

Improving knowledge of *Funiculina quadrangularis* and vulnerable marine ecosystems in the south Adriatic

Vesna MAČIĆ¹, Nikola ĐORĐEVIĆ¹, Mirko ĐUROVIĆ¹, Slavica PETOVIĆ¹ and Tommaso RUSSO^{2,3}

¹ Institute of Marine Biology, University of Montenegro, Put I Bokejske brigade 68, 85330 Kotor, Montenegro

² Laboratory of Experimental Ecology and Aquaculture, Department of Biology, University of Rome Tor Vergata, Rome, Italy

³ Consorzio Nazionale Interuniversitario Per Le Scienze Del Mare, Rome, Italy

Corresponding author: Vesna MAČIĆ; macic.v@ucg.ac.me

Contributing Editor: Vasilis GEROVASILEIOU

Received: 15 March 2022; 26 July 2022; Published online: 06 October 2022

Abstract

The Adriatic Sea is one of the most exploited areas in the Mediterranean; however, a large part of the South Adriatic Sea remains largely unexplored. Unfortunately, direct and indirect anthropogenic impacts are increasing exponentially, causing a loss of flagship species and threatening ecosystem functioning. This has led to several international conventions demanding better protection and management of sensitive species and areas worldwide. Soft mud facies bearing the sea pen *Funiculina quadrangularis* are indicators of vulnerable marine ecosystems (VMEs). This study aimed a) to contribute to the knowledge on the distributions of *F. quadrangularis* and VMEs, and b) to assess the main threats, thus enabling better identification of areas that need protection and ecosystem base management. The results show that *F. quadrangularis* are distributed from 160 m to 400 m depth, with the highest population density of 0.83 colony/m² reported in the Adriatic Sea at a 162 m depth. However, using video ground truthing, we registered stresses on deep-sea habitats originating from fishery and marine litter. Further research is needed to ascertain other potentially vulnerable areas. Moreover, international discussion is needed to determine future steps for the protection and sustainable use of resources beyond national jurisdictions.

Keywords: *Funiculina quadrangularis*; Vulnerable marine ecosystems; ROV; marine litter; Adriatic Sea.

Introduction

It is widely acknowledged that some marine species play crucial roles in providing ecosystem services and supporting biodiversity. To date, six of the 12 Mediterranean sea pen species have been recorded (Bastari *et al.*, 2018) in the Adriatic Sea, which is one of the most affected regions worldwide in terms of trawling (Amoroso *et al.*, 2018). These species play important ecological roles: they can create three-dimensional complexity (De Clippele *et al.*, 2015; Bastari *et al.*, 2018) in homogenous muddy habitats; act as substrates and refuges for eggs, larvae, juvenile fishes, and invertebrates; alter water current flow; increase food delivery; and decrease predation rates for closely associated species living in sea pen meadows with dense aggregations (Bastari *et al.*, 2018).

Unfortunately, basic information on sea pen ecology and distribution has only recently become available, and the deep sea (200 m depth) (despite being the largest Earth's ecosystem) is still largely unexplored (Greathead *et al.*, 2007; 2015; Wright *et al.*, 2015; Lauria *et al.*, 2017; Bastari *et al.*, 2018). However, these large pe-

lagic and deep-sea habitats are experiencing increasing cumulative anthropogenic impacts, both direct and indirect (Buhl-Mortensen *et al.*, 2010; Eigeerd *et al.*, 2017; Danovaro *et al.*, 2020). Sea pens (family Pennatulidae) and their habitats have been declining in the Adriatic Sea and worldwide because of the negative impacts of bottom trawling (Salvati *et al.*, 2014). The four major threats to deep-sea ecosystems are deep-sea fisheries, hydrocarbon extraction and mining, climate change, contaminants and litter (Danovaro *et al.*, 2020). Owing to the different international conventions (such as the OSPAR and Barcelona Convention), organisations like the United Nations (UN), International Council for the Exploration of the Sea (ICES), General Fisheries Commission for the Mediterranean (GFCM), European Commission (EC), and International Union for Conservation of Nature (IUCN) are demanding better protection and management of sensitive species and areas (UNEP/MAP-RAC/SPA, 2015; GM MESPU, 2020).

Organisms in deep seas are usually characterised by low productivity, low fecundity, reaching maturity at older age, and high longevity, which makes them more

sensitive to various anthropogenic pressures (Fabri *et al.*, 2014). In addition, knowledge about the pristine conditions of many deep-sea regions is missing, which is an obstacle to the assessment of their sustainable use. However, some parts of the Adriatic Sea are relatively better explored areas of the Mediterranean, and unfortunately, a comparison with historical data has shown dramatic changes over the last 40 years in this region (Boero & Bonsdorff, 2007). Moreover, the loss of many flagship species and key functions of endangered ecosystems without any sign of recovery is adversely influencing not only the Adriatic but also the entire marine ecosystem (Boero & Bonsdorff, 2007; Danovaro *et al.*, 2020). Considering the importance of marine ecological services, the United Nations adopted (among others) resolution 61/105 to protect vulnerable marine ecosystems (VME) (Rogers & Gianni, 2010). Therefore, deep-sea bottom trawlers in the Mediterranean Sea are forbidden from going beyond the depth of 1000 m, and trawl fishing in the Jabuka (Pomo) pit in the Adriatic Sea has been prohibited (Thompson *et al.*, 2016; GFCM, 2017) since 2017. The GFCM has also issued a list of criteria for the identification of the sensitive habitats of relevance for the management of priority species in the Mediterranean Sea (GFCM SAC, 2008) and soft mud facies harbouring sea pens is one of them.

The tall sea pen *Funiculina quadrangularis* (Pallas, 1766) is a colonial cnidarian belonging to the subclass Octocorallia. Individuals, usually white in colour, can be up to 2 m in length. Polyps are situated on a calcareous axial rod that is square in section and is anchored by a peduncle at the base (Greathead *et al.*, 2007; Trainito & Baldacconi, 2016). Nearly a quarter of the axis remains embedded in the sediment. The organism inhabits sheltered muddy substrates between 20 and 2000 m depth, spanning from the North Atlantic, Gulf of Mexico, and

the Mediterranean Sea to New Zealand and Japan (Wright *et al.*, 2015).

In the Mediterranean Sea, soft mud habitats with *F. quadrangularis* also harbour two additional species of great economic importance, i.e., the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) and the rose shrimp *Parapenaeus longirostris* (H. Lucas, 1846). Often, such habitats are under intensive bottom-trawling pressure (Martinelli *et al.*, 2013). Moreover, the European Commission (EU, 2006) reported that beds of *F. quadrangularis* and *Isidella elongata* (Esper, 1788) in the Mediterranean have largely disappeared from many areas due to bottom trawling. Therefore, mapping of *F. quadrangularis* could serve as an indicator of the state of these vulnerable marine ecosystems (VMEs) and also contribute to the assessment of the distribution of VMEs in the Mediterranean. Moreover, targets of the European Marine Strategy Framework Directive (EU Directive 2008/56/EC) towards achieving and maintaining good environmental status cannot be achieved without an initial assessment of deep-sea benthic ecosystems and evaluation of the types and intensities of major threats therein.

