP: FAST ASSESSMENT PINNA NOBILIS CONSERVATION STATUS
- SFM: PHOTOGRAMMETRY AS MONITORING TOOL FOR BENTHIC HABITATS STRUCTURE AND DYNAMICS

Citizen Science (CS) is intended as the participation of general public in scientific processes that generates new knowledge and understanding enabling participants to make a direct contribution to research and valuable data collection, but also representing an opportunity for education, local community engagement and support (Lucrezi et al. 2018). The most obvious advantage of citizen science lies in the contribution to scientific research through the collection of data, which volunteers can guarantee on large spatial and/or temporal scales. Moreover, CS represent a precious and novel source of information in support to local, regional, and EU environment-related policies.

The Management Bodies of MPAs are often partners or primary actors in CS projects in which citizens are involved in environmental monitoring actions and/or in the collection of data related, for example, to the presence and distribution of threatened plant and animal species.

In the framework of the MPA Engage project, three out of the eleven protocols have been selected to be performed through CS activities, specifically the Mass Mortality assessment and monitoring, the FVC, and the POFA. Citizen scientists that express their willingness to collaborate with the project, receive specific theoretical and practical trainings aiming at explaining the importance of the protocol application to evaluate the impacts of CC, the methods for the data collection and to share the data in online dedicated repositories. *Observadores del Mar* is an online repository dedicated to citizen science where anyone can upload data collected during field activities. These data are subsequently validated and confirmed by a team of more than 50 researchers.

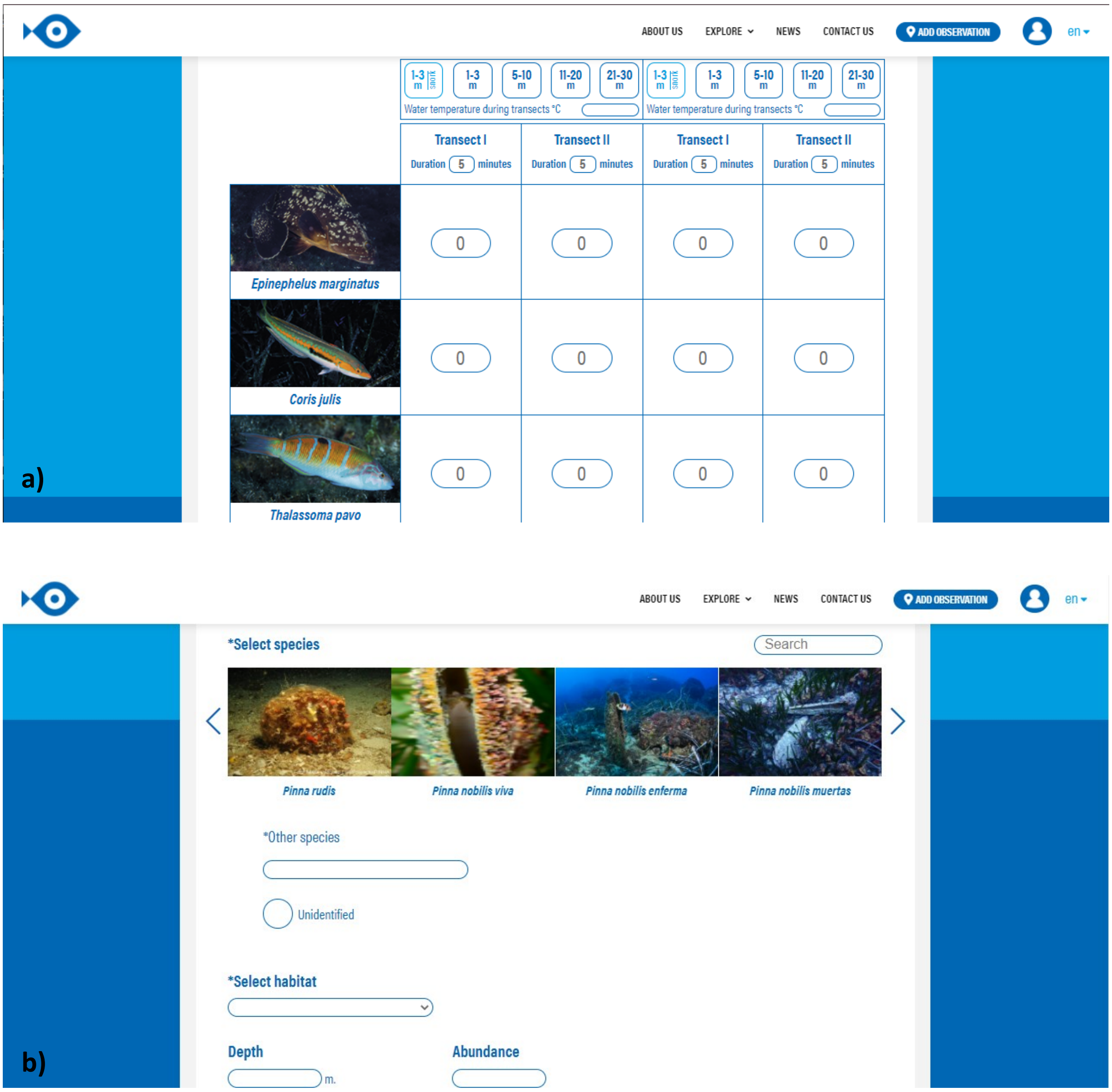


Fig. 3 Online repository *Observadores del Mar*. a) Webpage dedicated to Fish visual census data upload. b) Webpage dedicated to *Pinna nobilis* data upload (Source: observadoresdelmar.es).

