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## Spatio-temporal distribution of *Isidella elongata*, a vulnerable marine ecosystem indicator species, in the southern Adriatic Sea --Manuscript Draft--

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<b>Abstract:</b>	<p>The bamboo coral <i>Isidella elongata</i> is a forest-forming alcyonacean that dwells on deep-sea soft bottoms and is presently found in the Otranto Channel (Southern Adriatic Sea). In this study, we explored the spatial distribution and the demographic structure of the <i>I. elongata</i> facies (IF) over time, along with the diversity of IF-associated fauna in the southern Adriatic Sea, by using a time-series data of nine years (2012–2020) from the Mediterranean International Bottom Trawl Survey (MEDITS). The spatial distribution of <i>I. elongata</i> was confronted with the fishing effort in the study area over time. This facies showed a constant spatial extent but a decreasing biomass over time. Changes in the basal diameter distribution of the colonies were also observed, with a significant decrease in the sizes from 2014 to 2020. These results suggest an increasing fishery-related impact from a developing deep-sea fishery occurring in the study area. The bamboo coral in the study area represents a hotspot of biodiversity in the context of the bathyal environment. A fisheries-restricted area was recently proposed in the context of the General Fisheries Commission for the Mediterranean at the Otranto Channel to help the conservation of <i>I. elongata</i> in the southern Adriatic Sea.</p>	

**Response to Reviewers:**

Please find the response to the reviewer comments in the file attached to the submission.

1 **Spatio-temporal distribution of *Isidella elongata*, a vulnerable marine ecosystem indicator species, in the southern**  
2 **Adriatic Sea**

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14 **Abstract**

15 The bamboo coral *Isidella elongata* is a forest-forming alcyonacean that dwells on deep-sea soft bottoms and is presently  
16 found in the Otranto Channel (Southern Adriatic Sea). In this study, we explored the spatial distribution and the  
17 demographic structure of the *I. elongata* facies (IF) over time, along with the diversity of IF-associated fauna in the  
18 southern Adriatic Sea, by using a time-series data of nine years (2012–2020) from the Mediterranean International Bottom  
19 Trawl Survey (MEDITS). The spatial distribution of *I. elongata* was confronted with the fishing effort in the study area  
20 over time. This facies showed a constant spatial extent but a decreasing biomass over time. Changes in the basal diameter  
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22 suggest an increasing fishery-related impact from a developing deep-sea fishery occurring in the study area. The bamboo  
23 coral in the study area represents a hotspot of biodiversity in the context of the bathyal environment. A fisheries-restricted  
24 area was recently proposed in the context of the General Fisheries Commission for the Mediterranean at the Otranto  
25 Channel to help the conservation of *I. elongata* in the southern Adriatic Sea.

26

27 **Keywords:** Vulnerable marine ecosystem; *Isidella elongata*; Spatial distribution; Time series; Colonies basal diameter;  
28 southern Adriatic Sea; Fisheries

## 29 **Introduction**

30 Vulnerable Marine Ecosystems (VMEs) are overall defined as a group of species, communities, or habitats  
31 characterised by hotspots of biodiversity and ecosystem functioning in the deep sea that may be exposed to the impacts  
32 of fishing activities (FAO, 2009). Moreover, VMEs are characterised by a high vulnerability to fishing disturbances  
33 (Ashford et al., 2019). This vulnerability is related to its weakness in terms of the renewal of population and/or habitat  
34 stability as a result of fishing impacts. The set of criteria to class an ecosystem as VME was detailed in FAO (2009) for  
35 fisheries management, following the discussion detailed in United Nations General Assembly (UNGA) Resolution 61/105  
36 in 2006. Indeed, fishery activities performed in the deep sea can compromise the physical and functional stability of the  
37 ecosystem, triggering negative effects on both living marine resources and the ecosystem itself. Moreover, there is  
38 increasing concern linked with the overfishing of the target species associated with VMEs, where continuous removal  
39 may impact the overall functionality of the ecosystem (Maynou & Cartes, 2012; FAO, 2018). This is of primary interest,  
40 since VMEs are considered hotspots of biodiversity that provide healthy ecosystem functioning and several ecosystem  
41 services in the deep sea (FAO, 2009; Ashford et al., 2019; Chimienti et al., 2019b; Carbonara et al., 2020; Maiorano et  
42 al., 2022).

43 The *Isidella elongata* facies (IF) was added to the list of VMEs by the General Fisheries Commission for the  
44 Mediterranean Sea (GFCM) (GFCM, 2017), and several international actions have been implemented for their protection.  
45 This facies has been included in the “Deep Water Engineering benthic invertebrate assemblages” in the “Dark Habitats  
46 Action Plan” of the Barcelona Convention (UNEP-MAP-RAC-SPA, 2015). This inclusion has also been supported by  
47 the previous addition of the species to the International Union for Conservation of Nature (IUCN) red list for the  
48 Mediterranean, with *Isidella elongata* being the only Mediterranean anthozoan species classified as critically endangered  
49 (<http://www.iucn.it/scheda.php?id=789649086>). Recently, *Isidella elongata* has been also recognised as a deep-water  
50 VME representative taxon of the VME habitat type "Soft bottom coral garden: Soft bottom gorgonian and black coral  
51 gardens" by the International Council for the Exploration of the Sea (ICES, 2020). In the context of the Marine Strategy  
52 Framework Directive, only recently has it been recognised that the IF needs protection as a habitat (coral garden)  
53 (Palialexis et al., 2018; Danovaro et al., 2020).

54 The Mediterranean Sea is considered a major biodiversity hotspot due to its high species richness coupled with a  
55 considerable proportion of endemic species (Coll et al., 2010; Granger et al., 2015). The bamboo coral *I. elongata* is an  
56 alcyonacean species (family Keratoisididae), near-endemic to the Mediterranean Sea (Grasshoff, 1989), that dwells on  
57 soft bottoms at depths between 100 m and 1500 m (Bellan-Santini, 1985; Laubier & Emig, 1993; Bo et al., 2015a), where  
58 it can form very dense aggregations (Mastrototaro et al., 2017) called coral gardens or coral forests (FAO, 2009; Rossi et

59 al., 2017; Chimienti et al., 2019b). The coral forests of *I. elongata* represent the true facies of the bathyal mud in the  
60 Mediterranean Sea (*sensu* Pérès and Picard 1964).

61 *I. elongata* has a candelabrum-like shape and can reach 80 cm in height (Bo et al., 2015a; Carbonara et al., 2020),  
62 acting as a structuring organism that is able to generate a complex and three-dimensional heterogeneity on otherwise flat  
63 muddy bottoms where the species dwells. The IF can consist of dense bamboo coral colonies constituting a habitat that  
64 hosts several fishes and crustacean species (Mastrototaro et al., 2017; D’Onghia, 2019; Carbonara et al., 2020), as in the  
65 Balearic Sea where up to 2300–2683 colonies/ha and 50 taxa have been identified (Mastrototaro et al., 2017). Several  
66 studies have demonstrated that the presence of bamboo coral influences both the biodiversity and the abundance of several  
67 species (Buhl-Mortensen et al., 2010; Maynou & Cartes, 2012; Mytilineou et al., 2014; Carbonara et al., 2020; Cartes et  
68 al., 2022). These studies have been mainly focused on megafauna (fish and large invertebrates), and much less on  
69 macrofauna associated with the IF (Cartes et al., 2017, 2022; Carbonara et al., 2020). Predator species take advantage of  
70 the presence of IF, as they find a higher density of prey within bamboo coral (D’Onghia, 2019; Carbonara et al., 2020).  
71 Moreover, the arborescent complexity of *I. elongata* colonies’ could further provide a shelter effect against predators  
72 (Mastrototaro et al., 2017). Some of the typical species associated with the bamboo coral, such as the red shrimps  
73 *Aristaeomorpha foliacea* and *Aristeus antennatus* and the Norway lobster *Nephrops norvegicus* (Maynou & Cartes, 2012;  
74 Mastrototaro et al., 2017; D’Onghia, 2019), are of high commercial value and targeted by professional fisheries (Spedicato  
75 et al., 1998; Maynou & Cartes, 2012; Cartes et al., 2013; Lauria et al., 2017; D’Onghia, 2019; EUMOFA, 2019).

76 In the last decades, trawling activities in the Mediterranean have enlarged their operational bathymetric range,  
77 causing a greater impact on deep-sea bottoms (FAO, 2009, 2018) and consequently on deep-sea VMEs such as IF. In this  
78 context, determining the presence and understanding the spatial distribution of VMEs, including bamboo coral facies, are  
79 crucial to implementing actions of management or protection (Lauria et al., 2017; FAO, 2018; Chimienti et al., 2019a;  
80 Otero & Marin, 2019). IF occurrences have been studied in the western Mediterranean (Fabri et al., 2014; Mastrototaro  
81 et al., 2017; Chimienti et al., 2019a), in the central-eastern (D’Onghia et al., 2003; Vafidis et al., 2006; Mytilineou et al.,  
82 2014; Pierdomenico et al., 2016, 2018, 2019; Lauria et al., 2017; D’Onghia, 2019; Carbonara et al., 2020) and in the  
83 eastern part of the basin (Gerovasileiou et al., 2019). More recently, Carbonara et al. (2020) reported the occurrence of  
84 IF in the southern Adriatic Sea, where the IF was the least impacted by fishery among the study areas (i.e., Sardinian Sea,  
85 south-central Tyrrhenian, southern Adriatic, and north-western Ionian). It is to note that some populations are also in good  
86 condition in other areas, such as those around the Balearic Islands (Mastrototaro et al., 2017). While acknowledging how  
87 bottom trawl surveys are not the optimum way of sampling for benthic biocoenosis, opportunistic data collected during  
88 the Mediterranean International Bottom Trawl Survey (MEDITS) (Spedicato et al., 2019) could, nevertheless, be useful

89 for setting up spatial-temporal analysis of IF and associated communities on trawled soft bottoms (Farriols et al., 2015;  
90 Granger et al., 2015; Petović et al., 2016; Chimienti et al., 2018; Gerovasileiou et al., 2019).

