ADRIREEF Project

Final report

CROREEF

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Innovative exploitation of Adriatic Reefs in order to strengthen blue economy

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Table of Contents

Executive summary

This document reports on the results of coralligenous and infralitoral reef research carried out in the scope of the ADRIREEF project by a project partner the NGO Sunce (Croatia). Data were collected during the field work carried out from September 12 until September 20 2020 on the Dugi Otok Island (location Lagnići) and from September 29 until October 6 2020 on the Vis Island (location Stupišće). Both locations were selected *a priori* and they form part of the marine Natura 2000 network in Croatia.

The main aims of the undertaken activities were:

1) to characterize the coralligenous assemblages dominated by gorgonians at the selected sites and to assess current level of disturbances, if present, in order to set the baselines for future monitoring
2) to test several recently proposed protocols applicable for monitoring the effects of climate change on infralitoral reefs
3) to provide on-site practical training in reef monitoring for the employees and volunteers of the NGO Sunce

The underwater work performed by SCUBA diving included photosampling and visual census to collect data for the characterisation of the coralligenous biodiversity and structure as well as to collect data on potential disturbances acting upon these circalitoral reefs (developing below 25 m depth) such as data on mass mortalities, mucilaginous algal aggregates, invasive species, sedimentation and fishing impacts. Related to potential stressors on infralitoral (shallow water) reefs, we tested two protocols which were recently proposed within the other Interreg project, MPA Adapt, and have carried out fish visual census for climate change indicators as well as the assessment of conservation status and climate-related responses of sessile benthic macroinvertebrates. For the first time, we have also tested the Lost Fishing Gear protocol (developed within a GhostMed programme) in the Eastern Adriatic, both on coralligenous and infralitoral reefs. This protocol is designed to assess both the impact of the lost fishing gear as

well as to inform decision making process of whether it is feasible to remove it from the marine environment. In addition to natural reefs, we used an exquisite opportunity to also assess two artificial reefs (steamboat shipwrecks) found at our study locations, the shipwreck Michele next to the Dugi Otok Island and the shipwreck Teti next to the Vis Island.

During field work, 14 dives were performed on the Dugi Otok Island and 8 on the Vis Island. In total, 12 divers participated in the field activities.

In summary, results revealed rich coralligenous assemblages dominated by *Eunicella cavolini* at Lagnići and Stupišće location. Beside *E. cavolini* assemblage, at Stupišće location there is also present a mixed *Paramuricea clavata* and *Eunicella cavolini* assemblage. Coralline encrusting algae, the foundation builders of coralligenous outcrops, were more abundant on the Vis Island, where often a representative plate-like thalli of *Lithophyllum stictaeforme* were additionally contributing to the structural complexity of the outcrops and created additional micro-habitats. On the Vis Island, notable was abundance of erect bryozoans, one of the main animal builders of coralligenous outcrops, with large colonies of *Pentapora fascialis* especially abundant on Sika 6 site. In contrast, sponges, usually the most species rich group of sessile macroinvertebrates within the coralligenous, were relatively poorly represented on the Vis sites, whereas they were much more abundant on the Dugi Otok sites. On the latter, besides *Chondrosia reniformis*, as one of the most abundant sponges, notable was the presence of the strictly protected sponge *Aplysina cavernicola,* contributing to the intermediate layer of the coralligenous habitat. In addition, coralligenous outcrops on the Dugi Otok sites were characterized by a dominance of encrusting red algae belonging to family of Peyssonneliaceae.

Denser populations and larger colonies of the yellow gorgonian *Eunicella cavolini* were recorded at the Vis Island than at the Dugi Otok Island, whereas *Paramuricea clavata* was present only at the studied sites on the Vis Island, where it formed a mixed facies of coralligenous assemblages with *E. cavolini*. Its demography was similar to the demography of other Adriatic populations studied to date that were characterized by great proportion of large colonies (> 40 cm in height).

However, due to low number of assessed colonies, data on *P. clavata* should be interpreted with caution.

All descriptors selected to evaluate coralligenous ecological status that were related to assessment of its understorey (i.e. its basal and intermediate layer) suggest good conditions at our study sites. These include species richness and heterogeneity comparable to other Adriatic sites with low levels of anthropogenic pressure, high sensitivity levels indicating presence of high number of sensitive species and/or their high abundance such as in the case of erect-branchy bryozoans for example, positive balance in bioconstruction and generally low percent cover of sediment and algal turf. However, related to the erect layer, the extent of injury of erect anthozoans (i.e. gorgonians that exclusively form that layer within assessed assemblages) is considerable at most sites, indicating disturbances that can specifically and/or more adversely affect these more exposed (since they are the tallest within the community) and among the most sensitive organisms.

The mean extent of injury of *E. cavolini* colonies was almost twice as high on the Vis Island than on the Dugi Otok Island. The most affected was population at Sika 3 site followed by Sika 6 (with mean extent of injury of 39 and 31%, respectively) and almost 80% of affected colonies (i.e. with injury levels >10% of colony surface) at Sika 3 and 66% of affected colonies at Sika 6 site. Meanwhile, on the Dugi Otok Island mean extent of injury of *E. cavolini* colonies was 23% at Lagnići 1 site and 16% at Lagnići 2 with 57% and 41% of colonies affected at each site, respecitvely.

Mean extent of injury of *P. clavata* colonies was higher at Sika 3 than at Sika 6 site (36 vs. 27%), and overall they were fairly similar to the ones observed for *E. cavolini* at the same location. Likewise, high proportion of affected colonies of *P. clavata* was noted, being 66% of colonies affected at Sika 3 and 85% at Sika 6. Although most of the injuries were old, we also noticed recent injuries (i.e. denuded axes, mainly on apical tips) that would imply they occurred less than 1 month ago, which could have coincided with the increased seawater temperatures. Other putative causes may include past and present mechanical abrasion by the fishing gear (either lost or still in use; Lagnići sites were especially notorious in that respect) and mucilaginous algal aggregates. Although the latter were not observed during this study, their occurrence in the past was confirmed by the owner of a local diving center.

Besides gorgonian conservation status, the fish visual census also revealed more pronounced effects of climate change and seawater temperature increase on the Vis Island, located cca. 80 km south of the Dugi Otok Island. Thermophilic alien fish species *Sparisoma cretense* was recorded only on the Vis Island and the abundance of another thermophilic alien fish, *Thalassoma pavo,* was much higher on the Vis Island in comparison to the Dugi Otok Island.

On the other hand, medium (30-60%) to high percentage (>60%) of affected black keratose sponges were observed both on natural and artificial shallow reefs (3-5 m depth) on the Lagnići location, whereas another assessed sponge, *Chondrosia reniformis*, appeared unaffected. Putative cause or trigger could include increased seawater temperature over summer period, however other causes or even multiple causes of such affectation cannot be excluded. Unfortunately such an assessment could not be performed on the Vis Island due to low number of individuals/colonies of target species in comparable depth ranges.

Related to other potential stressors, high abundance of invasive green algae *Caulerpa cylindracea* poses a threat to infralitoral communities on the Vis Island, whereas its abundance was low at the Dugi Otok Island. Likewise, its presence was noted also within the coralligenous habitat at the Vis Island, however its abundance was low in the gorgonian dominated assemblages at 35-40 m depth.

Besides assessment of natural reefs, preliminary assessment of biodiversity on the unintentional artificial reefs - the shipwrecks in this study represents a valuable step forward in the evaluation of their role. Both visited shipwrecks provide permanent or temporary habitat for many marine species. Whereas the diversity and abundance of macrobenthos was outstanding on the shipwreck Michele, diversity and abundance of fish species was notable on the shipwreck Teti. They both advocate the value of artificial reefs in supporting biodiversity (when used as a tool in conservation), provide undisputable diving attraction and offer a great potential for education.

In conclusion, activities conducted within this study enabled the acquisition of data relevant both for monitoring of coralligenous and infralittoral reefs, as well as for valorization of shipwrecks as artificial reefs. Assessment of current status allows for evaluation of changes in the future, either due to natural or human-induced causes and informs conservation and management plans.

1. STUDY SITES

We carried out field activities at two locations belonging to the marine Natura 2000 network in Croatia that were selected *a priori* by project partners: location Lagnići on the Dugi Otok Island (Fig. 1.1.1) and location Stupišće on the Vis Island (Fig. 1.1.2). Location Stupišće was selected as a viable alternative to another location that was primarily envisaged for reef assessment on the island of Vis, plić Seget. During the field trip at the Vis Island, several attempts were made to dive at plić Seget, including dives by experienced technical divers. However, during the study period, strong currents were continuously present at the site, and finally decision was made to omit it as any underwater activities performed there were jeopardizing divers' safety. Being only few kilometres away, hence on the geographical scale where we did not expect significant differences in biodiversity patterns of coralligenous assemblages, as shown previously for the Eastern Adriatic Sea (Kipson 2012), and in line with the project proposal by the partner NGO Sunce, Cape Stupišće was selected as an alternative location with more favourable local hydrological conditions. In addition, we managed to carry out few specific activities nearby as well as to include three additional sites on the island of Vis (a cove close to the Oključna bay, Kamenica and Pločica) to perform fish visual census on shallow reefs (Fig. 1.1.2), otherwise halted at our project location due to adverse weather conditions. Geographic coordinates of all studied sites are indicated in Table 1.1.1, and a quick overview of the methods applied at each site is provided in Table 1.1.2. Visualisation of the studied sites if provided in Figs. 1.1.3 to 1.1.6.

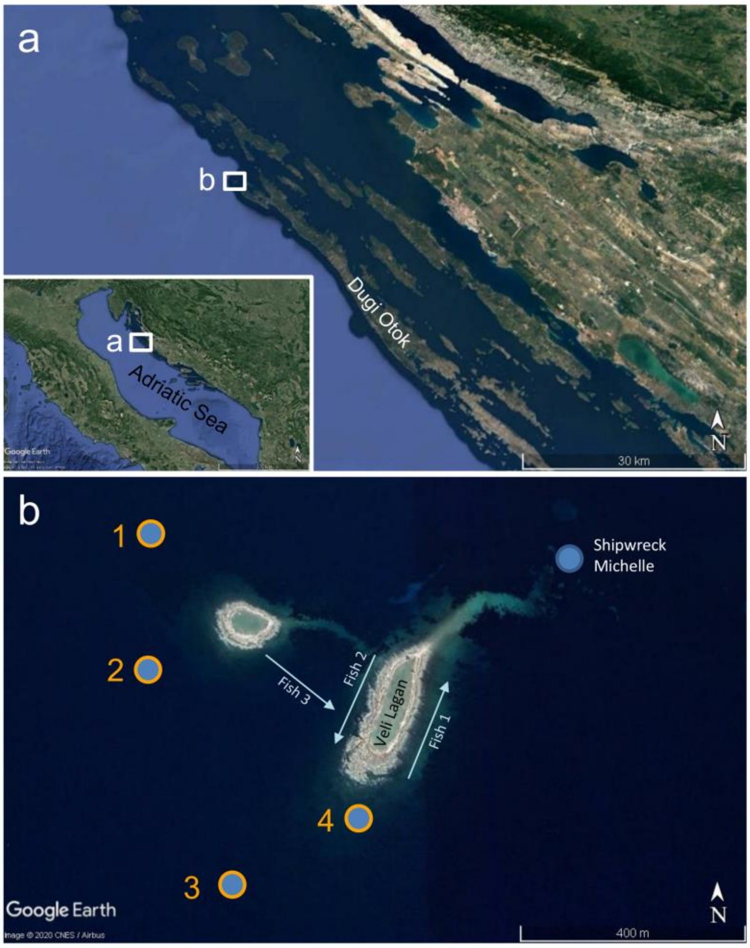


Fig. 1.1.1. Map of study sites on the Dugi Otok Island, location Lagnići.

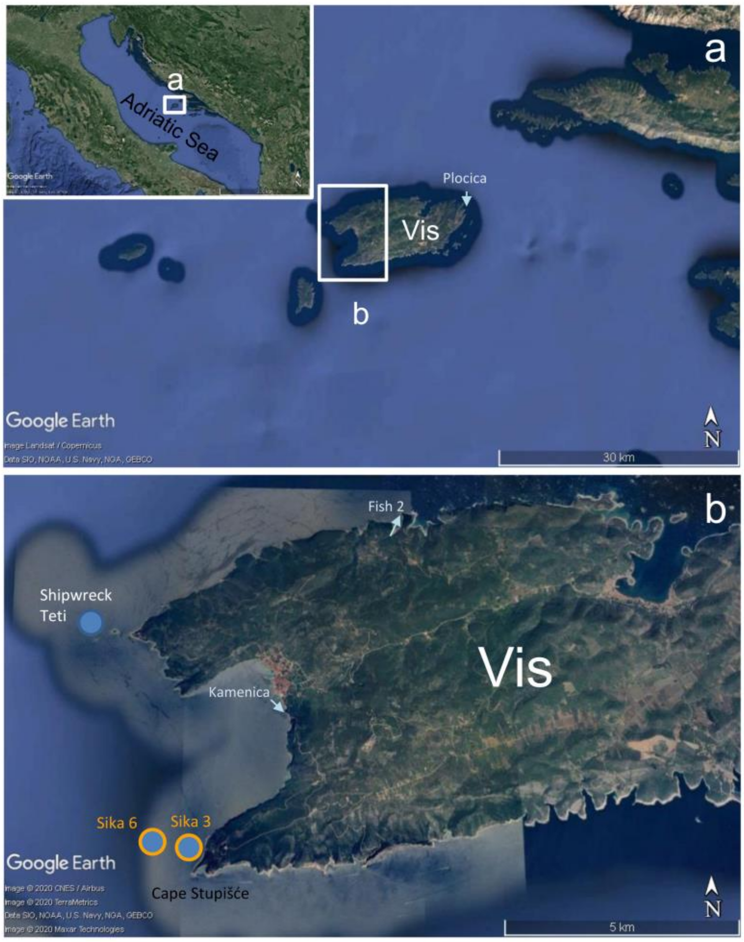


Fig. 1.1.2. Map of study sites on the Vis Island, indicating the main project location Stupišće and four other sites where few specific field activities were undertaken.

Table 1.1.1. Geographic coordinates (longitude and latitude) of study sites at both locations on the Dugi Otok and Vis Islands.

Location	Site	Latitude	Longitude
Dugi Otok	Lagnici 1	44° 10' 12.75"N	14° 48' 03.19"E
	Lagnici 2	44° 09' 59.38"N	14° 48' 03.10"E
	Lagnici 3	44° 09' 37.3"N	14° 48' 10.0"E
	Lagnici 4	44° 09' 44.7"N	14° 48' 29.3"E
	Michele	44° 10' 02.42"N	14° 48' 46.11"E
	Fish 1	44° 09' 48.74"N	14° 48' 33.20"E
	Fish 2	44° 09' 57.52"N	14° 48' 30.69"E
	Fish 3	44° 09' 56.86"N	14° 48' 16.68"E
Vis	Sika 3	43° 00' 35.5"N	16° 04' 06.2"E
	Sika 6	43° 00' 31.2"N	16° 03' 54.1"E
	Teti	43° 03' 12.50"N	16° 02' 28.01"E
	Fish 2	43° 04' 26.25"N	16° 07' 22.88"E
	Kamenica	43° 02' 14.10"N	16° 05' 30.17"E
	Plocica	43° 03' 55.43"N	16° 15' 37.45"E

Table 1.1.2. Overview of underwater activities/methods carried out at each study site. Photo = photo sampling, VCRT = visual census along random transect, Video = video transect, MME = assessment of sessile macroinvertebrates' mass mortalities, LFG = assessment of lost fishing gear.

Location	Site	Photo	VCRT	Video	Fish census	MME	LFG
Dugi Otok	Lagnici 1						
	Lagnici 2						
	Lagnici 3						
	Lagnici 4						
	Michele						
	Fish 1						
	Fish 2						
	Fish 3						
Vis	Sika 3						
	Sika 6						
	Teti						
	Fish 2						
	Kamenica						
	Plocica						

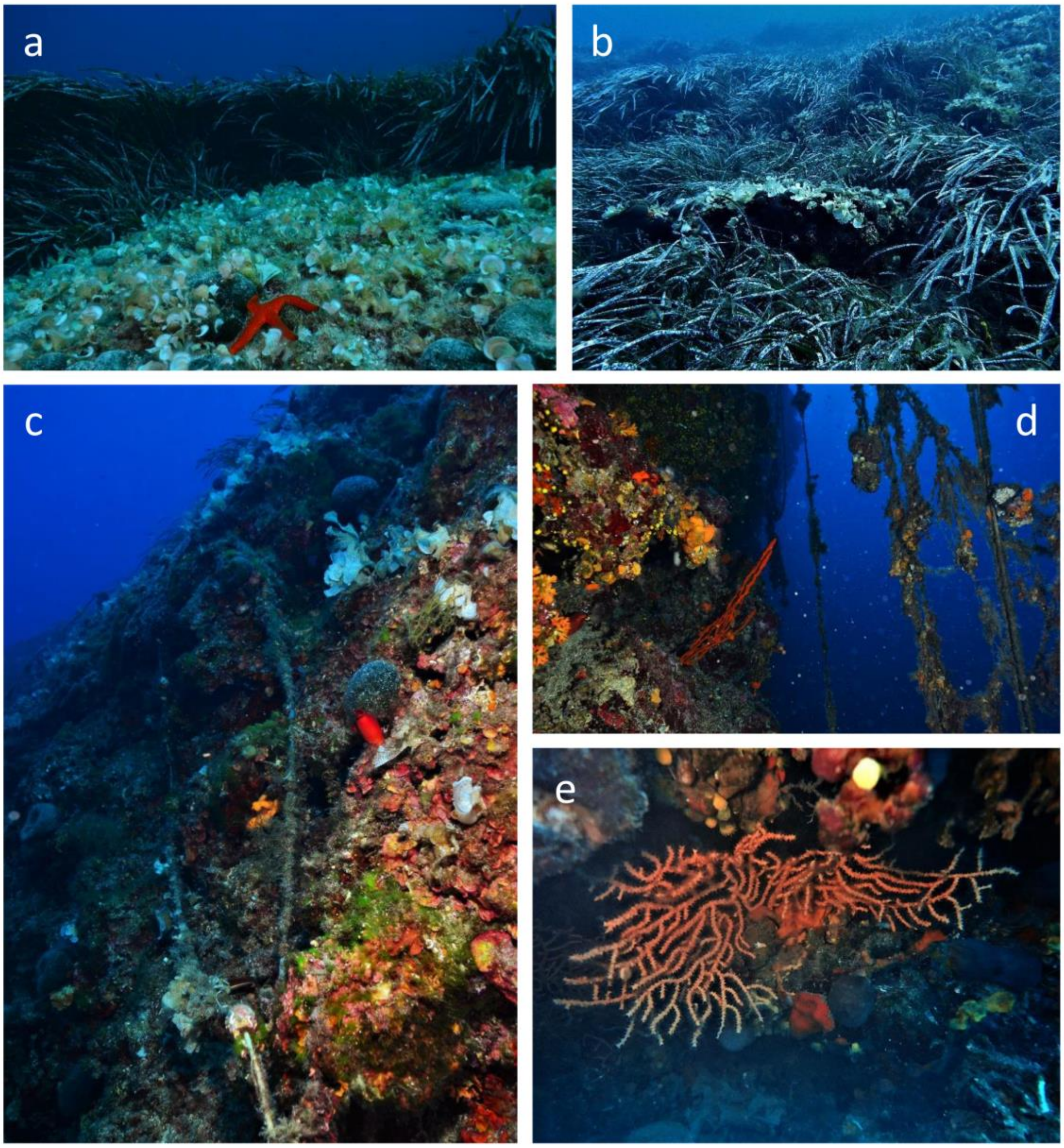


Fig. 1.1.3. Study site Lagnići 1 (Lagnići location, NW of the Dugi Otok Island): a) a fairly flat infralitoral rocky botom is replaced by a *Posidonia oceanica* meadow at 8 m depth which remains interspersed by shallow reefs down to 17 m (b), a depth which marks the top of a vertical west-facing wall full of lost fishing gear, either covering the substrate (c) or hanging from the wall (d) which is full of holes and crevices and hosts rich coralligenous assemblages with considerable structural complexity contributed by large erect sponges, e.g. *Axinella cannabina* (d) or abundant yellow gorgonian *Eunicella cavolini* population (e) that thrives down to 39 m depth, i.e. till the end of the wall which is being replaced by a detritic bottom. Photo credit: M. Belošević, except (b) and (e) by S. Kipson.

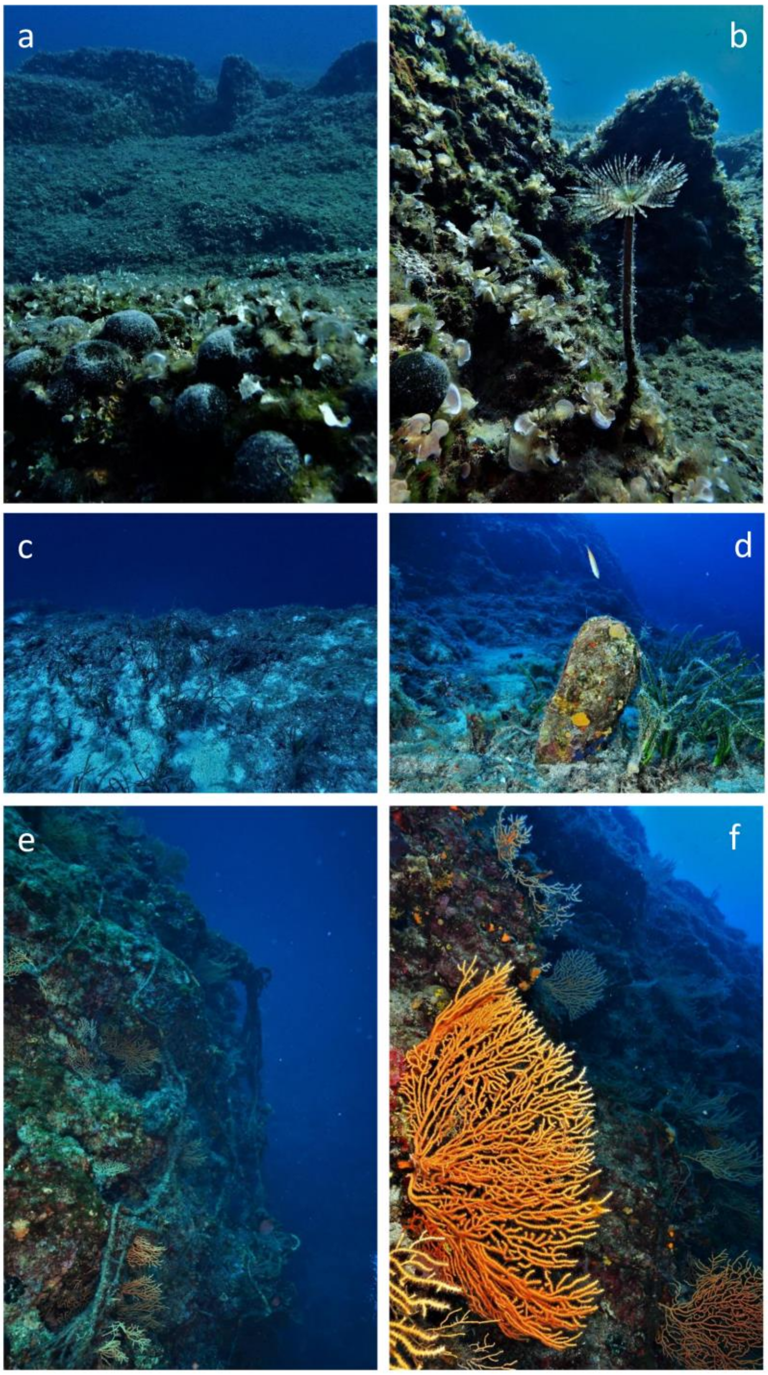


Fig. 1.1.4. Study site Lagnići 2 (Lagnići location, NW of the Dugi Otok Island): a) attractive shallow reefs are found between 5 and 8 m depth hosting photophillic algae such as *Padina pavonica* and *Codium bursa* and some of typical sedentary filter-feeders such as a polychaete *Sabella spalanzani* (b); *Posidonia oceanica* meadow interspersed by infralitoral reefs extends down to 18 m, the edge of a vertical wall (c); At 23 m depth the wall extends to a small ledge where few shoots of *Posidonia oceanica* are found on the sediment bottom as well as an empty shell of *Pinna nobilis* (d). After the ledge, the wall extends down to 40.5 m depth (e) and hosts a well developed coralligenous asssemblage dominated by the yellow gorgonian *Eunicella cavolini* (f). Photo credit: a) to c) S. Kipson, d) to f) M. Belošević.

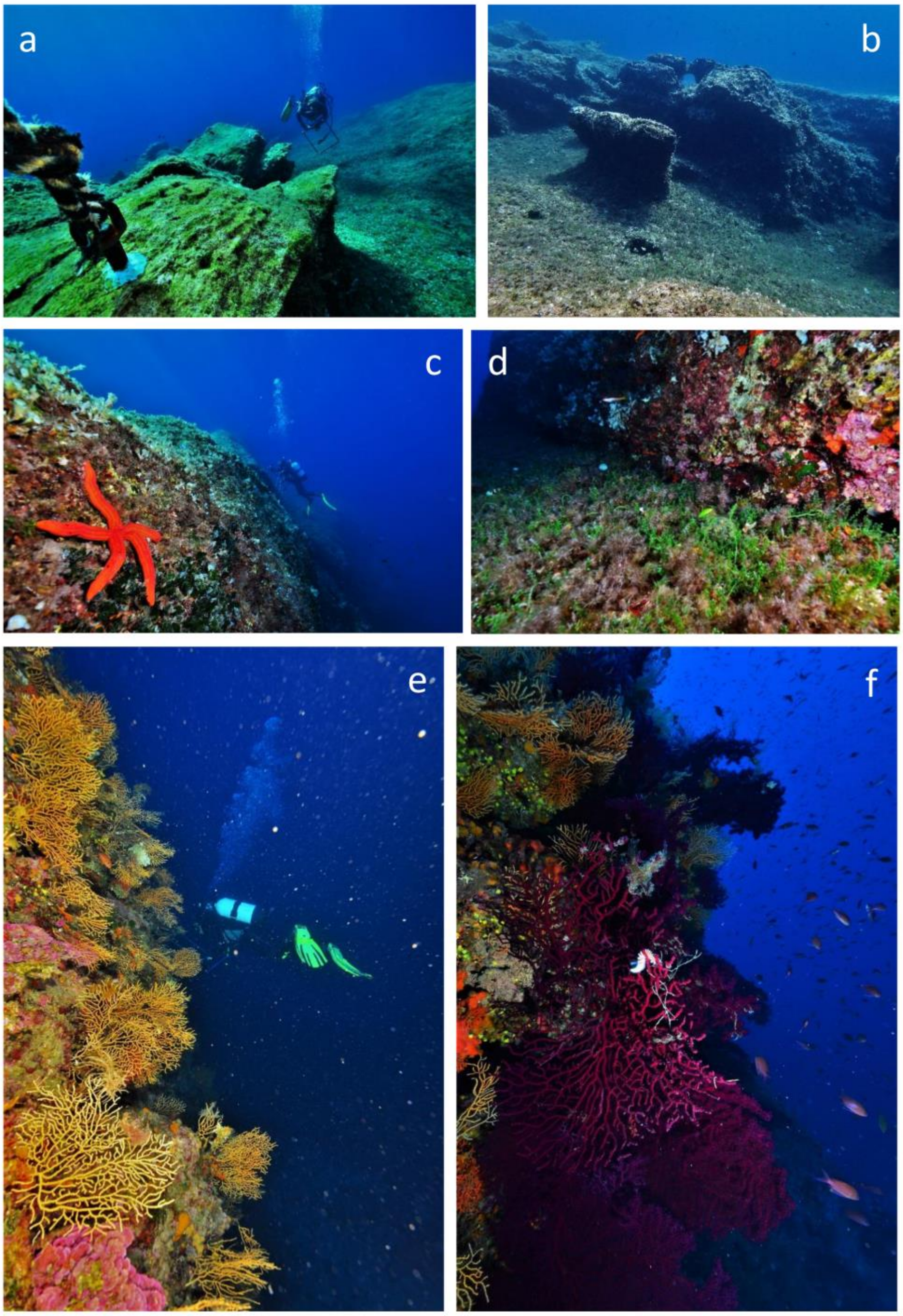


Fig. 1.1.5. Study site Sika 3 (Stupišće location, SW of the Vis Island): a) a shallow starts at 3 m depth; b) top of the shallow is characterized by attractively shaped infralitoral reefs adding to the seascape value of the site; c) the vertical wall extends from 4 till 42 m depth; d) infralitoral reefs are dominated by algae, both autochtonous as well as the invasive alien green algae *Caulerpa cylindracea*; e) from 25 m depth coralligenous assemblage is dominated by the yellow gorgonian *Eunicella cavolini* and there is a notable presence of a large plate-like thalli of red calcareous algae; f) from 35 m depth the red gorgonian *Paramuricea clavata* contribute to the structural complexity of the rich coralligenous assemblage and forms a mixed facies with *Eunicella cavolini*. Photo credit: M. Belošević except b) S. Kipson.

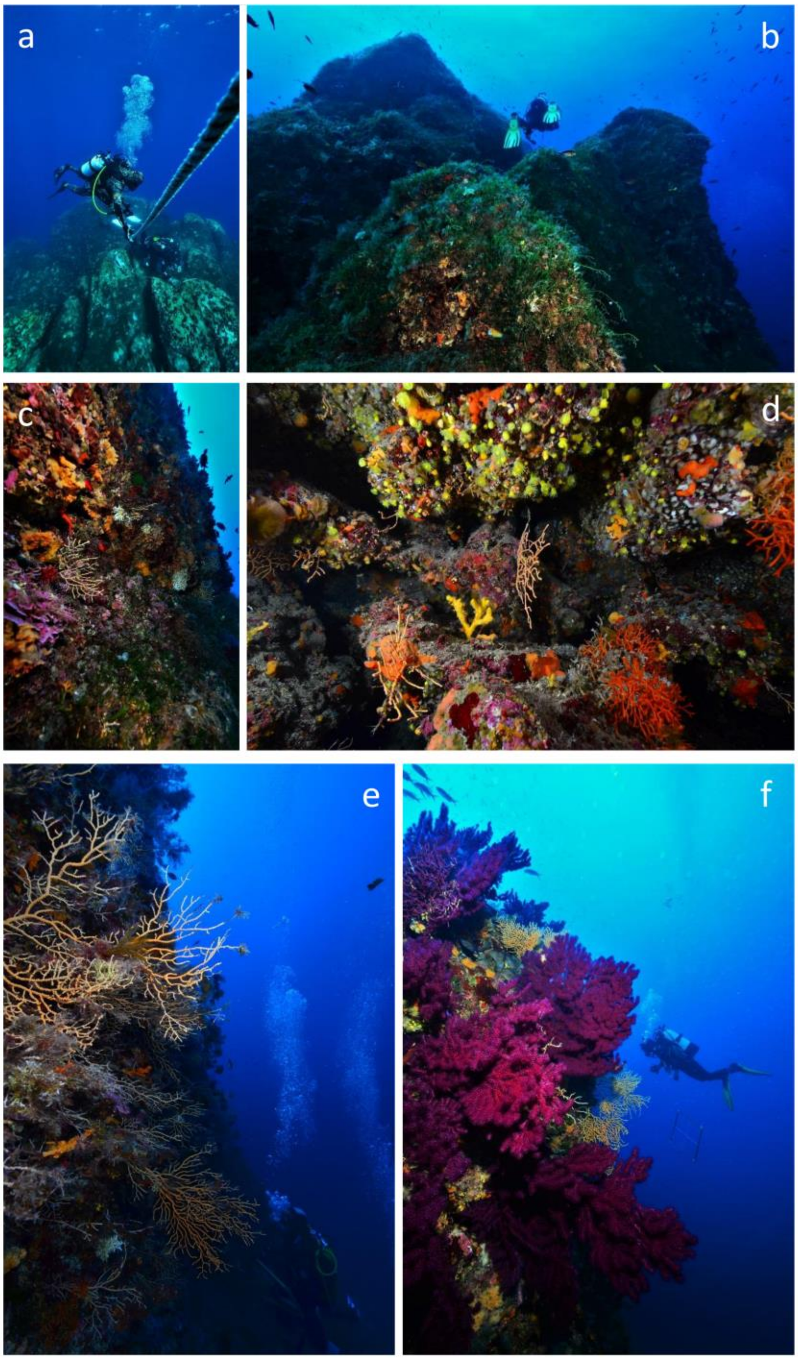


Fig. 1.1.6. Study site Sika 6 (Stupišće location, SW of the Vis Island): a) a shallow starts at 6 m depth; b) infralitoral reefs are heavily overgrown by the invasive green algae *Caulerpa cylindracea*; c) yellow gorgonian *Eunicella cavolini* population thrive from 18 m depth on infralitoral rocky bottom till the end of the West facing wall at 45 m depth; d) coralligenous outcrops developing on the vertical wall are characterized by many microhabitats such as holes and crevices; e) *Eunicella cavolini* is the most abundant contributor to the structural complexity of the coralligenous assemblages found here and from 35 m depth it is also present in the form of mixed facies with the red gorgonian *Paramuricea clavata* (f), making this location a prime diving attraction. Photo credit: M. Belošević.

2. MONITORING ACTIVITIES

Related to the coralligenous assemblages, at each selected location and two sites within, photosampling was combined with visual census to gather the information on habitat structure and function as well as on the degree of impact of the main disturbances (for more details see Garrabou *et al.* 2015). Random transects used for visual census were also video recorded, to provide additional permanent documentation. Moreover, since at our selected sites coralligenous assemblages were dominated by gorgonians - important habitat structuring organisms and useful indicators, we also performed assessment of their demography and conservation status (see section 2.2).

Related to infralitoral (shallow water) reefs, we used the opportunity to test two protocols which were recently proposed to track the impact of climate change. Hence, we carried out fish visual census for climate change indicators as well as the assessment of conservation status and climate-related responses of sessile benthic macroinvertebrates.

For the first time, we have also tested the Lost Fishing Gear protocol (developed within the GhostMed programme) in the Eastern Adriatic, both on coralligenous and infralitoral reefs, when possible.

Besides the assessment of natural reefs at our locations, both deep and shallow ones, we had an exquisite opportunity to assess also two artificial reefs i.e. accessible shipwrecks nearby. Since artificial reefs are also in the focus of the ADRIREEF project, we documented their associated biodiversity by underwater photography and videography.

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

2.1 Assessment of coralligenous assemblages

2.1.1. Overview of the methods

Photosampling

Within coralligenous assemblages, a minimum of three areas of 2,5 m^2 (comprised of 10 contiguous photos of 50 x 50 cm quadrats to ensure species identification; Figs. 2.1.1, 2.1.2 a) were photosampled within the same depth range. Photos were taken with Nikon D7000 digital SLR camera fitted with a 10-24 mm lens and housed in a SEACAM housing. Lighting was provided by two electronic strobes fitted with diffusers. Such sampling enables further acquisition (through subsequent photo analysis) of data on: (i) the presence and abundance of typical (target) species, (ii) the structural complexity based on the cover of species/categories contributing to basal layer (including encrusting organisms, boring sponges, turf, bare rock and sediment) and intermediate layer (massive or bush-like organisms below 15 cm in height) (see below the description for assessment of the third, erect layer), (iii) bioconcretion (through estimation of cover of encrusting calcareous algae and macroinvertebrates contributing to build-up of the coralligenous outcrops) and (iv) bioerosion (through estimation of the cover of boring sponge *Cliona* spp. and enumeration of bioeroding molluscs *Rocellaria dubia* and *Lithophaga lithophaga* as well as estimation of the effects of bioeroders from their grazing marks). Besides acquisition of data on habitat structure and function, photoquadrats obtained by photosampling furnish information on disturbances through estimates of abundance of invasive species and sediments (already available from the analysis of basal layer, as described above).

Since the goal was also to characterize these coralligenous sites for the first time, we additionally performed photosampling of a minimum of 3 replicates of 0.5 m^2 using 25 x 25 cm subquadrats (Fig. 2.1.2 b) to ensure a more reliable identification of organisms (as suggested previously by Kipson et al. 2011), since certain level of detail (useful for species identification) may be lost when 50 x 50 cm subquadrats are used.

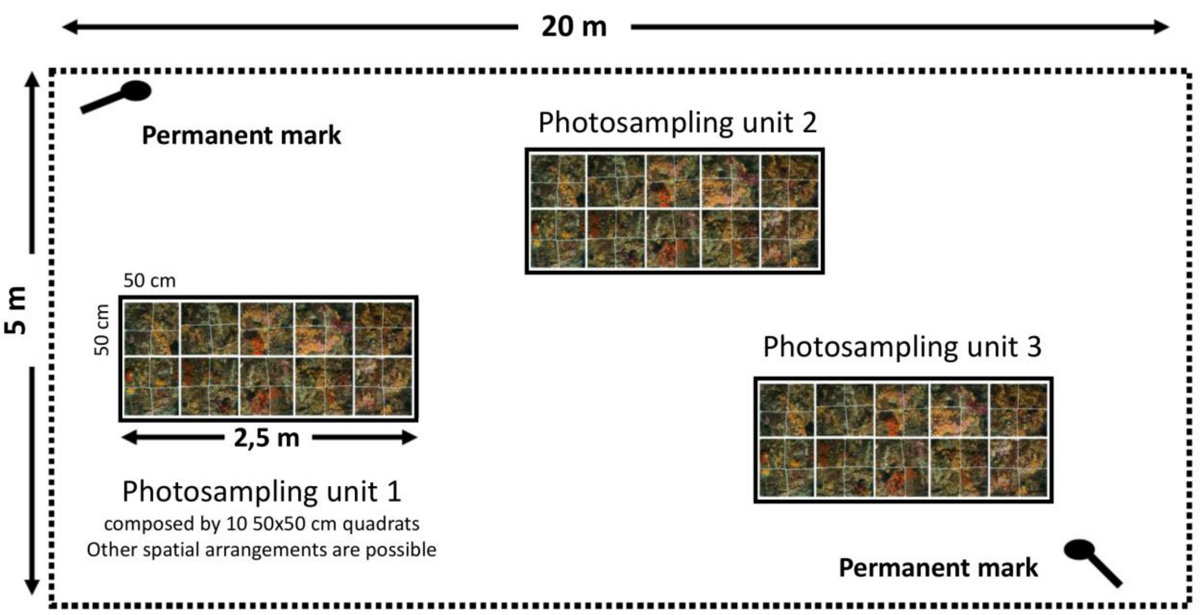


Fig. 2.1.1. Scheme of photo sampling carried out within coraligenous assemblages dominated by gorgonians at the selected sites (adapted from Garrabou et al. 2015).

Furthermore, to comply with the most integrated assessment of coralligenous ecological status so far (Piazzi et al. 2018), analysis was based on the following descriptors:

1. The percentage cover of the conspicuous taxa/morphological groups was evaluated for each sample using a Photoquad software (Trygonis & Sini 2012). Analysis of percent coverage was based on the grid method, with 625 cell division per 50 x 50 cm quadrat, assigning at least one category (species/taxa or bare substrate type) to each 4 cm^2. Following Piazzi et al. (2018), the overall Sensitivity level (SL) was calculated by multiplying the value of the SL of each taxon/group (see Appendix 3) for its class of abundance and then by summing up all the final values. The cover values of each taxon/morphological group was assigned to eight classes of abundance (see Piazzi et al. 2018 and references therein): (1) 0 to ≤0.01%; (2) 0.01 to ≤0.1%; (3) 0.1 to ≤1%; (4) 1 to ≤ 5%; (5) 5 to ≤25%; (6) 25 to ≤50%; (7) 50 to ≤75%; (8) 75 to ≤100%). Hence, a higher overall score would indicate a more pristine site.

2. The richness (α-diversity, i.e. the mean number of the taxa/ groups per replicate) was computed.

3. The β-diversity was evaluated as the mean distance of all replicates within each site from centroids calculated through PERMDISP procedure (Anderson 2006, Anderson et al. 2006). In undisturbed conditions, high values of β-diversity would be expected for coralligenous, due to its high variability at smaller spatial scales - stemming mainly from the patchy distribution of the organisms thriving there. Disturbances such as the loss of structuring perennial species and the proliferation of ephemeral algae lead to widespread biotic and to a consequential reduction of β-diversity (see Piazzi *et al*. 2018 and references therein).

4. The percentage cover of sediment and invasive algae was estimated for each sample.

5. The thickness of the calcareous layer was measured using a hand-held penetrometer with a minimum of six replicated measures per site. Since the calcareous accretion of biogenic reefs such as coralligenous may be impaired by human-induced impacts, the thickness and consistency of the calcareous deposit can be considered as a good indicator of the occurrence of a positive balance in the bioconstruction process, i.e. bioconstruction prevails over bioerosion. Thus, null penetration of penetrometer is indicative of a hard rock and suggests that either the biogenic substrate is absent or the bioconstructional process is no longer active; a millimetric penetration indicates the presence of active bioconstruction resulting in a calcareous biogenic substrate; and a centimetric penetration reveals a still unconsolidated bioconstruction (see Piazzi *et al*. 2018 and references therein).

6. The size (mean height) and the percentage of necrosis and epibiosis of erect anthozoans (gorgonians) was assessed *in situ* (see section 2.2).

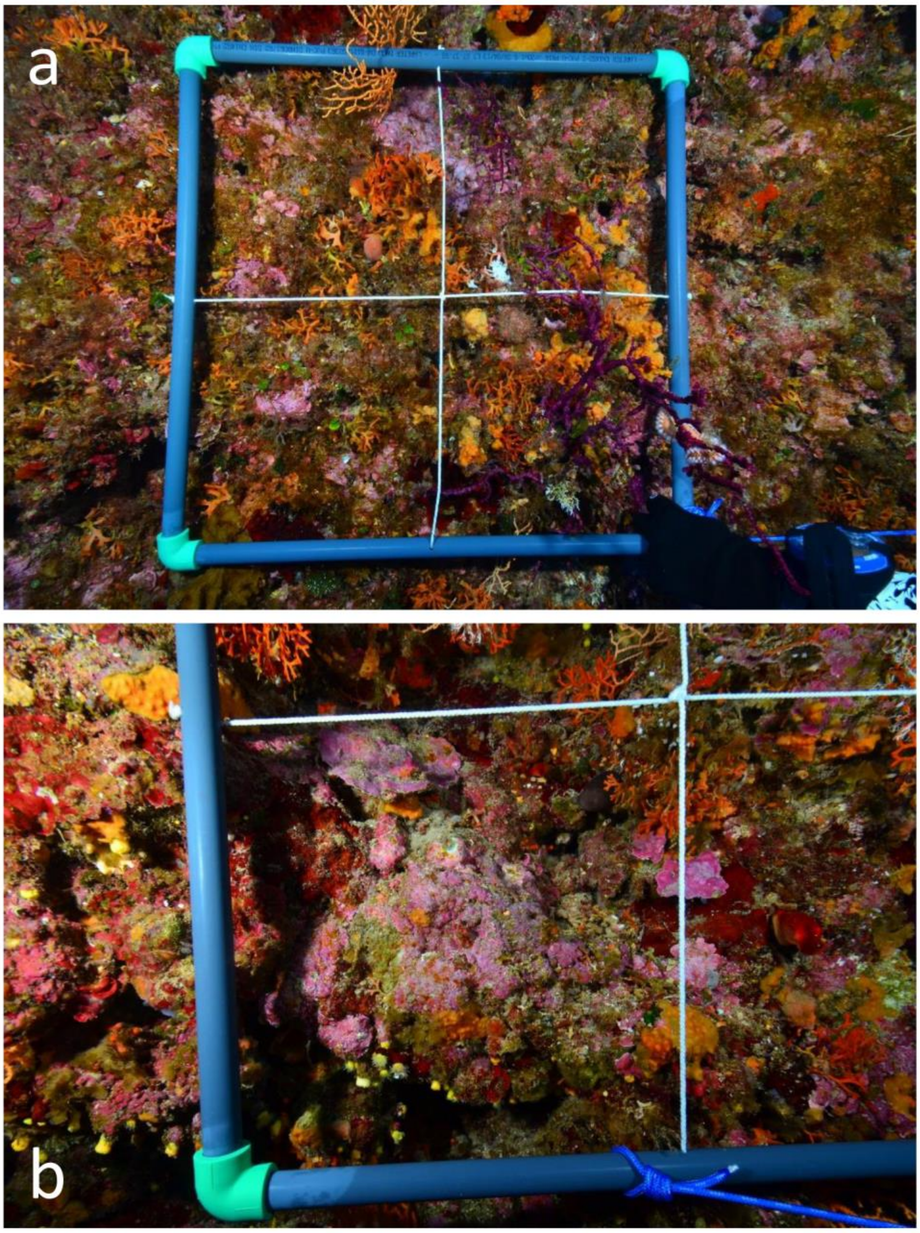
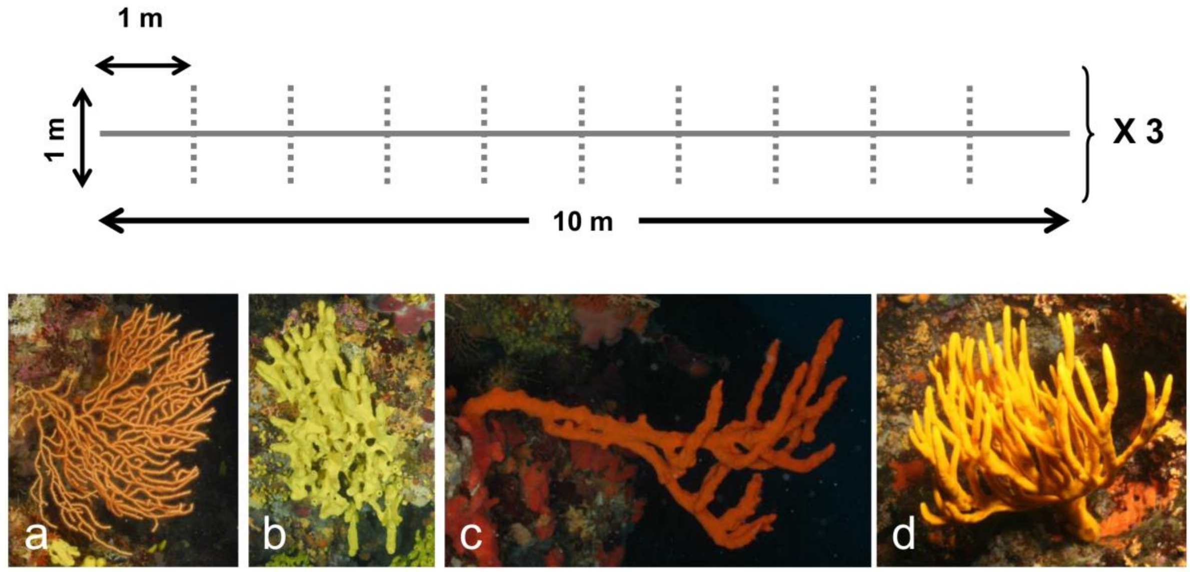


Fig. 2.1.2. Example of: a) 50x50 cm subquadrat and b) 25x25 cm subquadrat used during photo sampling within coralligenous assemblage at Stupišće location, Vis Island (photo credit: M. Belošević).

Visual census and video along random transects

Visual census along three 10 x 1 m horizontal transects was carried out to assess the third component of degree of structural complexity - the erect layer (by estimating the abundance of arborescent and massive species that can reach heights and/or diameters above 15 cm). One diver set the transect using a reel (marked at each 1 m length) whereas the other one estimated density of the organisms belonging to the erect layer, i.e. the ones higher than 15 cm. The latter diver observed the surface that extends 50 cm over and 50 cm below the transect, and afterwards he/she moved to the next m^2. Hence, the estimates were made within each 1 m^2 of the transect and the categories of density were used as indicated in Fig. 2.1.3) to avoid counting of all colonies, and hence, to speed up the work underwater (usually around 35 m depth).



The erect layer (> 15 cm in height)

for each 1 m section	Category				
	1	2	3	4	5
N of colonies/m2	0	1-2	2-10	10-20	> 20

Fig. 2.1.3. Visual census along random transect performed to aid the assessment of structural complexity, through estimation of of the erect layer. Images illustrate species usually forming the erect layer in the coralligenous: a) gorgonians, such as *Eunicella cavolini* and big sponges such as b) *Aplysina cavernicola*, c) *Axinella cannabina* and d) *Axinella polypoides*. To avoid counting of all colonies, categories of density were used as indicated in the scheme.

The total cover of the erect layer for each transect is obtained by summing up the values of scores for each category determined for each quadrat:

	Category				
	1	2	3	4	5
Score	0	1	2	3	4

Finally, the cover of the erect layer is obtained from the total score per transect (i.e. the sum of the scores of ten quadrats). The total score can range from 0 to 40. The estimate of erect layer cover in each transect is determined according to the following categories:

	Total score value			
	0	1-10	11-20	> 20
Cover	Null	Low	Medium	High

Besides estimation of the erect layer, visual census along the same transects allowed for estimation of abundance of macro-bioeroders such as sea urchins *Sphaerechinus granularis* and *Echinus* sp. (by counting total N of individuals of each species in each quadrat along the same transects, Fig. 2.1.4) as well as estimation of the cover of mucilaginous aggregates, using the categories as indicated in Fig. 2.1.5. Estimates of the cover of mucilaginous aggregates can be made in each quadrat to cope with the potential heterogeneity, however usually this phenomenon is quite homogenous, at least at the scale of the 10 m^2 transects, hence a single estimate for the whole transect may be provided.

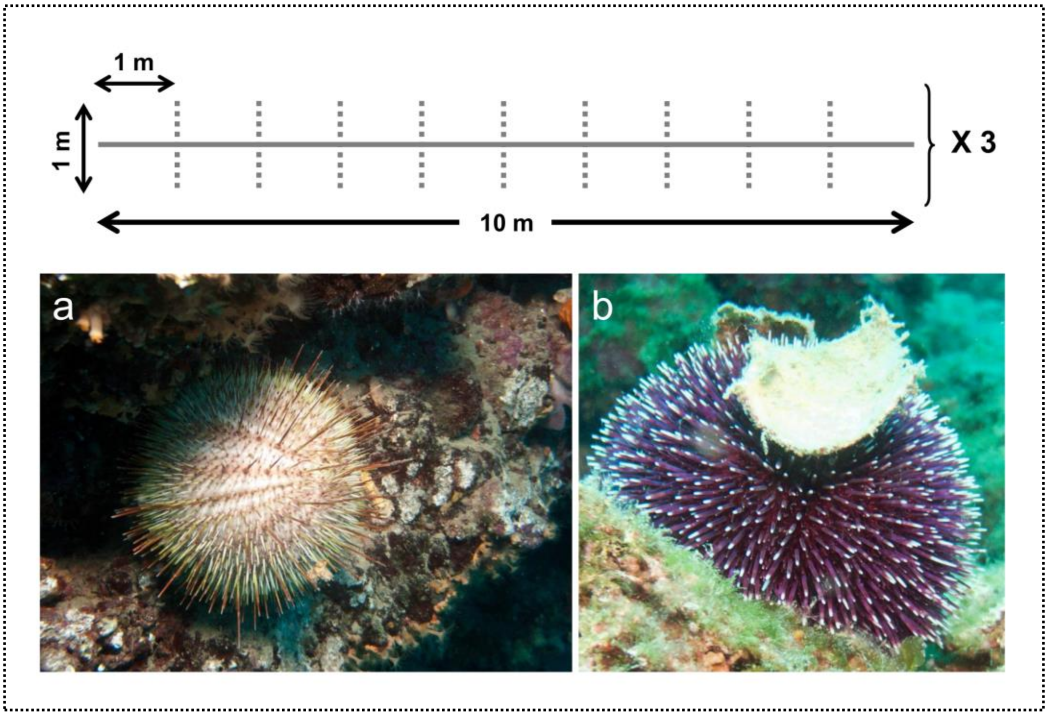
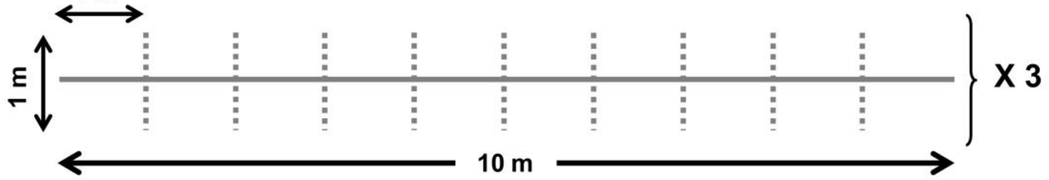


Fig. 2.1.4. Visual census along random transect to assess the efect of macrobioeroders.



Category 0 (Null): 0% cover of the transect

Category 1 (Low): low abundance in the basal-intermediate layers **AND/OR** in the erect layer

Category 2 (Medium): High abundance **EITHER** in the basal-intermediate layers **OR** in the erect layer

Category 3 (High): High abundance both in the basal-intermediate layers **AND** in the erect layer

Fig. 2.1.5. Visual census along random transect to assess the impact of mucilaginous outbreaks.

To provide additional permanent records of a greater surface of coralligenous and to compare assessment *in situ vs*. assessment based on video analysis, marked transects used by divers for visual census (Fig. 2.1.6) were also recorded with a GoPro 7 camera fitted with external lighting (BigBlue Black Molly V). Both photo sampling and video recording methods applied allow for the extraction of data that can be used for characterization of assemblages as well for estimation of descriptors aimed to assess coralligenous ecological status.

Fig. 2.1.6. A diver performing visual census along the random transect within a coralligenous assemblage dominated by the yellow gorgonian *Eunicella cavolini* at the Lagnići location (Dugi Otok Island, Croatia). Photo credit: Z. Jakl.

2.1.2. Main results

Characterization of coralligenous assemblages: community composition and habitat structural complexity

Overall, 120 images of 50 x 50 cm subquadrats (a surface of 30 m^2 in total, 7.5 m^2 per site) have been examined and a total of 99 macrobenthic taxa (i.e. categories of sessile organisms) were identified from photographs: 26 macroalgae (9 Chlorophyta, 14 Rhodophyta, 3 Ochrophyta), 1 protozoan, 33 sponges, 10 anthozoans, 1 hydrozoan, 4 polychaetes, 1 bivalve, 1 gastropod, 12 bryozoans and 10 ascidians (see Appendix 4). Based on this photosampling effort, the highest number of taxa was recorded at Sika 3 site on the Vis Island (74 taxa) and the lowest at Lagnići 2 (63 taxa; Fig.2.1.7). These values are in general slightly higher than the ones reported for the Adriatic Sea so far, using the same sampling effort (e.g. 46 to 65 taxa were recorded for sites in the Northern Adriatic, Kipson 2015). Approximately 1/3 of recorded taxa (36) were present at all surveyed sites. The most species rich taxonomic group was the one of sponges at both sites on Lagnići location (Dugi Otok Island), while the group of algae was the most species rich at both Stupišće sites (Vis Island). Other main taxonomic groups, i.e. anthozoans, bryozoans and tunicates showed comparable species richness at all sites (Fig.2.1.8).

The analysis of additional images of quadrats 25 x 25 cm (8 images within the replicate of 0.5 m^2, 5 replicates per site, in total 40 images - 2.5 m^2 per site) revealed another 10 species/groups at each site on the Vis Island, 6 new ones at Lagnići 1 site and 17 at Lagnići 2 site on the Dugi Otok Island (Fig. 2.1.7). These were mainly small, more conspicuous species whose identification was enabled by more close-up images that revealed more details and/or by inspection of larger overall surface, e.g. bryozoan *Beania sp.*, hard corals *Caryophyllia inornata* and *Hoplangia durotrix*, white encrusting colonial ascidians belonging to the family of Didemnidae, etc.

Whereas presence and/or abundance of algal and animal bioconstructors depended on the location or the site (see below a detailed characterization of coralligenous assemblage at each site), agglomerative species such as sponge *Fasciospongia cavernosa* and bryozoan *Beania* sp.

were commonly found. Likewise, the main bioeroders at all sites were boring sponges *Cliona* spp., and the endolithic bivalve *Rocellaria dubia*.

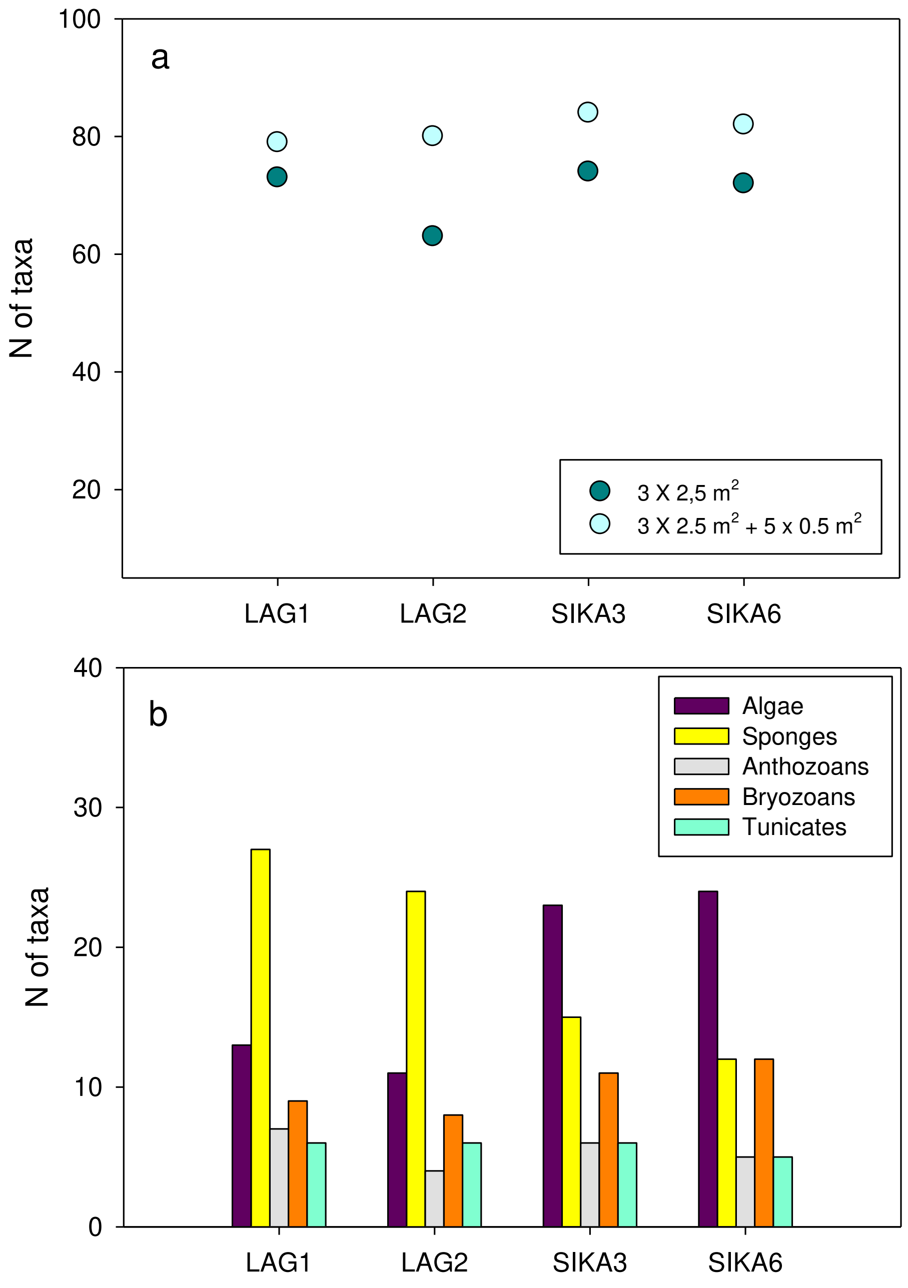


Fig. 2.1.7. Number of taxa recorded within coralligenous assemblages (30 - 40 m depth range) at each of the 4 studied sites: a) results of photosampling of three replicates of 2,5 m^2 per site and photosampling of three replicates of 2,5 m^2 and five replicates of 0,5 m^2 ; b) richness of the 5 main subsets of taxa (based on photosampling of three replicates of 2.5 m^2 per site).

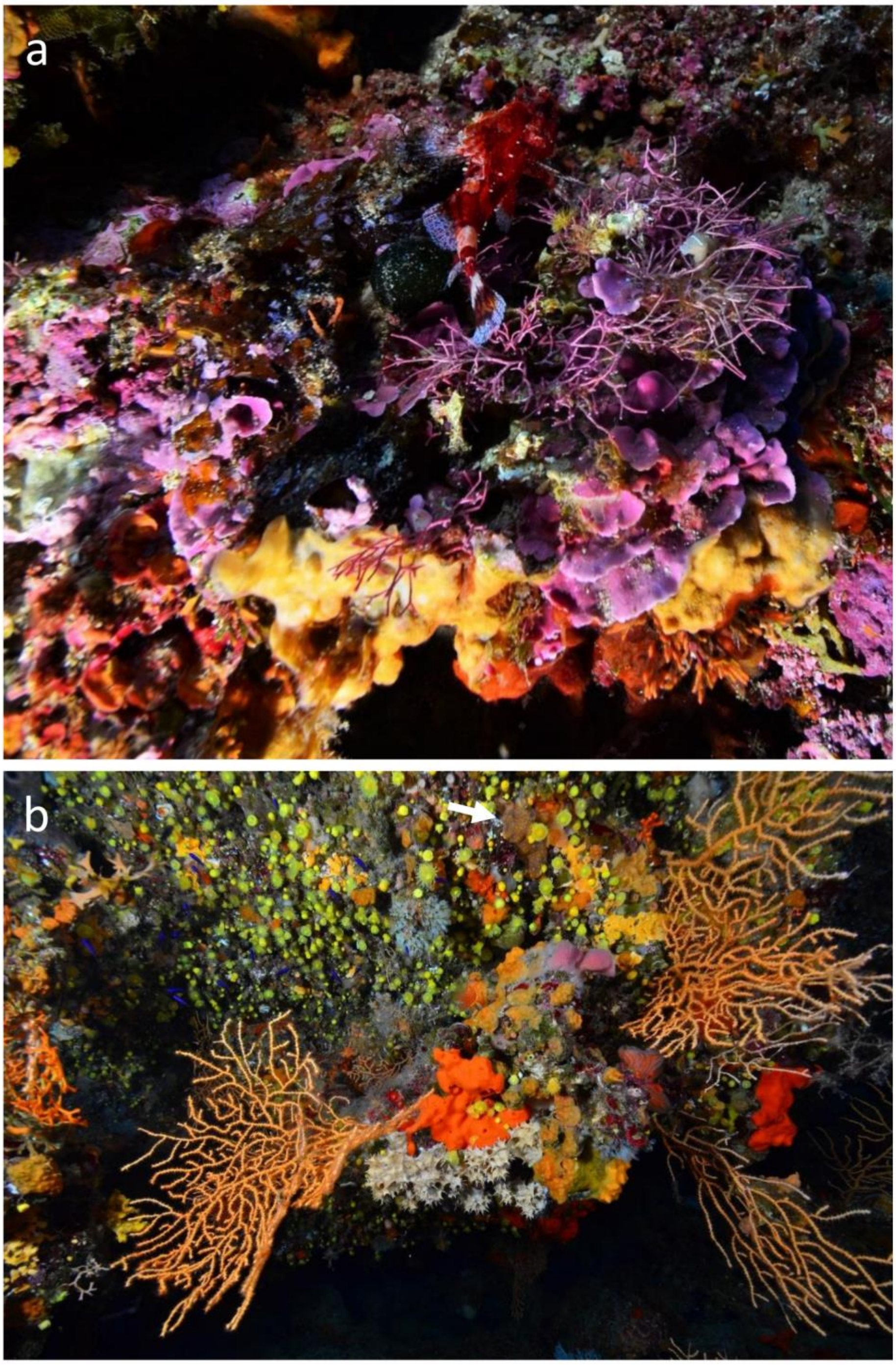


Fig. 2.1.8. Additional photo and video analysis revealed several previously undetected species in the coralligenous habitat: a) unidentified branchy coralline algae, b) unidentified sponge or tunicate (no expert consensus at the moment of report preparation) – indicated by a white arrow. Photo credit: M. Belošević.