The categories of citizens targeted by the project are mainly diving centers and recreational divers together with fishermen.

The monitoring protocols have been designed to be easily applied (after the training) also by non-scientists and since most of them are focused on charismatic species (e.g. gorgonians) and on species of interests (e.g. fishes), they are usually found attracting for either divers and fishers.

Divers are already somehow capable of distinguishing the fish species targeted on the protocol and gorgonians are already the focus of several underwater photographers. If an accurate theoretical and practical scientific training is provided, divers may become a powerful ally to detect and report the effects of climate change. In this sense, it is extremely useful to rely on the collaboration of fishermen as well since they are working at the sea almost every day, therefore they are among the first ones to detect the shift in fish populations by noticing the presence or absence of certain fish species. Having a well-constructed network of citizen scientists (both recreational divers and fishermen) can provide a constant influx of information useful for future predictions and for the development of mitigation and adaptation strategies.

1.2. AIM OF THE THESIS

This thesis focuses on three monitoring protocols that were implemented during a period of five months, from July to October 2021 along the Croatian coast. The three protocols that were applied for the data collection were: Fish Visual

Census (FVC), Sea urchins Populations (URCH), and Fast Assessment *Pinna nobilis* Conservation Status (FAP). There was an attempt to implement the Fast Assessment on Meadows Conservation (POFA) but it was unsuccessful. Most of these protocols were conducted with SCUBA diving at a maximum depth of 10 m. Fish visual census in snorkeling was performed once, following the exact same methods used with SCUBA diving (see below).

These three protocols were chosen primarily for the presence of the target organisms in the study area. Fish visual census is already greatly used in citizen science because it can be performed even during snorkeling activities (no need of a scuba diving certificate).

Studying the population structure of sea urchins is important because they contribute to the formation of "barrens" by grazing macroalgal covers and contributing to a loss of biodiversity. The lack of their natural predators is contributing greatly to their population growth (Sala 1997).

Sea urchins such as *Paracentrotus lividus* pose a significant influence on the structure and dynamics of algal assemblages. It has been demonstrated that the exclusion of sea urchins produced about 25% increase in non-crustose algal cover (Palacin et al., 1998). Moreover, the change in the species composition of sea urchin populations towards thermophilic species (i.e. *Arbacia lixula*) is an early sign of the undergoing effects of CC.

The noble pen shell protocol is mostly interested in finding and measuring alive individuals or in the detection of any sign of recovery of this species after the spreading of a parasite that caused a mass mortality event in the entire Mediterranean basin.

An additional goal of my thesis was the involvement of some of the local diving centers in the data collection and in the implementation of the MPA Engage protocols. For this purpose, I introduced to the diving center staff, the project and its overall importance, also explaining the methods of the monitoring protocols.

2. MATERIALS AND METHODS

2.1. STUDY AREA

This research was conducted along the Croatian coast but mostly concentrated on the northern part which includes Kvarner bay and the Istrian peninsula (Fig. 4). The northern part of the Adriatic Sea is characterized by shallow rocky shores, and moderately cold waters even during summer especially in Smokvica, near the city of Novi Vinodolski. The winter Bora is responsible for the modification of conditions in the area and for the generation of a cold, dense water mass (Orlić et al., 1991).

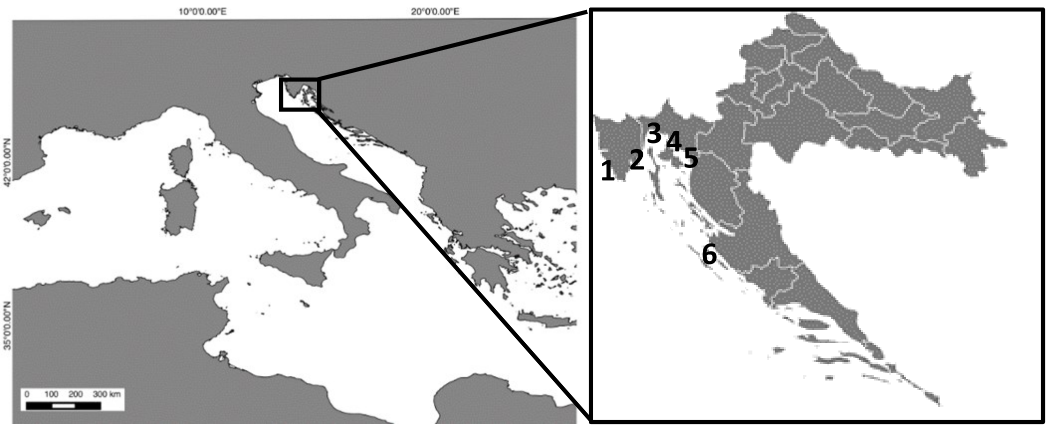


Fig. 4 Map of the Mediterranean basin with the insert of the study area (Croatia) and all the monitored sites: Vrsar (1), cove Kala (2), Rijeka (3), Novi Vinodolski (4), Smokvica (5) and island of Prvić (6). Photo credit: simplemaps.com

The Istrian peninsula, especially the western part, is a bit warmer. The areas of Rijeka, the Velebit channel and more specifically the area around Novi Vinodolski, in which most of the field activities were concentrated, tend to be colder because of cold currents, wellsprings and strong winds during winter.

2.2. FIELDWORK ACTIVITIES

Diving activities were performed from July to November 2021. Most dives regarding the fish visual census and the sea urchin protocol were performed in the bay of Rijeka but also in the Velebit channel, more specifically the area around Novi Vinodolski and surrounding cities (Fig. 4 and Table 1). An attempt to conduct the POFA protocol was made in the Šibensko-kninska county, but it ended up without success. In the eastern part of the Istrian peninsula in the Ližnjan area some shoots where present but not enough to extract sufficient data.