91 Thus, the aim of this study was to detect over a time-series data of nine years (2012–2020) the potential changes  
92 of: i) occurrence, spatial distribution and abundance of the *I. elongata* facies in the southern Adriatic Sea (GSA 18; *sensu*  
93 FAO GFCM); ii) biodiversity of the fauna associated with the IF; iii) the bamboo coral colonies demography; and iv) the  
94 overlap of IF with trawler fishing effort in the study area from 2015 to 2020.

## 95 **Materials and Method**

### 96 *Sampling method*

97 The data analysed in this work were collected in the context of the MEDITS trawl surveys in the years from 2012  
98 to 2020 in the geographical subarea GSA 18: southern Adriatic Sea (**Fig. 1**). The primary objective of the MEDITS trawl  
99 survey is to estimate the abundance, distribution, and population structure of commercial fish and invertebrate species in  
100 the Mediterranean Sea. The MEDITS survey is carried out annually between late spring and midsummer, and it covers  
101 the depth range from 10 to 800 m (AAVV, 2017). Trawl stations were allocated in five bathymetrical strata (10–50, 50–  
102 100, 100–200, 200–500 and 500–800 m) on the trawlable bottom (i.e. flat and soft-bottom) and consistently carried out  
103 in the same position according to a random-stratified sampling design (AAVV, 2017). The survey design, as well as the  
104 details of the sampling methodology, are reported by Spedicato et al. (2019). The standard sampling gear used was the  
105 bottom trawl GOC 73 (Bertrand et al., 2002) designed for experimental fishing. Briefly, it has a vertical opening slightly  
106 larger than that of the most common professional gear used in the area and the stretched mesh size of 20 mm at the codend.  
107 The haul duration was 30 minutes on the continental shelf (10–200 m depth) and 60 minutes on the slope (201–800 m  
108 depth), and hauls were allowed only during daytime. The standard fishing speed was 3 knots on the ground. Other specific  
109 details can be found in the study of Spedicato et al. (2019).

### 110 *Analysis of the occurrence, distribution, and biomass of I. elongata in GSA 18 and the associated biodiversity*

111 The occurrence of *I. elongata* was computed as the percentage of hauls with the presence of IF over the total  
112 number of hauls carried out by year. For each haul in which *I. elongata* was observed, its total weight was measured (kg;  
113 at gram resolution) and standardized to the swept surface unit (km<sup>2</sup>). As the trawling activity caused mechanical damage  
114 to the colonies of bamboo coral, its biomass caught in each haul consisted of various parts of fragmented colonies,  
115 including the basal part and/or the arms. Thus, the biomass of *I. elongata* was proportional to the number of colonies and  
116 their size. The biomass (kg km<sup>-2</sup>) index was computed according to Souplet (1996) by weighting the mean index by depth  
117 stratum or macro stratum (e.g. 200-800).

118 The spatial distribution analysis of *I. elongata* biomass was conducted using a spatial modelling approach based  
119 on a generalised additive model (GAM). In particular, biomass data were modelled using the Gaussian family distribution  
120 and link function identity, with longitude (Lon) and latitude (Lat) as explanatory variables. Three different GAM models  
121 were estimated to display *I. elongata* biomass distribution in two bathymetric strata from the MEDITS protocol (AAVV,  
122 2017) where the species was present (200–500 m and 500–800 m), for three periods (2012–2014, 2015–2017 and 2018–  
123 2020), as reported in the following formula:

$$124 \quad \text{Biomass} \sim s(\text{Lon}_{ij}, \text{Lat}_{ij}) + \varepsilon_{ij}$$

125 where  $i$  is the observation in the  $j$  time period, and  $\varepsilon$  is the error term.

126 The prediction maps were generated by using a regular grid with a point resolution of  $0.01^\circ$ . The data used to  
127 resize the grid to the selected depth range were derived from the EMODnet-Bathymetry portal (grid resolution of  $1/16^*$   
128  $1/16$  arc minutes, circa  $115 \times 115$  metres). All the GAM analyses were performed using the R software (R Development  
129 Core Team, 2021) and the *mgcv* package, while distribution maps were generated with QGIS software (QGIS  
130 Development Team, 2017).

131 For the analysis of the biodiversity of associated assemblages, we considered only those hauls performed on  
132 substrates (fine muddy bottom), where *I. elongata* was typically found (Mytilineou et al., 2014; Pierdomenico et al., 2016,  
133 2018; Mastrototaro et al., 2017; Chimienti et al., 2019a; Gerovasileiou et al., 2019; Carbonara et al., 2020; Cartes et al.,  
134 2022) for which the presence or the absence of the species was recorded (Carbonara et al., 2020). In this way, it is possible  
135 to minimise in the analysis the influence of the hauls where IF generally does not occur (e.g., gravel, coarse sand) (Lauria  
136 et al., 2017). From each haul, all the faunal specimens larger than 1 cm (Spedicato et al., 2019) were classified at the  
137 lowest possible taxonomic level, and for each Operational Taxonomic Unit (OTU) data on weight and number of  
138 specimens were collected according to the MEDITS protocol (AAVV, 2017). The Margalef richness index (D) (Margalef,  
139 1958) and Shannon-Wiener index ( $H'$ ) (Shannon, 1948) were computed for each sampling station carried out deeper than  
140 200 m, which represents the area where the probability of IF occurrence was higher (Carbonara et al., 2020). These  
141 indexes were used to detect the potential effects of the presence of *I. elongata* on the associated assemblages. The hauls  
142 performed on suitable substrate types (fine muddy) were pooled into two categories: “presence,” if the bamboo coral was  
143 caught in the haul, and “absence” for all remaining hauls on the muddy bottom. The analysis was carried out separately  
144 by year for the benthic and demersal species, and the differences in biodiversity between the two assemblages with  
145 (associated) and without (not associated) *I. elongata* for each year were evaluated using the non-parametric Wilcoxon–  
146 Mann–Whitney test. Moreover, the presence of a temporal trend in the biomass index ( $\text{kg}/\text{km}^2$ ) of *I. elongata* was tested  
147 by Spearman's rho correlation test.



148 ***Spatial distribution of fishing efforts***

149 Fishing effort in the study area was estimated by means of the aggregated Automatic Identification System (AIS)  
150 data provided by Global Fishing Watch (<https://globalfishingwatch.org/>). Annual fishing activities referred to those from  
151 2015 to 2020 (expressed in hours) and were aggregated in the same temporal frame of the MEDITS *I. elongata* spatial  
152 analysis (three years): 2015–2017 and 2018–2020. The first years (2012–2014) were not considered in the analysis  
153 because of the limited coverage of the active fishery by the AIS system (Global Fishing Watch;  
154 <https://globalfishingwatch.org/data-download/datasets/public-fishing-effort>) that came into effect progressively from  
155 May 2012 to May 2014 (2002/59/CE) in Italy. Moreover, the data were averaged over a grid with a resolution of 0.01° x  
156 0.01°. The hotspot of fishing activities was calculated as the 75th percentile of effort data distribution. The resulting  
157 fishing polygon bordering the effort hotspot was superimposed with the polygon bordering the 75th percentile of the  
158 estimated coral bamboo distribution, in order to assess the percentage of *I. elongata* hotspot surface area overlapping the  
159 main fishing ground in the southern Adriatic Sea (GSA18). The analysis was conducted using R software (R Core Team,  
160 2021) by means of the raster library (Hijmans, 2021).

161 ***Morphological parameter measurements and analyses***

162 During the survey, the morphological parameters of *I. elongata* colonies were collected in terms of basal diameter  
163 (BD, in millimetres) to infer information on the population demographic structure in the southern Adriatic Sea from 2014  
164 to 2020. The trawl survey caused mechanical damage to the colonies and it did not allow us to collect specific data, such  
165 as maximum height, branching patterns (colony width), and/or colony vitality (Carbonara et al., 2020). However, the  
166 basal diameter is often used to infer the size of the colonies (Andrews et al., 2009; Carbonara et al., 2020; Cartes et al.,  
167 2022), as it is correlated to the height (Carbonara et al., 2020). Basal diameter was measured only when the base of the  
168 colony was present, established by the presence of at least part of the root-shaped bases (Carbonara et al., 2020). In all  
169 the hauls where the presence of bamboo coral specimens was detected, the bases of the colonies were collected and  
170 subsequently measured. On each base colony, as close as possible to the root, at least three measurements of the diameter  
171 were taken by caliper (resolution of 0.1 mm), and the final diameter was the average of these measurements (Coefficient  
172 of Variation between 0 and 9.75). The annual average values of colony BD were used to test the trend of the colony sizes  
173 over seven years (2014–2020) using Spearman’s correlation. In addition, the eventual differences during the study period  
174 were tested using a Kruskal–Wallis one-way analysis of variance by ranks. The Nemenyi *post hoc* test was used to  
175 evaluate the significance of such median differences between years. In order to analyse the change in the basal diameter  
176 distribution (BDD), the years were grouped into three periods as follows: 2014, 2015–2017 and 2018–2020. The years

177 were grouped according to the time periods selected for the GAM analysis. The different BDDs were compared by  
178 Kolmogorov–Smirnov (K–S) test.