In addition to sampling of photoquadrats, all available images and videos were inspected and hence, additional taxa, not previously recorded at the respective site were noted. The number of taxa recorded in this way ranged from 2 to 18 per site. A list of all taxa is provided in Appendix 4.

Lagnići 1

The basal layer of coralligenous assemblage at Lagnići 1 site (Figs. 1.1.3 d,e; 2.1.9) is largerly dominated by several species of encrusting Peyssonneliales, which present the main algal builders here, reaching considerable cover of 36.52% (Fig. 2.1.9 a, Fig. 2.1.13). These algae are to a much lesser extent followed by encrusting coralline algae (8.6% cover) attaining around 45% cover in total (Figs. 2.1.13, 2.1.16). Out of other algal categories, green algal turf is found here with 6.7% cover, similar to the category of mixed turf (i.e. mixed algal and animal turf-forming species intermingled with sediment grains; e.g. some species of small hydrozoans may classify as animal turf; 6.45% cover; Fig.2.1.15). Other algae are either absent or they are present in low abundance.

In the intermediate layer, *Flabellia petiolata* is the main algal contributor (0.34% cover, Fig. 2.1.15). The most important group constituting this layer is the one of massive sponges, which are present here in the highest abundance of any studied site (7.5% cover, Fig.2.1.15). Species such as *Chondrosia reniformis*, *Aplysina cavernicola*, *Ircinia oros*, *Ircinia* sp. and category of black keratose sponges represent this group (Fig. 2.1.9 b,c). Whereas encrusting bryozoans are the main animal builders in the basal layer (3.2% cover), branchy bryozoans (4% cover) such as *Myriapora truncata* and *Smittina cervicornis*/*Aedonella pallasi* fulfill this role in the intermediate layer (Fig. 2.1.14).

The erect layer is formed exclusively by the yellow gorgonian *Eunicella cavolini* (Figs. 1.1.3 e; 2.1.9 c)., contributing to the upper-medium level of structural complexity (Fig. 2.1.18).

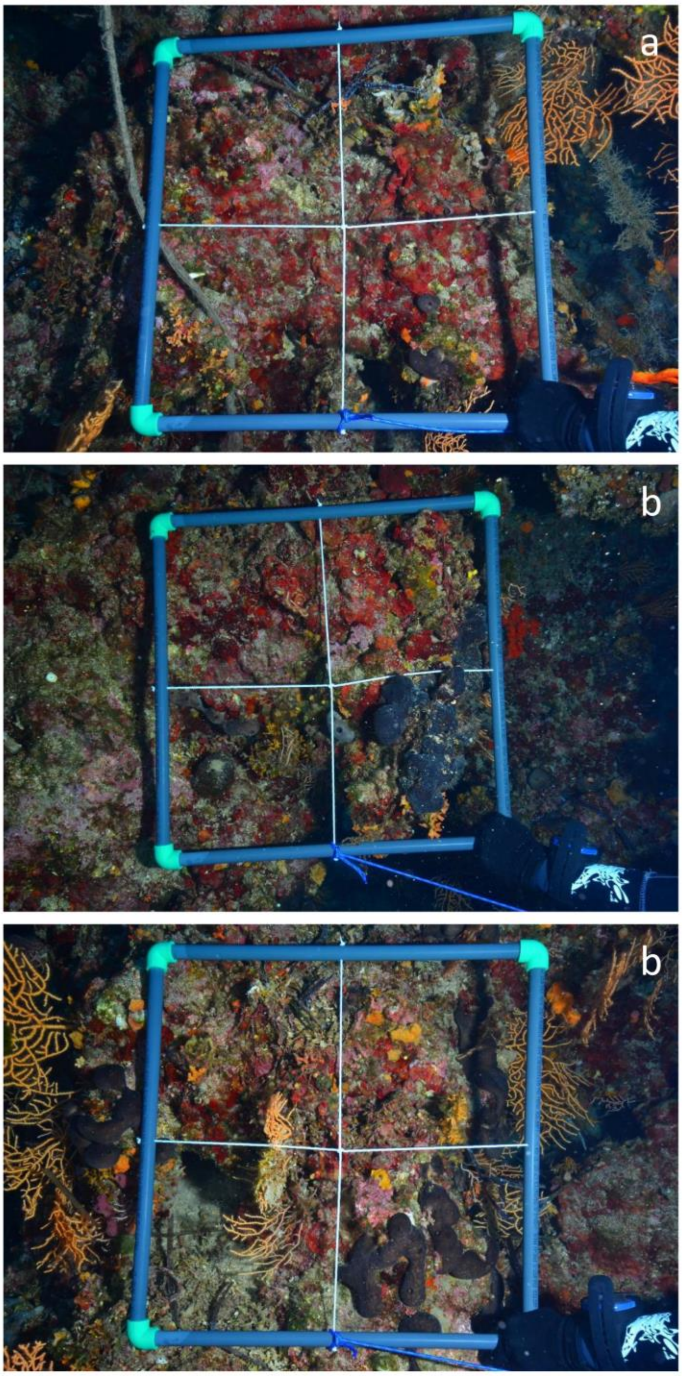


Fig. 2.1.9. Illustration of the coralligenous assemblage at Lagnići 1 site (location Lagnići, NW Dugi Otok Island) assessed at 35 m depth: a) several species of encrusting Peyssonneliales present major algal builders in the coralligenous basal layer where it seems as the occassional organic matter input may be provided by fallen posidonia leaves, b) intermediate layer is largerly constituted by massive sponges such as this keratose one or c) *Chondrosia reniformis*; yellow gorgonian *Eunicella cavolini* forms the erect layer and adds to the structural complexity of the assemblage. Likewise, holes and crevices create additional microhabitats here, convenient also for sediment retention. Photo credit: M. Belošević.

Lagnići 2

In terms of composition and structure of coralligenous assemblage this site (Figs. 1.1.4 e,f; 2.1.10) is very similar to Lagnići 1 site. Almost identical percent cover of *Peyssonnelia* spp. was recorded here as at Lagnići 1 site (36.56%), followed by encrusting coralline algae with 7% cover (Fig. 2.1.13). Hence, these species represent the main algal builders in the basal layer of coralligenous, reaching similar total values (43.5% cover, (Fig. 2.1.16). Likewise, encrusting bryozoans as the main animal bioconstructors are present in similar abundance as at Lagnići 1 site (3% cover; Fig.2.1.14). Green algal turf (4.4% cover) and mixed turf (8.3% cover) are also found among constituents of the basal layer (Fig.2.1.15).

Again, massive sponges, represented primarily by species such as *Chondrosia reniformis*, *Ircinia* sp. and category of black keratose sponges are the main contributors to the intermediate layer (5.2% cover, Fig. 2.1.10 b,c; Fig. 2.1.15). Sponge *Aplysina cavernicola*, a strictly protected species by national legislation and international conventions, thrives in the coralligenous assemblage here but finds also adequate substrate on the lost fishing gear (see section 2.5, Fig. 2.5.4 b). Branchy bryozoans as the main bioconstructors in the intermediate layer are present with 4% cover (Fig. 2.1.14). Of these, the most abundant species were *Myriapora truncata* and *Smittina cervicornis* / *Aedonella pallasi*.

As at the Lagnići 1 site, the erect layer is formed exclusively by the yellow gorgonian *Eunicella cavolini* (Figs. 1.1.4 f; 2.1.10 c) contributing to the slightly lower (i.e. medium *vs*. upper medium) level of structural complexity here (Fig. 2.1.18).

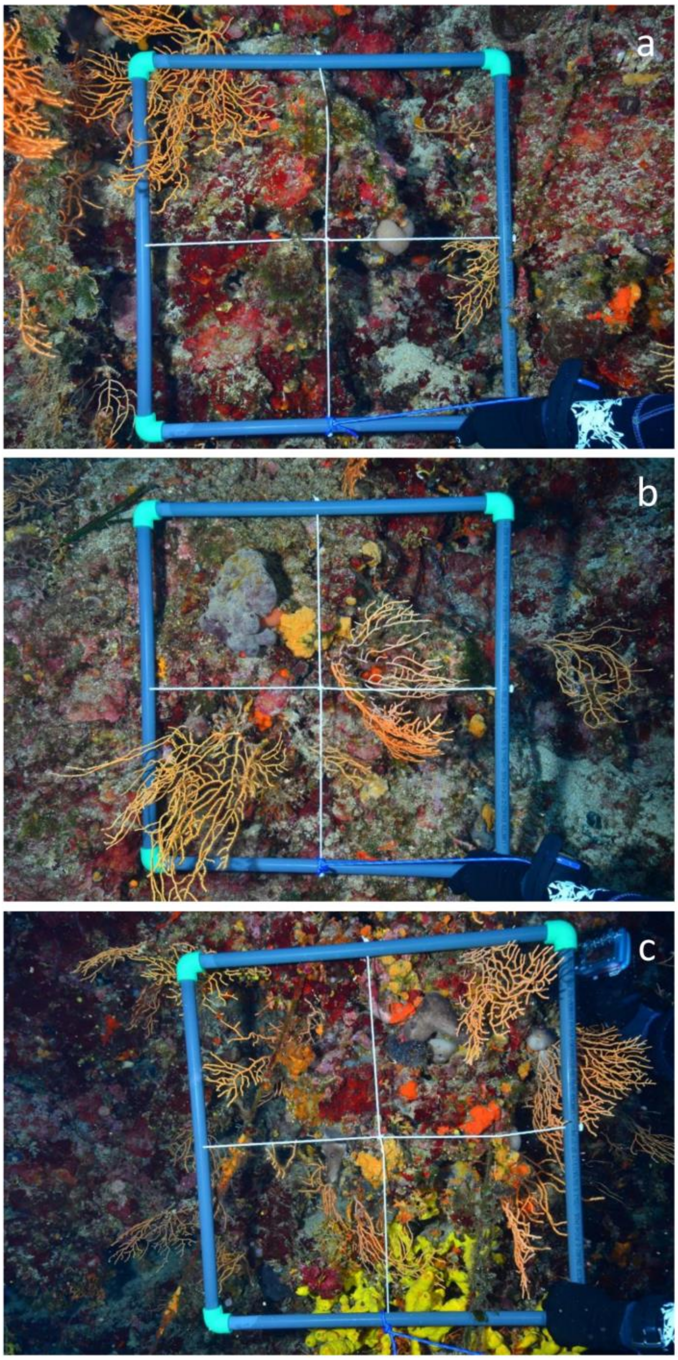


Fig. 2.1.10. Illustration of the coralligenous assemblage at Lagnići 2 site (location Lagnići, NW Dugi Otok Island) assessed at 35 m depth: a) in the basal layer encrusting *Peyssonnelia* spp. are predominant species and the main algal builders; green algal and mixed turf as well as sediment are present here with an overall of 15% cover; b) within the basal layer encrusting bryozoans represent the main animal builders whereas massive sponges are among the main contributors to the intermediate layer such as *Ircinia* sp., c) *Chondrosia reniformis* and occassionaly *Aplysina cavernicola*; the yellow gorgonian *Eunicella cavolini* forms the erect layer. A lot of diverse lost fishing gear was observed at Lagnići location, such as longlines present in this image. Photo credit: M. Belošević.

Sika 3

The basal layer of coralligenous assemblage at Sika 3 site is predominantly formed by encrusting coralline algae, amounting to the highest percent cover of all investigated sites (29.3%, Fig. 2.1.13). Some of the algae belonging to this group, namely *Lithophyllum sticteaforme* occassionaly form representative plate-like layered thalli (with approximately 4% cover) that add to the structural complexity of the assemblage and create additional microhabitats, as well as contribute to attractiveness of recreational diving there (Fig. 2.1.11 b,c). Other constituents of the basal layer include several species of encrusting Peyssonnaliacea that are present with 18.3 % cover (Fig.2.1.13). Hence, encrusting red algae account in total for almost 50% cover at this site (Fig. 2.1.16). Out of other algal species in the basal layer, notable is the frequency of occurence of *Palmophyllum crassum*. Encrusting bryozoans (4.1% cover) and serpulids (0.56% cover) are the most abundant animal species in the basal layer and the main animal builders here (Fig. 2.1.14). Interestingly, sponges (otherwise one of the most diverse taxonomic group within coralligenous) were rarely present in the samples, and if they were, they were never abundant (Fig. 2.1.15). The exception is the bioeroding sponge *Cliona* spp., whose presence (evident only from the oscula on the surface of the substrate) was frequent. On the contrary, another main bioeroder, the mollusk *Rocellaria dubia* was fairly rarely spoted. In the basal layer there was also considerable abundance of mixed algal and animal (e.g. Hydrozoans) turf forming species intermingled with sediment, that often covered encrusting red algae (this category was coined as “mixed turf” and amounted to 11% cover at this site, Fig.2.1.15, see example in Fig. 2.1.12 b).

In the intermediate layer the main animal builders were branchy bryozoans (2.6% cover) such as *Smittina cervicornis*/*Adeonella pallasi* and *Myriapora truncata* (Fig.2.1.14), although present as relatively small colonies – the ones of *M. truncata* rarely exceeded 3-4 cm in height whereas the ones of Smittina *cervicornis* /*Adeonella pallasi* rarely exceeded 10 cm. Of other animals, mainly ascidians such as *Halocynthia papillosa* and *Aplidium* cf. *tabarquensis* were noted. From algae within intermediate layer *Codium bursa* and C. cf. *effusum* (0.33% cover) were present as well as *Flabelia petiolata* (2.3% cover), with rare appearance of erect Rhodophyta (0.07 % cover; Fig.2.1.15).

The erect layer was formed exclusively by gorgonians, both the yellow gorgonian *Eunicella cavolini* and the red gorgonian *Paramuricea clavata* (Figs. 1.1.5 e,f; Fig.2.1.11 c), whereas no erect sponges were observed, either within image samples nor video transects. The level of structural complexity was evaluated as the upper medium, occassionaly even as high (Fig. 2.1.19)

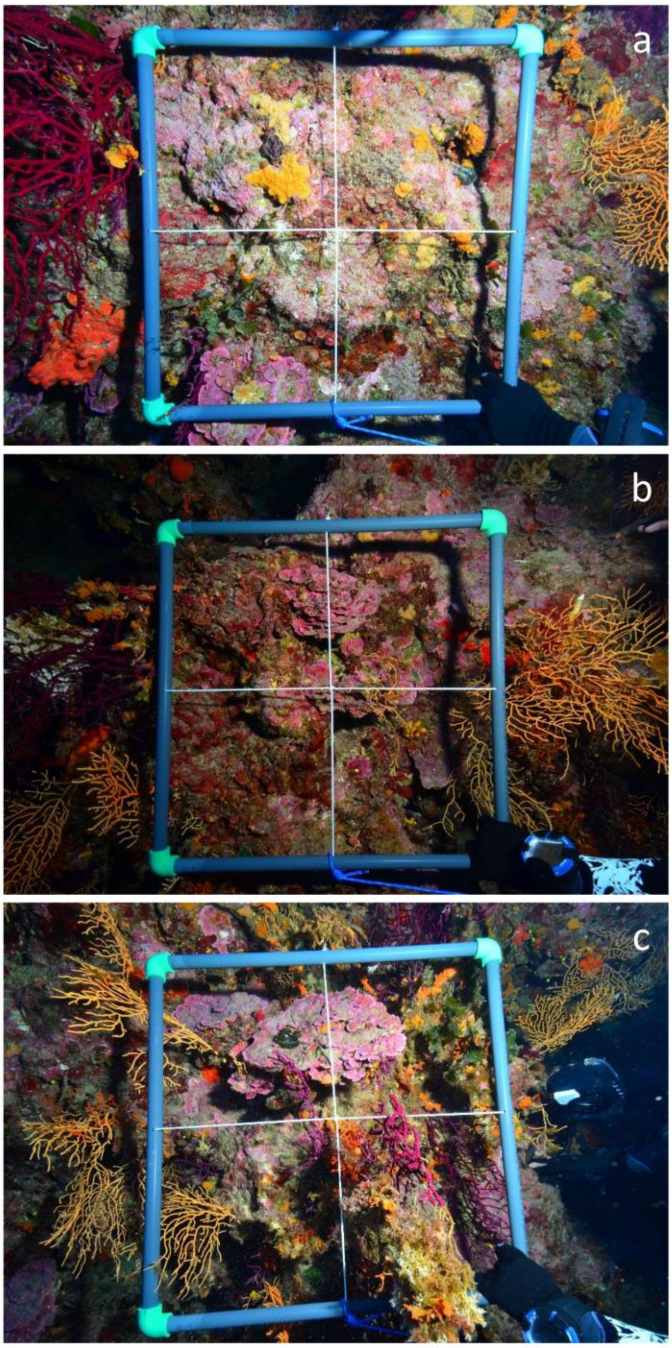


Fig. 2.1.11. Illustration of the coralligenous assemblage at Sika 3 site (location Stupišće, Vis Island) assessed at 35 m depth: a) in the basal layer encrusting Corallinales and *Peyssonnelia* spp. present the main algal builders, reaching almost 50% cover, whereas encrusting bryozoans are the main animal builders; b) coralline algae often form representative plate-like layered thalli at this site, contributing to the structural complexity and formation of additional microhabitats; c) in such plate-like formation, coralline algae even contribute to the intermediate layer, alongside green algae *Flabellia petiolata* and the main animal builder there - branchy bryozoan *Smittina cervicornis/Adeonella* sp.; the erect layer is formed by gorgonians *Eunicellla cavolini* and *Paramuricea clavata*, occassionaly considerably injured and overgrown by epibionts. Photo credits: M. Belošević.

Sika 6

The basal layer of coralligenous assemblage at this site (Fig. 1.1.6 e,f; Fig.2.1.12) is primarily formed by encrusting coralline algae and several species of Peyssonneliales, however they are present in lower total percent cover than at other Vis Island site, Sika 3 (around 28% *vs*. 50%,Fig. 2.1.13, Fig. 2.1.16). The abundance of encrusting coralline algae here was still almost twice as high as on the Dugi Otok Island sites (18.5%), whereas the lowest percent cover of *Peyssonnelia* spp. was recorded here of all investigated sites (9.3%; Fig.2.1.13). On the other hand, percentage of mixed turf was the highest here (20%, Fig. 2.1.15), 2 to 4-fold higher than on other sites. Other constituents of the basal layer include encrusting bryozoans (3.8% cover) and serpulids (1.7% cover) as the main animal builders (Fig.2.1.14). Again surprisingly, sponges were almost completely absent from photo samples (Fig. 2.1.15), except of bioeroding *Cliona* spp.. The mollusk *Rocellaria dubia* was another main bioeroder here.

One of the most characteristic and representative aspects at Sika 6 site was high abundance of large colonies of bryozoan *Pentapora fascialis* (with 9.7% cover) in the intermediate layer (Fig. 2.1.12 c), as well as of other erect bryozoans (reaching 13.5% cover in total, Fig. 2.1.14) such as *Smittina cervicornis*/*Adeonella pallasi* and *Myriapora truncata*, adding considerably to the overall bioconstruction. Related to other animals within this layer, not only massive sponges, but massive animals in general were rarely present here (Figs. 2.1.15, 2.1.16). From algae within intermediate layer, *Codium bursa* and C. cf. *effusum* (0.37% cover) were noted. Likewise, taxa considered as rather sensitive according to Piazzi *et al*. (2018; see Appendix 3) such as *Flabelia petiolata* and erect Rhodophyta were present here in the highest abundance of all sites (2.4% and 0.43 % cover, respectively, Fig. 2.1.15).

Like at Sika 3 site, the erect layer was formed exclusively by gorgonians, both the yellow gorgonian *Eunicella cavolini* and the red gorgonian *Paramuricea clavata* (Figs. 1.1.6 e,f; 2.1.12 c), resulting in the level of structural complexity evaluated as the upper medium to high (Fig. 2.1.19).

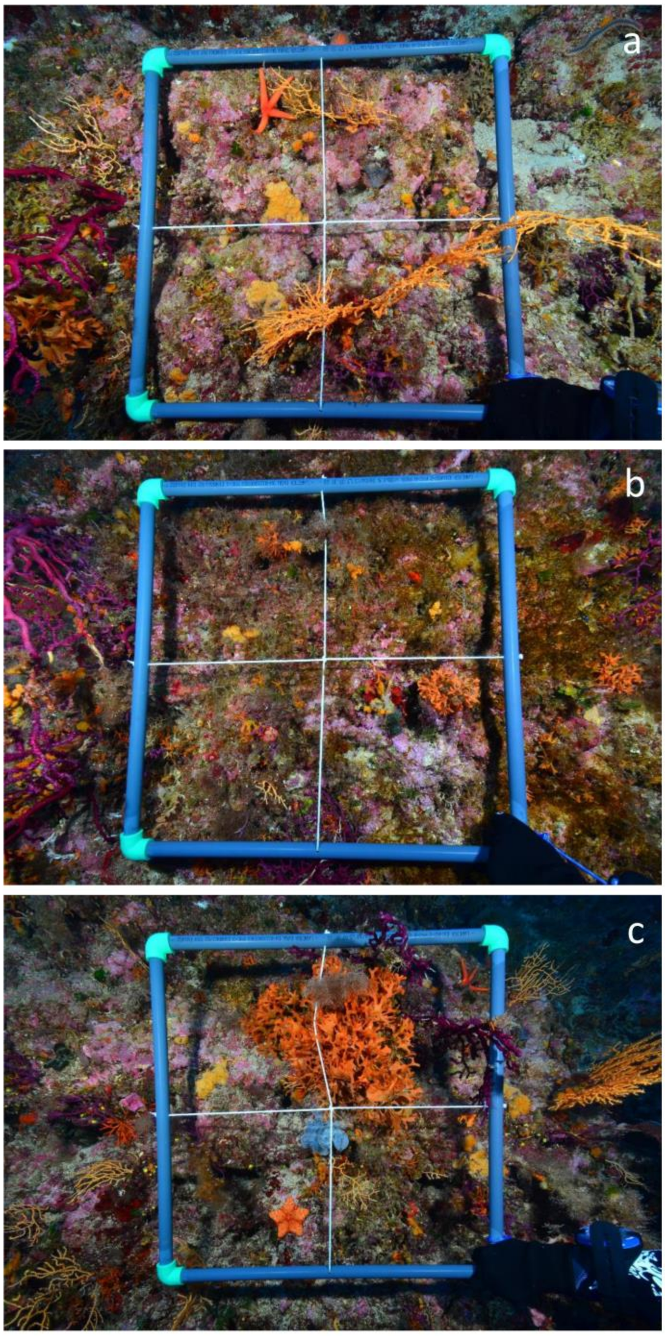


Fig. 2.1.12. Illustration of the coralligenous assemblage at Sika 6 site (location Stupišće, Vis Island) assessed at 35 m depth: a) encrusting Corallinales and Peyssonnelia spp. were the main algal builders and encrusting bryozoans were the main animal builders in the basal layer; b) category of mixed turf was the most abundant here from all studied sites (cca 20% cover) however one of the most prominent aspects of this site was high abundance of erect bryozoans in the intermediate layer such as Pentapora fascialis, present as small or c) large colonies, whereas the erect layer was formed by gorgonians Eunicellla cavolini and Paramuricea clavata – on this image present also as juveniles (< 15 cm in height) contributing to the intermediate layer. Photo credit: M. Belošević.

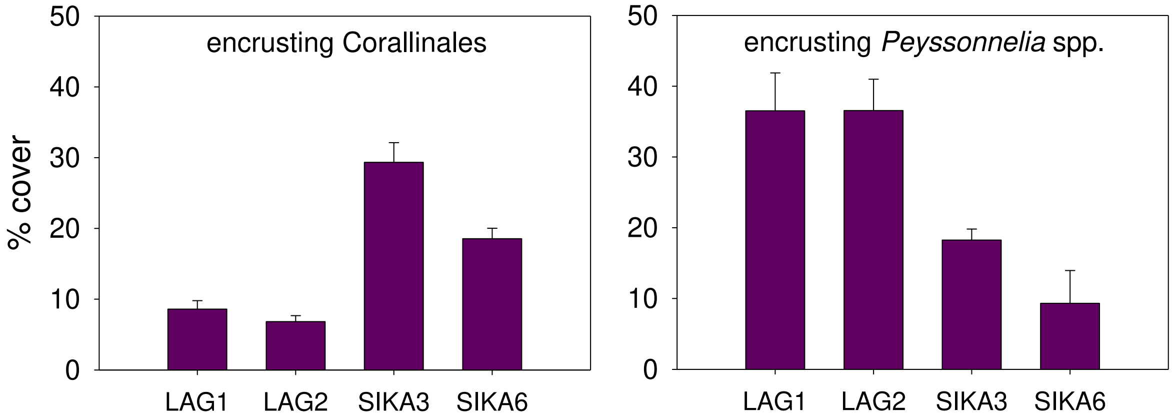


Fig. 2.1.13. Abundance (percent cover) of the main algal builders within coralligenous assemblages at sites on the Vis and Dugi Otok Island. Data are shown as a mean ± SE.

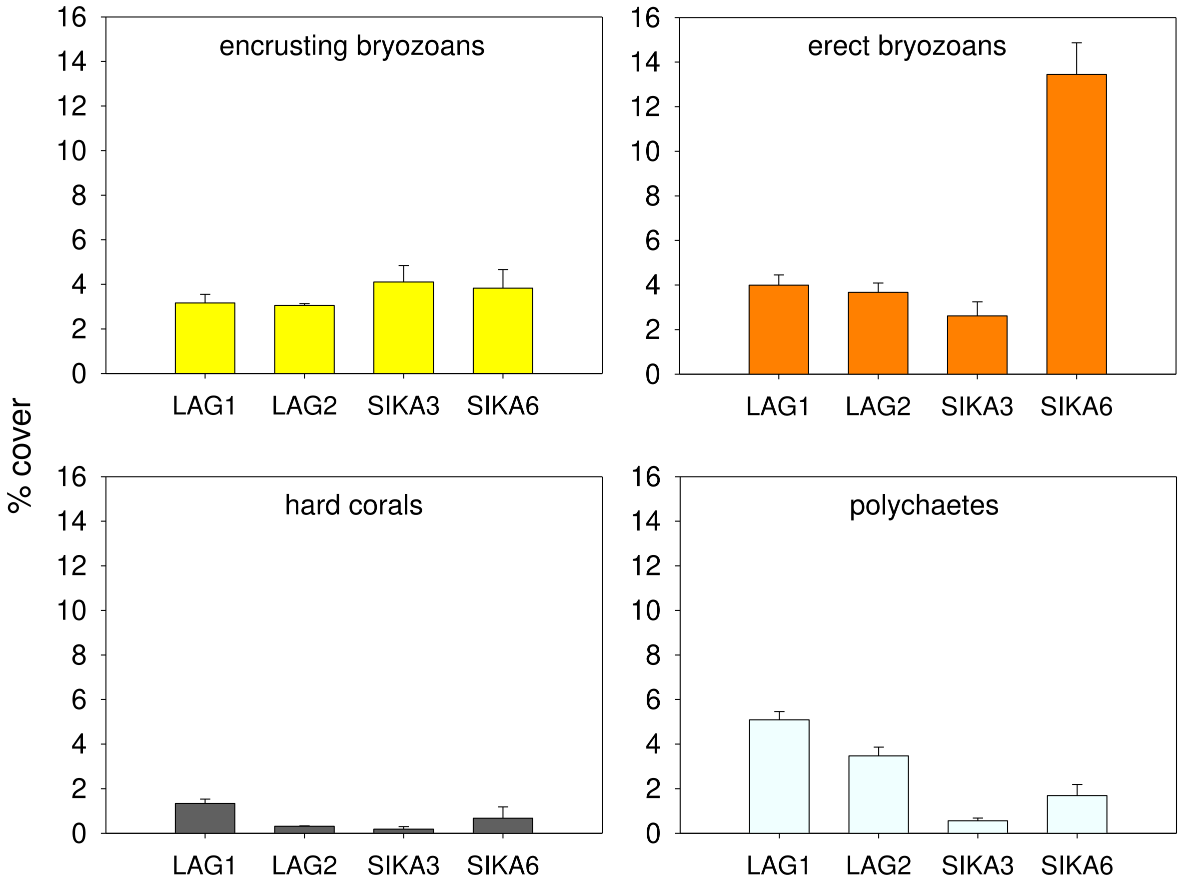


Fig. 2.1.14. Abundance (percent cover) of the main animal builders within coralligenous assemblages at sites on the Vis and Dugi Otok Island. Data are shown as a mean ± SE.

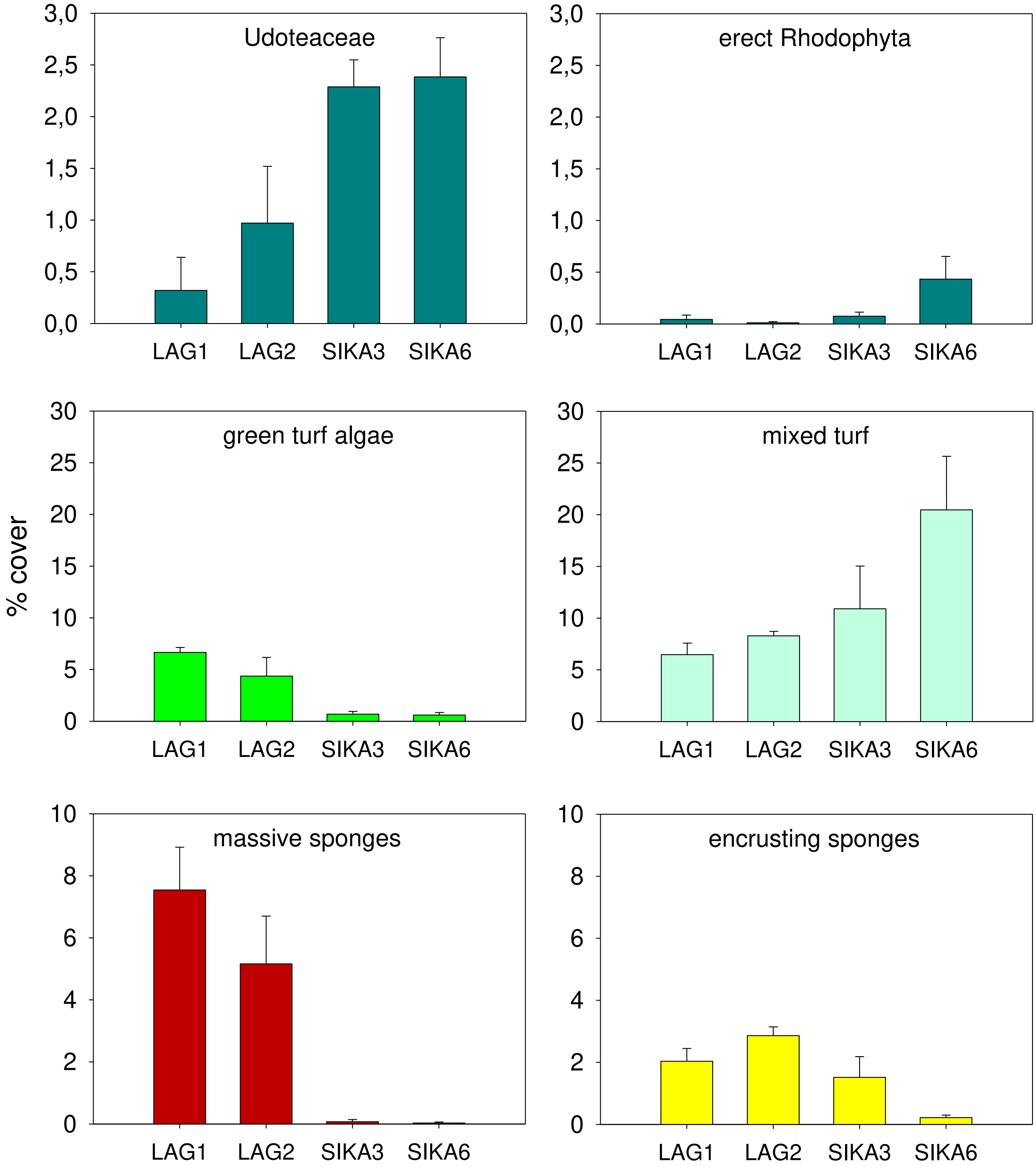


Fig. 2.1.15. Abundance (percent cover) of selected morpho-taxonomic or functional groups within coralligenous assemblages at sites on the Vis and Dugi Otok Island. Data are shown as a mean ± SE.

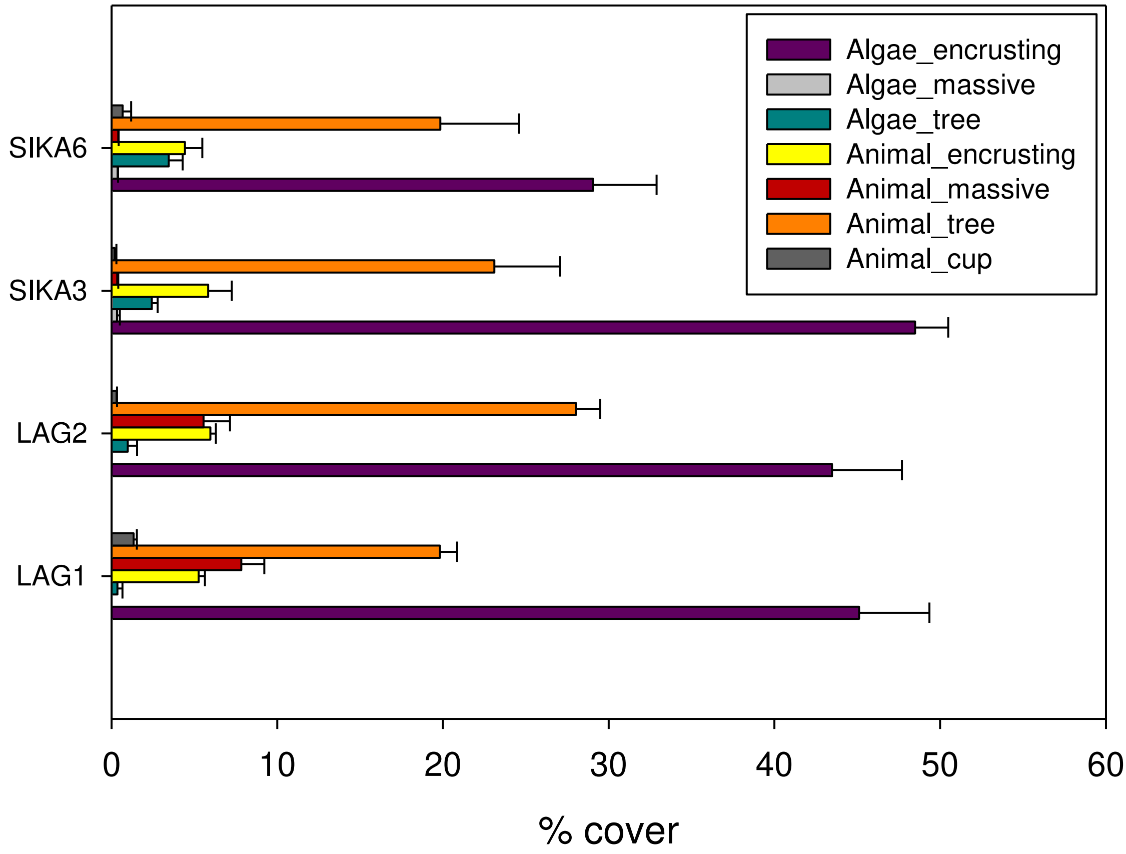


Fig. 2.1.16. Abundance (percent cover) of the main morphological groups of macrobenthos within coralligenous habitat at each study site. Data are shown as a mean ± SE.

Beside sessile marcrobenthos, additional photos and videos taken at each site were used to note the presence of vagile fauna in the coralligenous (see Appendix 4) without any intention to compare sites, since no equal sampling effort was applied. Some of recorded species are shown in Fig.2.1.17.

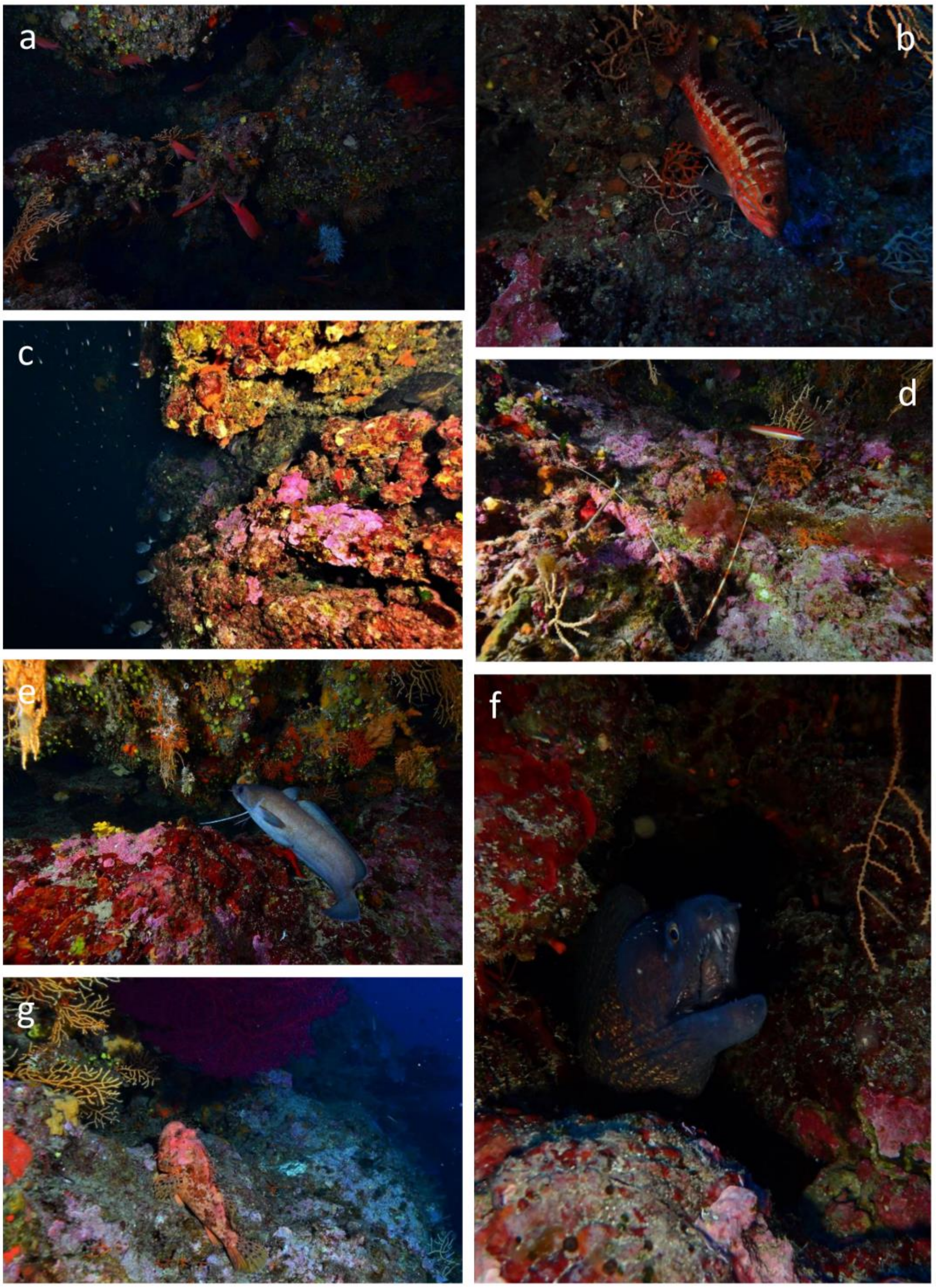


Fig. 2.1.17. Example of vagile fauna observed within coralligenous habitat that all present diving attraction, some are endangered or are commercially valued: a) *Anthias anthias*, b) *Serranus cabrilla*, c) *Ephinephelus marginatus*, d) *Palinurus elephas*, d) *Phycis phycis*, f) *Muraena helena, g) Scorpaena scrofa.* Photos are taken at Sika 3 and Sika 6 sites, Vis Island. Photo credit: M. Belošević except b) and f) S. Kipson.

The degree of complexity of coralligenous habitat: comparison of the assessment undertaken by different observers or by different methods – visual census in situ vs. video analysis

The estimates of the erect layer as a part of the assessment of coralligenous structural complexity in majority of cases did not differ considerably among the different observers who undertook assessment either *in situ* or from video recordings (Figs. 2.1.18, 2.1.19). Although estimations could differ for individual subquadrats, in most cases the final score assigned (as a sum of scores for 1 x 1 m subquadrats along a transect, see methodological section) was similar for different observers and it usually indicated a medium to upper medium complexity (total score between 10 and 20, Figs. 2.1.18, 2.1.19). Likewise, there were no considerable differences between assessment *in situ* and the subsequent one from videos, except in the case of Lagnići 1 site where medium complexity was estimated by most observers, and high complexity by Observer 5 based on video. Nevertheless overall high compatibility of scores from *in situ* and video analysis imply the underwater work could be reduced and a reliable estimate could be obtained from a desktop analysis, if the quality video is provided, i.e. the marked transect is positioned *in situ* to follow the relief of the substrate, the appropriate video lighting is in place and the distance of camera from the substrate is right (i.e. as close as possible to catch the video frame including 50 cm above and 50 cm below the marked transect – the reel's rope).

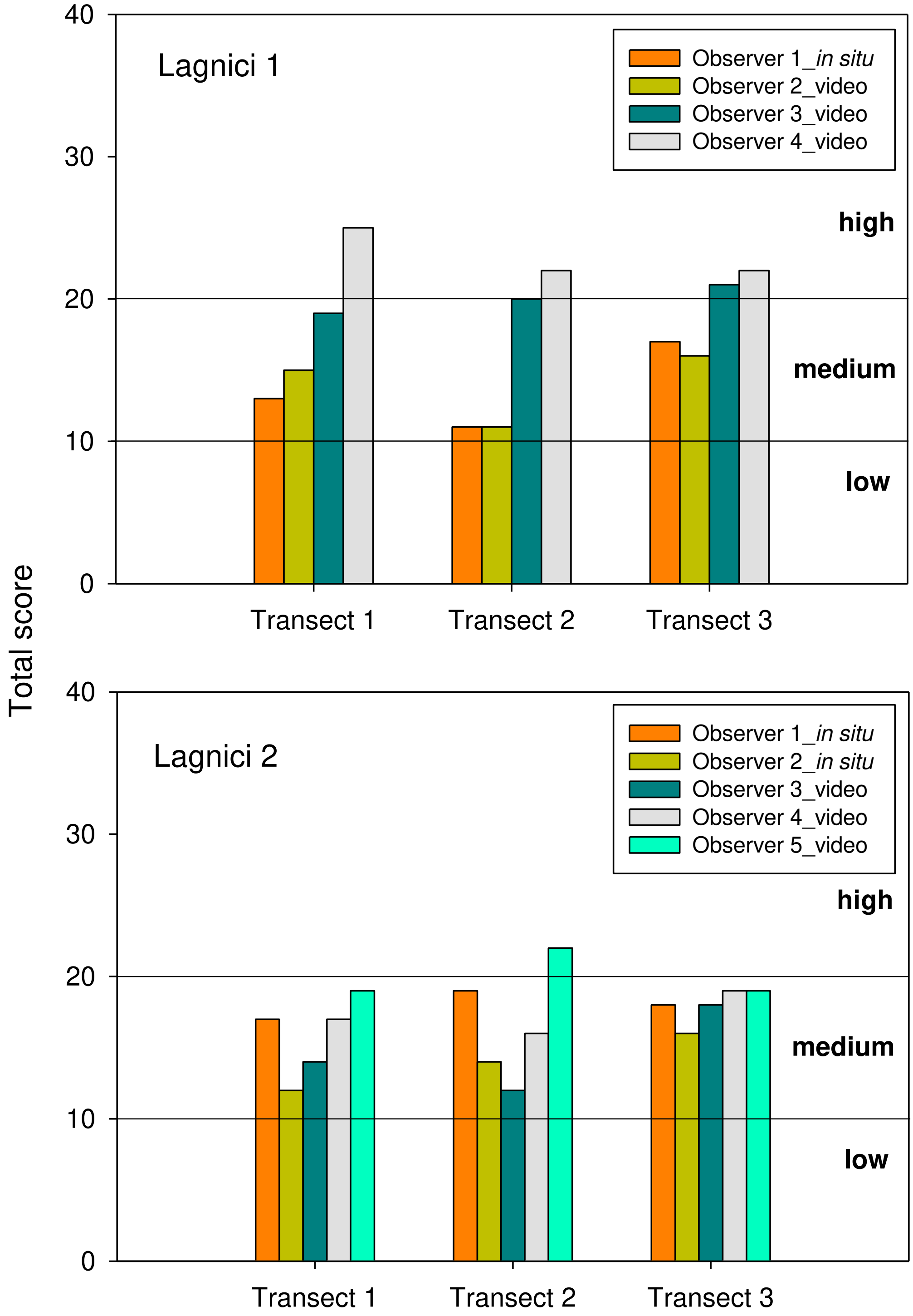


Fig. 2.1.18. Location Lagnići, NW Dugi Otok. Estimation of the erect layer (as a part of the assessment of coralligenous structural complexity) by multiple observers *in situ* along 3 marked transects (10 x 1 m) and/or subsequently from video recordings. All transects were set at 35 m depth.

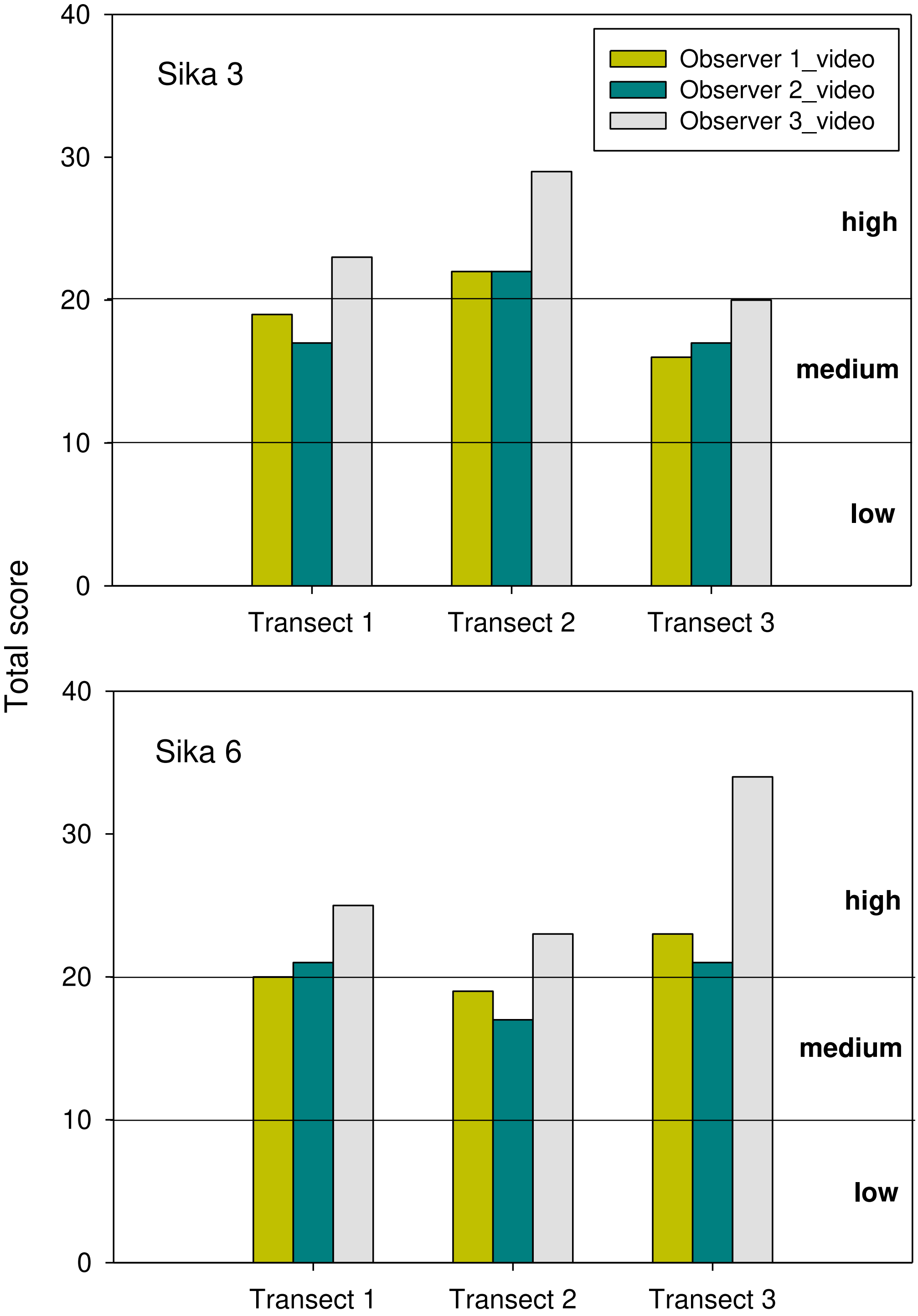


Fig.2.1.19. Location Stupišće, SW Vis Island. Estimation of the erect layer (as a part of the assessment of coralligenous structural complexity) by multiple observers from video recordings along 3 marked transects (10 x 1 m). All transects were set at 35 m depth.

Estimates on the abundance of macro-bioeroders (sea urchins)

Surprisingly, during both field trips, not a single sea urchin was observed along transects.

Estimate of coverage of mucilaginous aggregates

During field survey in September and October 2020 no mucilaginous algal aggregates were noted at any of the monitored sites on the Dugi Otok or the Vis Island.

Assessment of coralligenous ecological status

Similar species richness (alpha diversity, i.e. number of species/taxa per sampling unit/replicate; mean number of 51 to 57 species per 2.5 m^2) was observed at all study sites whereas heterogeneity (beta diversity, i.e. variability in species composition among sampling units/replicates within a study site, see methods section for further explantation) was slightly higher at Sika 3 site with the mean value of distance to centroids of 23, as opposed to 19 at Lagnići 1, and 15 at Lagnići 2 and Sika 6 (Fig. 2.1.20). This result would indicate Sika 3 was the most heterogeneous, that is, the least prone to potential homogenization due to human induced impacts. Since these descriptors are influenced by the size of the sampling unit/replicate, values reported here cannot be directly compared to the ones reported by Piazzi et al. 2018 (i.e. these authors considered 0.2 m^2 as a replicate *vs*. 2.5 m^2 considered here) but it can be compared to the ones previously obtained from low impact sites in the Eastern Adriatic by applying the same sampling approach (Kipson *et al*., unpubl. data).

As opposed to these 2 diversity descriptors, all of the others can be compared to Piazzi *et al*. (2018) because they are based on percentages or punctual field measurements. Hence, besides comparing our study sites among themselves, they can be also put in the perspective with sites in the west Mediterranean exposed to low or high human-induced impact, evaluated in the same way.

Sensitivity levels varied from 506 ± 16 at Lagnići 2 site to 617 ± 19 (mean ± SE) at Sika 6 site. Such values are 2 to 3-fold higher than the ones previously reported for sites exposed to high human impact and even slightly higher than the ones reported for sites with low human impact along western Italian coast (mean total score between 350-450, Piazzi et al. 2018). This would indicate greater number of more sensitive species (see Appendix 3) and/or their greater abundance at our study sites, with Sika 6 being especially remarkable in this regard. Among others, the percent cover of erect bryozoans, important animal bioconstructors within coralligenous and species sensitive to human induced impacts, especially mechanical abrasion, was 3 to even 12-fold higher at our study sites than at low impact sites reported by Piazzi *et al*. (2018). However, it should be also noted that some colonies were partially damaged.

Thickness of calcareous deposit, i.e. penetration of the penetrometer into the substrate ranged from 0.5 to 1.5 cm, with mean value around 1 cm at all studied sites (Fig. 2.1.20). Such values are 3 to 5-fold greater than the ones reported for coralligenous at stressed sites and even slightly higher (sometimes even 2-fold) than the ones reported for pristine sites so far (Piazzi *et al*. 2018). As the millimetric penetration of the penetrometer indicates the presence of active bioconstruction resulting in a calcareous biogenic substrate, and centimetric one indicates bioconstruction that is still not consolidated, our values lie somewhere in between and in any case, reveal positive balance in bioconstruction process (Gatti *et al*. 2012).

Since coralligenous assemblages were dominated by the yellow gorgonian *Eunicella cavolini* at 35 m depth where the main assessment was made at all our study sites, the extent of injury/necrosis was estimated *in situ* for this species. Colonies were twice as affected at sites on the Vis Island than at sites on the Dugi Otok Island, with the mean values varying between 15% and 38% (Fig. 2.1.20). Such considerable level of damage to colony (with the mean extent of injury of gorgonian tissue > 20%) for all sites except Lagnići 2 (where it was slightly lower but not low, 15%) would be comparable to the levels previously reported for the Central East Adriatic Sea (13-26%; Sini *et al*. 2015) as well as to the ones observed on disturbed sites by Piazzi *et al*. (2018).

Although sediment coverage varied 2 to 5-fold between sites (from 1.6 to 11%), being higher on Lagnići location (Figs. 2.1.20, 2.1.21 a), in all sites sedimentation was comparable to the level observed at sites with low human pressure conditions, as reported by Piazzi *et al*. (2018).

Out of other potential stressors, the presence of the invasive green algae *Caulerpa cylindracea* was noted at sites on the Vis Island, however its percent cover (at 35 m depth) was still very low: 0.01 ± 0.01 at Sika 3 and 1.13 ± 1.07 (mean ± SE) at Sika 6 site (Fig. 2.1.21).

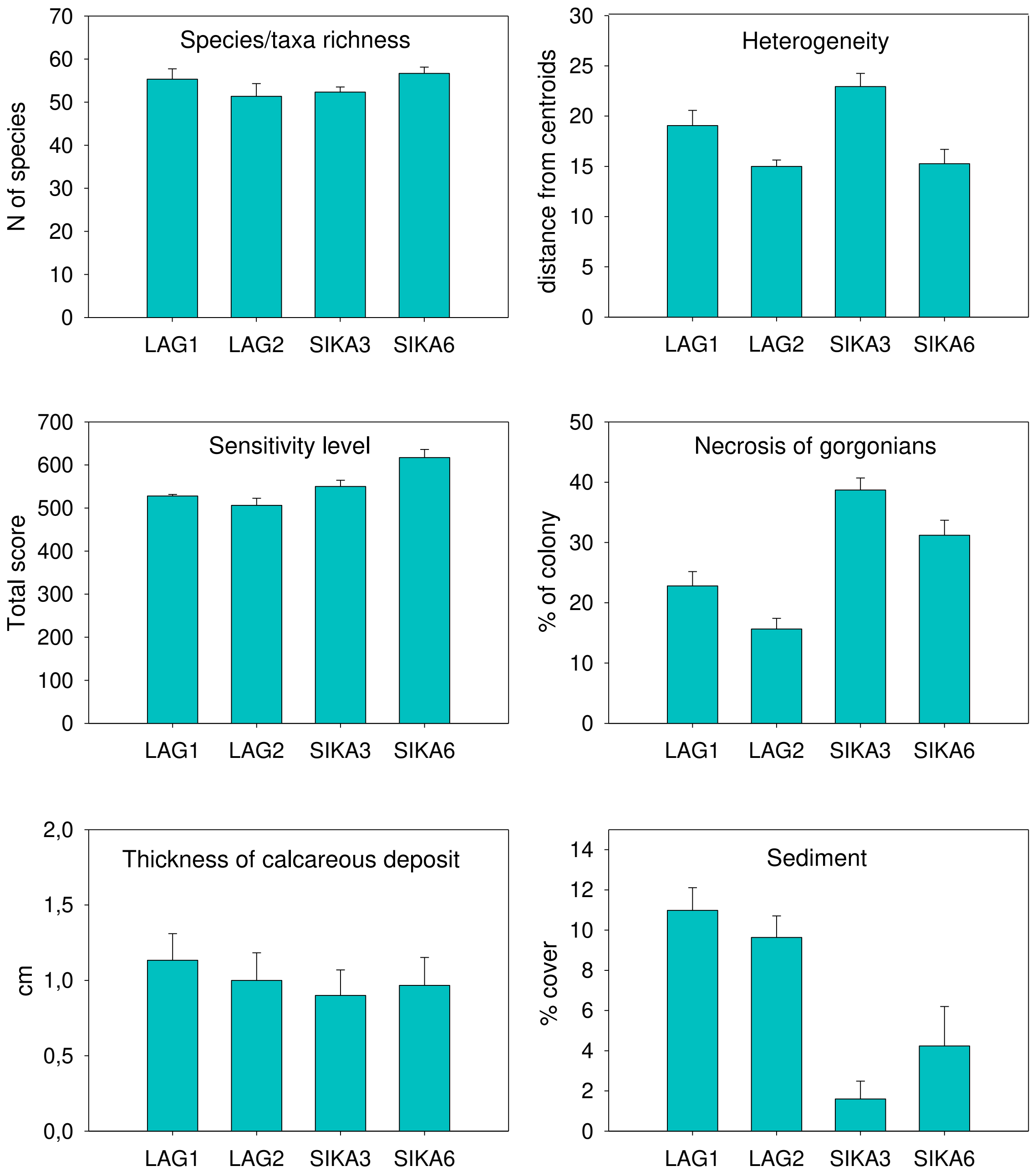


Fig.2.1.20. Descriptors of coralligenous ecological status at each study site. Data are shown as mean ± SE.

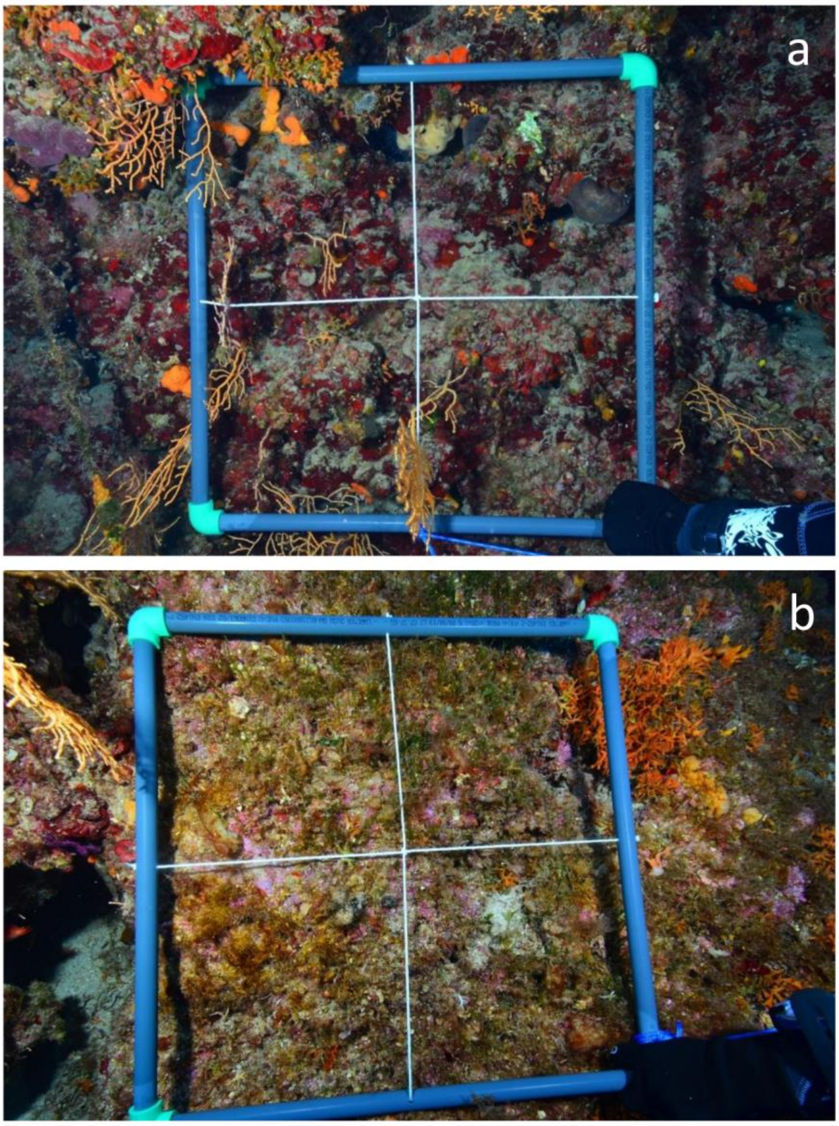


Fig. 2.1.21. Assessment of potential stressors within the coralligenous habitat: a) the level of sedimentation occassionaly observed at Lagnići sites (Dugi Otok Island) and b) the presence of invasive green algae *Caulerpa cylindracea* at Sika 3 and Sika 6 sites on the Vis Island. Photo credit: M. Belošević.

2.1.3. Conclusions

In the depth range from 30 to 40 m, 12 to 15 min of bottom-time was needed to carry out photosampling of 3 sets of 10 contiguous photos of 50 x 50 cm (i.e. to take 30 photographs) at each site. Thus, it was confirmed once again such sampling can be carried out in a reasonable (no-deco) time frame at these considerable depths.

Analysis of video transects, given a good quality of footage, yields results comparable to *in situ* assessment of the erect layer, carried out as a part of the assessment of coralligenous structural complexity. Hence, underwater work may be further reduced. Moreover, such videos provide valuable permanent records and serve as a source for detection of additional species, not observed within photo samples. Affordability, quality and practicality make the use of GoPro or similar cameras with additional video lights highly recommendable for characterization and monitoring of coralligenous habitat.

There was a clear regional pattern in the composition of understorey of studied coralligenous assemblages dominated by gorgonians: assemblages at sites within the same location were similar but they notably differed between locations on Dugi Otok and Vis Islands. Coralligenous assemblage on Lagnići location (Dugi Otok Island) was characterized by dominance of encrusting Peyssonneliales, greater abundance of massive sponges as well as greater presence of green algal turf and sediment than on Stupišće location (Vis Island). On the other hand, encrusting Corallinales dominated on Stupišće location, often developing more structurally complex, plate-like, laminar thalli. Likewise, greater abundance of erect bryozoans, especially *Pentapora fascialis* and *Smittina cervicornis* / *Aedonella* sp. was noted there as well as greater abundance of green algae *Flabellia petiolata*. *Codium bursa* and *Codium* cf. *effusum* were observed only on Stupišće location (Vis Island), as well as the invasive green algae *Caulerpa cylindracea*, still present in low abundance. Neither mucilaginous algal aggregates or macroeroders were observed at any site.

Majority of descriptors selected to evaluate coralligenous ecological status suggest good conditions at our study sites. These include species richness and heterogeneity comparable to other Adriatic sites with low levels of anthropogenic pressure, high sensitivity levels indicating presence of high number of sensitive species and/or their high abundance such as in the case of erect-branchy bryozoans for example, positive balance in bioconstruction and generally low percent cover of sediment and algal turf. However, all of these descriptors concern mainly basal and intermediate layer within coralligenous. Related to the erect layer, the extent of injury of erect anthozoans (i.e. gorgonians that form that layer) is considerable at most sites, indicating disturbances that can specifically and/or more adversely affect these more exposed (since they are the tallest within the community) and among the most sensitive organisms. Such disturbances may include mechanical abrasion by the fishing gear (either lost or still in use; Lagnići sites were especially notorious in that respect, see section 2.5), mucilaginous algal aggregates and increased seawater temperature due to climate change. Gorgonian conservation status is discussed in more detail in section 2.2.

The sampling approach adopted in this study is the one already proposed by the Croatian coralligenous monitoring protocol (Garrabou *et al*. 2015). As originally envisaged, it is robust enough to accommodate subsequent evaluation of different descriptors used for the assessment of coralligenous ecological status. With addition of the physical measurements of the thickness and consistency of calcareous accretion *in situ,* it enabled application of integrated STAR assessment summarized by Piazzi *et al*. (2018). The results of this assessment would fall more into perspective with additional sites being evaluated, especially in terms of only recently applied descriptor such as sensitivity levels, hence it is highly recommendable to apply the same standardized approach in future studies aimed at characterization and monitoring of coralligenous assemblages.

2.2. Assessment of gorgonian demography and conservation status

2.2.1. Overview of the methods

To assess gorgonian population structure and conservation status we followed the methodology used by Linares et al. (2008a) in the Western Mediterranean and readily applied in other parts of the Mediterranean, including the Adriatic Sea (e.g. Kipson et al. 2015, Sini et al. 2015). The density, size, biomass and injury rates were chosen as the main descriptors. At each site, data to assess these descriptors were collected within randomly placed 50 x 50 cm quadrats (see Fig. 9 for illustration of the method). For each colony within a quadrat, the maximum height was measured as the distance between the colony base and the tip of the most distant apical branch.