Table 1 List of all the locations where the protocols were performed during the sampling period between 30.07.2021 and 20.11.2021. Protocol abbreviation: FVC (Fish Visual Census) URCH (Sea Urchins Populations) and FAP (Fast Assessment *Pinna nobilis* Conservation Status). The "s" indicates FVC protocol performed with snorkeling.

DATE	SITE	LATITUDE	LONGITUDE	PROTOCOL	TEMPERATURE (°C)	DEPTH (m)
30.07.2021	Vrsar, Croatia	45,156223 N	13,606338 E	FVC	28	5-10
28.08.2021	Rijeka, Croatia	45,340382 N	14,372141 E	FCV	22	5-10
21.09.2021	Novi Vinodolski, Croatia	45,123153 N	14,792732 E	FVC	19	5-10
21.09.2021	Novi Vinodolski, Croatia	45,129886 N	14,769846 E	FVC	18	5-10
22.09.2021	Smokvica, Croatia	45,086028 N	14,849039 E	FVC	19	5-10
22.09.2021	Smokvica, Croatia	45,085608 N	14,850972 E	FVC	18	3-10
27.09.2021	Prvić island, Croatia	43,736192 N	15,792397 E	FVC	21	1-3 s
14.10.2021	Kala cove, Croatia	44,852617 N	13,984084 E	FVC	18	5-10
16.10.2021	Rijeka, Croatia	45,340382 N	14,372141 E	FVC	17	5-10
16.10.2021	Rijeka, Croatia	45,340382 N	14,372141 E	FVC	18	1-3
17.10.2021	Rijeka, Croatia	45,340092 N	14,373792 E	FVC	17	5-10
20.11.2021	Vrsar, Croatia	45,156223 N	13,606338 E	FVC	15	1-3 s
22.09.2021	Smokvica, Croatia	45,086028 N	14,849039 E	URCH	18	3-10
22.09.2021	Smokvica, Croatia	45,085608 N	14,850972 E	URCH	18	3-10
08.10.2021	Rijeka, Croatia	45,351844 N	14,333011 E	URCH	17	3-10
10.10.2021	Bakarac, Croatia	45,279779 N	14,574156 E	FAP	16	5-7
20.11.2021	Vrsar, Croatia	45,156223 N	13,606338 E	FAP	15	3-8

The usual SCUBA recreational equipment was used for every dive. Two types of diving protocols were used for the monitoring: one with scuba tanks and one without (snorkeling). Mares Puck Pro dive computer was used to measure the depth and temperature in each dive site.

2.3. PROTOCOLS DESCRIPTION

Each of the monitoring performed requires specific material to be implemented and follows a precise protocol.

FISH VISUAL CENSUS (FVC)

Certain types of fish species are used as reliable indicators of climate change. These species are chosen following scientific studies and are: *Epinephelus marginatus* (Dusky grouper), *Coris julis* (Mediterranean rainbow wrasse), *Thalassoma pavo* (Ornate wrasse), *Sarpa salpa* (Salema), *Serranus scriba* (Painted comber), *Serranus cabrilla (*Comber), *Sparisoma cretense* (Parrotfish), *Epinephelus costae (*Goldblotch grouper), *Sphyraena viridiensis* (Mediterranean barracuda), *Sciaena umbra* (Brown meagre), *Siganus* spp *(*Rabbitfishes) and *Fistularia commersonii* (Cometfish). A maximum of four other species can be added according to monitoring needs and to the characteristics of each location.

 *Epinephelus marginatus*	 *Sarpa salpa*
 *Sparisoma cretense* ♀	 *Sparisoma cretense* ♂

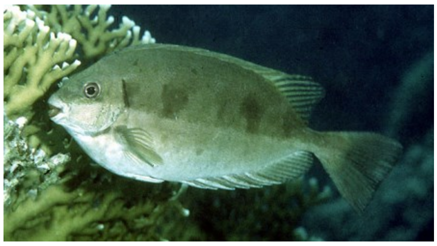
Serranus scriba

Serranus cabrilla

Epinephelus costae

Sciaena umbra

Fistularia commersonii



Siganus sp

Fig. 5 Pictures of all the fish species that could be used as a reliable indicator of climate change. Photo credit: fishbase.de, marinespecies.org

The target habitats for this protocol implementation are rocky bottoms with moderate slope. This protocol can be applied in different bathymetrical ranges: 1-3 m, 5-10 m, 11-20 m, 21-30 m. This protocol is suitable also for snorkelers and can be applied at 1-3 m of depth.

At least 3 permanent locations separated by a minimal distance of about 0.5 Km must be selected. At each location and for each depth layer, each diver must perform 4 consecutive transects of 50 m of length and 5 m of width swimming very slowly underwater (speed about 10 m/minute) for 5 minutes. All the species and individuals observed must be counted in the sampling area (50x5m). Fishes smaller than 2 cm should not be counted. The observation should be reported in a pre-printed (with the list of target species) underwater board (Fig. 6). A waterproof watch and a dive computer/thermometer are also needed to measure the 5 minutes intervals and to measure water temperature.

