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Assessment and Monitoring proposal for infralittoral reefs: Final report of the Interreg Italy-Croatia project HABI

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Dr. sc. Silvija Kipson
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Obala hrvatskog narodnog preporoda 7/III, HR – 21000 Split

NARUČITELJ

Javna ustanova Rezervat Lokrum

Put od Bosanke 4, 20000, Dubrovnik

VODITELJ PROJEKTA I KONTROLE KVALITETE

mr.sc. Matea Špika, mag. biol.

TUMAČENJE PODATAKA I PRIPREMA IZVJEŠĆA

Dr. Silvija Kipson, marine biologist – external expert

PRIKUPLJANJE PODATAKA

Dr. Silvija Kipson, Mirko Belošević, Fedra Dokoza, mag.ing agr., Lukša Gleđ, Denis Grubišić, Sara Nikšić, M.Sc. in marine ecology and nature preservation, Matea Martinović, mag.ing.maricult., Matea Špika, M.Sc. Management of protected areas.

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Executive summary

This document reports on the results of infralittoral reef research carried out in the scope of the Interreg Italy-Croatia HABI project within the Natura 2000 site Lokrum HR4000017 in collaboration with the subcontracted NGO Sunce (Croatia). Data were collected during the field work carried out from June 26 until June 28 2024 at 3 sites around the Lokrum Island down to 11-12 m, a depth that marked habitat transition from infralittoral rocky reefs to *Posidonia oceanica* beds.

The main aim of the undertaken activities was to assess the current status of shallow infralittoral assemblages based on methods suitable for application by the staff of the Public Institution "Lokrum reserve" and trained volunteers (snorkelers and SCUBA divers) that are not necessarily marine biologists, as requested in specification of the service. The acquired data should set the baseline for future monitoring.

To accommodate aforementioned request and to account for general lack of information on habitat type 1170 Reefs within Natura 2000 site Lokrum as well as to consider limited time frame of the study, the underwater work performed by SCUBA diving included visual census to collect data for the assessment of the *MedSens* biotic index and hence evaluate current ecological status based on methods suitable for citizen science initiatives. In addition, fish visual census for climate change indicators was performed to establish the baseline on the ratio between temperate and more thermophilic species and to check for potential alien fish species. Moreover, where possible or applicable general observations were made on the status of organisms non-targeted by forecited protocols which may be indicative of potential disturbances as well as on the biodiversity and presence of rare and/or protected marine species.

During field study, 6 dives were performed for assessment of the *MedSens* biotic index and 3 dives for fish visual census. In total, 7 divers participated in the field activities.

In summary, *MedSens* index revealed contrasting results for mean sensitivity to physical and chemical pressures versus biological pressures on infralittoral reefs of the Lokrum Natura 2000 site. Whereas the high abundance of species sensitive to biological pressures would be indicative of good status around the Lokrum Island related to this type of pressures (though the abundance of the invasive algae *Caulerpa cylindracea* should be re-examined at its vegetation peak in late summer/early autumn to get a more reliable assessment of this pressure), the abundance of species tolerant to physical and chemical pressures is not necessarily related to poor environmental conditions. Whether species sensitive to physical and chemical pressures were historically present but were subsequently lost or they were never present on the Lokrum Island, remains to be further investigated. Moreover, considering the application of the *MedSens* index, increased amount of data contributes significantly to the robustness of the method, hence there is a need for increased observational effort in space and time within the Lokrum Natura 2000 site.

The fish visual census confirmed great abundance of thermophilic species. The ornate wrasse *Thalassoma pavo* was among the most abundant fish species, and besides this species, the other two thermophilic species included in the protocol were observed, i.e. the Mediterranean parrotfish *Sparisoma cretense* and the dusky grouper *Ephinephelus marginatus,* although only few individuals were recorded of the latter one. Based on this data, according to the calculated Tropicalization index (amounting to 2) the Lokrum Island is positioned into the warm Mediterranean.

Although other stressors (or their synergistic act) cannot be excluded, some sessile benthic species such as the sponge *Petrosia ficiformis* around the Lokrum Island exhibited partial mortality indicative of the effect of marine heat waves which were recorded in the area with different duration and intensity since July 2023 till May 2024

Related to other potential stressors, almost no abandoned/lost fishing gear was observed and no mucilaginous algal aggregates were present at the time of the study, unlike in some other parts of the basin, i.e. the North Adriatic in the same period (author *pers. obs*.).

In conclusion, activities conducted within this study improved the knowledge on the Lokrum's infralittoral reefs and enabled the acquisition of data relevant for their monitoring. Such an assessment allows for evaluation of changes in the future, either due to natural or human-induced causes and informs conservation and management plans. However, it should be clearly noted that this information is still far from being truly comprehensive, and future efforts should be focused on increased spatio-temporal frequency of protocols applied here, inclusion of additional complementary protocols and inclusion of the circalittoral portion of the habitat type 1170 Reefs.

1. STUDY SITE

Lokrum Island is proclaimed as the special reserve of forest vegetation (representative of the southern phytogeographical area – Eumediterranean) in 1948, and as such is the third oldest nature protected area in Croatia whose basic phenomenon is forest vegetation covering about 90% of the island. Moreover, since 2018 the entire Island, including the area of the sea stretching around 150 meters from its coast, forms part of the Natura 2000 ecological network as the Natura 2000 site HR4000017 Lokrum – a conservation area of importance for species and habitat types (POVS) including 3 marine habitat types: 1120 Posidonia beds (Posidonion oceanicae), 1170 Reefs and 8330 Submerged or partially submerged caves. Due to its vicinity to the Old town of Dubrovnik, one of the main tourist attractions in Croatia, visits to this Natura 2000 site present a popular daily excursion and hence it provides a plethora of ecosystem, recreational and cultural services to both residents and tourists alike (Domijan & Medović 2023). The site is managed by the Public institution "Lokrum reserve".

Related to the marine environment, activities up till now in the Natura 2000 site Lokrum included mapping of marine habitats and assessment of *Posidonia oceanica* beds (the latter one parallel to this study), participation in the national project for conservation of *Pinna nobilis*, underwater and coastal cleanups of marine litter as well as various educational and awareness-raising campaigns (Domijan & Medović 2023). However there were no studies focused on the habitat type 1170 Reefs and the Interreg project HABI aimed to amend such lack of knowledge, at least for infralittoral reefs.

We carried out field activities at 3 sites around the island (Fig. 1.1), selected according to following criteria:

- Distance from the mainland and urbanized areas

- The width of infralittoral rocky habitat according to the National marine habitats map (Karta obalnih i pridnenih morskih staništa RH 2023 – ver. 1.1. 2024. Ministarstvo zaštite okoliša i zelene tranzicije, https://bioportal.hr/gis/) (Fig.1.1a)

- If plausible, combination of field research and establishment of monitoring at the same sites where Posidonia meadows are being monitored to optimize logistics (which was especially important during this time-constrained field work)

Geographic coordinates of all studied sites are indicated in Table 1.1. Visualisation of the studied sites are provided in Figs. 1.2 to 1.6.

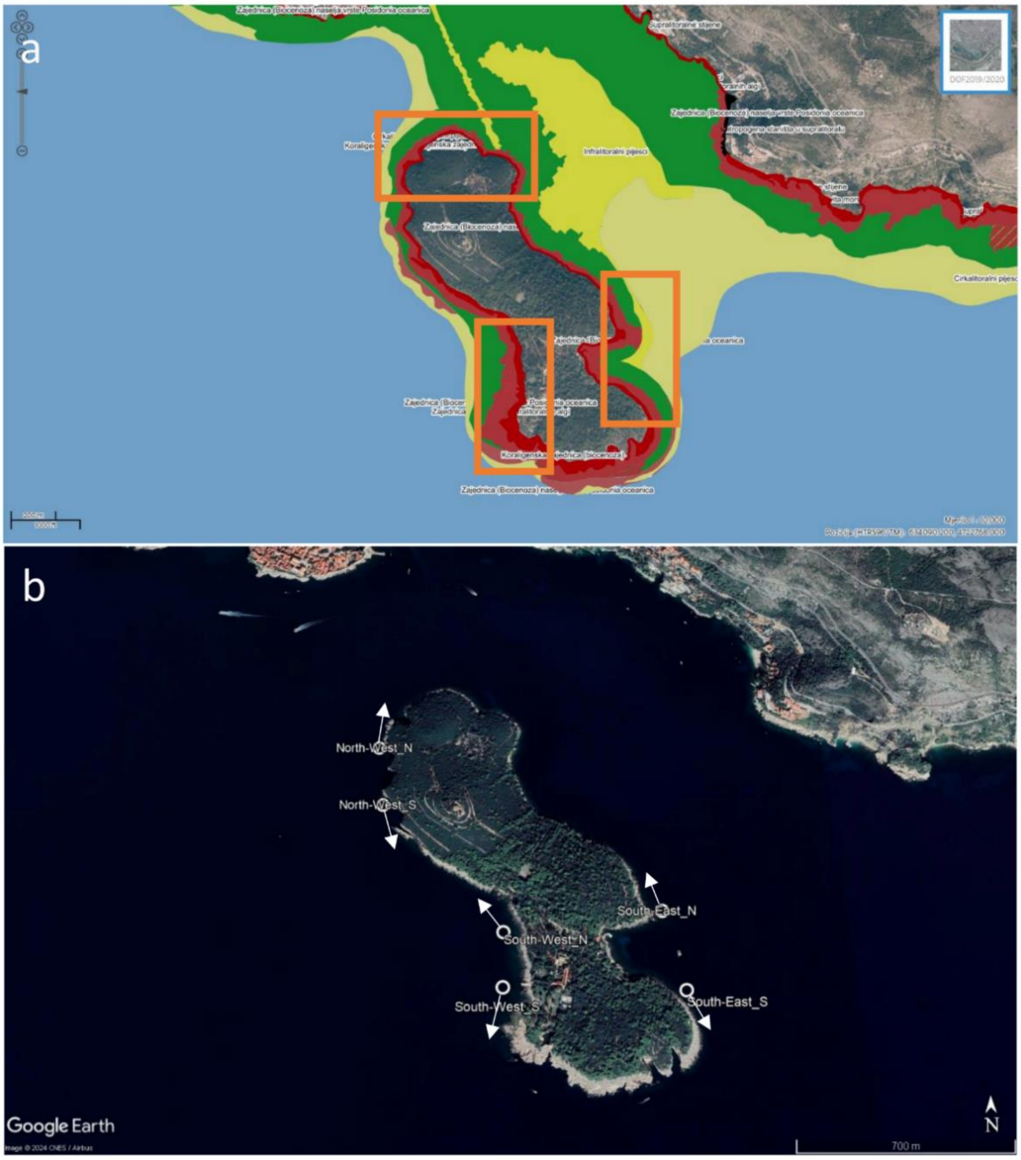

Fig. 1.1. Cartography of the Natura 2000 site Lokrum: a) 3 pre-selected areas based on the National marine habitat map (pale red belt indicates biocenosis of infralittoral algae, code G.3.6.1 according to the National classification of marine habitats) b) map of 3 study sites – at each site 2 stations (N-North and S-South) indicate initial points of visual census in deeper investigated depth range (7-12 m) and arrows point their direction; these stations are also the final points of visual censuses in shallower investigated depth range (3-7 m). Four visual censuses to assess *MedSens* biotic index were carried out at each station, 8 per study site. Fish visual censuses were carried out at South stations of each study site. Sources of maps: a) Karta obalnih i pridnenih morskih staništa RH 2023 – ver. 1.1. 2024. Ministarstvo zaštite okoliša i zelene tranzicije, https://bioportal.hr/gis/, b) Google Earth Image© 2024 CNES/Airbus.

At all investigated sites, rocky bottom with generally moderate slope dominated down to approximately 11 m depth. Below this depth, mosaic of hard bottom and *Posidonia oceanica* started to appear, except in one stretch of the North-West site (North station) where rocky bottom continued to cascade deeper, at least till 15-18 m depth (Fig. 1.6d). The hardscape was the most dynamic from the surface down to 4 m depth, highly structured with large boulders, smaller pillar-shaped rocks and crevices, especially in the cove Pod manastijerom (South-West site, Fig. 1.2a), whereas north of Portoć (South-East site) boulders were occassionaly interspersed with small to medium size spherical rocks (Fig. 1.3b).

At all investigated sites infralittoral hard bottom was covered by photophilic algae of fairly low structural complexity, forming turf to bushy macroalgal assemblages (e.g. Figs. 1.2c, 1.3c, 1.6c). The most abundant algae were *Padina pavonica*, *Acetabularia acetabulum* and *Codium bursa*. Particularly scenic were vast "fields" of *Acetabularia acetabulum* around 3-4 m depth north of the Portoć bay (North station of the South-East site, Fig. 1.4). No macroalgal forests formed by brown algae *Cystoseira* spp. were observed nor in fact, any individuals of *Cystoseira* spp. Barren grounds were occassionaly interspersed with described photophilic algae assemblages down to 5 m depth, but in general not overly present (e.g. Fig. 1.2b). Out of sessile benthic invertebrates, massive sponges *Petrosia ficiformis* and *Chondrosia reniformis* were more abundant from 3-5 m depth than deeper, whereas the abundance of encrusting *Crambe crambe* was fairly similar throughout the entire investigated depth range. Likewise, enclaves of biocenosis of semi-dark caves dominated by encrusting sponges, bryozoans as well as encrusting coralline algae and Peyssonneliaceae (closer to cavity openings) could be found throughout the entire investigated depth range at all 3 sites, linked to more sciaphilic environment of overhangs, cavities and crevices between rocks (see for example Figs. 2.5b, 3.5b, 3.10, 3.20f).

Table 1.1. Geographic coordinates (longitude and latitude) of study sites at the Lokrum Island.

	Coordinates	
	Latitude	Longitude
South-West_N	42°37'32.56"N	18° 7'7.18"E
South-West _S	42°37'26.43"N	18° 7'6.88"E
South-East_N	42°37'34.49"N	18° 7'31.39"E
South-East_S	42°37'25.56"N	18° 7'34.01"E
North-West _N	42°37'55.37"N	18° 6'47.89"E
North-West _S	42°37'48.08"N	18° 6'48.65"E

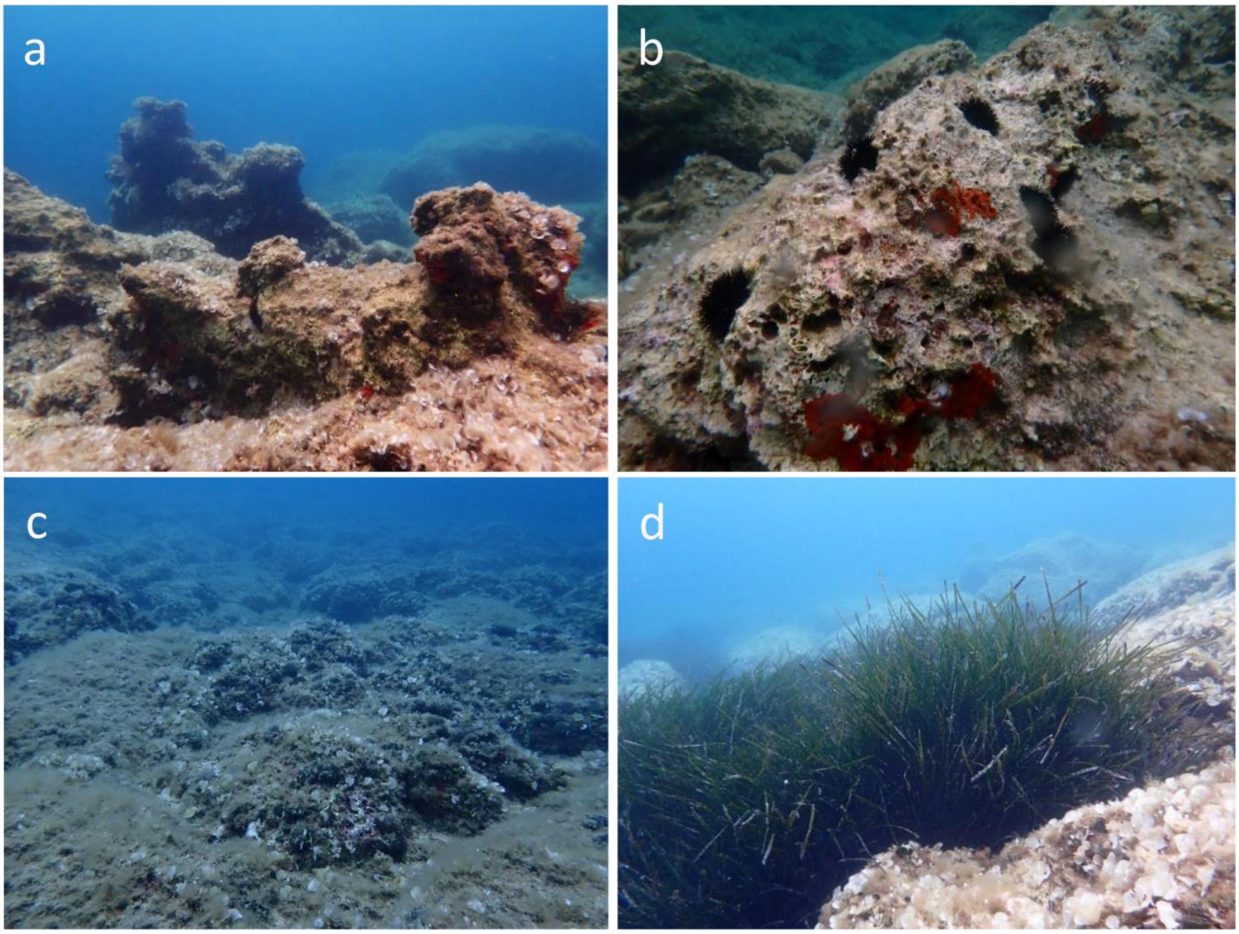

Fig. 1.2. Study site South-West (the cove Pod manastijerom): a) from the surface down to approximately 4 m depth, rocky botom was highly structured with many smaller pillar-shaped rocks and crevices, b) barrens were noted but were not overly present, c) a moderately-sloped rocky bottom stretched down to 11 m depth where it became interspersed with patches of *Posidonia oceanica* meadows (d). Photo credit: S. Kipson.

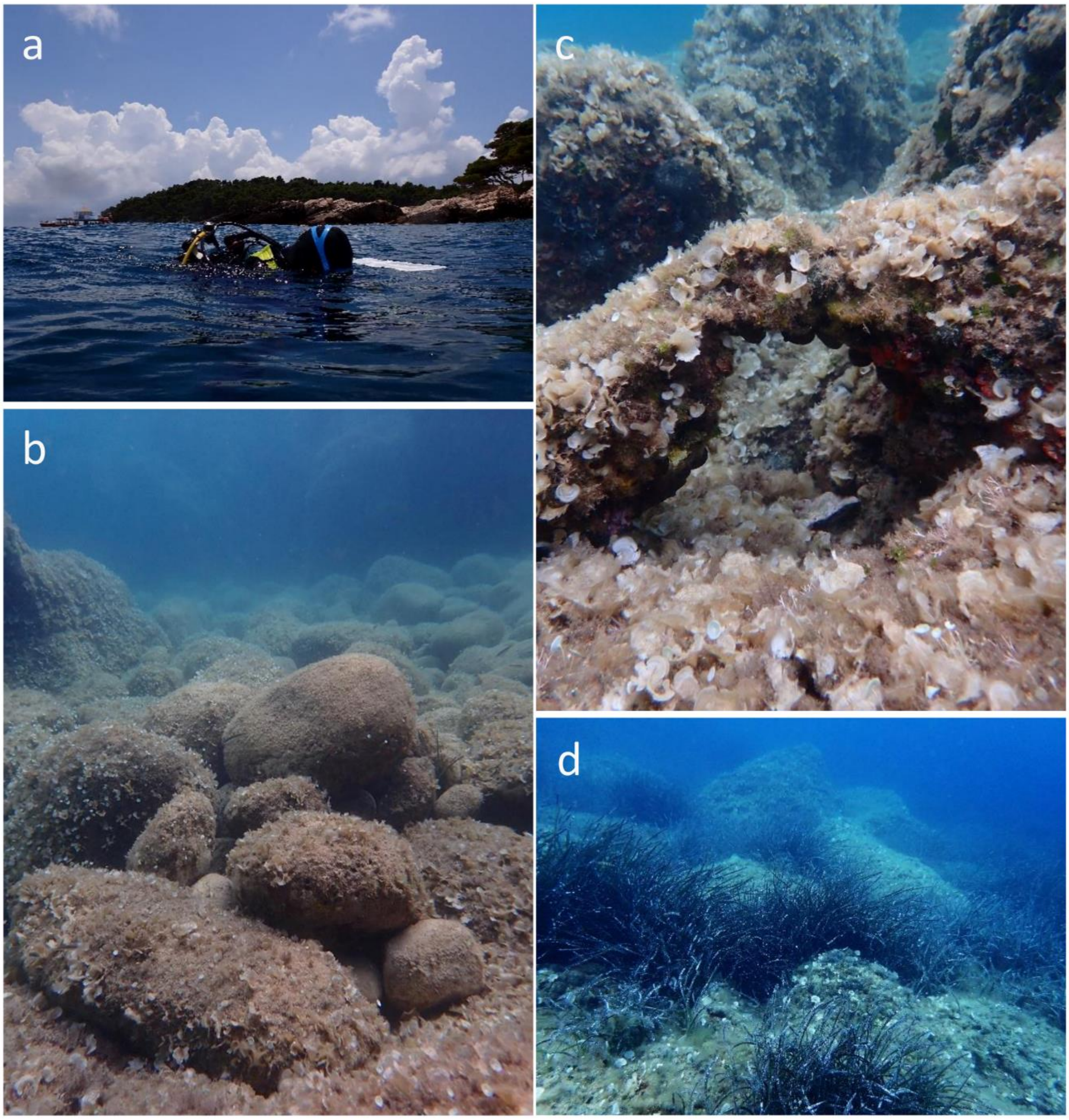

Fig. 1.3. Study site South-East (Portoć area): a) start of the dive in the North section of the site, b) down to 4-5 m depth the seascape was dynamic, comprised of vertical rocks with deep crevices, with occassional apearance of spherical stones and smaller boulders (b), followed by a moderately-sloped rocky bottom (c) down to 11-12 m depth where it became interspersed with patches of *Posidonia oceanica* meadows (d). Photo credit: S. Kipson.