To date, only two reports of *F. quadrangularis* in the south-east Adriatic Sea have been published. It was first observed during the MEDITTS survey 2011 (Petović *et al.*, 2016) on the soft-bottom depth stratum (200–500 m), and the population density was reported to be very low, i.e., 5.22 colonies/km² (Fig. 1). The second available report of this species in the south-east Adriatic Sea comes from a basic study for the environmental impact assessment of offshore exploratory wells in Montenegro (ENI, 2019), where an enclave of facies of soft muds with *F. quadrangularis* and *Aporrhais serresiana* (Michaud, 1828) were reported in a trawling-affected area. The aims of this study are to contribute to the existing knowledge of the

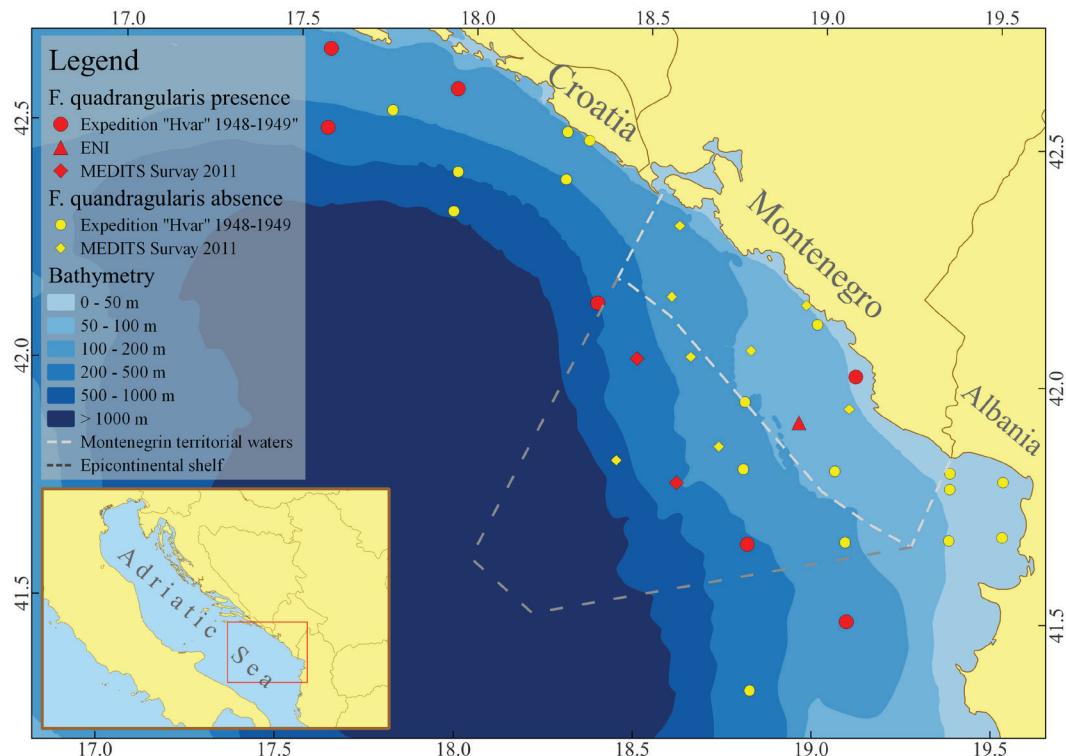


Fig. 1: *Funiculina quadrangularis* distribution map (published and “new” historic data from expedition “Hvar” (1948-1949).

distribution of the sea pen *F. quadrangularis* in relation to VMEs; to assess the major threats and to better identify areas requiring protection.

Materials and Methods

Study area and seabed habitat

The study area was the south-east Adriatic Sea (FAO Geographical Sub-Area 17; more precisely the Montenegrin territorial waters and epicontinental shelf), with a total area of ~6350 km². All available data on biotic and abiotic factors for the national waters of Montenegro as well as for the epicontinental shelf under Montenegrin jurisdiction were collected under the framework of the project GEF Adriatic, and surface areas of different habitats were calculated. Additional available data were included in the GIS database and provided as a baseline for further analysis (GM MESPU, 2020).

Historical data

One of the most important expeditions on the fishery resources in the Adriatic was the “Hvar” expedition performed during 1948–1949. Data from catch analyses spanning 28 sampling stations were entered on to data sheets; these included information not only on fish and edible invertebrates but also on the bycatch species and their approximate abundance (Ikica *et al.*, 2021). All original data were analysed for the presence of *F. quadrangularis*. Findings of *F. quadrangularis* from the “Hvar” expedition together with other available data for the south-eastern Adriatic Sea are presented in Figure 1.

Video collection

To lay down an underwater electric cable connecting Italy and Montenegro, various activities were carried out both on land and at sea. After the electric cable was laid down on the seafloor in 2017, it was buried (0.5–1 m) in the sea bottom (within a section deeper than 20 m). This was carried out with the help of a special Merlin WR200 ROV which deepens the channel in the sandy-muddy bottom with strong jets of seawater and buries the cable therein. The ROV had few HD cameras affixed on it for monitoring the realization of the planned work; HD cameras record at 1080p resolution with 25 fps. These recordings enabled the surveying of the seabed from a depth of 30 to 400 m and that includes a 46.2 km long transect.

Video analysis

Due to the low visibility in many parts of the recorded video material and the precautions taken to comply with ICES protocol (ICES, 2011), it was not possible to identify many organisms; therefore, we used video material

covering a total length of 14.2 km. *Funiculina quadrangularis* is a species that can be easily recognised; therefore, this study was limited to its populations. Analysis of the number of *F. quadrangularis* on the videos was performed by three readers. The camera placed on the top of the ROV recording the path of the laid cable (which was in the middle of the screen) was used to analyse the number of *F. quadrangularis* on videos. From the video, one frame was extracted and imported into ZEISS ZEN 3.2 software (2020). Using a simple proportion and the known width of the cable, we calculated the width of the filmed area. Thereafter, the width of the study area was multiplied by the length of the path, which was calculated from the latitude and longitude received from the Merlin WR200 ROV. At the start and end of each video, coordinates were written down and exported as CSV files that were later imported into QGIS software (2022), which calculated the distance covered between the start and end of the video. The average density was calculated for each video (track usually 140 m long) and entered into the GIS database.

Together with the assessment of *F. quadrangularis* abundance, the quantity of marine litter and marks of fishing pressure on seafloor integrity were evaluated using the ROV data. In particular, data on the amount, type, and spatial distribution of marine litter on the seabed in the Adriatic Sea were collected. According to the MEDITS protocol (MEDITS-Handbook. Version n. 8, 2016), marine litter is of six basic categories (plastic, rubber, textile, metal, glass/ceramics, and other). This classification was followed, and the average density by category was calculated for each video track and entered into the GIS database.

Fishing effort

The fishing effort deployed by trawlers operating in the area of interest was estimated using a suite of modelling approaches on automatic information system (AIS) data. AIS is an automatic tracking system used by vessel traffic services for safety purposes, but it has quickly become a source of information for the activities of fishing fleets (Russo *et al.*, 2016). The R-package VMSbase (Russo *et al.*, 2014, 2019) was used to process the AIS data. The workflow comprised the following steps: (1) data cleaning, (2) tracking (fishing trips by vessel) identification, (3) interpolation and standardisation of the AIS pings frequency, and (4) classification of fishing activities with respect to the standard EU classification (EU, 2016), cross-integrating with additional resources, including bathymetric databases and fleet registers (which report the characteristics of each fishing vessel). This procedure provided information on the fishing positions (without steaming or other activities) of trawlers operating in the area of interest. These fishing positions were spotted over a 1 × 1 km grid comprising 7459 cells.

The total annual fishing effort over the period 2016–2020 was then computed by aggregating fishing position by cell, year, and calendar date to obtain the number of

fishng days (as a measure of the effort). Finally, the mean annual fishing effort for the same period was computed and used for subsequent analyses.