Furthermore, we used three parameters to determine the level of impact. Firstly, for each measured colony within a quadrat we estimated the extent of injury of colony surface, i.e. the percentage of colony surface that displayed a denuded axes or overgrowth by other organisms (Fig. 9.2). Secondly, we noted the type of injury because the combined analysis of both parameters may be indicative of past disturbance events, including approximate time of their appearance (Coma et al. 2004; Linares et al. 2005). Therefore, depending on the presence/absence of different epibionts, three types of injury (related to the time of its origin) were recognized and noted (Fig. 10). The first type (type A) referred to a denuded axis, indicating a new injury (up to 1 month). The second type (type B) included overgrowth by pioneering species, filamentous algae and hydrozoans (indicating an approximately 1–12-month-old injury), while the third type (type C) included overgrowth mostly by bryozoans, sponges and/or algae and represented an old injury (approximately ≥12 months) (Linares et al. 2005). We considered colonies with <10% of injured surface to be healthy, as used in previous studies (Linares et al. 2008a; Garrabou et al. 2009).

For each gorgonian species encountered in the field, we have also noted its upper distribution limit and the lower one, when possible.

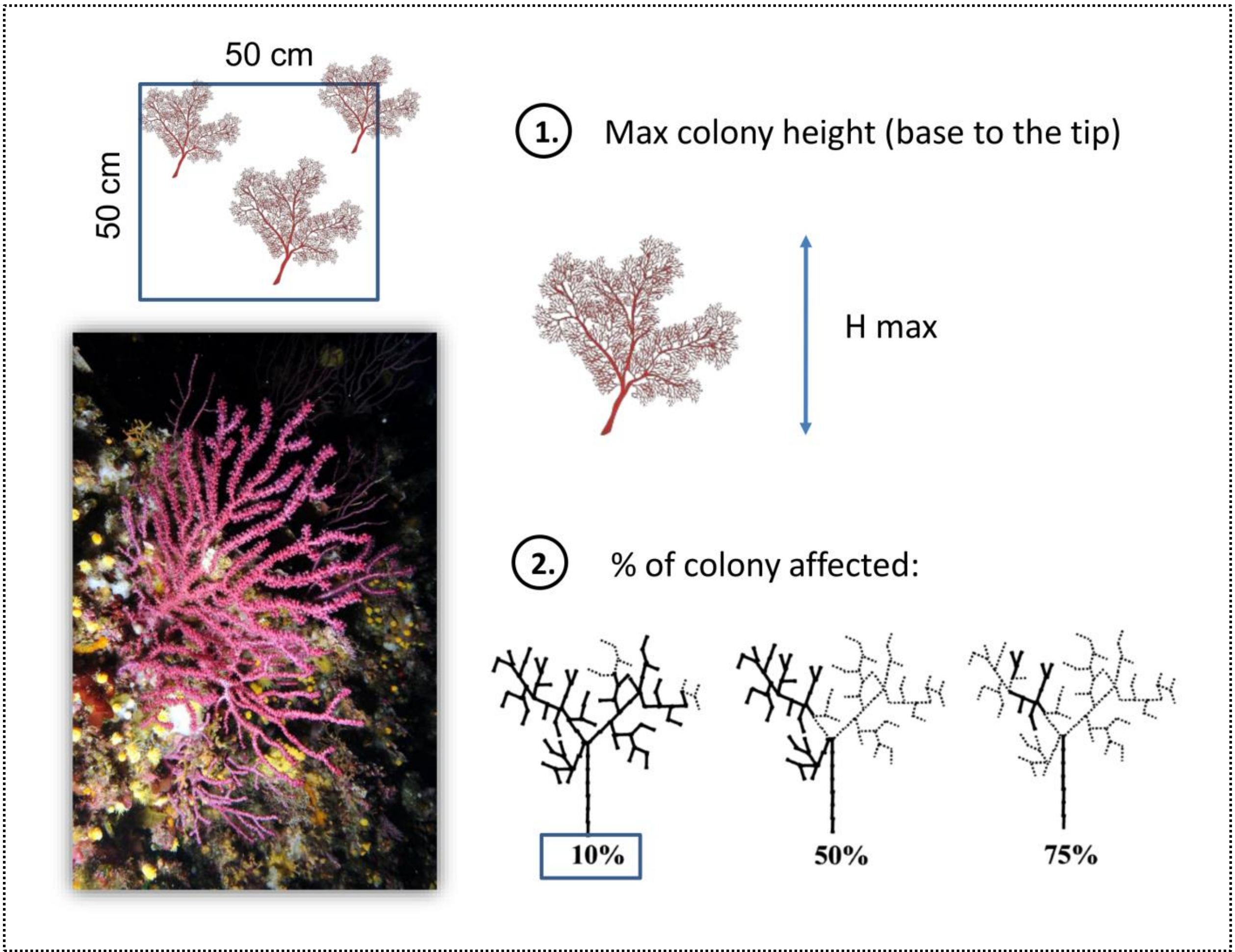


Fig. 2.2.1. Scheme of the assessment of gorgonian demography and conservation status.

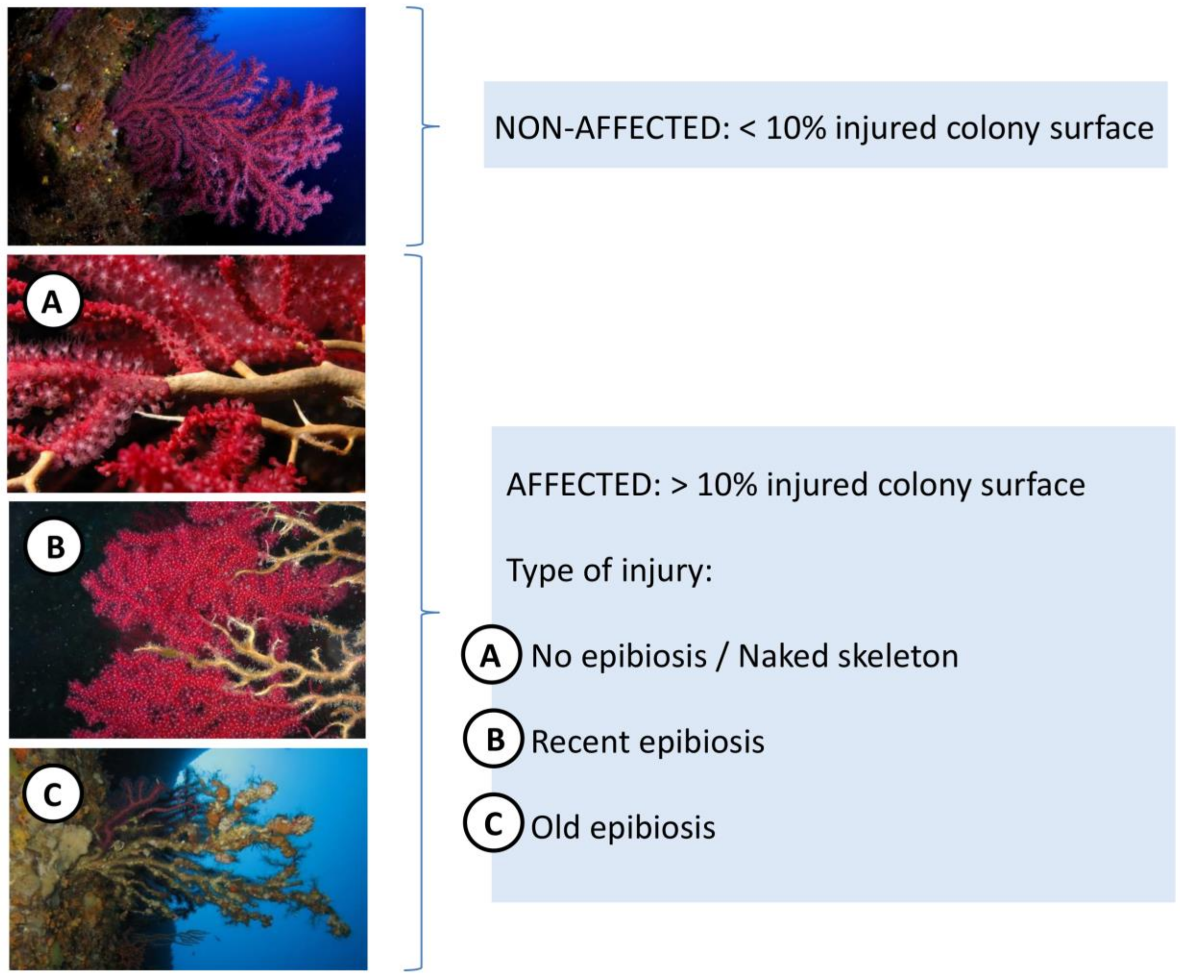


Fig. 2.2.2. Criteria used to assess whether gorgonian is affected or not and categories used for characterization of gorgonian tissue damage (adapted from Garrabou et al. 2015).

2.2.2. Main results

At the Lagnići location (Dugi Otok Island) we carried out the assessment of demography and conservation status of the yellow gorgonian *Eunicella cavolini*, whereas at the Stupišće location (Vis Island) we have assessed both the yellow gorgonian *E. cavolini* and the red gorgonian *Paramuricea clavata* (Fig. 2.2.3). Between 93 and 159 colonies of *E. cavolini* were assessed at each study site, whereas 29 and 26 colonies of *P. clavata* were assessed at Sika 3 and Sika 6 sites, respectively. When present (within this study in mixed assemblages with the yellow gorgonian *E. cavolini*) *P. clavata* was not as abundant, and hence, lower number of colonies could be measured and their health status estimated.

Gorgonian distribution

The upper distributional limit of *E. cavolini* at Lagnici 1 site was 20 m depth, whereas it was 25 m depth at Lagnici 2 site. However, at the latter site few individual colonies were present also at 22 m depth. At Sika 3 and Sika 6 sites on the Vis Island *E. cavolini* was mainly noted from 22 m depth, although few individual colonies could be observed at 18 m depth. In addition, at latter sites few individual colonies of *P. clavata* were present at 26 m depth, but their greater abundance was noted from 35 m depth. In all cases, the lower distributional limit of studied gorgonian species coincided with the end of the vertical wall, which was 39-40 m at Lagnici location and 42 and 45 m deoth at Sika 3 and Sika 6 sites on the Vis Island, respectively.

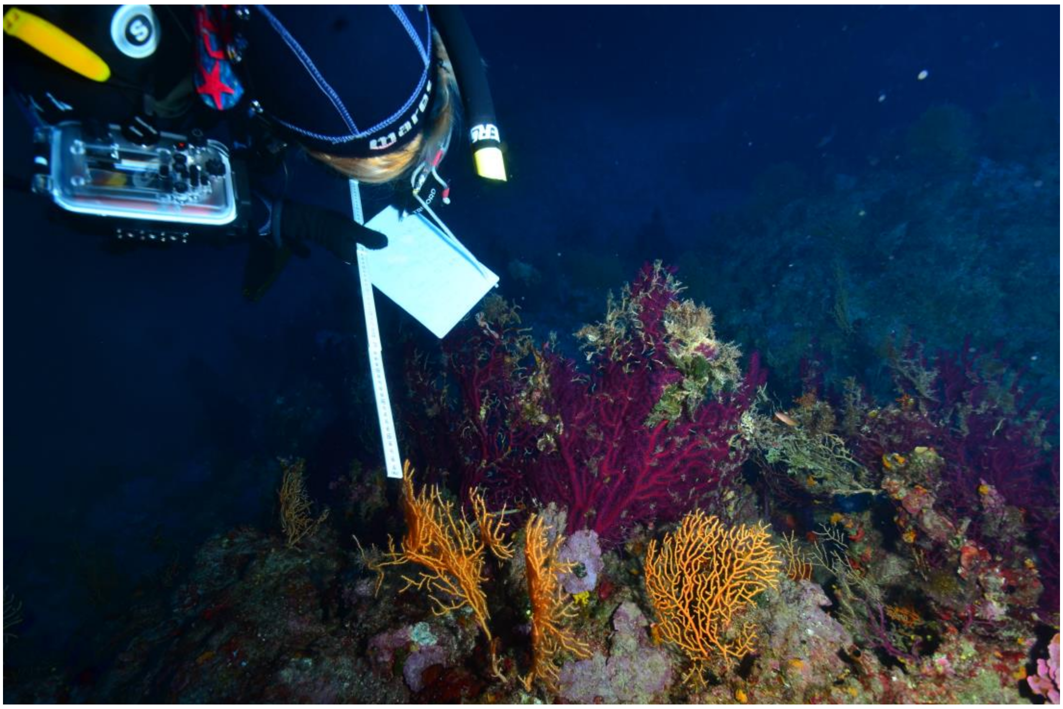


Fig. 2.2.3. A diver performing the assessment of demography and conservation status of the red gorgonian *Paramuricea clavata* and the yellow gorgonian *Eunicella cavolini* at the Stupišće location on the Vis Island (photo credit: M. Belošević).

Demography

Higher density of *E. cavolini* was recorded at the Vis Island than at the Dugi Otok Island. Almost twice as high was the density at the Sika 6 (18.83 ± 10.71, mean ± SD) and Sika 3 sites (17.67 ± 8.68, mean ± SD) than at the Lagnici 1 site (9.79 ± 4.71, mean ± SD), whereas density at the Lagnici 2 site (14.9 ± 8.61, mean ± SD) was only slightly lower than at the Vis sites (Fig. 2.2.4). Likewise, the mean colony height was larger at the Vis Island than at the Dugi Otok Island. Again, the highest values were recorded at Sika 6 site (27.69 ± 9.29, mean ± SD), followed by Sika 3 (25.65 ± 9.41, mean ± SD), while the mean colony height reached 21.93 ± 9.69 (mean ± SD) at Lagnici 2 and 18.82 ± 9.29 (mean ± SD) at the Lagnici 1 site (Fig. 2.2.4). The largest colony of E. cavolini (61 cm in height) was recorded at the Sika 3 site, whereas at the other sites the largest sizes measured between 40 and 45 cm. On the contrary, minimal recorded size was similar at all sites (6-7 cm).

Related to the populations' size frequency distribution, similar proportions of juvenile *E. cavolini* colonies (< 10 cm in height) were recorded at all sites. On the other hand, larger proportion of colonies bigger than 20 cm were present at Stupišće location on the Vis Island (79% at Sika 6 and 69% at Sika 3 site) than at Lagnići location on the Dugi Otok Island (around 50% at both sites, Fig. 2.2.5). Especially notable is a difference in proportion of the size class 31-40 cm that reaches 36% and 21% at Sika 6 and Sika 3 sites, respectively, in comparison to 4% at Lagnici 1 and 15% at Lagnici 2 sites (Fig. 2.2.5).

At Stupišće on the Vis Island, the only location where the red gorgonian *Paramuricea clavata* was recorded within this study, population density was slightly higher at Sika 3 site (10.55 ± 6.27, mean ± SD) than at Sika 6 (8.67 ± 3.75, mean ± SD, Fig. 2.2.4), whereas mean colony hight was almost 30% greater at the Sika 6 site (50.65 ± 17.38 vs. 36.66 ± 18.81, mean ± SD, Fig. 2.2.6). The largest *P. clavata* colony was recorded at Sika 3 site and it measured 98 cm in height, whereas minimal recorded size was similar at both sites (10-11 cm). Related to size frequency distribution of studied *P. clavata* populations, two patterns can be noted – a dominance of colonies smaller than 40 cm at Sika 3 site and the opposite at Sika 6 site: the proportion of colonies larger than 40 cm that was twice as high at the Sika 6 site in comparison to Sika 3 site (77 vs. 38%, Fig. 2.2.7). No juvenile colonies (<10 cm) were noted at Sika 3 site whereas only one juvenile colony was recorded at Sika 6 site (Fig. 2.2.7). Insights from the image analysis undertaken to assess coralligenous assemblages at respective sites confirm the existence of *P. clavata* recruits and juvenile colonies (visible for example within photoquadrats in Figs. 2.1.9 c and 2.1.10 b, section 2.1), hence results presented here (Fig. 2.2.7) are most probably the consequence of overlooking such small, more conspicuous colonies during *in situ* assessment. Moreover, in all analyses related to *P. clavata*, it should be kept in mind that number of colonies assessed was relatively low (26 colonies at Sika 6 and 29 colonies at Sika 3 site) so any conclusions related to this species must be made cautiously.

Disturbance impact levels

Mean extent of injury of *E. cavolini* colonies was almost twice as high at Stupišće location than at Lagnići. The most affected was population at Sika 3 site followed by Sika 6 (38.71 ± 24.98 and 31.19 ± 26.41, respectively, mean ± SD, Fig. 2.2.8). On the Dugi Otok Island mean extent of injury of *E. cavolini* colonies was 22.80 ± 22.85 at Lagnici 1 site and 15.64 ± 18.53 at Lagnici 2 (mean ± SD, Fig. 2.2.8). Fairly large standard deviations (SD) further indicate considerable differences in injury extent of colonies within a respective population.

Colonies of *E. cavolini* were highly affected at Stupišće location on the Vis Island (Fig.2.2.10). The highest levels were noted at Sika 3 (almost 80% of affected colonies, i.e. with injury levels >10% of colony surface), followed by Sika 6 (66% of affected colonies). In addition to the *in situ* observations, at Sika 6 site *E. cavolini* was also assessed at the upper limit of its depth distribution (at 25 m depth) from video transects (3 transects of 1 x 10 m). Out of 138 colonies recorded along transects, 64% was affected i.e. the proportion almost identical to the one stemming from the assessment of deeper colonies at the same site. Populations on the Dugi Otok Island showed considerable, but more moderate injury levels with 57% and 41% of colonies affected at Lagnici 1 and Lagnici 2 sites, respectively (Fig. 2.2.8). The proportion of dead colonies did not surpass 3% in any studied population.

Mean extent of injury of *P. clavata* colonies was higher at Sika 3 than at Sika 6 site (36.21 ± 30.90 and 27.31 ± 16.14, respectively, mean ± SD, Fig. 2.2.9), and overall they were fairly similar to the ones observed for *E. cavolini* at the same location. Likewise, high proportion of affected colonies of *P. clavata* was noted, being 66% of colonies affected at Sika 3 and 85% at Sika 6. No dead colonies were recorded (Fig. 2.2.9).

Regardless of the population or gorgonian species considered, majority of the affected colonies (with ≥10% of injured surface) showed an old epibiosis stage, characterized by colonized algae, sponges, bryozoans and other calcareous organisms (42 to 90.9%; Table 2.2.1). However, alongside old injuries, sometimes also recent ones (type A, i.e. denuded axes) were present. Among the highest proportion of such injuries on *E. cavolini* were noted at Lagnici 2 site where 15.9% colonies displayed only recent necrosis and additional 9.1% displayed recent injuries alongside old ones. Likewise, 15.8% of affected *P. clavata* colonies at Sika 3 site on the Vis Island displayed recent injuries alongside old ones (Table 2.1.1., Fig. 2.2.10).

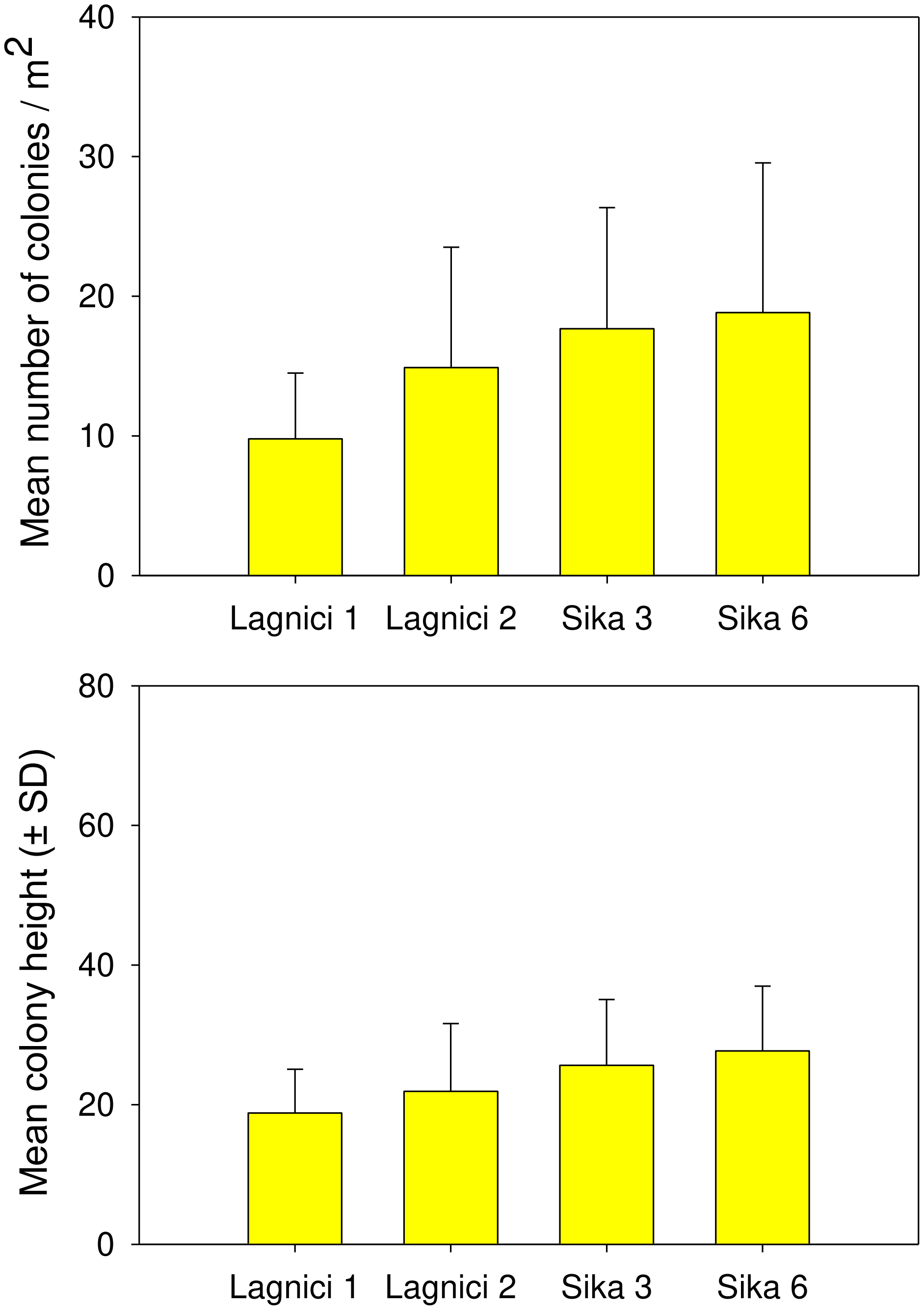


Fig.2.2.4. Density and mean colony height of the yellow gorgonian *Eunicella cavolini* at study sites on the Dugi otok Island (Lagnici 1 and Lagnici 2) and the Vis Island (Sika 3 and Sika 6).

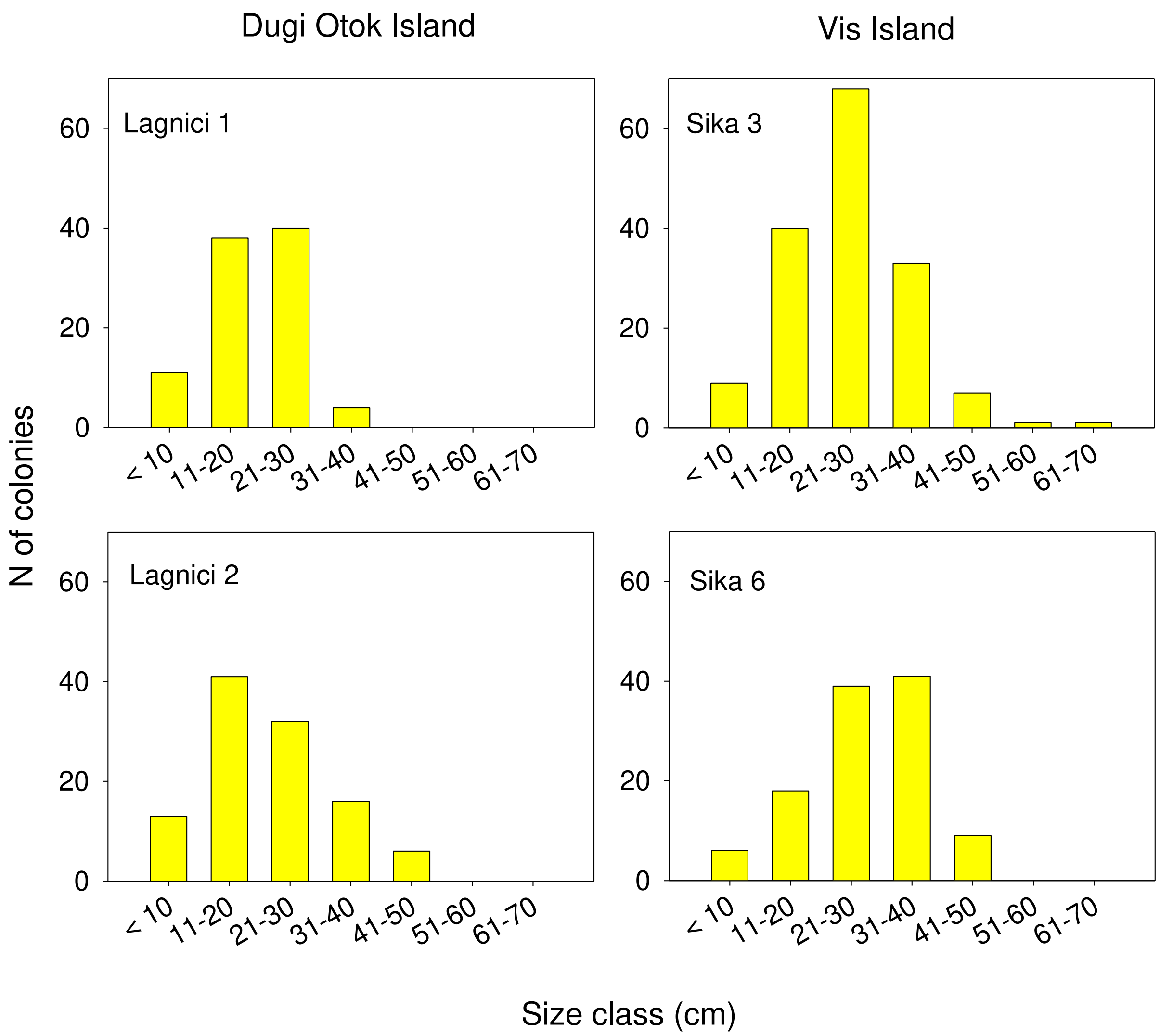


Fig.2.2.5. Size-frequency distribution of studied *Eunicella cavolini* populations.

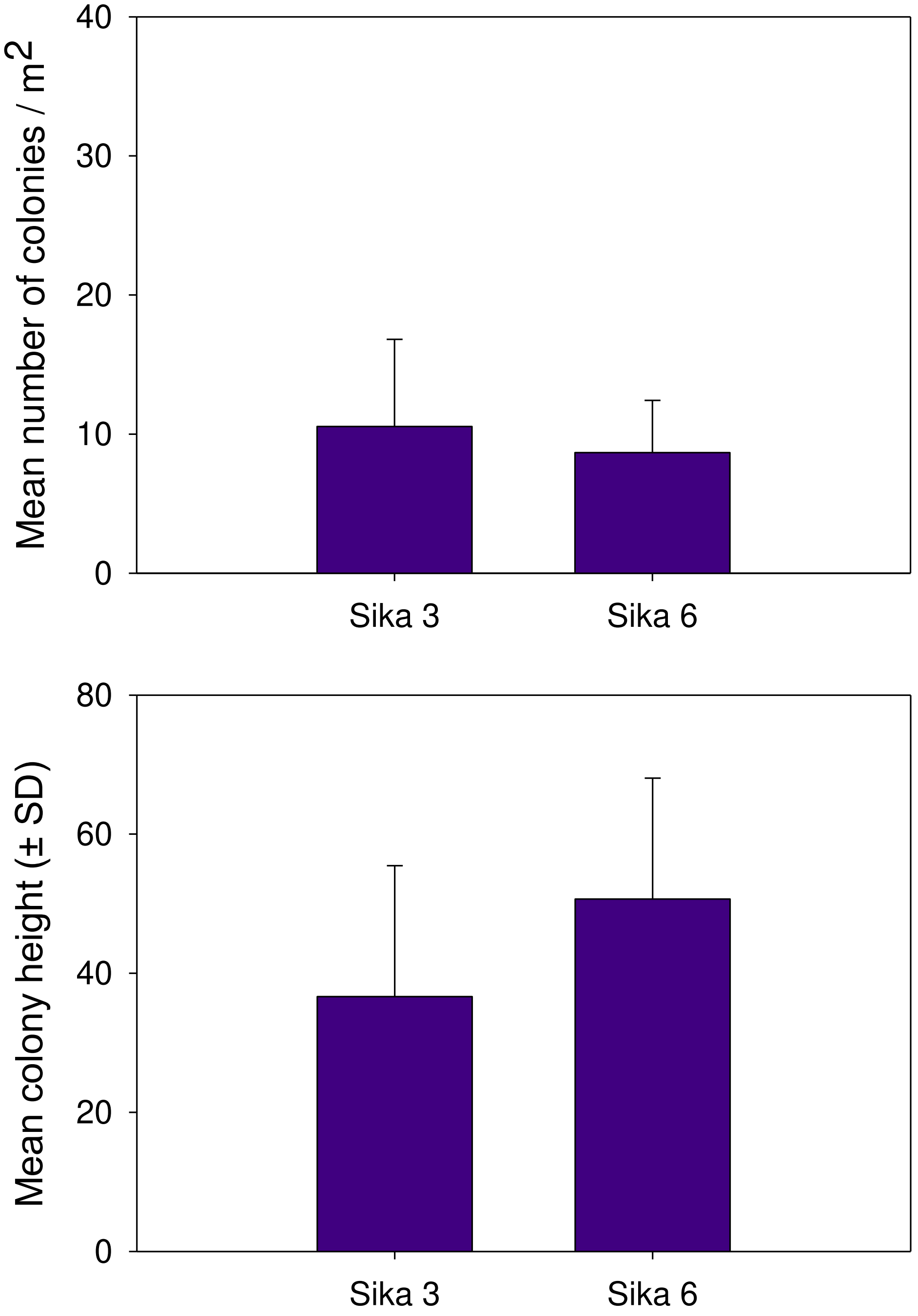


Fig. 2.2.6. Density and mean colony height of the red gorgonian *Paramuricea clavata* at study sites on the Vis Island (Sika 3 and Sika 6). Note: red gorgonians were not present at the study sites on the Dugi otok Island.

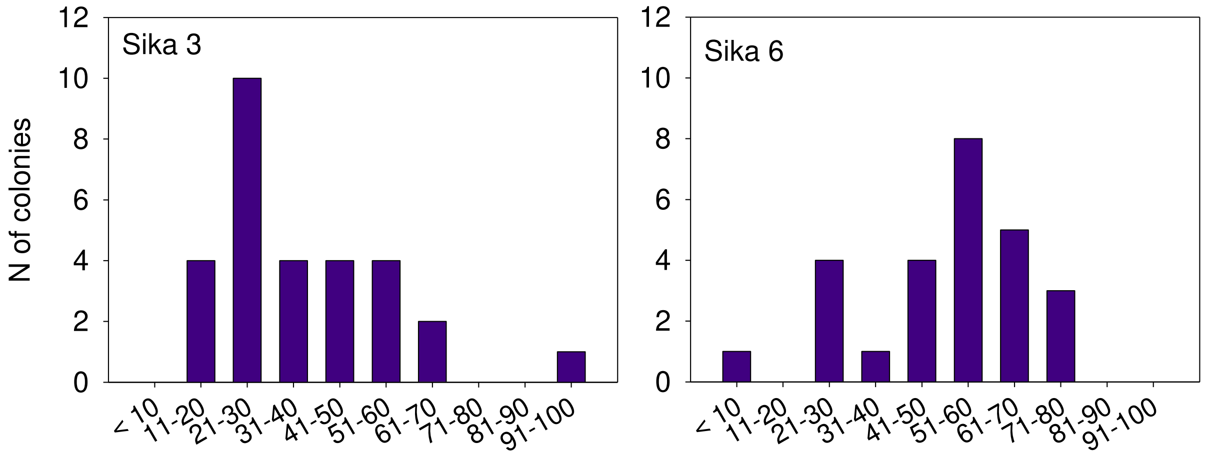


Fig. 2.2.7. Size-frequency distribution of studied *Paramuricea clavata* populations.

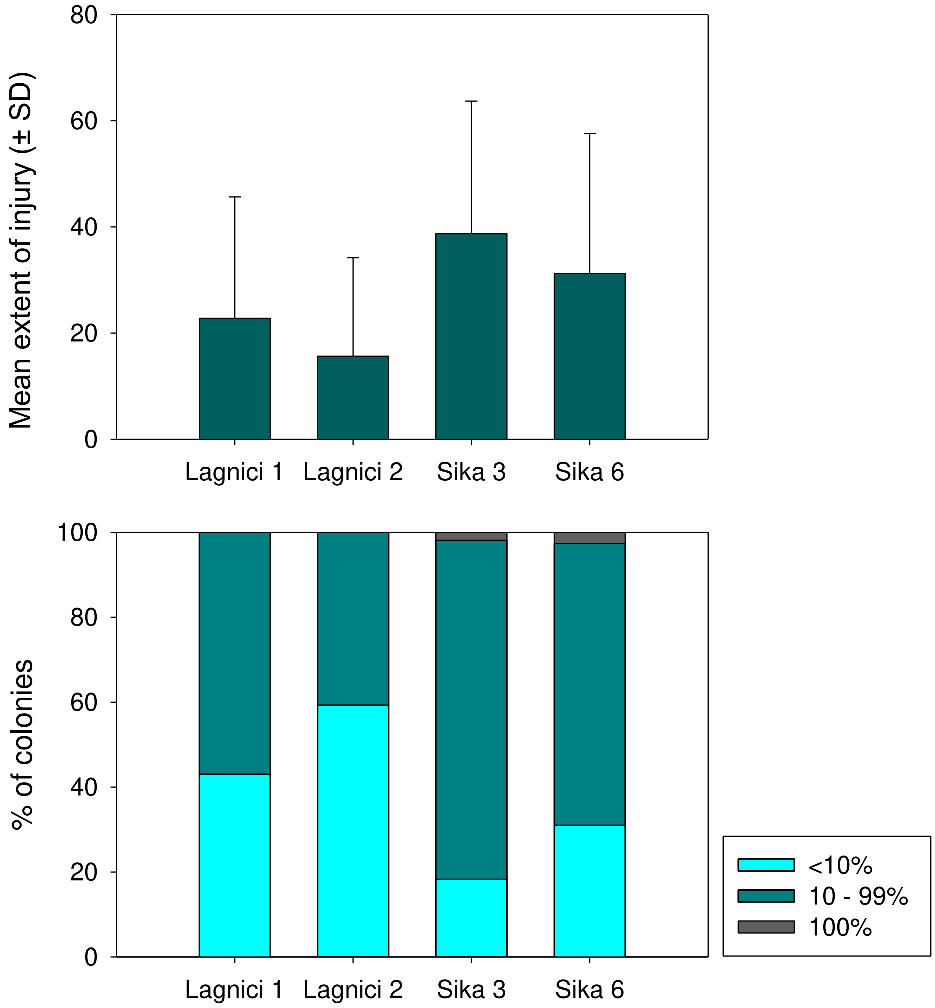


Fig. 2.2.8. Disturbance impact level parameters of the studied *Eunicella cavolini* populations on the Dugi otok Island (Lagnici 1 & 2) and Vis Island (Sika 3 & 6). Upper graph: Mean percentage of the extent of injury; lower graph: percentages of healthy colonies (with <10% injury extent), affected colonies (between ≥10% and ≤99% injury extent) and dead colonies (with 100% injury extent).

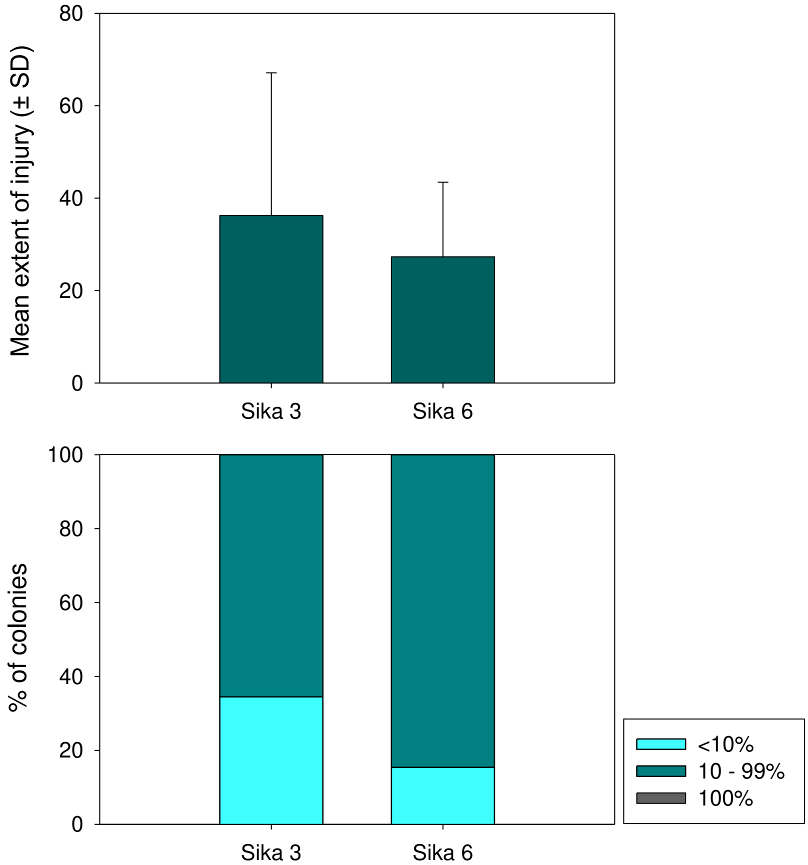


Fig.2.2.9. Disturbance impact level parameters of the studied *Paramuricea clavata* populations on the Vis Island. Upper graph: Mean percentage of the extent of injury; lower graph: percentages of healthy colonies (with <10% injury extent), affected colonies (between ≥10% and ≤99% injury extent) and dead colonies (with 100% injury extent).

Table 2.2.1. Percentage of affected *Eunicella cavolini* and *Paramuricea clavata* colonies (≥10% of surface injured) hosting different epibiosis types: 'type A' refers to a denuded axis, indicating a new injury (up to 1 month old); 'type B' includes overgrowth by pioneering species, filamentous algae and hydrozoans (indicating approximately 1–12-month-old injury); 'type C' includes overgrowth mostly by bryozoans, sponges and/or algae and represents an old injury (approximately ≥12 months) (see overview of the methods). Colony can host more than one epibiosis type.

	Type of injury						
%	**A**	**B**	**C**	**A+C**	**A+B**	**B+C**	**A+B+C**
Eunicella cavolini							
Lagnici 1	**1,9**	**35,8**	**47,2**	**1,9**	**5,7**	**7,5**	**0,0**
Lagnici 2	**15,9**	**15,9**	**43,2**	**9,1**	**0,0**	**15,9**	**0,0**
Sika 3	**0,0**	**11,0**	**66,1**	**7,9**	**4,7**	**7,9**	**2,4**
Sika 6	**0,0**	**5,3**	**62,7**	**4,0**	**0,0**	**28,0**	**0,0**
Paramuricea clavata							
Sika 3	**0,0**	**5,3**	**42,1**	**15,8**	**0,0**	**36,8**	**0,0**
Sika 6	**0,0**	**0,0**	**90,9**	**4,5**	**0,0**	**4,5**	**0,0**

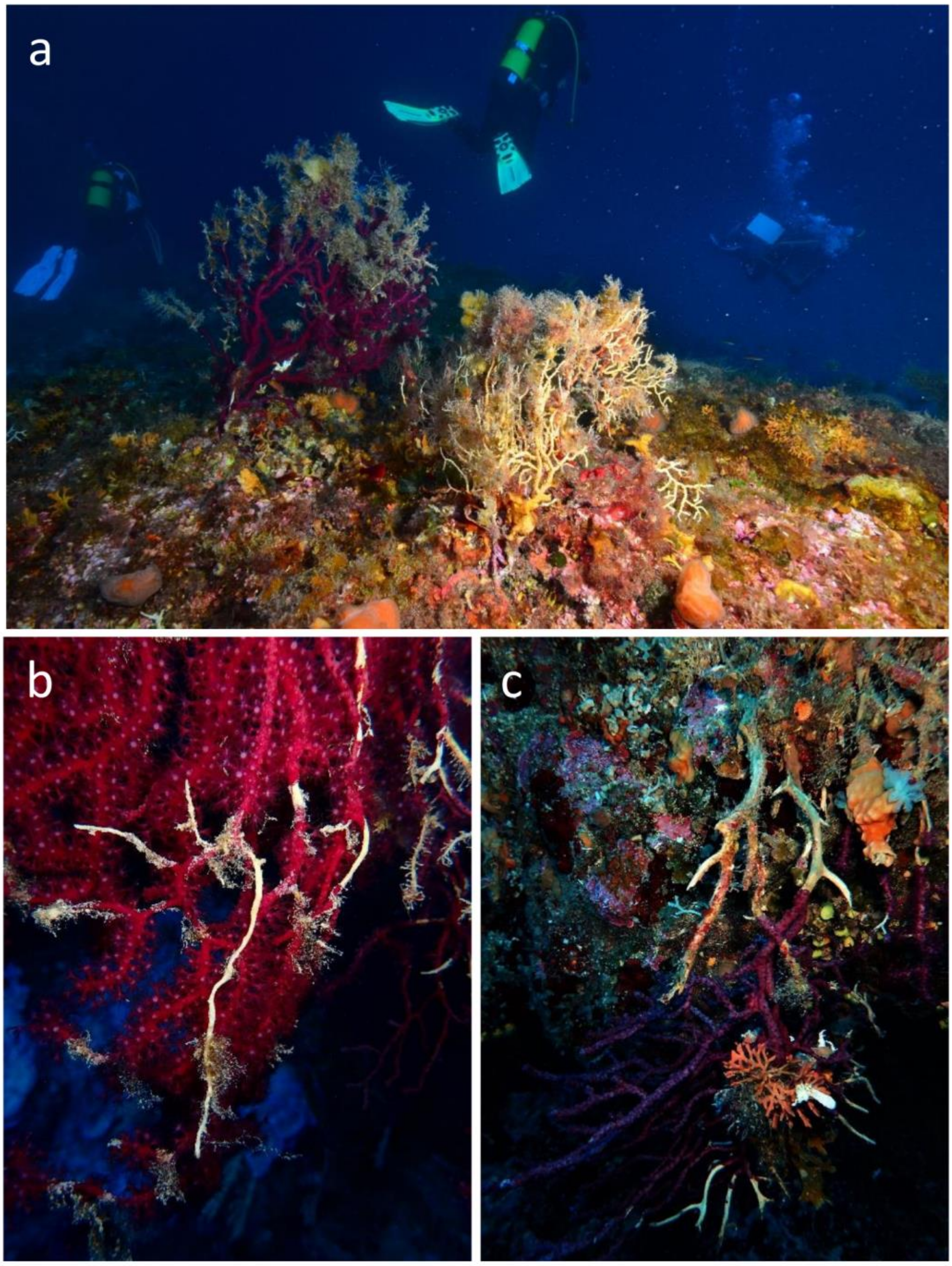


Fig. 2.2.10. Illustration of different types of injuries: a) the red gorgonian *Paramuricea clavata* and the yellow gorgonian *Eunicella cavolini* with B and C type of epibiosis, b) detail of *P. clavata* colony with recent – type A and slightly older (> 1 month) type B of epibiosis, c) *P. clavata* colony with all 3 types of epibiosis. All examples are from Sika 6 site, on the Vis Island. Photo credit: M. Belošević (a), S. Kipson (b,c).

2.2.3. Conclusions

Denser populations and larger colonies of the yellow gorgonian *Eunicella cavolini* were recorded on the Vis Island than on the Dugi Otok Island. All values related to their demography are within a range already reported from the other parts of the Adriatic and the Mediterranean Sea (Sini *et al*. 2015). The same applies for the populations of the red gorgonian *Paramuricea clavata*, comparable to the Adriatic populations studied to date (Kipson *et al*. 2015), characterized by a great proportion of large colonies (> 40 cm in height)

Whereas *E. cavolini* on the Dugi Otok Island displayed moderate proportion of affected colonies (i.e. with injury levels >10% of colony surface), their proportion was high both for *E. cavolini* and *P. clavata* on the Vis Island (> 60%). Although most of the injuries were old, we also noticed fresh injuries (i.e. naked skeleton, mainly on apical tips of colonies) that would imply they occurred less than 1 month ago. Hence, they could have coincided with the highest seawater temperatures usually recorded towards the end of summer. Related to the older injuries, although mucilaginous outbreaks were not recorded at the time of our assessment, their impact could be highly likely in the past, alongside elevated seawater temperatures. In fact, their occurrence in the past (the most persistent ones usually develop from May till July but other periods are not excluded) was confirmed by the owner of a local diving center and dive instructor in Komiža on the Vis Island. In addition, the abundance of lost fishing gear was especially high at the Lagnići sites (see section 2.5) and injuries caused by mechanical abrasion stemming from still active fishing practices or from the contact with abandoned fishing gear was highly likely in the past as well as it remains likely today.

2.3. Assessment of conservation status and climate-related responses of sessile benthic macroinvertebrates

2.3.1. Overview of the methods

As a part of the effort to monitor climate-related responses of a rocky bottom community, we have applied the protocol advocated by Garrabou, Bensoussan & Azzurro (2018) in the scope of the previous Interreg project MPA ADAPT, focused on sessile benthic macroinvertebrates. This protocol aims to reveal the conservation status of surveyed populations, while gathering baseline information to assess the impacts of mass mortality events when they occur.

Target species of this protocol are the ones sensitive to climate-related stressors, are easy to identify underwater and are sufficiently abundant in the surveyed area. Our target species included sponges *Petrosia ficiformis*, *Chondrosia reniformis*, *Ircinia* sp. and a broader category of black keratose sponges (Fig. 2.3.1) which may include species such as *Scalarispongia scalaris*, *Sarcotragus foetidus*, *Spongia officinalis* and similar. The rationnale behind defining such a broad category was to avoid misidentification by observers who did not feel confident enough to identify those sponges underwater, but there was a need to carry out assessment as we have often noticed a high proportion of such sponges damaged. Furthermore, selected species included bryozoan *Myriapora truncata* as well as hard corals *Balanophyllia europaea* and *Cladocora caespitosa* (Fig. 2.3.1).

We have carried out the assessment at the upper distribution limit of the selected species at 3 sites separated by at least 200 m (as previously mentioned, the conservation status of gorgonians were assessed within random quadrats 50 x 50 cm in the scope of a demographic study, see section 2.2). Observations were made along the imaginary transect at the selected depth (± 1 m). Observer counted each specimen of selected species and noted if it is affected, i.e. if any tissue necrosis is present (e.g. Figs. 2.3.2, 2.3.3, 2.3.4) or polyps of hard corals are bleached/dead. Besides visual census *in situ*, data was ocassionaly collected also by using calibrated photos. Due to a very low abundance of targeted sessile macroinvertebrates on infralitoral reefs at Stupišće location on the Vis Island, this assessment was omitted there. Hence, it was performed solely at location Lagnići on the Dugi Otok Island.

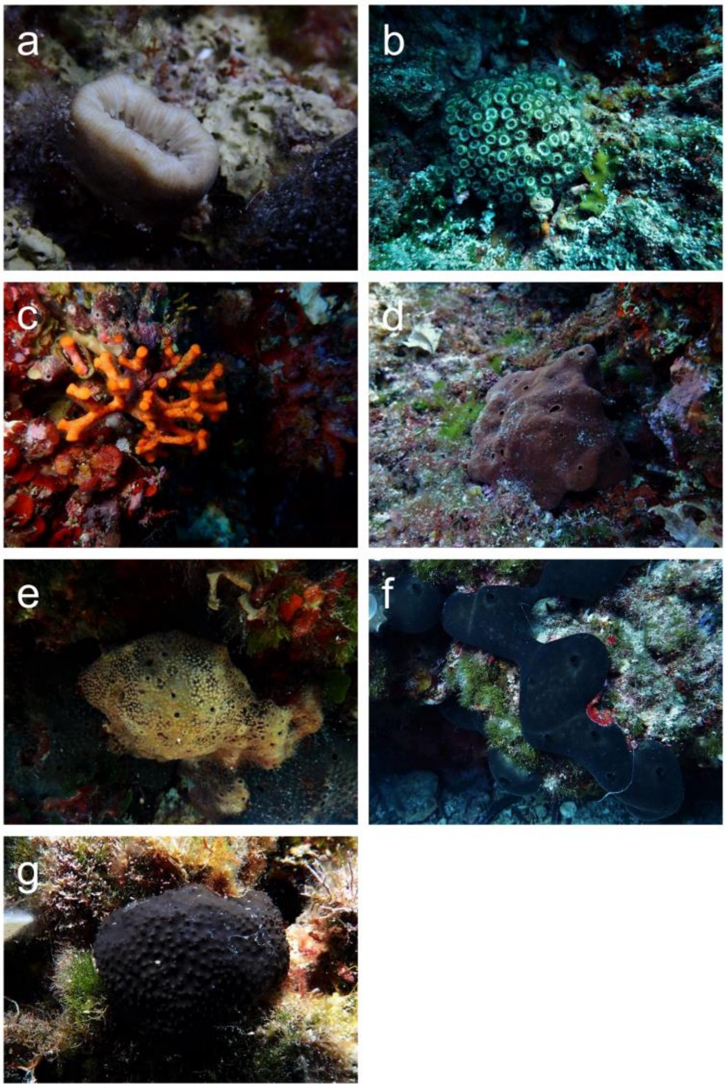


Fig. 2.3.1. Target species/groups selected for the assessment of conservation status and climate-related responses of sessile benthic macroinvertebrates: a) *Balanophyllia europaea*, b) *Cladocora caespitosa*, c) *Myriapora truncata*, d) *Petrosia ficiformis*, e) *Ircinia* sp., f) *Chondrosia reniformis* and g) black keratose sponge. Photo credit: S. Kipson.

2.3.2. Main results

Out of target species/taxa only sponge *Chondrosia reniformis* and black keratose sponges were present in sufficient numbers (around 50 or more specimens per dive/transect) at pre-selected depths to carry out a proper assessment according to the selected MME protocol. While *Chondrosia reniformis* was assessed at 3 sites within Lagnići location, black keratose sponges could not be assessed at more than two, but in that case at least 3 separate transects were observed. In total, over 600 individuals of black keratose sponges and over 260 individuals of *Chondrosia reniformis* were examined. Percent of affected black keratose sponges examined per transect by different observers in the depth range 3-5 m on natural rocky bottom (within individual transects spanning from 3-4 m, or from 4-5 m depth) at Lag 4 site varied between 11 and 62% (5 transects in total, Fig. 2.3.2). However, only 1 of these assessments reports percent of affected black keratose sponges below 30%. During that particular assessment almost 2-3 fold higher number of specimens were examined compared to all others, precluding that the observer potentially overlooked more subtle signs of damage/sickness, such as the one illustrated in Fig.2.3.3 a,b). On that note, the signs of damage/sickness observed on black keratose sponges varied from the appearance of a thin layer over sponge's surface, with affected tissue becoming evident only after gentle brushing off by hand (Fig.2.3.3 a,b), to more evident signs such as visible sponge's skeleton – spongin fibers protruding from a tissue in the probable process of healing (renewing pinacoderm) or clearly necrosed/dead parts (Fig.2.3.3 c-e). On one occassion, a black keratose sponge with a clearly visible white bacterial layer over its surface was observed (Fig.2.3.3 f). In addition, exclusively on the steamboat wreck Michele, in the depth range 3-5 m a specific "rust colored" damage/sickness on black keratose sponges was observed (Fig. 2.3.4), resulting in 48 to 55% of affected individuals (Fig. 2.3.2). It is known that keratose sponges can accumulate iron into their fibers, hence specific coloration in this case could stem from such a process (C. Cerrano *pers. comm*). On the methodological note, assessment of black keratose sponges on the shipwreck was carried out both by visual census *in situ* and from the subsequent analysis of video transects by different observers, yielding comparable results.

Contrary to large proportions of affected black keratose sponges on natural infralitoral reefs, *Chondrosia reniformis* appeared to be healthy, with less than 3,5% of affected individuals. The same result was obtained for sponges on artificial reef – the shipwreck Michele based on the analysis of video transects (Fig. 2.3.2) whereas reported amount of affected individuals by one observer *in situ* was considerable 30 % (Fig. 2.3.2). Later clarification of this result revealed that more varied coloration of *Chondrosia reniformis* (grey with more of white specks) was interpreted as a sign of affectation. Once again, it should be reminded that *C. reniformis* pigmentation can vary depending on its habitat – from dark grey specimens in more photophilic zone (see for example Fig. 2.3.6) to completely white specimens in biocenosis of semi-dark caves (e.g. Fig. 2.6.5 b), with lot of variation in between.

Although other target species were not present in sufficient numbers to be assessed within a single dive, and hence to be evaluated according to the protocol, I have nevertheless summarized results of the data that could be collected (Table 2.3.1). Due to low numbers, no attempt to draw conclusions on their health status was made. However, it is worth to note that different signs of affected sponges *Petrosia ficiformis* were also observed (see Fig. 2.3.5 for more details).

As previously mentioned, this protocol was not applied at sites on the Vis Island due to insufficient abundance of target species at shallow depths. Nevertheless, occassional presence of extra large specimens of target species is noteworthy, such as the specimen of *Chondrosia reniformis* of approximately 60 cm in diameter at Sika 6 site (Fig. 2.3.6).

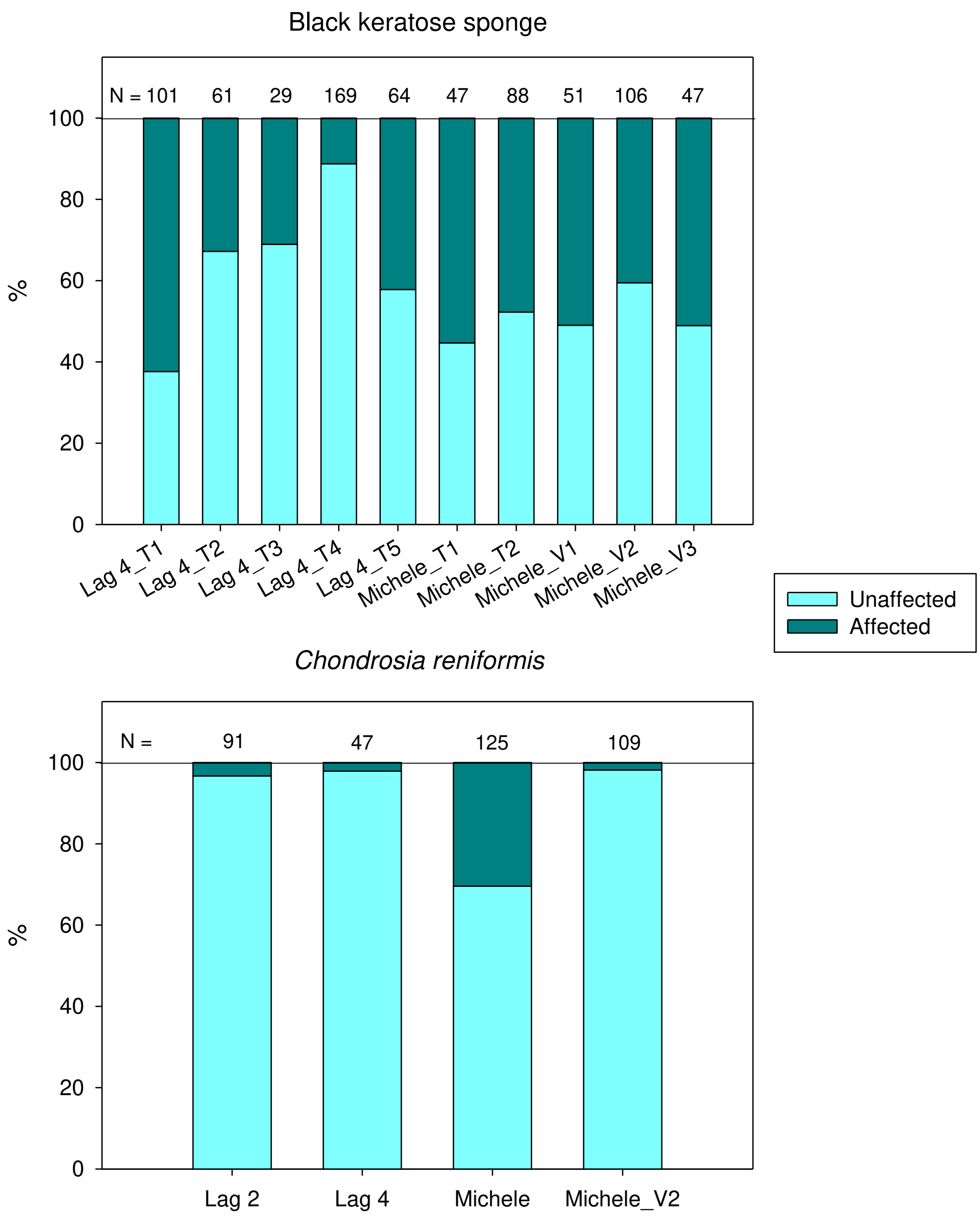


Fig. 2.3.2. Percentage of affected individuals of two target species/taxa within location Lagnići, NW Dugi Otok Island. N indicate total number of individuals assessed per dive/transect. V1 to V3 mark video transects, whereas other results are based on visual census *in situ*.

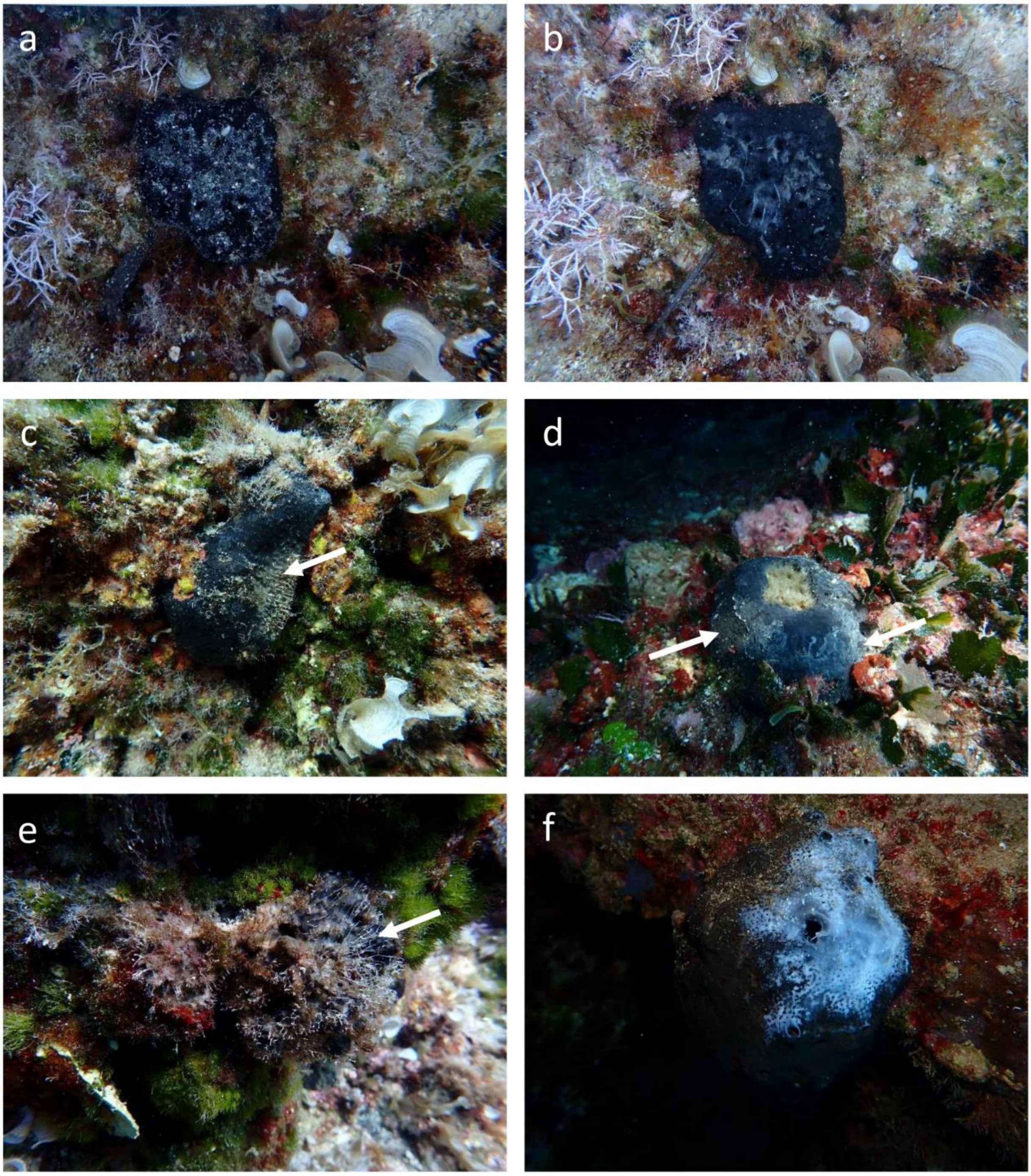


Fig. 2.3.3. Example of sick/affected specimens of black keratose sponges observed at the Lagnići location, NW Dugi Otok Island: a) thin layer covering sponge's pinacoderm, b) detail of sick tissue visible after gentle scrapping of the overlying layer, c) partially affected sponge showing signs of pinacoderm healing (white arrow) – no longer functional spongin remains visible, d) freshly affected sponge, white arrow points to the only remaining part that still looks unaffected, e) heavily damaged sponge (90%) with some signs of healing (white arrow), f) fresh white bacterial layer. Photo credit: S. Kipson except of f) M. Belošević.

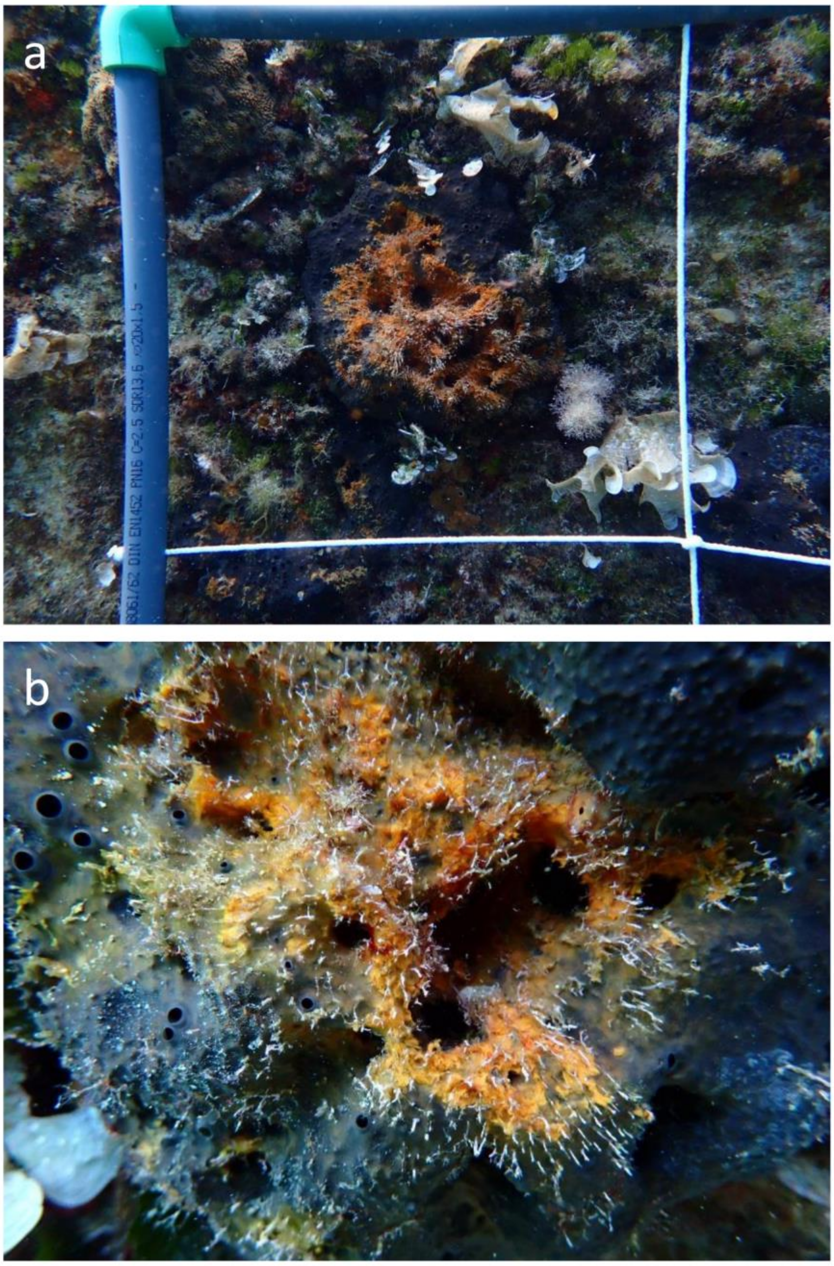


Fig. 2.3.4. Illustration of the specific “rust colored” damage observed on black keratose sponges thriving on the shipwreck Michele, NW Dugi Otok Island: a) the whole specimen, b) detail of affected part. Photo credit: S. Kipson.