Fig. 6 Board to collect FVC data underwater

SEA URCHINS' POPULATIONS (URCH)

Human activities (such as overfishing) but also climate change-related effects have changed the structure of rocky bottoms communities. These changes have also affected the structure of sea urchin populations which increase in number due to the reduction or the lack of predators. Consequently, the grazing on the forests *Cystoseira* has increased as well bringing to the formation, in some habitats of the so-called 'barrens', that are defined as marine deserts lacking in the structure and the associated biodiversity provided by the presence of *Cystoseira* forests. Indeed, *Cystoseira* forests are also important nurseries for some species of littoral fishes including species of commercial interest (Cheminée et al., 2012). Besides their importance of sustaining fish populations these forests are also crucial role in the structure and functioning of rocky benthic ecosystems. Monitoring the structure and abundance of sea urchin populations is therefore important to foresee future changes in the structure of benthic communities. Moreover, monitoring the species composition of sea urchin population can give insight of the undergoing effect of CC. As a point of fact, the most common sea urchin species in the shallow rocky reefs display a differential thermal affinity and foraging behaviour, *Paracentrotus lividus* is considered a “cold-water” species in contrast with *Arbacia lixula* that is a “warm-water” species.

The objective of the URCH protocol is to determine the structure and the dynamics of sea urchin populations in the shallow rocky habitats taking into account the presence of two target species *Paracentrotus lividus* and *Arbacia lixula*, although other sea urchin species (such as *Sphaerechinus granularis*) are also considered (Fig. 7). The sea urchin protocol can be performed together with fish visual census because of the invasion of *Siganus* spp. that is also causing the transition to impoverished barrens.

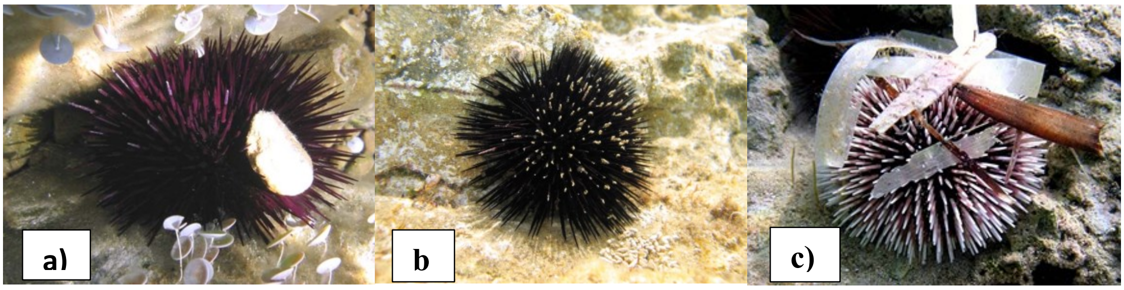


Fig. 7 a) *Paracentrotus lividus,* b) *Arbacia lixula* and c) *Sphaerechinus granularis*
Photo credit: marinespecies.org, sealifebase.ca

The material needed to implement this protocol is a caliper or ruler for measuring the urchins, a slate, a pencil, a measuring tape and gloves with a thickness of at least 3 mm.

The abundance and population structure of *Paracentrotus lividus* and *Arbacia lixula* are determined by SCUBA diving along three parallel horizontal transects (50 m×1 m each) between 3 and 10 m depth at each selected study site. Transects are divided into five 10 m^2 subtransects. In each transect, *P. lividus* and *A. lixula* >1 cm in diameter are counted and their diameters (test without spines) are measured with a caliper or ruler. Once the measurement of 100 urchins has been accomplished, only the abundance should be noted. To obtain the size structure of the population, all the sea-urchins measured are grouped in 6 categories: 1-2 cm=1, 2-3 cm=2, 3-4 cm=3, 4-5 cm=4, 5-6 cm=5, >6 cm=6.

FAST ASSESSMENT *Pinna nobilis* CONSERVATION STATUS (FAP)

Pinna nobilis is the largest endemic Mediterranean bivalve. Its populations reduced drastically in the last 40 years for various reasons such as trawling, fishing, anchoring and decreasing of *Posidonia* meadows, and climate change-related effects. One of the most impactful causes of their rapid decline is the presence of the parasite *Haplosporidium pinnae* identified for the first time in Spain in late 2016 together with a bacterial infection with a *Mycobacterium* sp. strain similar to the human mycobacterium *M. sherrissii* (Čižmek et al., 2020). The parasite spreads rapidly causing in some places a 100% mortality.

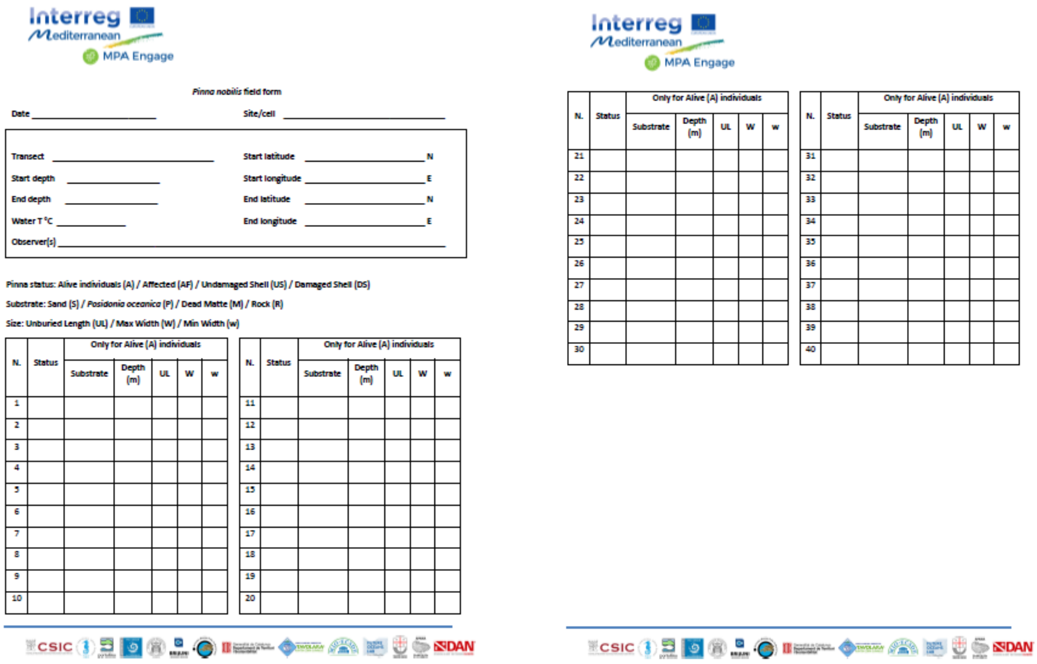
P. nobilis is mostly used for its byssus, flesh but it's most notable for large shells that are used as souvenirs. In recent years its populations started declining rapidly for the above-mentioned reasons. Because of these threats, this species is placed in the IUCN Red List under the category 'Critically Endangered'. Various experts are trying to preserve noble pen shells because of their great ecological importance. This species can filter a high quantity of water (Trigos et al., 2014) also providing an excellent substrate for colonization of numerous flora and fauna species acting as an ecosystem engineering species (Basso et al., 2015).