Modelling of *Funiculina abundance and its drivers*

Generalised additive models (GAMs) (Hastie & Tsibarani, 1990) were used to model the trends for and the relationships between *F. quadrangularis* and the following variables (predictors):

- sea bottom depth;
- density of plastics items (number of objects per area);
- density of metal items (number of objects per area);
- fishing effort (mean total annual trawling effort in days during the period 2016–2020);
- sea bottom habitats according to the EUNIS classification (GM MESPU, 2020)

GAMs are non-parametric regression techniques that allow the modelling of relationships between variables without specifying any particular form for the underlying regression function. The use of smooth functions as regressors gives GAMs greater flexibility than parametric (including linear) types of models. The R “mgcv” library (Wood, 2021) implements cubic regression splines function to model the smoothness of predictors. Residuals for all models were graphically inspected to detect eventual departures from the model assumptions or other anomalies in the data (Cleveland, 1993). The model outputs were evaluated by comparing the predictions and observations graphically.

Results

Analysis of videos and historical data

An important contribution to the knowledge of the distribution of *F. quadrangularis* before the mass exploitation during the second half of the XX century is the yet-unpublished historic data originating from the “Hvar” expedition (1948–1949). Positions of the trawling surveys recorded during this expedition are shown in Figure 1. Out of the 28 trawling positions, *F. quadrangularis* was found in only seven (25% of the cases). Clay or sandy-clay bottoms were reported from all these seven locations wherein *F. quadrangularis* was found. The highest number of individuals was found at 196–204 m depth in Montenegrin waters (six individuals), while at other locations, only one individual was reported or the species lists did not contain additional information.

The most important finding of *F. quadrangularis* is along the submerged electrical cable. Individuals of this species were recorded from 160 m to 400 m depth, but the highest densities were recorded from 162 m to 214 m (Fig. 2). In total, we observed 2515 colonies along 14.2 km long transects. Two areas of an exceptionally high density of *F. quadrangularis* were found at 162 m and 210 m depths with a maximum density of 0.83 col-

onies/m². According to the SPA/RAC–UN Environment/MAP (2019) habitat classification, these specimens were inhabiting mostly MD6.51 Offshore terrigenous sticky muds and ME6.51 Upper bathyal muds (Fig. 2).

Moreover, along the surveyed transect, we observed 397 items of plastic and 22 items of metal marine litter (Fig. 3); the mean seafloor litter density was 5.72 items/km². The highest concentration of marine litter was found at a depth range of 310–350 m where marine litter aggregated in the channel approximately 0.5 m deep. Other areas with the highest concentration of marine litter were closer to the coast, and marine litter temporarily aggregated along the electrical cable. Higher concentrations of plastic marine litter were observed, especially in the coastal part up to 200 m depth, and this type of litter consisted mostly of plastic bags and bottles, whereas almost all metal items were cans (beverage). Rubber and textiles were not found, and we recorded only a few glass items. Therefore, the quantity and distribution of only the plastic and metal items are presented.

Fishing pressure

Along the surveyed transect, we observed 1064 marks on the disturbed sea bottom (Fig. 4). Moreover, analysis of the AIS data allowed the reconstruction of the activity of 87 trawlers operating in this area. The mean fishing footprint of this fleet, along with the limits of the Montenegrin territorial waters and the main bathymetries, is represented in Figure 4. According to our visual observations along the surveyed transect and the AIS data, the fishing effort is mainly concentrated from 200 m to 500 m, which is outside the Montenegrin territorial waters (Fig. 5).

Modelling of *F. quadrangularis abundance and its drivers*

The application of the GAM allowed us to obtain the results summarised in Figure 6 and Table 1. GAM captured over 84% of the observed variability in the density of *F. quadrangularis* along the transect surveyed by the ROV. There are a few points for which the observed densities are lower than the predicted densities, but the overall pattern indicates a good fit. All predictors, except for habitats, were significantly associated with the response variable (i.e. the density of *F. quadrangularis*); however, sea bottom depth had a negative and linear effect on *F. quadrangularis*. Conversely, the number of plastic items at the sea bottom is positively related to the density of *F. quadrangularis* (see Discussion). The number of plastic items has non-linear correlation but shows substantial negative effects. Finally, trawling effort is associated with a more complex effect which is positive for low or medium values of effort but negative for high values.

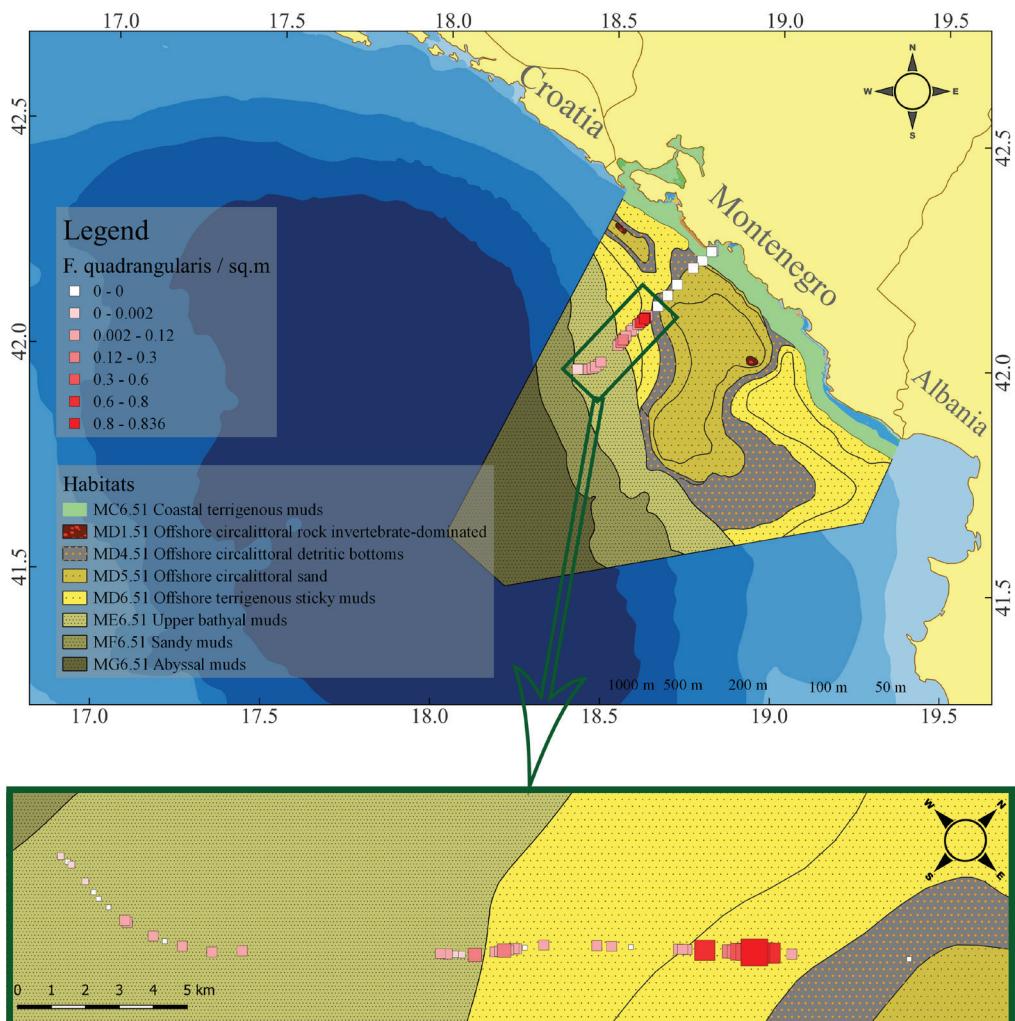


Fig. 2: *Funiculina quadrangularis* findings along surveyed transect (note: legend of habitats is presented only for areas below 30 m depth).