179 The skewness index (SK) of BD distribution was calculated to measure the distributions' deviation from symmetry.  
180 The skewness describes three scenarios: (i) distribution dominated by smaller colonies ( $SK > 0.5$ ), (ii) distribution  
181 dominated by larger colonies ( $SK < 0$ ), and (iii) no size dominance ( $0 \leq SK \leq 0.5$ ).

182

## 183 **Results**

### 184 ***Occurrence, distribution, and abundance of *I. elongata* in GSA 18***

185 The percentage and depth range of occurrence of *I. elongata* per haul in GSA 18 is reported in **Table 1**. The  
186 percentage of occurrence (number of hauls positive with the presence of IF out of the total number of hauls carried out)  
187 showed from a maximum of 9.41% in 2017 to a minimum value of 4.44% in 2019. The depth range of occurrence was  
188 between 410 m (2017) and 625 m (2016).

189

190 **Table 1.** Percentage (%) (number of positive hauls to the presence of bamboo coral) and depth range (m) of occurrence  
191 of *Isidella elongata* in each year, total number of hauls carried out in the survey, and their depth range.

Year	Percentage of occurrence (%)	Depth range of <i>I. elongata</i> occurrence (m)	Total number hauls carried out in the survey	Depth range of hauls carried out in the survey (m)
2012	6.67	520–618	90	12–678
2013	7.78	517–617	90	11–713
2014	6.67	447–617	90	11–715
2015	6.67	515–619	90	10–693
2016	6.67	429–625	90	10–739
2017	9.41	410–601	85	10–733
2018	6.74	518–596	89	10–701
2019	4.44	569–621	90	10–749
2020	7.14	551–600	70	10–759

192

193 In all the three models tested to describe the effect of the spatial displacement on the biomass of *I. elongata* (kg  
194 km<sup>-2</sup>), the bidimensional splines of the geographic coordinates included in the Gaussian GAM model were significant for  
195 the three monitored periods ( $p < 0.05$  for all), explaining the following amount of deviance: 86.2% in 2012–2014, 72.4%  
196 in 2015–2017 and 59.7% in 2018–2020. These splines (**Fig. 2**) indicate the combined effects of northing and easting on  
197 the biomass, locating the hotspot bamboo coral biomass in the Otranto Channel (**Fig. 3**). The extension of the area, in  
198 which the hotspots (75th percentile of the estimated biomass distribution) of IF were estimated to be located, is similar  
199 for the three periods considered (1436.7 km<sup>2</sup> for 2012–2014, 1520.4 km<sup>2</sup> for 2015–2017 and 1421.6 km<sup>2</sup> for 2018–2020).  
200 Similarities were seen in its spatial distribution pattern as well, although with a clear decreasing biomass of the bamboo  
201 coral over the three groups of study periods (**Fig. 3**). A significant decreasing trend ( $p < 0.05$ ) in the biomass index of *I.*  
202 *elongata* was also detected over the time series considered (2012–2020). The biomass index of *I. elongata* was around 25  
203 kg/m<sup>2</sup> in both 2012 and 2013, with a peak in 2014 ( $65.7 \pm 47.1$  kg/m<sup>2</sup>), while very low biomass indexes below 1.3 kg/m<sup>2</sup>  
204 were observed from 2018 to 2020 (**Fig. 4**).

205 The list of the OTUs found in the different years in the study area and their relative biomass were provided in the  
206 supplementary material. In **Figures 5 and 6**, the analyses of the Margalef and Shannon-Wiener biodiversity indexes for  
207 the hauls associated or not associated with the presence of *I. elongata* are reported. The results of the diversity indexes  
208 for the assemblages “associated” and “not associated” were compared within each year. For the benthic species, no  
209 significant difference between the assemblages with and without the bamboo coral was observed, except in 2016 for the  
210 Shannon diversity index. In contrast, for the demersal species the assemblage associated with *I. elongata* showed  
211 significantly higher values for both indices in most of the years analysed ( $p < 0.05$ ), excepted in 2016 for the Shannon  
212 diversity index and from 2018 to 2020 for the Margalef one ( $p < 0.05$ ; **Fig. 5**). A significant decreasing trend ( $p < 0.05$ )  
213 of the Margalef index was detected for the demersal “associated” assemblage over the study period (**Fig. 6**). No significant  
214 trend over time was observed in both indices of both benthic assemblages nor in the Margalef index of “not associated”  
215 demersal assemblages and in the Shannon diversity index of both demersal assemblages (**Fig. 5, 6**).

### 216 *Spatial distribution of fishing efforts*

217 **Figure 7** reports the hotspot of fishing activities (75th percentile) for the two-time frames analysed (2015–2017  
218 and 2018–2020). Moreover, the overlap between the area hotspot of fishing activities and the hotspot (75th percentile) of  
219 bamboo coral distribution is indicated and quantified in terms of percentage. The percentage of overlapping between the  
220 area hotspot of fishing activities and bamboo coral was found to be 8.84% between 2015–2017 while it reached 12.27  
221 between 2018–2020, with a resulting increase of 38.8 % between the two periods considered.

## 222 *Morphological parameters*

223 Significant differences were detected between BDD distributions in the three considered periods ( $p < 0.05$ ; **Fig.**  
224 **8**). In 2014, the BDD skewed towards larger-sized colonies (basal diameters  $> 4$  mm;  $SK = -0.275$ ) than in the 2015–  
225 2017 period when no dominance of any size was observed ( $SK = 0.26$ ) and two small peaks (1.5–2 mm and 3.5–4 mm)  
226 were observed. In 2018–2020, the BDDs skewed towards smaller-size colonies (peak at 1.5–2 mm;  $SK = 846$ ).

227 This pattern is more evident while analysing the yearly distribution (**Fig. 9**), where the Spearman's rho test revealed  
228 a significant decreasing trend in BD over the years ( $p < 0.05$ ) (**Fig. 9**). Indeed, in 2014, BD was significantly larger (mean  
229  $\pm$  SE,  $4.02 \pm 0.096$  mm) than in other years ( $p < 0.05$ ). Between 2016 and 2019, the BD values were overall similar over  
230 the years (from 0.58 mm to 6.0 mm), while the BD measured in 2020 was found significantly smaller than all other years  
231 ( $1.86 \pm 0.095$  mm) ( $p < 0.05$ ) (**Fig. 9**).

232

## 233 **Discussion**

234 The present study investigated the spatial and temporal distributions of *I. elongata* in the southern Adriatic Sea  
235 (GSA 18) through time-series data of nine years (2012–2020) collected during MEDITS surveys. The biodiversity of the  
236 assemblages associated with *I. elongata* was also investigated. In the study area, the depth range of species occurrence in  
237 the present study was from 410 m to 625 m, which is in line with data already published for the southern Adriatic Sea  
238 (Carbonara et al., 2020) as well as with other areas of the western and central Mediterranean (Cartes et al., 2009; Lauria  
239 et al., 2017; Mastrototaro et al., 2017; Pierdomenico et al., 2018; Ingrassia et al., 2019). However, it is difficult to define  
240 the maximum depth of the species distribution in the study area due to both the depth range of sampling of the MEDITS  
241 protocol (i.e., down to maximum 800 m in depth) and the bottom morphology of the study area around the IF presence,  
242 that are unsuitable to trawling at depths greater than 650 m. Despite the overall depth range of the investigated area was  
243 larger than the bamboo coral occurrence (**Table 1**) in the GSA 18 (north part of the GSA18), some deeper areas around  
244 the *I. elongata* presence are not trawlable and thus not explored during the MEDITS surveys. Consequently, it is not  
245 excluded that the IF in the southern Adriatic Sea could extend even deeper than that already observed (625 m depth)  
246 (Rueda et al., 2016; González-García et al., 2020) in a more pristine area unexplored by trawl-survey, as shown by the  
247 results of the GAM model (**Fig. 3**) (Carbonara et al., 2020). The spatial analysis performed in this study confirms that the  
248 Otranto Channel represents a suitable area with a consistent presence of IF (Carbonara et al., 2020), probably due to a  
249 combination of all required environmental factors (i.e. compact/semi consolidated mud with a low slope angle, low  
250 seasonal variation in term of salinity and temperature and high concentration of near bottom zooplankton) (Cartes et al.,

251 2013; Bo et al., 2015a; Lauria et al., 2017; Ingrassia et al., 2019). In addition, the presence of IF can be enhanced by the  
252 upwelling of nutrient-rich deep waters, which has been documented in the area (Vilibic & Orlic, 2002). Moreover, the  
253 fishing activity of the southern Adriatic trawlers fleet is more concentrated on the continental shelf and on the upper slope  
254 (Spedicato & Lembo, 2011), resulting in a lesser impact on the deeper bottoms where IF can be found. Although modelling  
255 *I. elongata* quantitative data was affected by the limitations of the MEDITS trawl net which is not the best sampler for  
256 exploring the benthic communities (González-García et al., 2020), the systematic collection of data on the macrobenthic  
257 species caught during the MEDITS trawl survey could represent a valuable source of information, particularly for  
258 understanding their distribution patterns and potential significant changes over time (Fernandez-arcaya et al., 2019), as  
259 well as the impact of human-related activities, such as trawling (Mangano et al., 2013). Mechanical damage from trawling  
260 rarely allows the collection of unbroken complete individual colonies, limiting morphological study; however, the  
261 biomass data of the bamboo coral collected during the MEDITS trawl survey are proportional to the number of colonies  
262 and their size (Carbonara et al., 2020). Moreover, in the absence of other systematic sources of information, these  
263 opportunistic data have proven to be useful sources of information for benthic communities in general and, in particular,  
264 for the IF (Lauria et al., 2017; Carbonara et al., 2020).