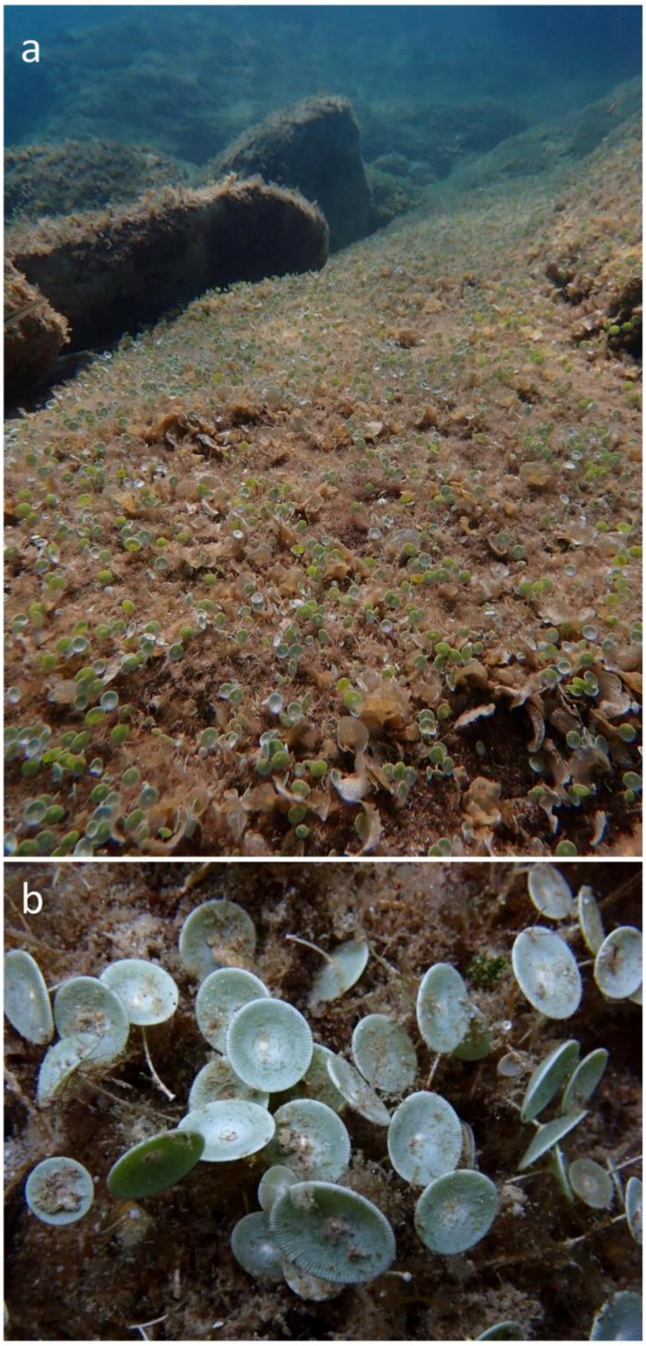

Fig. 1.4. Scenic macroalgal „fields“, a community dominated by *Acetabularia acetabulum* and *Padina pavonica* especially notable in the North section of the Portoć site (South-East) around 3-4 m depth (a) and b) detail of fairly extensive colony of *Acetabularia acetabulum* exhibiting maximum reported disc diameters of 10-12 mm (according to Falace *et al*. 2013). Photo credit: S. Kipson.

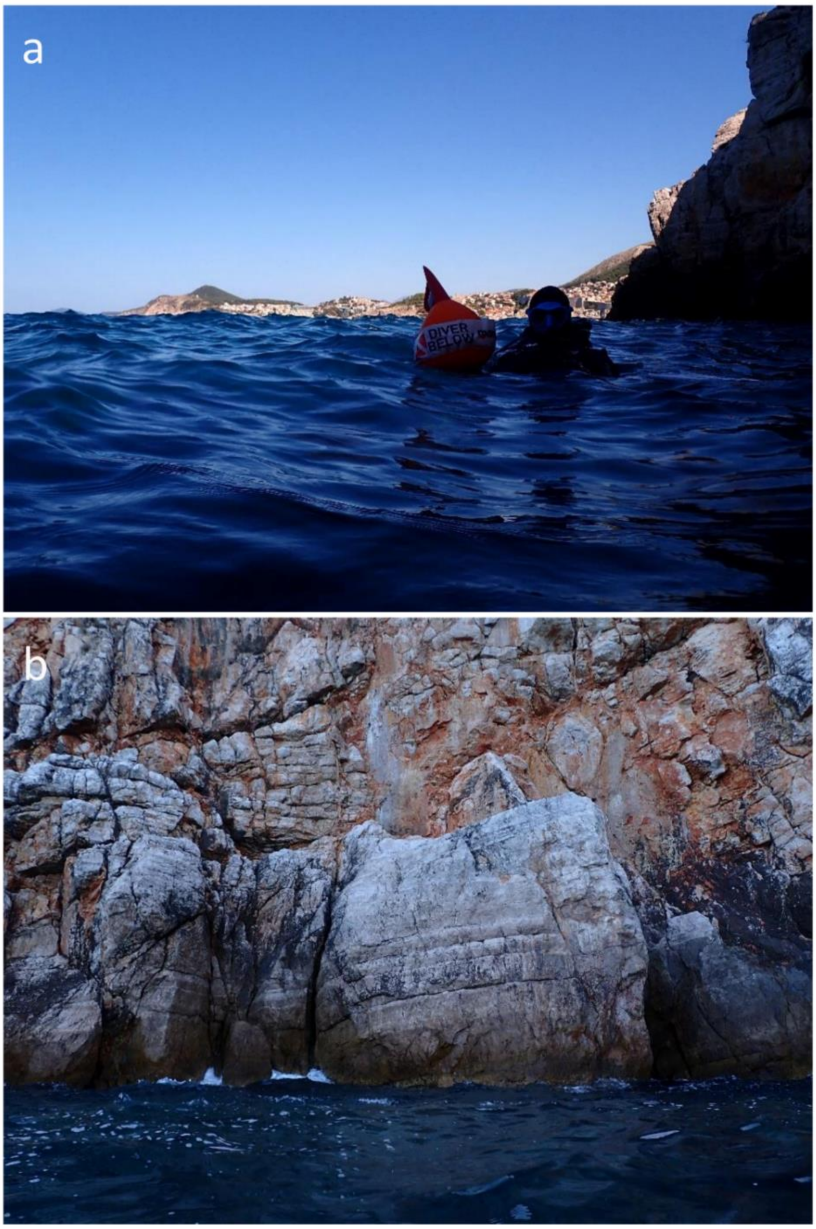

Fig. 1.5. Initiation of the dive in the North section of the North-West site: a) the town of Dubrovnik in the background, b) a characteristic rock on the Lokrum's shore that serves as a useful landmark. Photo credit: S. Kipson.

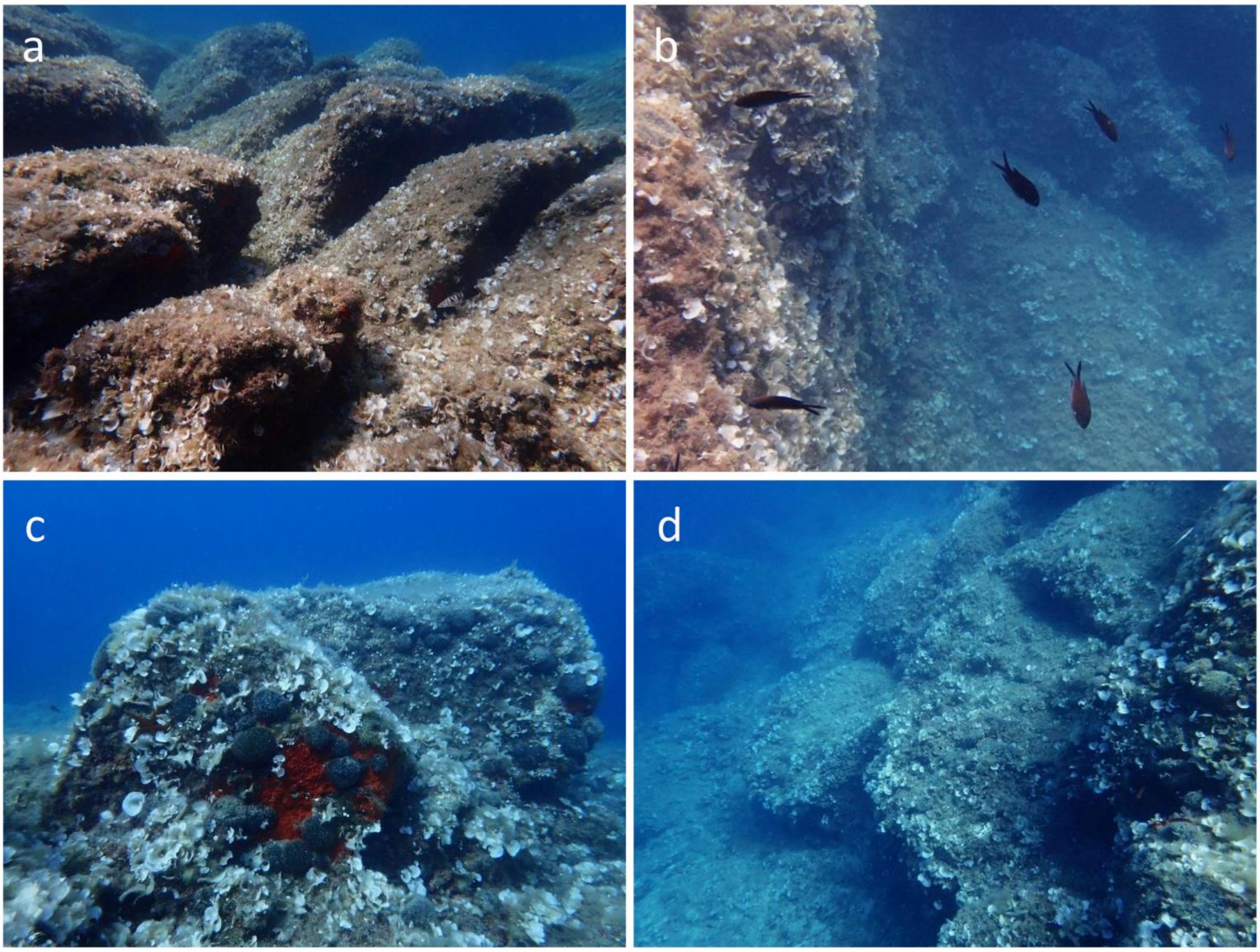

Fig. 1.6. Study site North-West: a) from the surface down to approximately 4-5 m depth, rocky botom was highly structured with many rocks and crevices, at times forming also smaller vertical walls that would stretch down to 9 m (b) on otherwise moderately-sloped rocky bottom with occassional boulders (c) that would become interspersed with patches of *Posidonia oceanica* meadows around 11-12 m; (d) in the North section of the site rocky bottom is cascading further down to at least 18 m depth . Photo credit: S. Kipson.

2. MONITORING ACTIVITIES

Following the explicit request from the employees of the Public Institution Lokrum to propose monitoring methods for infralittoral reefs that could be carried out by the staff themselves with potential assistance of volunteers that are not necessarily marine biologists, we used the opportunity to test a recently proposed biotic index *MedSens*, specifically developed to provide information on the environmental status of subtidal rocky coastal habitats, filling a gap between marine ciztizen science and coastal management in the Mediterranean Sea (Turicchia *et al.* 2021). The *MedSens* index is based on 25 selected species, incorporating their sensitivities to the pressures indicated by the European Union's Marine Strategy Framework Directive (MSFD) and open data on their distributions and abundances, collected by trained volunteers (scuba divers, free divers and snorkelers) using the Reef Check Mediterranean Underwater Coastal Environment Monitoring (RCMed U-CEM) protocol. The species' sensitivities were assessed relative to their resistance and resilience against physical, chemical, and biological pressures, according to benchmark levels and a literature review.

Besides assessment of *MedSens* index, we carried out fish visual census for climate change indicators, previously tested in several other Interreg projects e.g. Med ADAPT and ADRIREEF (Stagličić et al. 2021, Kipson 2021).

Overview of the methodology, main results and conclusions are outlined below for each of these activities.

2.1. Assessment of infralittoral reef status

2.1.1. Overview of the methods

Visual census according to the Reef Check Mediterranean U-CEM protocol/assessment of MedSens index

Since there was no previous field data on which we could base our selection, target species that could be expected to occur on infralittoral reefs of Lokrum were pre-selected from the list provided by Turicchia *et al*. 2021 (see Table XX). This list originally includes 25 species but out of these 13 were kept as species that could be expected considering the investigated depth range and habitat type.

Team of divers, equipped with the diving buoy for additional safety (Fig. 2.1°), recorded the abundance of target organisms on the plastic slate (Fig. 2.1b). Not encountered but actively searched taxa were recorded as absent. The diving sites were localised by global positioning system (GPS) . Geographical coordinates (WGS84) are recorded with ± 6 arc-seconds (i. e. 185 m in latitude) accuracy. Besides presence/absence and abundance of recorded species observations included site name, date and time, underwater visibility and survey depth range (min and max).

In total, 6 dives were performed at 3 sites on the Lokrum Island, separated by at least 1 km. At each site, 2 dives were performed by 2 observers who carried out 2 visual censuses each during one dive. Each visual census was a priori determined to last for 25 min, covering depth ranges as indicated in Fig. 2.2. For illustration, swimming in parallel, 1 diver performed visual census in a depth range 10-12 m, whereas the other one performed visual census in a depth range 8-10 m. After 25 min, both divers moved upwards, and 1 diver performed second visual census in a depth range 5-7 m and another one in a depth range 3-5 m, swimming back to the initial immersion point. During entire dive, divers could see each other and a rule of buddy diving was respected. Clearly, such a diving protocol was possible due to great underwater visibility. Dives lasted between 60 and 66 min. At each site, 8 observations were made, in total 24 within the Lokrum Natura 200 site.

RCMed species sensitivity (MedSens) index

The *MedSens* index provides the mean sensitivity of the species assemblages recorded by EcoDivers within a TU and TF. It can be calculated for the physical ($MedSens_{phy}$), chemical ($MedSens_{che}$), biological ($MedSens_{bio}$), and overall pressures ($MedSens_{tot}$) on the species (for enlisted pressures see Table 2.1), based on the corresponding mean sensitivity values (MSV, Table), weighted for the abundance classes of the taxa. For each observation, the abundance class was converted to an abundance score (Sc) of 0 to 6 (Table 2.2).

The index is calculated as:

MedSensx=Σ(Sci×MSV(x)i)/ΣSci

where x is the chosen pressure typology (phy, che, bio, or tot), and MSV(x) i refers to the taxon in the ith observation having an abundance score Sci in the selected TU and TF.

The minimum requirements for the index calculation are:

- TU size ≥0.08 km2,
- EcoDivers ≥3,
- number of observations (including absences) ≥20,
- and searched taxa ≥10.

The index values increase with increasing sensitivity means of the species recorded and, to a lesser extent, with their abundance.

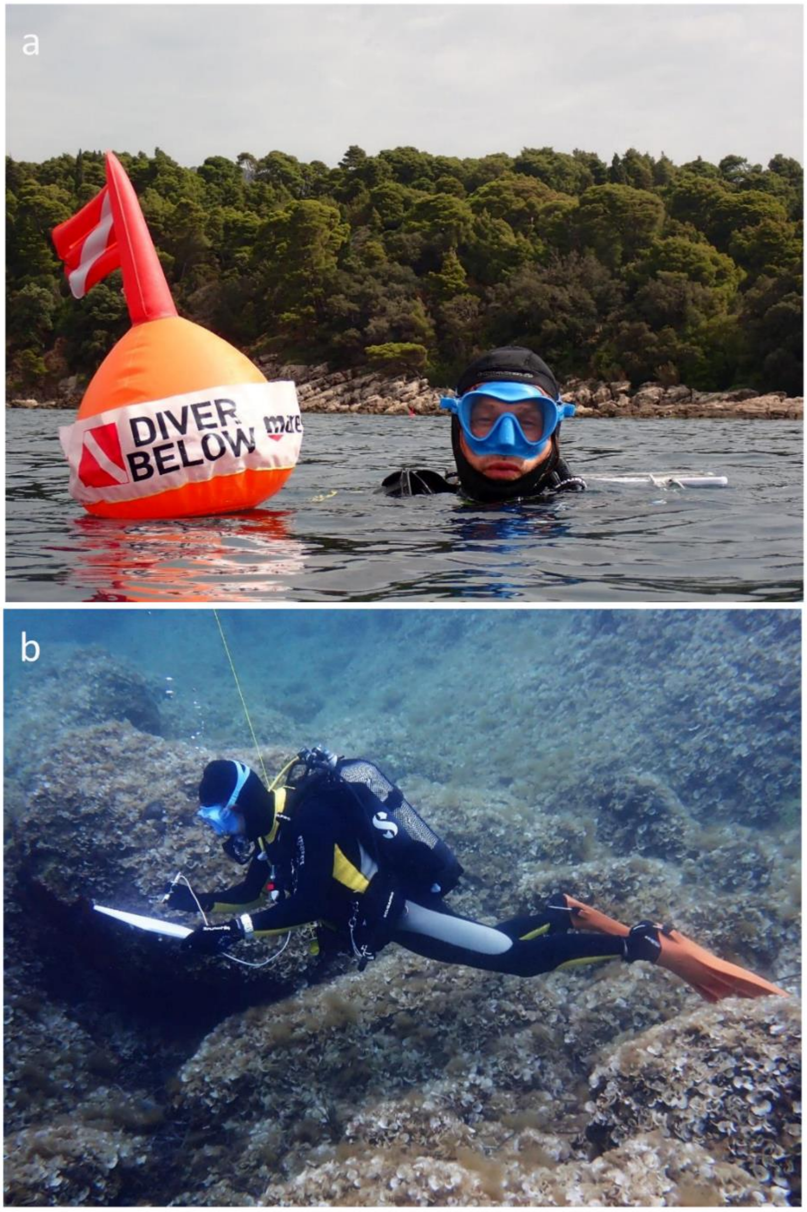

Fig. 2.1. Collecting data for calculation of the *MedSens* biotic index: a) team of divers, equipped with the diving buoy for additional safety due to intense boat traffic, b) diver recording the abundance of target organisms on the plastic slate. Photo credit: S. Kipson.