The objective of this protocol is to determine the abundance, the size structure and the health status of *Pinna nobilis* populations.

Fig. 8 Noble pen shell (*Pinna nobilis*). Photo credit: Borut Furlan

Monitoring should be done during late summer/early autumn. Three transects (100 m long and 6 m wide) should be performed. All individuals observed inside the transects should be counted and measured. Once an individual of *P. nobilis* is recorded, the health status should be reported as well: alive, affected by infection (still alive but with slow valves-closure reaction when disturbed), recently dead (buried parts with no epibiosis or byssus still abundant), not recently dead individuals (broken shells and evident epibiosis of buried parts) or dead individuals found still buried into the substrate

Each individual should be carefully measured. When measuring an individual, the diver should record these measurements: unburied length (UL) for alive individuals or total length for death individuals; maximum width (W); minimum width (w). The material needed to perform this protocol is: an underwater compass, a GPS, a buoy, a deco buoy, a metric-tape, a plastic sheet (Fig. 9), a pencil, a rule or frame with subquadrats and an underwater camera with housing and electronic strobes or focus providing continuous lighting.



Interreg Mediterranean
MPA Engage

Pinna nobilis field form

Date ____________ Site/cell ____________

Transect ____________ Start latitude ____________ N
Start depth ____________ Start longitude ____________ E
End depth ____________ End latitude ____________ N
Water T °C ____________ End longitude ____________ E
Observer(s) ____________

Pinna status: Alive individuals (A) / Affected (AF) / Undamaged Shell (US) / Damaged Shell (DS)
Substrate: Sand (S) / *Posidonia oceanica* (P) / Dead Matte (M) / Rock (R)
Size: Unburied Length (UL) / Max Width (W) / Min Width (w)

N.	Status	Only for Alive (A) individuals				
		Substrate	Depth (m)	UL	W	w
1						
2						
3						
4						
5						
6						
7						
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10						
11						
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32						
33						
34						
35						
36						
37						
38						
39						
40						

Fig. 9 Board to collect FAP data underwater

2.4. DATA TREATMENT

All the collected data were reported and elaborated in Excel spreadsheets provided by the head scientists of the MPA Engage project.

FVC seasonal data were also analyzed with *t-test*, using Microsoft Excel to detect any significant difference in the abundance of the species recorded at the same site in August and October.

Data obtained from FVC and FAP protocols were further uploaded on the website platform *Observadoresdelmar.es*.

3. RESULTS

3.1. FISH VISUAL CENSUS (FVC)

FVC was performed 12 times. Of the eight target species of the protocol, three were commonly recorded, namely: *Coris julis, Sarpa salpa* and *Serranus scriba.* In all the sites monitored, *S. salpa* was the most abundant species (Fig. 10a). The highest number of *C. julis* were recorded in Kantrida-Rijeka on the 28th August; a total of with 23 individuals where recorded and the water temperature at this site was 22°C. Only 1 individual was counted in two monitoring in Smokvica in September, cove Kala and Kantrida in October; the water temperature was 18°C, 19°C, 18°C and 17°C, respectively. As for *C. julis*, the highest number of *S. salpa* (64 individuals) were recorded in Kantrida-Rijeka while the lowest amount counted was 2 individuals in Smokvica. For *S. scriba* the highest number of individuals counted was 9 in the island Prvić (in September, water temperature: 21°C). In this site, the monitoring was implemented by the staff of the diving club "Roniti se mora" after receiving my training. For *S. scriba*, the places with the least amount (1 individual recorded) were Vrsar, Novi Vinodolski, Smokvica and Kantrida, Rijeka.

For the three recorded species, most of the individuals were found at 5-10 m depth. The only exception was the high abundance of *S. scriba* at the island Prvić site at 1-3m depth. Regardless the location investigated, the average density of the 3 recorded species was 5,7±4,6 individuals/250 m^2; 8,4±15,3 individuals/250 m^2; 2,3±1,9 individuals/250 m^2 for *C. julis*, *S. salpa*, and *S. scriba*, respectively.

The result of the *t-test* analysis did not show significant difference (p-value=0,31) in the abundance of the three species among season (August-October) in the same site. This result was obtained also considering the the abundance of the three species separately (*C. julis*, p-value= 0,32; *S. salpa*, p-value=0,48; *S. scriba*, p-value=0,58).