265 The temporal analysis performed through the spatial distribution of biomass within the IF shows a clear decreasing  
266 trend of abundance (biomass index) over the study period, but no difference in distribution extent. The decreasing trend  
267 of the IF in the southern Adriatic Sea in the last nine years (2012–2020) could be related to the cumulative impact of  
268 trawlers on the bamboo coral, produced by the increasing fishing effort detected at least in the area over time (**Fig. 7**) at  
269 least on the area monitored by the MEDITS survey (until about 630 m of depth; see **Table 1**). Despite the traditional  
270 fishing habits of the trawler fleet in southern Adriatic Sea, that mostly exploited the continental shelf and the upper slope  
271 (Spedicato & Lembo, 2011), in recent years, there has been an increase in trawler numbers capable of fishing even at  
272 great depths in the eastern part of the southern Adriatic Sea (Albania) (STECF, 2019), with an increasing impact on the  
273 IF. *Isidella elongata* is often associated with the main targets species of deep fishery trawlers, such as the red shrimps  
274 (*Aristaeomorpha foliacea* and *Aristeus antennatus*) and the Norway lobster (*Nephrops norvegicus*) (Maynou & Cartes,  
275 2012) in areas where a high overlap with the Essential Fish Habitat (EFH) was recently documented for both shrimps,  
276 particularly in the Otranto Channel (Carbonara et al., 2020). Pulcinella et al. (2021) analysed the AIS data and the fishing  
277 ground in the central-east Mediterranean and revealed how the number of fishing vessels dedicated to deep fishing in the  
278 GSA 18 increased from 23 in 2017 to 29 in 2018. Furthermore, the area of the Otranto Channel seems to be the most  
279 frequented area by deep sea fishing vessels with a monthly trawling haul frequency at depths greater than 400 m (deep  
280 water fishery) ranging from 12 to 24 over three years (2015–2018) and a mean number of fishing hours per km<sup>2</sup> ranging

281 from 6 to 16 in the east and from 44 to 118 in the west part of the Otranto channel in the same time series. The combined  
282 analysis with spatial distribution of the fishing effort and the distribution of IF, showed an increase in the fishing effort  
283 on the hotspot of bamboo coral presence over time. Indeed, the overlap between the IF presence and the hotspot (75th  
284 percentile) deep (depth > 200 m) fishing ground increased from 8.84% to 12.27% over time, resulting in an increase of  
285 38.8% between the two periods considered (2015-2017 vs 2018-2020; **Fig. 7**). Consequently, the increasing fishing effort  
286 for commercial fishery activity registered in recent years in the southern Adriatic Sea (IUCN, 2019) could deeply affect  
287 the distribution of the IF with fishing being considered one of the most impactful human activities on the VME, including  
288 the IF (Cartes et al., 2013; FAO, 2018; Fanelli et al., 2021).

289         The biodiversity associated with the colonies of the bamboo coral is significantly higher for both diversity indices  
290 used (i.e., Margalef and Shannon-Wiener) considering all time series (2012–2020) for the demersal OTU whereas no  
291 difference in the benthic OTU was found between the two assemblages considered (i.e., with and without bamboo coral).  
292 Based on these results, *I. elongata* seems to generate an important effect on the associated assemblage, more evident on  
293 the demersal than benthic fauna. Indeed, due to its candelabrum-like shape, the bamboo coral is one of the soft-bottom  
294 cold-water corals (CWCs) with a distinct frame-building ability to construct coral forests (Chimienti et al., 2019b) that  
295 also act as trophic areas where many species, such as the sharks *G. melastomus* and *E. spinax*, the teleost fishes *M.*  
296 *merluccius*, *M. poutassou*, *P. blennoides*, *H. dactylopterus*, *H. mediterraneus* and *Lepidorhombus boscii*, find preys  
297 swimming among the colonies or climbing on them (Mastrototaro et al., 2017; Carbonara et al., 2020; Cartes et al., 2022).  
298 Several studies on CWCs reveal a link between habitat complexity formed by corals and species diversity, particularly in  
299 bathyal zones (D’Onghia, 2019; Rueda et al., 2019; Cartes et al., 2022) where habitat-forming species variety is generally  
300 limited (Gage and Tyler, 1992). The presence of the bamboo coral, as a passive feeder, is often associated with the high  
301 abundance of zooplankton (Cartes et al., 2013). This biological feature, in turn, qualitatively and quantitatively influences  
302 IF-associated fauna in terms of demersal species within and around the colonies (Mastrototaro et al., 2017; Rueda et al.,  
303 2019; Carbonara et al., 2020). The large amount of zooplankton can produce a positive effect on several species in various  
304 food web positions, from the lower trophic levels to the higher of top predators (Rueda et al., 2019; Carbonara et al.,  
305 2020). For example, the presence of red shrimps in the IF (Cartes et al., 2013; Carbonara et al., 2020) seems to be linked  
306 with the presence of the Pandalidae species, which probably shares the same prey as bamboo coral (zooplankton), and, in  
307 turn, they are the favourite prey of both *A. foliacea* and *A. antennatus* (Kapiris, 2012). Moreover, the high concentration  
308 of pandalids and other crustaceans, including Aristeidae, attracts several bony fishes and ommastrephid cephalopods,  
309 producing a cascade of effects that could modify the fauna in the areas characterized by the presence of *I. elongata* and  
310 the energy flow through the food web (Eddy et al., 2021). Fishing is known to remove individuals at various trophic levels

311 from natural habitats, thus affecting the overall energy flow through the food web (Watling & Norse, 1998; Pauly &  
312 Palomares, 2005; Sieben et al., 2011; Carbonara et al., 2022), making the environment less efficient in supporting a certain  
313 level of biodiversity (Hooper et al., 2005; Thompson et al., 2012; Ullah et al., 2018). Finally, the typical candelabrum-  
314 like shape of *I. elongata* also plays a role in terms of refuge from predators (Mastrototaro et al., 2017) and spawning area  
315 for several elasmobranch species (e.g., *Galeus melastomus* and *Scyliorhinus canicula*) (Carbonara et al., 2020). This effect  
316 was already observed in other CWC habitats in both the Ionian and southern Adriatic Seas (D’Onghia et al., 2010, 2012,  
317 2016; Sion et al., 2019).

318 In this study, the time-series analysis also highlighted a temporal effect on biodiversity of assemblages with *I.*  
319 *elongata*, but with different results for the two indices. The richness index shows a significant negative trend over time  
320 in the assemblages with *I. elongata* whereas the Shannon-Wiener index exhibits no clear trend. The Margalef index has  
321 a good discriminatory ability towards species richness (Yeom & Kim, 2011; Magurran, 2013) that probably presents a  
322 faster response to the impact whereas the Shannon index is influenced by both species richness and species abundance  
323 distribution (evenness) that can exhibit a slower change in deep-sea communities. Moreover, the significant negative  
324 trend for the Margalef index could be linked to a decrease of the IF consistency over time (see **Fig. 2,3**). Indeed, the IF  
325 can play its role as a biodiversity hotspot (Cartes et al., 2013; Mytilineou et al., 2014; Mastrototaro et al., 2017) when it  
326 shows a certain level of integrity (Carbonara et al., 2020). When the IF is strongly impacted by fishery activity and consists  
327 of a few young and isolated colonies (Mastrototaro et al., 2017; Pierdomenico et al., 2018), it cannot fulfil its role as an  
328 attractive three-dimensional habitat (e.g., food riches, refuges) (Rueda et al., 2016).

329 Furthermore, the analyses carried out on the assemblages (with and without *I. elongata*) in the Otranto Channel  
330 showed significant differences in their specific compositions. The complete list of the OTUs found in the different years  
331 of the study is available in the supplementary material. In particular, among the most abundant species in the areas  
332 associated with IF, key species determine these differences more than others (Carbonara et al., 2020). Included in these  
333 key species are the osteichthyes *Hoplostethus mediterraneus mediterraneus* Cuvier, 1829, *Phycis blennoides* (Brünnich,  
334 1768) *Helicolenus dactylopterus* (Delaroche, 1809), and *Coelorinchus caelorhincus* (Risso, 1810); the elasmobranchs  
335 *Etmopterus spinax* (Linnaeus, 1758), *Dalatias licha* (Bonnaterre, 1788), and *Chimaera monstrosa* (Linnaeus, 1758); the  
336 crustaceans *Aristaeomorpha foliacea* (Risso, 1827), *Aristeus antennatus* (Risso, 1816), and *Paromola cuvieri* (Risso,  
337 1816); and the cephalopod *Todarodes sagittatus* (Lamarck, 1798). Several of these species are commercial (see  
338 supplementary material), in particular the red shrimps (*A. foliacea* and *A. antennatus*) that are the target of the trawlers  
339 fishing on the slope (deeper than 200 m) (Maiorano et al., 2010; Russo et al., 2017).