Table 2.3.1. Report on the assessment of other target species present in insufficient numbers to satisfy propositions of the applied MME protocol. Species were observed in 4 to 5 m depth range. N = total number of individuals; V1 to V3 mark video transects, whereas other results are based on visual census *in situ*.

	% of individuals		
	unaffected	affected	N
Petrosia ficiformis			
Lag 4_T1	96.43	3.57	28
Lag 4_T2	53.85	46.15	13
Ircinia sp.			
Lag 4_T1	86.67	13.33	15
Michele_V1	93.33	6.67	15
Michele_V2	30.91	69.09	55
Michele_V3	37.93	62.07	29
Ballanopyllia europaea			
Lag 4_T1	100	0	18

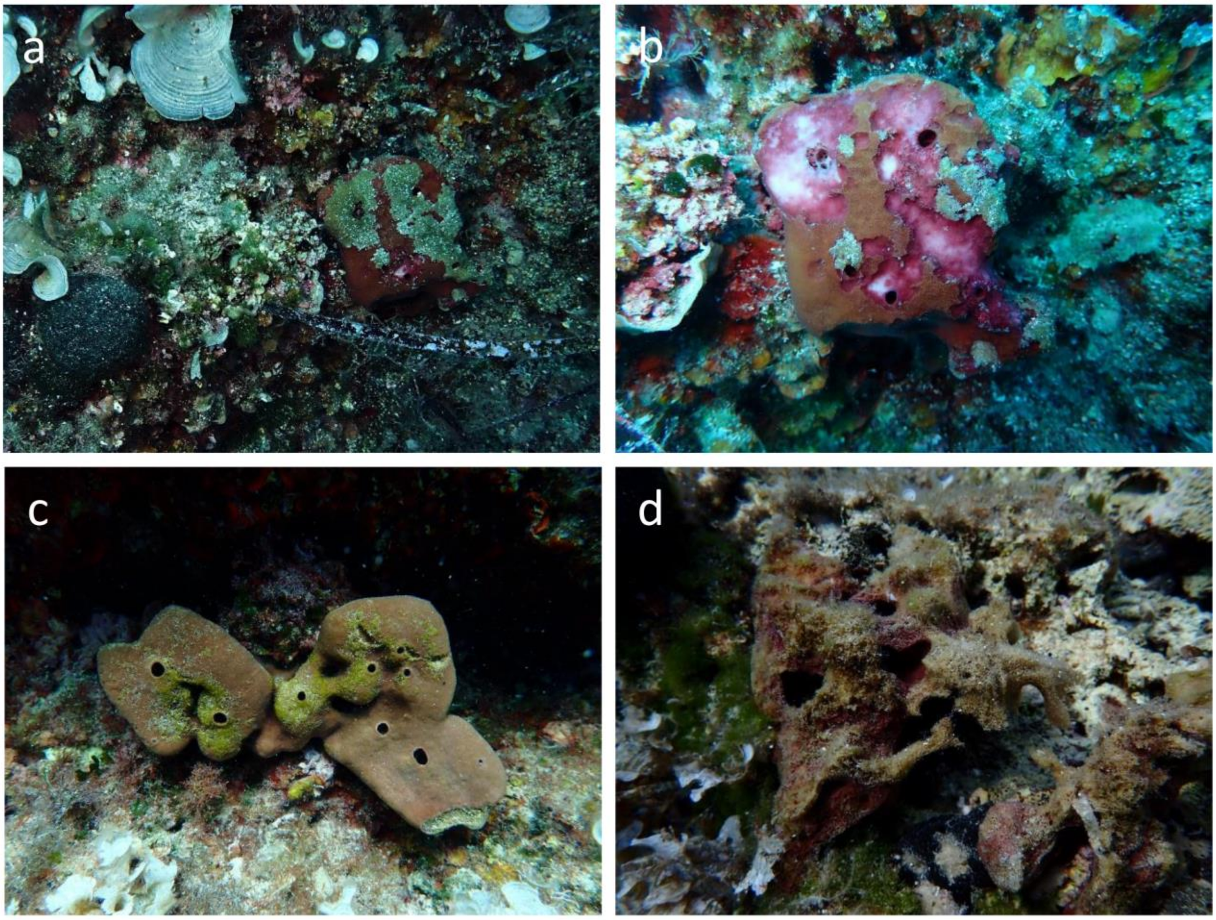


Fig. 2.3.5. Example of differently affected specimens of the sponge *Petrosia ficiformis* observed at the Lagnići location, NW Dugi Otok Island: a) specimen with partial "crust" on the surface, b) the same specimen after "crust" removal, c) partially damaged, overgrown specimen – healthy individuals are not prone to epibiosis and d) dead sponge. Photo credit: S. Kipson.

Fig. 2.3.6. One of the largest specimens of target sponge *Chondrosia reniformis* was observed at Sika 6 site, on the Vis Island (approx. 15 m depth). Approximate horizontal width of the sponge is 60 cm and it is surrounded by a dense population of the invasive green algae *Caulerpa cylindracea*. Photo credit: M. Belošević.

2.3.3. Conclusions

Out of all target species/taxa, abundance (in terms of number of individuals/colonies that need to be assessed) of sponges *Chondrosia reniformis* and black keratose sponges satisfied propositions of the selected MME protocol on the Lagnići location. However, whereas sponge *Chondrosia reniformis* appeared healthy, medium (30-60%) to high percentage (>60%) of affected black keratose sponges were observed both on natural and artificial shallow reefs (3-5 m depth). Putative cause could include increased seawater temperature over summer period, however other causes or even multiple causes of such affectation cannot be excluded. Hence, further studies are needed that would ideally include also continuous seawater temperature monitoring on location Lagnići.

This focused hands-on field excercise enhanced our ability to recognize different signs of sponge sickness/damage and such an experience will undoubtedly improve divers/observers training in the future. Besides *in situ*, assessment can be successfuly carried out from underwater images and video.

2.4. Fish visual census of climate change indicators

2.4.1. Overview of the methods

Beside the effort to monitor current conservation status and potential climate-related responses of a rocky bottom sessile macroinvertebrates, 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, Bensoussan & Azzurro 2018). These target species are: *Sparisoma cretense, Epinephelus marginatus, Thalassoma pavo, Sarpa salpa, Serranus scriba, Coris julis, Serranus cabrilla, Siganus spp.* and *Fistularia commersonii* (Fig. 14). In addition, local targets (max. 4) may be added according to local monitoring needs (e.g. exotic species), easiness of recognition, interaction with fisheries, increase/decrease in the area and potential impacts on the environment/fisheries/ human activities. At our locations we have opted to include also a striped red mullet *Mullus surmuletus* (Fig. 15a), fairly abundant and easy to recognize species with an important functional role, providing ecosystem services such as sediment resuspension (Pavičić et al. 2018 and references therein) and having a commercial value. Furthermore, we included common two-banded sea bream *Diplodus vulgaris* (Fig. 15), 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.

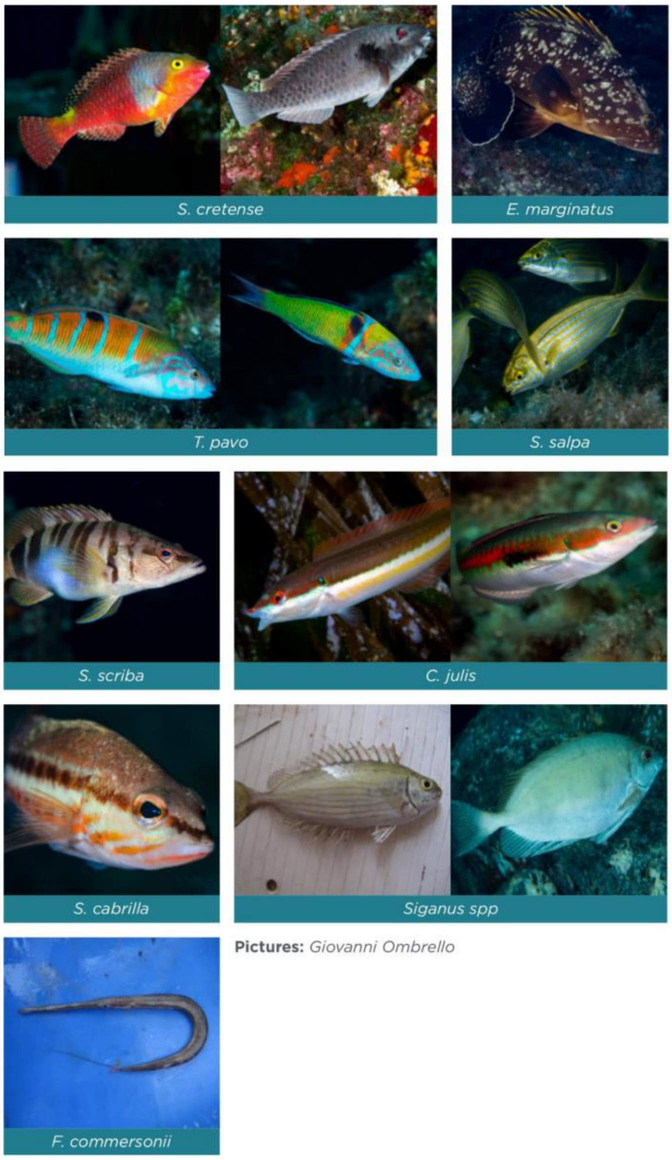


Fig. 2.4.1. Target species selected for fish census of climate change indicators (figure adopted from Garrabou, Bensoussan & Azzurro 2018).

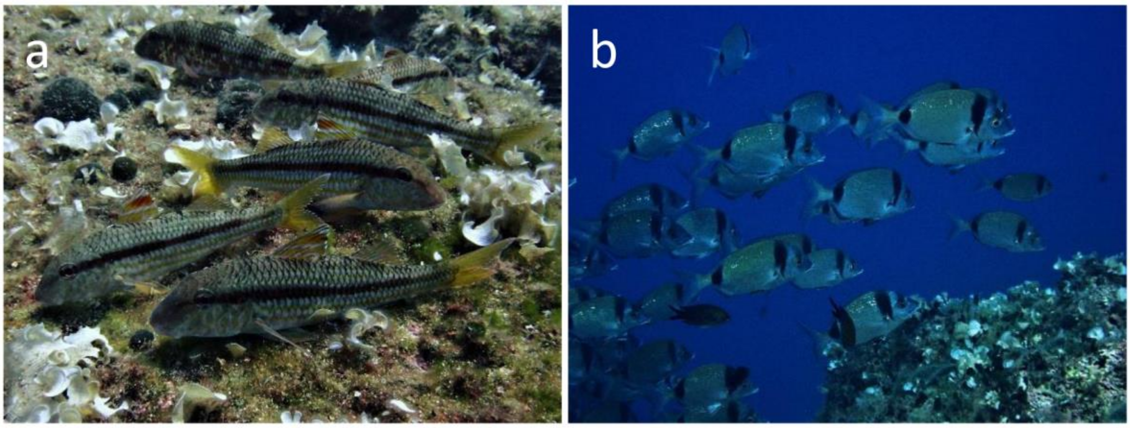


Fig. 2.4.2. Additional local target fish species selected for this study: a) striped red mullet *Mullus surmuletus* and b) common two-banded sea bream *Diplodus vulgaris*. Photo credit: S. Kipson.

Due to time constraints, at the Lagnići location (Dugi Otok Island) visual census was carried out only within one of the proposed depth ranges but advocated as the most important one in order to observe potential climate related changes (1-3 m depth range, Garrabou, Bensoussan & Azzurro 2018) at 3 sites. At the Stupišće location (Vis Island), visual census was carried out at 2 sites within 5-10 m depth range, since there were no sufficient rocky substrate above 5 m depth, whereas at the site next to Oključna bay, census was made both within 5-10 m and 1-3 m depth ranges. 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. 16). 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.

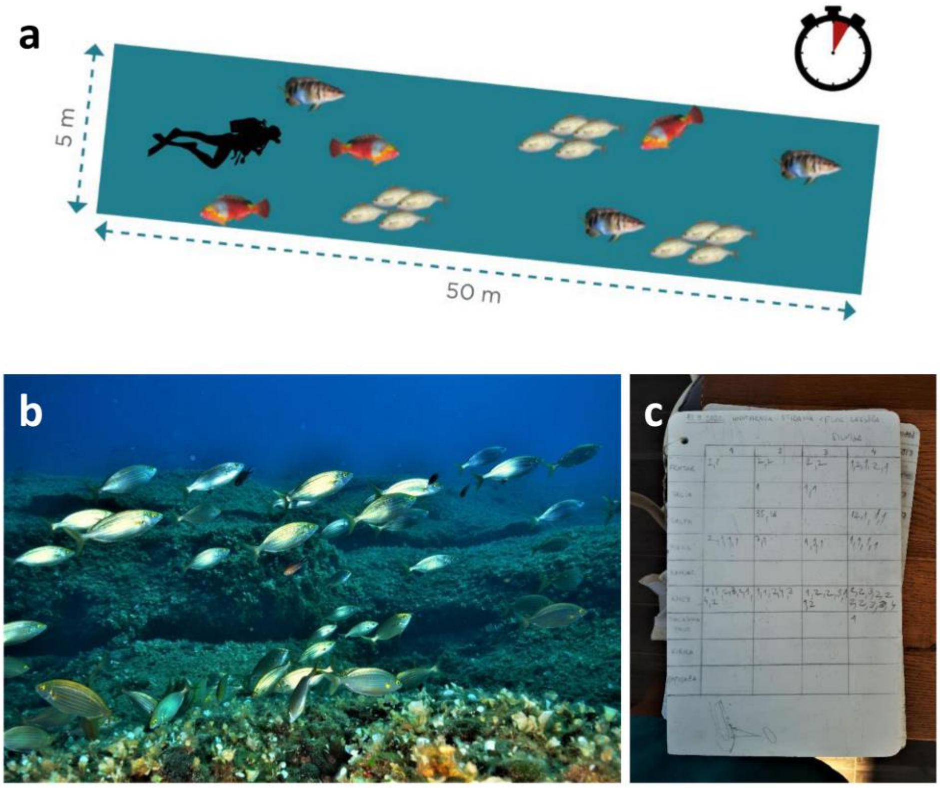


Fig. 2.4.3. Fish visual census of climate change indicators: a) scheme of a protocol (adopted from Garrabou, Bensoussan & Azzurro 2018); b) illustration of an underwater scene during visual census at the Lagnići location; c) an example of data recording.Photo credit: b) M. Belošević and c) S. Kipson.

2.4.2. Main results

Between 1-3 m depth, out of 9 target fish species, 6 were detected at all sites, except at Kamenica site (Vis Island) where 5 species were recorded. Common species at all sites included *Diplodus vulgaris*, *Sarpa salpa*, *Serranus scriba* and *Coris julis* (Fig.2.4.4). In this shallowest depth range (1-3 m), out of autochtonous species, *Diplodus vulgaris* was more abundant on the Dugi Otok sites and *Coris julis* was more abundant on the Vis Island sites (Fig. 2.4.5). Regarding thermophilic alien species, while few individuals of *Thalassoma pavo* were recorded on the Dugi Otok Island, its abundance was much higher on the Vis Island (Figs. 2.4.5, 2.4.7). In addition, another thermophilic alien species, *Sparisoma cretense,* was recorded exclusively on the Vis Island (Figs. 2.4.4, 2.4.5, 2.4.7).

Between 5-10 m depth (a depth range that was only assessed on the Vis Island) 8 out of 9 target species were recorded on Sika 3 site, 7 at Fish 2 site and 4 at Sika 6 site (Fig. 2.4.4). Out of autochtonous species, *Diplodus vulgaris* was dominant on Sika 3 site and *Coris julis* was dominant on Fish 2 (cove next to the Oključna bay) site. Notable also is the record of *Ephinephelus marginatus* at Sika 3 site and *Ephinephelus costae* on the Fish 2 site (Figs. 2.4.6, 2.4.8). Thermophilic alien species *Thalassoma pavo* was present at all sites within this depth range, but its abundance was the greatest at Sika 6 site: almost twice as high as at the Sika 3 site , and almost 3-fold higher than at the Fish 2 site (Fig. 2.4.6).

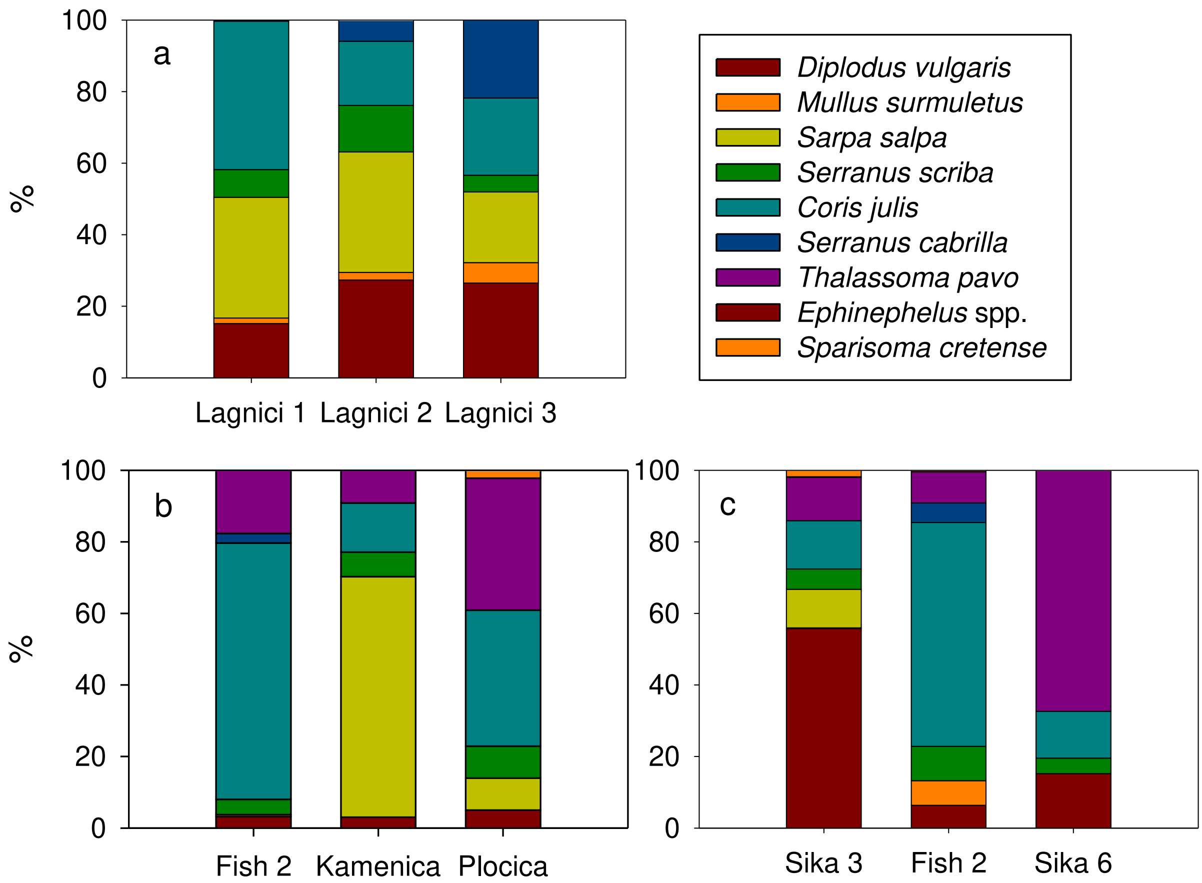


Fig. 2.4.4. Composition of target fish assemblages within: a) 1-3 m depth at the Dugi Otok Island, b) 1-3 m depth and c) 5-10 m depth on the Vis Island sites. Note: at Sika 6 site only 2 transects were censed.

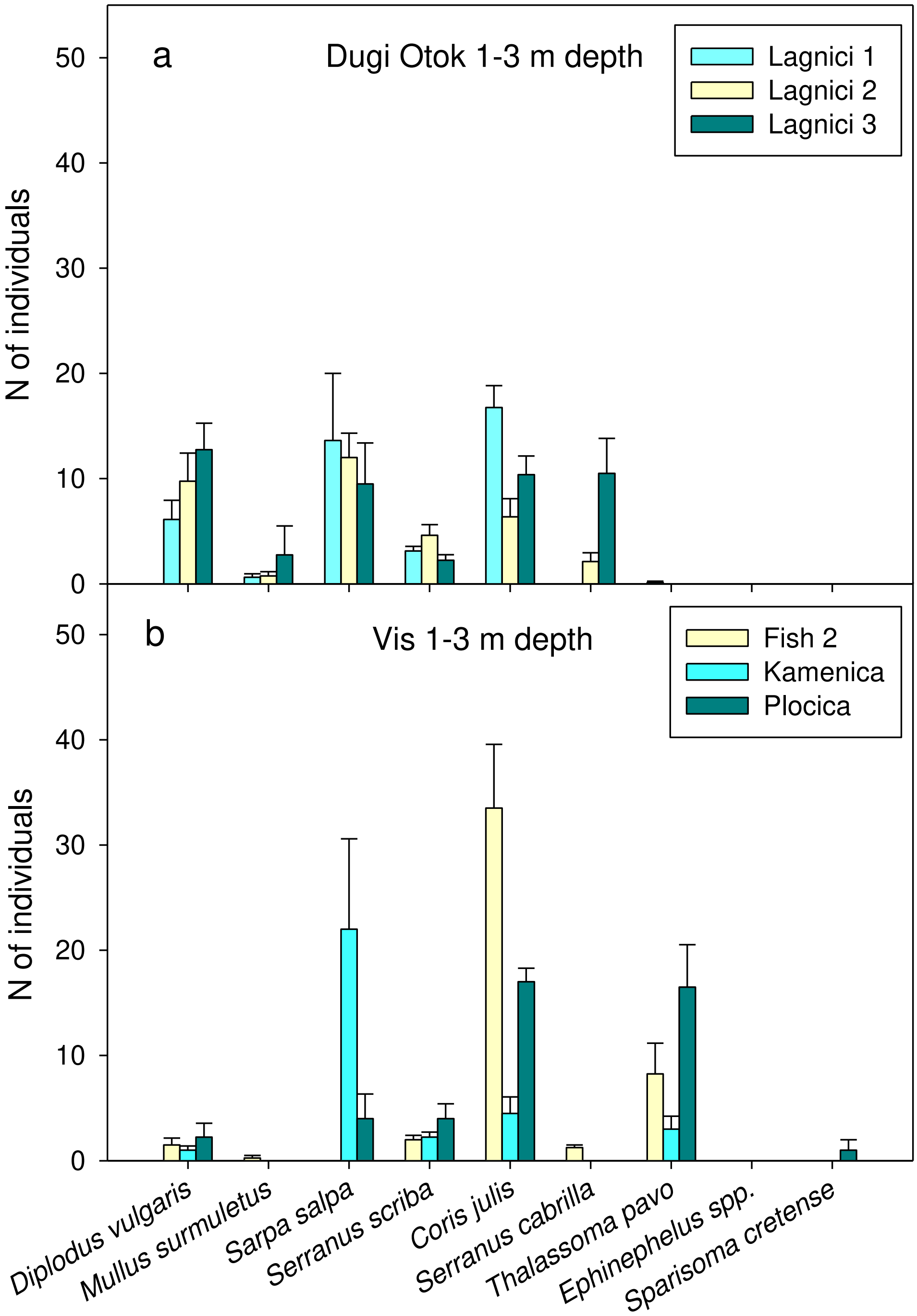


Fig. 2.4.5. Abundance of target fish species assessed by visual census between 1 and 3 m depth on: a) Dugi Otok Island and b) Vis Island. Data are shown as mean ± SE.

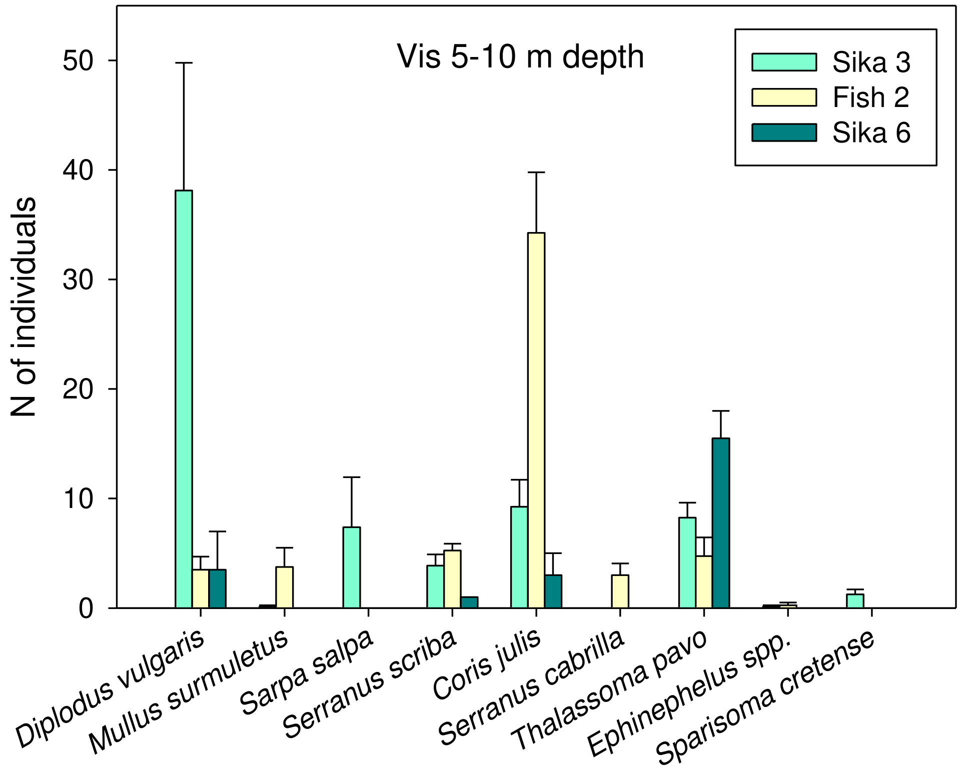


Fig. 2.4.6. Abundance of target fish species assessed by visual census between 5 and 10 m depth on the Vis Island. Data are shown as mean ± SE.

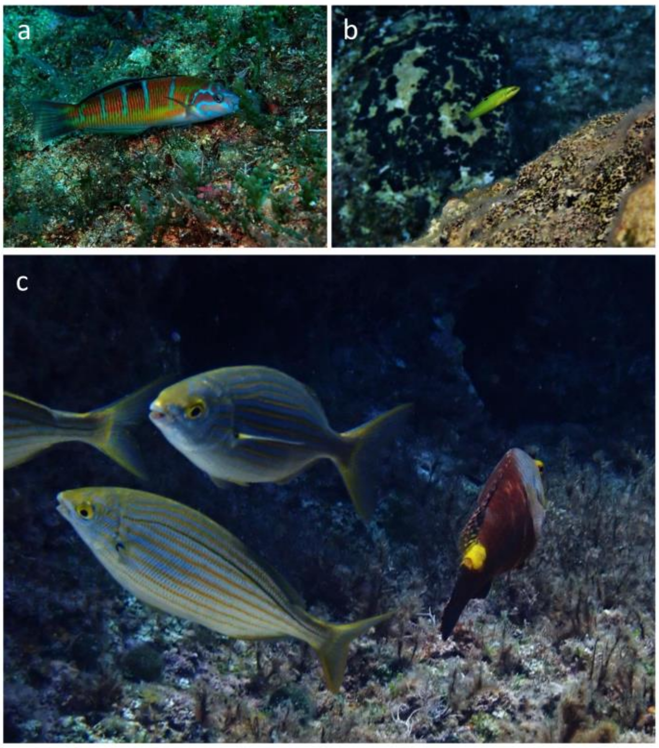


Fig.2.4.7. Thermophilic alien species observed on the Vis Island sites: a) adult and b) juvenile specimen of *Thalassoma pavo,* c) the Mediterranean parrotfish *Sparisoma cretense* (male) next to the autochtonous *Sarpa salpa.* Photo credit: S. Kipson.

Fig. 2.4.8. Goldblotch grouper *Ephinephelus costae* observed during the assessment at Fish 2 site (the cove next to the Oključna bay, the Vis Island) at 5 m depth. Photo credit: S. Kipson.

2.4.3. Conclusions

Fish abundance and diversity (including species not targeted by this protocol) was remarkable at the Vis Island sites, especially at Stupišće location (Sika 3 and Sika 6 sites), contributing considerably to the attractiveness of recreational diving there, an important aspect of a local tourism offer.

Overall, in comparison to the Dugi Otok Island, both the presence of thermophilic *Sparisoma cretense* and higher abundance of *Thalassoma pavo* on the Vis Island clearly indicate more pronounced effects of climate change on the latter island, located cca. 80 km south of the Dugi Otok Island. Hence, based on the data provided here and regular future monitoring, it will be possible to detect additional effects of seawater warming i.e. the consequent biological response in terms of changes in composition and structure of fish assemblages.

2.5. Assessment of the impact of the lost fishing gear (LFG)

2.5.1. Overview of the methods

To assess the impact of the lost fishing gear on the marine environment and to assist managers in their decisionmaking for the removal of nets, we have followed the protocol developed in the scope of the Ghost Med programme (Ruitton et al. 2019). This is the first time such protocol has been applied along the Croatian Adriatic coast. To assess the LFG impact, 3 criteria are used and they include environmental (EI) and seascape impact (SI) as well as the technical risk involved in the LFG removal. Each criterion is quantified by a set of relevant parameters and each parameter is assessed by a semi-quantitative or a qualitative scale. Moreover, scores are assigned and the criterion is assessed using the procedure described by Ruitton et al. (2019). Finally, based on the evaluation of all 3 criteria, a Removal Aid Index (RAI) is calculated (Ruitton et al. 2019).

Parameters for assessment of the LFG environmental impact (EI) include:

- **The colonization of the fishing gear** (evaluation of the colonization stage: (0) without epibiosis; (1) by filamentous algae; (2) by macroalgae and hydrozoa; and (3) by encrusting epibiosis (bryozoa, macroalgae, annelida, etc.). It is considered that the more developed the colonization is, the less the removal of the gear would be appropriate.

- **The trapped mobile fauna** (a semi-quantitative estimation of the number of individuals trapped in the LFG)

- **The removed fixed species** (number of individuals of all the benthic species fixed to the substrate that have been torn off by the action of the fishing gear)

- **The damaged fixed species** (number of individuals that undergo necrosis or breakage due to contact with the LFG)

- **The presence of outstanding species** (observation or not of species with heritage value, such as protection status and rarity, and/or commercial value that have colonized the LFG)

- **The obstructed cavities** (the number of cavities that are no longer accessible for mobile fauna)

- **The abrasion of the substrate** (observation or not of a friction effect of the LFG on the substrate which would consequently damage the colonization)

- **The habitat creation** (observation or not of the potential ecological role of LFG such as nursery, hideout or pantry for the marine fauna)

Furthermore, parameters for the assessment of the LFG seascape impact (SI) include:

- **The distance of visibility** (the estimation of a distance at which the LFG is visible)

- **The extent of impact** (the surface concerned by the LFG - usually the surface area occupied by the gear on the bottom)

- **The seascape alteration** (the recognition or not that there is an alteration of the seascape)

- **The qualifying adjective** (overall impression if and how LFG alters the seascape - could be

neutral, positive or negative

- **The relief created** (evaluation whether the natural relief of the site is altered by the LFG – e.g.

if the gear is lying on a rocky scree, it tends to detract from the relief, whereas

if it is deployed in the water column, it enhances the relief).

Lastly, parameters for the assessment of the technical risk (TR; i.e. taking into consideration the diver’s intervention or the technical equipment required for the removal of the LFG) include:

- **The depth of the LFG**;

- **Attachment of the LFG to the bottom** (evaluation to what extent the LFG is attached to the bottom and if its removal is relatively easy or it is difficult and time-consuming; this parameter presents a criterion that modulates the time spent by divers on the bottom and the use or not of specific tools).

The formulaire containing the parameters briefly outlined above was printed out and attached onto the slate (Fig. 2.5.1) and divers carried out the assessment underwater, first time (on September 14 2020) as a part of the trainning at the shallower depth (12-16 m), i.e. within an infralitoral rocky bottom interspersed by patches of *Posidona oceanica* at the location Lagnići (Fig. 2.5.1, 2.5.5) and later also within the coralligenous community (on September 16 and 17 2020) at two sites in the same location (Fig. 2.5.3). Unfortunately, due to time constraints, such assessment could not be performed *in situ* at the Stupišće location on the Vis Island.

Fig. 2.5.1. Data recording during a lost fishing gear impact assessment on an infralitoral reef at the Lagnići location, Dugi Otok Island (photo credit: S. Kipson).

2.5.2. Main results

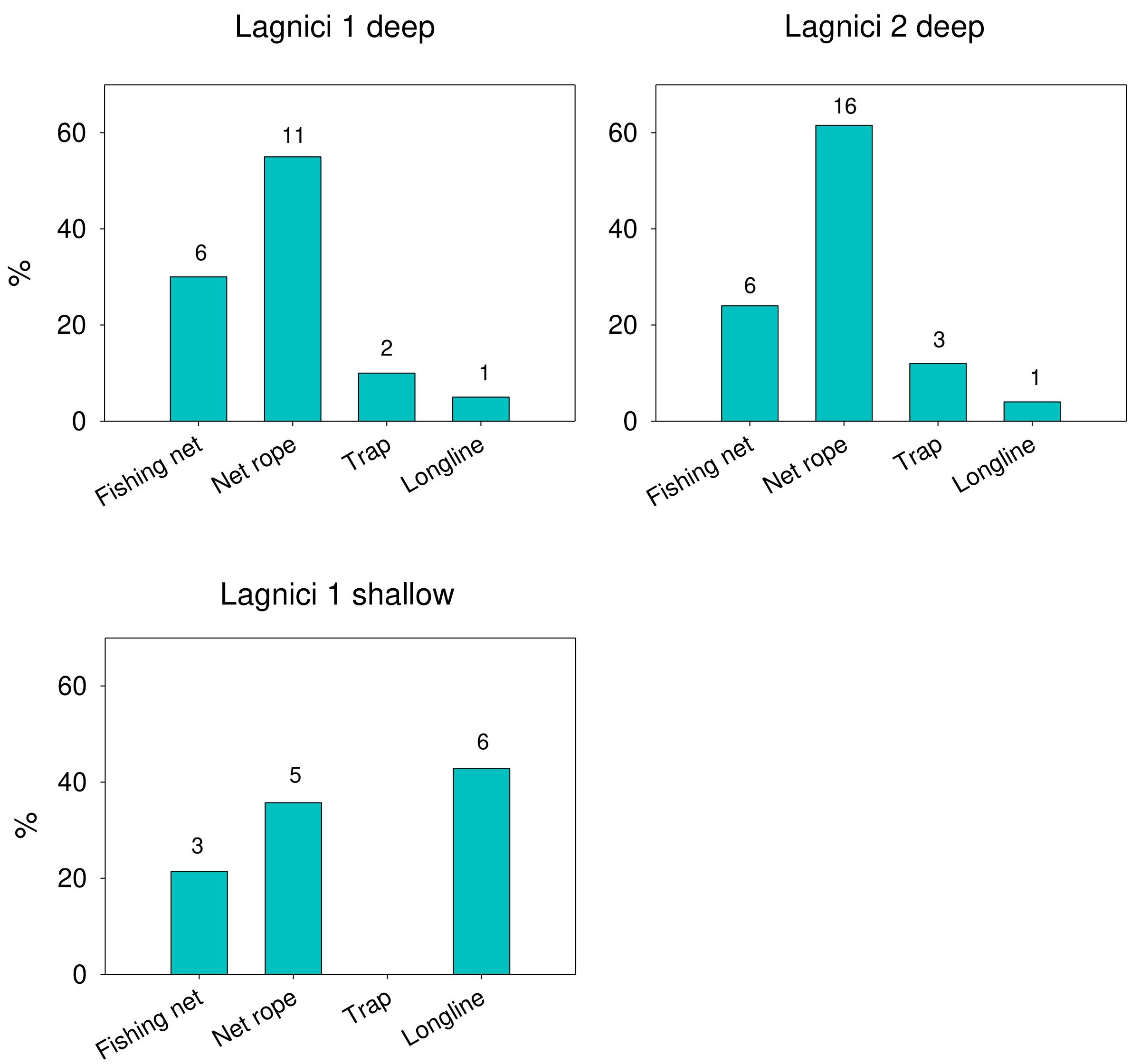


Fig. 2.5.2. Type and number of recorded lost fishing gear at the Lagnići location (NW Dugi Otok Island). “Deep” assessment was carried out within coralligenous habitat (30-39 m depth), whereas “shallow” one was carried out on infralitoral rocky bottom (12-16 m depth).

At the deep Lagnići sites where coralligenous habitat is found, predominant type of lost fishing gear were remnants of net ropes (55-61% of all LFG), followed by actual fishing nets (Fig.2.5.2).

Moreover, abandoned traps were noted only on deep sites. Related to environmental impact, all gear was heavily colonized by organisms (stage 3, e.g. Figs.2.5.3, 2.5.4a), whereas there were no trapped or removed individuals. However, some fixed organisms were damaged, in most cases in moderate number (between 1 and 10 individuals/colonies), whereas in the case of several nets this impact was larger (>10 ind.). Outstanding species were not associated with LFG, except on one occasion when a strictly protected sponge *Aplysina cavernicola* was recorded on a suspended rope (Fig.2.5.4b). There was no abrasion impact on the substrate and no obstructed cavities were noticed. In all cases LFG created additional habitat. Related to the impact on the seascape, all of LFG altered the seascape and these alterations were very visible (from > 5 m distance). When hanging from the wall, suspended in the water column, LFG did enhance the relief but the overall impression of their impact was negative. However, being mostly ropes, broken hanging nets and longlines, their impact mainly affected a surface below 5 m^2, and only in several cases, when nets were involved, their impact extended to a surface between 5-20 m^2.

Since large amount of LFG below 20 m depth actually hangs from the wall, i.e. for most part they are not attached to the substrate, their removal, at least partial, could be relatively easy. Nonetheless, technical risk is still influenced by the actual depth. On the contrary, at Lagnici 1 site LFG was also assessed on the shallow reefs (12-16 m depth) and accordingly, technical risk of LFG removal there (as far as depth is concerned) will be lower. However, at the same time, LFG was mainly attached to the substrate on the shallow reefs, making its removal more laborious from that aspect.

In general, on the shallow reefs, dominant LFG were longilines and net ropes, followed by the remnants of the fishing nets (Figs. 2.5.2, 2.5.5). Results of the assessment indicate that the impacts on species in the shallow were occassionaly more adverse than on deep reefs. They involved also removed fixed species and trapped mobile fauna, and sometimes the quantity of damaged fixed species has been estimated in the highest category. On the contrary, being more attached to the substrate, LFG in the shallow were occassionaly less visible and the observers did not have impression of relief alteration. For the same reason, the overall impression in those cases was more neutral, although the majority of assessments still included a diminished relief.

A Removal Aid Index (RAI) for LFG within coralligenous habitat at Lagnići sites ranged from 0.25 to 2, indicating that removal of some LFG is not a priority or its priority is low (1). Likewise, the RAI for LFG within shallow, infralitoral reefs at the same location ranged from 0.33 to 2.3, leading to the same recommendations of either non removal or its low priority.

Unlike at Lagnici location where LFG were abundant at both study sites, much less of LFG was present at studied Vis sites. Only one LFG was assessed there, at the study site Sika 3 and it was a net rope in the most advanced colonisation stage that has created habitat, whereas it did not remove or trap fauna, nor it had an abrasive effect on the substrate or significant impact on the seascape. In addition, since it was at depth below 20 m and was evaluated as difficult to remove, its overall RAI of -1 advised against its removal, giving it priority of 0.

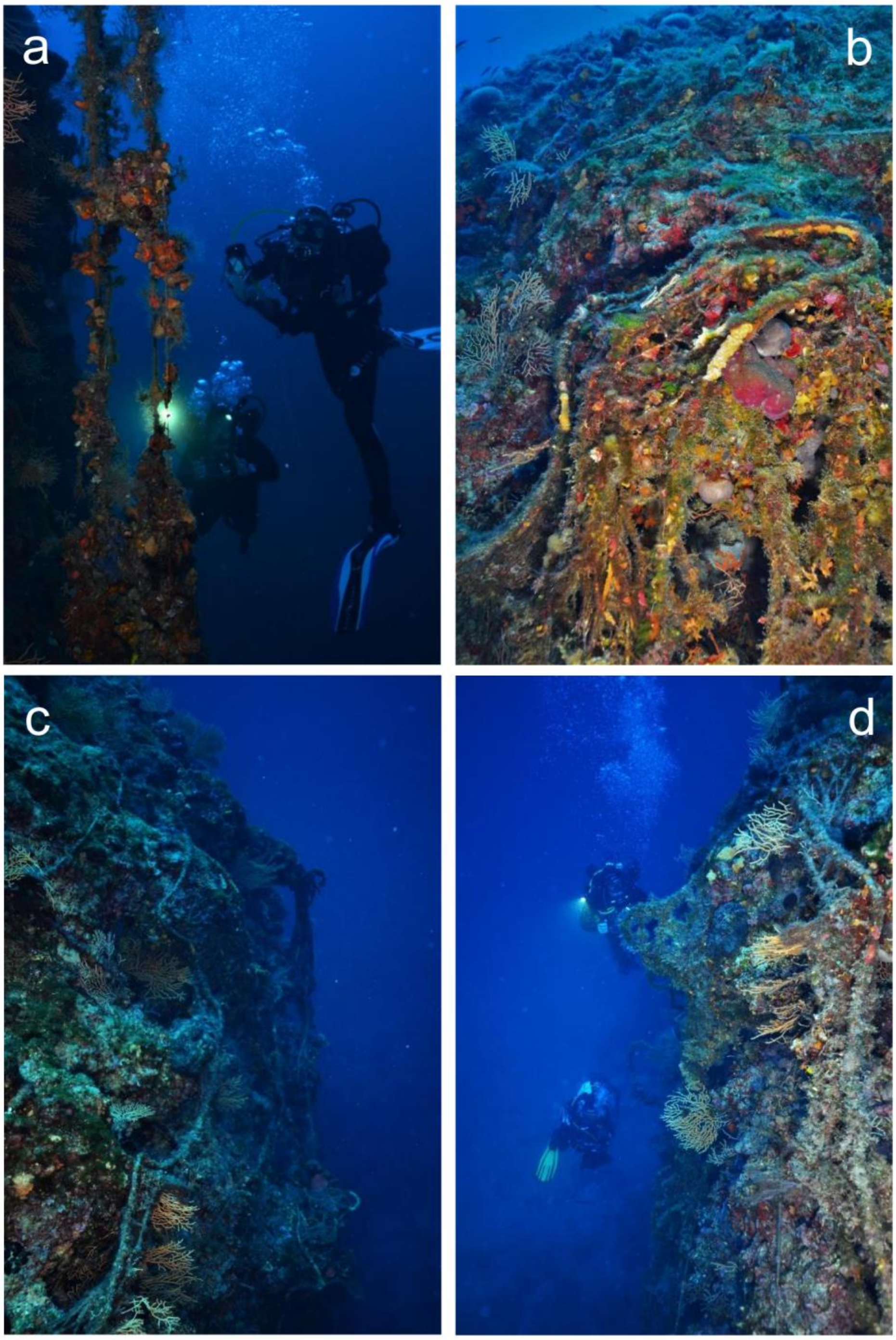


Fig. 2.5.3. Examples of lost fishing gear within coralligenous assemblages at deep Lagnici sites (30-39 m depth, Lagnici location, NW Dugi Otok Island): a) a very visible fishing net hanging from the wall and considerably altering the seascape; b) less visible fishing net covering the substrate; c) numerous remnant net ropes and longlines are interspersed over coralligenous reefs, and d) sometimes they are covering colonies of *Eunicella cavolini*. Photo credits: M. Belošević.

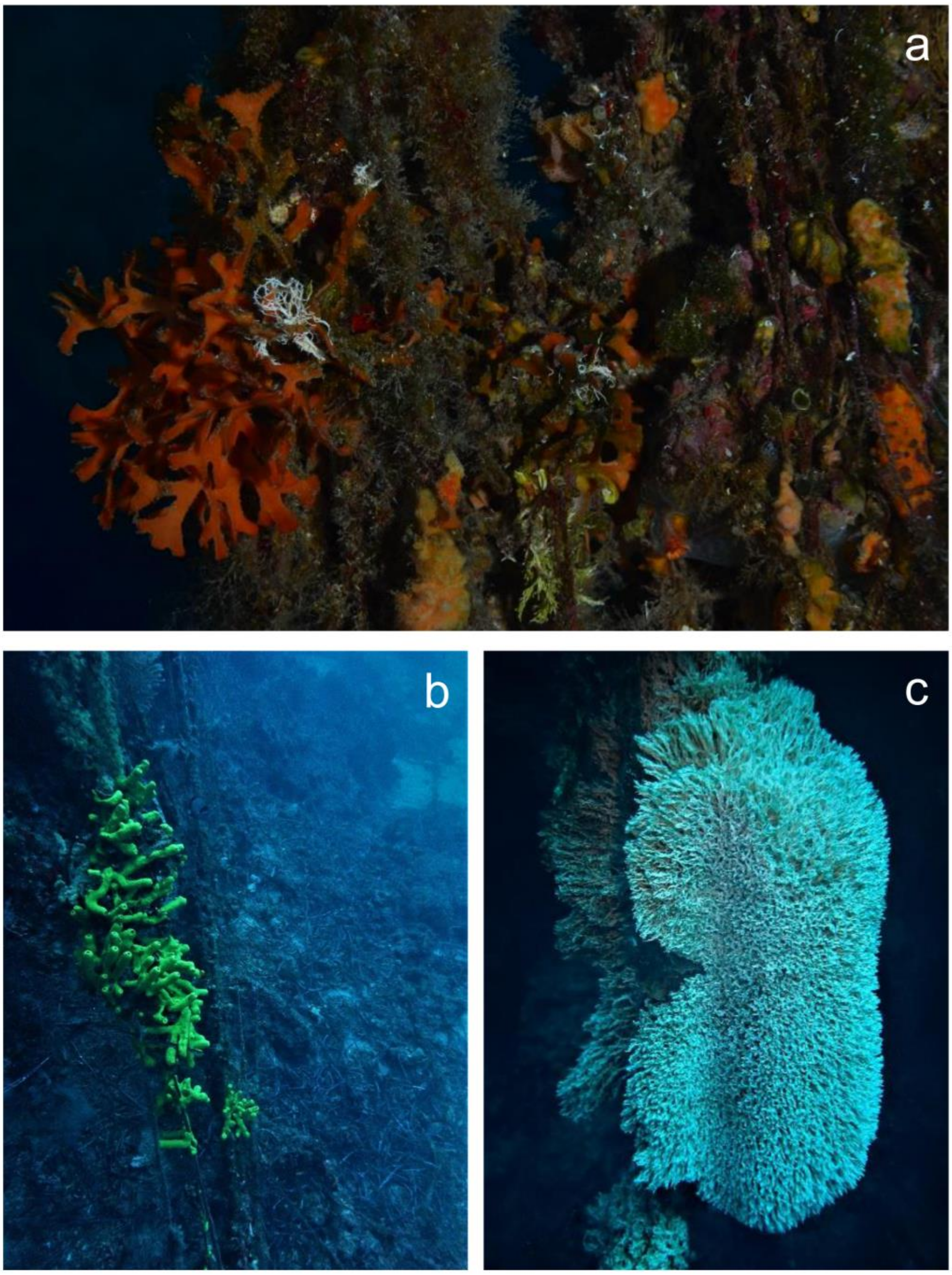


Fig. 2.5.4. Examples of sessile marine organisms attached to the lost fishing gear within coralligenous assemblage at Lagnici location: a) a fishing net suspended in the water column and heavily overgrown by diverse benthic species, including animal calcareous buliders such as branchy bryozoans *Pentapora fascialis* and *Reteporella* sp., as well as several encrusting bryozoan species; b) a strictly protected sponge *Aplysina cavernicola* attached to the ropes; c) a large aggregation of another calcareous animal builder attached to a remnant net rope – a polychaete belonging to *Filograna implexa*/*Salmacina dysteri* complex. Photo credits: M. Belošević (a), S.Kipson (b,c).

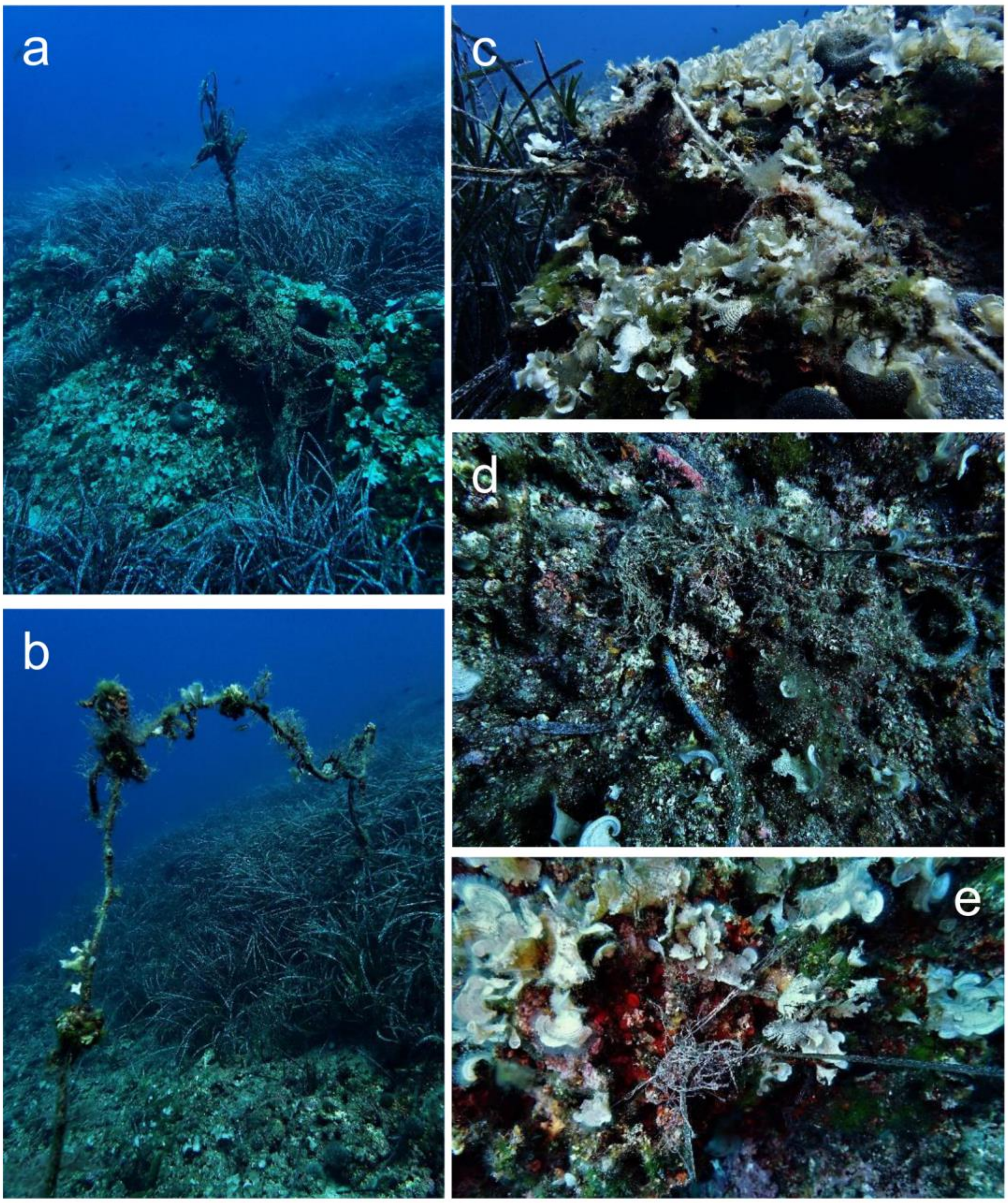


Fig. 2.5.5. Examples of lost fishing gear within infralitoral reefs at Lagnići sites (12-16 m depth, Lagnići location, NW Dugi Otok Island): a) the largest LFG found at shallow depths (13.7 m, Lagnići 1 site) - a fishing net partially attached to the rocky bottom and partially suspended in the water column, altering the seascape; b) a visible longline suspended in the water column and thus enhancing the relief c) less visible longline stretched over the rocky bottom and partially overgrown by photophilic algae; d) a remnant of the fishing net attached to the substrate; e) a bundle of monofilaments on the infralitoral rocky bottom. Photo credits: S.Kipson.

2.5.3. Conclusions

Assessment of the lost fishing gear at the Lagnići study sites indicated a low priority for their removal. Such an outcome partially stems from the fact that majority of LFG were remnants of net ropes. As such, they did not cover a large surface, obstruct cavities nor they trapped or removed many organisms. Most of them were laid over the substrate and showcased the most advanced colonization stage. Likewise, even when fishing nets were present, which do cover larger surface, they were often hanging from the wall, not directly affecting the substrate and/or they were heavily overgrown by sessile organisms and hence were contributing to habitat creation. Likewise, since large amount of LFG was located below 20 m depth, even when their removal could be relatively easy because they were not attached to the substrate, the technical risk is still influenced by the need for underwater work at considerable depth. Lastly, it should be taken into account that this protocol was applied for the first time by observers, therefore potential inexperience in detecting all of impacts may have also contributed to such an outcome.

Assessment of the lost fishing gear following the protocol proposed by Ruitton et al. (2019) proved to be practical and feasible to conduct underwater, even when observations are made within coralligenous assemblages, hence at depths below 25 m at our sites. However, as the protocol individually evaluates the priority for removal of each LFG, the sensation remains that overall quantity and cumulative effects of LFG, especially on covered surface, seascape alteration and diminution of relief at the particular site should also be taken into account as they may have additional environmental and seascape impacts and may considerably affect diving experience. In the current form, this protocol does not seem to account for that aspect.

2.6. Preliminary biodiversity assessment on unintentional artificial reefs – shipwrecks Michele and Teti

2.6.1. Overview of the methods

Since an accessible shipwreck (i.e. stretching down to the limits of recreational diving at 40 m depth) was present close to each of our study locations on the Dugi Otok and Vis Islands, we have used this extraordinary opportunity to visit them and carry out a preliminary biodiversity assessment in an attempt to contribute to the future, more comprehensive evaluation of their current role as artificial reefs, although their original placement underwater was not intentional (unlike when artificial reefs are specifically used as a conservation/restoration tool).

Whereas shipwreck Michele could be visited on 3 occassions (one of them being the assessment of potential mass mortalities of target sessile macroinvertebrates, see section 2.4), shipwreck Teti could be visited only once and on that occasion the sea current was fairly strong, limiting the underwater work. Hence, due to different sampling effort the results obtained for these 2 shipwrecks – artificial reefs are not comparable, and there is no intention to do so, but merely to document marine habitats and species/taxa so far associated to them. Likewise, results are based on the divers observations *in situ* and underwater photography and videography. Since reliable identification of many benthic taxa require a physical sample to be examined by a specialist (and collecting species samples was not predicted in the scope of our work and we have not requested official permissions for it), the list provided within this report is by no means a comprehensive species list for the respective sites.

2.6.2. Main results

2.6.2.1 The shipwreck Michele

Steamboat Michele was sunken in 1983 few 100s of meters north-east from Lagnići (NW Dugi Otok Island, Fig.1.1.1b). The shipwreck sits on the sea bottom at 6 m depth, it is 76 m long and 10 m wide and has NE-SW orientation (Frka & Mesić 2012). At first and for almost 3 decades a large proportion of the ship was still positioned above the water (to the extent that it was possible to walk onboard) but in the meantime it has corroded considerably and today only a tiny part still protrudes from the sea (Fig. 2.6.1a). Since it was a cargo ship, the greatest part of the hull was dedicated to storage (Fig.2.6.1 i,j), whereas cabins, utility and engine rooms were located at the rear end (Fig. 2.6.1. c,e,h). A more complete visualisation of the shipwreck is provided in Fig.2.6.1.

Being still well preserved, the shipwreck Michele acts as a premium artificial reef that provides a variety of marine habitats (Fig. 2.6.2). The most photophilic biocenosis develops on the outer hull with E-SE exposure (Fig.2.6.2 b) and it is characterized by a typical infralitoral algae *Padina pavonica* (Fig.2.6.2 b, Fig.2.6.3 a) but also sponges such as *Ircinia oros*, *Ircinia* sp., black keratose sponges and *Crambe crambe* (Fig. 2.6.3 a,h). Zooxanthellate (i.e. with symbiotic algae) scleractinian coral *Ballanophyllia europaea* also thrives there, as well as several species of encrusting bryozoans (Fig. 2.6.3 c-e) and a thorny oyster *Spondylus gaederopus* (Fig. 2.6.3 g). Due to the current position of the ship, the part of the hull with W-NW exposure creates a slight overhang and hence it is slightly more shaded (Fig.2.6.2 a). This part is dominated by more sciaphilic green algae *Flabelia petiolata* and *Halimeda tuna*, as well as non-calcifying red algae *Peyssonelia rubra* (Fig. 2.6.3 f). Sponge *Agelas oroides* (Fig. 2.6.3 b) is also noted there.

Furthermore, on the walls and ceilings of the inner part of the hull a truly sciaphillic biocenosis develops (Fig. 2.6.2 c,d), dominated by sessile macroinvertebrates, primarily massive and encrusting sponges (Fig. 2.6.4 a-c). The intermediate layer is formed by massive sponges such as *Chondrosia reniformis*, black keratose sponges, *Chlatrina* sp., *Ircinia* oros, *I. dendroides*, *Ircinia* sp. and the ascidian *Aplidium tabarquensis* (Fig. 2.6.4 a-d). Encrusting sponges such as *Phorbas tenacior*, *Spirastrella cunctatrix* and *Crambe crambe* contribute to the basal layer together with small branchy bryozoan, possibly *Scrupocellaria* sp. Besides animals, dominant organisms in the basal layer are both soft red algae *Peyssonnelia rubra* as well as several species of encrusting calcifying Peyssonnaliacea (Fig. 2.6.4 e,f).

Moving to the opposite end of the light gradient, within enclosed spaces such as cabins and engine rooms (Figs. 2.6.1 h, 2.6.2 e,f) the biocenosis of semi-dark caves develops and algae are no longer present there. Biocenosis is dominated by both encrusting and massive sponges (Fig. 2.6.5). Some of these species are lacking the usual pigments, due to the conditions of diminished light, such as *Spongia* cf*. officinalis*, *Chondrosia reniformis* and *Dysidea* sp. (Fig. 2.6.5a-c). Other abundant species include sponges *Spirastrella cunctatrix, Phorbas tenacior, Terpios fugax, Aplysina aerophoba and* several as yet unidentified yellow and red encrusting sponges (Fig. 2.6.5 c,d,f,g). Besides sponges other abundant species included foraminiferan *Miniacina miniacea* and a cup coral *Caryophillia inornata* (Fig. 2.6.5 e)*.*

Lastly, many fish used habitats provided by the shipwreck such as *Coris julis*, *Diplodus annularis*, *Serranus scriba*, *Diplodus vulgaris*, *Chromis chromis* and *Scorpaena scrofa* (Fig. 2.6.6). A list of all species associated to the shipwreck Michele is provided in Appendix 5.

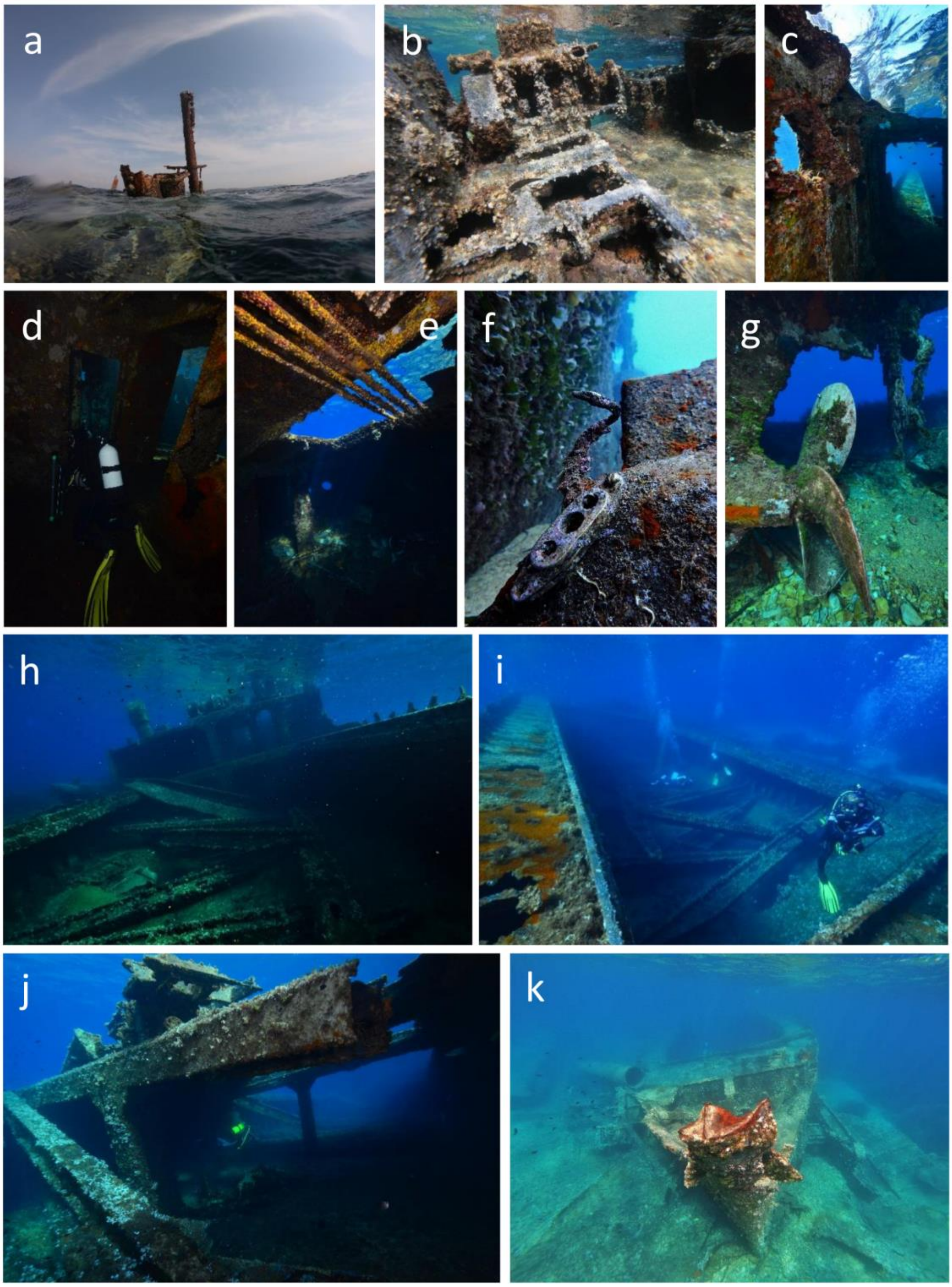


Fig.2.6.1. Shipwreck Michele – an artificial reef, NW Dugi Otok Island (0-6 m depth). From a) to k) details of the wreck are shown from its rear towards the frontal part. Photo credits M. Belošević except (a,b,k) Z. Jakl and (f) S. Kipson.