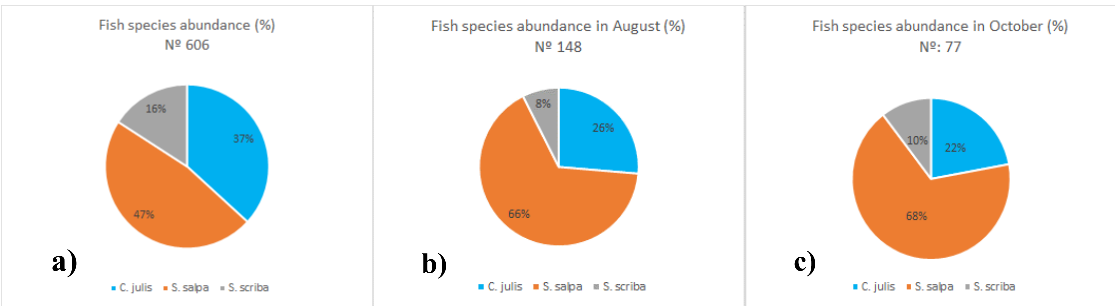
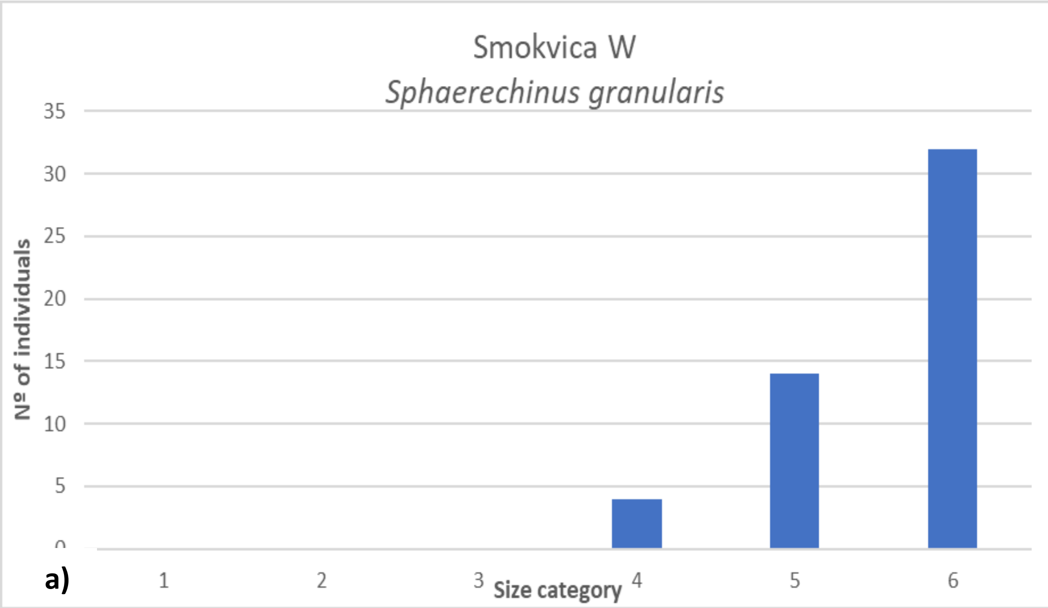


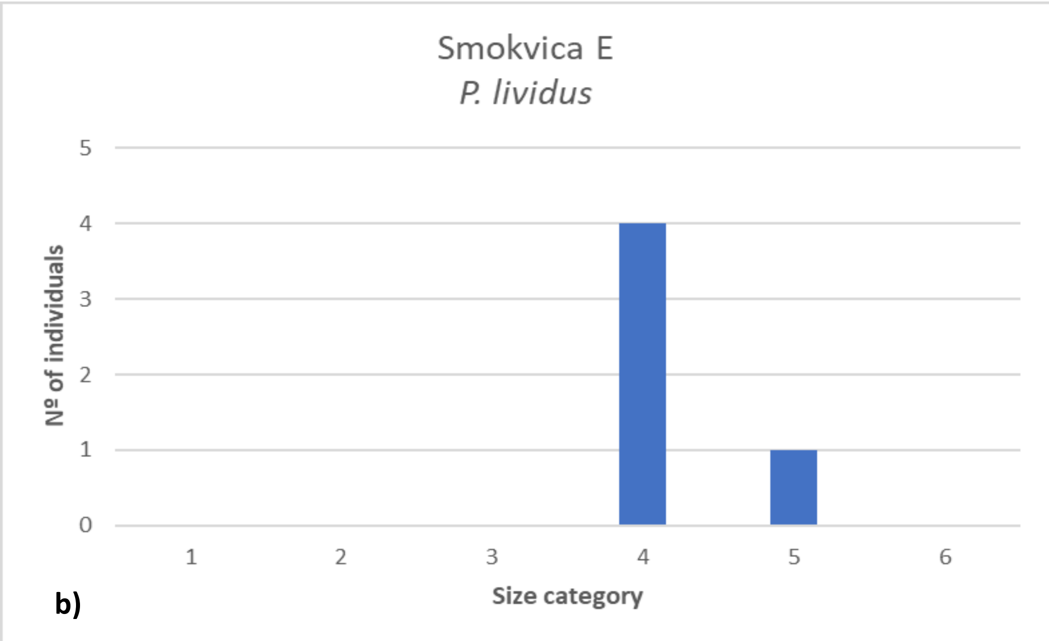
Fig. 10 Pie charts representing a) percentage of individuals for each species recorded in all the dive sites where the protocol was implemented (Total number of sites = 12; total number of fish counted= 606). b) and c) dives where most of the 3 noted species were counted at 28.08.2021 and 17.10.2021.