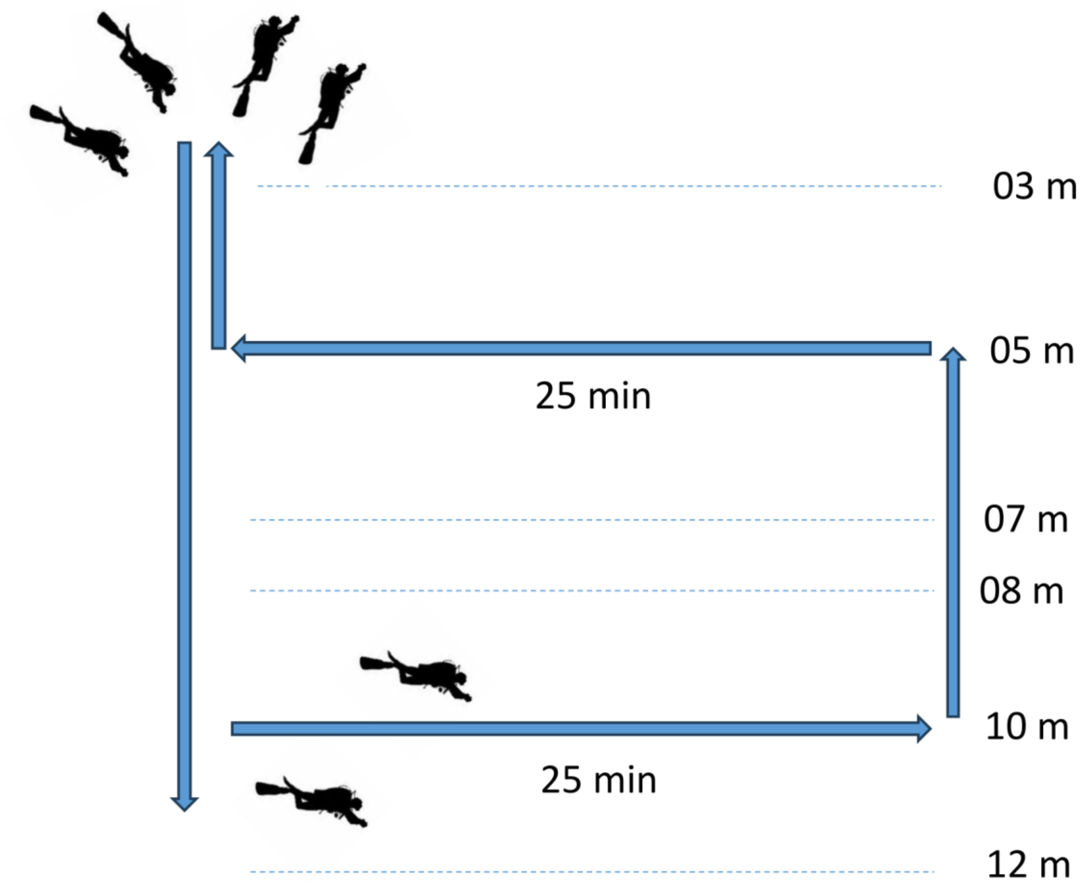

Fig. 2.2. Diving plan to assess target species' abundances for calculation of *MedSens* index (see Section 2.1.1 for more detailed description).

Table 2.1. Pressures likely to affect marine species and considered in the evidence-based sensitivity assessment according to the MarESA approach and the needs of the MSFD (Tyler Walters *et al*. 2018, adopted from Turicchia *et al*. 2021).

Pressure type	Pressure
Physical	Emergence regime changes
	Salinity changes (increase)
	Salinity changes (decrease)
	Temperature changes (increase)
	Temperature changes (decrease)
	Water flow (tidal current) changes
	Wave exposure changes
	Changes in suspended solids (water clarity)
	Habitat structure changes - removal of substratum (extraction)
	Abrasion/ disturbance at the surface of the substratum
	Penetration and/or disturbance of the substratum below the surface
	Smothering and siltation rate changes (light)
	Smothering and siltation rate changes (heavy)
	Physical change
	Physical loss
	Barrier to species movement
	Electromagnetic changes
	Death or injury by collision
	Introduction of light
	Litter
	Noise changes
	Visual disturbance
Chemical	Organic enrichment
	De-oxygenation
	Introduction of other substance (solid, liquid or gas)
	Nutrient enrichment
	Hydrocarbon and PAH contamination
	Radionuclide contamination
	Synthetic compound contamination
	Transition elements & organo-metal contamination
Biological	Genetic modification and translocation of indigenous species
	Introduction of microbial pathogens
	Introduction or spread of invasive non-indigenous species
	Removal of non-target species
	Removal of target species

Table 2.2. Abundance classes and their converted scores (Sc) (adopted from Turicchia *et al*. 2021)

Numerical class	Descriptive class	*Sc*
0	absent	0
1	isolated specimen	1
2	some scattered specimens	2
3–5	several scattered specimens	3
6–10	a crowded area	4
11–50	some crowded areas	5
>50	several crowded areas	6

Table 2.3. Mean sensitivity values of the physical (MSVphy), chemical (MSVche), and bio logical (MSVbio) pressures, and the overall mean (MSVtot) of the selected taxa (adopted from Turicchia *et al*. 2021)

Taxa	*MSVphy*	*MSVche*	*MSVbio*	*MSVtot*
★*Caulerpa cylindracea*	0.643	0.571	0.333	0.583
★*Caulerpa taxifolia*	0.643	0.571	0.333	0.583
★*Axinella* spp.	1.231	0.714	1.333	1.087
★*Aplysina* spp.	1.538	0.714	1.333	1.261
Geodia cydonium	1.769	1.571	1.667	1.696
Corallium rubrum	2.308	2.333	3.000	2.409
Paramuricea clavata	2.462	2.667	2.750	2.565
Eunicella cavolini	2.462	2.500	2.750	2.522
Eunicella singularis	2.231	2.500	2.500	2.348
Eunicella verrucosa	1.692	2.333	2.750	2.043
★*Parazoanthus axinellae*	1.769	1.833	0.667	1.636
Savalia savaglia	2.385	2.000	2.000	2.217
★*Cladocora caespitosa*	2.154	2.500	2.333	2.273
Astroides calycularis	1.769	2.500	1.000	1.826
★*Balanophyllia europaea*	1.769	2.333	1.333	1.864
★*Leptopsammia pruvoti*	1.692	2.000	1.000	1.682
Pinna nobilis	1.923	1.500	2.750	1.957
★*Arca noae*	1.308	2.167	2.250	1.696
Palinurus elephas	1.214	1.857	2.500	1.600
Homarus gammarus	1.214	1.857	2.750	1.640
★*Scyllarides latus*	1.231	1.857	2.500	1.625
★*Paracentrotus lividus*	1.462	1.429	2.250	1.583
★*Hippocampus* spp.	1.933	1.143	2.250	1.769
★*Diplodus* spp.	1.133	0.714	2.250	1.192
★*Sciaena umbra*	1.267	1.286	2.000	1.385

Table 2.4. MedSens index classification of the physical, chemical, biological, and overall pressures (adopted from Turicchia *et al*. 2021).

Mean sensitivity	$MedSens_{phy}$	$MedSens_{che}$	$MedSens_{bio}$	$MedSens_{tot}$
Very low	$\leq$1.5106	$\leq$1.4381	$\leq$1.5554	$\leq$1.5305
Low	$\leq$1.6275	$\leq$1.6342	$\leq$1.7908	$\leq$1.6432
Moderate	$\leq$1.7206	$\leq$1.7806	$\leq$1.9168	$\leq$1.7431
High	$\leq$1.8456	$\leq$1.9621	$\leq$2.0594	$\leq$1.8921
Very high	$>$1.8456	$>$1.9621	$>$2.0594	$>$1.8921

2.1.2. Main results

Initially 13 species out of 25 proposed ones by Turicchia *et al*. 2021 were pre-selected to assess *MedSens* index, based on the expectations of their occurrence on infralittoral reefs around Lokrum Island. Additionaly, based on the field observation, slippery lobster *Scyllarides latus* was added to the list. Since it is an elusive decapod crustacean, fairly rarely to be seen and mainly active by night (Miller *et al*. 2023), initially it was deemed as an unlikely observation during our dives. However, it was observed on June 28 during daytime while sheltering in a rocky crevice of the shallow infralitoral at the North-West site, which is in fact in line with a seasonal pattern of adults and marks their inshore reproductive season. Following this period, in the fall they are suspected to migrate into deeper water (Miller *et al*. 2023).

Fig. 2.3. Strictly protected slippery lobster *Scyllarides latus*, a species of Community Interest listed in the Habitat Directive and one of the target species for assessment of *MedSens* index. Photo credit: S. Kipson.

Although the noble pen shell *Pinna nobilis* was common around the Lokrum Island in the past, especially around Portoć area (Jakl & Špika 2019), it was not considered in the visual census. Such decision was based on the knowledge of the mass mortalities in the whole Mediterranean and in the Adriatic, including the Lokrum Island, since Public Institution is one of the partners in the national project „Conservation of the Noble pen shell *Pinna nobilis* in the Adriatic Sea“ and it is monitoring regularly the status within this Natura 2000 site. To the best of our knowledge, the sighting of live specimen was considered unlikely.

Out of all selected species, in total 7 were recorded during investigated period. These included a solitary coral *Balanophyllia europaea*, bivalve *Arca noae*, sea urchin *Paracentrotus lividus*, fish *Diplodus* spp. and *Sciaena umbra*, already mentioned crustacean *Scyllarides latus* as well as the invasive green algae *Caulerpa cylindracea*. It should be noted that another sea urchin, *Arbacia lixula*, was present at all sites in similar abundances as *Paracentrotus lividus* (Fig. 2.4). However, since it is not included on the species list to calculate *MedSens* index, it was not further considered. Likewise, sea urchin *Sphaerechinus granularis* was also present, but expectedly less abundant, accounting to several scattered individuals at each site. Out of fish belonging to the Diplodus genus, the most abundant one was *Diplodus vulgaris*, sometimes forming schools of considerable size, comprised of cca 150 individuals. Besides this fish species, several specimens of *Diplodus puntazzo* and *Diplodus sargus sargus* were observed. Interestingly, one of the most common infralitoral sponges, *Aplysina aerophoba*, was not recorded on the Lokrum Island. Likewise, no *Axinella* spp. were noted. Out of coral species, only *Balanophyllia europaea* (Fig. 2.5a) was observed whereas common species such as *Cladocora caespitosa*, *Parazoanthus axinellae* and *Leptopsammia prouvoti* were absent. Noteably, although not a target species, the only other observed hard corals besides *B. europaea* were *Madracis pharensis* and *Caryophillia inornata,* found at the ceiling of an overhang (presenting in fact an enclave of the biocenosis of semi-dark caves within the biocenosis of photophilic algae, Fig. 2.5b). Out of target bivalves, *Arca noae* was rarely observed (Fig. 2.6). Invasive green algae *Caulerpa cylindracea* was present on all sites, however, it was observable only upon closer inspection of the substrate, as it was present in very low abundances (see for example Fig. 2.7). Other invasive algae belonging to the same genus, *Caulerpa taxifolia* was not present.

Fig. 2.4. The most common sea urchins sharing the same habitat on infralittoral reefs around Lokrum Island: a) *Paracentrotus lividus* and b) *Arbacia lixula.* Photo credit: S. Kipson.

Fig. 2.5. Some of the observed hard coral species: a) *Balanophyllia europaea*, partially bleached (affected by the loss of symbiotic zooxanthellae, hence partially pale in colour) and b) *Madracis pharensis*, found in a more sciaphilic habitat – the ceiling of an overhang, in fact an enclave of the biocenosis of semi-dark caves within the infralitoral reefs. Photo credit: S. Kipson.

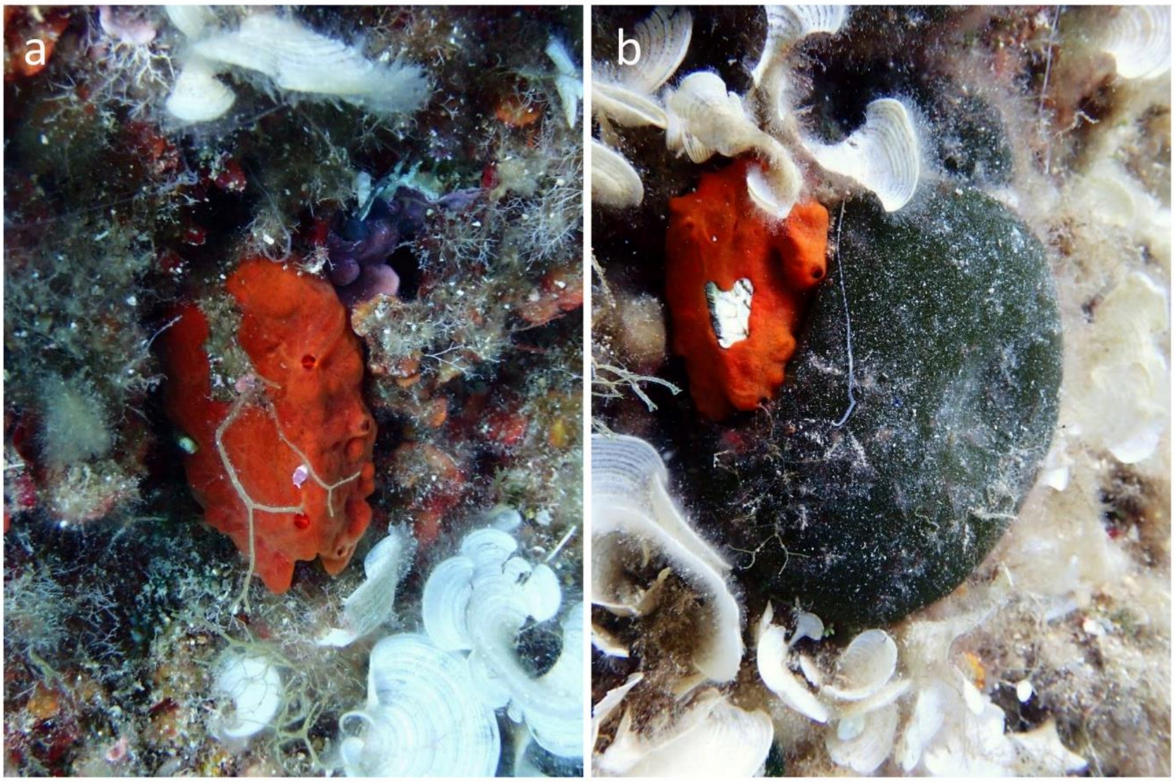

Fig. 2.6. Rarely observed, well camouflaged bivalve *Arca noae*: a) completely covered by encrusting sponge *Crambe crambe* and b) a speciemen with partially visible shell. Photo credit: S. Kipson.

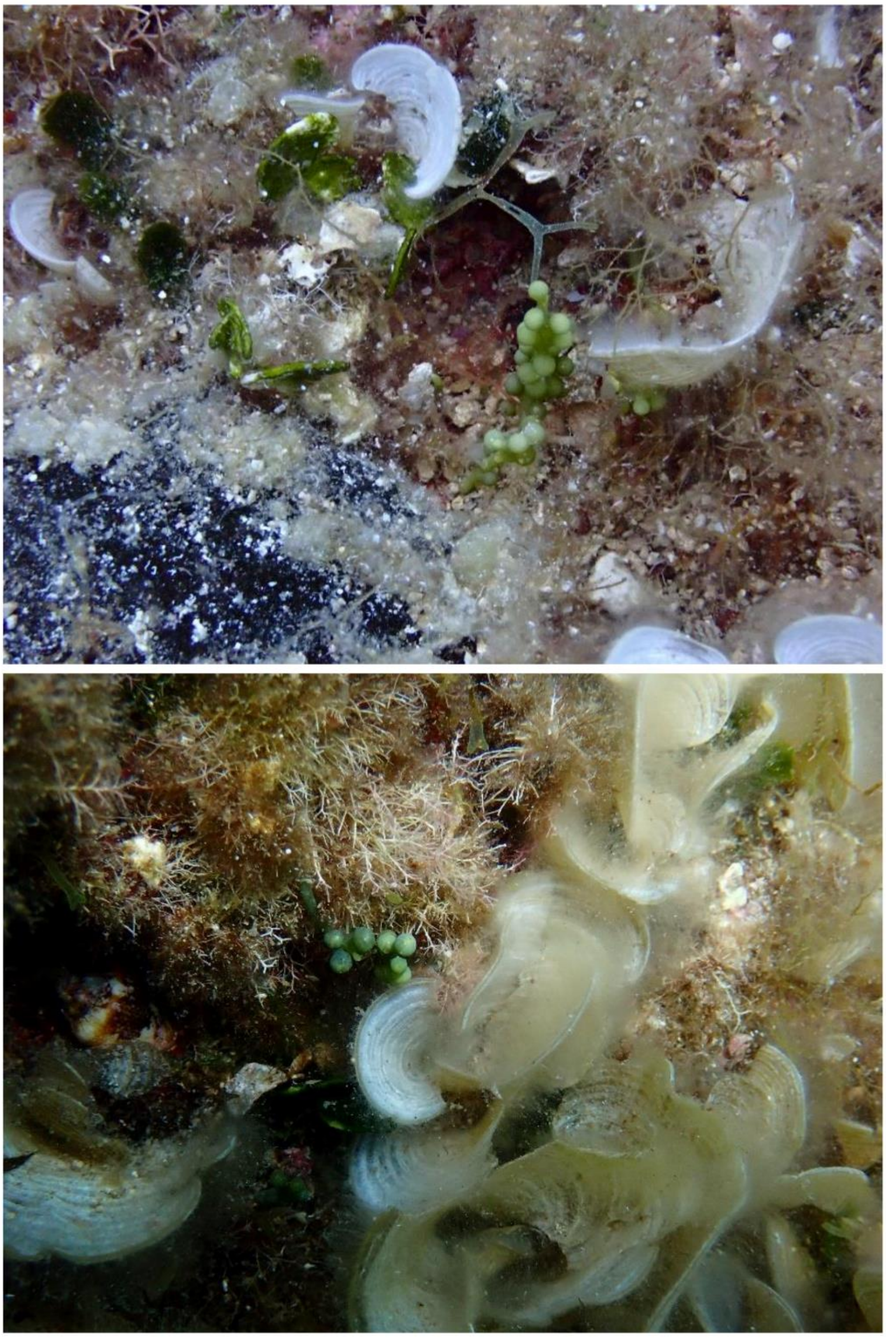

Fig. 2.7. Invasive green algae *Caulerpa cylindracea* was present on all sites, but at the end of June still in very low abundances, hardly noticeable. Photo credit: S. Kipson.

Recorded numbers of individuals per species were converted to appropriate Scores (Sc) according to Table 2.2. These scores are indicated in Table 2.5 for each species and each observation. Using these scores and Mean sensitivity Values indicated in Table 2.3 for each species and each type of pressure, the values of *MedSens* index for physical, chemical, biological and total pressures were calculated based on the formula provided in the Section 2.1.1. The results are presented in Table 2.6.

Table 2.5. Numerical classes converted to scores (according to Table 2.2) for each target species and for each observation.

SITE	Census N	Date	Depth min	Depth max	Start time	*Caulerpa cylindracea*	*Caulerpa taxifolia*	*Aplysina aerophoba*	*Axinella* spp.	*Balanophyllia europaea*	*Cladocora caespitosa*	*Leptopsammia prouvoti*	*Parazoanthus axinellae*	*Arca noae*	*Scylarides latus*	*Paracentrotus lividus*	*Diplodus* spp.	*Hippocampus* sp.	*Sciaena umbra*
Lokrum SW	1	26/06/2024	10	12	10:07	2	0	0	0	0	0	0	0	0	0	3	4	0	0
	2	26/06/2024	5	6	10:40	2	0	0	0	0	0	0	0	0	0	4	6	0	0
	3	26/06/2024	8	11	11:52	0	0	0	0	0	0	0	0	0	0	3	5	0	0
	4	26/06/2024	4	5	12:25	0	0	0	0	0	0	0	0	0	0	6	1	0	0
	5	26/06/2024	8	9	10:07	0	0	0	0	0	0	0	0	0	0	1	5	0	0
	6	26/06/2024	6	7	10:40	0	0	0	0	0	0	0	0	0	0	1	6	0	0
	7	26/06/2024	6	8	11:52	0	0	0	0	0	0	0	0	0	0	1	6	0	1
	8	26/06/2024	5	6	12:25	0	0	0	0	0	0	0	0	0	0	5	5	0	0
Lokrum SE	1	27/06/2024	11	13	09:50	3	0	0	0	0	0	0	0	1	0	0	6	0	1
	2	27/06/2024	4	5	10:23	3	0	0	0	0	0	0	0	0	0	6	5	0	0
	3	27/06/2024	9	12	13:25	2	0	0	0	1	0	0	0	1	0	0	1	0	0
	4	27/06/2024	4	5	13:59	3	0	0	0	0	0	0	0	0	0	5	3	0	0
	5	27/06/2024	7	9	09:50	0	0	0	0	0	0	0	0	0	0	5	5	0	0
	6	27/06/2024	5	7	10:23	0	0	0	0	0	0	0	0	0	0	4	6	0	0
	7	27/06/2024	7	9	13:25	0	0	0	0	0	0	0	0	0	0	0	6	0	0
	8	27/06/2024	5	6	13:59	0	0	0	0	0	0	0	0	0	0	3	6	0	0
Lokrum NW	1	28/06/2024	10	12	10:10	3	0	0	0	0	0	0	0	1	1	3	6	0	0
	2	28/06/2024	4	5	10:42	3	0	0	0	0	0	0	0	0	0	5	5	0	0
	3	28/06/2024	10	12	13:53	3	0	0	0	1	0	0	0	0	0	1	6	0	0
	4	28/06/2024	4	5	14:32	3	0	0	0	0	0	0	0	0	0	5	5	0	0
	5	28/06/2024	7	9	10:10	0	0	0	0	0	0	0	0	0	0	0	6	0	0
	6	28/06/2024	5	7	10:42	0	0	0	0	0	0	0	0	0	0	5	5	0	0
	7	28/06/2024	7	9	13:53	0	0	0	0	1	0	0	0	0	0	0	6	0	0
	8	28/06/2024	5	7	14:32	0	0	0	0	0	0	0	0	1	0	2	4	0	0

Table 2.6. *MedSens* index values toward physical ($MedSens_{phy}$), chemical ($MedSens_{che}$), biological ($MedSens_{bio}$) and overall pressures ($MedSens_{tot}$) in the Lokrum Natura 2000 site. Red color indicates very low values and green color indicates high value, for reference see Table 2.4.