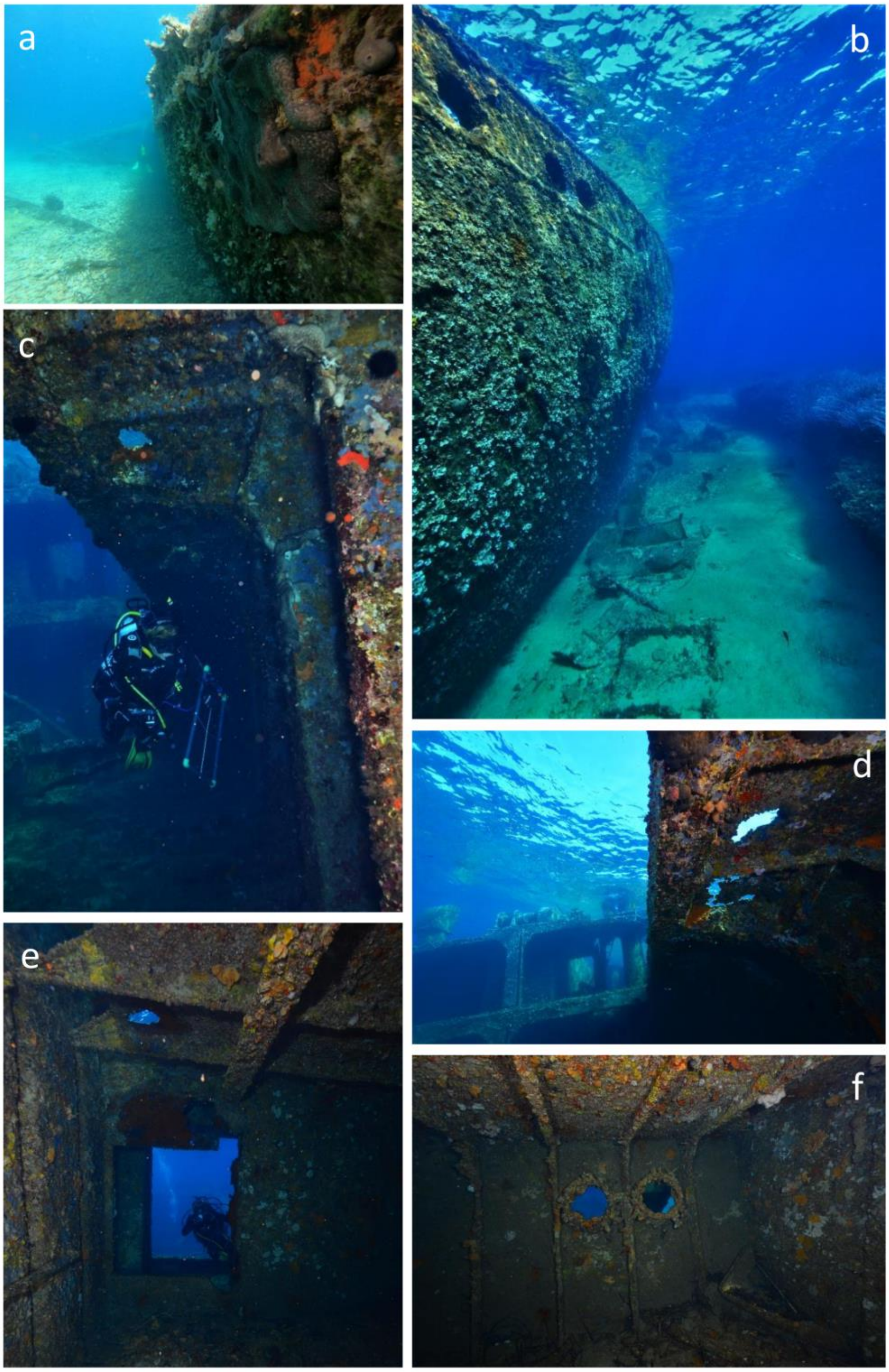


Fig. 2.6.2. Diversity of marine habitats found on an artificial reef - the shipwreck Michele (3-6 m depth, NW Dugi Otok Island): a-b) infralitoral biocenosis dominated by photophilic algae developing on the outer ship's hull; c-d) sciaphilic biocenosis developing on the more shaded vertical walls and ceilings of the inner ship's hull; e-f) biocenosis of the semi-dark caves developing in the interior of the cabin/engine room. Photo credits: M. Belošević except a) Z. Jakl.

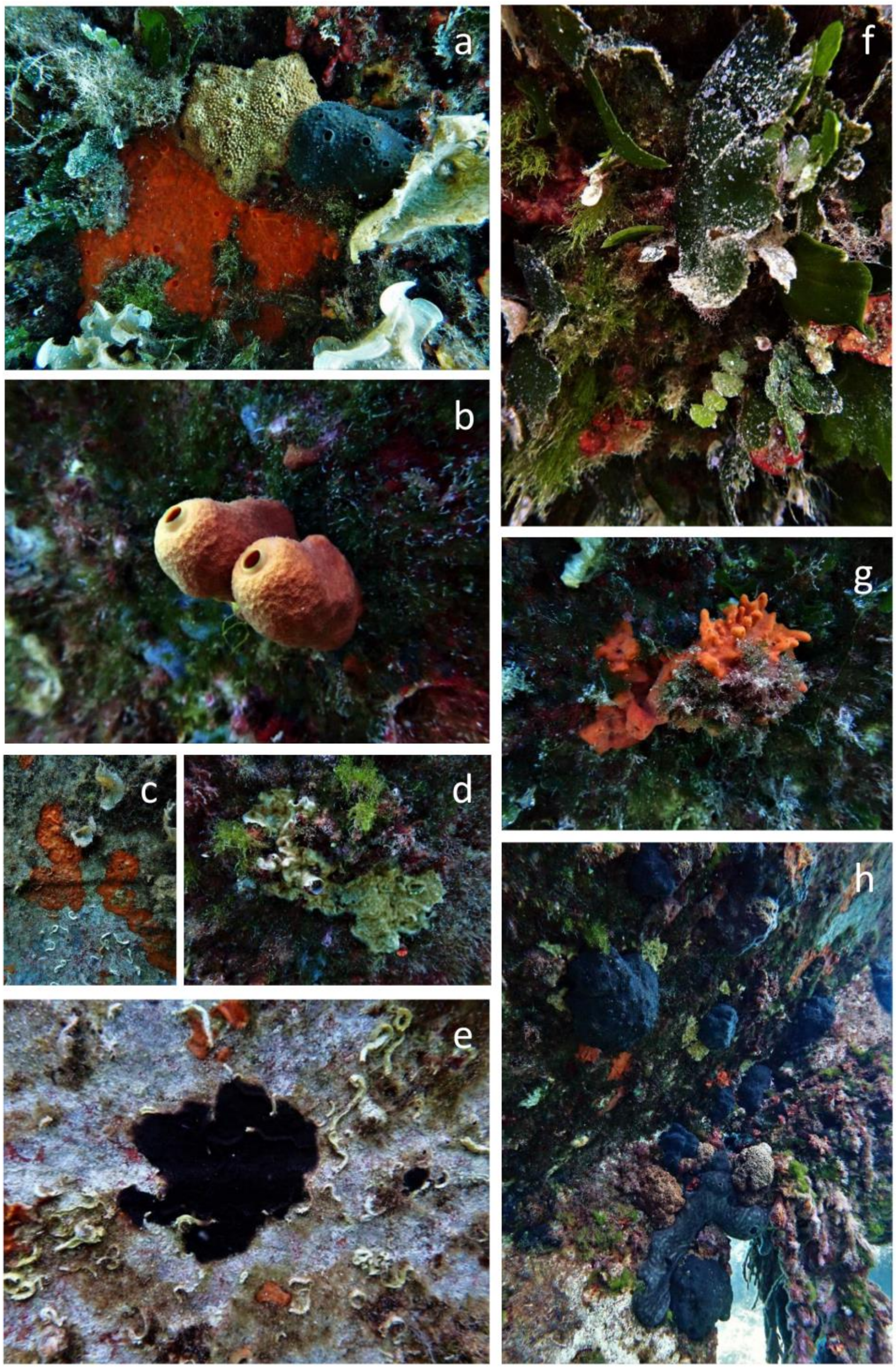


Fig. 2.6.3. Organisms characterizing the infralitoral biocenosis dwelling on the outer, more light exposed part of the ship's hull: a) black keratose sponge, Ircinia oros, Crambe crambe and photophilic algae *Padina pavonica*; b) sponge *Agelas oroides*; c–e) diversity of encrusting bryozoans; f) algae *Flabelia petiolata*, *Halimeda tuna*, *Peysonnelia rubra* thriving in slightly lower light at the overhanged part of the outer hull exposed to W-SW; g) thorny oyster *Spondylus gaederopus* covered by encrusting sponge *Crambe crambe*; h) detail of the outer hull showing great abundance of massive sponges. Photo credits: S. Kipson.

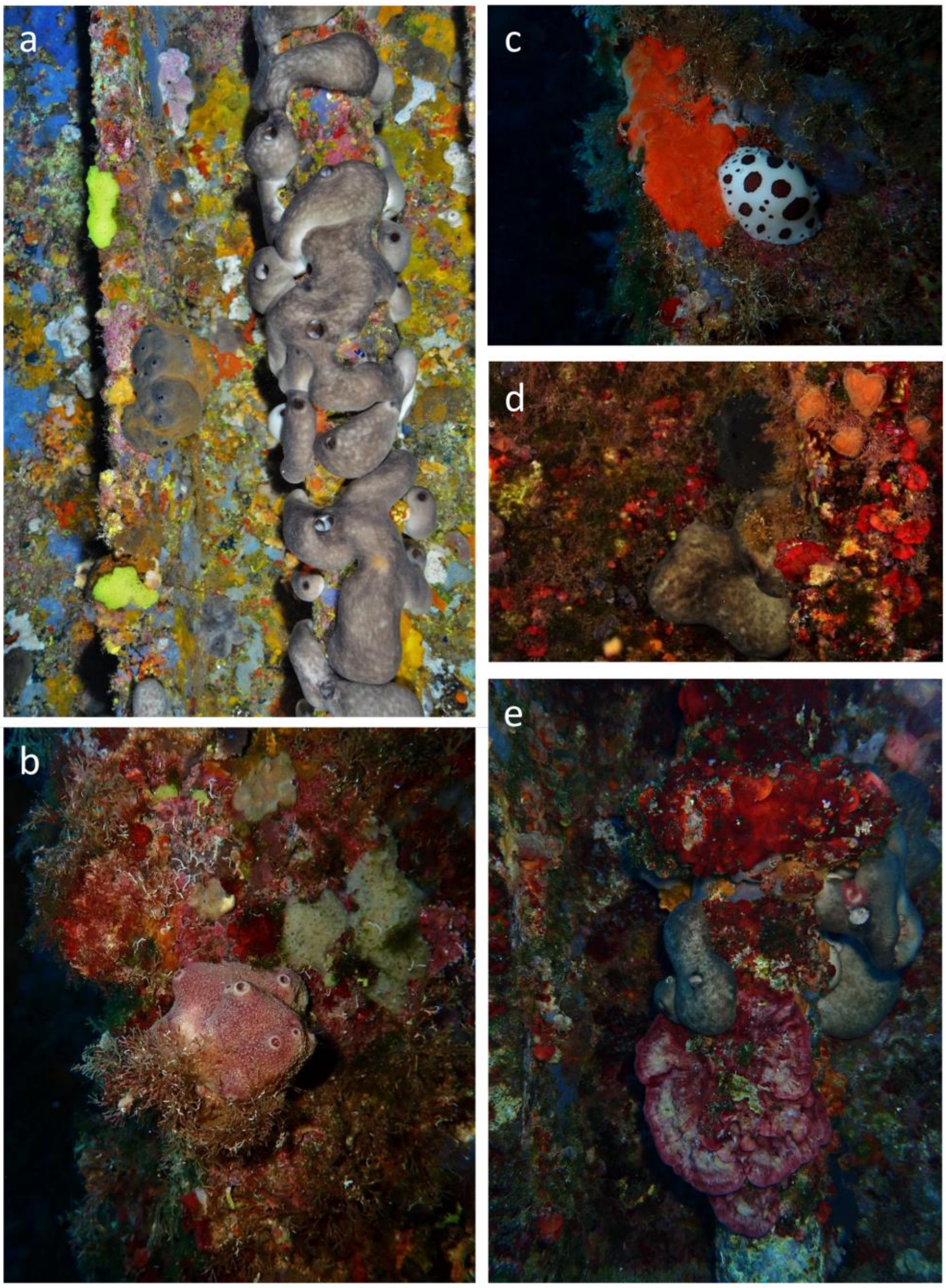


Fig. 2.6.4. Organisms characterizing the sciaphilic biocenosis dwelling on the vertical wall – the inner, shaded part of the ship's hull: a) both massive and encrusting sponges were dominant organisms here, such as *Chondrosia reniformis*, black keratose sponges, *Chlatrina* sp., *Phorbas tenacior* as well as b) *Ircinia* sp. and c) *Crambe crambe*, whereas nudibranch *Peltodoris atromaculata* was one of mobile macroinvertebrates present. Another common sessile filter feeder was d) the ascidian *Aplidium tabarquensis* and the basal layer was largerly formed by small branchy bryozoan, possibly *Scrupocellaria* sp. Besides animals, dominant organisms in the basal layer were both soft red algae *Peyssonnelia rubra* (d) as well as several species of encrusting calcifying Peyssonnaliacea (e). Photo credits: M. Belošević (a,d), S. Kipson (b,c,e).

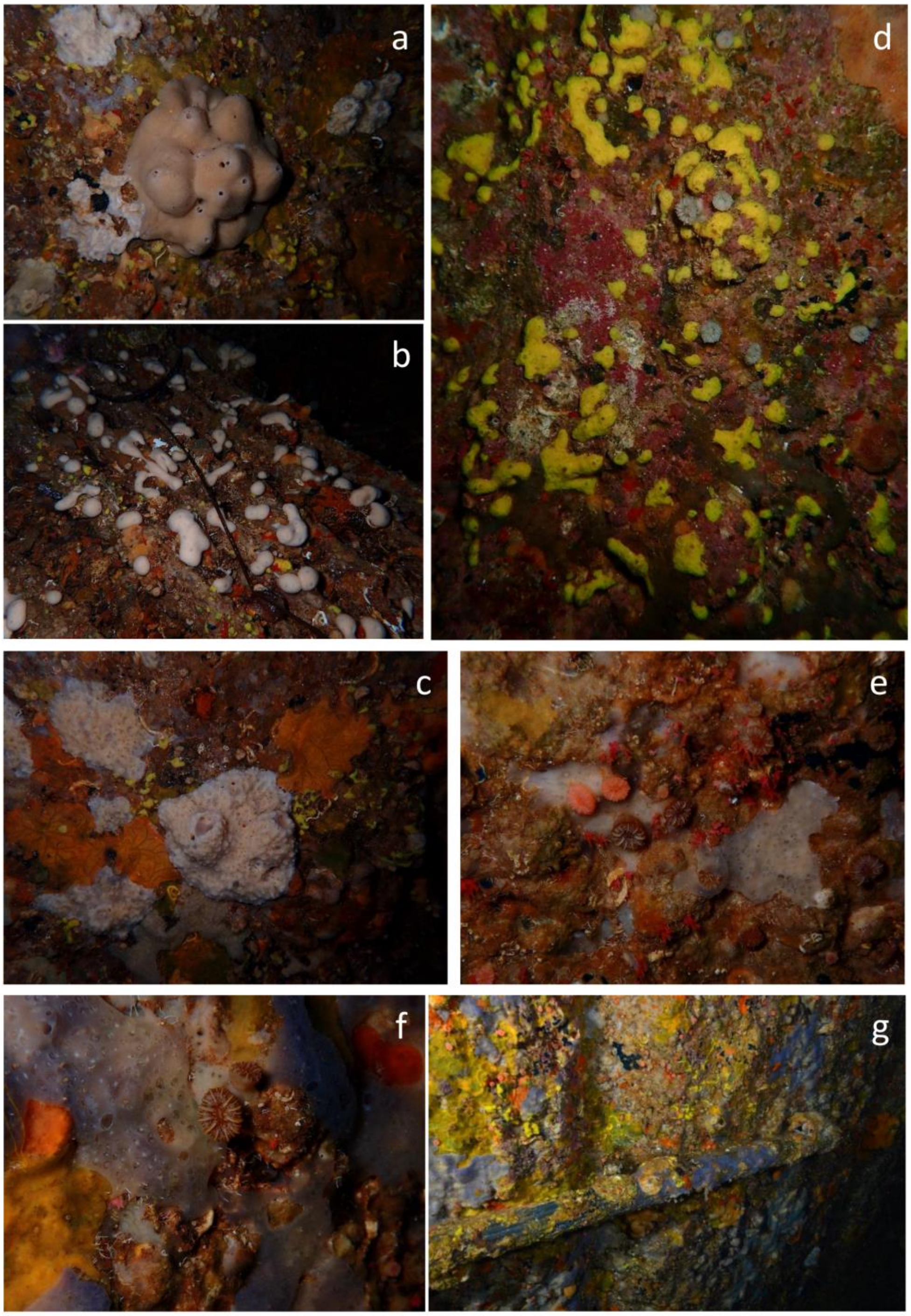


Fig. 2.6.5. Organisms characterizing the biocenosis of semi-dark caves dwelling within a former steamboat cabins and engine rooms. Biocenosis is dominated by encrusting and massive sponges with some species lacking the usual pigments, due to the lack of light, such as: a) *Spongia officinalis*, b*) Chondrosia reniformis* and c) *Dysidea* sp.; Other abundant species included: c) orange encrusting sponge *Spirastrella cunctatrix,* d) *Aplysina aerophoba,* e) foraminiferan *Miniacina miniacea*, cup coral *Caryophillia inornata* and sponge *Terpios fugax*, e) *Phorbas tenacior* and as yet unidentified yellow and red encrusting sponge; f) detail of the colorful wall covered by diverse encrusting sponges. Photo credits: S. Kipson (a-f), M. Belošević (g).

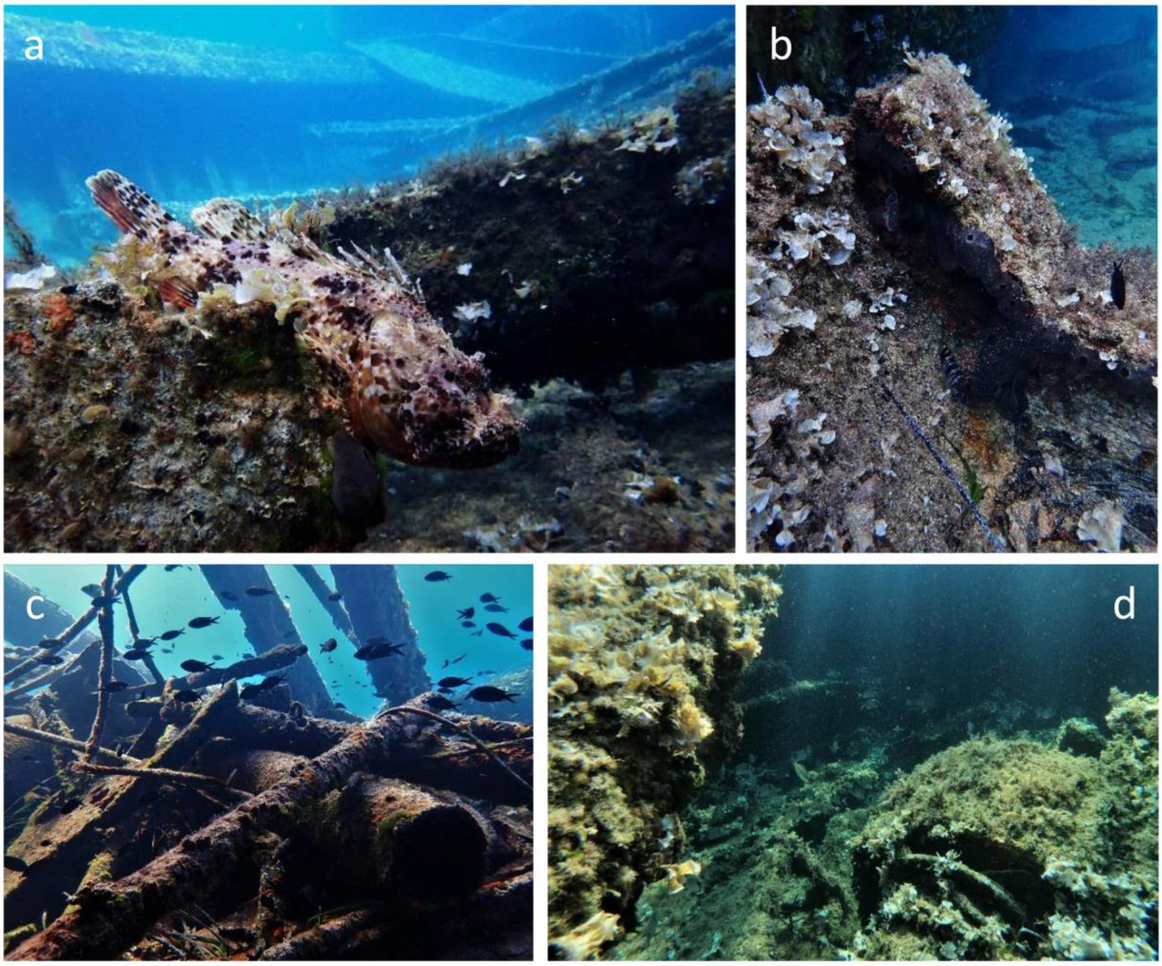


Fig. 2.6.6. Fish inhabiting the shipwreck Michele such as: a) *Scorpaena scrofa*, b) *Serranus scriba*, and large schools of c) *Chromis chromis* and d) *Diplodus vulgaris*. Photo credits: S. Kipson (a,b), M. Belošević (c) and Z. Jakl (d).

2.6.2.2. The shipwreck Teti

Similar to Michele, Teti was also a cargo steamboat, but sunken 50 years earlier, in 1930, next to the Islet Mali Barjak (NW part of the Vis Island). It was 72 m long and 8 m wide. The shipwreck streches from 10 till 34 m depth and it has NE – SW orientation (Frka & Mesić 2012). Deeper part is much better preserved than the shallower one. A more complete visualisation of the shipwreck is provided in Fig.2.6.7.

In the shallowest, light exposed part of the shipwreck a typical infralitoral biocenosis dominated by photophilic algae such as *Padina pavonica* and Dyctiotales is developed (Fig.2.6.8 a). Encrusting coralline algae, putatively belonging to *Lithothamnion* genus are also present around 15 m depth as well as the invasive green algae *Caulerpa cylindracea* Fig.2.6.8 c). Besides algae, sponge *Ircinia* sp. is present. More sciaphilic species are found both in shaded shipwreck parts in the shallow, such as encrusting sponge *Phorbas tenacior*, a cup scleractinian coral *Leptopsammia pruvoti* and green algae *Flabellia petiolata.* At greater depths, below 25 m, a sea star *Peltaster placenta*, sponge *Haliclona mediterranea* and branchy bryozoan *Smittina cervicornis*/*Adeonella pallasi* were recorded (Fig.2.6.8).

Likewise, a diversity and great abundance of fish species is using this artificial reef as a permanent or occassional habitat and by doing so, they enhance the diving experience at the site. Large schools of *Boops boops* and *Diplodus vulgaris* were observed in the shallows, along with *Spondyliosoma cantharus*, *Diplodus vulgaris, Labrus merula, Symphodus mediterraneus* (Fig.2.6.9). Besides native species, alien *Thalassoma pavo* also dwells there (Fig.2.6.9 f). In total, 21 fish species were observed within a single dive (see Appendix 5).

A list of all species associated to the shipwreck Teti that could be observed during this study is provided in Appendix 5.

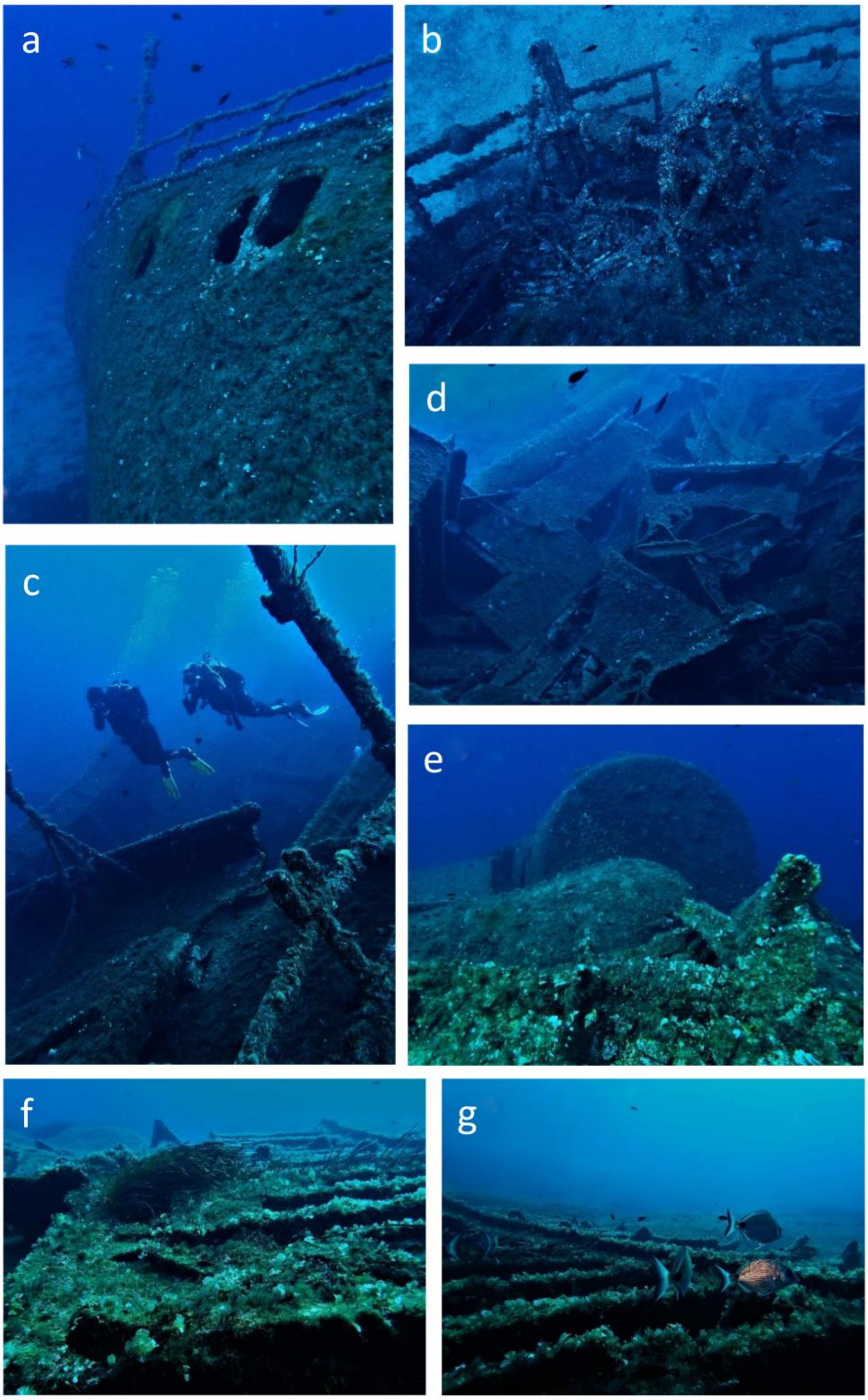


Fig.2.6.7. Shipwreck Teti – an artificial reef (10-34 m depth, NW Vis Island). From a) to g) details of the wreck are shown from its rear towards the frontal part and from its deepest to the shallowest part. Photo credit: S. Kipson.

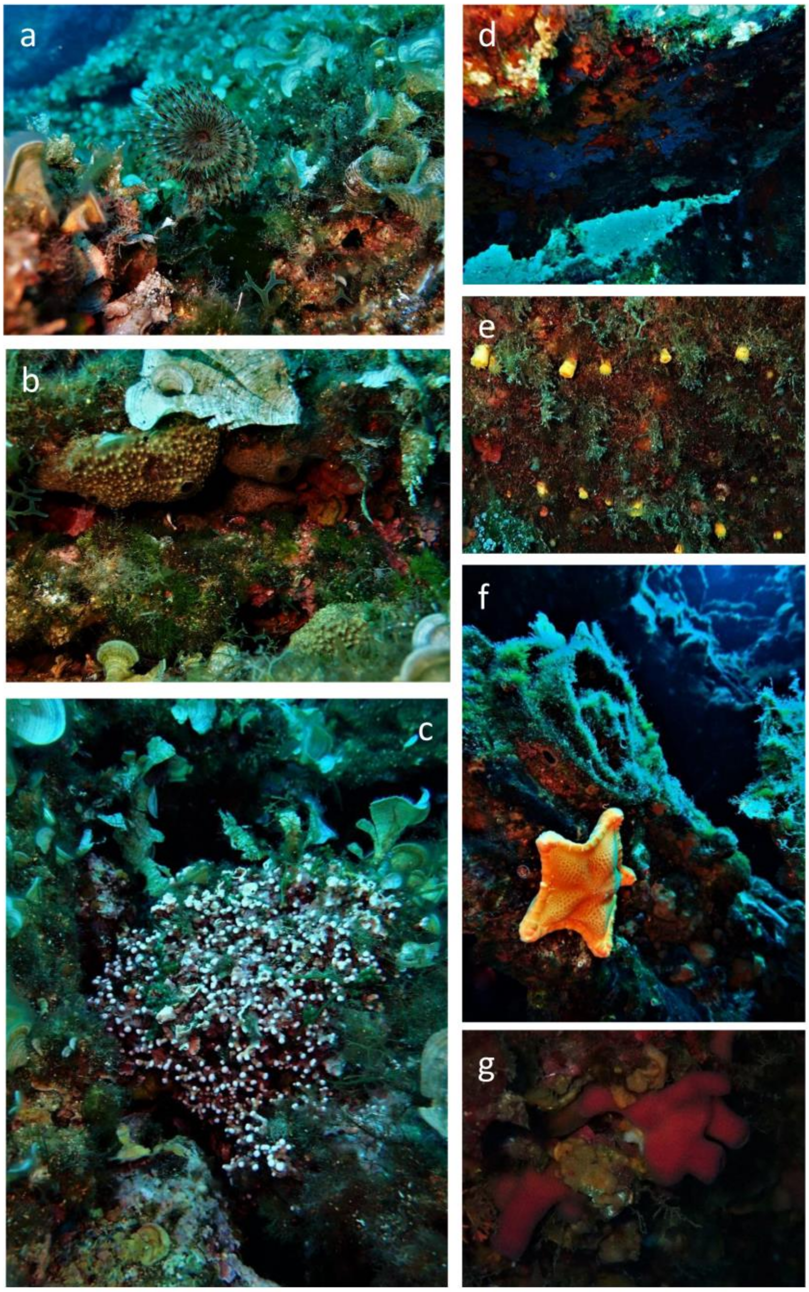


Fig. 2.6.8. Example of benthic organisms dwelling on the shipwreck Teti – an artificial reef (10-34 m depth, NW Vis Island). A typical infralitoral biocenosis dominated by photophilic algae is developed in the shallowest, light exposed part of the shipwreck: a) algae *Padina pavonica* and Dyctiotales, polychaete *Sabella spalanzani*, b) sponge *Ircinia* sp. c) coralline algae *Lithothamnion* cf. *crispatum* with the runners of invasive green algae *Caulerpa cylindracea*. More sciaphilic species are found both in shaded shipwreck parts in the shallow such as: d) encrusting sponge *Phorbas tenacior*, e) cup scleractinian coral *Leptopsammia pruvoti* and green algae *Flabellia petiolata* as well as at greater depths, such as sea star *Peltaster placenta* and g) sponge *Haliclona mediterranea*, branchy bryozoan *Smittina cervicornis*/*Adeonella pallasi.* Photo credit: S. Kipson.

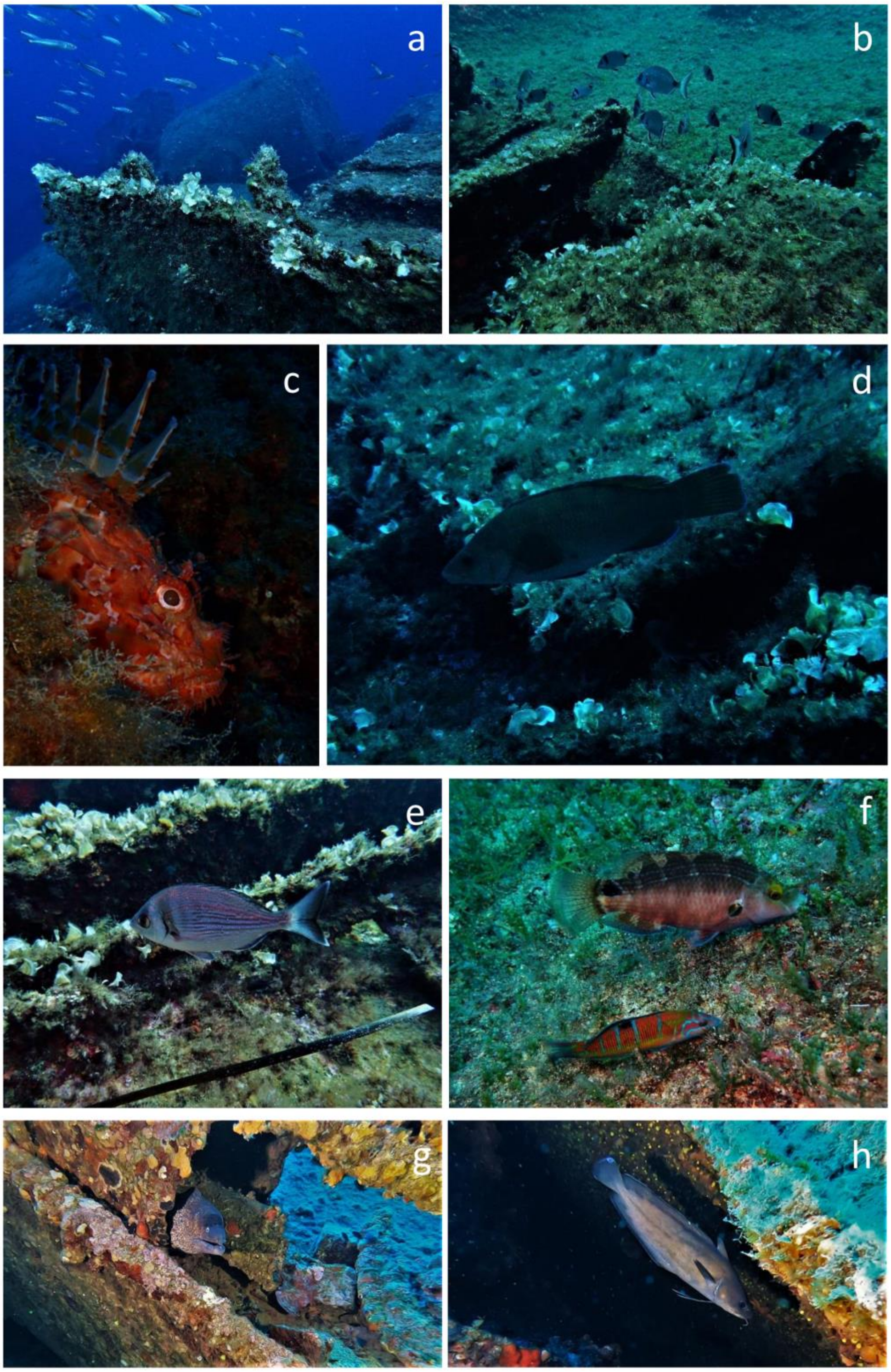


Fig. 2.6.9.Some of fish species using shipwreck Teti and its surroundings as their habitat: a) *Boops boops*, b) *Diplodus vulgaris*, c) *Scorpaena scrofa*, d) *Labrus merula*, e) *Spondyliosoma cantharus*, f) *Symphodus mediterraneus* and *Thalassoma pavo,* g) *Muraena helena* and h) *Phycis phycis*. Photo credit: S. Kipson (a-f), B. Plazonić (g,h).

2.6.3. Conclusions

Biodiversity associated to the shipwrecks in the Croatian part of the Adriatic Sea and their role as artificial reefs has been largerly overlooked. The fact that there are many shipwrecks scattered along our coast (Frka & Mesic 2012) makes this neglect even more unjustified. In that respect even a preliminary work done in the scope of this study is a valuable step forward.

Both visited shipwrecks provide permanent or temporary habitat for many marine species. Whereas the diversity and abundance of macrobenthos was outstanding on the shipwreck Michele, diversity and abundance of fish species was notable on the shipwreck Teti. They both advocate the value of artificial reefs in supporting biodiversity (when the placement of artificial reefs is justified by marine conservation agenda) and their attraction for diving tourism is undisputable.

However, I would like to emphasize another role/use that I feel it could be especially relevant for the shipwreck Michele. Its super shallow position and hence accessibility to both snorkelers as well as to all levels of divers-including the beginners, diversity of marine habitats and species both on the wreck itself and in its surroundings, where a representative *Posidonia oceanica* meadow is developed, as well as plethora of still preserved technical details makes this shipwreck not only a top notch attraction for diving tourism but also an exquisite site for education. In that respect, its potential is huge, both in terms of gaining knowledge on diverse marine species as well as on the marine ecology, e.g. the effect of abiotic factors such as light on development of different biocenoses (while eliminating all issues related to depth unsuitable for divers-beginners), but also on the effect of potential stressors (see section 2.4). Hence, it could serve as a field study site for marine biology courses aimed to students or interested citizens. Likewise, it can serve as an excellent field site for classes of underwater photography/videography. Clearly, since a shipwreck is located away from the coast, a prerequisite (and sometimes a limiting factor) for all these activities is the availability of the boat to access the site.

Overall conclusions and recommendations

- Coralligenous habitat is a priority habitat type "1170 Reefs" by the EU Habitat Directive (92/43/EEC). In addition, it has been included among the "special habitats types" that should be assessed under the Marine Strategy Framework Directive of the European Union as well as in the European Red List of marine habitats, where it is still classified as "data deficient". In order to enhance our knowledge on this habitat and its current ecological status over broad geographical scale, it is pivotal to apply a standardized methodology that would allow for direct comparisons between different areas or times of monitoring. The methodology used in this study, previously proposed within the national protocol for monitoring of coralligenous, proved to be robust enough to accommodate evaluation of diverse descriptors of its status and to enable both biocenotic and seascape approaches.
- All of protocols used in this study demonstrated their applicability in the field and provided valuable data. Hence, their application should be encouraged over broader geographical scales and over time. Further implementation will yield comparable data and it will improve knowledge and detection capacity of observers (e.g. to recognize different signs of species sickness/damage, to detect juvenile gorgonians in population studies, to properly assess the impact of certain LFG) and such an experience will undoubtedly improve training of future divers/observers.
- Likewise, wider application of these protocols by multiple users may lead to their improvements and hence creation of more effective tool for decision-making. For example, after application of LFG protocol and being aware of the situation in the field, Lagnići location in particular, the sensation remains that protocol should assess also cumulative impacts of abundant LFG (that were assessed individualy) as they may have additional environmental and seascape impacts and may considerably affect diving experience (if a site is valorized from the aspect of dive tourism).
- joining international collaborative networks, such as for example T-MEDNet for reporting 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 visibility of individual or organisation involved in the monitoring efforts

- During this study, one of the most adverse impacts was observed on gorgonians, especially on the Vis Island. Unfortunately, gorgonian populations are declining throughout the Mediterranean, leading to decreased complexity of ecosystems and loss of associated functions and services, including the service they provide to dive tourism. And without concerted action, this downward trajectory will continue. 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 gorgonian-dominated assemblages, every effort should be made to relieve such sensitive organisms from more manageable stressors and hence to putatively improve their resilience when faced with marine heat waves. Preventing mechanical abrasion from contact with abandoned/lost fishing gear by their targeted removal (i.e. removal of at least those LFG parts that are in direct contact with gorgonians), regulating fishing activities in zones within Natura 2000 sites where gorgonians are present and organizing clean ups of colonies from dense mucilaginous aggregates (i.e. during periods of their outbreak) by volunteer divers could present some of plausible actions
- Both Lagnići and Stupišće locations have great value for local tourism, offering very attractive recreational dive packages that can include both natural and cultural heritage. Beyond that, diversity of marine habitats and environmental conditions and accessibility of super shallow shipwreck make the Lagnići location particularly suitable as a field site for educational purposes, particularly for marine biology courses as well as courses in underwater photography/videography
- Engagement of citizen science, i.e. volunteer divers or snorkelers (e.g. for assessment in the depth range 1-3 m) is a highly valuable way to enhance underwater work effort, increase awareness and the sense of stewardship for marine environment. The prerequisite for such an engagement is a comprehensive training and expert supervision.

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Appendix 1.

Form used for visual census along the random transects, to assess the erect layer as a part of evaluation of structural complexity of the coralligenous assemblages, as well as to note the presence of macrobioeroders, mucilaginous algal aggregates and fishing gear, following the protocol proposed by Garrabou et al. (2015).

Observer: Species:		Date: Depth:						Site:				
		Sectors (1 m)										
Transect	**Parameter**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**TOTAL**
	Erect layer											
1	**Bioeroders**											
	Mucilaginous											
	Erect layer											
2	**Bioeroders**											
	Mucilaginous											
	Erect layer											
3	**Bioeroders**											
	Mucilaginous											
	Fishing nets:											
	Comments:											

Appendix 2.

Form used underwater for lost fishing gear (LFG) assessment, following the protocol developed by Ruitton et al. (2019).

Page 1:

Observer:	Date:	Site:				
Parameter	Assessment	1	2	3	4	5
Fishing gear type						
1. ENVIRONMENTAL IMPACT (EI)						
Colonization	Stage 0					
	Stage 1					
	Stage 2					
	Stage 3					
Trapped mobile fauna	0					
	1 to 5 ind					
	> 5 ind					
Removed fixed species	0					
	1 to 10 ind					
	> 10 ind					
Damaged fixed species	0					
	1 to 10 ind					
	> 10 ind					
Presence of outstanding species	Yes					
	No					
Obstructed cavities	0					
	1 to 10 cavities					
	> 10 cavities					
Abrasion of the substrate	No					
	Yes					
Habitat creation	Yes					
	No					

Page 2:

Parameter	Assessment	1	2	3	4	5
2. SEASCAPE IMPACT (SI)						
Distance of visibility	< 1 m					
	1 m - 5 m					
	> 5 m					
Extent of impact	< 5 m^2					
	5 m^2 - 20 m^2					
	> 20 m^2					
Seascape alteration	No					
	Yes					
Qualifying adjective	Neutral					
	Negative					
	Positive					
Relief	No alteration					
	Diminution					
	Enhancement					
3. TECHNICAL RISK (TR)						
Depth	≤ 20 m					
	20 to 50 m					
	> 50 m					
Attachment to the bottom	Relatively easy					
	Difficult					

Appendix 3.

Sensitivity levels (SL) of the main taxa/morphological groups in the coralligenous assemblages (see Piazzi et al. 2018 and references therein). Values range from 1 = the lowest sensitivity to 10 = the highest sensitivity.

Taxon/group	SL
Algal turf	1
Hydrozoans (e.g. *Eudendrium* spp.)	2
Pseudochlorodesmis furcellata	2
Perforating sponges (e.g. *Cliona* spp.)	2
Dyctiotales	3
Encrusting sponges	3
Encrusting bryozoans	3
Encrusting ascidians (also epibiontic)	3
Encrusting Corallinales, articulated Corallinales	4
Peyssonnelia spp.	4
Valonia spp., *Codium* spp.	4
Sponges prostrate (e.g. *Chondrosia reniformis, Petrosia ficiformis*)	5
Large serpulids (e.g. *Protula tubularia, Serpula vermicularis*)	5
Parazoanthus axinellae	5
Leptogorgia sarmentosa	5
Flabellia petiolata	6
Erect corticated terete Ochrophyta (e.g. *Sporochnus pedunculatus*)	6
Encrusting Ochrophyta (e.g. *Zanardinia typus*)	6
Azooxantellate individual scleractinians (e.g. *Leptopsammia pruvoti*)	6
Ramified bryozoans (e.g. *Caberea boryi, Cellaria fistulosa*)	6
Palmophyllum crassum	7
Arborescent and massive sponges (e.g. *Axinella polypoides*)	7
Salmacina–Filograna complex	7
Myriapora truncata	7
Erect corticated terete Rodophyta (e.g. *Osmundea pelagosae*)	8
Bushy sponges (e.g. *Axinella damicornis, Acanthella acuta*)	8
Eunicella verrucosa, Alcyonium acaule	8
Erect ascidians	8
Corallium rubrum, Paramuricea clavata, Alcyonium coralloides	9
Zooxantellate scleractinians (e.g. *Cladocora caespitosa*)	9
Pentapora fascialis	9
Flattened Rhodophyta with cortication (e.g. *Kallymenia* spp.)	10

Halimeda tuna	10
Fucales (e.g. *Cystoseira* spp., *Sargassum* spp.), *Phyllariopsis brevipes*	10
Eunicella singularis, Eunicella cavolini, Savalia savaglia	10
Aedonella calveti, Reteporella grimaldii, Smittina cervicornis	10

Appendix 4.

List of all species/taxonomic groups recorded within coralligenous assemblage (30 – 40 m depth) at each study site based on photographic sampling of 50 x 50 cm subquadrats (3 replicates of 2.5 m^2, in total 7.5 m^2 per site, indicated by +). In addition, +* indicates taxa recorded within 25 x 25 cm subquadrats (5 replicates of 0.5 m^2, in total 2.5 m^2 per site) whereas +** indicates taxa recorded from photos and videos, taken independently of photosampling. Note: sites are directly comparable based on + and +* because the same sampling effort is applied, but are not comparable based on taxa marked as +**.

Taxa	LAG1	LAG2	SIKA 3	SIKA 6
CHLOROPHYTA				
Acetabularia acetabulum (Linnaeus) P.C.Silva, 1952			+	+
Caulerpa cylindracea Sonder, 1845			+	+
Codium bursa (Olivi) C. Agardh, 1817			+	+**
Codium cf. *effusum*			+	+
Flabellia petiolata (Turra) Nizamuddin 1987	+	+	+	+
Green filamentous algae	+	+	+	+
Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux 1816			+	+
Palmophyllum crassum (Naccari) Rabenhorst 1868	+	+	+	+
Pseudochlorodesmis furcellata (Zanardini) Børgesen, 1925				+**
Valonia macrophysa Kützing 1843	+	+	+	+
OCHROPHYTA				
Brown erect algae			+	+
Dictyotaceae	+*	+*		+
Zanardinia typus (Nardo) P.C.Silva, 2000		+*	+	+
RHODOPHYTA				
Botryocladia sp.			+	+
Branchy Corallinales (*Amphiroa* sp.?)				+**
branchy red algae sp. 1				+
branchy red algae sp. 2				+
Lithophyllum stictaeforme (J.E. Areschoug) Hauck, 1877			+	
Mesophyllum macroblastum (Foslie) Adey, 1970	+	+	+	+
Peyssonnelia polymorpha (Zanardini) F. Schmitz, 1879	+	+	+	+
Peyssonnelia rubra (Greville) J. Agardh, 1851	+	+	+	+
Peyssonnelia sp.	+	+	+	+
Peyssonnelia squamaria (S. G. Gmelin) Decaisne, 1842	+	+	+	+
Red erect algae	+		+	+
Red filamentous algae			+	+
Red foliose algae			+	+
Rodriguezella strafforelloi F. Schmitz, 1895	+			

Taxa	LAG1	LAG2	SIKA 3	SIKA 6
Unidentified Corallinales	+	+	+	+
FORAMINIFERA				
Miniacina miniacea (Pallas, 1766)	+	+	+	+
PORIFERA				
Acanthella acuta Schmidt, 1862	+	+*		
Agelas oroides (Schmidt, 1864)	+	+	+	
Aplysina cavernicola (Vacelet, 1959)	+	+	+	
Axinella cannabina (Esper, 1794)	+**			
Axinella damicornis (Esper, 1794)			+**	+**
Axinella polypoides Schmidt, 1862	+**		+**	
Axinella sp.	+	+	+	+
Chondrosia reniformis Nardo, 1847	+	+		+**
Cliona schmidti (Ridley, 1881)	+	+	+	+
Cliona sp.	+	+	+	+
Cliona viridis (Schmidt, 1862)				+**
Crambe crambe (Schmidt, 1862) / *Spirastrella cunctatrix Schmidt, 1868*		+	+	+
Fasciospongia cavernosa (Schmidt, 1862)	+	+	+*	
Grey sponge	+	+		+
Haliclona (Reniera) mediterranea Griessinger, 1971	+	+**		+**
Haliclona (Halichoclona) fulva (Topsent, 1893)	+	+	+*	
Haliclona (Soestella) mucosa (Griessinger, 1971)	+	+	+*	
Haliclona sp.	+	+*		+**
Hemimycale columella (Bowerbank, 1874)	+*			
Ircinia dendroides (Schmidt, 1862)		+		
Ircinia oros (Schmidt, 1864)		+*		
Ircinia sp.	+	+		
Keratose sponge	+	+		
Orange encrusting sponge	+	+	+	+
Orange massive sponge	+		+	+
Petrosia ficiformis (Poiret, 1789)	+			
Phorbas tenacior (Topsent, 1925)	+	+		+**
Pink sponge (net like)				+**
Pleraplysilla spinifera (Schulze, 1879)				+**
Raspaciona aculeata (Johnston, 1842)		+	+	
Red encrusting sponge	+	+	+	+
Haliclona (Rhizoniera) sarai (Pulitzer-Finali, 1969)		+**	+**	+*
Salmon encrusting sponge	+			
Sycon sp.				+
Terpios fugax Duchassaing et Michelotti, 1864	+	+		+**

Taxa	LAG1	LAG2	SIKA 3	SIKA 6
Thin orange encrusting sponge		+*		
Unidentified white sponge	+	+		+*
White Dendroceratida	+	+	+	+
White sponge with yellowish crust		+		
Yellow encrusting sponge	+	+	+	+
Yellow sponge	+		+	+
ANTHOZOA				
Alcyonium coralloides (Pallas, 1766)			+	+
Balanophyllia (Balanophyllia) europaea (Risso, 1826)	+			
Caryophyllia inornata (Duncan, 1878)	+	+*	+*	+*
Caryophyllia smithii Stokes et Broderip, 1828	+	+		+
Epizoanthus sp.	+	+*		
Eunicella cavolini (Koch, 1887)	+	+	+	+
Hoplangia durothrix Gosse, 1860				+**
Leptopsammia pruvoti Lacaze-Duthiers, 1897	+	+	+	+
Madracis pharensis (Heller, 1868)			+	+**
Paramuricea clavata (Risso, 1826)			+	+
Parazoanthus axinellae (O. Schmidt, 1862)	+	+	+	+*
small colonial Scleractinia		+*	+*	+*
solitary Scleractinia	+	+	+	+
HYDROZOA				
Unidentified Hydrozoa	+	+	+	+
ANNELIDA				
Eupolymnia nebulosa (Montagu, 1819)	+			
Filograna implexa Berkeley, 1835 / *Salmacina dysteri* (Huxley, 1855)	+	+	+	+
Hermodice carunculata (Pallas, 1766)			+**	+**
Protula sp.	+	+	+	+
Sabella sp.			+	
Serpulidae	+	+	+	+
MOLLUSCA				
Rocellaria dubia (Pennant, 1777)	+	+	+	+
Vermetidae	+	+	+	+**
Pteria hirundo (Linnaeus, 1758)			+**	
ARTHROPODA (CRUSTACEA)				
Palinurus elephas (J.C. Fabricius, 1787)				+**
Dardanus sp.				+**
BRYOZOA				
Beania sp.	+*		+*	+*
Branchy bryozoan	+	+	+	+

Taxa	LAG1	LAG2	SIKA 3	SIKA 6
Caberea boryi (Audouin, 1826)	+*	+*		
Cellaria sp.				+
Dentiporella sp. / *Schizomavella* sp.	+	+	+	+
Encrusting beige bryozoan	+	+	+	+
Encrusting orange bryozoan	+	+	+	+
Myriapora truncata (Pallas, 1766)	+	+	+	+
Pentapora fascialis (Pallas, 1766)		+*	+	+
Schizobrachiella sanguinea (Norman, 1868)			+	+
Schizoporella sp.	+	+*	+	+
Reteporella sp.	+	+	+	+
Schizotheca serratimargo (Hincks, 1886)	+	+	+	+
Scrupocellaria sp.	+*	+*	+	+
Smittina cervicornis (Pallas, 1766) / *Adeonella pallasii* (Heller, 1867)	+	+	+	+
ECHINODERMATA				
Echinaster (Echinaster) sepositus (Retzius, 1783)				+**
Hacelia attenuata Gray, 1840			+**	+**
Holothuria sp.		+**		
Ophidiaster ophidianus (Lamarck, 1816)				+**
Peltaster placenta (Müller & Troschel, 1842)			+**	+**
CHORDATA (TUNICATA)				
Aplidium tabarquensis Ramos-Espla, 1991			+	+
Aplidium elegans (Giard, 1872)	+	+*		
Aplidium sp.		+*		
Didemnum sp. (dark orange)			+*	+*
Didemnum sp. (orange)		+	+	+*
Didemnum sp. (white)	+	+*	+*	+*
Diplosoma sp.	+	+	+	+
Halocynthia papillosa (Linnaeus, 1767)	+	+	+	+
Orange colonial Synascidia	+*	+	+	+
Orange-grey marmorated tunicate (or sponge?)				+**
Polycitor adriaticus (Drasche, 1883)	+	+		
Pycnoclavella sp. / *Perophora* sp./*Clavelina nana*			+	+
White Synascidia	+	+	+*	+*
CHORDATA (PISCES)				
Anthias anthias (Linnaeus, 1758)			+**	+**
Apogon imberbis (Linnaeus, 1758)				+**
Chromis chromis (Linnaeus, 1758)			+**	+**
Coris julis (Linnaeus, 1758)	+**	+**	+**	+**
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)	+**			

Taxa	LAG1	LAG2	SIKA 3	SIKA 6
Muraena helena Linnaeus, 1758			+**	+**
Odondebuenia balearica (Pellegrin & Fage, 1907)			+	+**
Parablennius rouxi (Cocco, 1933)	+		+	+**
Phycis phycis (Linnaeus, 1766**)**			+**	+**
Scorpaena porcus Linnaeus, 1758				+**
Scorpaena scrofa Linnaeus, 1758	+**	+**	+**	+**
Serranus cabrilla (Linnaeus, 1758)			+**	+**
Spicara maena (Linnaeus, 1758)			+**	+**
Thorogobius ephippiatus (Lowe, 1839)			+	+**

Appendix 5.

List of species/taxonomic groups recorded on shipwrecks Michele (NW Dugi Otok Island, 4-6 m depth) and Teti (NW Vis Island, 10 – 34 m depth) based on underwater photos and videos.

Taxa	Michele	Teti
CHLOROPHYTA		
branchy green algae		+
Caulerpa cylindracea Sonder, 1845		+
Codium bursa (Olivi) C.Agardh, 1817	+	+
Flabellia petiolata (Turra) Nizamuddin 1987	+	+
Green filamentous algae	+	+
Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux 1816	+	+
Palmophyllum crassum (Naccari) Rabenhorst 1868	+	+
Pseudochlorodesmis furcellata (Zanardini) Børgesen, 1925	+	+
Valonia macrophysa Kützing 1843	+	+
OCHROPHYTA		
Dictyotaceae	+	+
Padina pavonica (Linnaeus) Thivy, 1960	+	+
Taonia sp.		+
Zanardinia typus (Nardo) P.C.Silva, 2000		+
RHODOPHYTA		
branchy red algae		+
Lithothamnion crispatum Hauck, 1878		+
Mesophyllum sp.		
Peyssonnelia polymorpha (Zanardini) F.Schmitz, 1879	+	+
Peyssonnelia rubra (Greville) J. Agardh, 1851	+	+
Peyssonnelia sp.	+	
Rodriguezella strafforelloi F.Schmitz, 1895	+	
Unidentified Corallinales	+	+
TRACHEOPHYTA		
Posidonia oceanica (Linnaeus) Delile, 1813	+	+
FORAMINIFERA		
Miniacina miniacea (Pallas, 1766)	+	
PORIFERA		
Agelas oroides (Schmidt, 1864)	+	
Aplysina aerophoba (Nardo, 1833)	+	
black keratose sponge	+	+
Chlatrina sp.	+	

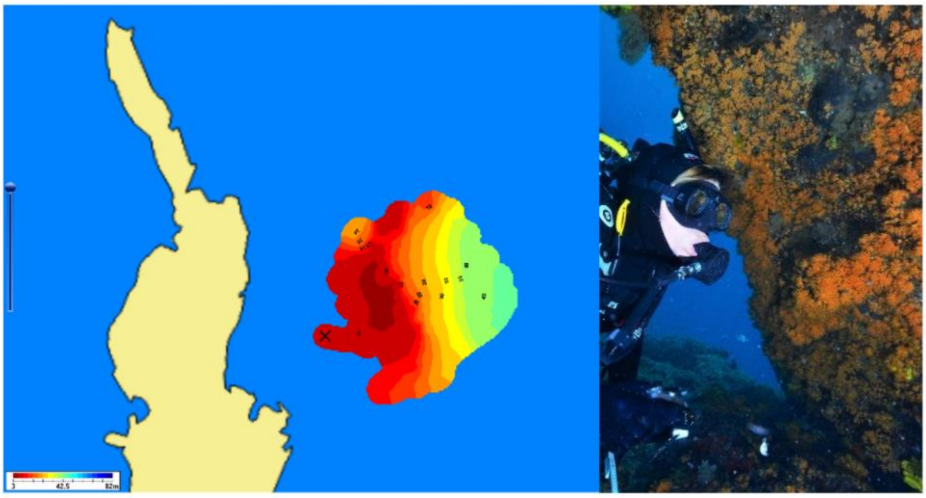
Taxa	Michele	Teti
Dysidea sp.	+	
Chondrosia reniformis Nardo, 1847	+	+
Cliona schmidti (Ridley, 1881)	+	+
Cliona sp.	+	
Cliona viridis (Schmidt, 1862)	+	
Crambe crambe (Schmidt, 1862)	+	+
Haliclona (Reniera) mediterranea Griessinger, 1971		+
Ircinia dendroides (Schmidt, 1862)	+	
Ircinia oros (Schmidt, 1864)	+	
Ircinia sp.	+	+
Orange encrusting sponge	+	+
Petrosia ficiformis (Poiret, 1789)	+	
Phorbas tenacior (Topsent, 1925)	+	+
Scalarispongia scalaris (Schmidt, 1862)	+	
Spirastrella cunctatrix Schmidt, 1868	+	
Spongia (Spongia) officinalis Linnaeus, 1759	+	
Terpios fugax Duchassaing et Michelotti, 1864	+	
Yellow sponge		+
ANTHOZOA		
Balanophyllia (Balanophyllia) europaea (Risso, 1826)	+	
Caryophyllia inornata (Duncan, 1878)	+	
Caryophyllia smithii Stokes et Broderip, 1828	+	
Leptopsammia pruvoti Lacaze-Duthiers, 1897		+
solitary Scleractinia	+	+
HYDROZOA		
Unidentified Hydrozoa	+	+
ANNELIDA		
Eupolymnia nebulosa (Montagu, 1819)		+
Filograna implexa Berkeley, 1835 / *Salmacina dysteri* (Huxley, 1855)	+	+
Hermodice carunculata (Pallas, 1766)	+	+
Protula sp.		+
Sabella sp.	+	+
Serpulidae	+	+
MOLLUSCA		
Cerithium sp.	+	+
Hexaplex trunculus (Linnaeus, 1758)		
Peltodoris atromaculata Bergh, 1880	+	
Rocellaria dubia (Pennant, 1777)	+	+
Spondylus gaederopus Linnaeus, 1758	+	

Taxa	Michele	Teti
Vermetidae	+	+
ARTHROPODA (CRUSTACEA)		
Chthamalus *sp.*	+	
BRYOZOA		
black encrusting bryozoan	+	+
Branchy bryozoan		+
Dentiporella sp. / *Schizomavella* sp.		+
Encrusting beige bryozoan	+	+
Encrusting orange bryozoan	+	+
Myriapora truncata (Pallas, 1766)		+
Schizobrachiella sanguinea (Norman, 1868)		+
Reteporella sp.		+
Scrupocellaria sp.		+
Smittina cervicornis (Pallas, 1766) / *Adeonella pallasii* (Heller, 1867)		+
ECHINODERMATA		
Arbacia lixula (Linnaeus, 1758)	+	
Echinaster (Echinaster) sepositus (Retzius, 1783)	+	
Hacelia attenuata Gray, 1840		+
Holothuria sp.	+	
Paracentrotus lividus (Lamarck, 1816)	+	
Peltaster placenta (Müller & Troschel, 1842)		+
Ophidiaster ophidianus (Lamarck, 1816)		+
Sphaerechinus granularis (Lamarck, 1816)		+
CHORDATA (TUNICATA)		
Aplidium tabarquensis Ramos-Espla, 1991	+	+
Aplidium sp.		+
Halocynthia papillosa (Linnaeus, 1767)	+	+
Pyura sp.		+
CHORDATA (PISCES)		
Apogon imberbis (Linnaeus, 1758)		+
Chromis chromis (Linnaeus, 1758)	+	+
Coris julis (Linnaeus, 1758)	+	+
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)	+	+
Muraena helena Linnaeus, 1758		+
Parablennius rouxi (Cocco, 1933)		+
Phycis phycis (Linnaeus, 1766*)*		+
Scorpaena scrofa Linnaeus, 1758	+	+
Serranus cabrilla (Linnaeus, 1758)		+
Spicara maena (Linnaeus, 1758)		+

Taxa	Michele	Teti
Serranus scriba (Linnaeus, 1758)	+	+
Diplodus annularis (Linnaeus, 1758)	+	+
Diplodus sargus sargus (Linnaeus, 1758)		+
Boops boops (Linnaeus, 1758)		+
Sparisoma cretense (Linnaeus, 1758)		+
Thalassoma pavo (Linnaeus, 1758)		+
Labrus merula Linnaeus, 1758		+
Symphodus tinca (Linnaeus, 1758)		+
Spondyliosoma cantharus (Linnaeus, 1758)		+
Centrolabrus melanocercus (Risso, 1810)		+
Symphodus mediterraneus (Linnaeus, 1758)		+

ADRIREEF Project:

the assessment of reefs on the location Konjsko (the Krk Island)



June 2021

Dr. Silvija Kipson

Project: **ADRIREEF**
Innovative exploitation of Adriatic Reefs in order to strengthen blue economy

Title: ADRIREEF Project: the assessment of reefs on the location Konjsko (the Krk Island)

Author: Dr. Silvija Kipson, marine biologist – external expert

Contracting Entity: Udruga za prirodu, okoliš i održivi razvoj Sunce
Obala HNP 7/III, 21 000 Split

Contract: N°01/19

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This report has been produced in the framework of the project **ADRIREEF** *"*Innovative exploitation of Adriatic Reefs in order to strengthen blue economy*", under the contract N. 2/2021 assigned to SeaFan – marine research and consultancy.*

Reef monitoring expertize, field work coordination, data collection and analysis, underwater photography and videography, report preparation: Dr. Silvija Kipson, marine biologist-external expert

Data collection: Fedra Dokoza, Anamarija Paradinović

Dive logistics: Dive City diving center, Crikvenica

Underwater photography/videography: Mirko Belošević, Fedra Dokoza

Authors of cover images: Lovre Maglić (left) and Mirko Belošević (right)

Contents

Introduction

This document reports on results of field activities aimed at assessment and establishment of reef monitoring on the project location plić Konjsko (Krk Island, Primorsko-goranska County). Maximum depth at the project location is around 20 m, hence no circalittoral reefs including coralligenous were found there. Therefore, field activities focused on the infralittoral zone. The general approach to this task included application of protocols and collection of data that are comparable (to the maximum extent possible) to data already collected at two other project locations within the ADRIREEF project, i.e. location Lagnići next to the Dugi Otok Island and location Stupišće next to the Vis Island (for further information see Kipson 2021). Bearing in mind the above stated, field activities on the location plić Konjsko included:

- Assessment of conservation status and climate-related responses of sessile benthic macroinvertebrates, including gorgonians
- Fish visual census of climate change indicators
- Assessment of the impact of the lost fishing gear (LFG)

Moreover, similarly to the previous study within the ADRIREEF project (see Kipson 2021) we have used the exquisite opportunity to visit the nearby shipwreck Peltastis and to make a preliminary assessment of biodiversity associated to this unintentional artificial reef.