3.2. SEA URCHIN POPULATIONS (URCH)

URCH was performed 3 times at a depth range of 3-8 m. Two were performed in two different sites in Smokvica (Smokvica E and Smokvica W) whilst only one was performed in Rijeka. Only one individual of *Arbacia lixula* was found in Smokvica E while 5 individuals of *Paracentrotus lividus* were found at the same sampling site. The size of *Arbacia lixula* was 4 cm and assigned to the size category 4. Of the five *Paracentrotus lividus* individuals, four had a size of 4 cm and one was 5 cm. They were assigned to the categories 4 and 5, respectively. The most abundant species was *Sphaerechinus granularis* with 71 individuals recorded. Most of the encountered *S. granularis* were larger than 6 cm. The largest *S. granularis* had a size of 12 cm and was assigned to the category 6 (Fig 11 a-b). All together the average size of the recorded *S. granularis* was 7,33±1,90 cm.

The average density of the *S. granularis* recorded was also measured in the monitored locations: the site Smokvica showed the highest density with 1,7±2,4 individuals/10m^2, followed by Rijeka in which the density was 1,6±1,9 individuals/10m^2 (Fig. 11c).


Smokvica W
Sphaerechinus granularis
35
30
25
20
15
10
5
Nº of individuals
a)
1
2
3
Size category
4
5
6


Smokvica E
P. lividus
5
4
3
2
1
0
Nº of individuals
1
2
3
4
5
6
Size category
b)

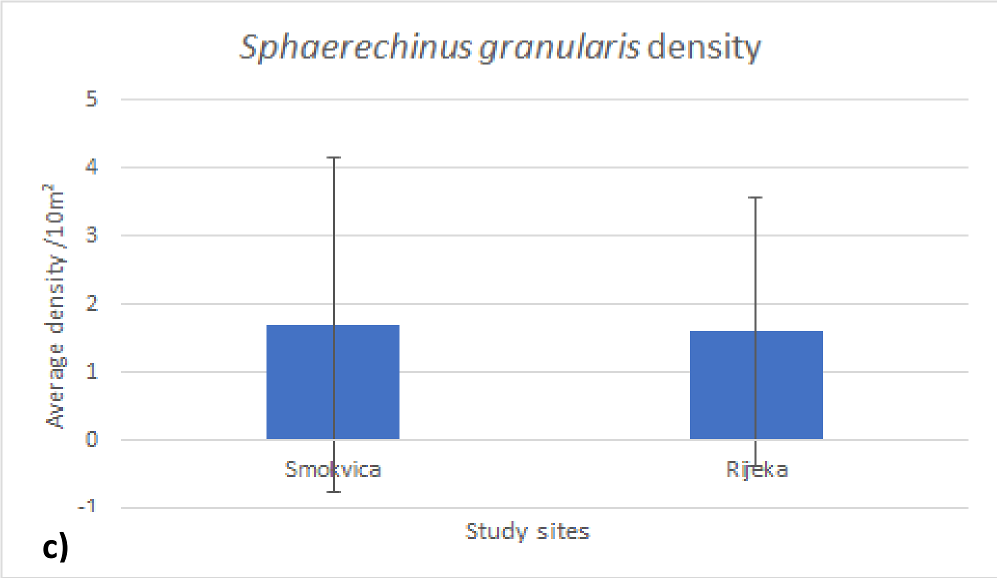


Fig. 11 Bar charts representing the population size structure of a) *Sphaerechinus granularis* and b) *Paracentrotus lividus;* c) Bar chart representing the average density of *Sphaerechinus granularis* and SD in 2 of the sites monitored

3.3. FAST ASSESSMENT *Pinna nobilis* CONSERVATION STATUS (FAP)

FAP was performed twice, one in Bakarac and one in Vrsar. Both the sites showed a sandy bottom substrate, with a depth range of 3-7m. In Bakarac only one noble pen shell was found. It was dead, with an undamaged shell. Measurements for the unburied lenght (UL), maximum width (W) and minimum width (w) were 22cm, 17cm, 7cm, respectively. A total of 20 noble pen shells were found in Vrsar. All of them were dead; 80% of them showed an undamaged shell whilst 20% showed damaged shells.

Table 2
Pinna nobilis measurements collected in Vrsar, Croatia. UL= Unburied Lenght, W= Maximum Width and w= Minimum Width. The symbol (±) indicates standard deviation.

Pinna nobilis	UL ± SD (cm)	W ± SD (cm)	w ± SD (cm)
Nº =20	26±5	15±3	10±2

4. DISCUSSION

Climate change is already affecting and reshaping the structure of Mediterranean communities facilitating thermophilic and thermo-tolerant species that are widening their geographical distribution. In particular, a poleward expansion (or latitudinal shift) of many species has been indicated as a response to the increase of sea water temperature. This northward expansion may cause severe effects on ecosystems causing local extinctions in sensitive areas (Cheung et al., 2009). Many alterations are already happening, and others are likely to occur highlighting the urgency to collect reference data on the current status of natural communities (Azzurro et al., 2013). In absence of historical data, present-day data may serve to set the baseline to evaluate future changes and scenarios (Sala et al., 2012). In this sense, the purpose of my thesis, realized in the framework of the MPA Engage project, was to determine the overall effects of climate change on various target organisms and to highlight the potential shifts towards thermophilic species in the Croatian coast. Three different protocols of the MPA Engage project were applied, specifically the Fish Visual Census (FVC), the assessment of the population structure of sea urchin (URCH) and the assessment of the health status of the sea pen shell

Pinna nobilis (FAP). An attempt to implement a fourth protocol to evaluate the health status of *Posidonia oceanica* meadow was also made without success.