MedSens_phy	MedSens_che	MedSens_bio	MedSens_tot
1.19	0.97	2.04	1.27

2.1.3. Conclusions

Contrasting results were obtained for mean sensitivity to physical and chemical pressures versus biological pressures on infralittoral reefs of the Lokrum Natura 2000 site. Whereas the first two were evaluated as being very low ($MedSens_{phy}$ = 1.19 and $MedSens_{che}$ = 0.97), sensitivity to biological pressures was evaluated as being high to very high (with a borderline $MedSens_{bio}$ mean sensitivity value of 2.04, while values >2.05 are considered as indicative of very high sensitivity to biologial pressures, Table 2.6). Influenced by the very low sensitivity values for physical and chemical pressures, the total mean sensitivity value was also very low ($MedSens_{tot}$ = 1.27).

Such results were reported also for the Italian Marine protected Area Miramare, western Coast of Istria and an artificial reef - the offshore platform Paguro, all located in the Northern Adriatic Sea (Turicchia *et al*. 2021, Supplementary material). The explanation given by the authors of the *MedSens* index was that those protected areas with the least sensitive species assemblages were characterised by artificial habitats, such as shipwrecks in Malta and the offshore platform wreck 'Paguro' in the northern Adriatic Sea, whose benthic assemblages are found to be simplified compared to natural rocky bottoms. Low to very low mean species sensitivities were also found at 'Tegnùe di Chioggia', a northern Adriatic no-take zone characterised by mesophotic coralligenous banks, and these results were considered as being consistent with high anthropogenic disturbance in the area, including several dystrophic crises and intense trawling that may diminish the abundance of species sensitive to physical and chemical pressures (Turicchia *et al*. 2021 and references therein).

However, the very low sensitivity to physical pressures calculated for Lokrum Island may be also putatively explained by the intrinsic lack (natural absence) of erect or massive coral species such as gorgonians and bushy coral *Cladocora caespitosa*, whose sensitivity to such type of pressure is the highest (MSV phys > 2, Table 2.3). Among these species, considering their habitat preferences and a sole focus on the infralittoral depth range of reefs within this study, only *Cladocora caespitosa* and *Eunicella singularis* could occur in the first place, less likely also *Eunicella cavolinii* in a shaded environment, like some overhangs and crevices. Indeed, absence of *Cladocora caespitosa* was unexpected, however based on the available knowledge of the area (gathered from local recreational divers), *Eunicella* spp. were currently not expected (and indeed, not observed). It remains to be investigated if these species were historically present or maybe they still are, just in some other areas, not covered by this study. Similar conclusion may be drawn for a result indicating very low sensitivity to chemical pressures. The same above-mentioned species (reportedly absent here) are the most sensitive to chemical pressures, with addition of the solitary hard coral *Balanophyllia europaea* and a bivalve *Arca noae* that were observed at the Lokrum Island, but in very low abundance (i.e. a single specimen per site).

As a reminder, the *MedSens* index values increase with increasing sensitivity means of the species recorded and, to a lesser extent, with their abundance (Turicchia *et al*. 2021). Moreover, "**whereas the high abundance of sensitive species is likely a witness of reduced pressures, the high abundance of tolerant species is not necessarily related to poor environmental conditions – this should be considered when interpreting the results"** (Turicchia *et al*. 2021). And although *MedSens* index is an effective monitoring tool that can help to identify the main pressures acting upon subtidal rocky coastal habitats and support the implementation of appropriate marine biodiversity conservation measures, it also has some recognized shortcomings. Its main weaknesses are considered to be a reduced number of considered species (which though could be increased in the future), and the need for large amounts of data from many well-trained volunteers (Turicchia *et al*. 2021).

Related to the assessment of the target invasive algae during the *MedSens* census, although *Caulerpa cylindracea* was present at all investigated sites within the Lokrum Natura 2000 site, its abundance was very low at the end of June. In fact, the algae was observable only upon closer inspection of the substrate, and less experienced observers may easily ommit it in their censuses (as it was the case here with one observer who was not biologist). Hence, it should be noted that both observers' training and season may influece results of visual census. Hence, to assess the highest abundance of *C. cylindracea*, the census should be repeated later in the summer (or in the early autumn) when the growth rate of this invasive alga is at its maximum (Ruitton *et al*. 2006). A drastic regression during winter months would not be expected on the Lokrum Island, located in the warmer South Adriatic (in comparison to the North Adriatic), similarly as reported for other warmer Mediterranean regions, where the layer of stolons and sparse fronds were maintained throughout the colder season (Iveša *et al*. 2015 and references therein).

C. cylindracea may grow at depths from 0 to 70 m (Klein & Verlaque, 2008) and pressures such as mechanical destruction of habitats, enhanced sedimentation rate and nutrients loading directly promote its spread. In general, factors that promote the spread of turf-forming algae at the expense of canopy-forming species and hence result in a decrease of substrate complexity contribute to the *C. cylindracea* invasion (Piazzi *et al*. 2016).

Although there are insights that the Lokrum Island hosted canopy-forming Cystoseira species (A. Žuljević, *pers. comm*.), they were not observed at our investigated sites. Photophilic algae assemblages exhibited fairly low structural complexity and were dominated by the species such as *Padina pavonica*, *Acetabularia acetabulum* and *Codium bursa*. It would be of interest to try to gather further information on historical photophilic algae assemblages and to understand if macroalgal forests were present here and if so, to what extent.

Ultimately, the spread of *C. cylindracea* seems to be regulated by a complex web of interactions between abiotic and biotic factors and although some of the factors regulating the establishment and spread of *C. cylindracea* have been identified, other aspects are still not well understood (Piazzi *et al*. 2016). For example, it appears that herbivores may have both negative and positive effects on *C. cylindracea* spread. Positive effect on algal reduction would be evident through its direct removal, however herbivores can also enhance its spread by fragmenting the algae and by stimulating its growth through grazing on autochtonous macroalgal assemblages (Piazzi *et al*. 2016 and references therein). Indeed, several marine organisms that may consume *C. cylindracea* such as sea urchins *Paracentrotus lividus* and *Sphaerechinus granularis* as well as sparid fishes *Sarpa salpa* and *Diplodus* *sargus* (Klein & Verlaque 2008; Gorbiet *et al*. 2014) were observed here. With the exception of *Sphaerechinus granularis* (several individuals were recorded, author's *pers. obs*.), they were all fairly abundant.

Competition for space might also be critical for the late spring recovery of extant *C. cylindracea* mats and the spread to new sites. In May, when new *C. cylindracea* fronds are formed from the surviving fragments of thalli, perennial autochthonous macrophytobenthic species are at the vegetative maximum. The available experimental evidence from the other warmer Mediterranean regions indicate that algal canopies act as a barrier against the invasion of *C. cylindracea* (Bulleri *et al*. 2011), hence the protection of autochthonous macrophytobenthic species seems to be pivotal for containing *C. cylindracea* expansion (Iveša *et al*. 2015).

In conclusion, whereas the high abundance of species sensitive to biological pressures would be indicative of good status around the Lokrum Island related to this type of pressures (however the abundance of the invasive algae *Caulerpa cylindracea* should be re-examined at its vegetation peak in late summer/early autumn to get a more reliable assessment of this pressure), the abundance of species tolerant to physical and chemical pressures is not necessarily related to poor environmental conditions considering those types of pressures. Whether species sensitive to physical and chemical pressures were historically present but were subsequently lost or they were never present on the Lokrum Island in the first place, remains to be further investigated. Moreover, considering the application of the *MedSens* index, increased amount of data contributes significantly to the robustness of the method (E. Turicchia *pers. comm.*), hence there is a need for increased observational effort in space and time within the Lokrum Natura 2000 site.

To further note, in order to facilitate the application and calculation of the *MedSens* index, a plugin for QGIS was developed in Python language and made freely available in the QGIS plugin repository (see Turicchia *et al*. 2021 for instructions).

2.2. Fish visual census of climate change indicators

2.2.1 Overview of the methods

Beside the effort to monitor current ecological status we have also conducted a survey of selected fish species that may be indicative of climate related changes, following the protocol adopted within the Interreg project MPA ADAPT (Garrabou *et al*. 2022). These target species included native fish *Sparisoma cretense, Epinephelus marginatus, Thalassoma pavo, Sarpa salpa, Serranus scriba, Coris julis* and *Serranus cabrilla* (Fig. 2.8). Moreover, attention was dedicated to potential sightings of alien species *Siganus spp., Fistularia commersonii, Pterois miles, Torquigener flavimaculosus and Parupeneus forskali* (Fig. 2.9). In addition, local targets (max. 4) may be added according to local monitoring needs, easiness of recognition, interaction with fisheries, increase/decrease in the area and potential impacts on the environment/fisheries/ human activities. At our location we have opted to include also the common two-banded sea bream *Diplodus vulgaris* (Geoffroy Saint Hilaire, 1817) (Fig. 2.12e), due to its abundance, easiness of recognition, importance in food webs and trophic cascades (i.e. among others, as a predator of sea urchins it may prevent their overgrazing and habitat degradation in form of barrens creation; Guidetti & Dulčić 2007) and lastly, importance for fishery. Moreover, the saddled sea bream *Oblada melanurus* (Linnaeus, 1758) was also selected due to its local abundance, easiness of recognition and importance to fishery (Fig. Fig. 2.12f).

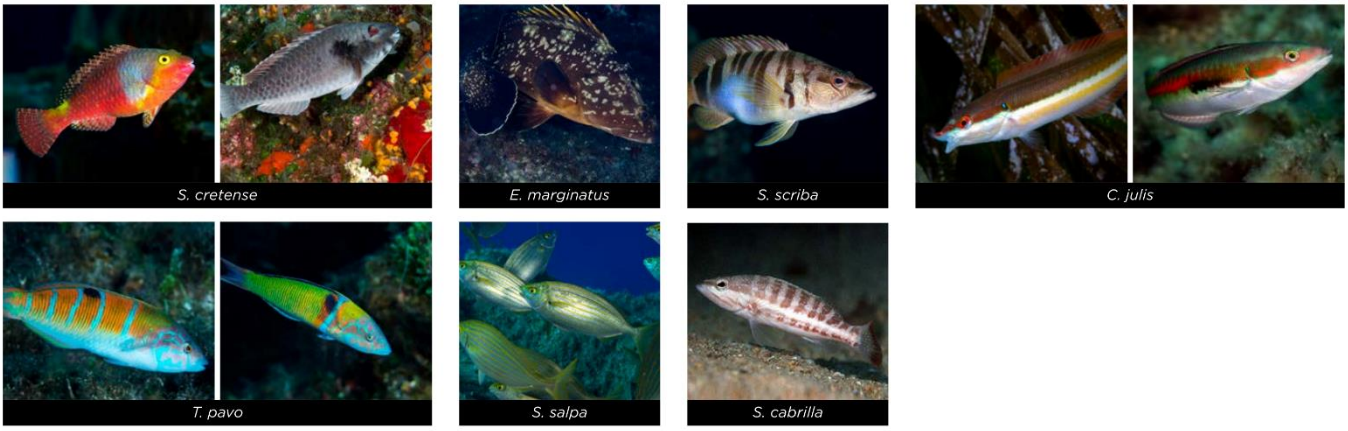

Fig. 2.8. Native target species selected for fish census of climate change indicators (adopted from Garrabou *et al*. 2022).

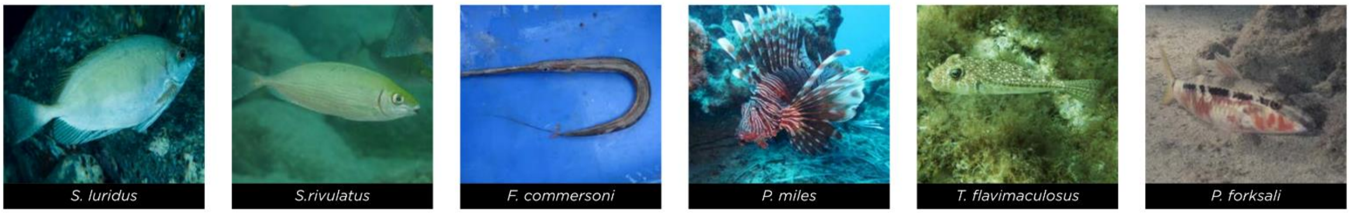

Fig. 2.9. Alien target species selected for fish census of climate change indicators (adopted from Garrabou *et al*. 2022).

Visual census was carried out within the two proposed depth ranges: 1-3 m and 5-10 m (Garrabou *et al*. 2022) at the same sites where protocol to assess *MedSens* index was carried out. At each site 4-8 transects were surveyed per depth range. Visual census consisted of slow forward swimming (at a speed of approximately 10 m/min for 5 minutes, covering a distance of about 50 m) and counting all the individuals of target species observed within a 5 m-wide transect (i.e. 2.5 m at each side of the imaginary transect, Fig. 2.10). Small individuals (less than 2 cm) were not counted. After an observation period of 5 min, a diver proceeded to swim in the same direction and after a pause of approximately 10 m (1 min), he/she started a new census/transect.

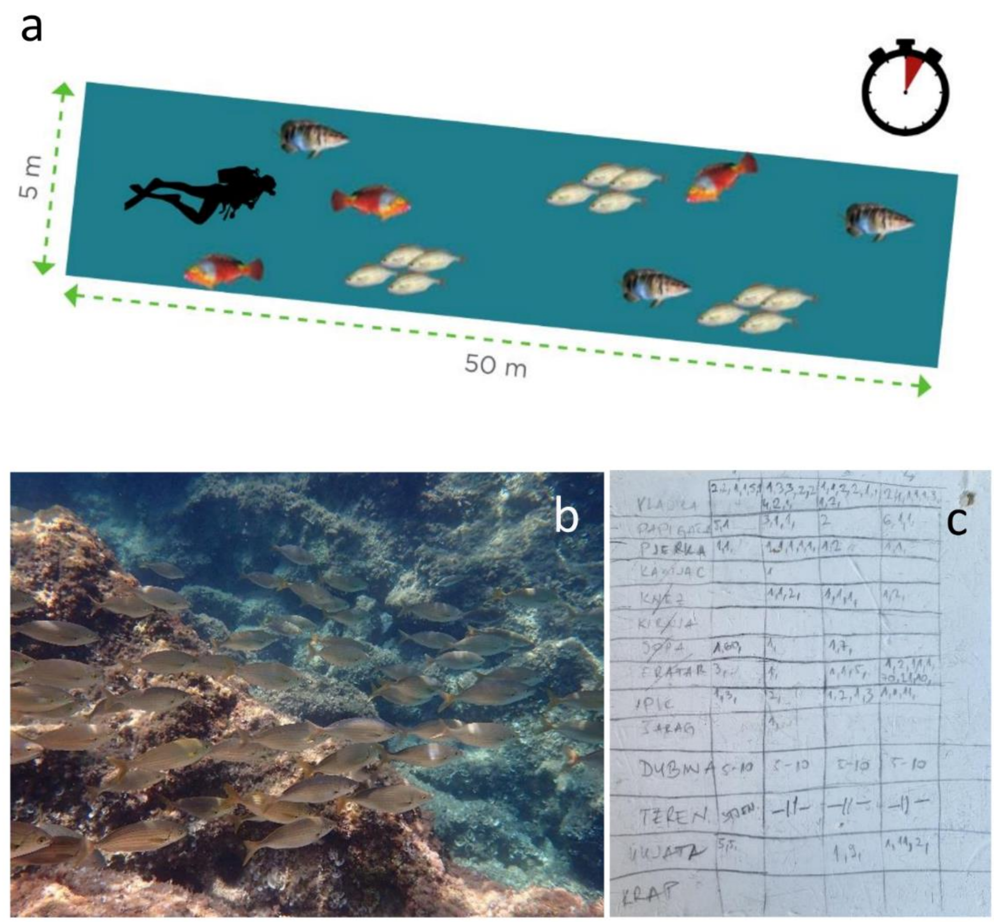

Fig. 2.10. Fish visual census of climate change indicators: a) scheme of a protocol (adopted from Garrabou *et al*. 2022); b) illustration of an underwater scene during fish visual census; c) an example of data recording. Photo credit: S. Kipson.

Species considered in the specific estimations related to climate change included: *Coris julis, Thalassoma pavo, Sarpa salpa, Sparisoma cretense* and all potential alien species (*e.g. Siganus spp*., *Fistularia commersonii, Pterois miles*). For that purpose, abundance data were transformed to presence/absence matrix and were used as such to calculate tropicalization index, according to the conceptual scheme presented in Fig. 2.11.

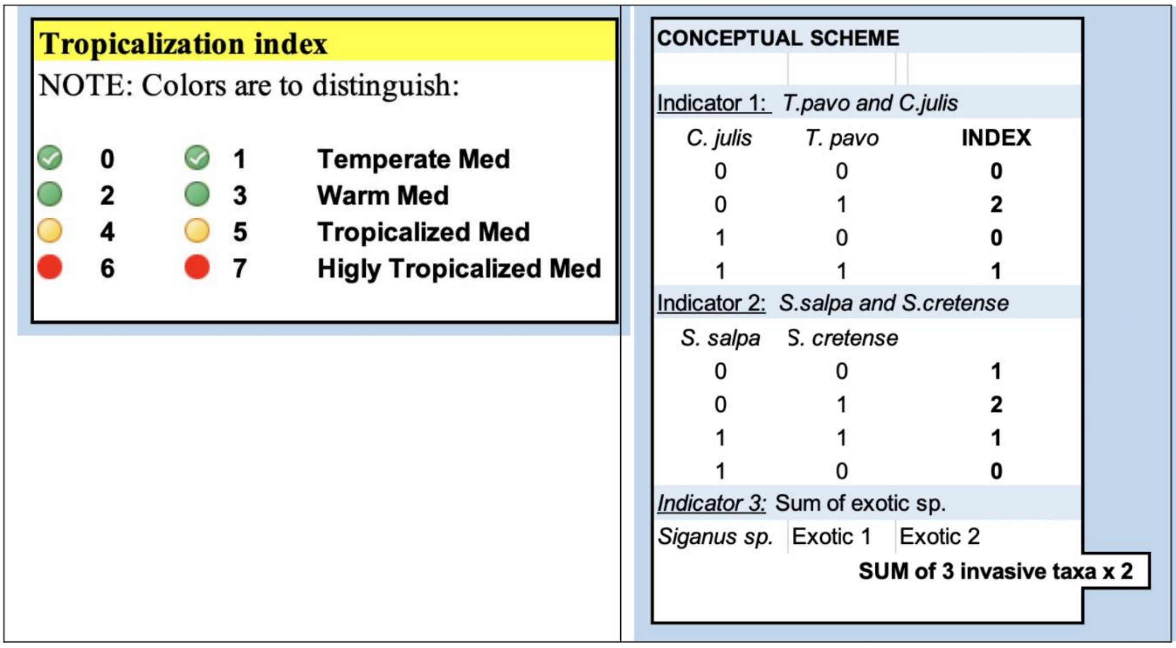

Fig. 2.11. Tropicalization index calculated as the sum of 3 different indices, based on transformed presence/absence matrix for: 1) *Coris julis* and *Thalassoma pavo*; 2) herbivore fish *Sarpa salpa* and *Sparisoma cretense* and 3) invasive alochtonous (alien) species (such as *Siganus* spp., *Fistularia commersonii*). Adopted from Stagličić *et al*. 2021.

2.2.2. Main results

Out of 11 target fish species, 6 were detected at all sites. Common species included *Diplodus vulgaris*, *Sarpa salpa*, *Serranus scriba*, *Coris julis* and thermophilic *Thalassoma pavo* and *Sparisoma cretense* (Figs. 2.12, 2.15). The most abundant species at all sites were *Sarpa salpa*, *Diplodus vulgaris* and *Thalassoma pavo*. Whereas the former one was more abundant in a depth range 1-3 m (accounting to 38-52% of all fish recorded, Fig. 2.13), the latter ones were more abundant in the depth range 5-10 m (Figs. 2.13 and 2.14). *Thalassoma pavo* was particularly abundant in the cove Pod manastijerom (South-West site), accounting for more than 40% of all fish recorded there. Moreover, at the South-West and North-West sites in particular, large schools of *Diplodus vulgaris,* numbering more than 100 individuals, have been observed, accounting to more than 30% of all observed fish (Fig. 2.13). Likewise, large school of *Oblada melanurus,* numbering more than 200 individuals, was observed along the south part of Portoć Bay (South station of the South-East site) in a 5 – 10 m depth range (Fig. 2.14). Clearly, for such large schooling fish variability in abundance per transect (250 m^2) was very high (resulting in pronounced standard errors of the means; Fig. 2.14, 5-10 m depth) as in some transects 100 or more individuals were observed, whereas in others at the same site and depth there were none or a few. Besides *Thalassoma pavo*, *Sparisoma cretense* as another thermophilic fish species was observed at all sites and depth ranges but in much lower abundance, accounting for 1-11% of recorded fish. Contrary to the North-West site where no *Ephinephelus marginatus* was spotted, up to 4 individuals - 2 in each depth range were recorded during census in the cove Pod manastijerom (South-West site).