340 Mature colonies (i.e., larger colonies with higher density) of *I. elongata* exhibit a higher macrofaunal community  
341 diversity (Cartes et al., 2022), also highlighting the importance of the colony size in the species diversity. Basal diameter  
342 is correlated with the height and width of the colonies in *I. elongata* (Carbonara et al., 2020) and with the age of colonies  
343 in congeneric species (Roark et al., 2005; Andrews et al., 2009). In this study, we observed that the BDD was different  
344 among the three periods considered (2014, 2015–2017 and 2018–2020). In the first period (2014), the BDDs are  
345 dominated by larger and older colonies (basal diameters > 4 mm), while in the 2015–2017 period, the BDDs are  
346 characterized by two similar peaks (1.5–2 mm and 3.5–4 mm) with a lower peak at 5 mm. In the last period considered  
347 (2018–2020), the BDD is quite monomodal with a peak at 1.5–2 mm. Thus, in the time series considered, the colony  
348 dimension/age seems to shift from a non- or little-impacted population (2012–2014) to a highly impacted population  
349 (2018–2020). The records from the last period considered (2018–2020) are similar to those found by Carbonara et al.  
350 (2020) in a population that was highly impacted by fishing (Tyrrhenian Sea). The population dominated by colonies with  
351 BD greater than 4 mm, found in 2014, could be considered quite pristine or minimally impacted by fishing (Bo et al.,  
352 2015a, 2015b; Carbonara et al., 2020). Colonies with BD greater than 4 mm correspond to the overall size of colonies  
353 (Carbonara et al., 2020) in pristine areas (Bo et al., 2015a, 2015b) with a capacity for reproduction and the renewal of the  
354 population still untouched (Bo et al., 2015a, 2015b). These changes over time are also confirmed by the significant trend  
355 of a decrease in yearly average BD. In this case, as well as for the spatial analysis, the reason for this decrease (average  
356 BD) could be the increased pressure of deep-sea fishing (FAO, 2018; IUCN, 2019). Although the age and growth for the  
357 *I. elongata* data are missing, congeneric species have been found to have a slow growth rate (Roark et al., 2005; Andrews  
358 et al., 2009). In the Gulf of Alaska, it was estimated that the lifespan for *Isidella spp.* was 75 to 126 years, with a slow  
359 growth rate ranged between about 0.1–0.05 mm of radial basal diameter per year (Roark et al., 2005; Andrews et al.,  
360 2009). From the perspective of *I. elongata* VME conservation, such fast decreasing trend detected in few years together  
361 with the low growth of *Isidella sp.* (Andrews et al., 2009) could be a dangerous warning sign of a severe progressive  
362 impact on IF. This biological characteristic of *Isidella sp.* coral indicates that the recovery of areas impacted by fishing  
363 (Mastrototaro et al., 2017; Pierdomenico et al., 2018) can take a very long time, ranging from a few decades to a century  
364 (Roark et al., 2005). Furthermore, it should be emphasised that studies on the growth and age of *I. elongata* have not yet  
365 been carried out and should be implemented as soon as possible not only for the purpose of biological and ecological  
366 knowledge but also for the conservation of this species (Carbonara et al., 2020).

### 367 **Concluding remarks**

368 In conclusion, the consistent presence of an *I. elongata* VME was confirmed in the Otranto Channel in the southern  
369 Adriatic Sea (Carbonara et al., 2020). Over a period of nine years, this VME seems to have a constant extension, but with



370 a significant decrease in biomass and changes in demographic structure. The overlap between the spatial distribution of  
371 the bamboo coral and that of the fishing effort, indicates a severe impact clearly highlighted by the results of this study.  
372 Furthermore, other signs of the increasing impact of anthropogenic activities, such as fishing, are the changes in BDD  
373 (skewed towards smaller colonies in the last period from 2018 to 2020, unlike in 2014, where it skewed more towards  
374 large sizes) and the significant decrease in BD over time (from 2014–2020). It is also important to emphasise that IF  
375 represents biodiversity hotspots for deep environments in the study area (Cartes et al., 2009; Chimienti et al., 2019a,  
376 2019b) that could be compromised by fisheries (Cartes et al., 2013). Until now, no fishing restrictions have been  
377 implemented in the area where the presence of IF (Otranto Channel) has been detected; indeed, as shown by our analysis,  
378 this area is a fishing ground for deep trawlers. Thus, it was recently proposed, in the context of the GFCM Subregional  
379 Committee for the Adriatic Sea, that the process to establish a fisheries restricted area (FRA) at the Otranto Channel  
380 (GFCM, 2021) must be started in order to help the conservation of *I. elongata* in the southern Adriatic Sea, achieving the  
381 goal of protecting VMEs set by various international organizations/conventions (FAO, 2009; UNEP-MAP-RAC-SPA,  
382 2015).

383

#### 384 **Statements and Declarations**

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#### 390 **Data availability**

391 All data generated or analysed during this study are included in this published article and its supplementary information  
392 files, further inquiries can be directed to the corresponding author.

393 **Competing of Interest:** The authors declare that they have no conflicts of interest.

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646 **Captions for figures**

647 **Figure 1.** The study area is the southern Adriatic Sea (Geographical sub-area GSA 18 *sensu* GFCM-FAO). The  
648 geographic allocation of the hauls explored during the MEDITS trawl surveys in GSA 18 are indicated by black points.

649 **Figure 2.** The bidimensional splines of the Gaussian GAM models used to describe the spatial distribution of *Isidella*  
650 *elongata* biomass (kg/km<sup>2</sup>) in (a) 2012–2014, (b) 2015–2017, and (c) 2018–2020. X: Longitude, Y: Latitude.

651 **Figure 3.** The spatial distribution of *Isidella elongata* biomass (kg/km<sup>2</sup>) in the three considered periods: (a) 2012–2014,  
652 (b) 2015–2017, and (c) 2018–2020. The green line borders the 75th percentile of the estimated biomass distribution.

653 **Figure 4.** *Isidella elongata* biomass index (kg/km<sup>2</sup>) (blue trend in macrostratum at depths of 200–800 m). The Spearman's  
654 rho correlation test showed a significant decrease ( $p < 0.05$ ). The solid line represents the biomass index, while the dashed  
655 lines represent the standard deviation.

656 **Figure 5.** Benthos OTU box plots of the (a) Margalef (D) and (b) Shannon (H') indexes of assemblages with (associated)  
657 and without (not associated) *Isidella elongata* per year. The solid line of each boxplot indicates the median, and the boxes  
658 on either side represent the quartiles, with the whiskers covering 95% of the values. Outlier values are represented by  
659 black dots. Statistics reported in the figure are based on Wilcoxon–Mann–Whitney tests carried out within each period  
660 (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; NS = not significant). The Spearman's rho correlation test was not significant ( $p > 0.05$ ) for  
661 both assemblages (associated and not associated) and both indices (Margalef and Shannon).

662 **Figure 6.** Demersal OTU box plots of the (a) Margalef (D) and (b) Shannon (H') indexes of assemblages with (associated)  
663 and without (not associated) *Isidella elongata* per year. The solid line of each boxplot indicates the median, and the boxes  
664 on either side represent the quartiles, with the whiskers covering 95% of the values. Outlier values are represented by  
665 black dots. Statistics reported in the figure are based on Wilcoxon–Mann–Whitney tests carried out within each period  
666 (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; NS (not significant):  $p > 0.05$ ). The Spearman's rho correlation test was not significant ( $p >$   
667  $0.05$ ) for both assemblages (associated and not associated) for Shannon's index. For the Margalef's index the “associated”  
668 assemblage shows a significant decreasing trend (Spearman's rho correlation test  $p < 0.05$ ), while that of the “not  
669 associated” assemblage was not significant ( $p > 0.05$ ).

670 **Figure 7.** Fishing effort in the southern Adriatic Sea in (A) 2015–2017 and (B) 2018–2020, estimated using the aggregated  
671 AIS data provided by Global Fishing Watch (<https://globalfishingwatch.org/>). The hotspot of fishing activities was  
672 calculated as the 75th percentile of effort data distribution. Moreover, the spatial and percentage overlap between the  
673 hotspot of *Isidella elongata* presence and fishing efforts is also shown.

674 **Figure 8.** Basal diameter (mm) distribution of *Isidella elongata* colonies in (a) 2014, (b) 2015–2017, and (c) 2018–2020.

675 **Figure 9.** Box plots of the colony basal diameter (mm) measured from 2014 to 2020 in the GSA 18. For boxplot  
676 representation, the central line of each boxplot indicates the median, and the boxes on either side represent the quartiles,  
677 with the whiskers covering 95% of the values. Outlier values are represented by black dots. Different letters indicate  
678 significant differences among groups between the years (Kruskal–Wallis and Nemenyi post-hoc tests,  $p < 0.05$ ).

679

1 **Spatio-temporal distribution of *Isidella elongata*, a vulnerable marine ecosystem indicator species, in the southern**  
2 **Adriatic Sea**

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14 **Abstract**

15 The bamboo coral *Isidella elongata* is a forest-forming alcyonacean that dwells on deep-sea soft bottoms and is presently  
16 found in the Otranto Channel (Southern Adriatic Sea). In this study, we explored the spatial distribution and the  
17 demographic structure of the *I. elongata* facies (IF) over time, along with the diversity of IF-associated fauna in the  
18 southern Adriatic Sea, by using a time-series data of nine years (2012–2020) from the Mediterranean International Bottom  
19 Trawl Survey (MEDITS). The spatial distribution of *I. elongata* was confronted with the fishing effort in the study area  
20 over time. This facies showed a constant spatial extent but a decreasing biomass over time. Changes in the basal diameter  
21 distribution of the colonies were also observed, with a significant decrease in the sizes from 2014 to 2020. These results  
22 suggest an increasing fishery-related impact from a developing deep-sea fishery occurring in the study area. The bamboo  
23 coral in the study area represents a hotspot of biodiversity in the context of the bathyal environment. A fisheries-restricted  
24 area was recently proposed in the context of the General Fisheries Commission for the Mediterranean at the Otranto  
25 Channel to help the conservation of *I. elongata* in the southern Adriatic Sea.