The results of the FVC highlighted that the only 3 species recorded (out of the 8 monitored with this protocol) were autochthonous species of the Mediterranean Sea. The presence of these species during all the sampling period and with no significant difference in the abundance of these species, confirm that these species are excellent bioindicators (Piazzi et al., 2012). The density calculated in this study for the 3 fish species in the bathymetrical range 5-10 m is in accordance with previous results from the south of Italy (Azzurro et al., 2013). Compared with a previous study performed in the Prvić island, the sea water temperature recorded during the monitoring activities was slightly higher in July and August and in line from September to November (Novosel et al., 2004), although a continuous monitoring of the temperature is needed to confirm this results in the long-term. The lack of records of thermophilic species might suggest that the meridionalization of the northern part of the Adriatic Sea might temporally lag with respect to the Tyrrhenian Sea (Sbragaglia et al., 2020). As a point of fact, this process is already undergoing in southern Croatia, with one specimen of lionfish spotted near Komiža, Vis on August 13th 2021. Lionfish (*Pterois miles*) is a Lessepsian migrant that was recorded near Cyprus and recently also in the Ionian and Adriatic seas. Up to now, this species has not spread towards the norther parts of the Adriatic Sea as the water tend to be much colder still representing an obstacle for its further invasion (Dragičević et al., 2021).

About the URCH protocol, the results shows that the most dominant species was *Sphaerechinus granularis* with only few specimens of *Arbacia lixula* and *Paracentrotus lividus* recorded. A wide range of reasons could be attributed for the lack of these two species: the juveniles of *Paracentrotus lividus* are highly predated by different species of fishes such as *Coris julis* and *Thalassoma pavo* (Guidetti, 2004), the first of which present in the study area. It is also important to consider the type of habitats where the monitoring where performed: in Rijeka and Smokvica the recorded sea urchin (mostly *S. granularis*) inhabited a rather horizontal substrata with limited or absent slope. Contrarily, *Arbacia lixula* is generally more abundant on vertical substrata (Regis, 1978) and at shallow depths (Ballesteros, 1981). The size of the measured *S. granularis* was never less than 4 cm, with the majority measuring more than 6 cm. A study conducted in Greece with *S. granularis* highlighted that this species reaches sexual maturity at around 5 cm in diameter and this data was also confirmed by histological analysis of its reproductive biology (Vafidis et al., 2020). According to this, we can assume that almost all the measured *S. granularis*, were adult individuals. The depth at which the sea urchins were recorded and measured might also explain their larger sizes. Indeed, my sampling activities concentrated at 8 m of depth and the juveniles usually inhabits shallower depth than the adults as reported by a previous study from the Balearic Island (Ballesteros 1981). Further investigations are needed to confirm this distinct bathymetrical distribution among juveniles and adults also in the Croatian coast. The densities of *S. granularis* observed in the sites of Rijeka and Smokvica are one order of magnitude higher than the value reported by Vafidis et al., (2020) in the Aegean Sea. However, it is known that the density of this species may significantly vary among different areas of the Mediterranean Sea related with the different environmental conditions (Soualili et al., 1999). The eutrophic conditions (Barausse et al., 2009) of the Adriatic Sea might therefore explain the higher density recorded in the Croatian coast.

About the FAP protocol, my research confirmed the undergoing massive mortality of *Pinna nobilis* in the Mediterranean Sea with the lack of recording of alive specimens. A high mortality rate caused by *Haplosporidium* in the rest of the Mediterranean basin might be also the cause in the Croatian study sites. Any visible signs of juveniles' resettlement or recovery was evident. The Ruđer Bošković Institute from Rovinj attempted to perform the technique of "caging" with a only surviving noble pen shell in the location Vrsar, but unfortunately, even this one succumbed to the disease before I was able to collect any data. High amount of undamaged and upright shells, together with the lack of epibionts on the shells might indicate that the mortality events were recent. A previous work from the Balearic Islands estimated an average growth rate for *Pinna nobilis* as high as 0.28 mm/day (Hendriks et al., 2012), showing a higher growth rate during the first month of life (up to 0.32 mm/day) which is directly influenced by the food availability, with food limitations causing a decrease in the growth rate. Considering these previous information, and being aware that the food availability in the Croatian area investigated might be different from the Balearic Islands, I can roughly estimate the age of the dead specimens recorded during my monitoring to be around 2 years of age.

Other threats for the survival and potential recover of the populations of *Pinna nobilis* are related to illegal fishing practices which are still present despite the existence of severe punishment.

Fig. 12 *Pinna nobilis* illegal fishing. Photo credit: ansa.it

About the attempt to implement the POFA protocol, my search of various locations for *Posidonia* meadows was proven to be fairly difficult because locals tend to confuse *Posidonia oceanica* with *Zostera marina*, another species of seagrass. Upon arrival at the destination, after a few minutes of diving, I noticed immediately that it was a meadow of *Zostera marina* or in some cases even *Cymodocea nodosa*. Meadows are starting to decrease with every passing year due to habitat destruction, high water turbidity, pollution, coastal works, vessel mooring, competition with allochthonous invasive algae (*Caulerpa* sp.) and climatic variations. Most of reasonably healthy meadows can be found only in protected areas such as national parks and nature parks but except for protected areas, *Posidonia oceanica* is really hard to find especially in the Northern Adriatic, although some spots are still present in the islands Plavnik and Rab.

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