In total, 2472 target fish were recorded at the Lokrum Island within 40 transects (of 250 m^2 each) in both depth ranges. Out of this, almost twice as much fish were observed in a depth range 5-10 m in comparison to 1-3 m (1557 *vs*. 915 individuals). None of the alien fish species were observed at any site around the Lokrum Island.

Due to the presence of *Coris julis, Thalassoma pavo, Sarpa salpa* and *Sparisoma cretense* at all investigated sites and in all assessed depth ranges, with concurrent absence of alien fish species, tropicalization index for each site and each depth range, and eventually for the Lokrum Island in total amounts to 2, indicating the warm Mediterranean area, but not yet considered as tropicalized (see the scheme in Fig. 2.11).

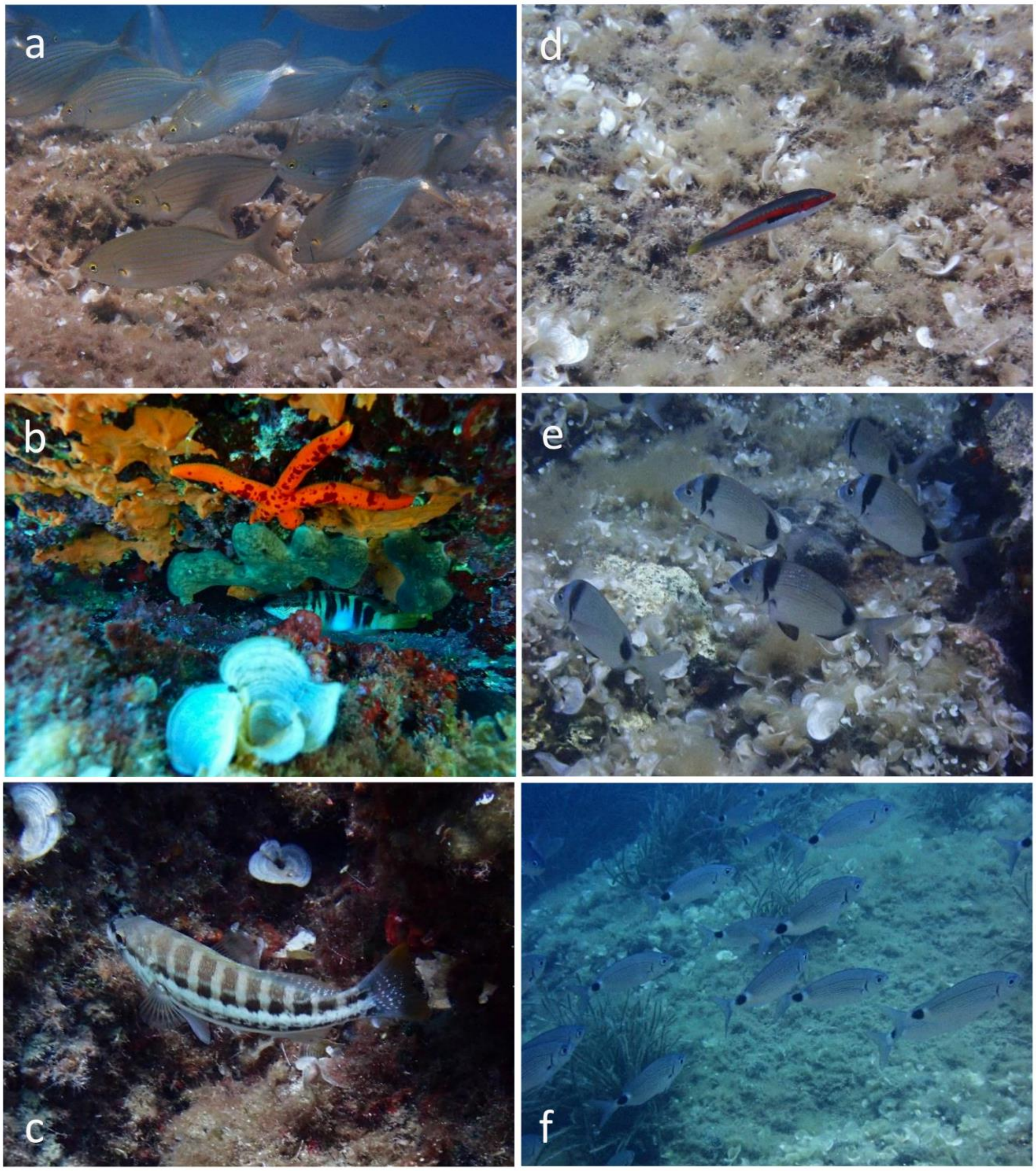

Fig.2.12. Observed native target fish species on the infralittoral reefs of the Lokrum Island: a) salema *Sarpa salpa, b)* painted comber *Serranus scriba, c)* comber *Serranus cabrilla, d)* Mediterranean rainbow wrasse *Coris julis, e)* common two-banded sea bream *Diplodus vulgaris* and f) saddled sea bream *Oblada melanurus*. Photo credit: S. Kipson.

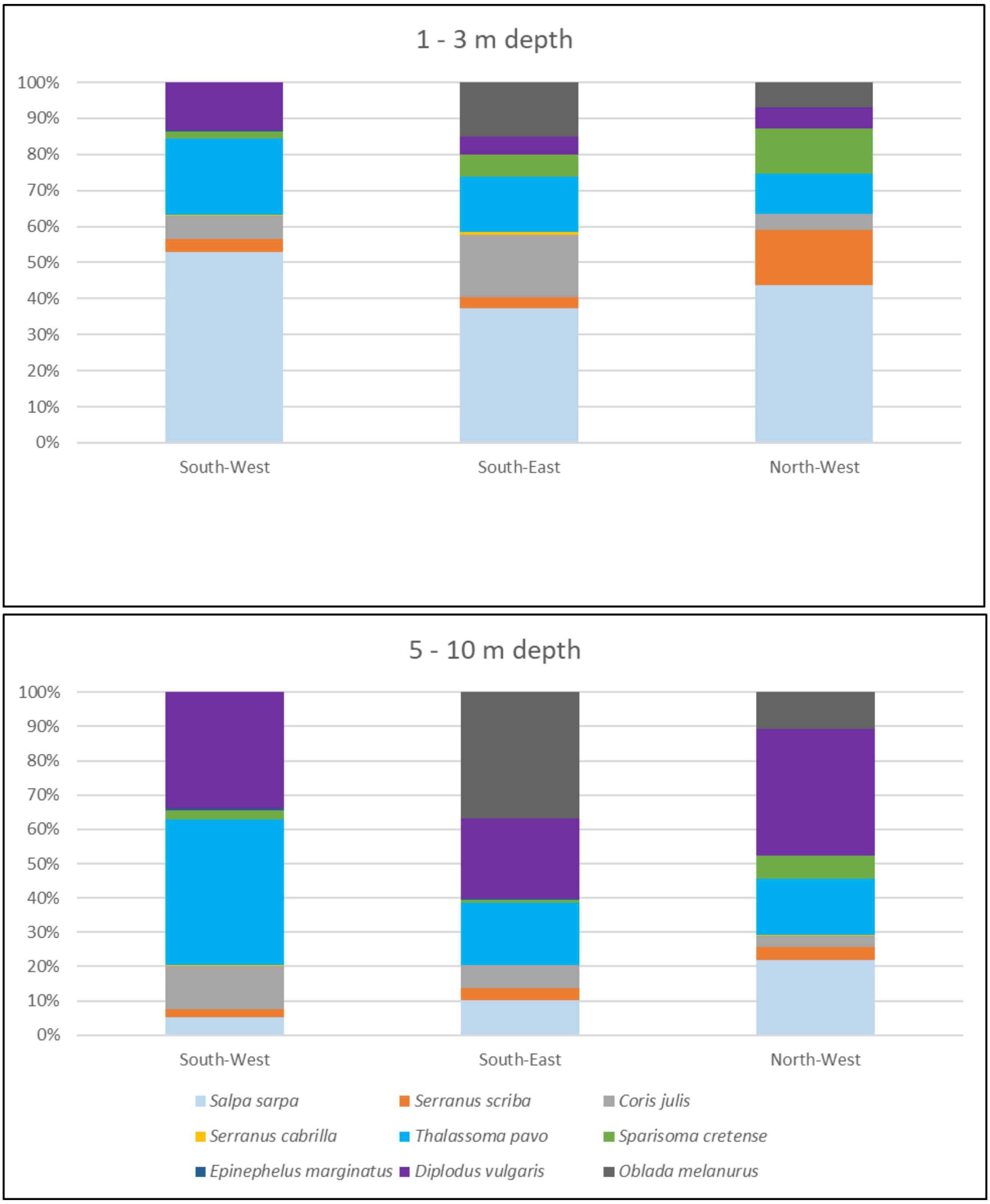

Fig. 2.13. Composition of target fish assemblages based on their presence and abundance at 3 sites around the Lokrum Island within two investigated depth ranges (1-3 m and 5-10 m).

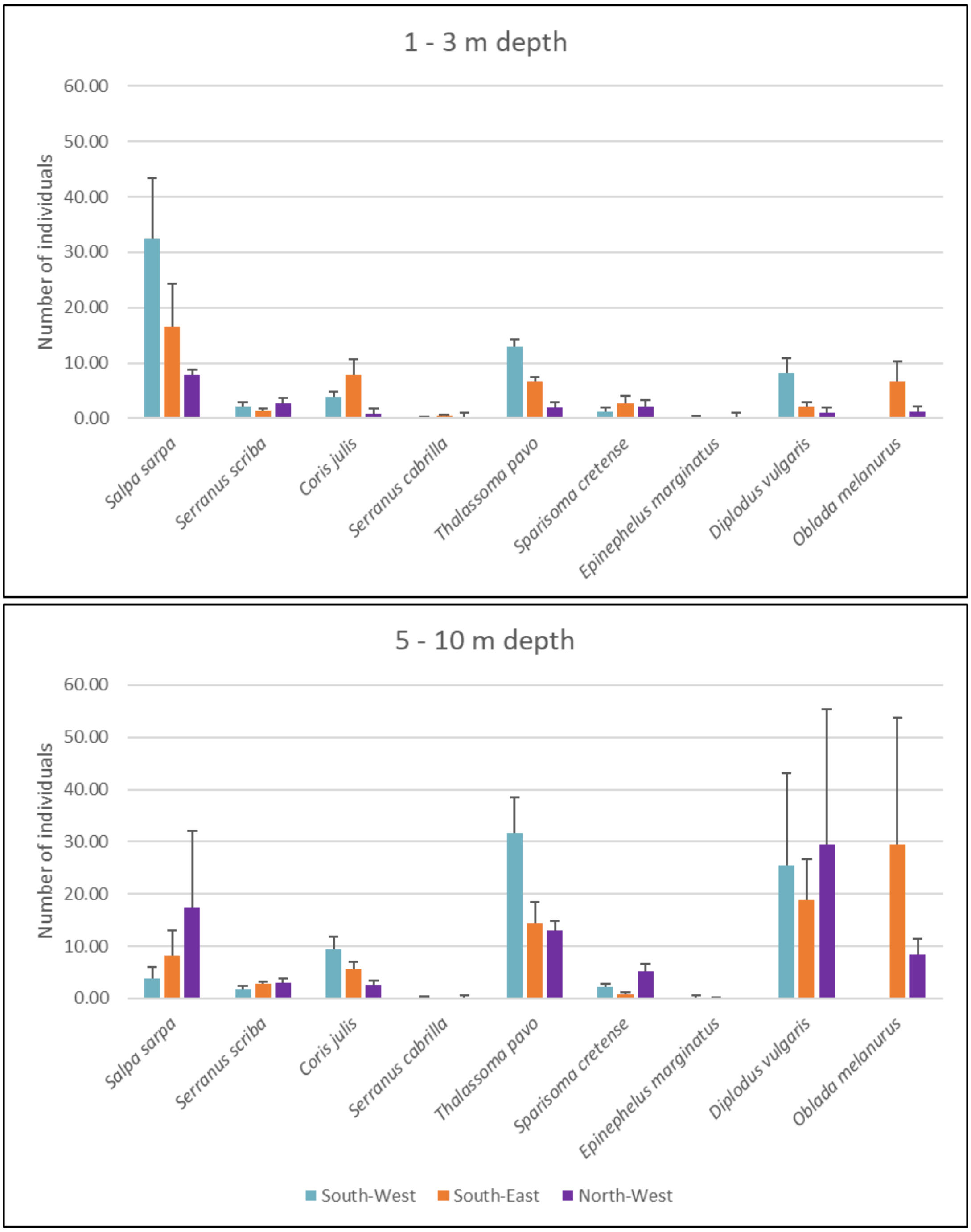

Fig. 2.14. Abundance of target fish species per 250 m^2 assessed by visual census in two depth ranges (1–3 m and 5–10 m) at 3 sites on the Lokrum Island. Data are shown as mean ± SE.

Fig. 2.15. Illustration of two out of three thermophilic species observed at the Lokrum Island sites: a) the Mediterranean parrotfish *Sparisoma cretense* (Linnaeus, 1758) and b) the Ornate wrasse *Thalassoma pavo* (Linnaeus, 1758). Photo credit: S. Kipson.

Fig. 2.16. Mediterranean moray eel *Muraena helena* Linnaeus, 1758 was frequently observed on shallow infralittoral reefs around Lokrum Island. Photo credit: S. Kipson.

Fig. 2.17. Abundant cardinal fish *Apogon imberbis* associated to infralittoral reefs of the Lokrum Island. Photo credit: S. Kipson.

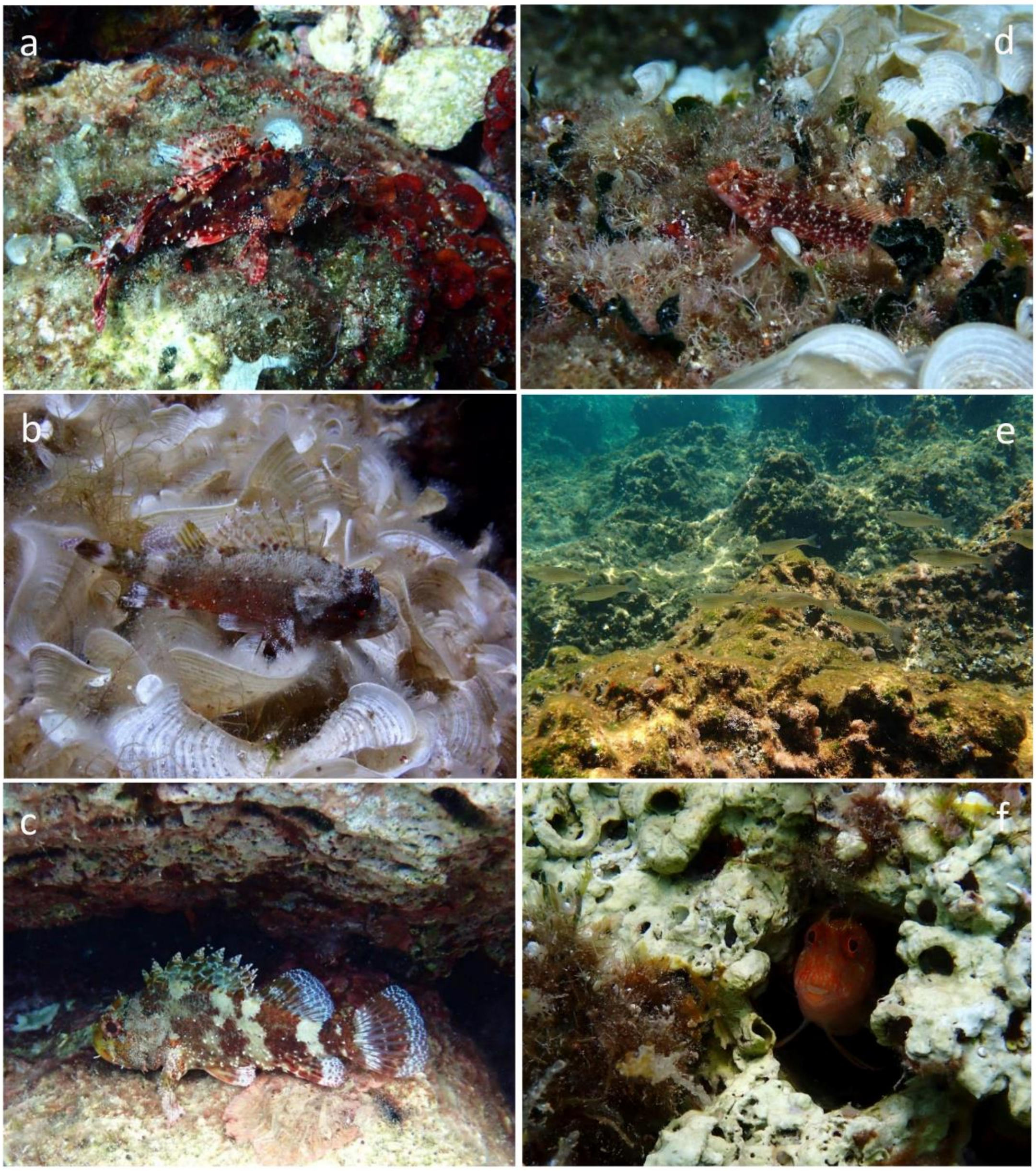

Fig. 2.18. Some of the fishes frequently encountered on infralittoral reefs around the Lokrum Island: a-c) Small red scorpionfish *Scorpaena notata* Rafinesque, 1810 was abundant and present in various colours, d) a female of Red black-faced triplefin *Tripterygion tripteronotum* (Risso, 1810), e) Thicklip grey mullet *Chelon labrosus* (Risso, 1827) and f) a blenny (family Blenniidae). Photo credit: S. Kipson.

2.2.3. Conclusions

Fish abundance and diversity (including species not targeted by this protocol) was notable at the Lokrum Island, contributing considerably to the attractiveness of recreational snorkeling and diving there, an important aspect of the local tourism offer (Figs. 2.12, 2.16-2.18). Especially attractive were large schools of the common two-banded sea bream *Diplodus vulgaris,* the saddled seabream *Oblada melanurus* and salema *Sarpa salpa* (Fig.2.12), abundance of the small red scorpionfish *Scorpaena notata* (Fig. 2.18a-c) and the cardinal fish *Apogon imberbis* (Fig. 2.17) often observed out in the water column, contrary to shady/dark habitats where this sciaphilous species usually reside during daytime (Bussotti *et al*. 2018), as well as fairily common sightings of the moray eel *Muraena helena* (Fig. 2.16) and occassional sightings of more elusive fish such as the brown meagre *Sciena umbra* and the dusky grouper *Epinephelus marginatus.*

Overall, all three thermophilic species assessed by this visual census, i.e. *Sparisoma cretense, Ephinephelus marginatus* and *Thalassoma pavo,* are present in the waters of the Lokrum Island, with the latter one reaching up to 40 % of recorded fish at some site s (e.g. the cove Pod manastijerom, South-West site). How their abundance changed with time is unknown, since there is no previous study. Hence, there is a need for longer term application of this simplified protocol at the Lokrum Island to enable reliable interpretation of the data. Based on the data provided here and regular future monitoring, throughout time it will be possible to improve the understanding of natural variability or detect additional effects of pressures such as climatic fluctuations i.e. the consequent biological response in terms of changes in composition and structure of fish assemblages. In addition to researchers, the engagement of citizen science, i.e. observations provided by trained and supervised volunteer SCUBA divers and/or snorkelers is especially valuable way to enhance underwater work effort and to assist limited staff of the Public Institution Lokrum. This may be especially true for the sightings of alien species around Lokrum. Although none of the alien fish were observed during this study, such sightings are highly likely here in the South - the forefront of biological invasions in the Adriatic basin but are also dependant on the observational capacity that should be thus increased in a most cost-effective way, such as through the application of citizen science.