26

27 **Keywords:** Vulnerable marine ecosystem; *Isidella elongata*; Spatial distribution; Time series; Colonies basal diameter;  
28 southern Adriatic Sea; Fisheries

## 29 **Introduction**

30 Vulnerable Marine Ecosystems (VMEs) are overall defined as a group of species, communities, or habitats  
31 characterised by hotspots of biodiversity and ecosystem functioning in the deep sea that may be exposed to the impacts  
32 of fishing activities (FAO, 2009). Moreover, VMEs are characterised by a high vulnerability to fishing disturbances  
33 (Ashford et al., 2019). This vulnerability is related to its weakness in terms of the renewal of population and/or habitat  
34 stability as a result of fishing impacts. The set of criteria to class an ecosystem as VME was detailed in FAO (2009) for  
35 fisheries management, following the discussion detailed in United Nations General Assembly (UNGA) Resolution 61/105  
36 in 2006. Indeed, fishery activities performed in the deep sea can compromise the physical and functional stability of the  
37 ecosystem, triggering negative effects on both living marine resources and the ecosystem itself. Moreover, there is  
38 increasing concern linked with the overfishing of the target species associated with VMEs, where continuous removal  
39 may impact the overall functionality of the ecosystem (Maynou & Cartes, 2012; FAO, 2018). This is of primary interest,  
40 since VMEs are considered hotspots of biodiversity that provide healthy ecosystem functioning and several ecosystem  
41 services in the deep sea (FAO, 2009; Ashford et al., 2019; Chimienti et al., 2019b; Carbonara et al., 2020; Maiorano et  
42 al., 2022).

43 The *Isidella elongata* facies (IF) was added to the list of VMEs by the General Fisheries Commission for the  
44 Mediterranean Sea (GFCM) (GFCM, 2017), and several international actions have been implemented for their protection.  
45 This facies has been included in the “Deep Water Engineering benthic invertebrate assemblages” in the “Dark Habitats  
46 Action Plan” of the Barcelona Convention (UNEP-MAP-RAC-SPA, 2015). This inclusion has also been supported by  
47 the previous addition of the species to the International Union for Conservation of Nature (IUCN) red list for the  
48 Mediterranean, with *Isidella elongata* being the only Mediterranean anthozoan species classified as critically endangered  
49 (<http://www.iucn.it/scheda.php?id=789649086>). Recently, *Isidella elongata* has been also recognised as a deep-water  
50 VME representative taxon of the VME habitat type "Soft bottom coral garden: Soft bottom gorgonian and black coral  
51 gardens" by the International Council for the Exploration of the Sea (ICES, 2020). In the context of the Marine Strategy  
52 Framework Directive, only recently has it been recognised that the IF needs protection as a habitat (coral garden)  
53 (Palialexis et al., 2018; Danovaro et al., 2020).

54 The Mediterranean Sea is considered a major biodiversity hotspot due to its high species richness coupled with a  
55 considerable proportion of endemic species (Coll et al., 2010; Granger et al., 2015). The bamboo coral *I. elongata* is an  
56 alcyonacean species (family Keratoisididae), near-endemic to the Mediterranean Sea (Grasshoff, 1989), that dwells on  
57 soft bottoms at depths between 100 m and 1500 m (Bellan-Santini, 1985; Laubier & Emig, 1993; Bo et al., 2015a), where  
58 it can form very dense aggregations (Mastrototaro et al., 2017) called coral gardens or coral forests (FAO, 2009; Rossi et



59 al., 2017; Chimienti et al., 2019b). The coral forests of *I. elongata* represent the true facies of the bathyal mud in the  
60 Mediterranean Sea (*sensu* Pérès and Picard 1964).

61 *I. elongata* has a candelabrum-like shape and can reach 80 cm in height (Bo et al., 2015a; Carbonara et al., 2020),  
62 acting as a structuring organism that is able to generate a complex and three-dimensional heterogeneity on otherwise flat  
63 muddy bottoms where the species dwells. The IF can consist of dense bamboo coral colonies constituting a habitat that  
64 hosts several fishes and crustacean species (Mastrototaro et al., 2017; D’Onghia, 2019; Carbonara et al., 2020), as in the  
65 Balearic Sea where up to 2300–2683 colonies/ha and 50 taxa have been identified (Mastrototaro et al., 2017). Several  
66 studies have demonstrated that the presence of bamboo coral influences both the biodiversity and the abundance of several  
67 species (Buhl-Mortensen et al., 2010; Maynou & Cartes, 2012; Mytilineou et al., 2014; Carbonara et al., 2020; Cartes et  
68 al., 2022). These studies have been mainly focused on megafauna (fish and large invertebrates), and much less on  
69 macrofauna associated with the IF (Cartes et al., 2017, 2022; Carbonara et al., 2020). Predator species take advantage of  
70 the presence of IF, as they find a higher density of prey within bamboo coral (D’Onghia, 2019; Carbonara et al., 2020).  
71 Moreover, the arborescent complexity of *I. elongata* colonies’ could further provide a shelter effect against predators  
72 (Mastrototaro et al., 2017). Some of the typical species associated with the bamboo coral, such as the red shrimps  
73 *Aristaeomorpha foliacea* and *Aristeus antennatus* and the Norway lobster *Nephrops norvegicus* (Maynou & Cartes, 2012;  
74 Mastrototaro et al., 2017; D’Onghia, 2019), are of high commercial value and targeted by professional fisheries (Spedicato  
75 et al., 1998; Maynou & Cartes, 2012; Cartes et al., 2013; Lauria et al., 2017; D’Onghia, 2019; EUMOFA, 2019).

76 In the last decades, trawling activities in the Mediterranean have enlarged their operational bathymetric range,  
77 causing a greater impact on deep-sea bottoms (FAO, 2009, 2018) and consequently on deep-sea VMEs such as IF. In this  
78 context, determining the presence and understanding the spatial distribution of VMEs, including bamboo coral facies, are  
79 crucial to implementing actions of management or protection (Lauria et al., 2017; FAO, 2018; Chimienti et al., 2019a;  
80 Otero & Marin, 2019). IF occurrences have been studied in the western Mediterranean (Fabri et al., 2014; Mastrototaro  
81 et al., 2017; Chimienti et al., 2019a), in the central-eastern (D’Onghia et al., 2003; Vafidis et al., 2006; Mytilineou et al.,  
82 2014; Pierdomenico et al., 2016, 2018, 2019; Lauria et al., 2017; D’Onghia, 2019; Carbonara et al., 2020) and in the  
83 eastern part of the basin (Gerovasileiou et al., 2019). More recently, Carbonara et al. (2020) reported the occurrence of  
84 IF in the southern Adriatic Sea, where the IF was the least impacted by fishery among the study areas (i.e., Sardinian Sea,  
85 south-central Tyrrhenian, southern Adriatic, and north-western Ionian). It is to note that some populations are also in good  
86 condition in other areas, such as those around the Balearic Islands (Mastrototaro et al., 2017). While acknowledging how  
87 bottom trawl surveys are not the optimum way of sampling for benthic biocoenosis, opportunistic data collected during  
88 the Mediterranean International Bottom Trawl Survey (MEDITS) (Spedicato et al., 2019) could, nevertheless, be useful

89 for setting up spatial-temporal analysis of IF and associated communities on trawled soft bottoms (Farriols et al., 2015;  
90 Granger et al., 2015; Petović et al., 2016; Chimienti et al., 2018; Gerovasileiou et al., 2019).

91 Thus, the aim of this study was to detect over a time-series data of nine years (2012–2020) the potential changes  
92 of: i) occurrence, spatial distribution and abundance of the *I. elongata* facies in the southern Adriatic Sea (GSA 18; *sensu*  
93 FAO GFCM); ii) biodiversity of the fauna associated with the IF; iii) the bamboo coral colonies demography; and iv) the  
94 overlap of IF with trawler fishing effort in the study area from 2015 to 2020.

## 95 **Materials and Method**

### 96 *Sampling method*

97 The data analysed in this work were collected in the context of the MEDITS trawl surveys in the years from 2012  
98 to 2020 in the geographical subarea GSA 18: southern Adriatic Sea (**Fig. 1**). The primary objective of the MEDITS trawl  
99 survey is to estimate the abundance, distribution, and population structure of commercial fish and invertebrate species in  
100 the Mediterranean Sea. The MEDITS survey is carried out annually between late spring and midsummer, and it covers  
101 the depth range from 10 to 800 m (AAVV, 2017). Trawl stations were allocated in five bathymetrical strata (10–50, 50–  
102 100, 100–200, 200–500 and 500–800 m) on the trawlable bottom (i.e. flat and soft-bottom) and consistently carried out  
103 in the same position according to a random-stratified sampling design (AAVV, 2017). The survey design, as well as the  
104 details of the sampling methodology, are reported by Spedicato et al. (2019). The standard sampling gear used was the  
105 bottom trawl GOC 73 (Bertrand et al., 2002) designed for experimental fishing. Briefly, it has a vertical opening slightly  
106 larger than that of the most common professional gear used in the area and the stretched mesh size of 20 mm at the codend.  
107 The haul duration was 30 minutes on the continental shelf (10–200 m depth) and 60 minutes on the slope (201–800 m  
108 depth), and hauls were allowed only during daytime. The standard fishing speed was 3 knots on the ground. Other specific  
109 details can be found in the study of Spedicato et al. (2019).

### 110 *Analysis of the occurrence, distribution, and biomass of I. elongata in GSA 18 and the associated biodiversity*

111 The occurrence of *I. elongata* was computed as the percentage of hauls with the presence of IF over the total  
112 number of hauls carried out by year. For each haul in which *I. elongata* was observed, its total weight was measured (kg;  
113 at gram resolution) and standardized to the swept surface unit (km<sup>2</sup>). As the trawling activity caused mechanical damage  
114 to the colonies of bamboo coral, its biomass caught in each haul consisted of various parts of fragmented colonies,  
115 including the basal part and/or the arms. Thus, the biomass of *I. elongata* was proportional to the number of colonies and  
116 their size. The biomass (kg km<sup>-2</sup>) index was computed according to Souplet (1996) by weighting the mean index by depth  
117 stratum or macro stratum (e.g. 200-800).