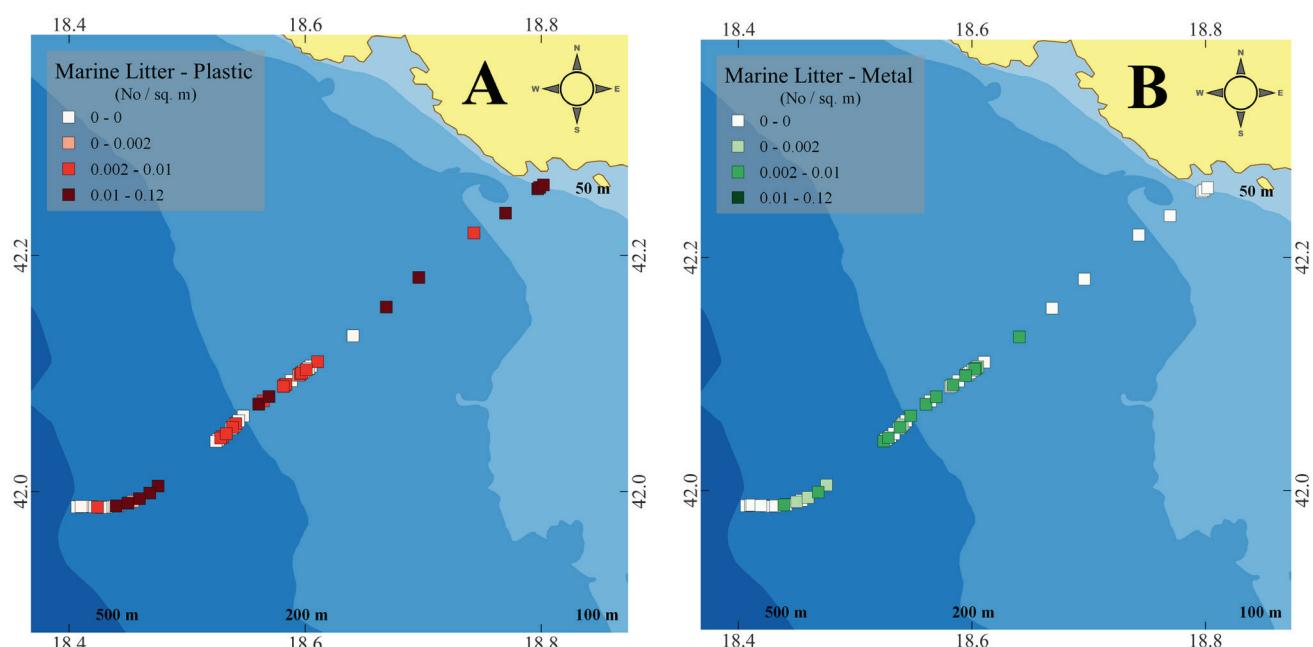


Fig. 3: Distribution of A) Plastic marine litter, B) Metal marine litter.

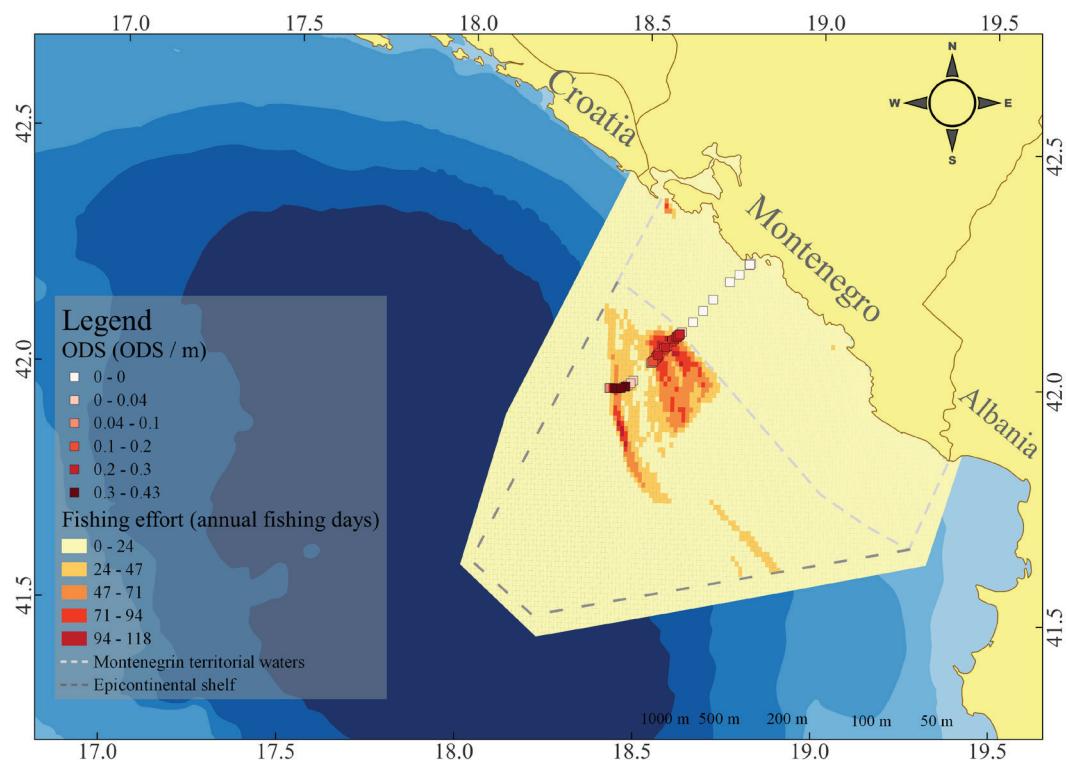


Fig. 4: Mean yearly fishing effort 2016-2020 (in annual fishing days) and number of observed disturbances of the seafloor (ODS) (in ODS per meter of surveyed transect).