3. ADDITIONAL MONITORING PROPOSALS AND OBSERVATIONS

3.1. Proposal of additional monitoring protocol: Assessment and monitoring of mass mortalities

Beside two protocols applied in this study (assessment of *MedSens* index and Fish Visual Census), as a part of the effort to further monitor responses of a rocky bottom community to pressures, we propose also to apply the protocol advocated by Garrabou *et al*. (2022) which includes a rapid assessment of conservation status of populations of selected sessile benthic macroinvertebrates, thus gathering also baseline information for the assessment of the impacts of mass mortality events if/when they occur. Although the mass mortality events are mainly associated with the onset of marine heatwaves, there are other stressors such as severe storms, blooms of mucilagenous algal species and sedimentation that can result in similar effects (Garrabou *et al*. 2022).
Target species of this protocol are easy to identify underwater and are sufficiently abundant in the surveyed area. Since there were no previous data on which we could base the selection of good target species for Lokrum area before this survey, they could be only proposed now, based on field observations. Fullfiling the above-mentioned criteria, the proposed species to observe within their upper depth limit include sponges *Petrosia ficiformis* and *Chondrosia reniformis as well as* bryozoan *Myriapora truncata* (Figs. 3.2, 3.4a, 3.5a). Whereas these two sponges may be found in greater abundance within photophilic algae assemblages around 3-5 m depth, bryozoan *Myriapora truncata* was equally abundant throughout the 5 – 12 m depth range but constricted to more shaded environments such as overhangs, cavities and crevices.

The protocol consists of observations being made along the imaginary transect at the selected depth (± 1 m), at minimum of 3 sites separated by at least 200 m. Observer counts each specimen of selected species and notes if it healthy (Figs. 3.2, 3.4a, 3.5a) or is affected, i.e. if any tissue necrosis is present (e.g. Figs. 3.4b, 3.6, 3.7-3.9; see Garrabou *et al*. 2022 and linked video tutorials for more details). Ideally, a minimum of 100 specimens would be assessed per mortality survey. However, if the species is not abundant in the monitoring site the total number of observed specimens per survey can be reduced to 20-30 (Garrabou *et al*. 2022). Due to severe time constraints of this field study, we were not able to dedicate sufficient time to appropriately execute this protocol. However, some observations can be made that would, hopefully, assist in future monitoring. For sponge *Petrosia ficiformis* in particular, various signs of unhealthy specimens were observed during this field study, ranging from more subtle changes in pinacoderm (external layer) evident upon closer inspection of the specimens to clearly visible damaged/necrosed tissue. Herein, in Figs. 3.7-3.9 I attempted to illustrate different examples of unhealthy sponges. It is noteworthy to emphasize that majority of observed specimens of *Petrosia ficiformis* (although not observed in sufficient number for a proper assessment) showed signs of affectation.

On the contrary, *Chondrosia reniformis* generally appeared unaffected, although in this case also more subtle signs of illness should not be overlooked, such as the presence of a thin exfoliating transparent layer observed in some specimens (Fig. 3.6). Related to this sponge, one should be cautioned that more varied coloration of *Chondrosia reniformis* (e.g. grey with more of white specks) should not be interpreted as a sign of affectation. As a reminder, pigmentation of this sponge can vary considerably depending on its habitat – from dark brown-grey specimens inhabiting more photophilic zone (see for example Fig. 3.5a) to completely white specimens thriving in biocenosis of semi-dark caves, with a lot of variation in between, (see for example Fig. 3.5b showing light greyish specimen thriving in a shaded cavity at the Lokrum Island).

Affectation of the bryozoan *Myriapora truncata* was mainly observable as the partial overgrowth of its skeleton by different epibionts (see Fig. 3.4b).

Focused hands-on field excercises are needed to train properly the observers and to enhance their ability to recognize different signs of organisms' sickness/damage. Besides *in situ*, assessment can be successfuly carried out from quality underwater images and/or video.

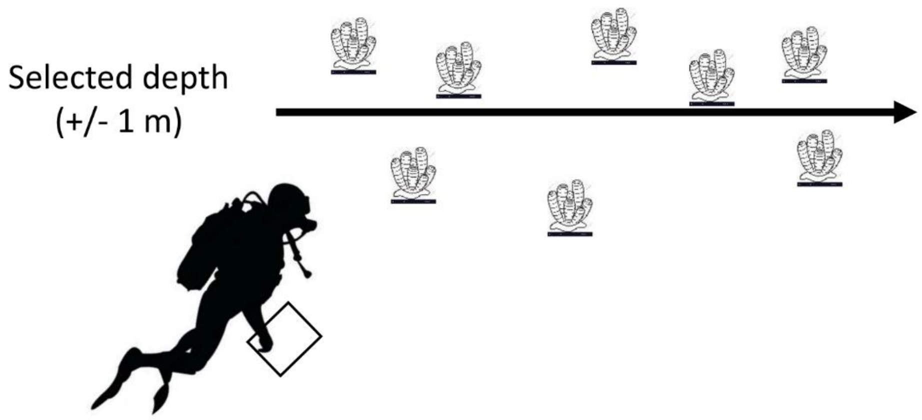

Fig. 3.1. Illustration of the protocol for the assessment and monitoring of mass mortalities of benthic species according to Garrabou *et al*. 2022.

Fig. 3.2. Example of the healthy specimen of the sponge *Petrosia ficiformis.* Photo credit: S. Kipson.

Fig. 3.3. Occasionally large specimens (or several specimens growing immediately next to each other) of sponge *Petrosia ficifirmis* were observed, as illustrated here for the South-West site (the cove Pod Manastijerom) at 5 m depth. Approximate horizontal width of this cluster of sponges is 80 cm. Photo credit: S. Kipson.

Fig. 3.4. Potential target bryozoan species *Myriapora truncata*: a) healthy specimen and b) affected specimen exhibiting old partial mortality, with portions of skeleton covered by epibionts. Photo credit: S. Kipson.

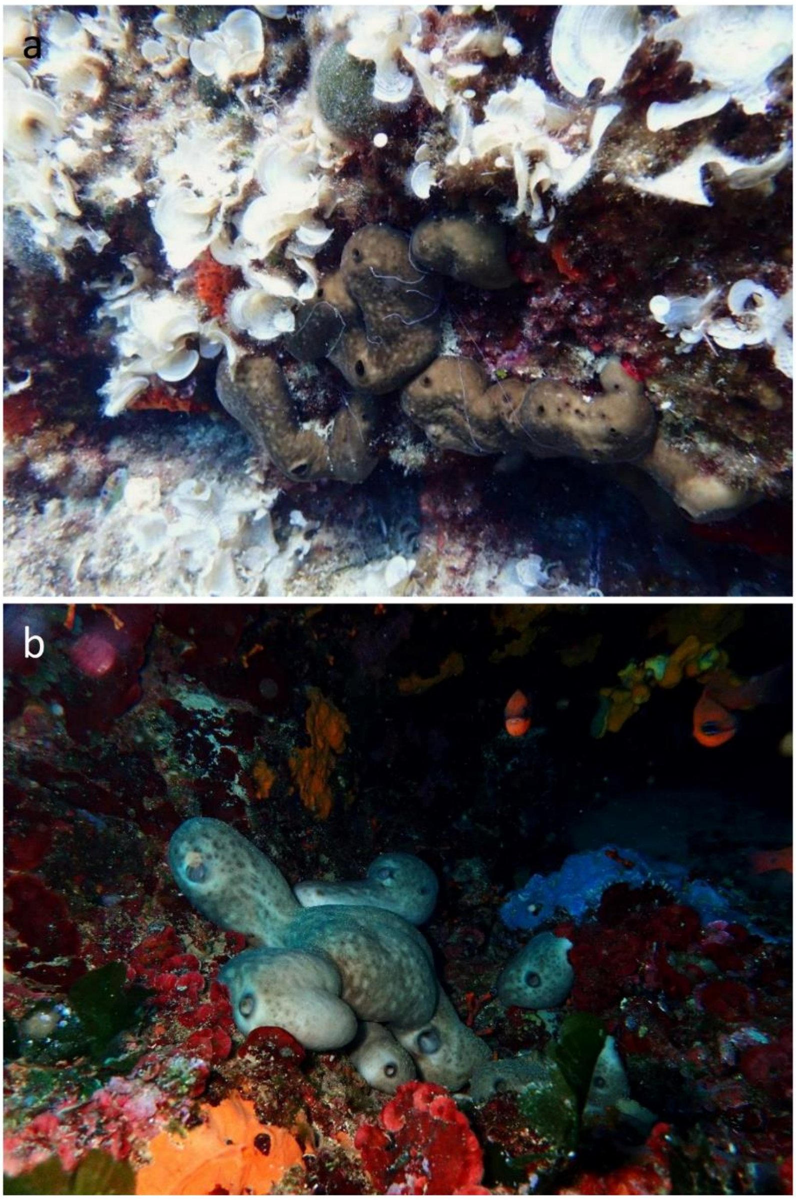

Fig. 3.5. Another potential target sponge species *Chondrosia reniformis*: a) healthy specimen of usual brownish color and b) healthy specimen with less pigment adapted to more sciaphilic environment within a crevice – individuals thriving in more pronounced dark habitats may be almost completely white. Photo credit: S. Kipson.

Fig. 3.6. Affected sponge *Chondrosia reniformis* – presence of a thin exfoliating transparent layer observed in some specimens. Photo credit: S. Kipson.

Fig. 3.7. Examples of affected specimens of the sponge *Petrosia ficiformis* observed at the Lokrum Island (illustration No. 1): damaged pinacoderm ranged from the one observable only upon closer visual inspection of the sponge (rough surface instead of „smooth“, e.g upper image row) to specimens with visible discoloured patches of epidermal cells. Photo credit: S. Kipson.

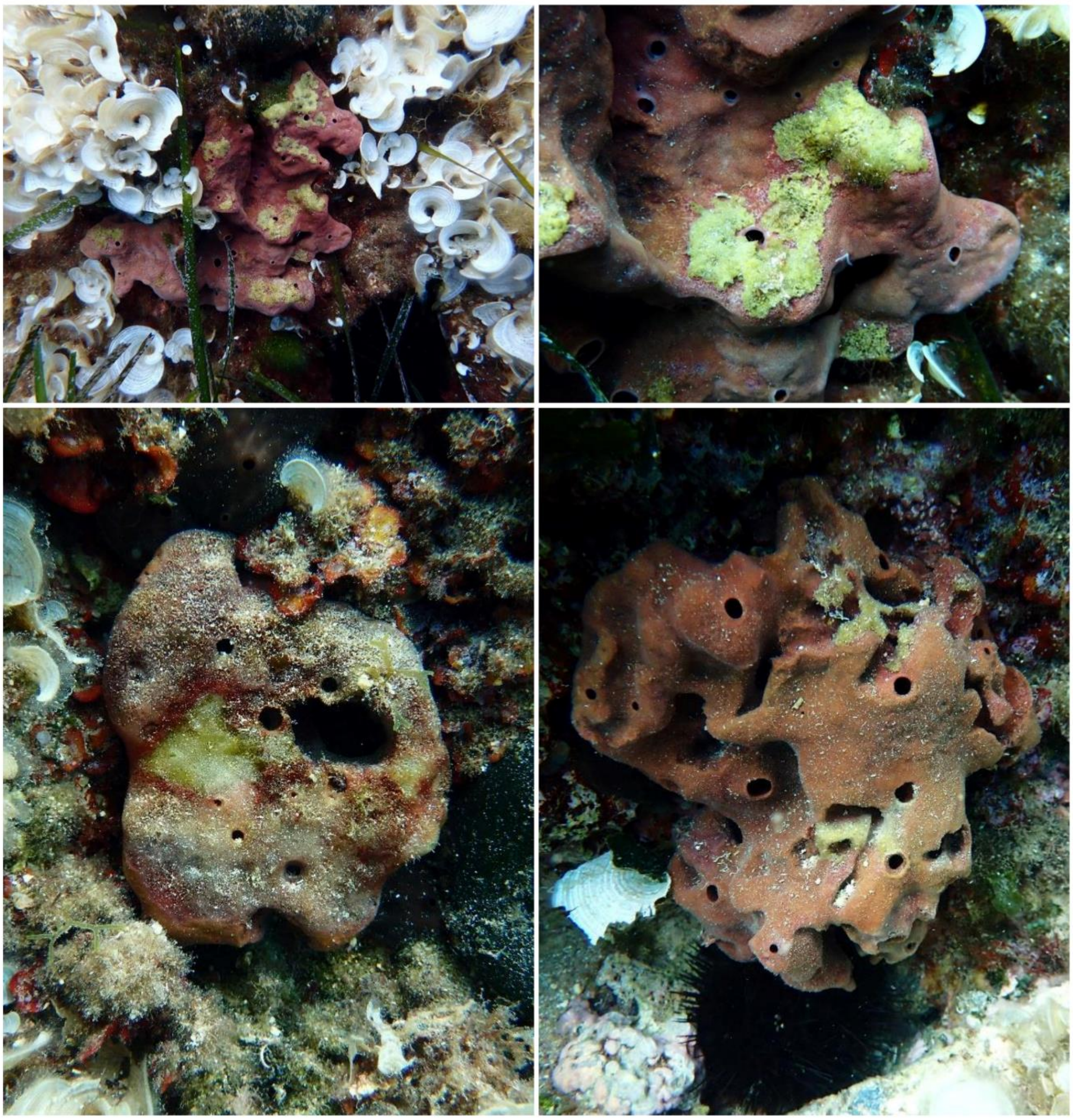

Fig. 3.8. Examples of affected specimens of the sponge *Petrosia ficiformis* observed at the Lokrum Island (illustration No. 2): specimens with partial “crust” on the surface and partial damage – healthy individuals are not prone to epibiosis. Photo credit: S. Kipson.

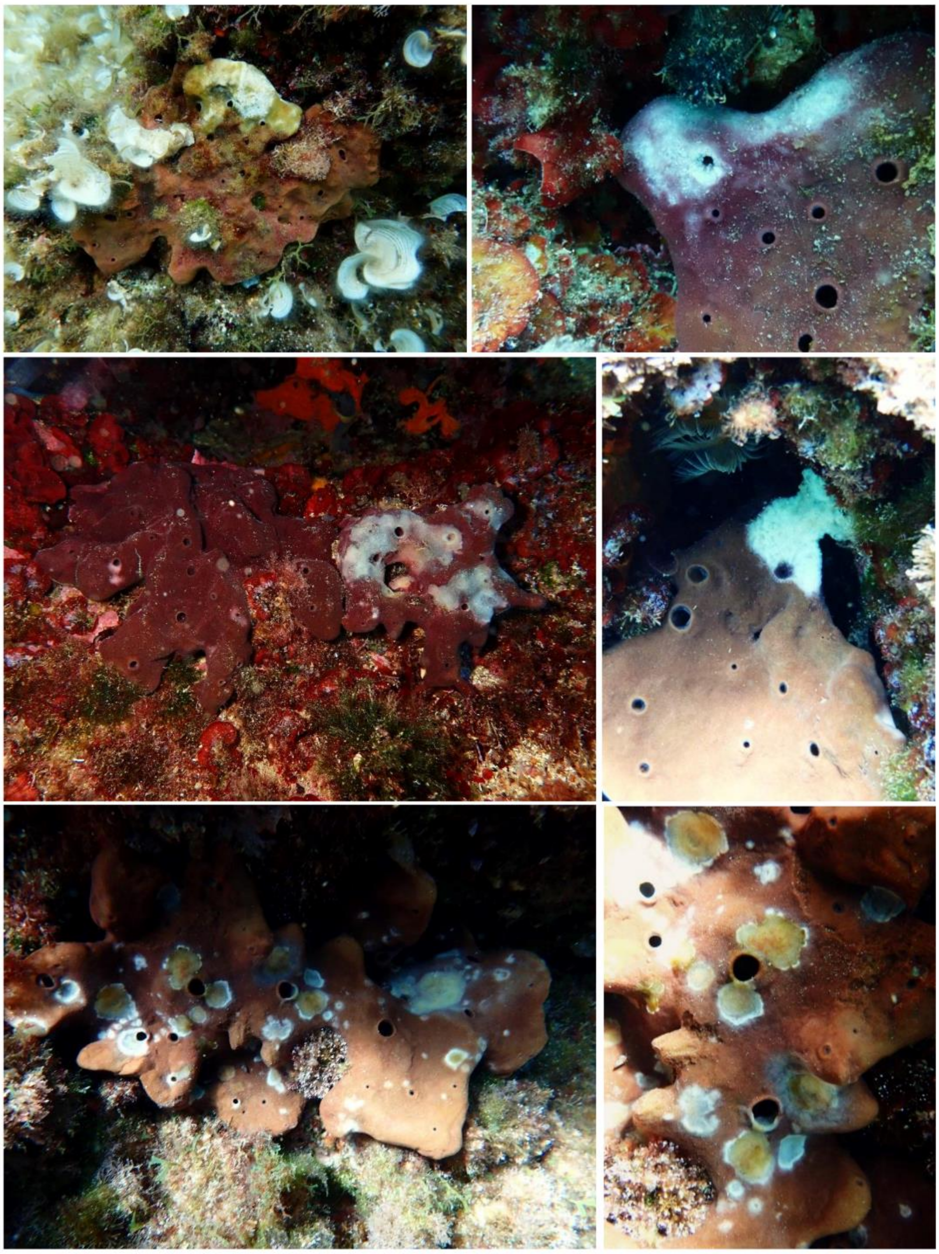

Fig. 3.9. Examples of affected specimens of the sponge *Petrosia ficiformis* observed at the Lokrum Island (illustration No. 3): specimens with white blotches on the surface and partial tissue damage. Photo credit: S. Kipson.

3.2. Other observations on biodiversity including rare, sensitive and/or protected marine species and pressures

Crustose coralline algae

It is noteworthy that several species of crustose coralline algae were observed during our dives and although most of them cannot be identified without the physical sample, some of them may be identified as belonging to the *Litophyllum stictaeforme* complex (S. Kaleb, *pers. comm*., Fig. 3.10). Unfortunately, many specimens of encrusting corallline algae between 5 and 12 m depth partially affected, bleached (Fig. 3.11). These algae showed heavy exfoliation of epithelium cells, and some of them also showed signs of grazing (Fig. 3.12). Putatively, exfoliation may be happening as an anti-grazing response, in an attempt to remove epiphytes that are of interest to grazers, but on the other hand exfoliation may increase vulnarability of CCA and make them even more palatable to predators (S. Kaleb, *pers. comm*.). As for bleaching, the exact causes are hard to tell, but elevated seawater temperature during marine heatwaves are known to affect this marine group (Garrabou *et al*. 2022) and in 2023 and 2024 many temperature records were broken. In fact, marine heatwaves were recorded in the area exhibiting different duration and intensity since July 2023 till May 2024 (see Figs. 3.21, 3.22). Related to potential predators, encrusting coralline algae present most frequent prey item in the starfish *Ophidiaster ophidianus* diet (Di Trapani *et al*. 2020) as well as it presents a notable portion in a diet of sea urchin *Arbacia lixula* (Wangensteen *et al*. 2011). Both species were frequently observed around Lokrum Island (see the section on echinoderms). Whatever the underlying reason, it is evident that many encrusting corallline algae show signs of multiple stress and did not appear healthy at the Lokrum Island in the investigated period.

Crustose coralline algae are macrobentic group of major importance for structure and function of benthic communities. They induce settlement and recruitment of numerous invertebrates and provide habitats for a variety of grazing and burrowing infauna (Chenelot *et al*. 2011). In addition, crustose coralline algae are also of significant importance in the carbon and carbonate cycles of shallow coastal ecosystems, being major contributors to CO_2 fluxes through high community $CaCO_3$ production and dissolution (McCoy & Kamenos 2015). Hence, the causes of their decline merrit a dedicated investigation in the future.

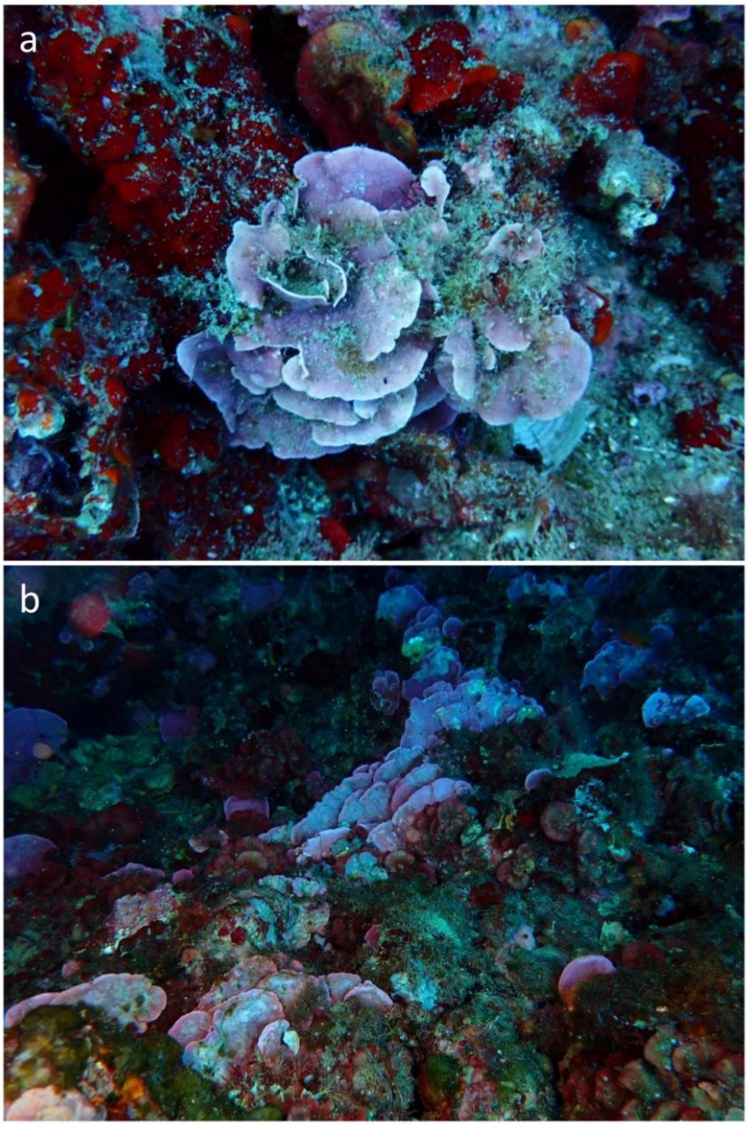

Fig. 3.10. Crustose coralline algae belonging to the *Litophyllum stictaeforme* complex found in: a) slightly shaded or b) sciaphilic environments of the rocky crevices and overhangs. Photo credit: S. Kipson.