118 The spatial distribution analysis of *I. elongata* biomass was conducted using a spatial modelling approach based  
119 on a generalised additive model (GAM). In particular, biomass data were modelled using the Gaussian family distribution  
120 and link function identity, with longitude (Lon) and latitude (Lat) as explanatory variables. Three different GAM models  
121 were estimated to display *I. elongata* biomass distribution in two bathymetric strata from the MEDITS protocol (AAVV,  
122 2017) where the species was present (200–500 m and 500–800 m), for three periods (2012–2014, 2015–2017 and 2018–  
123 2020), as reported in the following formula:

$$124 \quad \text{Biomass} \sim s(\text{Lon}_{ij}, \text{Lat}_{ij}) + \varepsilon_{ij}$$

125 where  $i$  is the observation in the  $j$  time period, and  $\varepsilon$  is the error term.

126 The prediction maps were generated by using a regular grid with a point resolution of  $0.01^\circ$ . The data used to  
127 resize the grid to the selected depth range were derived from the EMODnet-Bathymetry portal (grid resolution of  $1/16^*$   
128  $1/16$  arc minutes, circa  $115 \times 115$  metres). All the GAM analyses were performed using the R software (R Development  
129 Core Team, 2021) and the *mgcv* package, while distribution maps were generated with QGIS software (QGIS  
130 Development Team, 2017).

131 For the analysis of the biodiversity of associated assemblages, we considered only those hauls performed on  
132 substrates (fine muddy bottom), where *I. elongata* was typically found (Mytilineou et al., 2014; Pierdomenico et al., 2016,  
133 2018; Mastrototaro et al., 2017; Chimienti et al., 2019a; Gerovasileiou et al., 2019; Carbonara et al., 2020; Cartes et al.,  
134 2022) for which the presence or the absence of the species was recorded (Carbonara et al., 2020). In this way, it is possible  
135 to minimise in the analysis the influence of the hauls where IF generally does not occur (e.g., gravel, coarse sand) (Lauria  
136 et al., 2017). From each haul, all the faunal specimens larger than 1 cm (Spedicato et al., 2019) were classified at the  
137 lowest possible taxonomic level, and for each Operational Taxonomic Unit (OTU) data on weight and number of  
138 specimens were collected according to the MEDITS protocol (AAVV, 2017). The Margalef richness index (D) (Margalef,  
139 1958) and Shannon-Wiener index ( $H'$ ) (Shannon, 1948) were computed for each sampling station carried out deeper than  
140 200 m, which represents the area where the probability of IF occurrence was higher (Carbonara et al., 2020). These  
141 indexes were used to detect the potential effects of the presence of *I. elongata* on the associated assemblages. The hauls  
142 performed on suitable substrate types (fine muddy) were pooled into two categories: “presence,” if the bamboo coral was  
143 caught in the haul, and “absence” for all remaining hauls on the muddy bottom. The analysis was carried out separately  
144 by year for the benthic and demersal species, and the differences in biodiversity between the two assemblages with  
145 (associated) and without (not associated) *I. elongata* for each year were evaluated using the non-parametric Wilcoxon–  
146 Mann–Whitney test. Moreover, the presence of a temporal trend in the biomass index ( $\text{kg}/\text{km}^2$ ) of *I. elongata* was tested  
147 by Spearman's rho correlation test.

148 ***Spatial distribution of fishing efforts***

149 Fishing effort in the study area was estimated by means of the aggregated Automatic Identification System (AIS)  
150 data provided by Global Fishing Watch (<https://globalfishingwatch.org/>). Annual fishing activities referred to those from  
151 2015 to 2020 (expressed in hours) and were aggregated in the same temporal frame of the MEDITS *I. elongata* spatial  
152 analysis (three years): 2015–2017 and 2018–2020. The first years (2012–2014) were not considered in the analysis  
153 because of the limited coverage of the active fishery by the AIS system (Global Fishing Watch;  
154 <https://globalfishingwatch.org/data-download/datasets/public-fishing-effort>) that came into effect progressively from  
155 May 2012 to May 2014 (2002/59/CE) in Italy. Moreover, the data were averaged over a grid with a resolution of 0.01° x  
156 0.01°. The hotspot of fishing activities was calculated as the 75th percentile of effort data distribution. The resulting  
157 fishing polygon bordering the effort hotspot was superimposed with the polygon bordering the 75th percentile of the  
158 estimated coral bamboo distribution, in order to assess the percentage of *I. elongata* hotspot surface area overlapping the  
159 main fishing ground in the southern Adriatic Sea (GSA18). The analysis was conducted using R software (R Core Team,  
160 2021) by means of the raster library (Hijmans, 2021).

161 ***Morphological parameter measurements and analyses***

162 During the survey, the morphological parameters of *I. elongata* colonies were collected in terms of basal diameter  
163 (BD, in millimetres) to infer information on the population demographic structure in the southern Adriatic Sea from 2014  
164 to 2020. The trawl survey caused mechanical damage to the colonies and it did not allow us to collect specific data, such  
165 as maximum height, branching patterns (colony width), and/or colony vitality (Carbonara et al., 2020). However, the  
166 basal diameter is often used to infer the size of the colonies (Andrews et al., 2009; Carbonara et al., 2020; Cartes et al.,  
167 2022), as it is correlated to the height (Carbonara et al., 2020). Basal diameter was measured only when the base of the  
168 colony was present, established by the presence of at least part of the root-shaped bases (Carbonara et al., 2020). In all  
169 the hauls where the presence of bamboo coral specimens was detected, the bases of the colonies were collected and  
170 subsequently measured. On each base colony, as close as possible to the root, at least three measurements of the diameter  
171 were taken by caliper (resolution of 0.1 mm), and the final diameter was the average of these measurements (Coefficient  
172 of Variation between 0 and 9.75). The annual average values of colony BD were used to test the trend of the colony sizes  
173 over seven years (2014–2020) using Spearman’s correlation. In addition, the eventual differences during the study period  
174 were tested using a Kruskal–Wallis one-way analysis of variance by ranks. The Nemenyi *post hoc* test was used to  
175 evaluate the significance of such median differences between years. In order to analyse the change in the basal diameter  
176 distribution (BDD), the years were grouped into three periods as follows: 2014, 2015–2017 and 2018–2020. The years

177 were grouped according to the time periods selected for the GAM analysis. The different BDDs were compared by  
178 Kolmogorov–Smirnov (K–S) test.

179 The skewness index (SK) of BD distribution was calculated to measure the distributions' deviation from symmetry.  
180 The skewness describes three scenarios: (i) distribution dominated by smaller colonies ( $SK > 0.5$ ), (ii) distribution  
181 dominated by larger colonies ( $SK < 0$ ), and (iii) no size dominance ( $0 \leq SK \leq 0.5$ ).

182

## 183 **Results**

### 184 ***Occurrence, distribution, and abundance of *I. elongata* in GSA 18***

185 The percentage and depth range of occurrence of *I. elongata* per haul in GSA 18 is reported in **Table 1**. The  
186 percentage of occurrence (number of hauls positive with the presence of IF out of the total number of hauls carried out)  
187 showed from a maximum of 9.41% in 2017 to a minimum value of 4.44% in 2019. The depth range of occurrence was  
188 between 410 m (2017) and 625 m (2016).

189

190 **Table 1.** Percentage (%) (number of positive hauls to the presence of bamboo coral) and depth range (m) of occurrence  
191 of *Isidella elongata* in each year, total number of hauls carried out in the survey, and their depth range.

Year	Percentage of occurrence (%)	Depth range of <i>I. elongata</i> occurrence (m)	Total number hauls carried out in the survey	Depth range of hauls carried out in the survey (m)
2012	6.67	520–618	90	12–678
2013	7.78	517–617	90	11–713
2014	6.67	447–617	90	11–715
2015	6.67	515–619	90	10–693
2016	6.67	429–625	90	10–739
2017	9.41	410–601	85	10–733
2018	6.74	518–596	89	10–701
2019	4.44	569–621	90	10–749
2020	7.14	551–600	70	10–759

192

193 In all the three models tested to describe the effect of the spatial displacement on the biomass of *I. elongata* (kg  
194 km<sup>-2</sup>), the bidimensional splines of the geographic coordinates included in the Gaussian GAM model were significant for  
195 the three monitored periods ( $p < 0.05$  for all), explaining the following amount of deviance: 86.2% in 2012–2014, 72.4%  
196 in 2015–2017 and 59.7% in 2018–2020. These splines (**Fig. 2**) indicate the combined effects of northing and easting on  
197 the biomass, locating the hotspot bamboo coral biomass in the Otranto Channel (**Fig. 3**). The extension of the area, in  
198 which the hotspots (75th percentile of the estimated biomass distribution) of IF were estimated to be located, is similar  
199 for the three periods considered (1436.7 km<sup>2</sup> for 2012–2014, 1520.4 km<sup>2</sup> for 2015–2017 and 1421.6 km<sup>2</sup> for 2018–2020).  
200 Similarities were seen in its spatial distribution pattern as well, although with a clear decreasing biomass of the bamboo  
201 coral over the three groups of study periods (**Fig. 3**). A significant decreasing trend ( $p < 0.05$ ) in the biomass index of *I.*  
202 *elongata* was also detected over the time series considered (2012–2020). The biomass index of *I. elongata* was around 25  
203 kg/m<sup>2</sup> in both 2012 and 2013, with a peak in 2014 ( $65.7 \pm 47.1$  kg/m<sup>2</sup>), while very low biomass indexes below 1.3 kg/m<sup>2</sup>  
204 were observed from 2018 to 2020 (**Fig. 4**).