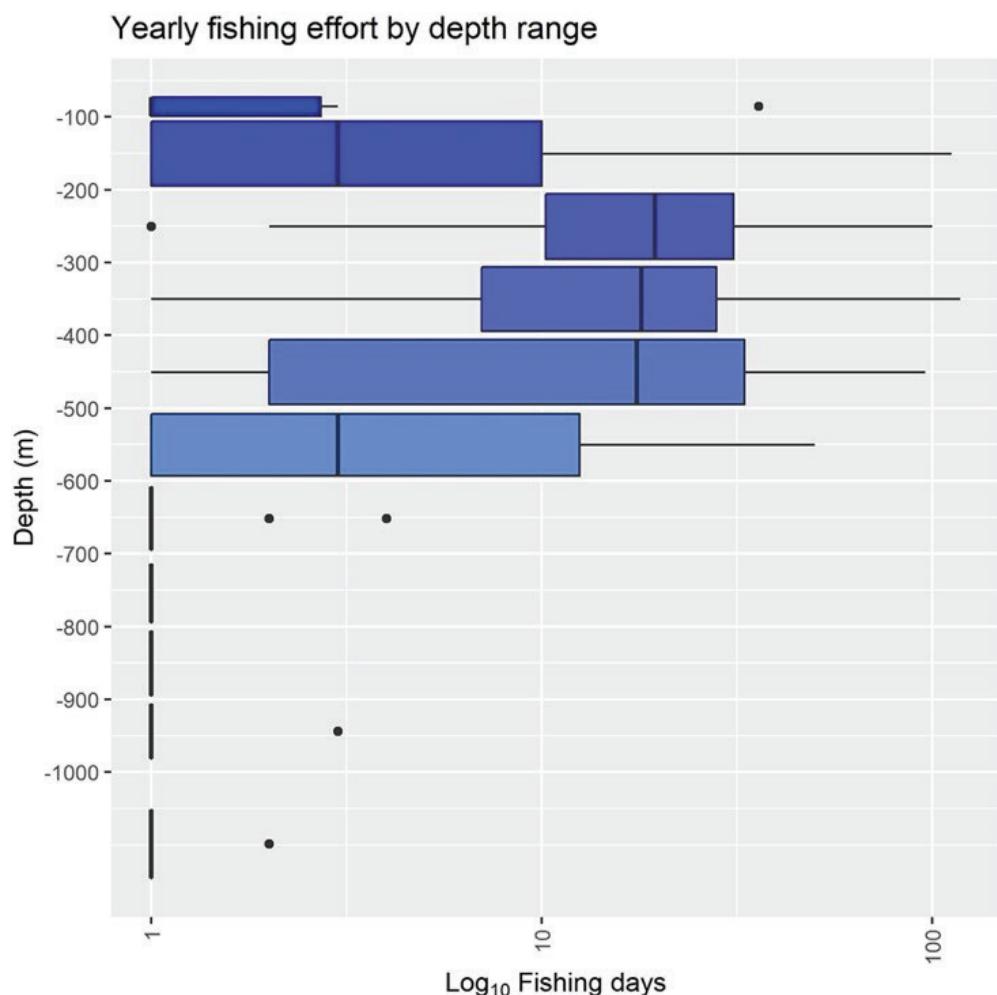


Fig. 5: Yearly fishing effort by depth range present in the study area.

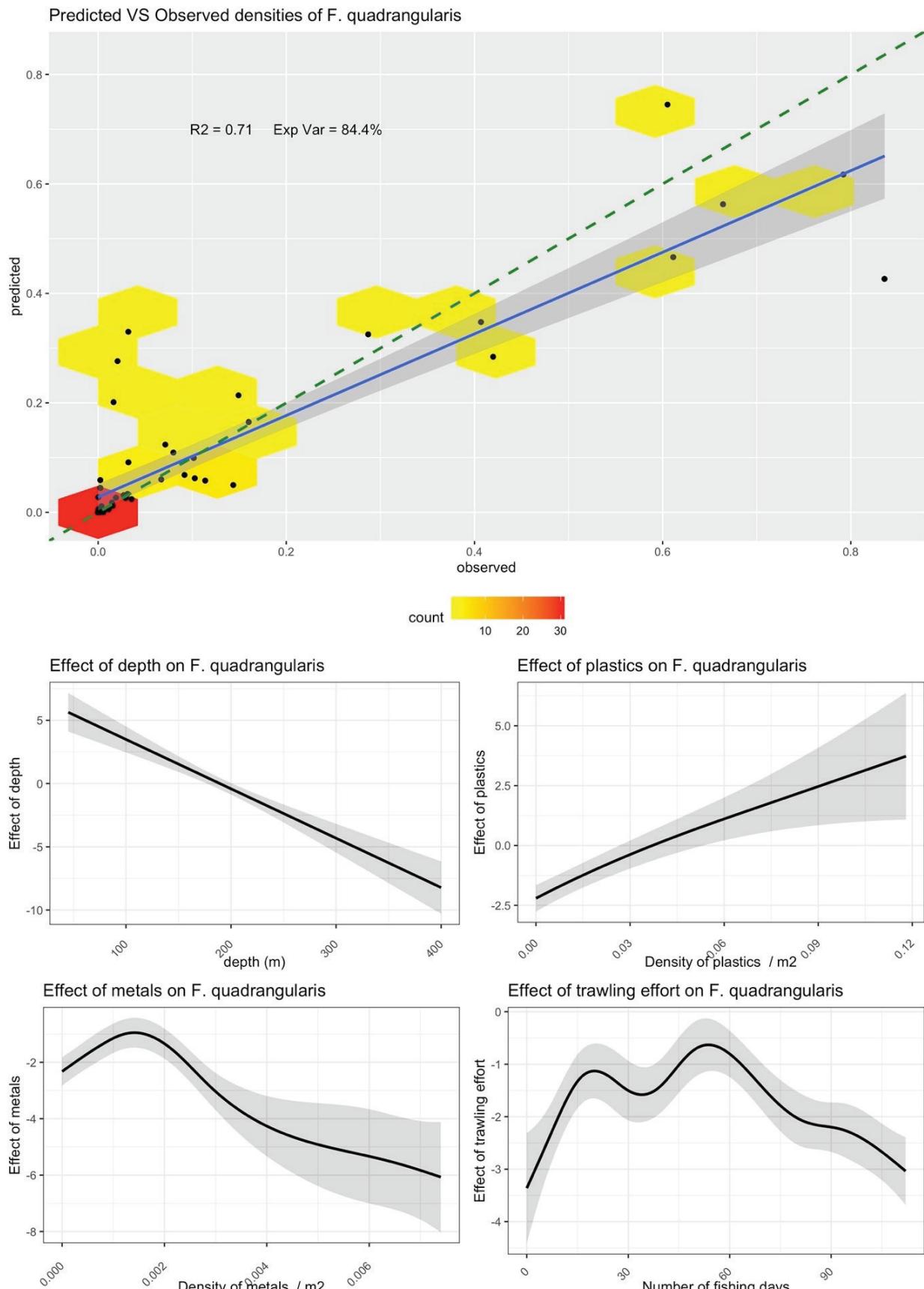


Fig. 6: Top panel: scatterplot of the predicted (by GAM) and observed values of *F. quadrangularis* density. R^2 and percentage of total variance explained by the model are presented, together with the estimated linear relationship (blue line with grey shaded area corresponding to the confidence interval at alpha = 0.05) between fitted (GAM) and observed values. The bisector of the first quadrant is also shown (green dashed line) as a reference of a perfect match. Bottom panel: effects of the different predictors, independent of the other predictors, are represented as response variable shapes. Confidence intervals (95%) around the response curve are represented by shaded areas (in grey).

Table 1. GAM (Generalized Additive Models) calculated relationships among *Funiculina quadrangularis* and the variables (predictors).

Predictor	Estimate	Std. Error	t value	Pr(> t)
(intercept)	-61.066.659	3466291	-17,61729e-05	0.9999860
habitatOffshore circalittoral mixed sediment	4.885.708	4902075	9.966612e-07	0.9999992
habitatOffshore circalittoral mud	57.933.423	3466291	1.671338e-05	0.9999868
habitatOffshore circalittoral sand	3.981.111	5480687	7.263891e-07	0.9999994
habitatUpper bathyal muds	57.247.117	3466291	1.651538e-05	0.9999869

Predictor	edf	ref.df	F	p-value
s(depth)	1.000020	1.000036	60.85258	0.00000e+00
s(plastics_m2)	1.247130	1.430614	15.34646	5.73901e-05
s(metals_m2)	3.417242	4.001859	26.78738	0.00000e+00
s(effort)	5.695278	6.464984	18.73106	0.00000e+01

deviance.explained	Adj.Rsq
84.36%	0.7141111

Discussion

There is a dearth of knowledge on deep-water habitats, and the available knowledge for all 12 known sea pen species in the Mediterranean Sea has been synthesised by Bastari *et al.* (2018), underlining the lack of information from the southern and eastern regions of the Mediterranean Sea. Although several studies have reported the presence of *F. quadrangularis* (mostly in the central and northern parts of the Adriatic Sea), this study substantially improved our knowledge of its distribution in the southern part of the Adriatic Sea. Seven new locations are reported from old, unpublished data originating from the “Hvar” expedition (1948-1949) in the depth range of 44-260 m. Four of these locations are in Croatian, two in Montenegrin, and one in Albanian waters (Fig. 1). Keeping in mind that during the “Hvar” expedition, 28 trawling surveys were performed and only on seven *F. quadrangularis* was found, it could be concluded that this species was rare.