Fig. 3.11. Affected crustose coralline algae exhibiting heavy exfoliation and partialy bleaching. Photo credit: S. Kipson.

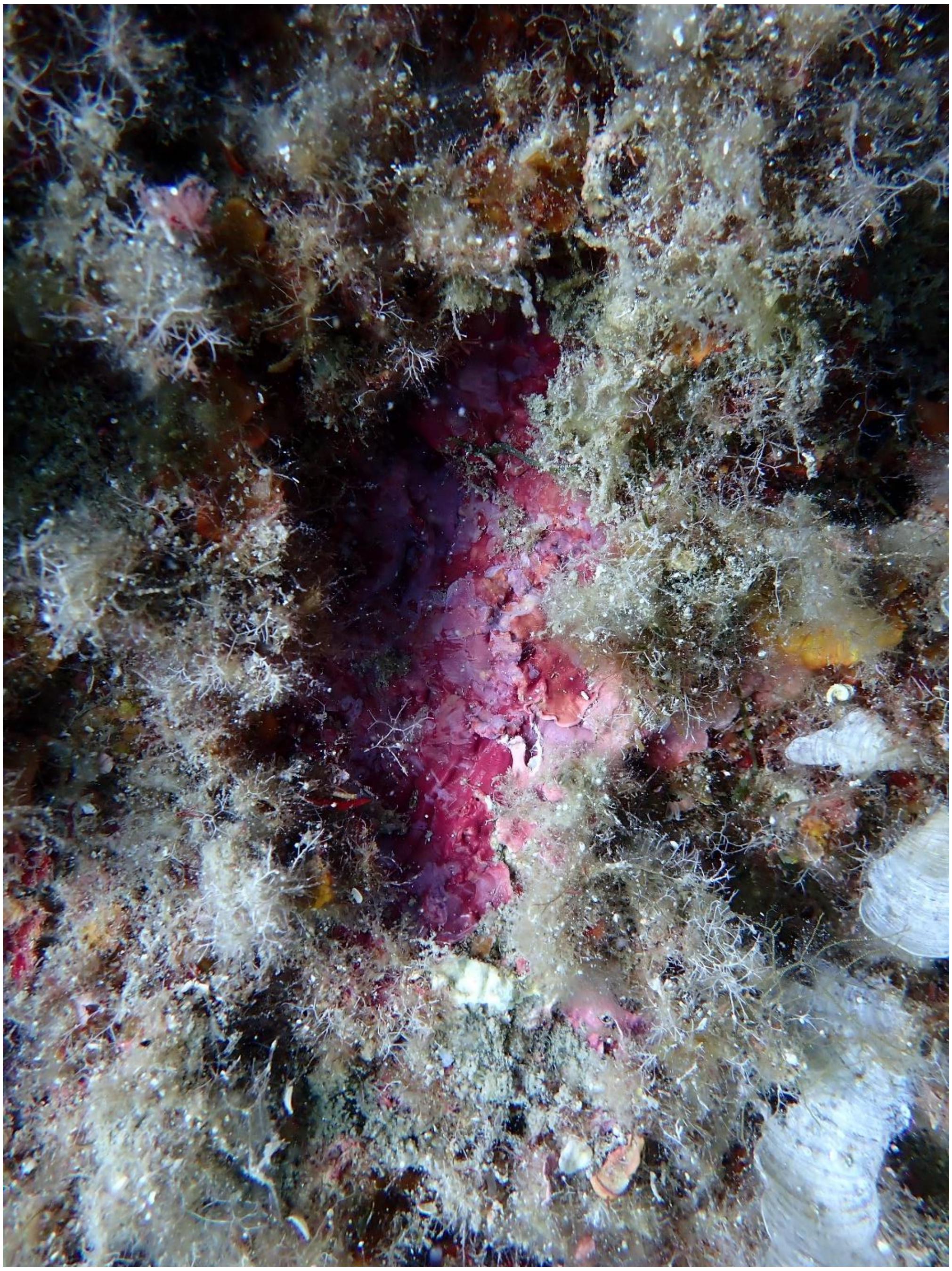

Fig 3.12. Affected crustose coralline algae exhibiting heavy exfoliation and signs of grazing (mark similar to the scratch with the fork in the central part of the algae). Photo credit: S. Kipson.

Echinoderms

Furthermore, as already mentioned, the abundance of the starfish *Ophidiaster ophidianus* (Lamarck, 1816), a strictly protected species by the national Nature Protection Act (OG 80/13) and the Ordinance on Strictly Protected Species (OG 73/16), was notable on the Lokrum Island. The species is also protected under the Barcelona and Bern Conventions. It is a large conspicuous starfish (tip-to-tip diameter up to 40 cm), easily recognizable by its small disc and five arms that are moderately long and cylindrical, with rounded tips. Individuals may vary from orange-red, with or without brown spots, to bright red (Fig. 3.13). This species occurs on rocky reefs from shallow (< 5 m deep) to deep waters (> 100 m deep). As a thermophilic species whose northward migration has been widely documented in the recent years supporting the linkage between climate change and latitudinal variation of the Mediterranean Sea biodiversity, it is considered to be a meaningful indicator of climate change and meridionalization of the northern Mediterranean Sea (see Di Trapani *et al*. 2020 and references therein).

Although not assessed as a target species, between 6 and 25 specimens were noted during *MedSens* visual censuses (hence in the time frame of 25 min) and by far it was the most abundant starfish observed during this field trip (with abundance being higher in the depth range 4-5 m than 10-12 m). Interestingly, putative feeding of polychaete *Hermodice carunculata* on the dead specimen of this starfish was also observed (Fig. 3.14).

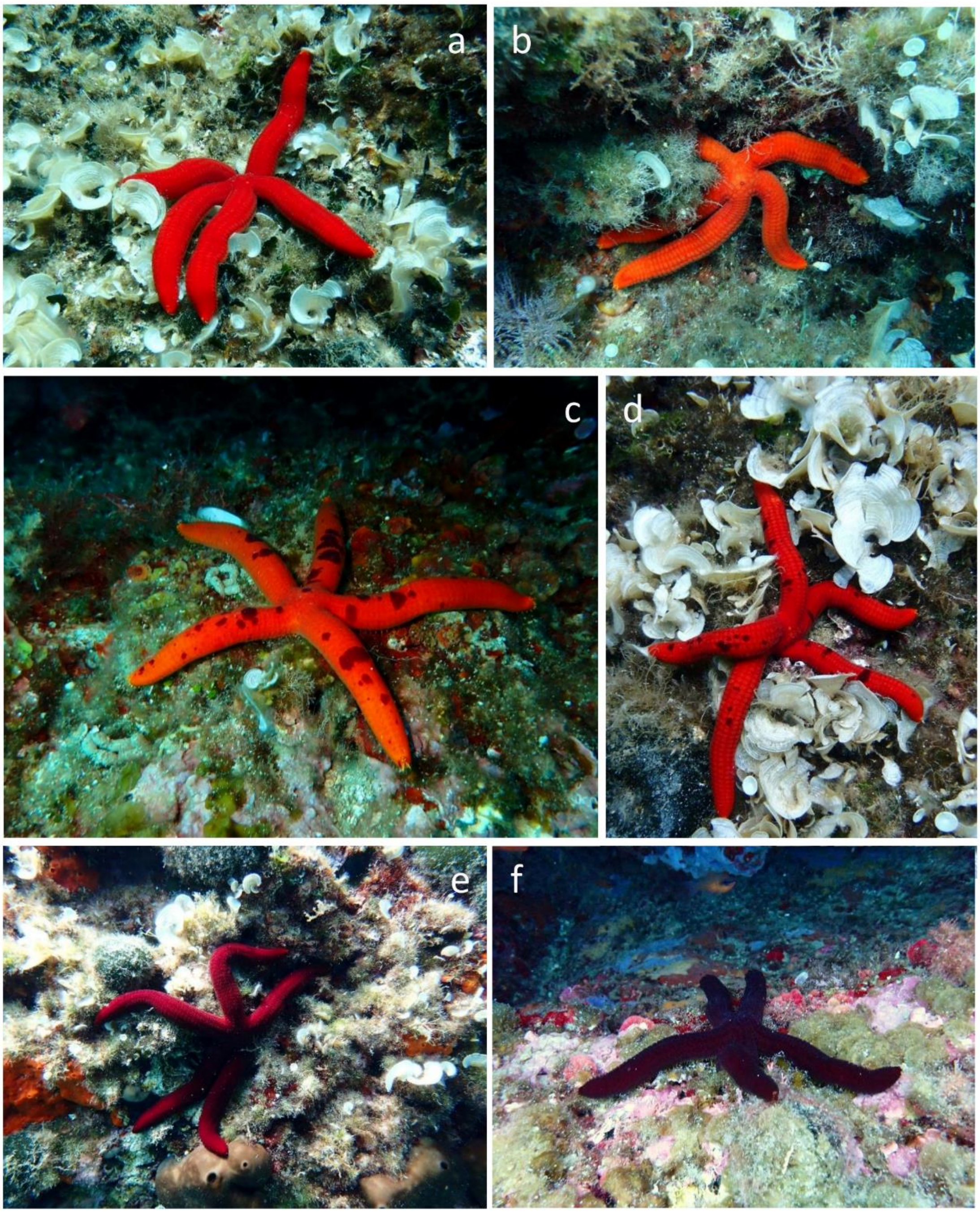

Fig. 3.13. Strictly protected starfish *Ophidiaster ophidianus* is a termophilic species present in all 3 color varieties at the Lokrum Island: a,b) orange; c,d) orange with brownish spots; e,f) dark red. Photo credit: S. Kipson.

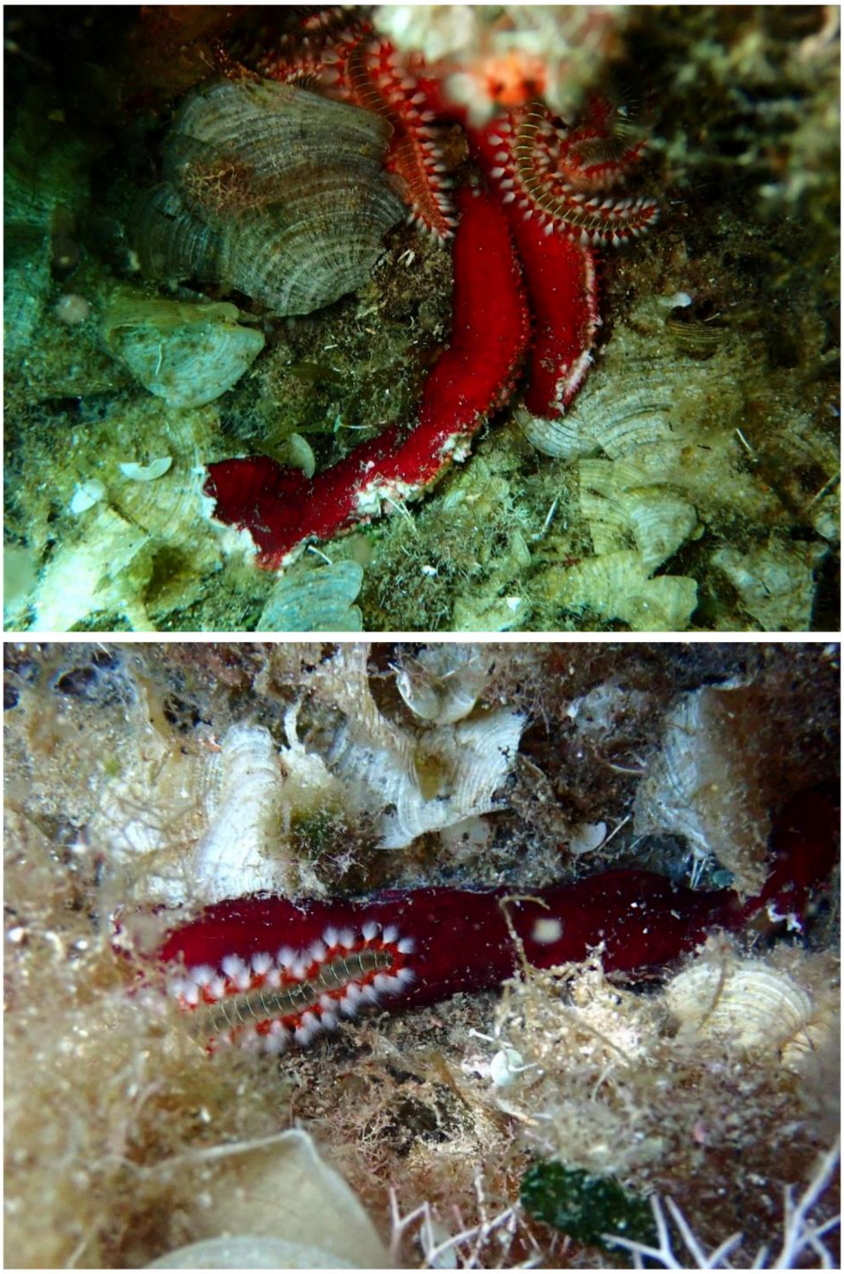

Fig. 3.14. Polychaete *Hermodice carunculata* gathering on a carcass of a starfish *Ophidiaster ophidianus* and likely feeding on it. Photo credit: S. Kipson.

Besides *O. ophidianus*, which was the most abundant starfish, *Echinaster sepositus* and *Hacelia attenuata* were also observed (Fig. 3.15).

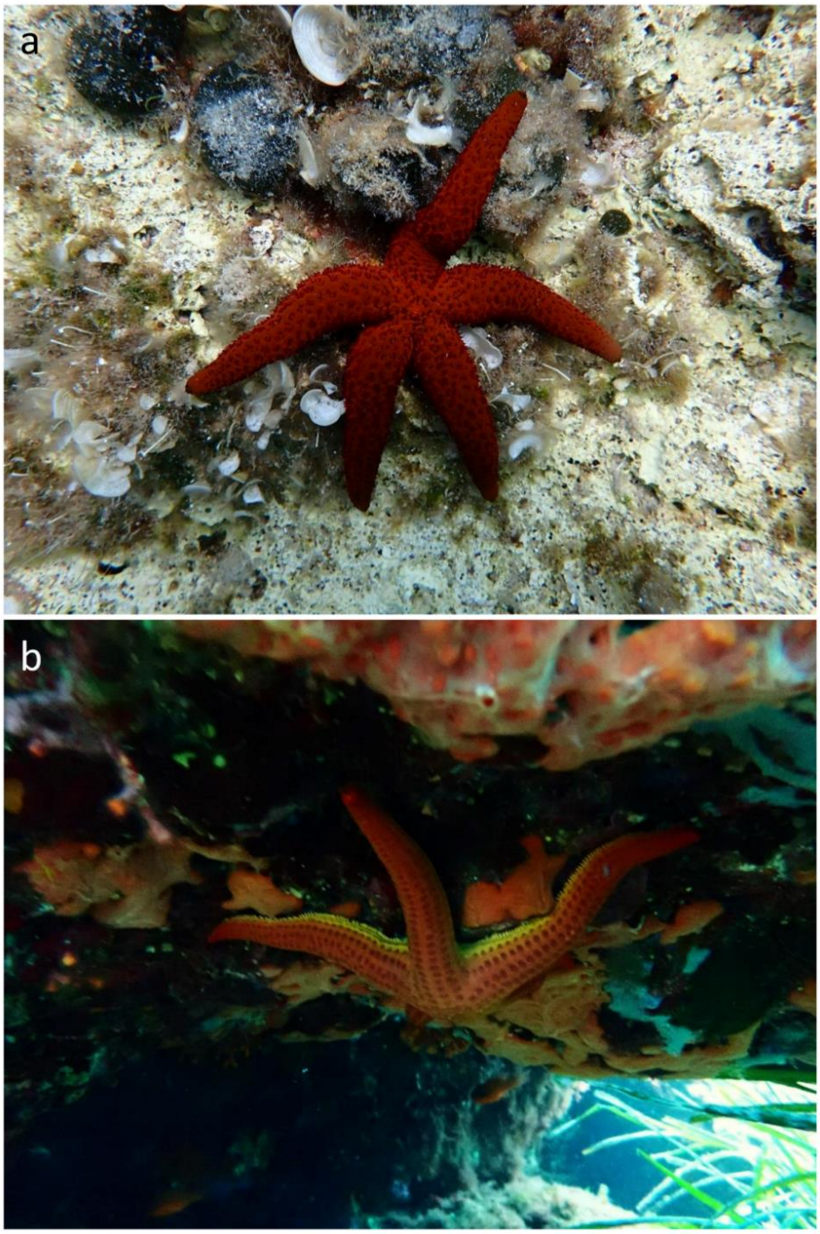

Fig. 3.15. Other frequently observed starfish: a) *Echinaster sepositus* and b) *Hacelia atenuata*. Photo credit: S. Kipson.

Out of other Echinodermata, black sea urchin *Arbacia lixula* (Linnaeus, 1758) was present in almost equal (high) abundance as *Paracentrotus lividus*, Fig. 2.4b). Their possible co-existence in the same habitat at high densities is mainly explained by their different feeding strategies. Whereas *Paracentrotus lividus* is predominantly a herbivore with preference for erect seaweeds, *Arbacia lixula* is an omnivore tending to carnivory in Mediterranean ecosystems. It feeds at least partially on sessile animals such as Cirripedia, Hydrozoa or Bryozoa and out of vegetal components, crustose coralline algae comprise a considerable portion of its diet (Wangensteen *et al*. 2011). As the above-mentioned starfish *Ophidiaster ophidianus*, *Arbacia lixula* is also a thermophilic species, and the ongoing warming of the Mediterranean is creating an environment increasingly favourable for its reproduction and larval development (Di Trapani *et al*. 2020 and references therein). In this context, it would be of interest to monitor populations of both species in the future and to improve also understanding of their potential impacts (through feeding habits or other types of species interactions) on community dynamics, biodiversity and ecosystem functioning.

Out of the other protected echinoderms, several Holothurians (sea cucumbers) were observed, with *Holothuria (Platyperona) sanctori* Delle Chiaje, 1823 being among the most abundant ones (Fig. 3.16). As a reminder, all species of sea cucumbers are protected by the national Nature Protection Act[1] (OG 80/13) and the Ordinance on Strictly Protected Species (OG 73/16).

[1] cro. *Zakon o zaštiti prirode*

Fig. 3.16. One of the most commonly observed sea cucumbers at the Lokrum Island was attractive *Holothuria (Platyperona) sanctori.* Photo credit: S. Kipson.

Bivalves

As already mentioned (see Section 2.1.2), although the noble pen shell *Pinna nobilis* is on the *MedSens* list of species, it was not considered in the visual census, due to Mediterranean-wide mass mortality events related to the disease and unlikely occurence of alive specimens (based on the data stemming from regular monitoring by the Public Institution Lokrum). However, out of observed bivalve species, two records of co-generic species, strictily protected *Pinna rudis* are noteworthy. Both alive individuals were observed on the South-East site, around Portoć bay: the first specimen (approximately 12 cm in maximum shell width) was found at 12 m depth south of Portoć (South station of the South-East site, Fig. 3.17) and the other specimen (approximately 9 cm in maximum shell width) was observed on the 5 m depth north of Portoć (North station of the South-East site; Fig. 3.18). It should be noted that stated shell widths are only approximative as no measuring tools were available during the dive. Hence, it is highly advisable to measure specimens in detail and establish their future monitoring.

Fig. 3.17. The specimen of the pen shell *Pinna rudis* found at 12 m depth south of Portoć area (South station of the South-East site). Photo credit: S. Kipson.

Fig. 3.18. The second specimen of the pen shell *Pinna rudis* found at 5 m depth north of Portoć area (North station of the South-East site). Photo credit: S. Kipson.