Brief description of visited sites, methods applied and results stemming from each of these activities are outlined below. Conclusions related to the specific activity are provided at the end of each section, whereas the overall conclusion and recommendations are provided at the end of this report.

1. STUDY SITES

We carried out field activities on the location Konjsko shallow, next to the eastern side of the Krk Island. This location was selected *a priori* and randomly by project partners (Fig. 1.1.1, Fig.1.1.2). The rationale behind its selection was the position in the Northern Adriatic Sea and complete absence of any previous information, both on its biological characteristics and the potential for blue economy. In addition, nearby Družinin site was added as a locally dived site, already contributing to the local economy and harbouring the population of the yellow gorgonian *Eunicella cavolini* in the same depth range as found at the Konjsko site, hence enabling the assessment of its health status (as an important indicator) on more than one site (see Section 2.2). Coordinates of all studied sites are indicated in Table 1.1.1 and the visualisation of sites is provided in Figs. 1.1.3 to 1.1.7. All dives were performed from the boat Irma (Fig.1.1.8).

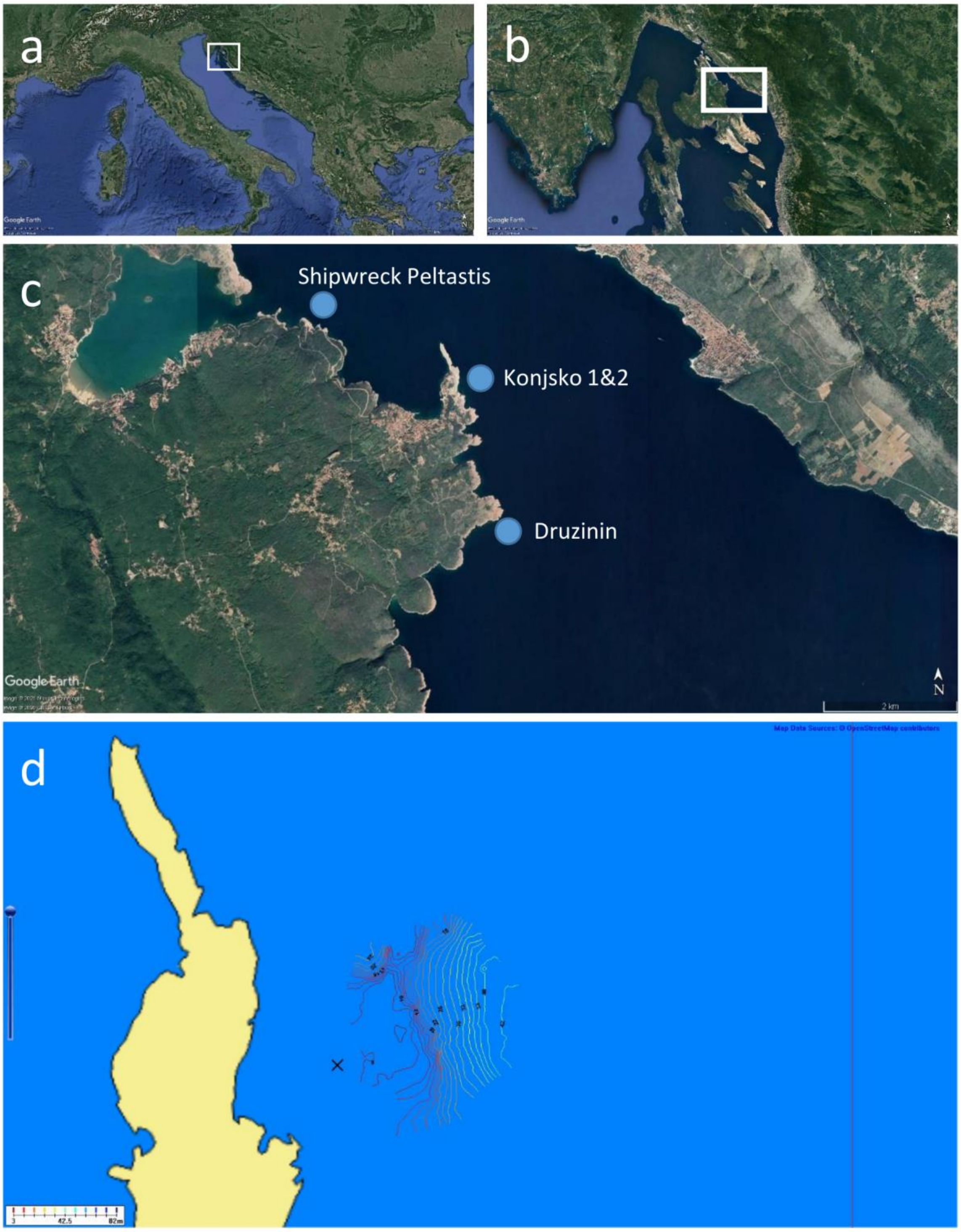

Fig. 1.1.1 Map of the studied location in relation to the whole Adriatic (a) and the Krk Island (b) in the Northern Adriatic; c) position of studied sites along the Eastern coast of the Krk Island and d) Konjsko shallow in relation to the Cape Šilo. Author of image d: Lovre Maglić.

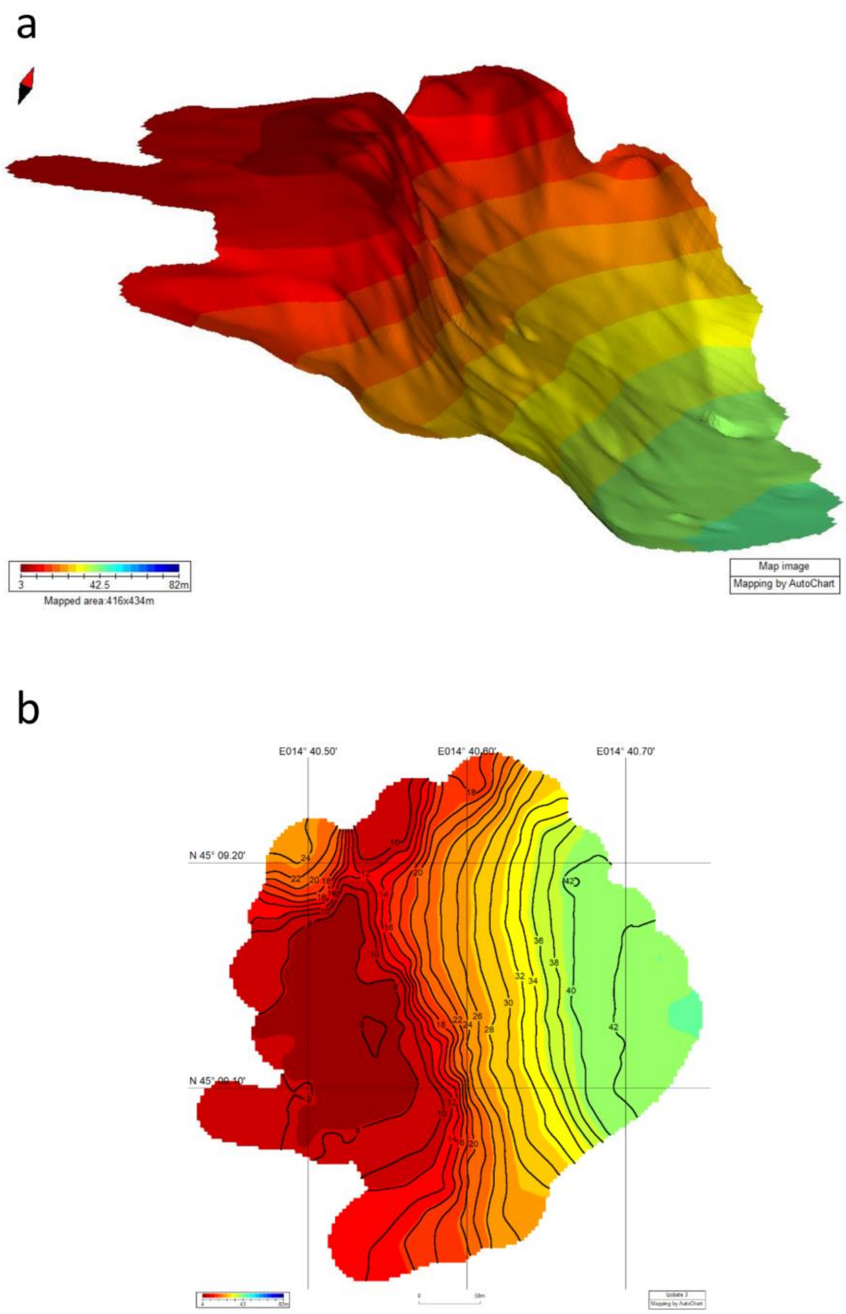

Fig. 1.1.2. Bathymetric model (a) and a map (b) of location Konjsko on the Eastern side of the Krk Island. Author: Lovre Maglić.

Fig. 1.1.3. The view of the Cape Šilo on the Krk Island, from our diving position on location Konjsko.

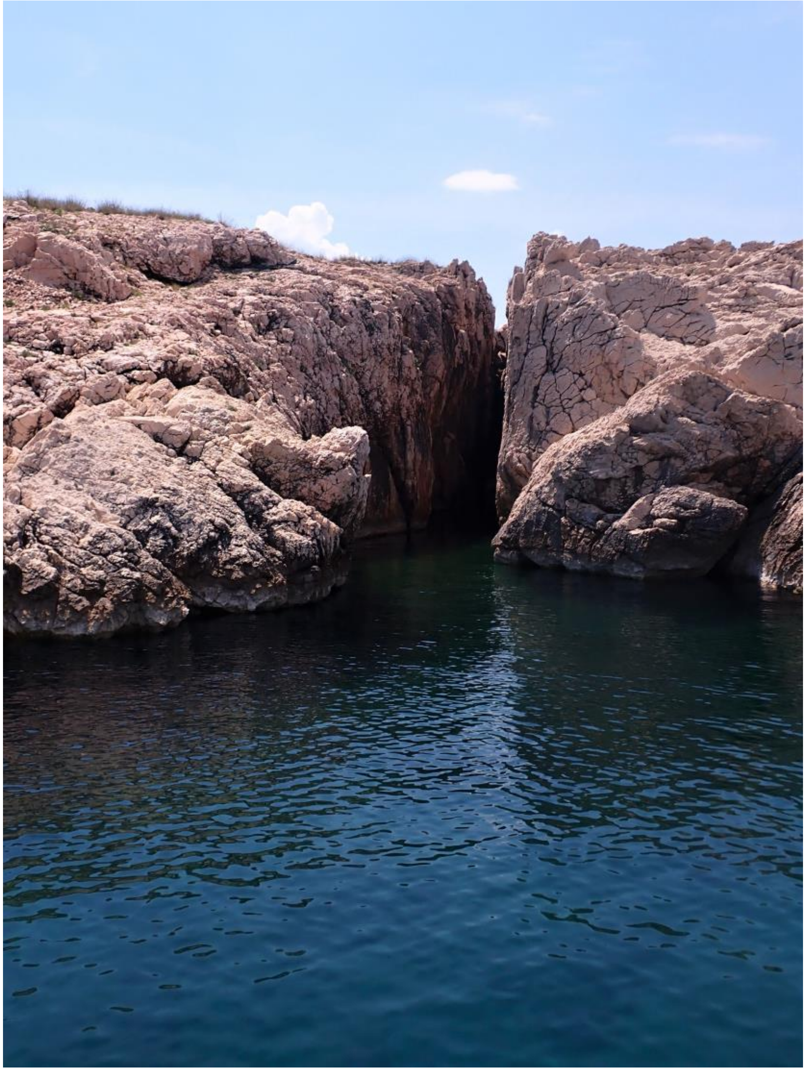

Fig. 1.1.4. A remarkable feature on the site Druzinin (the Krk Island) is a small canyon that stretches underwater to approximately 3 m depth.

Table 1.1.1. Geographic coordinates (longitude and latitude) of study sites on location Konjsko and Druzinin (the Eastern side of the Krk Island).

ident	Latitude	Longitude
KONJ01	45,15239800	14,67569200
KONJ 02	45,15338600	14,67596300
DRUZININ	45,13637700	14,67888800

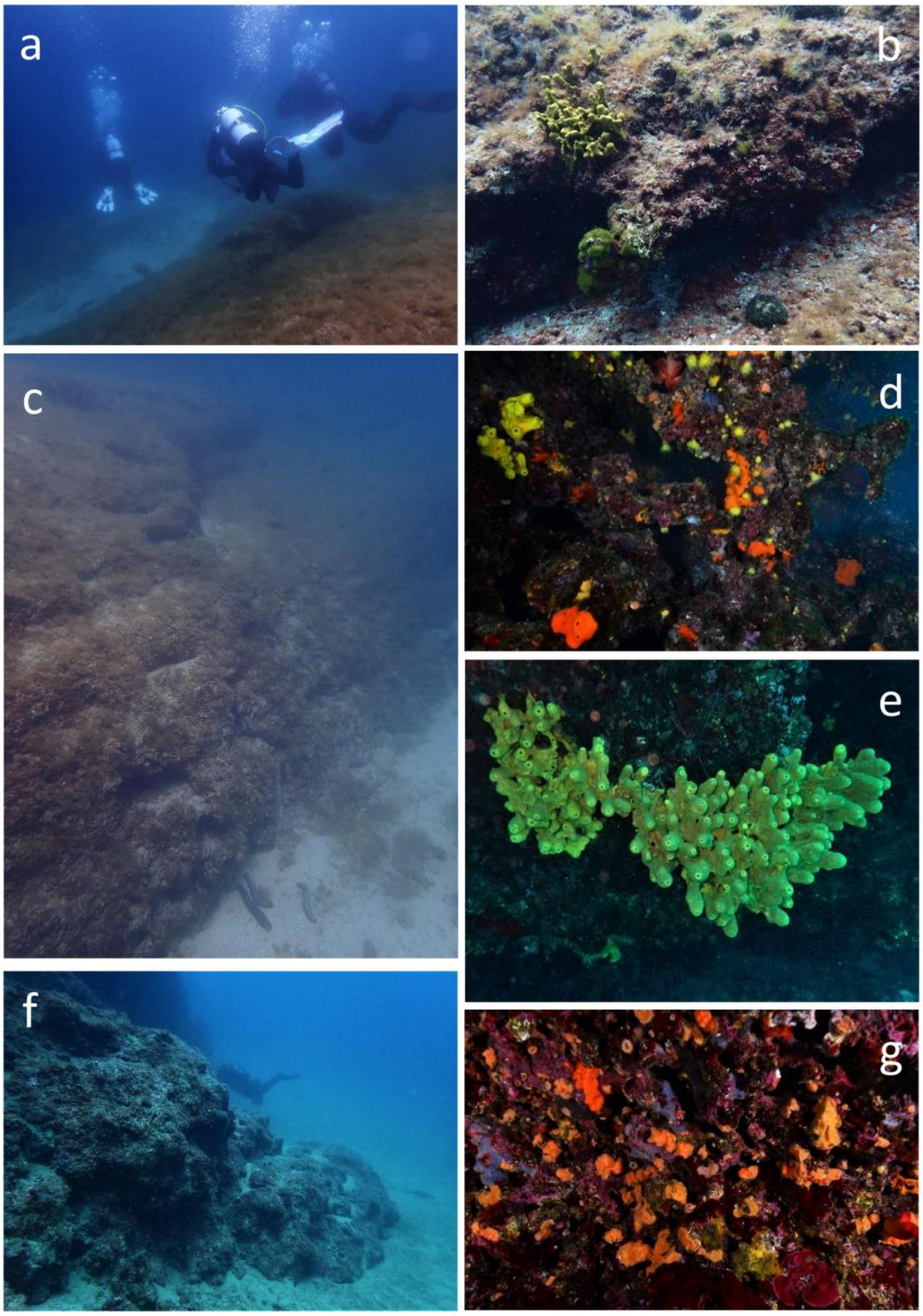

Fig.1.1.5. Konjsko 1 site: a) a rocky plateau at 5 m depth with biocenosis of infralittoral algae and abundant sponge *Aplysina aerophoba* turns deeper into small, well-lit cascading walls (b and c) with occasional crevices hosting more sciaphilic organisms such as solitary scleractinian *Leptopsammia pruvoti*, sponges *Haliclona fulva* and *Phorbas tenacior* (d) and abundant sponge *Aplysina* spp. that thrives both on the walls and inside crevices and overhangs from 12 m depth (d, e); f) rocky wall ends at 18.5 m depth and it is replaced by a soft bottom, g) a small semi-dark cave was also observed during a dive. Photo credits: S. Kipson except d) and g) M. Belošević.

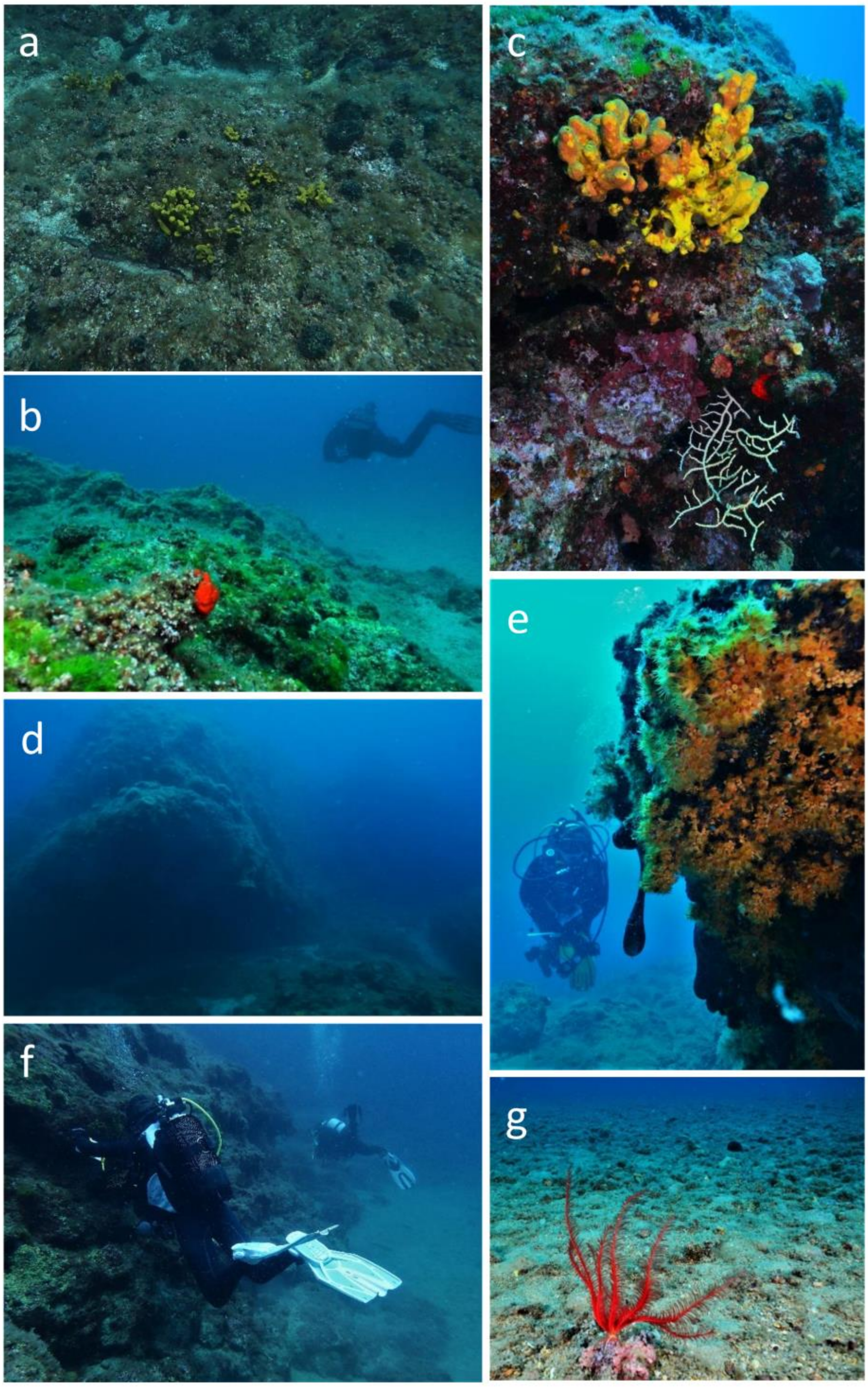

Fig. 1.1.6. Konjsko 2 site: a) similarly to Konjsko 1 site a rocky plateau at 5 m depth with biocenosis of infralittoral algae and abundant sponge *Aplysina aerophoba* turns deeper into small, well-lit cascading walls (b) hosting sparse population of the yellow gorgonian *Eunicella cavolini* from 11 m depth till the end of the reef (c); d) attractive boulders are present in one part of the reef around 12 m depth and are dominated by the yellow cluster anemone *Parazoanthus axinellae* (e); f) rocky wall ends at 19.5 m depth and it is replaced by a detritic bottom (g). Photo credits: M. Belošević except S. Kipson (a, f).

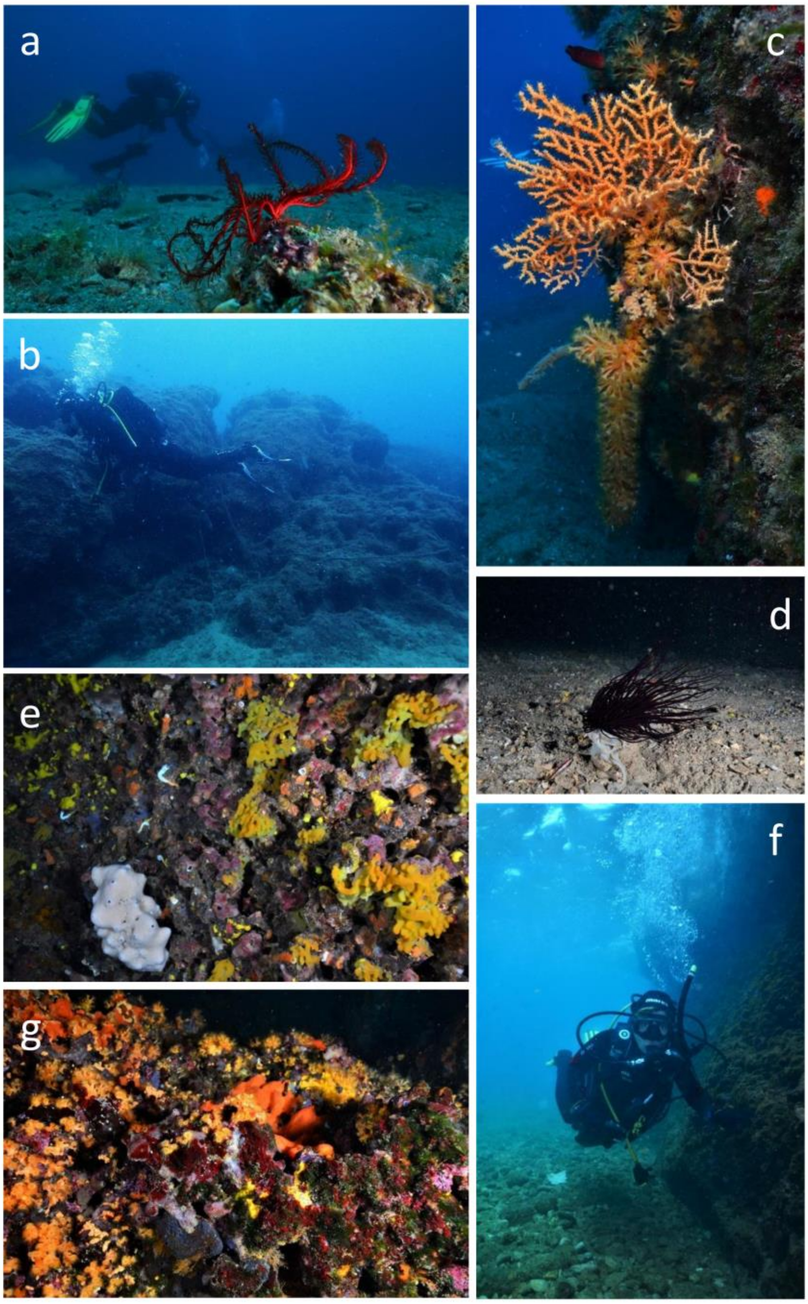

Fig. 1.1.7. Družinin site: a) dive starts over a soft bottom to reach an infralittoral reef stretching from 11 to cca 20 m depth (b) hosting sparse colonies of the yellow gorgonian *Eunicella cavolini*, ascidian *Halocynthia papillosa*, carpets of *Parazoanthus axinellae*, occasional colonies of *Alcyonium acaule*; d) after circling around the reef our dive continued over soft bottom with typical species such as *Cerianthus membranaceus* to reach the coastal infralittoral wall with few small semi-dark caves and overhangs with many typical species such as hard corals and encrusting and massive sponges including sponge *Oscarella* sp. (e); f) infralittoral reef turns at one point into a narrow canyon at 3 m depth with many crevices hosting sciaphilic organisms (g). Photo credits: M. Belošević except (b) S. Kipson.

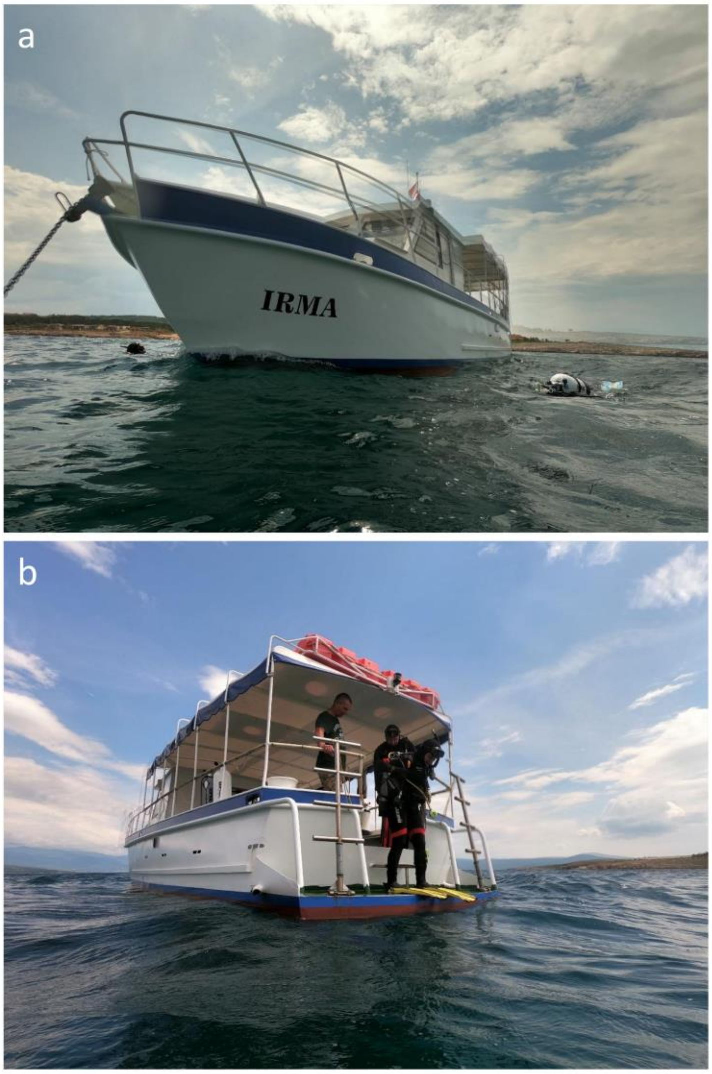

Fig. 1.1.8. Diving logistics for the location Konjsko: a) on board of the boat Irma and b) preparation for the dive. Photo credits: Fedra Dokoza.

2. MONITORING ACTIVITIES

2.1. Assessment of conservation status and climate-related responses of sessile benthic macroinvertebrates

2.1.1.Overview of the methods

As a part of the effort to monitor climate-related responses of a rocky bottom community, we applied the protocol advocated by Garrabou, Bensoussan & Azzurro (2018) in the scope of the previous Interreg project MPA ADAPT, focused on sessile benthic macroinvertebrates. This protocol aims to reveal the conservation status of surveyed populations, while gathering baseline information to assess the impacts of mass mortality events when they occur.

Target species of this protocol are the ones sensitive to climate-related stressors, are easy to identify underwater and are sufficiently abundant in the surveyed area. After the inspection of the actual abundance of sessile macroinvertebrates at the project location, sponges *Petrosia ficiformis* and *Aplysina* spp. were selected as target species as well as scleractinian solitary corals *Balanophylia europaea* and *Leptopsammia pruvoti* and the yellow cluster anemone *Parazoanthus axinellae* (now considered as a species complex, Ocana et al. 2019). Observations were made along the imaginary transect at the selected depth (± 1 m; Fig. 2.1.1). Observer counted each specimen of selected species and noted if it is affected, i.e. if any tissue necrosis is present or polyps of hard coral are bleached/dead. Besides visual census *in situ*, data were collected also by photographs.

Although *Aplysina cavernicola*, the sponge included in Annex II of the SPA / BD Protocol and listed in Annex II of the Bern Convention as a strictly protected species was most likely present at the location Konjsko (especially within deepeest crevices, holes and overhangs), due to its morphological similarity to *A. aerophoba* and the fact that sponges were present both on the cascading walls and within holes and crevices between 12 and 18 m depth (in fact, both species may be found down to 40 m depth according to Klöppel et al. 2009), we have decided to record it at the genus level, as *Aplysina* spp.

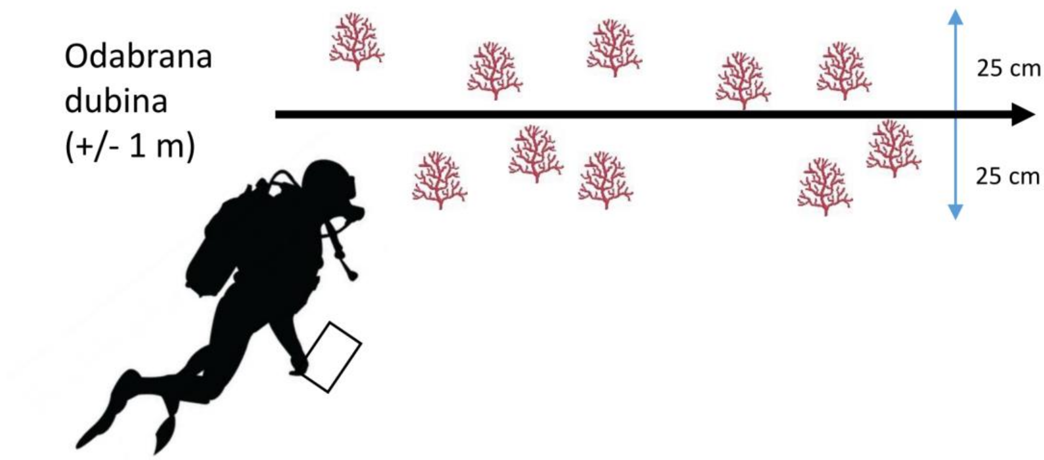

Fig. 2.1.1. Illustration of the standardized protocol aimed at assessment of conservation status and climate-related responses of sessile benthic macroinvertebrates following Garrabou, Bensoussan & Azzurro (2018). Odabrana dubina = selected depth.

Fig. 2.1.2. Divers recording data on marine sessile macroinvertebrates on location Konjsko (Krk Island). Photo credit: M. Belošević.

2.1.2.Main results

Due to the overall, relatively low numbers of targeted sessile macroinvertebrates and limited stretch of the reef at depths below 12 m, a bathymetric range in which most of them were assessed streched from 11 to 18 m at the Konjsko 1 site and 11-19 m depth at the Konjsko 2 and Družinin sites. Hence, such bathymetric range was wider than it would be envisaged by the standard MME protocol. The exception was the assessment of solitary hard coral *Balanophyllia europaea* which was examined around 5 m depth at the Konjsko 1 site.

At studied sites, sponge *Petrosia ficiformis* was present both on the well-lit cascading walls as well as within more sciaphilic crevices and overhangs (Fig.2.1.3). In starck contrast to other examined species, it showed the highest proportion of affected specimens (Table 2.1.1). Between 42 and 61% of individuals were damaged/sick, showing signs illustrated in Fig. 2.1.4. Instead of a smooth, intact pinacoderm (i.e. the outermost layer of body cells, pinacocytes) , purple-brownish in colour due to its symbiosis with cyanobacteria and free of any epibionts (Fig. 2.1.3a,b), damaged sponges were partially covered by a "crust", sometimes forming a greenish carpet (possibly formed by some algae, e.g. Fig. 2.1.4.a,b) or the pinacoderm was missing and spongin fibers were exposed (e.g. Fig. 2.1.4.c,d) All these injuries were clearly differentiated from the signs of natural predation, primarily by the nudibranch *Peltodoris atromaculata* - leaving more or less circular pale marks, as illustrated in Fig.2.1.3.c.

On the location Konjsko in the bathymetric range of 12-18 m sponge *Aplysina* spp. was present within more sciaphilic crevices and overhangs, as well as on well-lit vertical walls (Fig. 2.1.5). It was even observed on a mobile substrate, such as on the carapace of the crustacean *Maja verrucosa* (Fig. 2.1.5d). Following Pfannkuchen (2018), as signs of injury in this sponge we considered blue tissue discoloration and visible spongin skeleton exposure. Brownish spots on the tissue of othervwise healthy looking sponge (e.g. in Fig.2.1.5a) were not considered as a damage. Taking this in consideration, its conservation status was generally good, and the proportion of specimens where some damage could be identified did not surpass 20% at any site, being low 7% at the Konjsko 2 site (Table 2.1.1). We have assessed the size of this sponge both as the number of „chimneys" (i.e. tubularly shaped part of the sponge body with an osculum on the top, as in Pfannkuchen 2018) and as a maximum diameter. The largest recorded specimen had 155 chimneys, whereas the smallest one had only 1 chimney. The maximum diameter was cm, and the minimal was The size-frequency of this sponge taxa is shown both for the depth range of 12-18 m, as well as for the shallower depth range of 5 to 10 m (Fig. 2.1.6). In the latter case the sponge was assessed mainly on the well-lit walls and a plateau of the Konjsko 1 site, where most probably all specimens belonged to *Aplysina aerophoba*.

The last examined sponge in this study was *Chondrosia reniformis* (Fig. 2.1.7c) and it showed excellent conservation status, with no injured or sick specimens observed at 3 m depth, nor in a depth range from 11 to 19 m (Table 2.1.1).

Besides already mentioned sponges, other sponge species/categories that were selected or proposed for selection on the other ADRIREEF locations and/or within other initiatives were also present on the Konjsko location but in very low numbers, such as black keratose sponges, *Ircinia* spp. and *Dysidea avara* (Fig. 2.1.7). Additionally, within the study location Konjsko several specimens of sponge *Tethya* sp. were observed (Fig. 2.1.8). To my experience, this sponge seems to be more common in the Northern Adriatic, in comparison to other parts od the Croatian coast.

Out of target coral species, *Leptopsammia pruvoti* (Fig. 2.1.9) and *Parazoanthus axinellae* (Fig. 2.1.10), when found, were present in sufficient abundance over a relatively small area, which made their assessment fairly straightforward. Together with *Balanophyllia europaea* (Fig. 2.1.11), which was assessed on the rocky plateau of the Konjsko 1 site at 5 m depth, all of these coral species showed excellent conservation status at the time of assessment and affected specimens evident as bleached or dead corallites/polyps were not identified (Table 2.1.1). Although only few colonies of *Cladocora caespitosa* (Fig. 2.1.12), were recorded at the Konjsko 1 site, it is worth to mention it, as it is an endangered species (according to the IUCN Red list; Casado de Amezua et al. 2015) endemic to the Mediterranean and the only reef-building coral here - hence it has an important structuring role. All observed colonies, although harbouring portions with healthy corallites, were partially dead and these parts were overgrown by algae such as *Laurencia* sp., green and red turf algae and red algae *Peyssonnelia* sp. (Fig. 2.1.12).

Besides sponges and corals, *Halocynthia papillosa* (Fig. 2.1.13) was the only acidian species assessed. This species, although common, is usually present in very low density. In comparison to other ADRIREEF locations as well as many other parts of the Adriatic, the abundance of this ascidian in the Kvarner region is relatively high, and occassionaly several individuals may be found clumped together (e.g. Fig. 2.1.13c). It also showed great conservation status (Table 2.1.1), however this result should be considered with caution, as overall small number of individuals was examined. During this study I have personnaly observed dead specimens of this ascidian for the first time (see Fig. 2.1.13d). However, only 2 such cases were recorded.

Out of other observations related to the benthic macroinvertebrates on this study location, the most commonly observed macrobioeroder was the sea urchin *Sphaerechinus granularis*, present as quite large specimens (Fig. 2.1.14). Moreover, the presence of nudibranchs such as *Flabellina affinis* and *Paraflabellina ischitana* (Fig. 2.1.15) always offer an additional diving attraction to the site. It is also worth mentioning that at the time of our assessment (beginning of June 2021) we have observed the initial accumulation of mucilaginous algal aggregates over some branchy benthic organisms, such as hydrozoans in this case (Fig. Fig. 2.1.15b).

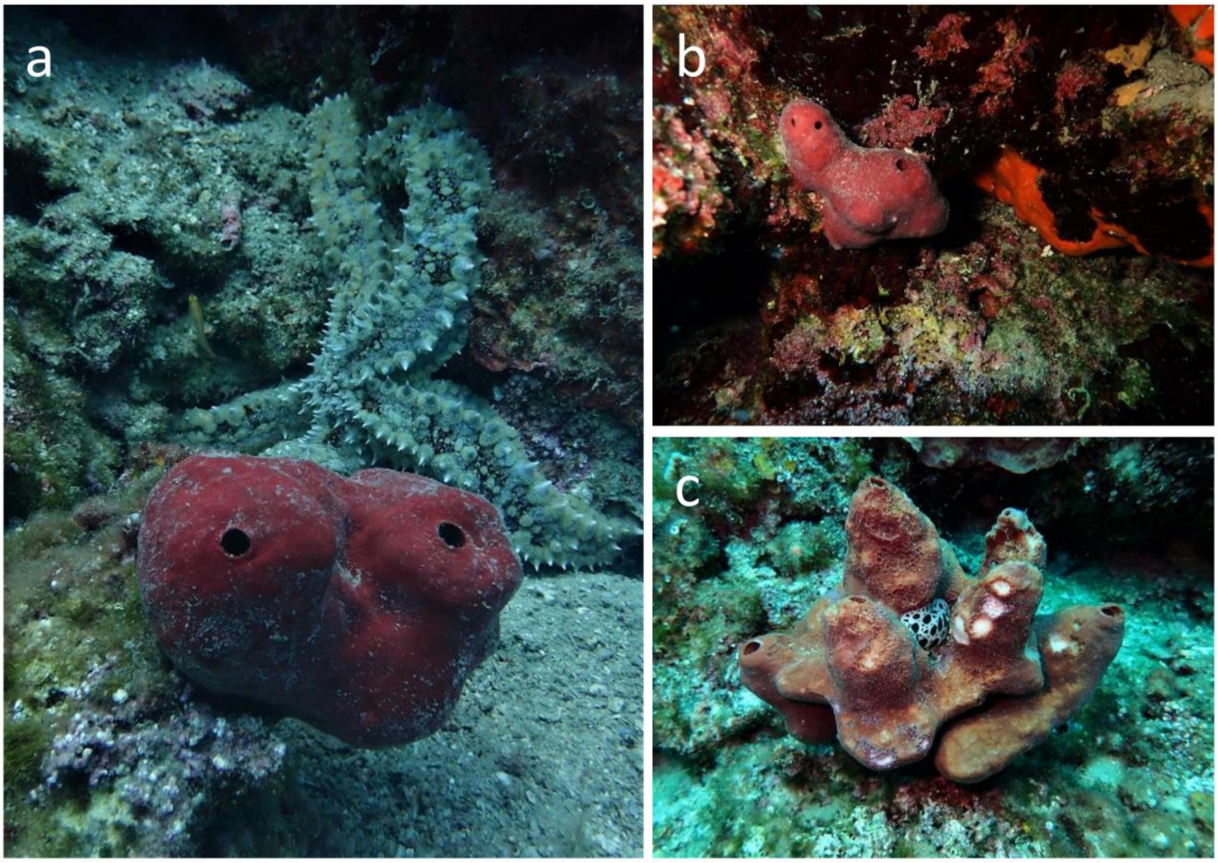

Fig. 2.1.3. A target sponge *Petrosia ficiformis:* a) healthy specimen on a well-lit rock at 18 m depth, b) a healthy specimen in a more sciaphilous habitat – within a crevice and c) a specimen showing signs of natural predation by a nudibranch *Peltodoris atromaculata*, also present in the picture. Photo credits: S. Kipson.

Fig. 2.1.4. Non-healthy specimens of a target sponge *Petrosia ficiformis.* In all images pinacoderm (the external sponge layer, purple-reddish in colour) is partially or completely affected, which is manifested either in overgrowth (putatively by some cyanobacteria, e.g. „greenish carpet“ under (a and b)) or exposed spongin fibers (e.g. under b) and c)). A healthy sponge have an intact pinacoderm and overgrowth is never present (see previous Fig. 2.1.3 a,b). Photo credits: S. Kipson.

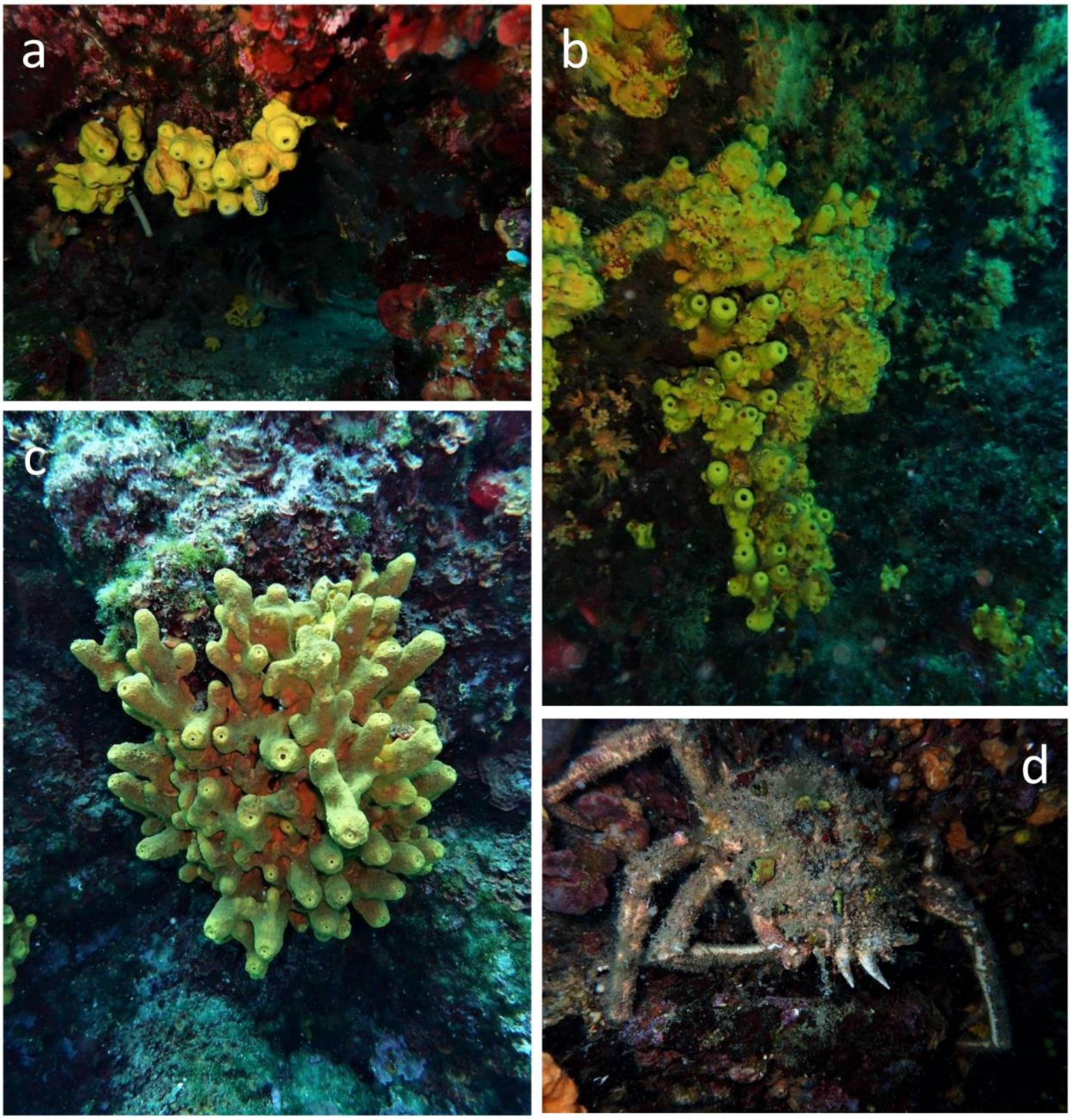

Fig. 2.1.5. On the location Konjsko in the bathymetric range of 12-18 m sponge *Aplysina* spp. was present within more sciaphilic crevices (a) and overhangs (b), as well as on well-lit vertical walls (c). It was even observed on a mobile substrate, such as on the carapace of the crustacean *Maja verrucosa* (d). Photo credits: S. Kipson.

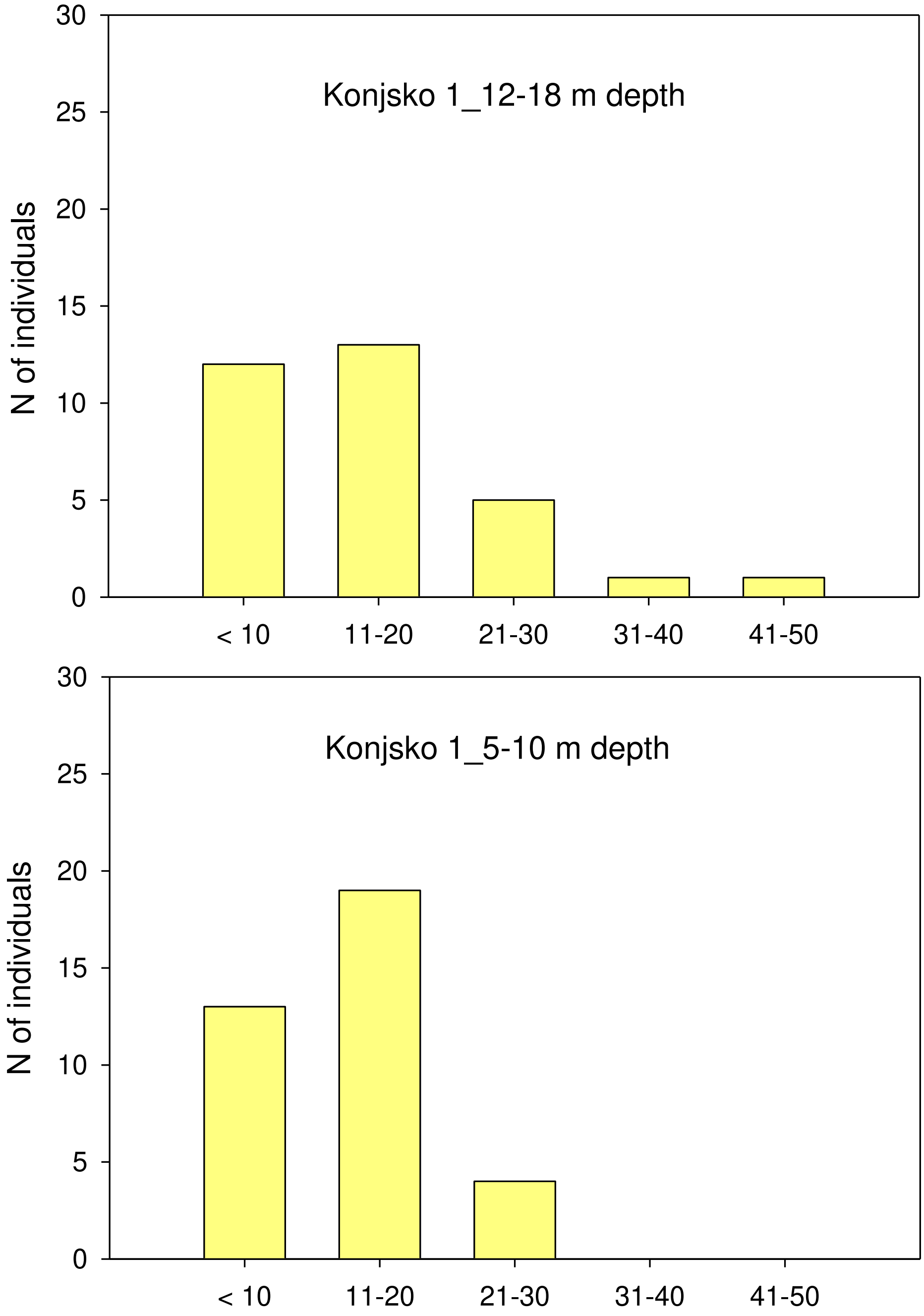

Fig. 2.1.6. Size-frequency of the sponge *Aplysina* sp. assessed within two depth ranges on the reef of the Konjsko 1 site. The size is expressed as the maximum diameter.

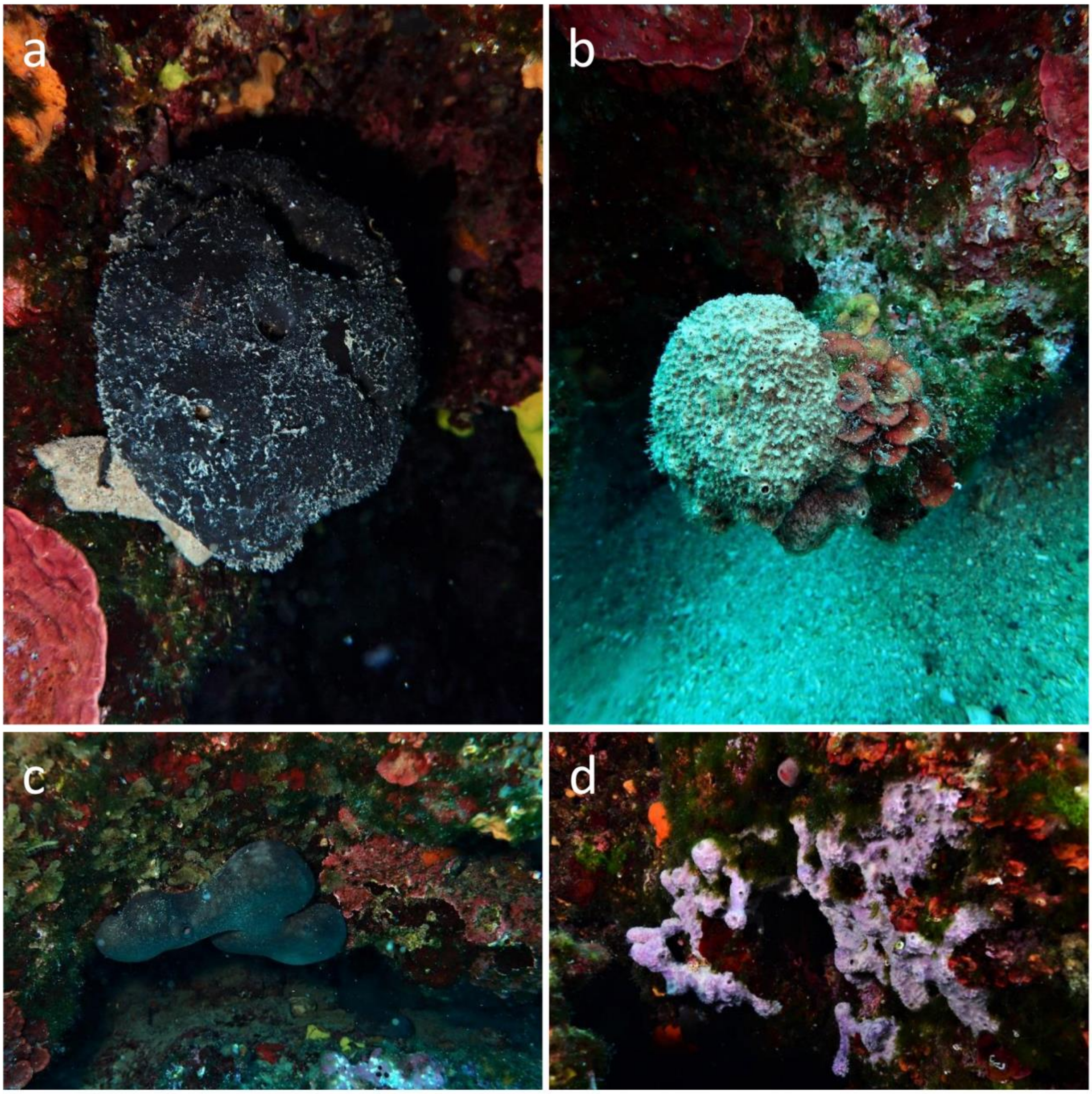

Fig. 2.1.7. Other sponges sparsley present on the Konjsko location within the examined depth range that were selected or proposed for selection on the ADRIREEF locations and/or within other initiatives: a) black keratose sponge, b) *Ircinia* spp., c) *Chondrosia reniformis* - also assessed within this study and d) *Dysidea avara*. Photo credits: S. Kipson.

Fig. 2.1.8. Within a study location Konjsko several specimens of sponge *Tethya* sp. were observed. Photo credits: M. Belošević.

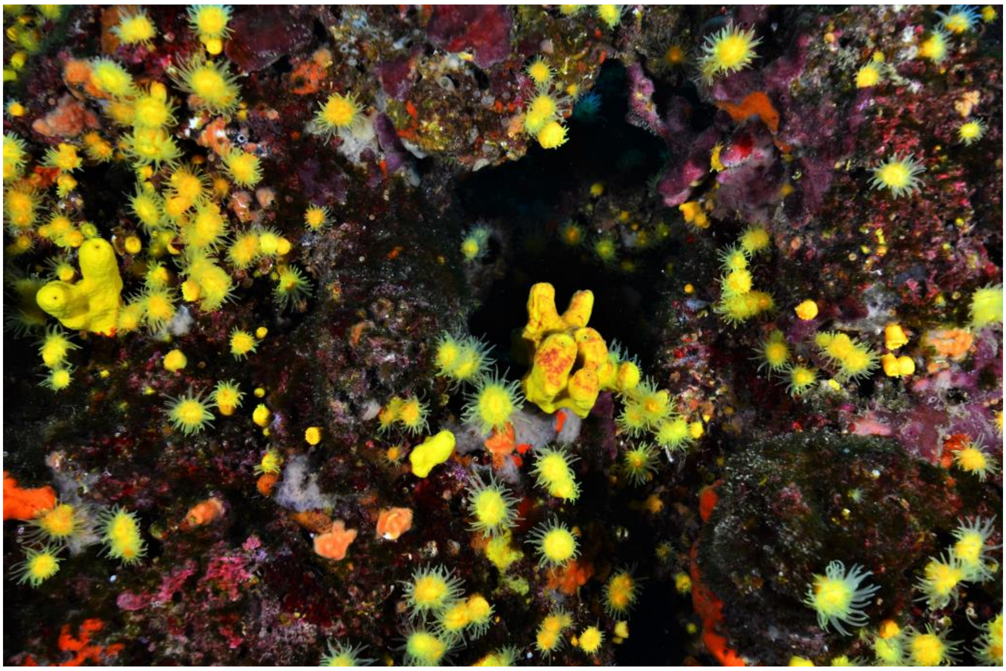

Fig. 2.1.9. Solitary hard coral *Leptopsammia pruvoti* at the site Konjsko 1 (and a small *Aplysina* spp). Photo credits: M. Belošević.

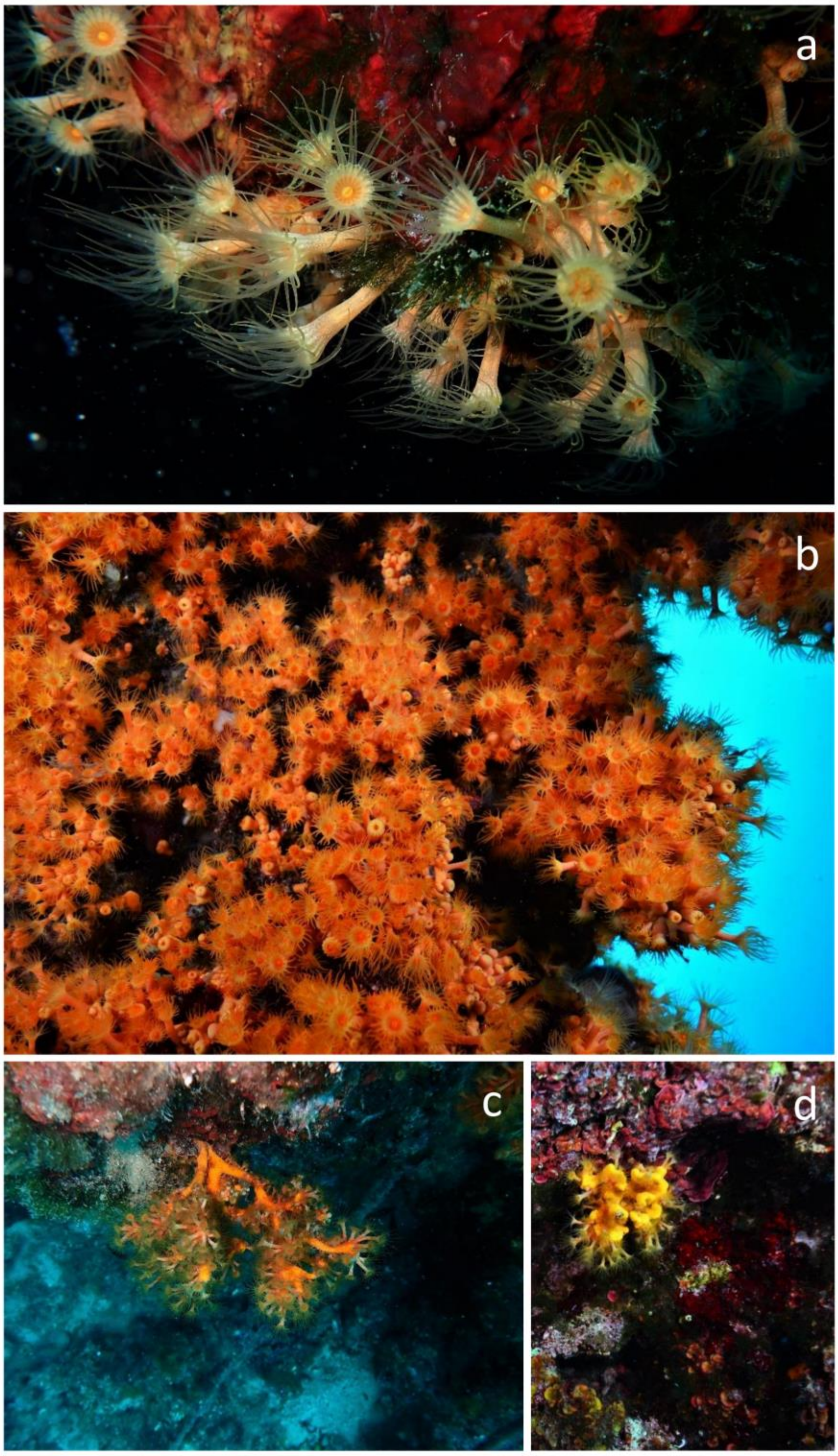

Fig. 2.1.10. The yellow carpet anemone *Parazoanthus axinellae*, now considered as a species complex, was thriving directly on the rocky substrate (a), occassionaly forming extensive carpets (b), as well as it was thriving as an epibiont on several sponge species (c,d). Photo credits: S. Kipson (a,c), M. Belošević (b,d).

Fig. 2.1.11. Solitary hard coral *Balanophyllia europaea* was the most abundant at the rocky plateau on the Konjsko 1 site around 5 m depth. Although it is a solitary corallite (a), sometimes two corallites may be found (b). Photo credits: S. Kipson (a), M. Belošević (b).

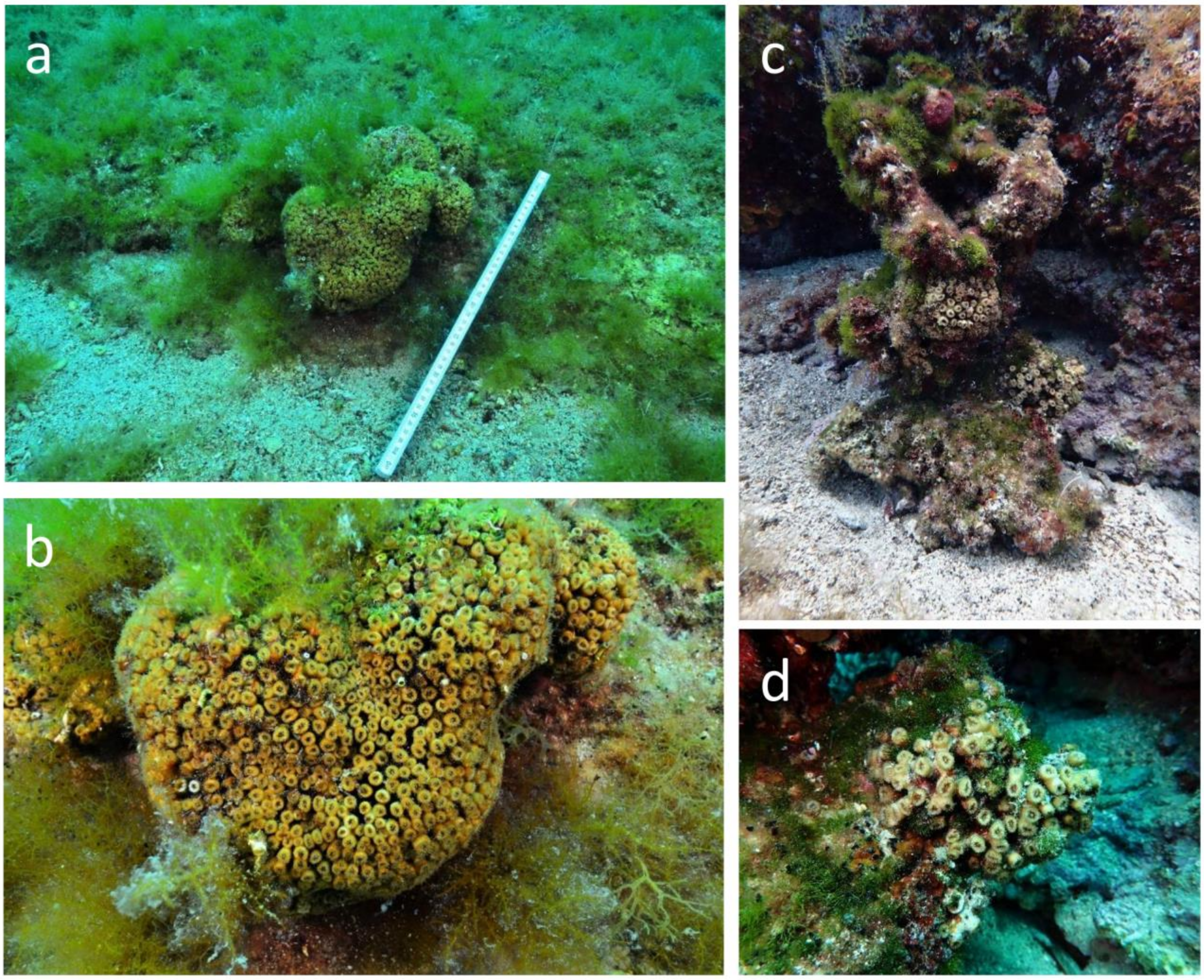

Fig. 2.1.12. Few colonies of a hard coral *Cladocora caespitosa,* the only reef-building coral in the Mediterranean and an endemic and threatened species, where recorded at the Konjsko 1 site (a,c, d). Image b) shows detail of the colony shown under a), partially overgrown by infralitoral algae such as *Laurencia* sp. on dead portions of the colony, but otherwise displaying healthy corallites. Colony on image c) and its detail under d) is also partially overgrown by green and red turf algae and red algae *Peyssonnelia* sp. Photo credits: M. Belošević (a,b), S. Kipson (c,d).