The possibility of using visual ground-truthing allowed us to determine and report the high density of this species in the depth range from 160 m to 400 m depth in the waters along the Montenegrin coast (Fig. 7A). Along the 14 km transect, we found 2515 colonies with the highest population density of 0.83 colony/m². According

to available information, this population has the highest density of *F. quadrangularis* in the Adriatic Sea, and considering the number of counted specimens, it is one of the largest reported populations in general (Greathed *et al.*, 2007; Martinelli *et al.*, 2013; Salvalaggio *et al.*, 2016; Lauria *et al.*, 2017; Bastari *et al.*, 2018; Pierdomenico *et al.*, 2018). Aggregation and patchy distribution are usually observed in benthic species, and it depends on many environmental factors, as well as on the ecology of the species. Moreover, several studies have reported that this species has a patchy distribution and prefers relatively stable environmental conditions (Greathed *et al.*, 2007; Lauria *et al.*, 2017; Bastari *et al.*, 2018). Considering this, we should be aware of and responsible for the protection of specific vulnerable locations, such as those in the south-eastern Adriatic Sea.

Unfortunately, in this study, we identified different types of stresses on deep-sea habitats. Currently, marine litter is ubiquitous. Fortibuoni *et al.* (2019) presented information on the harmonised assessment of seafloor litter, its abundance, and composition in the Adriatic-Ionian macro-region based on trawl and scuba visual surveys. Our results showing 5.72 items/km² is less than the average values recorded for the Adriatic-Ionian and Mediterranean regions; however, the situation is not quite promising. It is well known that seafloor litter shows uneven



Fig. 7: Images from ROV: A) Aggregation of *Funiculina quadrangularis*, B) plastic marine litter, C) mark of disturbance on the seafloor.

distribution (Fortibuoni *et al.*, 2019; UNEP/MAP, 2015) and along our surveyed transect, it was unevenly distributed (Fig. 7B). In our study, the quantity of metal marine litter calculated for some locations was up to 7400 items/km² while for the plastic items, our findings were up to 188,000 items/km², which is much higher than the values reported by Fortibuoni *et al.* (2019) and closer to that of some others (Galgani *et al.*, 2000; Pham *et al.*, 2014; Angiolillo *et al.*, 2015; Enrichetti *et al.*, 2020). The most probable reason for such big differences is that our survey was based on video-truthing while most of the others (like Fortibuoni *et al.*, 2019) dealt with trawl surveys and scuba diving in shallow areas. Moreover, it is likely that the electrical cable was a temporary obstacle for the drifting marine litter on the sea floor because it was placed on the sea-bed one month before its burial (when the ROV videos were recorded). The electrical cable is now buried and no longer forms an obstacle on the sea floor; therefore, the quantity of marine litter in the same area is expected to be less, not because it is no longer present, but because it drifts along the marine currents. In the present study, results show that seafloor marine litter is mostly hidden but is still a tremendous threat to the marine environment and living organisms.

Another source of stress on deep-sea habitats comes from fisheries. Using video truthing, we registered 1064 marks of disturbance on the bed (Fig. 4, 7C), which is not surprising because the muddy habitat that bears *F. quadrangularis* is also suitable for some commercially important species of fish, shrimp, and Norway lobster; therefore, these areas are under intensive fishing pressure (Thompson *et al.*, 2016; Pierdomenico *et al.*, 2018). There have also been many reports of disturbed integrity of bottom habitats and destruction of sessile organisms like corals and sea pens (Rossi, 2013; Lauria *et al.*, 2017;

Eigaard *et al.*, 2017; Bastari *et al.*, 2018; Danovaro *et al.*, 2020).

However, the analysis of the fishing effort in the Montenegrin territorial waters and epicontinental shelf during the period 2016–2020 indicated the highest fishing pressure in 200–500 m depth, which overlaps with the zones having the highest density of *F. quadrangularis* (Fig. 8). Bottom trawling outside the territorial waters of former Yugoslavian countries is almost negligible (Scarcella *et al.*, 2014), and although the majority of fishing pressures in European waters are concentrated at depths of less than 200 m, the fishing pressure inside the territorial waters of Montenegro is very low (Eigaard *et al.*, 2017, Ikica *et al.*, 2018). Close to the area with many *F. quadrangularis* is a military munition-dumping area, which is an important reason why this area is avoided by many (but not all) trawlers. Considering the analysis of the fishing effort and the overlap with the dumping area, it could be estimated that there are no obstacles for fishing nets in this area and/or such obstacles no longer exist, but considering the possible threats to the safety of the fishers and the environment, there must be strict and immediate enforcement of fishing prohibition. Moreover, it is well known that the protection of habitats from physical disturbance can benefit not only the biodiversity conservation efforts but also enhance the sustainability of fisheries (Greathead *et al.*, 2007; Rossi, 2013; Martinelli *et al.*, 2013; Thompson *et al.*, 2016).

Our new report on the large population of *F. quadrangularis* in the fishing ground confirms that this species is more resistant to fishing pressure compared to some other sea pen species, probably because it is attached to the bottom by a peduncle that penetrates the sediment down to approximately 50 cm, thus providing flexibility to the colony that can temporarily lie flat under the

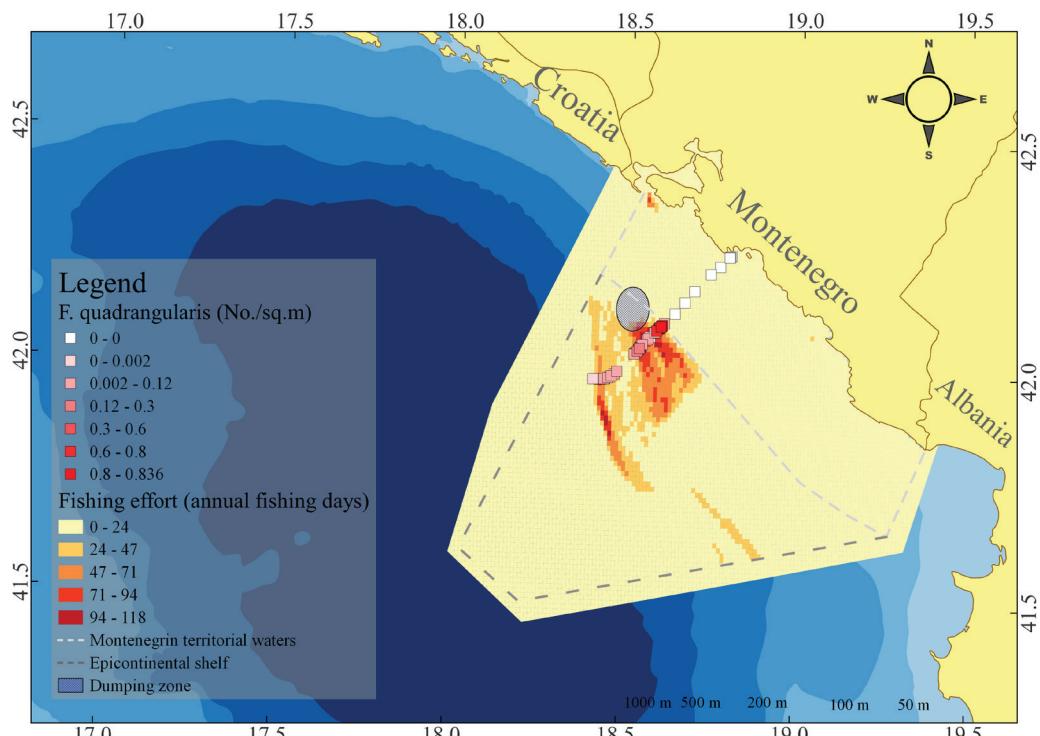


Fig. 8: Fishing effort and *Funiculina quadrangularis* population density.

passage of fishing gear (Lauria *et al.*, 2017). However, it must be noted that fishing, although economically important, is significantly harming the non-targeted species, and together with other anthropogenic factors is causing the decline of sea pens worldwide (Salvati *et al.*, 2014).