Sponges

Regarding rare species, the sighting of sponge *Calyx nicaeensis* (Risso, 1827) at 12.7 m within the north transect carried out in the cove Pod manastijerom (North station of the South-West site) is noteworthy (Fig. 3.19). It is an endemic Mediterranean sponge whose locus typicus is the Gulf of Nice (France), as depicted by its specific name. In the most recent publication on its distribution by Trainito *et al*. (2020) only 3 locations in Croatia are mentioned, and they are all concentrated in the Kvarner region (Schmidt 1862 generally reported it for the Kvarner Gulf, Frijsinger & Vestjens 2012 for Selce and Faresi 2018 for Orlec on the Cres Island, cited in Trainito *et al*. 2020) indicating that further information on its occurence in the Adriatic Sea is valuable to get a more comprehensive knowledge on its distribution. In general, species is considered to be a rare all-around generalist, with a high ecological tolerance range. It has been recorded in various habitats, from infralittoral rocky bottoms with photophilic algae (as recorded here) to coralligenous outcrops, as well as from shallow caves to deep sedimentation bottoms (Trainito *et al*. 2020).

Some other common but previously not shown sponge species, contributing to overall biodiversity of Lokrum's infralittoral reefs are illustrated in Fig. 3.20.

Fig. 3.19. Rare sponge *Calyx nicaeensis* (Risso, 1827) observed at 12.7 m, within the north transect in the cove Pod manastijerom (North station of the South-West site). Photo credit: S. Kipson.

Fig. 3.20. Illustration of some other observed sponge species: a) *Chondrilla nucula* and *Crambe crambe*, b) *Ircinia* sp. and the black keratose sponge, c) *Agelas oroides*, d) *Axinella* cf. *verrucosa* - for proper identification a physical sample would be needed, d) bioeroding sponge *Cliona virids*, with large encrusting specimens most frequently observed around 10 m depth at the North-West site and d) *Phorbas tenacior*, *Spirastrella cuntatrix*, *Terpios gelatinosus*, *Haliclona (Halichoclona) fulva* and *Haliclona* (*Reniera*) sp. on the ceiling of an overhang, an enclave of the biocenosis of semi-dark caves within the photophilic algal communities in the Portoć area (South-East site). Photo credit: S. Kipson.

Additional note on pressures

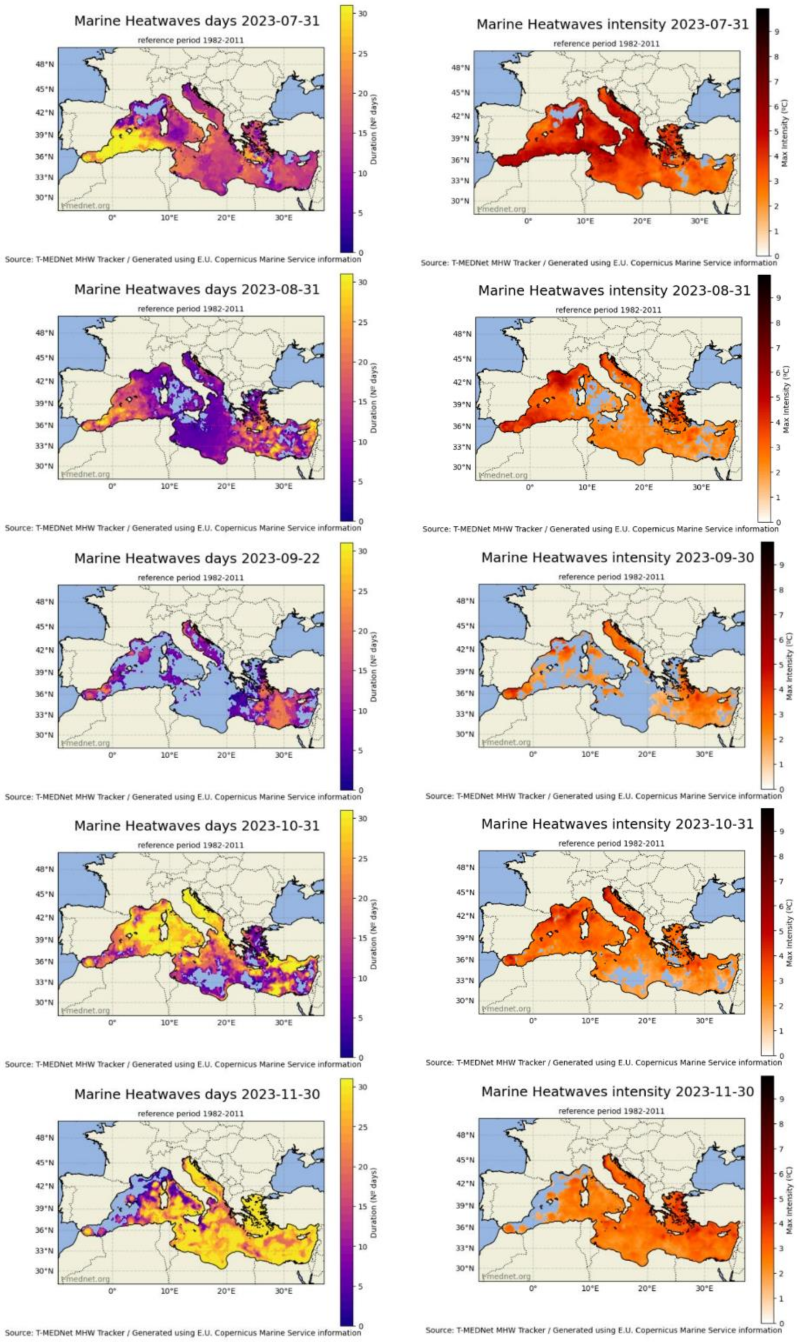

Fig. 3.21. Monthly maps showing duration and intensity of Marine Heat Waves across the Mediterranean Sea in the period from the beginning of July till the end of November 2023. Source: T MEDNet, 2023 Marine heatwaves (t-mednet.org).

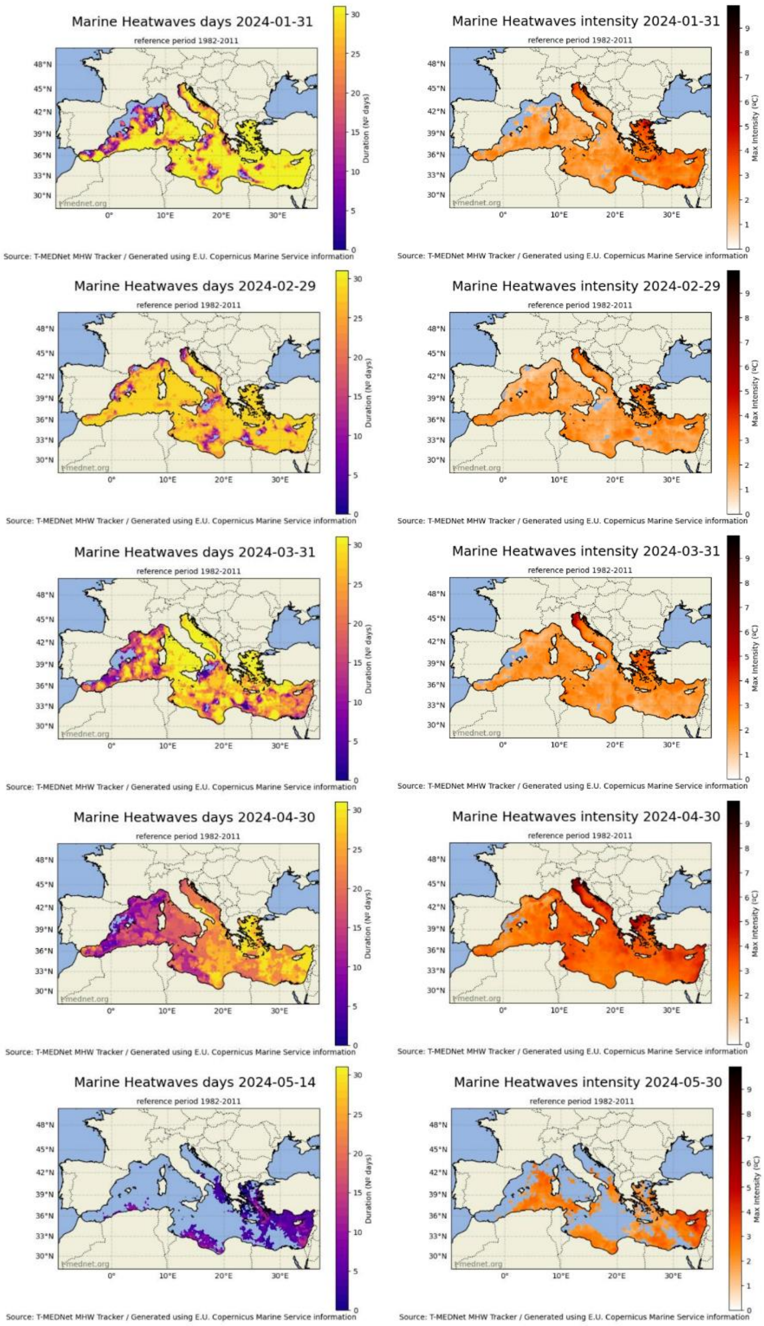

Fig. 3.22. Monthly maps showing duration and intensity of Marine Heatwaves across the Mediterranean Sea in the period from the beginning of January till the end of May 2024. Source: T MEDNet, 2024 Marine heatwaves (t-mednet.org).

Marine Heatwaves (i.e. events when seawater temperatures exceed a seasonally-varying threshold, usually the 90th percentile, for at least 5 consecutive days; successive heatwaves with gaps of 2 days or less are considered part of the same event, Hobday *et al*. 2016) of different duration and intensity were recorded around the Lokrum Island in the period from July 2023 till the beginning of May 2024 (Figs. 3.21, 3.22).

On the other hand, contrary to the situation in some other marine Natura 2000 sites where lost/discarded fishing gear could be observed on infralittoral reefs (Kipson 2021), at the investigated sites of the Lokrum Island down to 12 m depth we have observed almost none. The sole finding was one entangled monofilament covering the seabed surface of approximately 20 x 10 cm (Fig. 3.23).

Fig. 3.23. The only observed lost/abandoned fishing gear on shallow infralittoral reefs (down to 12 m depth) of the Lokrum Natura 2000 site. Photo credit: S. Kipson.

4. Overall conclusions and recommendations

- The *MedSens* index tested here intends to integrate the assessment of the environmental status of coastal Mediterranean areas threatened by multiple stressors while considering the protected and sensitive species, hence adhering to the requests of the European Union's Habitat Directive (92/ 43/EEC) and Marine Strategy Framework Directive (MSFD, 2008/56/ EC).
- Fish Visual Census is a simple tool for assisting MPAs to monitor and manage global warming effects, targeting easy recognizable fish species that are considered as appropriate indicators of climate change.
- Both protocols applied in this study demonstrated their applicability in the field and provided valuable information on the infralittoral portion of the "1170 Reefs", a habitat type of the EU Habitat Directive (92/43/EEC) targeted by the Natura 2000 site Lokrum that was severely understudied up till now. However, there is a need for a <u>wider spatio-temporal assessment</u> of the *MedSens* index and the application of simplified Fish Visual census for climate change indicators at the Lokrum Island to improve the reliability and interpretability of the acquired data. Long-term data are pivotal for improved understanding of the natural variability, relation to climatic fluctuations and other stressors and consequently the response of marine protected areas to them.
- In addition to researchers, the engagement of citizen science, i.e. observations provided by trained and supervised volunteer SCUBA divers and/or snorkelers is a highly valuable way to enhance underwater work effort, increase awareness and the sense of stewardship for marine environment. Quality data collected in such a standardized manner will enable the detection of the effects of seawater warming and the consequent biological responses, such as changes in composition and structure of fish and benthic assemblages.
- *MedSens* index revealed contrasting results for mean sensitivity to physical and chemical pressures versus biological pressures on infralittoral reefs of the Lokrum Natura 2000 site. Whereas the high abundance of species sensitive to biological pressures would be indicative of good status around the Lokrum Island related to this type of pressures (though the abundance of the invasive algae *Caulerpa cylindracea* should be re-examined at its vegetation peak in late summer/early autumn to get a more reliable assessment of this pressure, see below), the abundance of species tolerant to physical and chemical pressures is not

necessarily related to poor environmental conditions. Whether species sensitive to physical and chemical pressures were historically present but were subsequently lost or they were never present on the Lokrum Island, remains to be further investigated. Moreover, considering the application of the *MedSens* index, increased amount of data contributes significantly to the robustness of the method. Hence, as already emphasized, there is a need for increased observational effort in space and time within the Lokrum Natura 2000 site.

- The fish visual census confirmed considerable abundance of thermophilic species. The ornate wrasse *Thalassoma pavo* was among the most abundant fish species, and besides this one, the other two thermophilic species included in the protocol were observed, i.e. the Mediterranean parrotfish *Sparisoma cretense* and the dusky grouper *Ephinephelus marginatus,* although only few individuals were recorded of the latter one. Based on this data, according to the calculated Tropicalization index (amounting to 2) the Lokrum Island is clearly positioned into the warm Mediterranean.
- Although other stressors (or their synergistic act) cannot be excluded, some sessile benthic species such as the sponge *Petrosia ficiformis* around the Lokrum Island exhibits partial mortality indicative of the effect of marine heat waves which were evident in the area with different duration and intensity since July 2023 till May 2024 (Figs. 3.21, 3.22).
- Whereas impacts of climate change cannot be addressed locally without an attempt to tackle this issue at its source by reducing CO_2 emissions globally – an action that remains key for preventing irreversible change to the marine assemblages, every effort should be made to relieve sensitive organisms from more manageable stressors and hence to putatively improve their resilience when faced with marine heat waves. Such actions may include but are not limited to preventing physical damage/abrasion by boating and/or anchoring, preventing pollution, ensuring good water quality (which has to be done in collaboration with the surrounding municipalities), etc.
- IMPORTANT NOTE: It should be clearly understood that only preliminary and partial knowledge on the status of the habitat type 1170 Reefs could be obtained with this study. It is highly recommended to increase the number of observations and to extend the future study of the habitat type 1170 Reefs to depths beyond those ones included here, hence to assess also deeper infralittoral and circalittoral rocky habitats.

- Besides protocols applied here (assessment of *MedSens* index and Fish visual census), I highly recommend to implement also several other complimentary protocols that inform management actions, namely Assessment and Monitoring of Mass Mortality (for which sponges *Petrosia ficiformis* and *Chondrosia reniformis* as well as bryozoan *Myriapora truncata* are identified as good target species), URCH -Sea Urchins population, BHARA - Benthic Habitat Rapid Assessment and potentially SFM - Photogrammetry as monitoring tool for benthic habitats structure and dynamics (Garrabou *et al*. 2022a). Limited information on benthic communities prior to this study and severe time constraints for field work prevented us from considering these protocols on this occasion.
- It would be highly beneficial to establish at least one station for continuous, high-resolution monitoring of the seawater temperature throughout the water column, on a site where the habitat type "1170 Reefs" extends down to the 30-40 m depth (with temperature dataloggers set at every 5 m depth to depict also vertical stratification, according to the protocol described by Garrabou *et al*. 2022a, see examples for other Adriatic and Mediterranean sites at https://t-mednet.org/visualize-data/temperature).
- Due to their importance in the carbon and carbonate cycles of shallow coastal ecosystems, being major contributors to CO_2 fluxes through high community $CaCO_3$ production and dissolution (McCoy & Kamenos 2015), also inducing settlement and recruitment of numerous invertebrates and providing habitats for a variety of grazing and burrowing infauna (Chenelot *et al*. 2011), Crustose coralline algae should be monitored more closely and the underlying factors of their observed decline investigated by professional researchers.
- Moreover, demography and conservation status of many strictly protected species and species of Community interest remain unknown. On the Lokrum Island, the presence of starfish *Ophidiaster ophidianus* is remarkable and merits a further population study.
- Invasive algae *Caulerpa cylindracea* was present at all sites but in low abundance at the end of June - additional assessment is necessary at its vegetation peak is during summer. Irrespective of the relative importance of the different factors involved, there appears to be general consensus that the main cause of *C. cylindracea* spread is the loss of resistance of natural assemblages. Thus, conservation of coastal systems (e.g. maintenance of good water quality, prevention of physical degradation, protection of

canopy habitats where naturally present) represents the only effective instrument to contain the invasion, also because of the weak effectiveness of eradication efforts (Piazzi *et al*. 2016 and references therein).

- Although invasive alien species *Stypopodium schimperii* (Fig. 4.1) was not observed during our field research, due to its extraordinary capacity for invasion and quick spreading as noted on the not-so-far Vis Island (near Komiža, A. Žuljević, *pers. comm*.), it is advisable to regularly monitor infralittoral macroalgal community – in this context, involvement of well-trained volunteer divers through marine citizen science schemes are highly advisable to increase underwater observation effort.

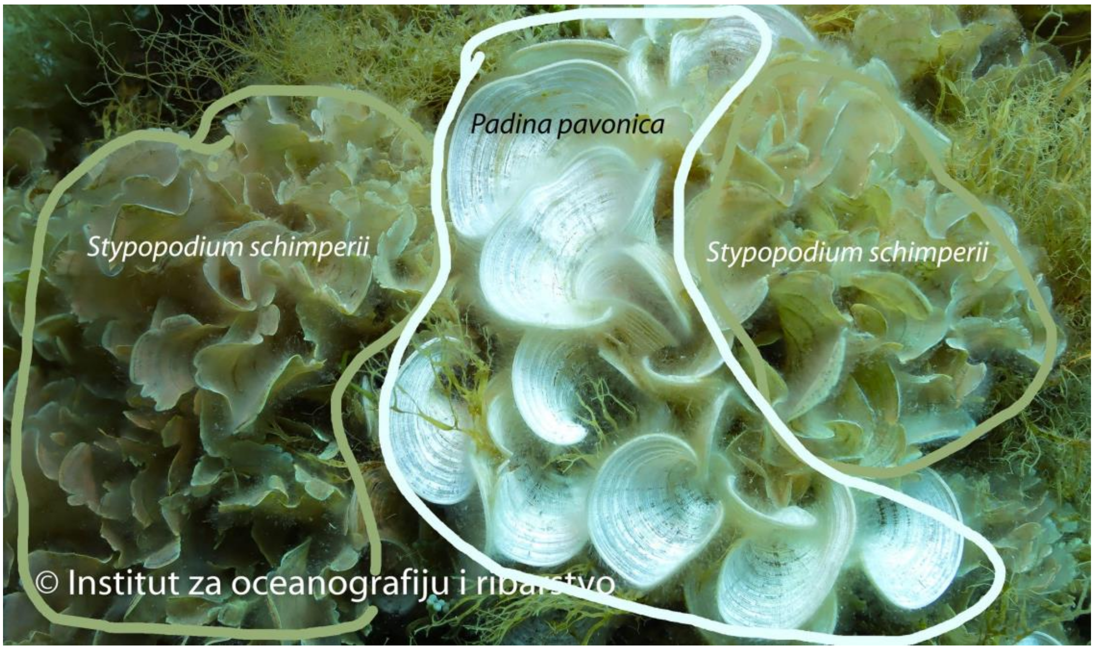

Fig. 4.1. Comparison between autochtonous algae *Padina pavonica* and the invasive alien species *Stypopodium schimperii.* Photo credit: Croatian Institute for Oceanography and Fisheries (IOR).

- In the future studies, it would be advisable to consider also some of the other relevant ecological indices that should be assessed by professional researchers e.g. EEI-c and reef-EBQI for infralittoral rocky habitat (Orfanidis *et al*. 2011, Thibaut *et al*. 2017) and INDEX-COR, ESCA, CBQI, ISLA, MACS for coralligenous assemblages (Sartoretto *et al*. 2017, Piazzi *et al*. 2017, Ferrigno *et al*. 2017, Montefalcone *et al*. 2017, Enrichetti *et al*. 2019) and to assess their relation to the results of the *MedSens* index.

- the "blitz biodiversity studies" may be organized involving a group of taxonomy specialists with complementary expertise which would carry out short but intensive fieldwork to create a comprehensive marine species checklist for the Lokrum Island.
- Due to its natural underwater value and vicinity to the old city of Dubrovnik, the Lokrum Island is of great significance for local tourism, offering very attractive recreational dive locations and fieldwork sites for educational purposes, particularly targeting the Youth (e.g. marine biology students of the nearby University of Dubrovnik, high and elementary school pupils, etc.).
- joining international collaborative networks, such as for example T-MEDNet (https://t-mednet.org/) for reporting seawater temperature data (if collected throughout the water column in a standardized manner, see the recommendation above) and mass mortalities of marine species (see Mass Mortality Events (t-mednet.org) for more info) would contribute to enhanced understanding of ecological impact of climate change and other disturbances on the Mediterranean level as well as it would increase the visibility of the Public Institution Lokrum involved in such monitoring efforts.

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