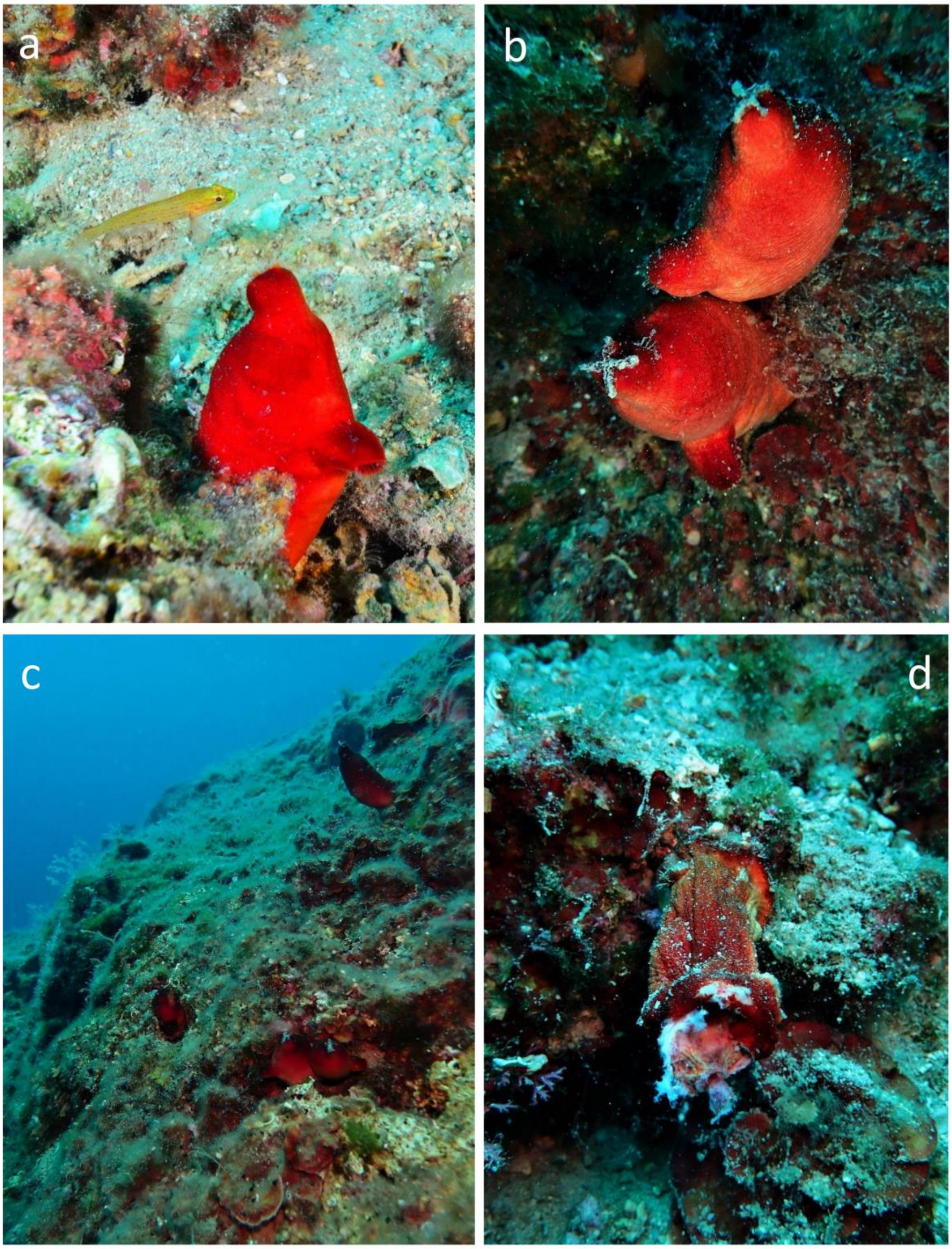

Fig. 2.1.13. Solitary ascidian *Halocynthia papillosa* (a) was fairly abundant on cascading walls (b,c) and occasionaly a dead specimen was noticed (d). Photo credits: M. Belošević (a), S. Kipson (b-d).

Table 2.1.1. The assessment of target macroinvertebrates. Species were observed in a depth range from 11 to 18 m (at the Konjsko 1 site), 11 to 19 m at the Konjsko 2 and Družinin sites, except *Balanophyllia europaea* which was assessed around 5 m depth. N = total number of individuals (or tufts of polyps in the case of *Parazoanthus axinellae*); results are based on photographic sampling except for *Aplysina* sp. that was assessed by visual census *in situ*.

	% of individuals		
	unaffected	affected	N
Petrosia ficiformis			
Konjsko 1	39.13	60.87	23
Konjsko 2	54.72	45.28	53
Družinin	58.33	41.67	24
Aplysina sp.			
Konjsko 1	81.32	18.68	91
Konjsko 2	92.96	7.04	71
Chondrosia reniformis			
Konjsko 2	100	0	28
Družinin 11-19 m	100	0	38
Družinin 3 m	100	0	46
Halocynthia papilosa			
Konjsko 1	92.60	7.40	27
Konjsko 2	100	0	13
Družinin	100	0	17
Balanopyllia europaea			
Konjsko 1	100	0	38
Leptopsammia pruvoti			
Konjsko 1	100	0	156
Družinin	100	0	213
Parazoanthus axinellae			
Konjsko 1	100	0	206
Konjsko 2	100	0	310
Družinin	100	0	131

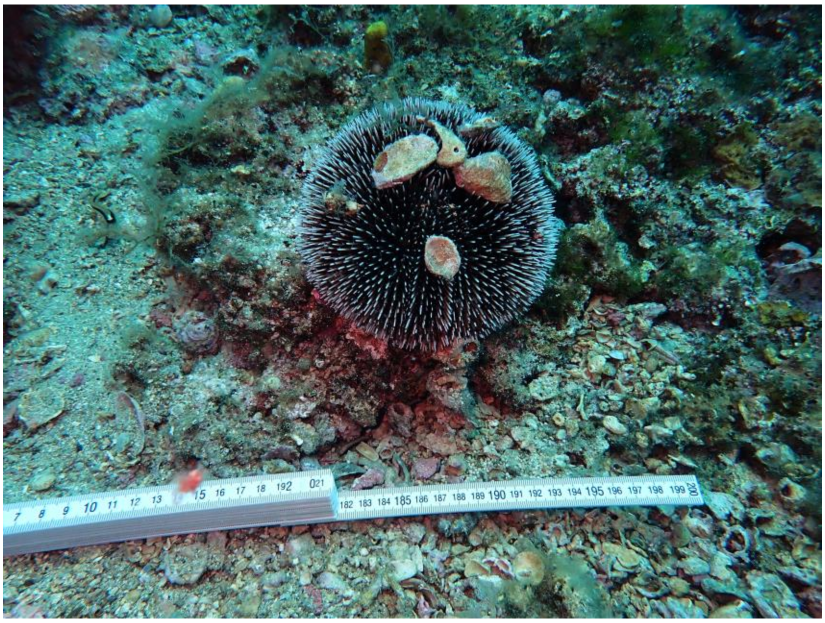

Fig. 2.1.14. The sea urchin *Sphaerechinus granularis*, one of the most common macrobioeroders observed at study sites. Photo credit: S. Kipson.

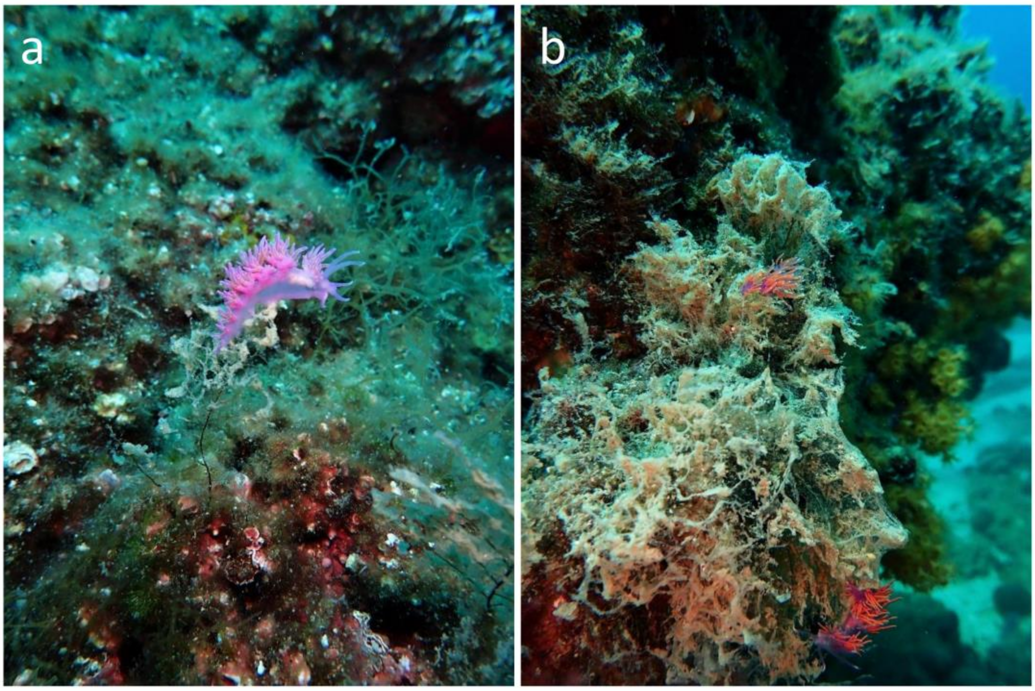

Fig. 2.1.15. Attractive nudibranchs feeding on hydrozoans: a) *Flabellina affinis* and b) *Paraflabellina ischitana.* In addition, first sights of mucilaginous algal aggregates were observed - accumulated over a hydrozoan in image (b). Photo credits: S. Kipson.

2.1.3.Conclusions

Out of all examined species, sponge *Petrosia ficiformis* was in the worst state, showing the highest proportion of affected specimens (from 42 to 61%, although results from Konjsko 1 and Družinin sites should be regarded with caution due to low number of examined individuals), and these unhealthy sponges were showing similar signs of affectation/sickness (see Fig. 2.1.4) as the ones previously observed on the Lagnići location (the Dugi Otok Island, Kipson 2021). The fact that similar signs are noticed within different regions of the Adriatic Sea (its Northern and Central part) and at different times of assessment (October 2020 and June 2021) imply that this phenomenon deserves further attention and should be monitored closely in the future, including as many sites as possible, to evaluate how wide-spread this phenomenon really is and to assess its potential causes.

Likewise, whenever possible considering time and sampling effort required, alongside the usual target species for the MME protocol (Garrabou, Bensoussan & Azzurro 2018) it is beneficial to document other sessile macroinvertebrate species, especially the ones whose individuals/tufts are aggregated in a small area, hence they are easy and quick to assess (by visual census *in situ* or from photographs). On the location Konjsko this was the case with the solitary hard coral *Leptopsammia pruvoti* and the yellow cluster anemone *Parazoanthus axinellae.* In general, if there is data on the effect of a certain stressor, e.g. marine heat waves (periods of elevated seawater temperature) on sessile marine organisms, information is limited to several species. Likewise, a certain species may showcase plasticity of thermotolerance in different geographical regions, shaped by processes such as acclimatization and/or local adaptation, hence it would be interesting to monitor them in the Adriatic ecoregion to see if patterns similar to the ones in other investigated Mediterranean regions emerge. For example, in the case of the two above mentioned coral species, *ex situ* experimental evidences so far from the NW Mediterranean imply they could have contrasting tolerance to elevated seawater temperature, with *L. pruvoti* being quite resistant whereas different morphotypes of *P. axinellae* showing low to moderate thermotolerance (Gomez-Gras et al. 2019). However, discrepancies in results of experiments *ex situ* and field observations are not uncommon in temperate corals (e.g. Kersting et al. 2015), and additionally, it remains to be seen if Adriatic populations show the same levels of sensitivity. It should be noted however, that in case of *P. axinellae* negative impacts may be assessed by the MME protocol only at time of actual death i.e. during mortality event, and not later on since these species have no hard skeleton. Especially in this case, changes over time in density and coverage could be assessed by comparing results of previous baseline demographic study in the same area (ideally using permanent transects) with newly collected data, during a dedicated monitoring effort.

Out of other macroinvertebrates present on the location Konjsko, although it was not nearly sufficiently abundant to carry out the MME assessment, it is worth noting the presence of several colonies of *Cladocora caespitosa* as it is an endemic coral with endangered status on the Mediterranean level according to the IUCN Red list (Casado de Amezua et al. 2015). In addition, as already mentioned in the Methods section, *Aplysina cavernicola*, the sponge included in Annex II of the SPA / BD Protocol and listed in Annex II of the Bern Convention as a strictly protected species was most likely present at the Konjsko reef, especially within its deepest crevices, holes and overhangs. Since according to Klöppel et al. (2009) this sponge cannot be reliably distinguished solely on morphology from the co-generic *A. aerophoba* at depths down to 40 m that both species may inhabit (when thriving on the more lit walls), we have recorded it at the higher, genus level.

Applying the same methodology as in the previous ADRIREEF study further improved our ability and confidence to recognize unhealthy/injured individuals, especially of sponges. This study further confirmed that the MME assessment can be successfuly carried out based on underwater images and/or video, as already concluded by Kipson (2021).

2.2. Assessment of gorgonian demography and conservation status

2.2.1. Overview of the methods

The yellow gorgonian *Eunicella cavolini* (an important structural species of the Adriatic reefs sensitive to many disturbances) was thriving on our study location, although in very low abundance. Therefore, for the assessment of its density we used a transect method – each colony was photographed along a horizontal transect. Conservation status of 68 colonies at the Konjsko 2 site and 56 colonies at the Družinin site was evaluated in a depth range from 11 to 19 m. Additionally, scuba divers managed to measure 30 colonies (max height – from colony base to the furthest tip) *in situ* only at the site Konjsko 2, whereas colonies at the Družinin site were not measured. Three parameters were used to determine the level of impact. Firstly, for each colony we estimated the extent of injury of colony surface, i.e. the percentage of colony surface that display a denuded axes and/or overgrowth by other organisms. Secondly, we noted the type of injury because the combined analysis of both parameters may be indicative of past disturbance events, including approximate time of their appearance (Coma et al. 2004; Linares et al. 2005). Therefore, depending on the presence/absence of different epibionts, three types of injury (related to the time of its origin) can be recognized in the field. The first type (type A) refers to a denuded axis, indicating a new injury (up to 1 month). The second type (type B) includes overgrowth by pioneering species, filamentous algae and hydrozoans (indicating an approximately 1–12-month-old injury), while the third type (type C) includes overgrowth mostly by bryozoans, sponges and/or algae and represents an old injury (approximately ≥12 months) (Linares et al. 2005; Fig.2.2.1). We considered colonies with <10% of injured surface to be healthy, as used in previous studies (Linares et al. 2008). Besides the already mentioned parameters, we also noted the upper and lower distribution limit of *E. cavolini* on the Konjsko location.

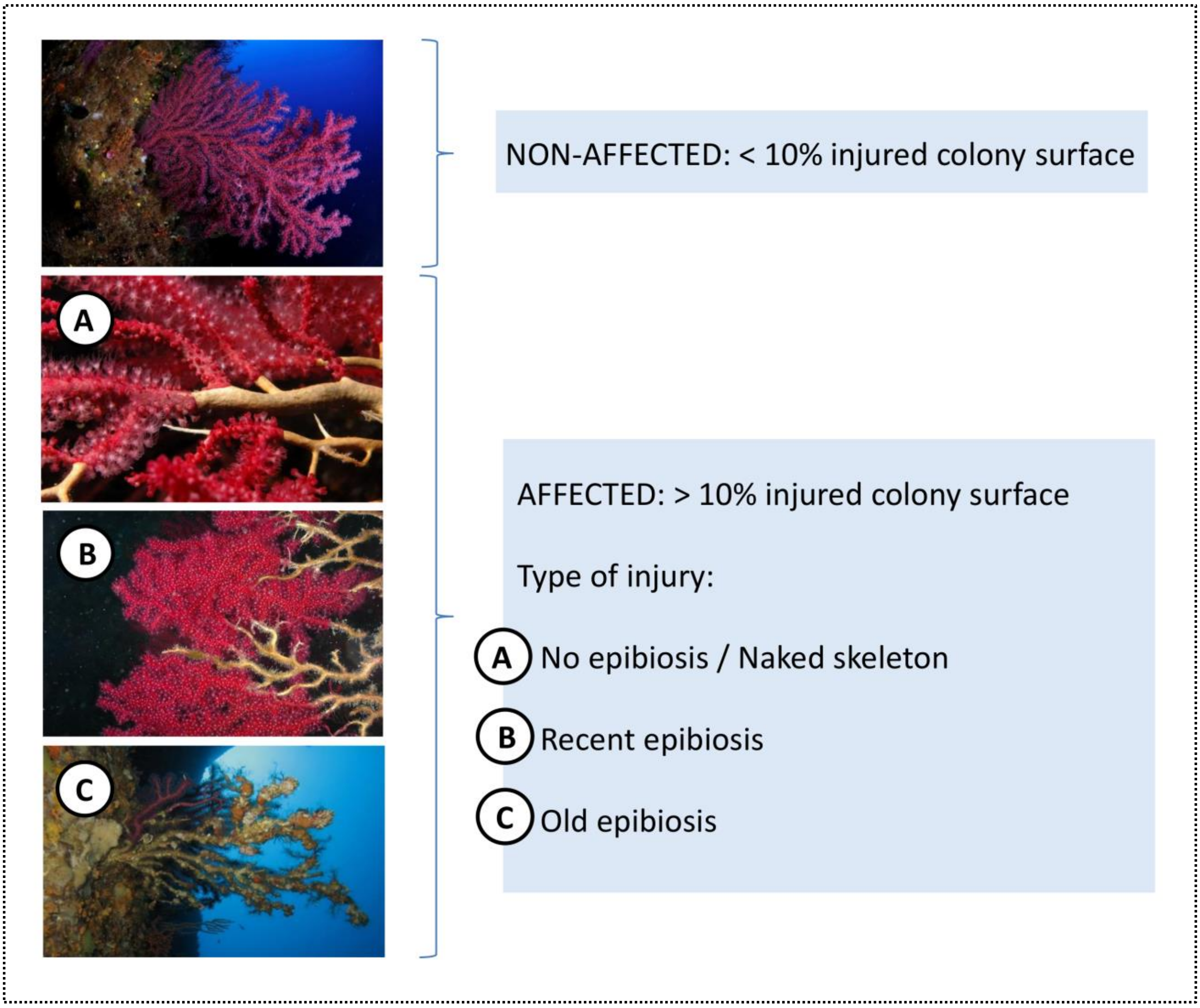

Fig. 2.2.1. Criteria used to assess whether gorgonian is affected or not and categories used for characterization of gorgonian tissue damage (adapted from Garrabou et al. 2015).

2.2.2.Main results

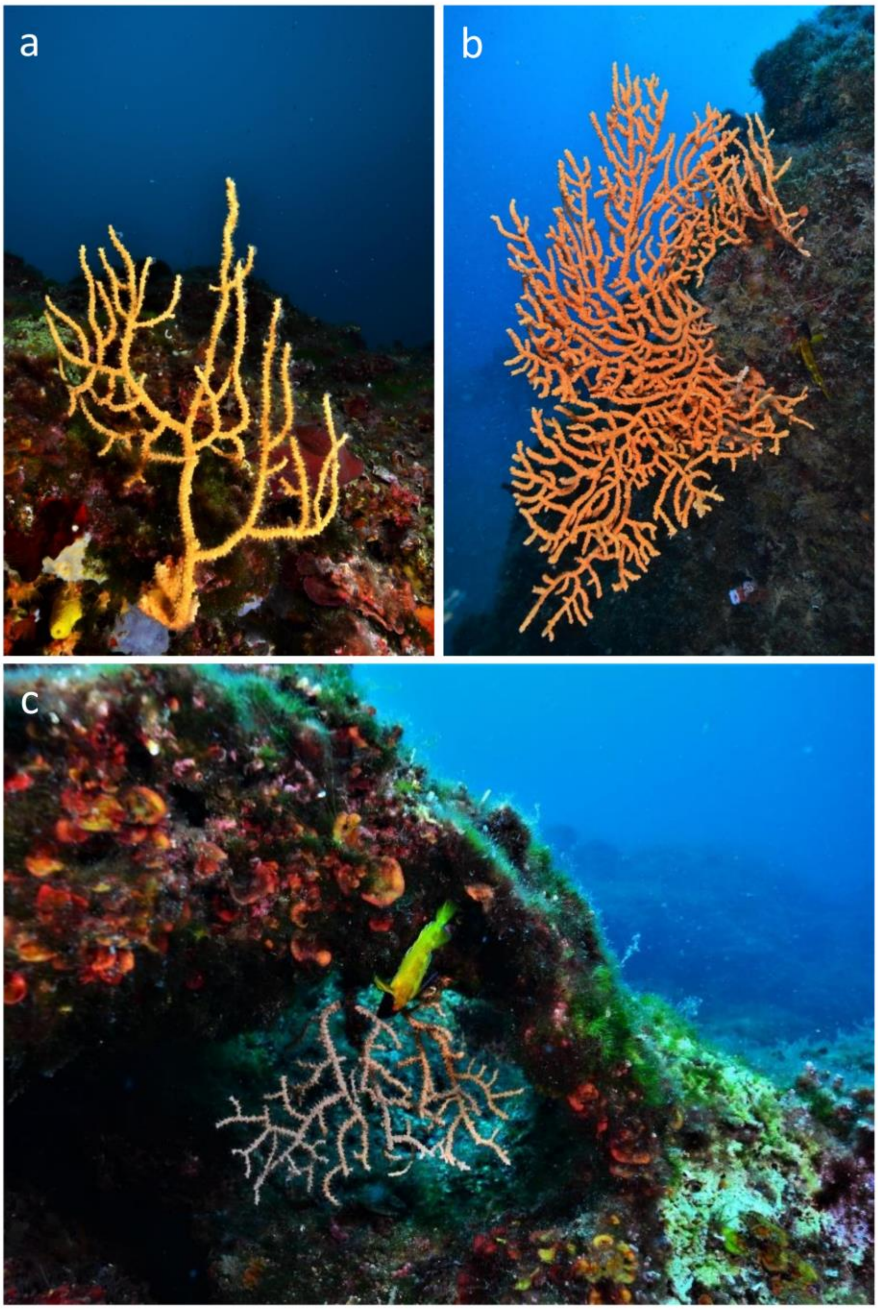

Fig. 2.2.2. Healthy specimens of the yellow gorgonian *Eunicella cavolini* present between 11 and 19 m depth on location Konjsko (Krk Island): a) and b) on well-lit cascading walls, c) within more sciaphilic crevices and overhangs. Photo credits: M. Belošević.

Gorgonian distribution

Only the yellow gorgonian *Eunicella cavolini* was present on the Konjsko location. It thrived both on the well-lit walls as well as within more sciaphilic crevices, passages and overhangs (Fig. 2.2.2). Its upper distributional limit was 11 m depth, whereas the lower limit coincided with the end of the cascading wall at 19.5 m depth at the Konjsko 2 site.

Demography

For demographic study, 30 colonies of the yellow gorgonian *Eunicella cavolini* were measured directly underwater by scuba divers at Konjsko 2 site. The mean colony height was 20 20 ± 7.42 (mean ± SD). The largest colony of *E. cavolini* was 36 cm tall, whereas the smallest one measured 8 cm. Related to the populations' size frequency distribution, 50% of colonies belonged to the size class 11-20 cm, whereas additional 33% belonged to the the next size class 11-30 cm. 10% of colonies were juveniles (<10 cm in size) and 7% were larger than 30 cm (36 cm in height precisely) (Fig. 2.2.3).

In the bathymetric range from 14 to 18 m depth at Konjsko 2 site gorgonian density, assessed along 100 m transect, was very low, only 0.17 ind./m^2. Colonies were not measured at Družinin site whereas similar gorgonian density was estimated there, amounting to 0.16 ind./m^2.

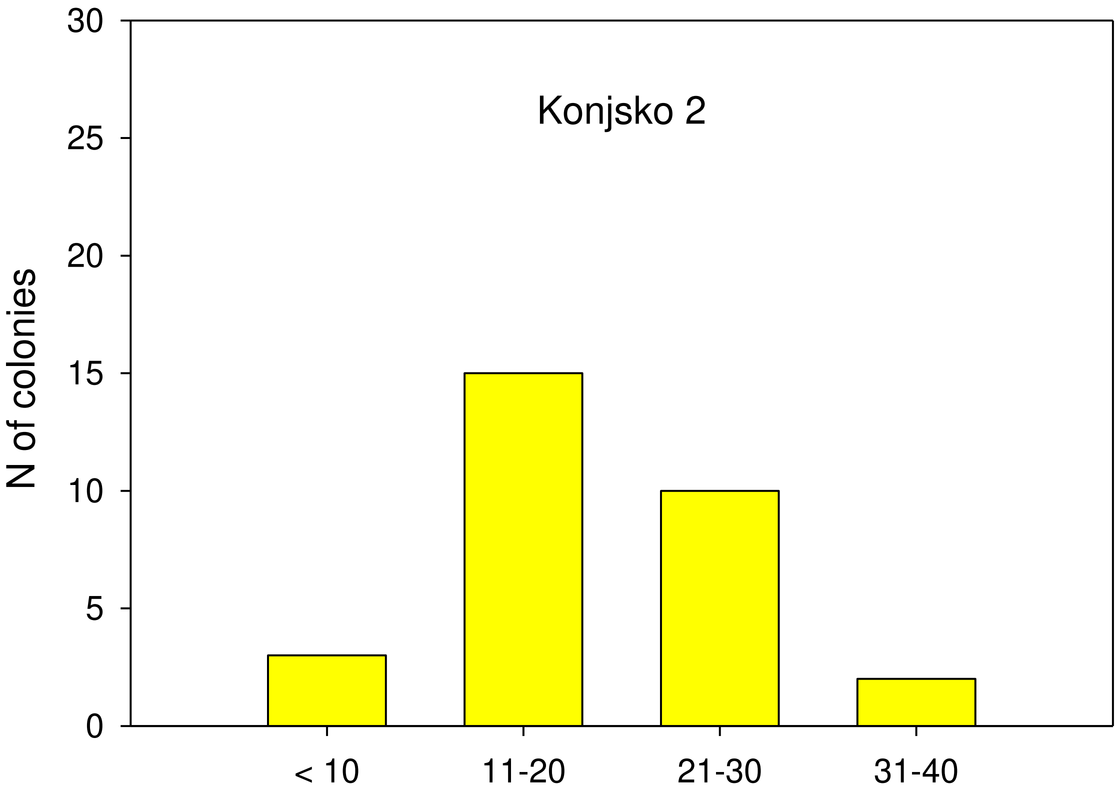

Fig.2.2.3. Size-frequency distribution of studied *Eunicella cavolini* population at the Konjsko 2 site.

Disturbance impact levels

Mean extent of injury of *E. cavolini* colonies measured directly underwater (N of colonies = 30) at Konjsko 2 site was 20.83 ± 22.48 (mean ± SD), whereas the same parameter assessed on the basis of photographs (N of colonies = 68) was slightly lower and amounted to 17.0 ± 28.3 (mean ± SD, Fig. 2.2.4). The Družinin population was assessed only from photographs (N of colonies = 56), resulting in the similar mean extent of injury (16.77 ± 30.48). The fairly large standard deviations (SD) in all cases indicate considerable differences in injury extent of colonies within a population (e.g. from 0 to 90%).

Out of 68 colonies recorded along a transect at Konjsko 2 site, 28% was affected i.e. showed more than 10% of injured surface, whereas 21.43% was affected at the Družinin site (out of 56 recorded colonies). Only 4.4% and 5.4% of colonies were completely dead at the Konjsko 2 and Družinin site, respectively (Fig. 2.2.4).

Types of gorgonian injuries observed in June 2021 during this study are visualized in Fig. 2.2.5. At the Konjsko 2 site the majority of affected colonies (with ≥ 10% of injured surface) showed type B epibiosis stage (up to 1 year old), characterized by colonized turf and filamentous algae, as well as hydrozoans (53.85%; Table 2.2.1, Fig.2.2.5c). Moreover, 30.77% showed an old epibiosis stage, characterized by encrusting algae and bryozoans (Fig.2.2.5e) and an additional 15.38% showed both injuries of type B and C (Table 2.2.1, Fig.2.2.5b,d). Identical proportion of colonies with both injury types B and C was recorded on the Družinin site, while type C was the dominant epibiosis type there, amounting to 61.54% and followed by type B (23%). Recent injury (type A, i.e. denuded axes) was noted in only one colony at the Konjsko 2 site and it did not surpass 5% of its surface (Fig.2.2.5a).

At the time of assessment, as major threats to gorgonians on Konjsko and Družinin sites we have noted mechanical abrasion by lost/abandoned fishing gear such as longlines and monofilament nylon lines (Fig.2.2.6a,b) as well as detachment of colonies due to unknown natural or anthropogenic causes (Fig.2.2.6c,d).

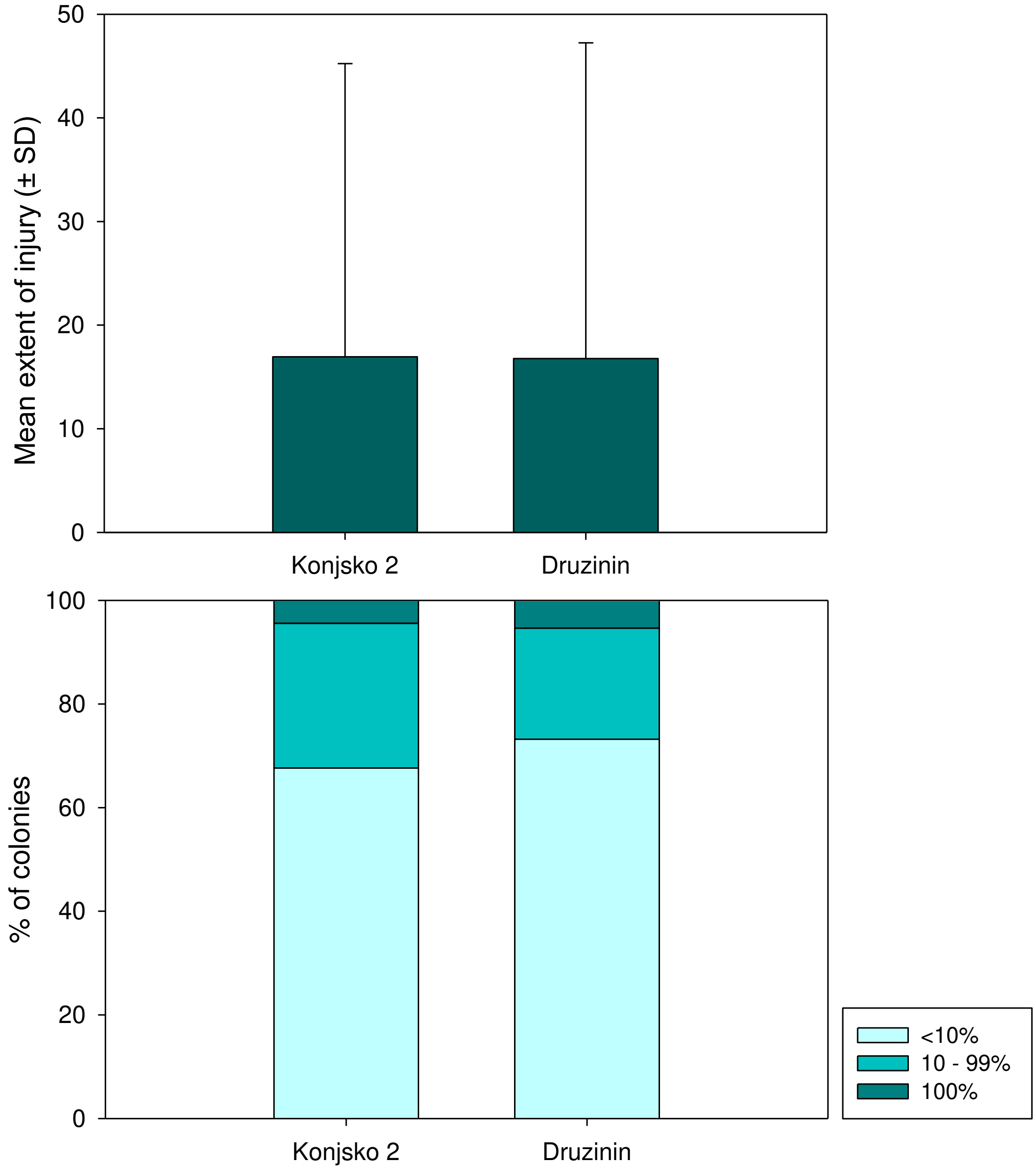

Fig. 2.2.4. Disturbance impact level parameters of the studied *Eunicella cavolini* population on the Konjsko location (E Krk Island) Upper graph: Mean percentage of the extent of injury; lower graph: percentages of healthy colonies (with <10% injury extent), affected colonies (between ≥10% and ≤99% injury extent) and dead colonies (with 100% injury extent).

Table 2.2.1. Percentage of affected *Eunicella cavolini* colonies (≥10% of surface injured) hosting different epibiosis types: 'type A' refers to a denuded axis, indicating a new injury (up to 1 month old); 'type B' includes overgrowth by pioneering species, filamentous algae and hydrozoans (indicating approximately 1–12-month-old injury); 'type C' includes overgrowth mostly by bryozoans, sponges and/or algae and represents an old injury (approximately ≥12 months) (see overview of the methods). Colony can host more than one epibiosis type.

	Type of injury						
%	A	B	C	A+C	A+B	B+C	A+B+C
Eunicella cavolini							
Konjsko 2	0	53.85	30.77	0.0	0.0	15.38	0.0
Družinin	0	23.08	61.54	0.0	0.0	15.38	0.0

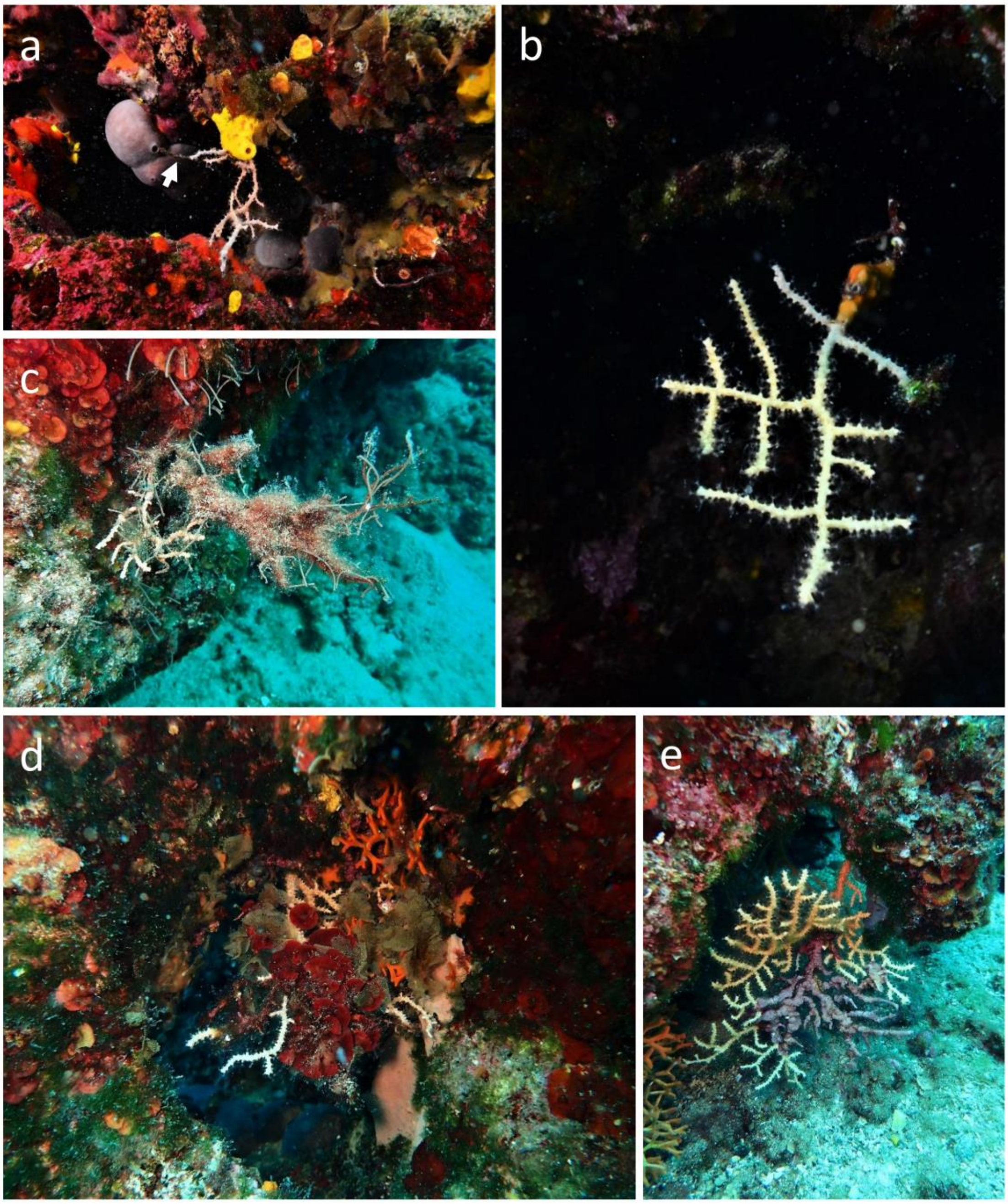

Fig. 2.2.4. Affected specimens of the yellow gorgonian *Eunicella cavolini* on location Konjsko (the Krk Island) with different types of epibiosis: a) damaged tips of colony, with denuded axis ('type A' epibiosis), b) base of the colony overgrown by encrusting orange bryozoan and encrusting red algae ('type C') while 1 tip is overgrown by filamentous algae and *Valonia* sp. ('type B'); c) colony mainly overgrown by filamentous and turf algae ('type B'); d) colony mostly overgrown by red algae *Peyssonnelia* sp. and bryozoan *Beania* sp. ('type C'), whereas several branches are partially overgrown by red turf algae ('type B'); e) central portion of the colony overgrown by red encrusting algae *Peyssonnelia* sp. ('type C' epibiosis). Photo credits: S.Kipson except (a and b) M. Belošević.

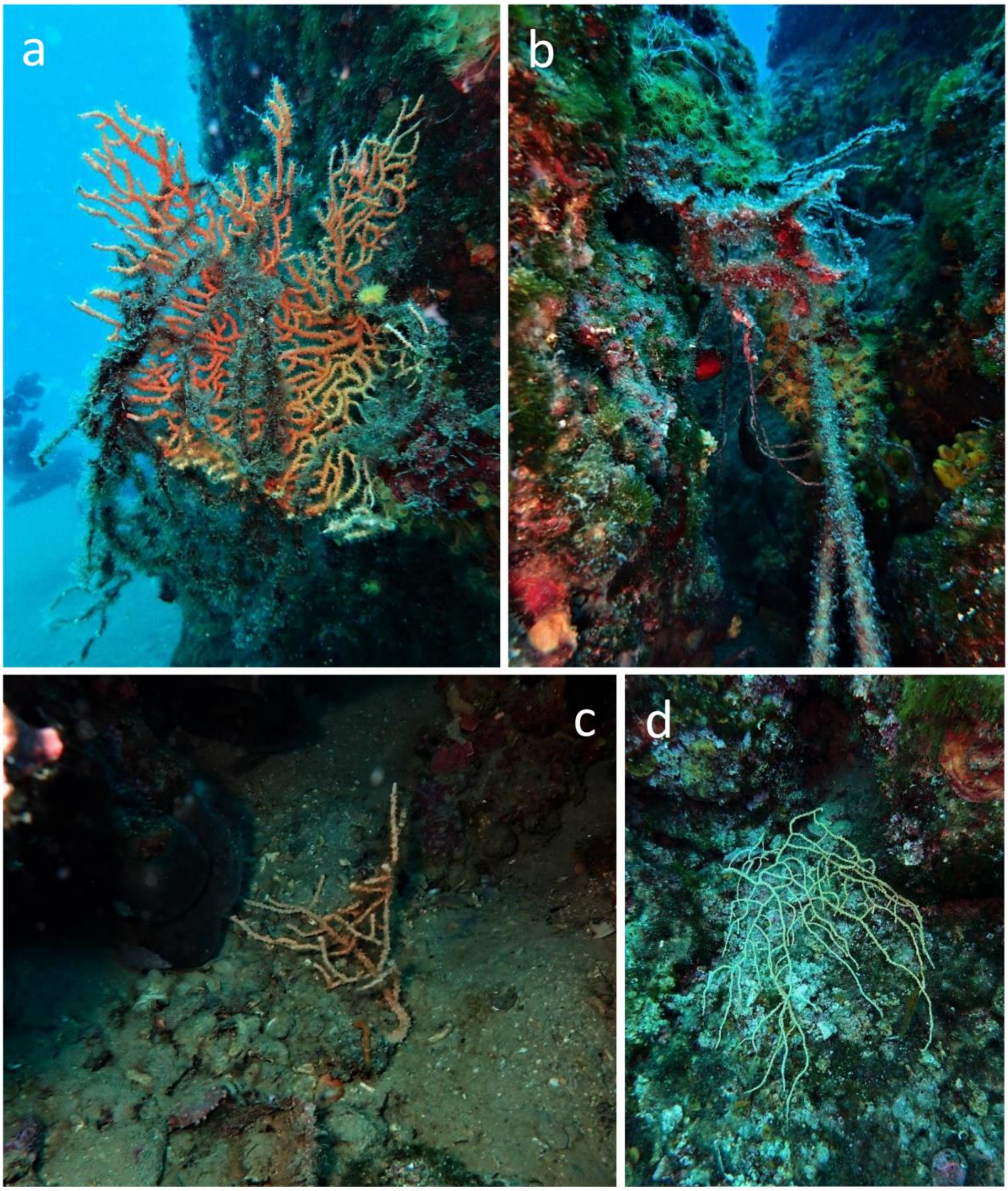

Fig. 2.2.5. Observed threats to the yellow gorgonian *Eunicella cavolini* on Konjsko and Družinin sites (the Krk Island): a) and b) entanglement by lost/abandoned fishing gear (e.g. long-lines and nylon monofilaments) causing mechanical abrasion and tissue damage; c) and d) detachment of colonies due to natural or human-induced causes. Photo credits: S. Kipson.

2.2.3.Conclusions

Results of this study are in concordance with my previous observations implying that the yellow gorgonian rarely (if ever) form “forests” (a well-formed facies) in the Kvarner region (at least to my knowledge). It is definitely a common species, present at many sites but in fairly low abundance. In comparison to the other two ADRIREEF locations, Dugi Otok and Vis Islands (as well as to the many other Mediterranean locations, e.g. Sini et al. 2015), density of *E. cavolini* on the Krk Island location (Konjsko) was almost two orders of magnitude lower (0.17 ind/m2 *vs*. 10 to 19 ind./m2, Kipson 2021). However, although indicative, it should be kept in mind that these results are not directly comparable since assessment depths (i.e. depths close to the upper distributional limit hosting a representative population for a proper quantitative assessment) were different: from 22 to 40 m depth on Dugi Otok and Vis locations *vs*. 14-18 m depth in this study.

With less than 1/3 (28% and 21.4%, at Konjsko 2 and Družinin site, respectively) of studied populations of *Eunicella cavolini* affected (i.e. with 10% of injured surface) and despite the fact that assessment of conservation status was carried out in the shallower depth range, this study recorded the healthiest population of the yellow gorgonian out of all studied ADRIREEF locations along the Croatian coast. In comparison, percentage of affected colonies of *E. cavolini* ranged between 41 and 57% on Lagnići location (the Dugi Otok Island) and high 66 to 80% on Stupišće location (the Vis Island). Meanwhile, proportion of dead colonies was similar at all sites (4.4-5.4% in this study *vs*. up to 3% on locations Lagnići and Stupišće) and within the natural range for gorgonian species (Linares et al. 2008, Sini et al. 2015). In June 2021 we did not observe any recent injuries of colony tissue. The majority of injuries were at least 1 year old or older. Similar to Lagnići location on the Dugi Otok Island, one of the major evidenced threats to gorgonians was lost/abandoned fishing gear causing mechanical abrasion of colony tissue and subsequent injuries. In addition, here we have observed several detached (but still alive) colonies lying on the sea bottom. Possible cause of their detachment may be also interaction with the fishing gear and/or other human-induced or natural (e.g. extreme storms) causes, at present unknown.

Related to the older injuries, besides potential effect of the elevated seawater temperatures (unfortunately there is no long-term, continuous seawater temperature monitoring at different depths in the area), one of the main causes would be the outbreaks of mucilaginous algal aggregates, covering primarily branchy erect sessile organisms such as gorgonians and preventing successful feeding of these suspension feeders as well as their gas exchanges with the surrounding water. Mucilaginous outbreaks were not recorded at the time of our assessment, although initial signs of mucilaginous algal aggregates formation were observed, see Fig.2.1.15b. However, there is ample evidence of their recurrent occurrence in the past - the most persistent ones usually develop from May till July but other periods are not excluded. These events are evidenced by the operators of the local diving centers (e.g. in Crikvenica and Baška) as well as personally by the author of this report at least on two occasions – in July 2013 and June 2018.

2.3. Fish visual census of climate change indicators

2.3.1. Overview of the methods

Beside the effort to monitor current conservation status and potential climate-related responses of a rocky bottom sessile macroinvertebrates, we 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, Bensoussan & Azzurro 2018), and the one already applied on two other ADRIREEF locations (Dugi Otok and Vis Islands, Kipson 2021). The original list of target species includes: *Sparisoma cretense, Epinephelus marginatus, Thalassoma pavo, Sarpa salpa, Serranus scriba, Coris julis, Serranus cabrilla, Siganus spp.* and *Fistularia commersonii.* In addition to this list, local targets (max. 4) may be added according to local monitoring needs (e.g. exotic species), easiness of recognition, interaction with fisheries, increase/decrease in the area and potential impacts on the environment/fisheries/ human activities.

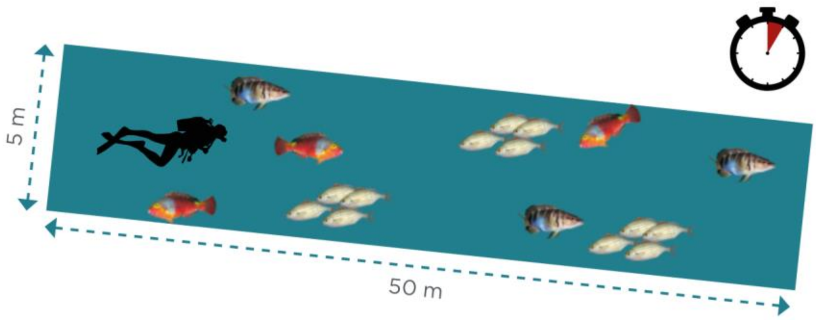

Fig. 2.3.1. Ilustration of the protocol aimed at fish visual census of climate change indicators (adopted from Garrabou, Bensoussan & Azzurro 2018). Census along one transect is shown.

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. 2.3.1). Small individuals (less than 2 cm) were not counted. After an observation period of 5 min, a diver proceeds to swim in the same direction and after a pause of approximately 10 m (1 min), he/she starts a new census/transect.

In relation to the available bathymetric range of the shallow Konjsko and in order to enable comparison with previous fish census data stemming from the other ADRIREEF locations along Croatian coast, the assessment was carried out from 5 to 10 m depth at the Konjsko 1 site.

2.3.2.Main results

From the original target fish species only *Coris julis* and *Serranus scriba* were observed on the location Konjsko (Fig. 2.3.2 b,c). In addition to these two original target species, local target *Diplodus vulgaris* was chosen for assessment due to its easiness of recognition and importance for small scale fishery (Fig. 2.3.2a). Out of these observed fish, *Coris julis* was the most abundant species in the examined 5-10 m depth range at the Konjsko 1 site (Fig. 2.3.3). Unlike on the two other ADRIREEF project locations (Dugi Otok and Vis Islands) termophilic alien species such as *Sparisoma cretense* and *Thalassoma pavo* were not recorded on the Krk Island, location Konjsko.

Besides fish species targeted for visual census, several other common species were observed on the Konjsko location, as shown in Fig. 2.3.4, as well as an occassionaly seen species such as gurnard from the family Triglidae, *Trigloporus lastoviza,* usually associated to sandy bottoms stretching in the vicinity of the rocky ones (Fig. 2.3.4g).

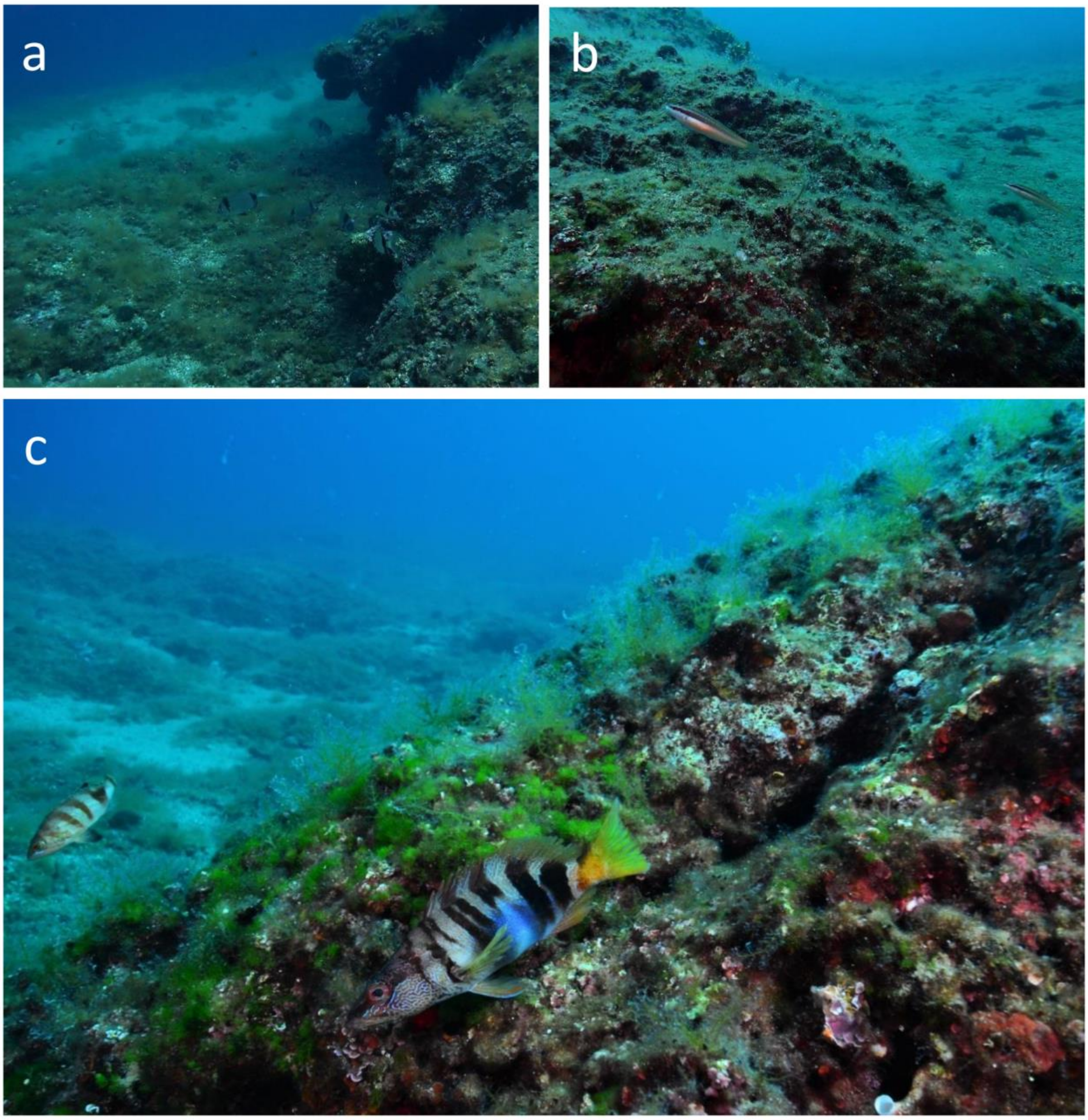

Fig. 2.3.2. Target fish species present on location Konjsko (Krk Island): a) *Diplodus vulgaris*, b) *Coris julis* and c) *Serranus scriba*.

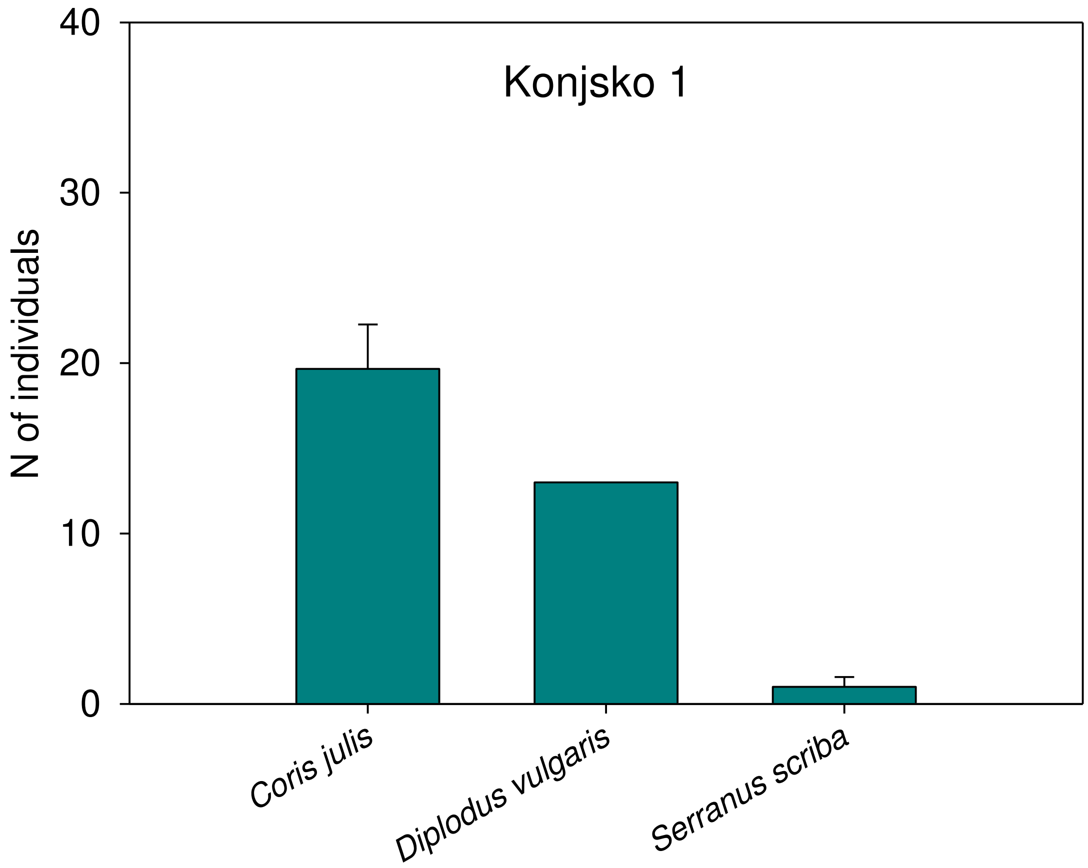

Fig. 2.3.3. Mean abundance of the target fish species at the Konjsko 1 site in a depth range from 5 to 10 m.

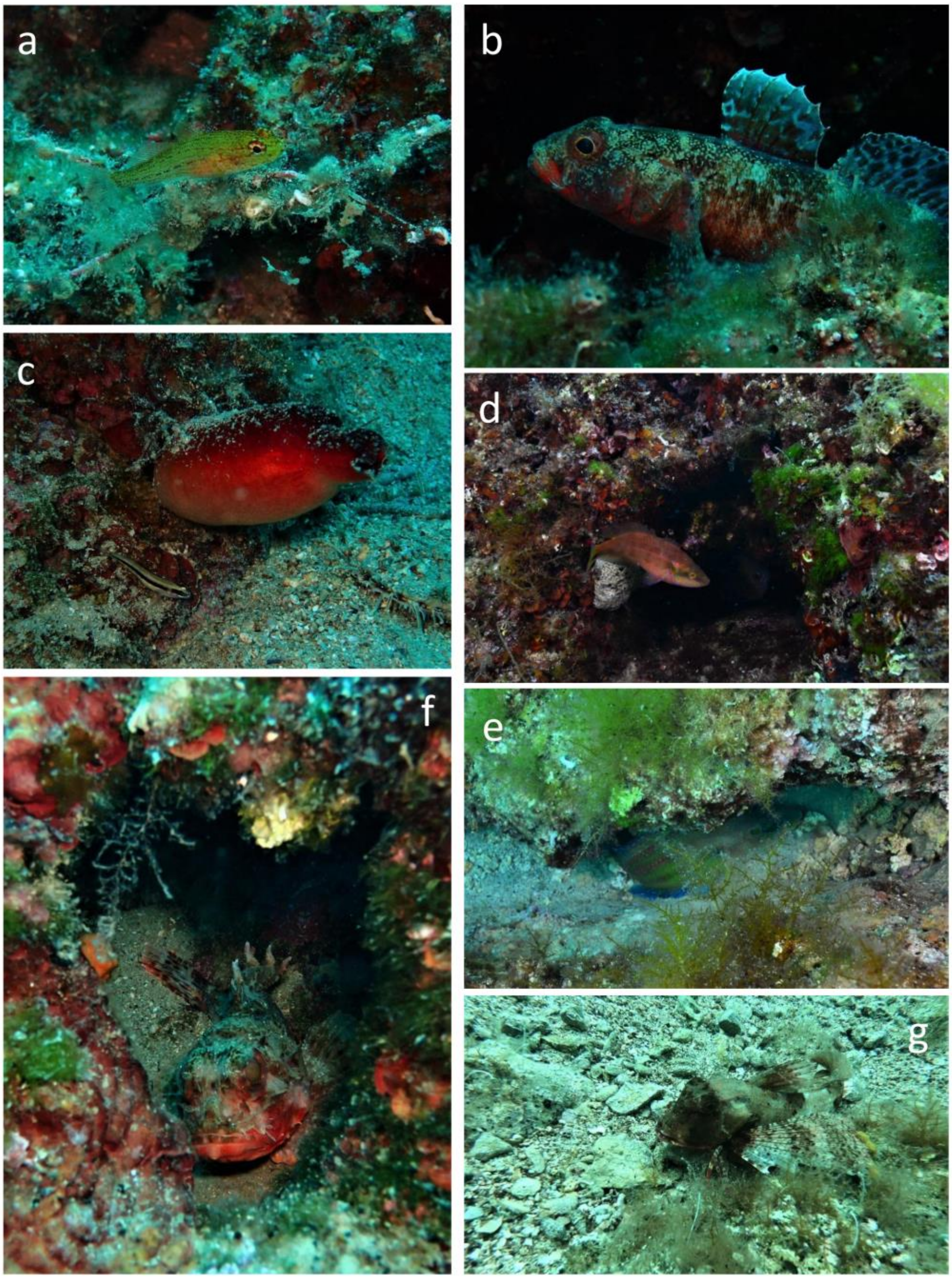

Fig. 2.3.4. Some of other common fish species observed on location Konjsko, Krk Island: a) the golden goby *Gobius auratus*, b) the red-mouthed goby *Gobius cruentatus*, c) the striped goby *Gobius vittatus*, d) the ocellated wrasse *Symphodus ocellatus*, e) the peacock wrasse *Symphodus tinca*, f) the small red scorpionfish *Scorpaena notata* and an occassionaly seen species - a fish from the family Triglidae, likely the streaked gurnard *Trigloporus lastoviza* (g). Photo credits: S. Kipson (a-e), Fedra Dokoza (f).

2.3.3. Conclusions

Fish abundance and diversity was modest on the location Konjsko. Out of 9 target species included in assessments on the other two ADRIREEF locations (on the Dugi Otok and Vis Islands), only 3 were recorded here. However, luckily alien thermophilic species *Sparisoma cretense* and *Thalassoma pavo* were not among them, implying the effects of climate change may be still less pronounced here in the Northern Adriatic. These data collected in a standardized manner enable comparison among different sites and will enable detection of upcoming effects of seawater warming i.e. the consequent biological response in terms of changes in composition and structure of fish assemblages.

2.4. Assessment of the impact of the lost fishing gear (LFG)

2.4.1. Overview of the methods

To assess the impact of the lost fishing gear on the project location and to assist managers in their decisionmaking for the removal of nets, we followed the protocol developed in the scope of the Ghost Med programme (Ruitton et al. 2019 and a methodological update by Ruitton et al. 2020). To assess the LFG impact, 3 criteria are used and they include environmental (EI) and seascape impact (SI) as well as the technical risk involved in the LFG removal. Each criterion is quantified by a set of relevant parameters and each parameter is assessed by a semi-quantitative or a qualitative scale. Moreover, scores are assigned and the criterion is assessed using the procedure described by Ruitton et al. (2019, 2020). Finally, based on the evaluation of all 3 criteria, a Gear Removal Index (GRI) is calculated (Ruitton et al. 2020).

Parameters for assessment of the LFG environmental impact (EI) that we evaluated in the field include:

- **The colonization of the fishing gear** (evaluation of the colonization stage: (0) without epibiosis; (1) by filamentous algae; (2) by macroalgae and hydrozoa; and (3) by encrusting epibiosis (bryozoa, macroalgae, annelida, etc.).

- **The trapped mobile fauna** (a semi-quantitative estimation of the number of individuals trapped in the LFG)

- **The removed fixed species** (number of individuals of all the benthic species fixed to the substrate that have been torn off by the action of the fishing gear)

- **The damaged fixed species** (number of individuals that undergo necrosis or breakage due to contact with the LFG)

- **The presence of outstanding species** (observation or not of species with heritage value, such as protection status and rarity, and/or commercial value that have colonized the LFG)

- **The obstructed cavities** (the number of cavities that are no longer accessible for mobile fauna)

- **The abrasion of the substrate** (observation or not of a friction effect of the LFG on the substrate which would consequently damage the colonization)

- **The habitat creation** (observation or not of the potential ecological role of LFG such as nursery, hideout or pantry for the marine fauna)

In addition, the methodological update (Ruitton et al. 2020) included few other parameters, namely:

- **The proximity of outstanding species** (observation or not of species with heritage value, such as protection status and rarity, and/or commercial value in the vicinity of the LFG)

- **Engagement of the impact** (i.e. surface of the substrate affected)

- **Fishing capacity** (whether or not LFG still has capacity to catch marine organisms)

Moreover, in the updated methodology (Ruitton et al. 2020), scores are also given to the habitat that has been involved in the assessment as well as to the site usage (e.g. fishing, diving/snorkeling, swimming, sailing/mooring, etc.).

Furthermore, parameters for the assessment of the LFG seascape impact (SI) that we evaluated in the field include:

- **The distance of visibility** (the estimation of a distance at which the LFG is visible)

- **The extent of impact** (the surface concerned by the LFG - usually the surface area occupied by the gear on the bottom)

- **The seascape alteration** (the recognition or not that there is an alteration of the seascape)

- **The qualifying adjective** (overall impression if and how LFG alters the seascape - could be

neutral, positive or negative

- **The relief created** (evaluation whether the natural relief of the site is altered by the LFG – e.g.

if the gear is lying on a rocky scree, it tends to detract from the relief, whereas

if it is deployed in the water column, it enhances the relief).

Lastly, parameters for the assessment of the technical risk (TR; i.e. taking into consideration the diver's intervention or the technical equipment required for the removal of the LFG) that we evaluated in the field include:

- **The depth of the LFG**;

- **Attachment of the LFG to the bottom** (evaluation to what extent the LFG is attached to the bottom and if its removal is relatively easy or it is difficult and time-consuming; this parameter presents a criterion that modulates the time spent by divers on the bottom and the use or not of specific tools).