As reported by the worldwide application of GAM, the distribution and abundance of *F. quadrangularis* along the inspected transect are mainly dependent on depth, while marine litter and trawling efforts have significant but more complex and not monotonic effects (see Table 1). The effects associated with plastics and metals (the two marine litter categories considered in this study) could be explained by considering this relationship as a pattern of co-occurrence rather than a direct causal effect. Probably, there are statistically significant associations between the gradient of *F. quadrangularis*, plastic items, and metal items, which lead to the response-variable shapes shown in Figure 6; however, further studies are needed to establish if the abundance of marine litter is likely to interfere with the presence of this species. However, the GAM-reconstructed effect of trawling effort is consistent with previous knowledge on this species, since it seems that only a high level of disturbance can have negative effects on the abundance of *F. quadrangularis*.

In the Adriatic Sea, deep-sea bottom trawlers are prohibited in the Pomo/Jabuka pits and below 1000 m depth (Thompson, 2016; GFCM, 2017), but marine animal forests (*sensu* Rossi *et al.*, 2017), such as the ones in south-eastern Adriatic, are left completely unprotected. This study contributes to the mapping of previously unknown VMEs and improves our knowledge of the distribution of this priority sea pen species. Our results could be used in monitoring and marine spatial conservation planning. Nevertheless, further research is needed to better determine other potentially important and vulnerable areas, and international discussions with decision-makers and stakeholders are needed to determine further steps toward the protection and sustainable use of resources, especially in areas beyond the national jurisdiction of the Adriatic Sea.

Acknowledgements

The authors are grateful to Michele Pantini and Damiano Borri from Terna Crna Gora d.o.o. who provided ROV video material. We also thank Zdravko Ikica who provided a historic data sheet used to record fishery data during the “HVAR” Expedition 1948/49.

References

- Amoroso, R.O., Pitcher, C.R., Rijnsdorp, A. D., McConaughay, R.A., Parma, A.M. *et al.*, 2018. Bottom trawl fishing footprints on the world’s continental shelves. *Proceedings of the National Academy of Sciences U.S.A.*, 115, E10275-E10282.
- Angiolillo, M., di Lorenzo, B., Farcomeni, A., Bo, M., Bavestrello, G. *et al.*, 2015. Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy). *Marine Pollution Bulletin*, 92 (1-2), 149-159.
- Bastari, A., Pica, D., Ferretti, F., Micheli, F., Cerrano, C., 2018. Sea pens in the Mediterranean Sea: habitat suitability and opportunities for ecosystem recovery. *ICES Journal of Marine Science*, 75 (5), 1722-1732.
- Boero, F., Bonsdorff, E., 2007. A conceptual framework for marine biodiversity and ecosystem functioning. *Marine Ecology*, 28 (s1), 134-145.
- Buhl-Mortensen, L., Vanreusel, A., Gooday, A.J., Levin, L.A., Priede, I.G. *et al.*, 2010. Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. *Marine Ecology*, 31 (1), 21-50.
- Cleveland, W. 1993. *Visualizing Data*. Summit, CA: Hobart Press, New Jersey, 360 pp.
- Danovaro, R., Fanelli, E., Aguzzi, J., Billett, D., Carugati, L. *et al.*, 2020. Ecological variables for developing a global deep-ocean monitoring and conservation strategy. *Nature Ecology & Evolution*, 4 (2), 181-192.
- De Clippel, L. H., Buhl-Mortensen, P., Buhl-Mortensen, L., 2015. Fauna associated with cold water gorgonians and sea pens. *Continental Shelf Research*, 105, 67-78.
- Eigaard, O.R., Bastardie, F., Hintzen, N.T., Buhl-Mortensen, L., Buhl-Mortensen, P. *et al.*, 2017. The footprint of bottom trawling in European waters: distribution, intensity, and seabed integrity. *ICES Journal of Marine Science*, 74 (3), 847-865.
- ENI, 2019. *Offshore Environmental Baseline Study (OEBS) for 4118-5-2 and 4118-5-1 Offshore Exploratory wells in Montenegro*. Torchia G., Djurović, M. (Ed). ENI Technical report, 343/2019 MD/ES, 183 pp.
- Enrichetti, F., Dominguez-Carrió, C., Toma, M., Bavestrello, G., Canese, S. *et al.*, 2020. Assessment and distribution of seafloor litter on the deep Ligurian continental shelf and shelf break (NW Mediterranean Sea). *Marine Pollution Bulletin*, 151, 110872.
- EU, 2006. Council Regulation (EC) No. 1967/2006 of 21 December 2006 concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, amending Regulation (EEC) No 2847/93 and repealing Regulation (EC) No 1626/94. *Official Journal of the European Communities* L409, 11-85.
- EU, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). *Official Journal of the European Communities* L164, 19-40.
- EU, 2016. Commission Implementing Decision (EU) 2016/1251 of 12 July 2016 adopting a multiannual Union programme for the collection, management and use of data in the fisheries and aquaculture sectors for the period 2017-2019 (notified under document C(2016) 4329). *Official Journal of the European Communities* L207, 113-177.
- Fabri, M.C., Pedel, L., Beuck, L., Galgani, F., Hebbeln, D. *et al.*, 2014. Megafauna of vulnerable marine ecosystems in French mediterranean submarine canyons: Spatial distribution and anthropogenic impacts. *Deep Sea Research Part II: Topical Studies in Oceanography*, 104, 184-207.
- Fortibuoni, T., Ronchic, F., Mačić, V., Mandić, M., Mazzottie, C. *et al.*, 2019. A harmonized and coordinated assessment