2.4.2.Main results

Predominant type of lost/abandoned fishing gear were longlines and monofilament nylon lines (Fig.2.4.1a-d), followed by traps (Fig.2.4.1e, Fig.2.4.2, Fig.2.4.3). Only one fishing net was observed at the site Konjsko 1.

Related to the evaluation of their environmental impact, longlines were overgrown mainly by filamentous algae (stage 1, e.g. Fig. 2.4.1. a-d), except traps that were either colonized by macroalgae or encrusting organisms such as bryozoans, red algae and annelids (stage 2 or 3 of epibiosis according to Ruitton et al. 2020, e.g. Fig.2.4.2b). No trapped, removed or damaged individuals were observed on the Konjsko 1 and 2 sites. However, some fixed organisms were damaged, in most cases in moderate number (between 1 and 10 individuals/colonies) on the Družinin site. There, longlines and/or monofilaments were occassionaly entangled around gorgonian colonies, causing abrasion and tissue injuries (see Fig.2.2.5a,b). Several colonies were found detached, potentially due to contact with a fishing gear, but this cannot be claimed with certainty. Outstanding species in terms of rare or protected organisms were not associated with LFG or found in their proximity. In the case of one trap, a squid, i.e. a commercially valuable species, was using it as a nursery (Fig. 2.4.2b). Other LFG, being mostly longlines and monofilament lines, did not create additional habitat with a role of a nursery, hideouts or pantry. There was no abrasion impact on the substrate and no obstructed cavities were noticed. Likewise, fishing capacity of predominant LFG found here was nil. Related to the impact on the seascape, since most of observed LFG were longlines and monofilament lines laid down on the substrate, they were moderately visible (from 1-5 m distance) and they did not considerably alter the seascape. For the same reason, they did not substantially enhance the relief (except of several traps found on the nearby detritic bottom) and their impact mainly affected a surface below 5 m^2.

When evaluating technical risk associated to the potential removal of observed LFG, positive points present the relatively shallow depth range where they were placed (not more than 20 m depth) as well as their fairly loose attachment to the bottom and hence, relatively easy removal.

Related to the site usages, whereas Konjsko sites have been used as fishing grounds (as evident from the LFG found there), Družinin site has been also used as a scuba diving site by the local dive centers.

Based on all parameters mentioned above, the calculated GRI (Gear Removal Index) ranged from -5 to 3 for Konjsko 1 and 2 sites and it amounted to 5 for the Družinin site. Hence, removal would be advised (priority 4) for longlines and monofilaments, especially on the Družinin site ($0 < GRI < 10$), whereas removal of traps on the Konjsko 2 site is not recommended ($GRI < 0$).

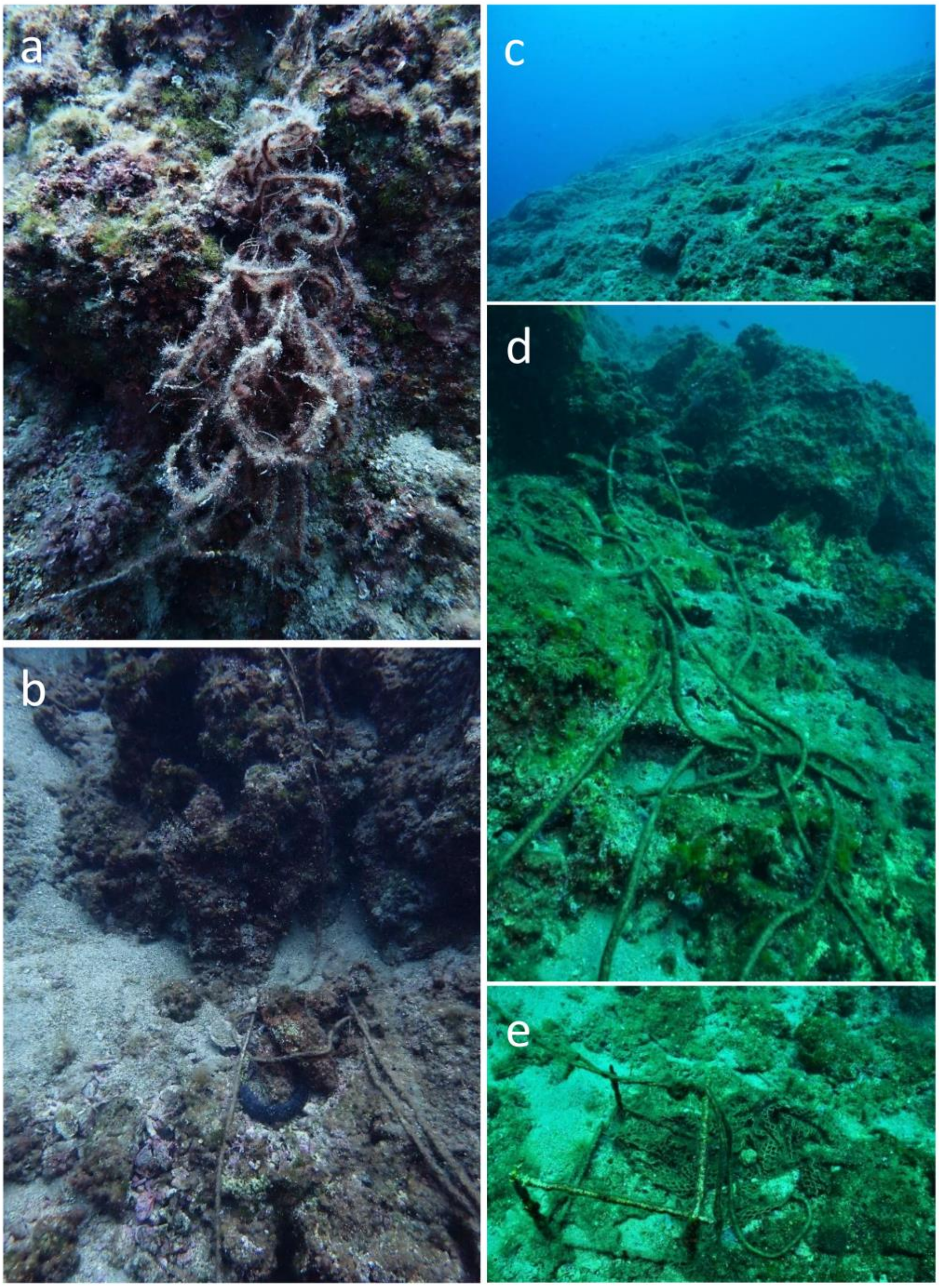

Fig. 2.4.1. Example of lost/abandoned fishing gear on location Konjsko next to the Krk Island: a) ropes on site Konjsko 1 and b) Druzinin, c-d) ropes on site Konjsko 2 and e) a trap fallen apart.

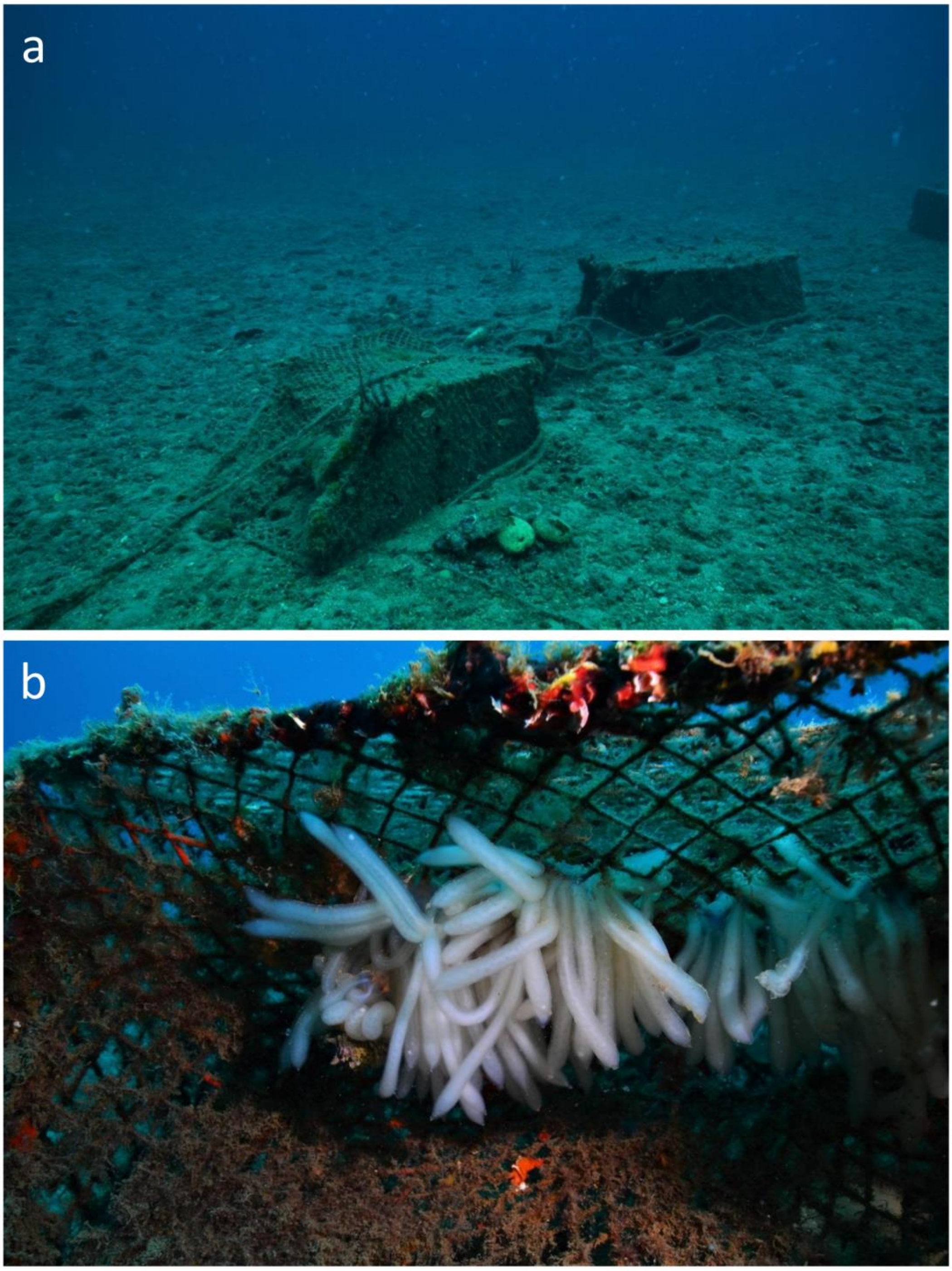

Fig. 2.4.2. Example of lost/abandoned traps on site Konjsko 2 (Krk Island, a) that host encrusting bryozoans, algae and serpulid polychaetes and serve also as a nursery for squid eggs (b).

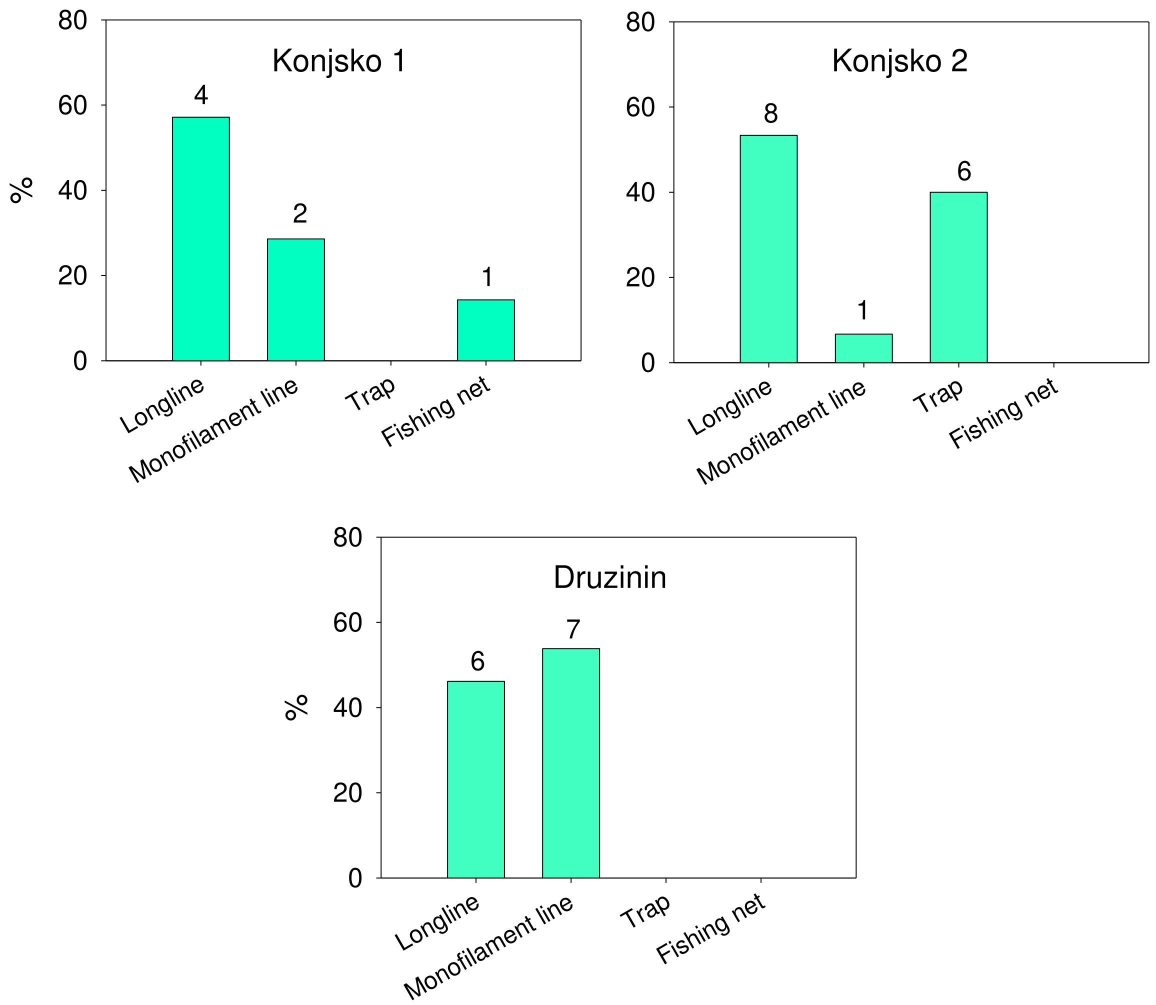

Fig. 2.4.3. Type and number of recorded lost/abandoned fishing gear at the Konjsko location (E Krk Island).

2.4.3. Conclusions

Results of this assessment confirm the observation that it is becoming increasingly difficult to find a reef site without a trace of human presence, most notably in the form of abandoned/lost fishing gear. In this study, LFG was noted at all 3 sites.

According to the evaluation following Ruitton et al. (2020), removal would be advised in the case of longlines and monofilaments on the Družinin site – a diving site visited by local dive centers, where, among others, they cause damage to gorgonians and are relatively easy to remove. On the contrary, removal of traps on the Konjsko 2 site is not recommended, likely because they create additional habitat and are in advanced stage of epibiosis, including calcifying, encrusting organisms. Moreover, they do not pose an imminent threat, as no damaged, removed or trapped organisms were associated to them.

2.5. Preliminary biodiversity assessment on unintentional artificial reef – a shipwreck Peltastis

2.5.1. Overview of the methods

Like on previous ADRIREEF locations on the Dugi Otok and Vis Islands, an accessible shipwreck (i.e. the one stretching down to the limits of recreational diving at 40 m depth) was present close to our study location Konjsko. Hence, once again we have used this extraordinary opportunity to visit it and carry out a preliminary biodiversity assessment in an attempt to contribute to the future, more comprehensive evaluation of the current role of Adriatic shipwrecks as artificial reefs, although their original placement underwater was not intentional (unlike when artificial reefs are specifically used as a conservation/restoration tool).

This shipwreck could be visited only once and on that limited occassion we documented marine habitats and species/taxa associated to it. Results are based on the divers observations *in situ* and underwater photography and videography. Since reliable identification of many benthic taxa require a physical sample to be examined by a specialist (and collecting species samples was not predicted in the scope of our work and we have not requested official permissions for it), the list provided within this report (see Appendix 1) is by no means a comprehensive check list of species associated with the shipwreck Peltastis.

2.5.2. Main results

A motor boat Peltastis was sunken in 1968 and it is located 50 meters from the East coast of the Krk Island, approximately 1.5 NM north-west from the Šilo settlement (Fig.1.1.1c; 45° 09,800'S, 14° 38,650'I). The deepest part of the shipwreck sits on the sea bottom at 33 m depth, whereas the shallowest frontal part is found at 15 m, facing the coast. The boat is 60 m long and 8.3 m wide and has NE-SW orientation (Frka & Mesić 2012). Since it was a cargo ship, the greatest part of the hull was dedicated to storage, whereas captain's bridge, utility and engine rooms were located at the rear end (Fig. 2.5.1 e-h, j). A more complete visualisation of the shipwreck is provided in Fig.2.5.1. Being situated in the Velebit channel, visibility on the shipwreck rarely surpass 10 m (Frka & Mesić 2012) and such conditions of fairly low visibility were present also during our visit on June 9, 2021.

The shipwreck Peltastis is still well preserved, hence it acts as a one of the premium diving locations in the Northern Adriatic. It also acts as an artificial reef that provides a variety of marine habitats (Figs. 2.5.2-2.5.5). On the outer hull from cca 26 to 33 m depth a sciaphilic biocenosis develops dominated by sessile benthic invertebrates such as encrusting and massive sponges, solitary hard corals *Caryophyllia inornata* and *C. smithii* as well as other colonial scleractinians, branchy and encrusting bryozoans, vermetid snails and serpulid polychaetes (Fig. 2.5.2). Besides them, ocassionaly blue mussels, solitary ascidian *Halocynthia papilosa*, soft coral *Alcyonium acaule* and polychaete *Sabella spallanzanii* could be seen (Fig. 2.5.3a-d) whereas on the more horizontal parts of the ship, on places where sediment could accumulate, one can find some organisms typical of sandy bottoms such as the cylinder anemone *Cerianthus membranaceus* and a polychaete worm *Myxicola infundibulum,* both anchored in the sediment (Fig. 2.5.3e-f). Within the hull, on walls and left-over structures which are still sciaphilic but receive slightly more light than the deepest outer part, red encrusting algae dominate, belonging to orders of Corallinales and Peyssonneliales (Fig. 2.5.4) and ocassionaly sciaphilic green algae *Palmophyllum crassum* is present (e.g. Fig. 2.5.4b). This part is also more sheltered and prone to sedimentation of fine particles Fig. 2.5.4a,b).

Furthermore, biocenosis of semi-dark caves develops close to the entrances of the enclosed spaces such as captain's bridge and adjecent utility rooms situated at the rear end of the ship (Fig. 2.5.5a), whereas deeper inside, including on ceilings, a biocenosis of caves and dark passages is present (Fig. 2.5.5.c). These habitats are dominated by encrusting and massive sponges, including *Aplysina cavernicola,* endangered sponge listed in Annex II of the Barcelona Convention and strictly protected species listed in Annex II of the Bern Convention (Fig. 2.5.5.b), as well as hard corals and serpulid polychaetes thriving on the ceilings (Fig. 2.5.5.c).

Besides sessile macrobenthos, mobile invertebrates inhabit the shipwreck, such as attractive nudibranchs, found while feeding and reproducing (Fig. 2.5.6), and Echinodermata such as sea star *Marthasterias glacialis* and sea cucumbers (Fig. 2.5.7 b,c). Morerover, 14 fish species were recorded during a dive on the shipwreck Peltastis (Fig.2.5.8, Appendix 1). All of these are common species associated to the natural infralitoral reefs in the area. In total, 61 species/taxa were recorded during a single dive on a shipwreck Peltastis (Appendix 1).

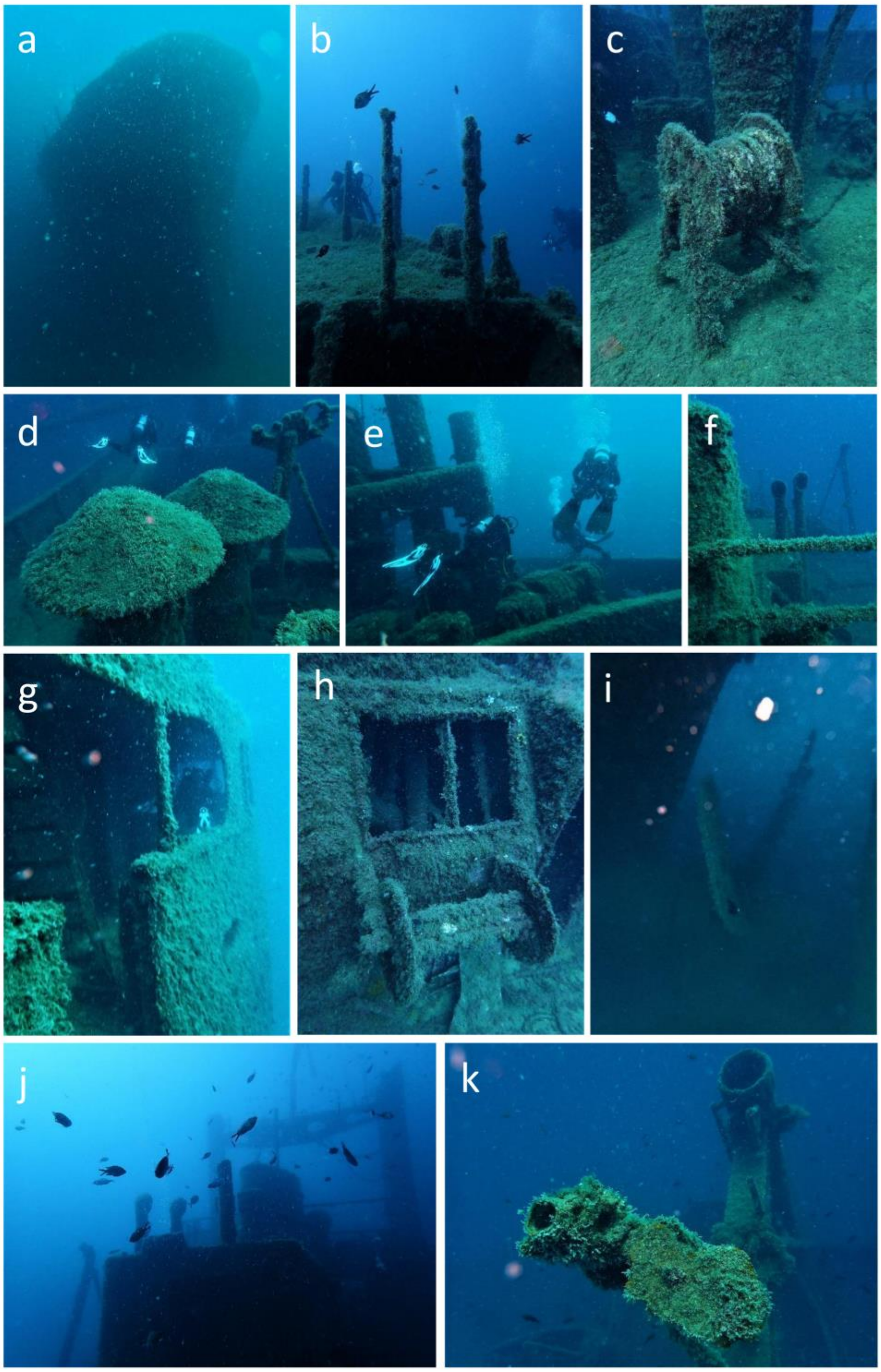

Fig.2.5.1. Shipwreck Peltastis – a premium diving location in the Northern Adriatic and an unintentional artificial reef on the Eastern side of the Krk Island (15 – 33 m depth). From a) to j) details of the wreck are shown from its frontal part towards the rear, and image k) shows the main mast that protrudes up to 6 m depth and usually presents the starting and the end point of a guided dive. Photo credits: S. Kipson.

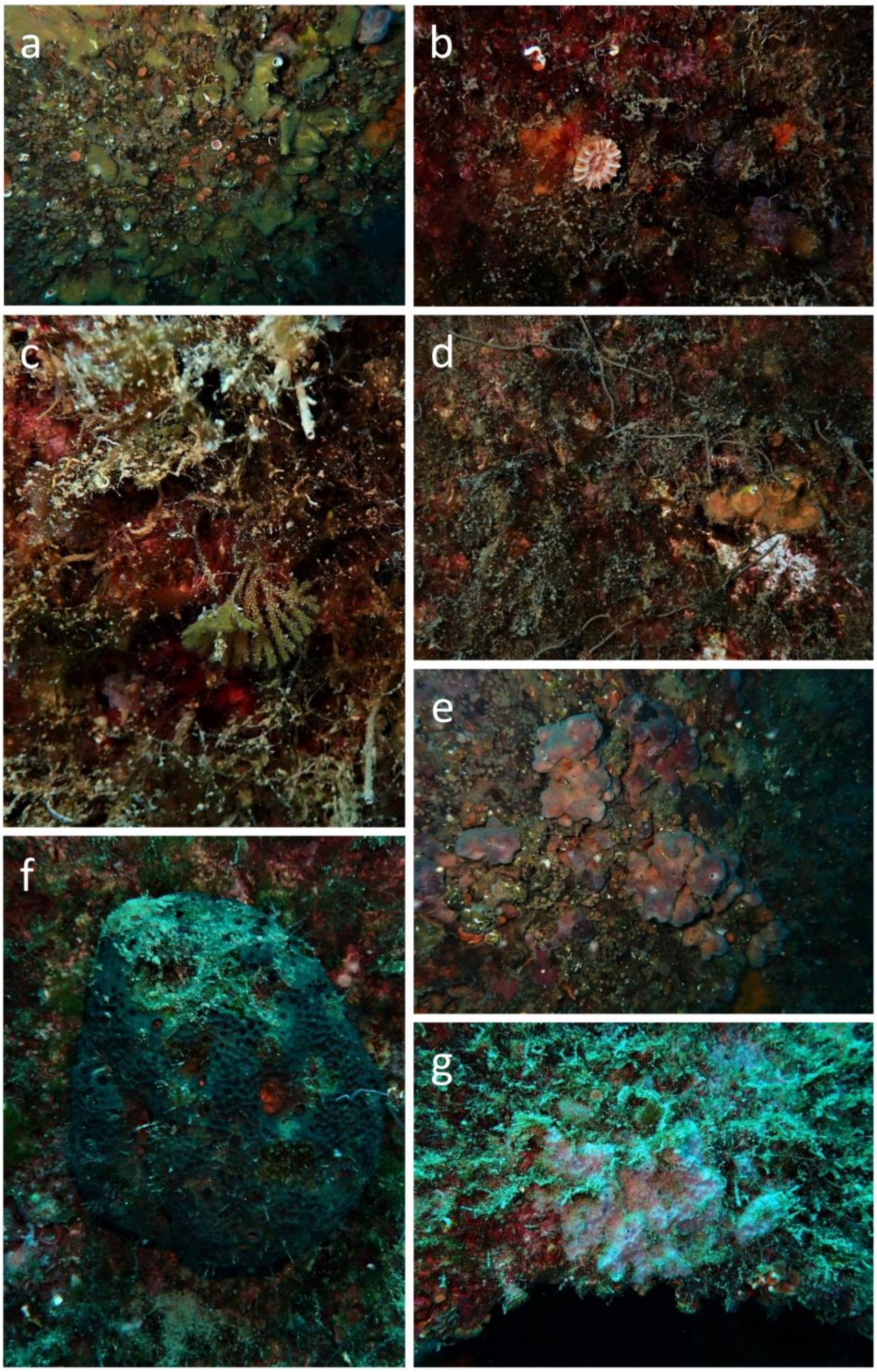

Fig. 2.5.2. Organisms characterizing the sciaphilic biocenosis dwelling on the outer part of the ship's hull from 26 to 30 m depth: a) solitary hard coral *Caryophyllia inornata*, colonial scleractinians, encrusting sponge, vermetid snails and serpulid polychaetes, b) solitary hard coral *Caryophyllia smithii*, c) branchy bryozoan, d) encrusting orange bryozoan and green algae *Valonia* sp., e) massive sponge *Ircinia* sp., f) massive black keratose sponge and g) sponge *Dysidea* sp. Photo credits: S. Kipson.

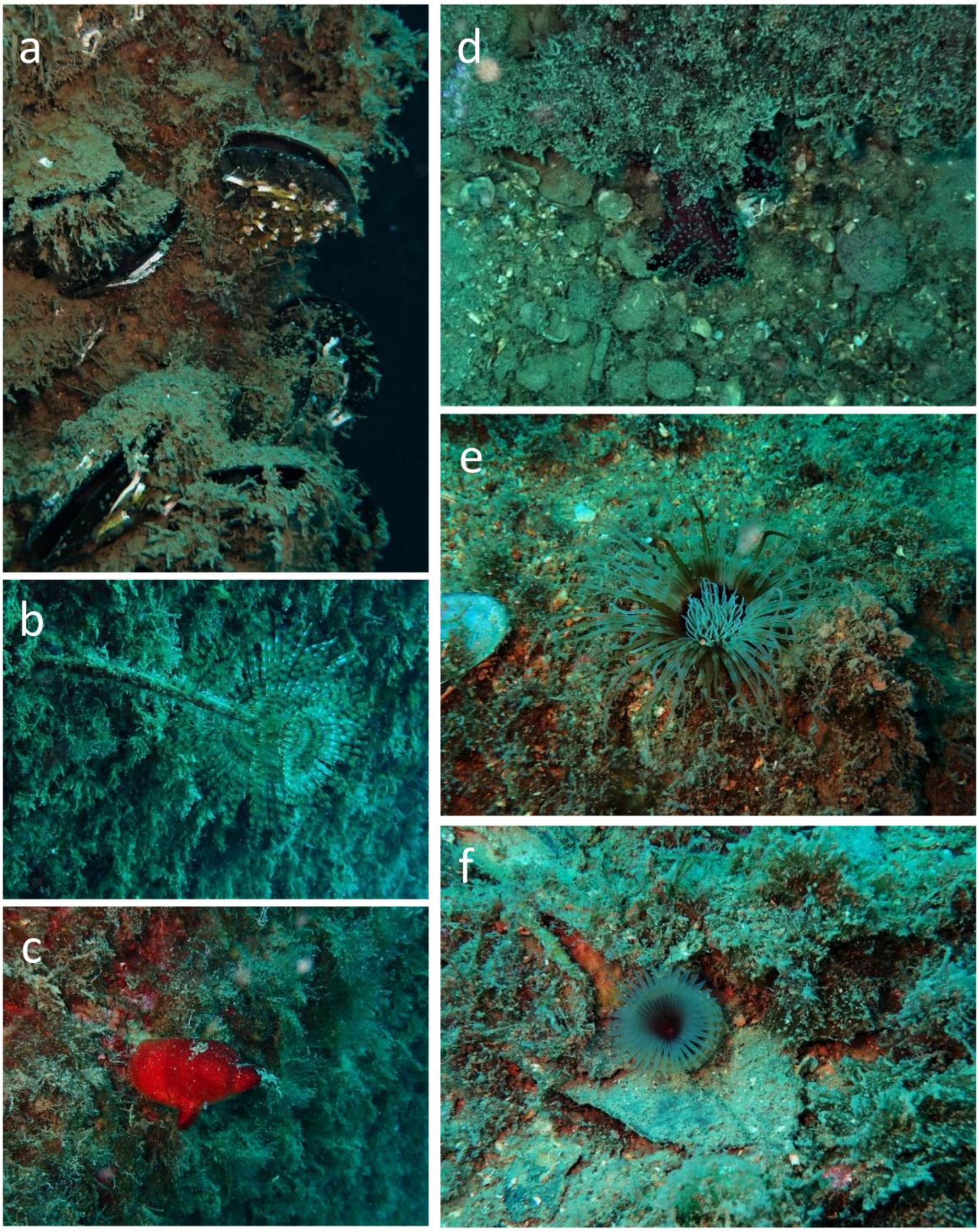

Fig. 2.5.3. On the outer haul of the ship Peltastis many other benthic species typical of hard-bottoms are present such as: a) blue mussel *Mytilus galloprovincialis*, b) polychaete worm *Sabella spallanzanii*, c) solitary tunicate *Halocynthia papilosa and* d) soft coral *Alcyonium acaule* whereas in parts of the wreck where sediment could accumulate, e.g. in parts of the deck typical species of sandy-muddy bottoms could be found such as e) cnidarian *Cerianthus membranaceus* and f) a polychaete *Myxicola infundibulum.* Photo credits: S. Kipson.

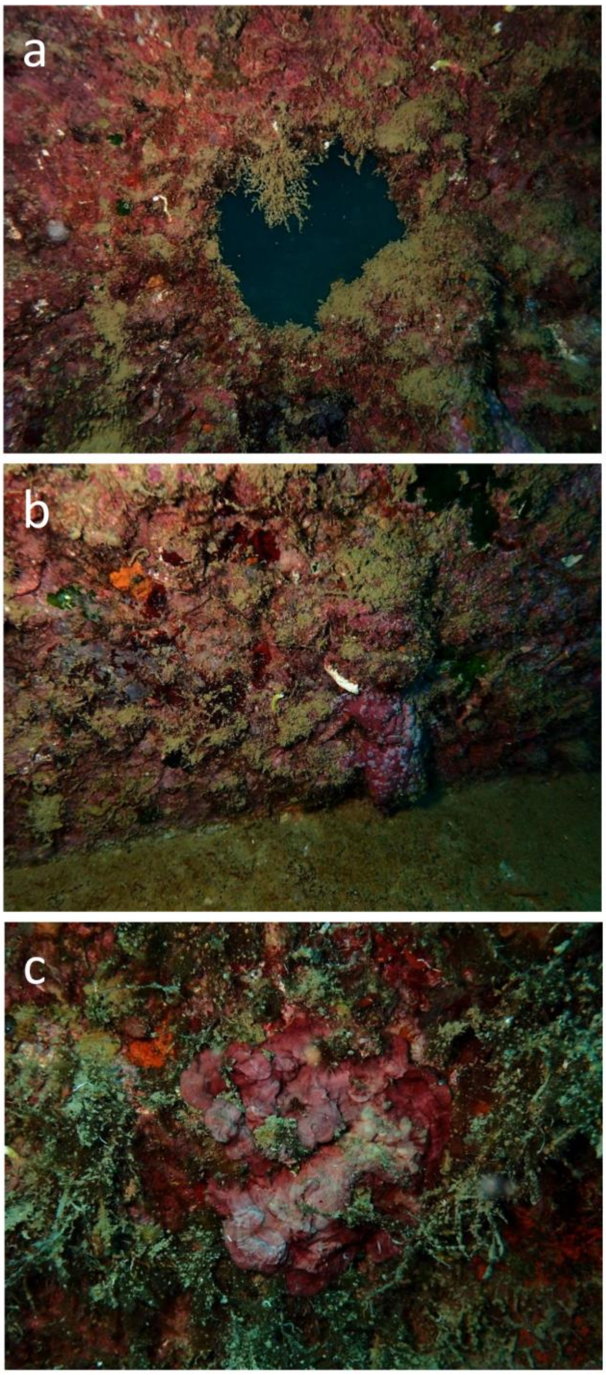

Fig. 2.5.4. Biocenosis on the inner part of the haul, receiving more light but still developing in sciaphilic conditions is dominated by red encrusting algae (a), belonging to order of Corallinales (b) and Peyssonneliales (c). Photo credits: S. Kipson.

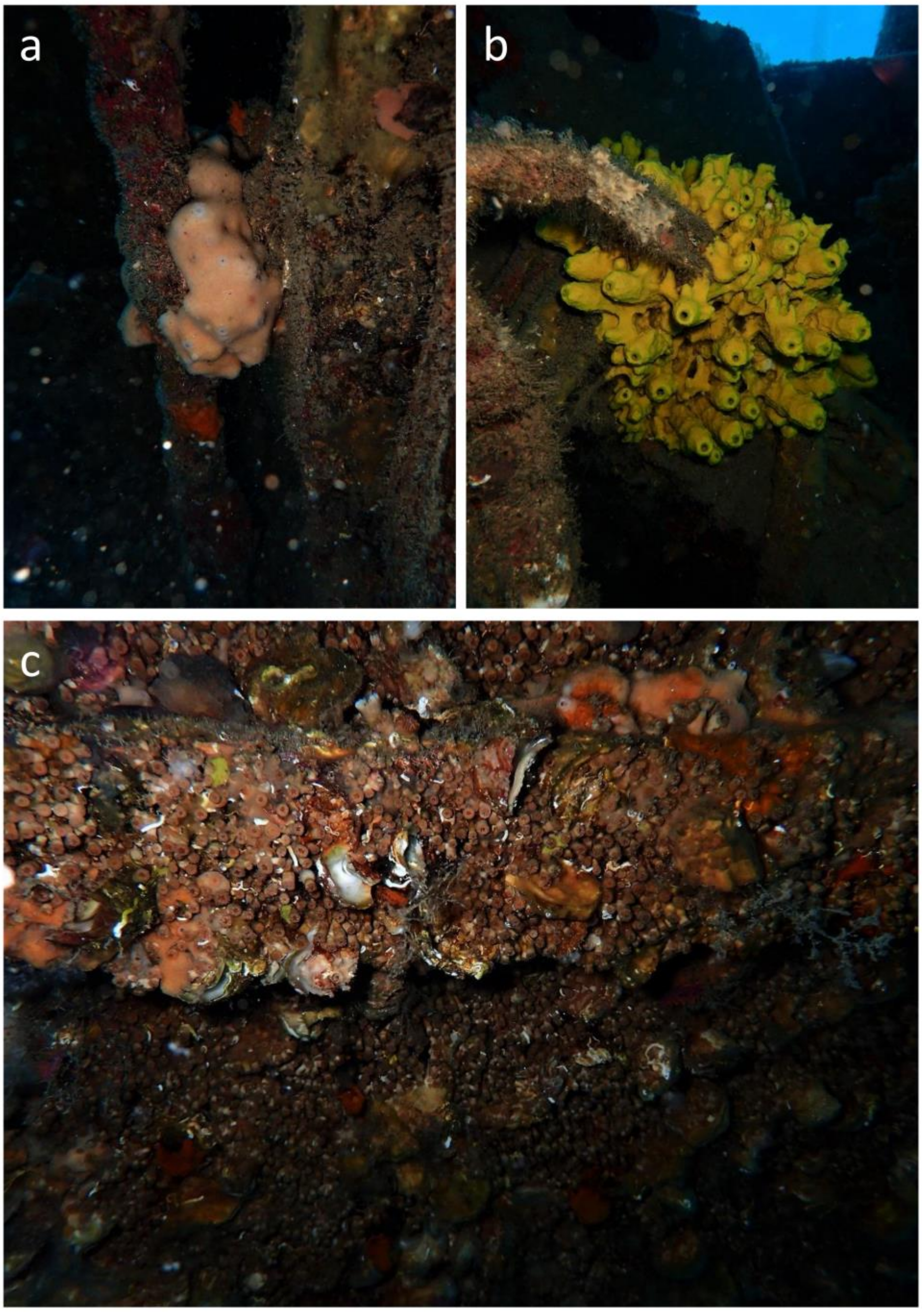

Fig. 2.5.5. Biocenoses of semi-dark caves as well as passages and dark caves develop within the enclosed spaces such as captain's bridge and adjecent utility rooms; they are dominated by encrusting and massive sponges (a), including *Aplysina cavernicola,* an endangered sponge listed in Annex II of the Barcelona Convention and strictly protected species by the Bern Convention (b) as well as hard corals and serpulid polychaetes thriving on the ceilings. Photo credits: S. Kipson.

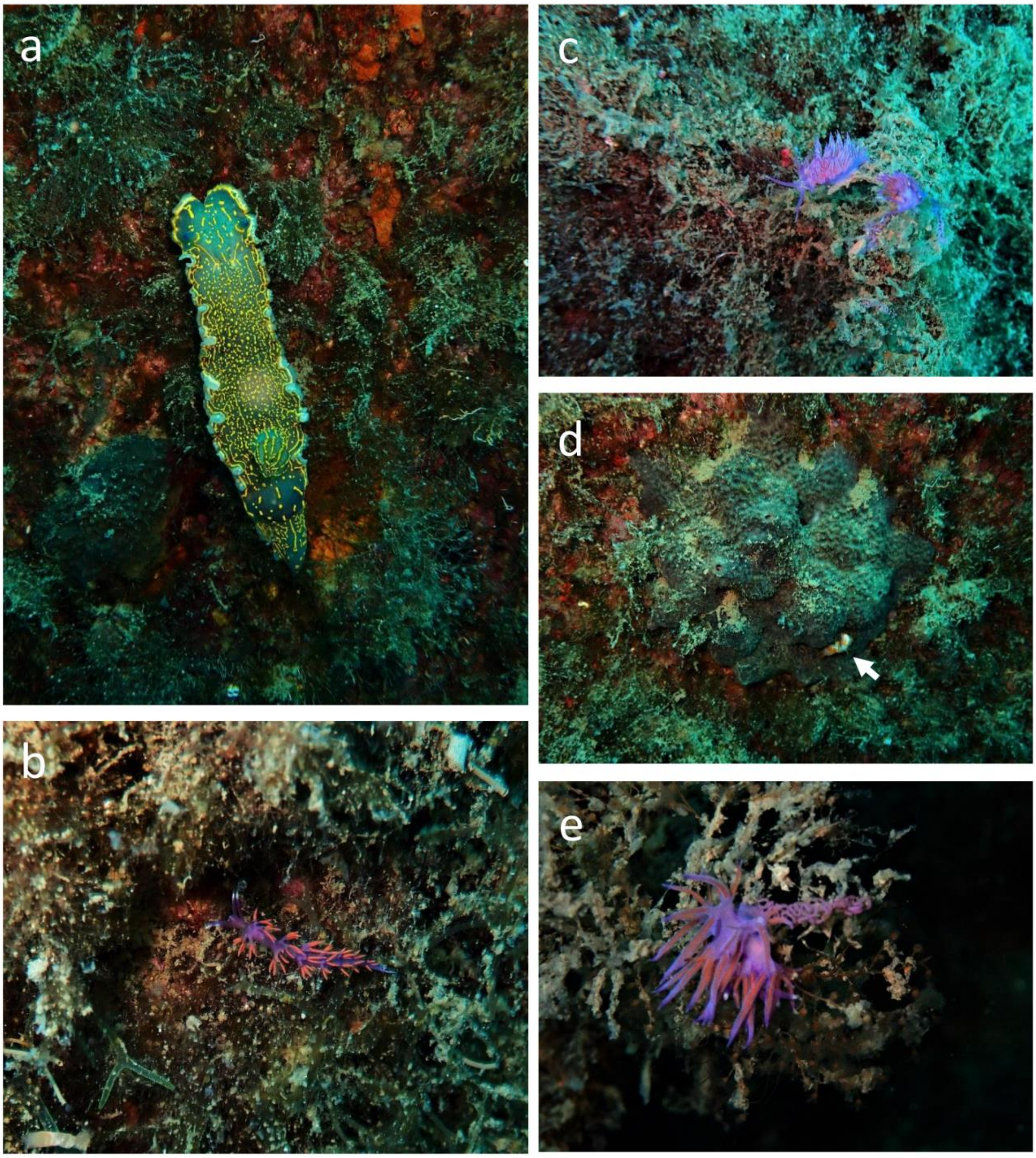

Fig. 2.5.6. Besides sessile macrobenthos, mobile invertebrates inhabit the shipwreck, such as attractive nudibranchs: a) *Felimare picta*, b) *Paraflabellina ischitana*, c) *Flabellina affinis* and d) unidentified white-orange nudibranch, possibly *Trapania maculata.* Some of these mollusks were observed while lying eggs (e). Photo credits: S. Kipson.

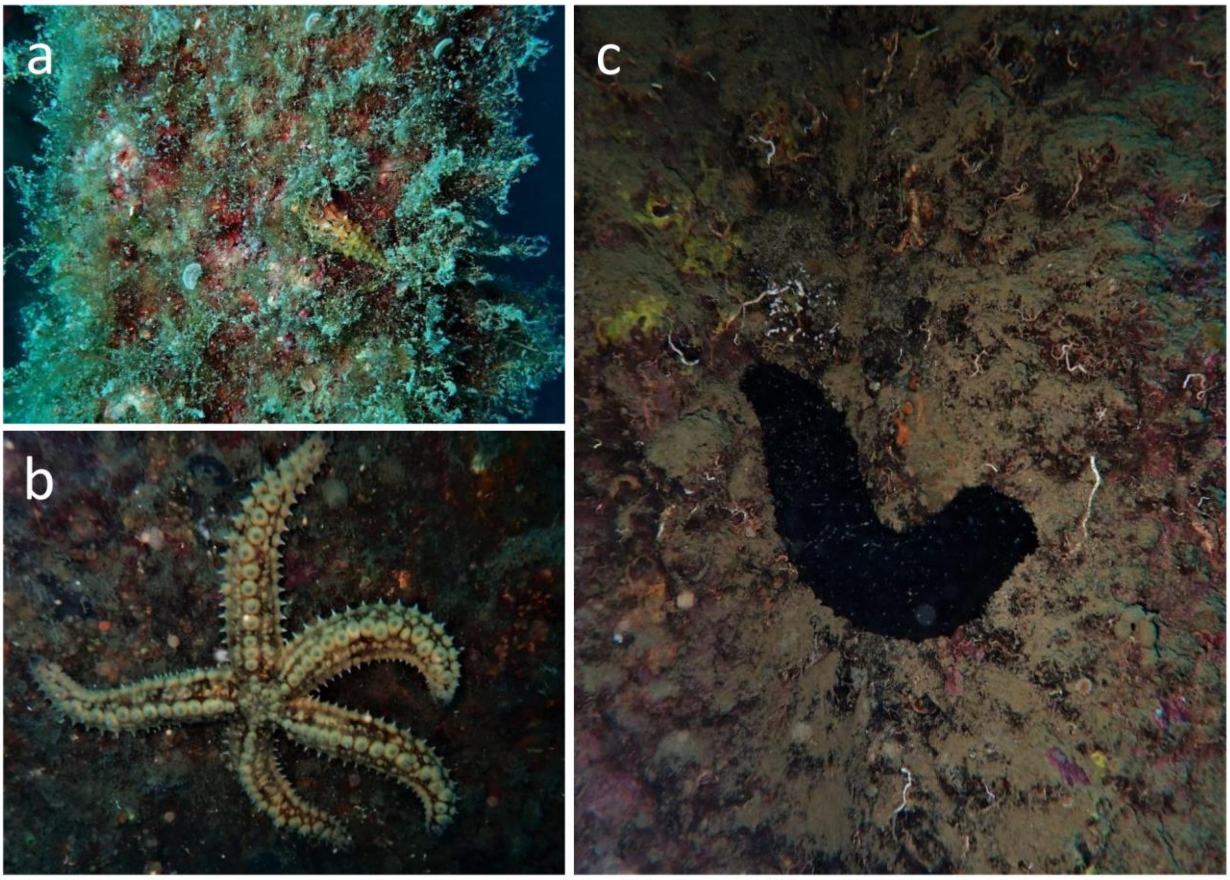

Fig. 2.5.7. Other mobile invertebrates were observed dwelling on the shipwreck such as: a) gastropod *Cerithium* sp., b) sea star *Marthasterias glacialis*, and c) a sea cucumber *Holothuria* sp. Photo credits: S. Kipson.

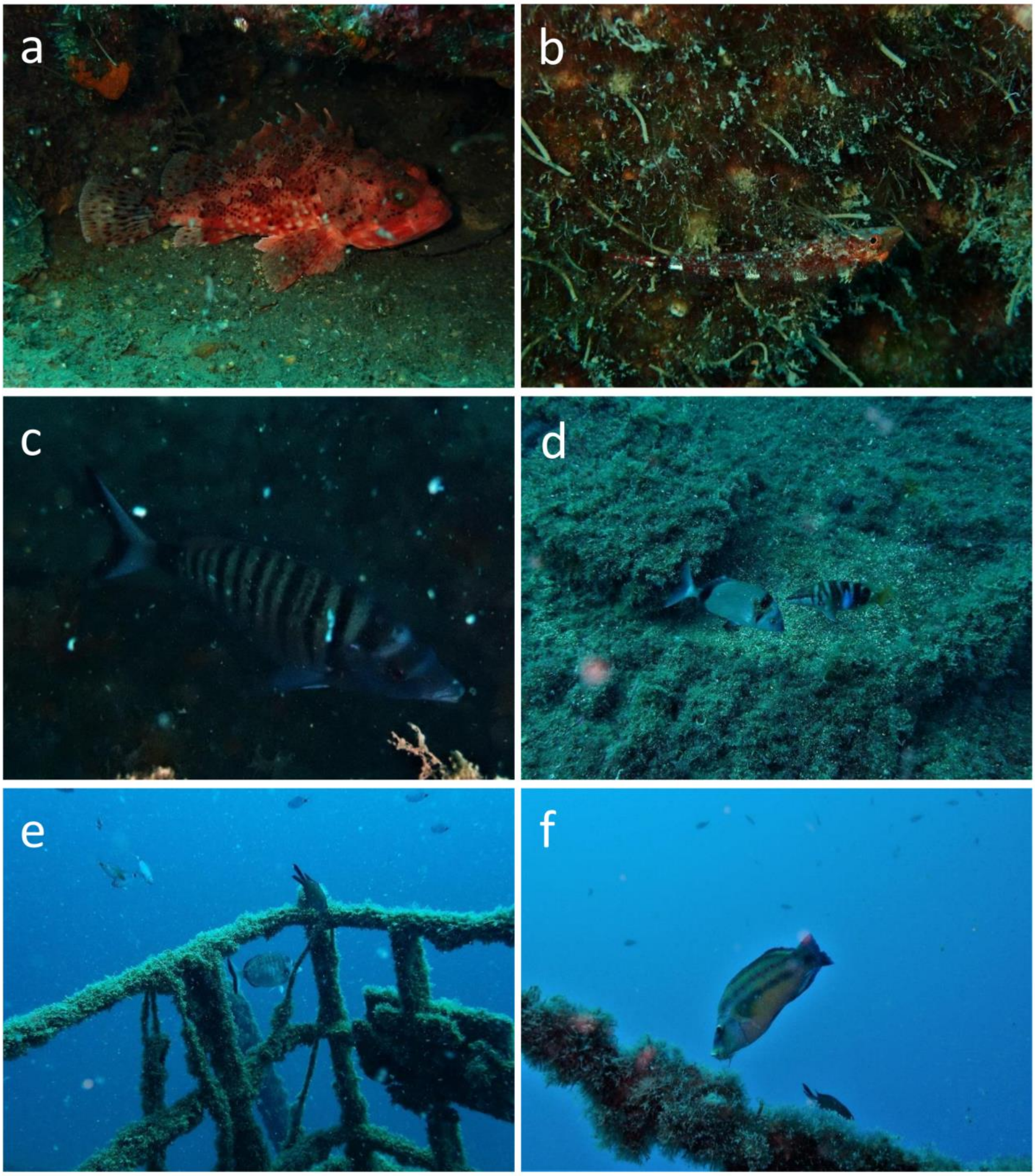

Fig. 2.5.8. Some of fishes observed on the shipwreck Peltastis such as: a) the small red scorpionfish *Scorpaena notata*, b) a female of the yellow black-faced triplefin *Tripterygion delaisi,* c) the sharpsnout seabream *Diplodus puntazzo*, d) the common two-banded seabream *Diplodus vulgaris* and the painted comber *Serranus scriba*, e) the saddled seabream *Oblada melanura* and damselfish *Chromis chromis*, f) a male of the peacock wrasse *Symphodus tinca* collecting algae for the nest construction. Photo credits: S. Kipson.

2.5.3.Conclusions

Without any doubt, the shipwreck Peltastis is one of the premium diving attractions in the Northern Adriatic Sea. Being still well preserved, the wreck also acts as an unintentional artificial reef surrounded by sediment bottom, that provides a variety of additional marine habitats, from well-lit walls and plateaus to dim-lit crevices, overhangs, passages and dark “caves“. This diversity of habitats further attracts a diversity of marine life, which encompasses common species found on the adjecent natural infra- and circalitoral reefs, within overhangs, semi-dark and dark caves as well as species typical of soft-bottom, which found appropriate substrate accumulated in holes and crevices on the horizontal deck. Even some protected species found home within a shipwreck, such as *Aplysina cavernicola,* endangered sponge listed in Annex II of the Barcelona Convention and strictly protected species listed in Annex II of the Bern Convention, thus protected also by the national legislation.

A clear limitation of the created list of species associated to the wreck is that it is based on the results of photographic sampling during a sole dive. There is only a number of species that could be reliably identified based on visual census or photographs (and even those are not likely to be spotted during a single dive, e.g. *Conger conger* is a permanent inhabitant of this shipwreck, however, it was not spotted during our visit). Additional sampling effort would be needed to create a more complete species list, including limited destructive sampling of those organisms that require a physical sample for their determination, such as most sponges, bryozoans, tunicates, red encrusting algae and some hard corals. All these efforts would lead to further evaluation of the Adriatic shipwrecks as unintentional artificial reefs.

Overall conclusions and recommendations

Assessing the additional reef located in the Northern Adriatic was a valuable initiative in the scope of the ADRIREEF project, enabling the comparison of at least some parameters with the two previously examined locations, Lagnići and Stupišće on the Dugi Otok and Vis Islands (Central and South Adriatic), respectively. In that sense, the most notable observation is less pronounced effect of climate change here, as evident from the biological response of indicator organisms such as gorgonians and fish. Unlike on locations in the Central and South Adriatic, no thermophilic alien species such as *Thalassoma pavo* and *Sparisoma cretense* were noted here, and the percentage of affected colonies of the yellow gorgonian *Eunicella cavolini* was two to three times lower than the ones observed at the other two locations. Another valuable input stemming from this study was the notion that damages observed on the sponge *Petrosia ficiformis* are a more widespread phenomenon, as very similar injury/sickness signs were observed here as on the Dugi Otok Island, irrespective of the different time of observation (October 2020 vs. June 2021). This implies a need for a closer follow up of these events and research on their relationship with various abiotic and biotic factors, in order to identify likely causes of the health status deterioration of this species.

The Konjsko shallow was selected randomly by one ADRIREEF project partner as a reef location within the Northern Adriatic and with the purpose to collect data on this site for which no prior information existed in terms of its natural value or its value for recreation and tourism.

Although some of the methods/protocols used so far for the assessment of reefs could be applied here, I do not recommend this location for future monitoring due to:

1. Lack of desireable number of individuals of sessile macroinvertebrates within more narrow depth ranges to apply MME protocol in a standardized manner and to appropriately assess mass mortality events (following Garrabou et al. 2018)
2. Lack of rocky bottom bellow 19 m depth that could host coralligenous community (as an especially valuable and endangered habitat included within priority "1170 Reefs" habitat type by the EU Habitat Directive and hence, an obligation of Croatia as the EU Member

State to monitor and report on) and hence enable assessment of more habitats within a single field visit. Only in such way field work efforts and costs may be optimized.

Other rationale behind investigation of the Konjsko location mentioned to us by a project partner was to explore its potential for boosting local blue economy, as an additional diving site in the area. When it was established that this site lacks attractiveness as a natural diving site (bearing in mind the taste/requirements of an average tourist-diver), some of the ideas how this could be done included development of a sort of artificial “underwater adrenaline park”. In my opinion, the major obstacle for any such use of this site as a diving location is its position in the middle of the navigation route and hence, exposing divers to unecessary safety risk. Moreover, as the idea would be to primarily increase diversity of diving offer of the Crikvenica area, as one of major tourist hubs along the Eastern Adriatic Coast, appropriate locations for the above-mentioned use could be found closer to the city, thus facilitating logistics as well as decreasing time and costs (e.g. of fuel consumption, etc.) needed to reach and return from a destination.

In the future, I would recommend a more thorough consultation and involvement of stakeholders such as local diving centers. A straightforward, one-time conversation with them revealed their actual needs and direction that selection of the ADRIREEF site could have taken in that particular area. For example, in order to increase diversity of diving sites and to enable local dive centers to guide trips even during bora conditions (and hence increase the amount of their operational days), detection of sites along the coast of the Krk Island, between the Cape Šilo and the Krk Bridge (hence, more to the North in comparison to our location Konjsko and more sheltered from bora wind) would be beneficial to them.

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Appendix 1.

List of species/taxonomic groups recorded on 3 shipwrecks in the scope of ADRIREEF project: Michele (NW Dugi Otok Island, 4-6 m depth), Teti (NW Vis Island, 10 – 34 m depth) and Peltastis (E Krk Island,15-33 m depth) based on underwater photos and videos. Note: due to unequal sampling effort, there is no intention to directly compare observed species richness among these shipwrecks – artificial reefs. Green highlights those species recorded so far only on the shipwreck Peltastis.

Taxa	Michele	Teti	Peltastis
CHLOROPHYTA			
branchy green algae		+	+
Caulerpa cylindracea Sonder, 1845		+	
Codium bursa (Olivi) C. Agardh, 1817	+	+	
Flabellia petiolata (Turra) Nizamuddin 1987	+	+	+
Green filamentous algae	+	+	+
Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux 1816	+	+	
Palmophyllum crassum (Naccari) Rabenhorst 1868	+	+	+
Pseudochlorodesmis furcellata (Zanardini) Børgesen, 1925	+	+	
Valonia macrophysa Kützing 1843	+	+	+
OCHROPHYTA			
Dictyotaceae	+	+	+
Padina pavonica (Linnaeus) Thivy, 1960	+	+	+
Taonia sp.		+	
Zanardinia typus (Nardo) P.C. Silva, 2000		+	
RHODOPHYTA			
branchy red algae		+	+
Lithothamnion crispatum Hauck, 1878		+	
Mesophyllum sp.			+
Peyssonnelia polymorpha (Zanardini) F. Schmitz, 1879	+	+	
Peyssonnelia rubra (Greville) J. Agardh, 1851	+	+	
Peyssonnelia sp.	+		+
Rodriguezella strafforelloi F. Schmitz, 1895	+		
Unidentified Corallinales	+	+	+
TRACHEOPHYTA			
Posidonia oceanica (Linnaeus) Delile, 1813	+	+	
FORAMINIFERA			
Miniacina miniacea (Pallas, 1766)	+		+
PORIFERA			
Agelas oroides (Schmidt, 1864)	+		
Aplysina aerophoba (Nardo, 1833)	+		

Taxa	Michele	Teti	Peltastis
Aplysina cavernicola (Vacelet, 1959)			+
black keratose sponge	+	+	+
Chlatrina sp.	+		
Dysidea sp.	+		+
Chondrosia reniformis Nardo, 1847	+	+	+
Cliona schmidti (Ridley, 1881)	+	+	
Cliona sp.	+		
Cliona viridis (Schmidt, 1862)	+		
Crambe crambe (Schmidt, 1862)	+	+	
Haliclona (Reniera) mediterranea Griessinger, 1971		+	
Ircinia dendroides (Schmidt, 1862)	+		
Ircinia oros (Schmidt, 1864)	+		+
Ircinia sp.	+	+	+
Orange encrusting sponge	+	+	+
Petrosia ficiformis (Poiret, 1789)	+		
Phorbas tenacior (Topsent, 1925)	+	+	
Red encrusting sponge			+
Scalarispongia scalaris (Schmidt, 1862)	+		
Spirastrella cunctatrix Schmidt, 1868	+		
Spongia (Spongia) officinalis Linnaeus, 1759	+		
Terpios fugax Duchassaing et Michelotti, 1864	+		
Yellow sponge		+	+
ANTHOZOA			
Alcyonium acaule Marion, 1878			+
Balanophyllia (Balanophyllia) europaea (Risso, 1826)	+		
Caryophyllia inornata (Duncan, 1878)	+		+
Caryophyllia smithii Stokes et Broderip, 1828	+		+
Cerianthus membranaceus (Gmelin, 1791)			+
Leptopsammia pruvoti Lacaze-Duthiers, 1897		+	
colonial Scleractinia			+
solitary Scleractinia	+	+	+
HYDROZOA			
Eudendrium sp.			+
Unidentified Hydrozoa	+	+	+
ANNELIDA			
Eupolymnia nebulosa (Montagu, 1819)		+	
Filograna implexa Berkeley, 1835 / *Salmacina dysteri* (Huxley, 1855)	+	+	+
Hermodice carunculata (Pallas, 1766)	+	+	
Myxicola infundibulum (Montagu, 1808)			+

Taxa	Michele	Teti	Peltastis
Protula sp.		+	+
Sabella spallanzanii (Gmelin, 1791)			+
Sabella sp.	+	+	
Serpulidae	+	+	+
MOLLUSCA			
Cerithium sp.	+	+	+
Felimare picta (Philippi, 1836)			+
Flabellina affinis (Gmelin, 1791)			+
Hexaplex trunculus (Linnaeus, 1758)	+		
Paraflabellina ischitana (Hirano & T.E. Thompson, 1990)			+
Peltodoris atromaculata Bergh, 1880	+		
Rocellaria dubia (Pennant, 1777)	+	+	+
Spondylus gaederopus Linnaeus, 1758	+		
White-orange nudibranch cf. *Trapania maculata* Haefelfinger, 1960			+
Vermetidae	+	+	+
ARTHROPODA (CRUSTACEA)			
Chthamalus *sp.*	+		
BRYOZOA			
black encrusting bryozoan	+	+	
Branchy bryozoan		+	+
Dentiporella sp. / *Schizomavella* sp.		+	
Encrusting beige bryozoan	+	+	
Encrusting orange bryozoan	+	+	+
Myriapora truncata (Pallas, 1766)		+	
Schizobrachiella sanguinea (Norman, 1868)		+	
Reteporella sp.		+	
Scrupocellaria sp.		+	+
Smittina cervicornis (Pallas, 1766) / *Adeonella pallasii* (Heller, 1867)		+	+
ECHINODERMATA			
Arbacia lixula (Linnaeus, 1758)	+		
Echinaster (Echinaster) sepositus (Retzius, 1783)	+		
Hacelia attenuata Gray, 1840		+	
Holothuria sp.	+		+
Marthasterias glacialis (Linnaeus, 1758)			+
Paracentrotus lividus (Lamarck, 1816)	+		
Peltaster placenta (Müller & Troschel, 1842)		+	
Ophidiaster ophidianus (Lamarck, 1816)		+	
Sphaerechinus granularis (Lamarck, 1816)		+	
CHORDATA (TUNICATA)			

Taxa	Michele	Teti	Peltastis
Aplidium tabarquensis Ramos-Espla, 1991	+	+	
Aplidium sp.		+	
Halocynthia papillosa (Linnaeus, 1767)	+	+	+
Pyura sp.		+	
CHORDATA (PISCES)			
Apogon imberbis (Linnaeus, 1758)		+	
Chromis chromis (Linnaeus, 1758)	+	+	+
Coris julis (Linnaeus, 1758)	+	+	+
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)	+	+	+
Muraena helena Linnaeus, 1758		+	
Parablennius rouxi (Cocco, 1933)		+	
Phycis phycis (Linnaeus, 1766)		+	
Scorpaena notata Rafinesque, 1810			+
Scorpaena scrofa Linnaeus, 1758	+	+	
Serranus cabrilla (Linnaeus, 1758)		+	
Spicara maena (Linnaeus, 1758)		+	
Serranus hepatus (Linnaeus, 1758)			+
Serranus scriba (Linnaeus, 1758)	+	+	+
Diplodus annularis (Linnaeus, 1758)	+	+	+
Diplodus sargus sargus (Linnaeus, 1758)		+	
Diplodus puntazzo (Walbaum, 1792)			+
Boops boops (Linnaeus, 1758)		+	
Oblada melanura (Linnaeus, 1758)			+
Sparisoma cretense (Linnaeus, 1758)		+	
Tripterygion delaisi Cadenat & Blache, 1970			+
Tripterygion tripteronotus (Risso, 1810)			+
Thalassoma pavo (Linnaeus, 1758)		+	
Labrus merula Linnaeus, 1758		+	
Symphodus cinereus (Bonnaterre, 1788)			+
Symphodus mediterraneus (Linnaeus, 1758)		+	+
Symphodus tinca (Linnaeus, 1758)		+	+
Spondyliosoma cantharus (Linnaeus, 1758)		+	
Centrolabrus melanocercus (Risso, 1810)		+	