- of the abundance and composition of seafloor litter in the Adriatic-Ionian macroregion (Mediterranean Sea). *Marine Pollution Bulletin*, 139, 412–426.
- Galgani, F., Leaute, J.P., Moguedet, P., Souplet, A., Verin, Y. *et al.*, 2000. Litter on the sea floor along European coasts. *Marine Pollution Bulletin*, 40 (6), 516-527.
- GFCM, 2017. Recommendation GFCM/41/2017/3 on the establishment of a fisheries restricted area in the Jabuka/Pomo Pit in the Adriatic Sea. GFCM/41/2017/3, 7 pp.
- GFCM SAC, 2008. Criteria for the identification of sensitive habitats of relevance for the management of priority species. General Fisheries Commission for the Mediterranean (GFCM), Scientific Advisory Committee (SAC), Marrakech, Morocco, 2008, 1-3.
- Government of Montenegro, Ministry of Ecology, Spatial Planning and Urbanism (GM MESPU), 2020. *Initial Assessment of the Status of Marine Environment of Montenegro*. GM MESPU, Technical report, 441 pp.
- Greathead, C.F., Donnan, D.W., Mair, J.M., Saunders, G.R., 2007. The sea pens *Virgularia mirabilis*, *Pennatula phosphorea* and *Funiculina quadrangularis*: distribution and conservation issues in Scottish waters. *Journal of the Marine Biological Association of the United Kingdom*, 87 (5), 1095-1103.
- Greathead, C., González-Irusta, J.M., Clarke, J., Boulcott, P., Blackadder, L. *et al.*, 2015. Environmental requirements for three sea pen species: relevance to distribution and conservation. *ICES Journal of Marine Science*, 72 (2), 576-586.
- Hastie, T., Tibshirani, R. 1990. *Generalized Additive Models*. Chapman and Hall, London, 352 pp.
- ICES, 2011. *Protocols for assessing the status of sea-pen and burrowing megafauna communities*. Report of the ICES Advisory Committee, 226 pp.
- Ikica, Z., Đurović, M., Joksimović, A., Mandić, M., Marković, O. *et al.*, 2018. Monitoring of the Montenegrin fisheries sector: Biological sampling (September 2007-August 2011). *Studia Marina*. Monograph Series No. 1. Institute of Marine Biology, University of Montenegro. Kotor, Montenegro. 106 pp.
- Ikica, Z., Isajlović, I., Pešić, A., Ćetković, I., Vrgoč, N., 2021. "HVAR" Expedition (1948–1949) in South-Eastern Adriatic (Croatia, Montenegro, Albania). p. 301-328. In: *The Montenegrin Adriatic Coast: Marine Biology*. Joksimović, A., Đurović, M., Zonn, I.S., Kostianoy, A.G., Semenov, A.V. (Eds). Springer Nature Switzerland, Cham.
- Lauria, V., Garofalo, G., Fiorentino, F., Massi, D., Milisenda, G. *et al.*, 2017. Species distribution models of two critically endangered deep-sea octocorals reveal fishing impacts on vulnerable marine ecosystems in central Mediterranean Sea. *Scientific Reports*, 7 (1), 1-14.
- Martinelli, M., Morello, E.B., Isajlović, I., Belardinelli, A., Lucchetti, A. *et al.*, 2013. Towed underwater television towards the quantification of Norway lobster, squat lobsters and sea pens in the Adriatic Sea. *Acta Adriatica*, 54 (1), 3-12.
- MEDITS-Handbook. Version n. 8, 2016, *MEDITS Working Group*, 177 pp.
- Petović, S., Marković, O., Ikica, Z., Đurović, M., Joksimović, A., 2016. Effects of bottom trawling on the benthic assemblages in the south Adriatic Sea (Montenegro). *Acta Adriatica*, 57 (1), 79-90.
- Pham, C.K., Ramirez-Llodra, E., Alt, C.H., Amaro, T., Bergmann, M. *et al.*, 2014. Marine litter distribution and density in European seas, from the shelves to deep basins. *Plos ONE*, 9 (4), e95839.
- Pierdomenico, M., Russo, T., Ambroso, S., Gori, A., Martorelli, E. *et al.*, 2018. Effects of trawling activity on the bamboo-coral *Isidella elongata* and the sea pen *Funiculina quadrangularis* along the Gioia Canyon (Western Mediterranean, southern Tyrrhenian Sea). *Progress in Oceanography*, 169, 214-226.
- Quantum GIS software, 2022. *QGIS A Free and Open Source Geographic Information System* <https://qgis.org/en/site/> (Accessed 5 May 2021)
- Rogers, A.D., Gianni, M., 2010. *The Implementation of UNGA Resolutions 61/105 and 64/72 in the Management of Deep-Sea Fisheries on the High Seas*. Report prepared for the Deep-Sea Conservation Coalition. International Programme on the State of the Ocean, London, United Kingdom, 97 pp.
- Rossi, S., 2013. The destruction of the 'animal forests' in the oceans: towards an over-simplification of the benthic ecosystems. *Ocean & Coastal Management*, 84, 77-85.
- Rossi, S., Bramanti, L., Gori, A., Orejas, C. (Ed.), 2017. *Marine animal forests*. Springer International, Cham, 505 pp.
- Russo, T., D'Andrea, L., Parisi, A., Cataudella, S., 2014. VMS-base: an R-package for VMS and logbook data management and analysis in fisheries ecology. *PLoS One* 9:e100195.
- Russo, T., D'Andrea, L., Parisi, A., Martinelli, M., Belardinelli, A., *et al.*, 2016. Assessing the fishing footprint using data integrated from different tracking devices: Issues and opportunities. *Ecological Indicators*, 69, 818-827.
- Russo, T., Franceschini, S., D'Andrea, L., Scardi, M., Parisi, A. *et al.*, 2019. Predicting fishing footprint of trawlers from environmental and fleet data: an application of artificial neural networks. *Frontiers in Marine Science*, 6, 670, 1-18.
- Salvalaggio, V., Fabi, G., Punzo, E., Santelli, A., Strafella, P. *et al.*, 2016. Distribution of the sea pens *Virgularia mirabilis* and *Funiculina quadrangularis* (Cnidaria Anthozoa) in the northern and central Adriatic Sea. p. 504. In *Rapport Du 41e Congrès De La CIESM—Commission Internationale Pour L'Exploration Scientifique De La Mer Méditerranée*, Monaco, Vol. 41.
- Salvati, E., Bo, M., Rondinini, C., Battistoni, A., Teofili, C., 2014. *Lista Rossa IUCN dei coralli italiani*. Comitato italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma, 132 pp.
- Scarella, G., Grati, F., Raicevich, S., Russo, T., Gramolini, R. *et al.*, 2014. Common sole in the northern and central Adriatic Sea: Spatial management scenarios to rebuild the stock. *Journal of Sea Research* 89, 12-22.
- SPA/RAC-UN Environment/MAP, 2019. *Updated Classification of Benthic Marine Habitat Types for the Mediterranean Region*. UNEP MAP, SPA RAC, Tunis Cedex, 15 pp.
- Thompson, A., Sanders, J., Tandstad, M., Carocci, F., Fuller, J., 2016. *Vulnerable marine ecosystems: processes and practices in the high seas*. FAO Fisheries and Aquaculture Technical Paper, 595, Rome, 188 pp.
- Trainito, E., Baldacconi, R., 2016. Coralli del Mediterraneo. Il Castello, Cornaro (MI), 176 pp.
- UNEP/MAP, 2015. *Marine Litter Assessment in the Mediterranean*. UNEP/MAP report, 45 pp.

- UNEP/MAP-RAC/SPA, 2015. *Adriatic Sea: Description of the ecology and identification of the areas that may deserve to be protected*. By Cerrano, C. Edited by Cebrian, D. Requena, S., RAC/SPA, Tunis, 92 pp.
- Wood, S. 2021. *Mixed GAM Computation Vehicle with Automatic Smoothness Estimation*. <https://cran.r-project.org/web/packages/mgcv/index.html> (Accessed 5 October 2021)
- Wright, E.P., Kemp, K., Rogers, A.D., Yesson, C., 2015. Genetic structure of the tall sea pen *Funiculina quadrangularis* in NW Scottish sea lochs. *Marine Ecology*, 36 (3), 659-667.
- ZEISS ZEN 3.2 software, 2020. *Manual ZEISS Group*, Zeiss, Germany, 28 pp.