205 The list of the OTUs found in the different years in the study area and their relative biomass were provided in the  
206 supplementary material. In **Figures 5 and 6**, the analyses of the Margalef and Shannon-Wiener biodiversity indexes for  
207 the hauls associated or not associated with the presence of *I. elongata* are reported. The results of the diversity indexes  
208 for the assemblages “associated” and “not associated” were compared within each year. For the benthic species, no  
209 significant difference between the assemblages with and without the bamboo coral was observed, except in 2016 for the  
210 Shannon diversity index. **In contrast**, for the demersal species the assemblage associated with *I. elongata* showed  
211 significantly higher values for both indices in most of the years analysed ( $p < 0.05$ ), excepted in 2016 for the Shannon  
212 diversity index and from 2018 to 2020 for the Margalef one ( $p < 0.05$ ; **Fig. 5**). A significant decreasing trend ( $p < 0.05$ )  
213 of the Margalef index was detected for the demersal “associated” assemblage over the study period (**Fig. 6**). No significant  
214 trend over time was observed in both indices of both benthic assemblages nor in the Margalef index of “not associated”  
215 demersal assemblages and in the Shannon diversity index of both demersal assemblages (**Fig. 5, 6**).

### 216 *Spatial distribution of fishing efforts*

217 **Figure 7** reports the hotspot of fishing activities (75th percentile) for the two-time frames analysed (2015–2017  
218 and 2018–2020). Moreover, the overlap between the area hotspot of fishing activities and the hotspot (75th percentile) of  
219 bamboo coral distribution is indicated and quantified in terms of percentage. The percentage of overlapping between the  
220 area hotspot of fishing activities and bamboo coral was found to be 8.84% between 2015–2017 while it reached 12.27  
221 between 2018–2020, with a resulting increase of 38.8 % between the two periods considered.

## 222 *Morphological parameters*

223 Significant differences were detected between BDD distributions in the three considered periods ( $p < 0.05$ ; **Fig.**  
224 **8**). In 2014, the BDD skewed towards larger-sized colonies (basal diameters  $> 4$  mm;  $SK = -0.275$ ) than in the 2015–  
225 2017 period when no dominance of any size was observed ( $SK = 0.26$ ) and two small peaks (1.5–2 mm and 3.5–4 mm)  
226 were observed. In 2018–2020, the BDDs skewed towards smaller-size colonies (peak at 1.5–2 mm;  $SK = 846$ ).

227 This pattern is more evident while analysing the yearly distribution (**Fig. 9**), where the Spearman's rho test revealed  
228 a significant decreasing trend in BD over the years ( $p < 0.05$ ) (**Fig. 9**). Indeed, in 2014, BD was significantly larger (mean  
229  $\pm$  SE,  $4.02 \pm 0.096$  mm) than in other years ( $p < 0.05$ ). Between 2016 and 2019, the BD values were overall similar over  
230 the years (from 0.58 mm to 6.0 mm), while the BD measured in 2020 was found significantly smaller than all other years  
231 ( $1.86 \pm 0.095$  mm) ( $p < 0.05$ ) (**Fig. 9**).

232

## 233 **Discussion**

234 The present study investigated the spatial and temporal distributions of *I. elongata* in the southern Adriatic Sea  
235 (GSA 18) through time-series data of nine years (2012–2020) collected during MEDITS surveys. The biodiversity of the  
236 assemblages associated with *I. elongata* was also investigated. In the study area, the depth range of species occurrence in  
237 the present study was from 410 m to 625 m, which is in line with data already published for the southern Adriatic Sea  
238 (Carbonara et al., 2020) as well as with other areas of the western and central Mediterranean (Cartes et al., 2009; Lauria  
239 et al., 2017; Mastrototaro et al., 2017; Pierdomenico et al., 2018; Ingrassia et al., 2019). However, it is difficult to define  
240 the maximum depth of the species distribution in the study area due to both the depth range of sampling of the MEDITS  
241 protocol (i.e., down to maximum 800 m in depth) and the bottom morphology of the study area around the IF presence,  
242 that are unsuitable to trawling at depths greater than 650 m. Despite the overall depth range of the investigated area was  
243 larger than the bamboo coral occurrence (**Table 1**) in the GSA 18 (north part of the GSA18), some deeper areas around  
244 the *I. elongata* presence are not trawlable and thus not explored during the MEDITS surveys. Consequently, it is not  
245 excluded that the IF in the southern Adriatic Sea could extend even deeper than that already observed (625 m depth)  
246 (Rueda et al., 2016; González-García et al., 2020) in a more pristine area unexplored by trawl-survey, as **shown by the**  
247 **results of the GAM model (Fig. 3)** (Carbonara et al., 2020). The spatial analysis performed in this study confirms that the  
248 Otranto Channel represents a suitable area with a consistent presence of IF (Carbonara et al., 2020), probably due to a  
249 combination of all required environmental factors (i.e. compact/semi consolidated mud with a low slope angle, low  
250 seasonal variation in term of salinity and temperature and high concentration of near bottom zooplankton) (Cartes et al.,

251 2013; Bo et al., 2015a; Lauria et al., 2017; Ingrassia et al., 2019). In addition, the presence of IF can be enhanced by the  
252 upwelling of nutrient-rich deep waters, which has been documented in the area (Vilibic & Orlic, 2002). Moreover, the  
253 fishing activity of the southern Adriatic trawlers fleet is more concentrated on the continental shelf and on the upper slope  
254 (Spedicato & Lembo, 2011), resulting in a lesser impact on the deeper bottoms where IF can be found. Although modelling  
255 *I. elongata* quantitative data was affected by the limitations of the MEDITS trawl net which is not the best sampler for  
256 exploring the benthic communities (González-García et al., 2020), the systematic collection of data on the macrobenthic  
257 species caught during the MEDITS trawl survey could represent a valuable source of information, particularly for  
258 understanding their distribution patterns and potential significant changes over time (Fernandez-arcaya et al., 2019), as  
259 well as the impact of human-related activities, such as trawling (Mangano et al., 2013). Mechanical damage from trawling  
260 rarely allows the collection of unbroken complete individual colonies, limiting morphological study; however, the  
261 biomass data of the bamboo coral collected during the MEDITS trawl survey are proportional to the number of colonies  
262 and their size (Carbonara et al., 2020). Moreover, in the absence of other systematic sources of information, these  
263 opportunistic data have proven to be useful sources of information for benthic communities in general and, in particular,  
264 for the IF (Lauria et al., 2017; Carbonara et al., 2020).

265 The temporal analysis performed through the spatial distribution of biomass within the IF shows a clear decreasing  
266 trend of abundance (biomass index) over the study period, but no difference in distribution extent. The decreasing trend  
267 of the IF in the southern Adriatic Sea in the last nine years (2012–2020) could be related to the cumulative impact of  
268 trawlers on the bamboo coral, produced by the increasing fishing effort detected at least in the area over time (Fig. 7) at  
269 least on the area monitored by the MEDITS survey (until about 630 m of depth; see Table 1). Despite the traditional  
270 fishing habits of the trawler fleet in southern Adriatic Sea, that mostly exploited the continental shelf and the upper slope  
271 (Spedicato & Lembo, 2011), in recent years, there has been an increase in trawler numbers capable of fishing even at  
272 great depths in the eastern part of the southern Adriatic Sea (Albania) (STECF, 2019), with an increasing impact on the  
273 IF. *Isidella elongata* is often associated with the main targets species of deep fishery trawlers, such as the red shrimps  
274 (*Aristaeomorpha foliacea* and *Aristeus antennatus*) and the Norway lobster (*Nephrops norvegicus*) (Maynou & Cartes,  
275 2012) in areas where a high overlap with the Essential Fish Habitat (EFH) was recently documented for both shrimps,  
276 particularly in the Otranto Channel (Carbonara et al., 2020). Pulcinella et al. (2021) analysed the AIS data and the fishing  
277 ground in the central-east Mediterranean and revealed how the number of fishing vessels dedicated to deep fishing in the  
278 GSA 18 increased from 23 in 2017 to 29 in 2018. Furthermore, the area of the Otranto Channel seems to be the most  
279 frequented area by deep sea fishing vessels with a monthly trawling haul frequency at depths greater than 400 m (deep  
280 water fishery) ranging from 12 to 24 over three years (2015–2018) and a mean number of fishing hours per km<sup>2</sup> ranging



281 from 6 to 16 in the east and from 44 to 118 in the west part of the Otranto channel in the same time series. The combined  
282 analysis with spatial distribution of the fishing effort and the distribution of IF, showed an increase in the fishing effort  
283 on the hotspot of bamboo coral presence over time. Indeed, the overlap between the IF presence and the hotspot (75th  
284 percentile) deep (depth > 200 m) fishing ground increased from 8.84% to 12.27% over time, resulting in an increase of  
285 38.8% between the two periods considered (2015-2017 vs 2018-2020; **Fig. 7**). Consequently, the increasing fishing effort  
286 for commercial fishery activity registered in recent years in the southern Adriatic Sea (IUCN, 2019) could deeply affect  
287 the distribution of the IF with fishing being considered one of the most impactful human activities on the VME, including  
288 the IF (Cartes et al., 2013; FAO, 2018; Fanelli et al., 2021).

289 The biodiversity associated with the colonies of the bamboo coral is significantly higher for both diversity indices  
290 used (i.e., Margalef and Shannon-Wiener) considering all time series (2012–2020) for the demersal OTU whereas no  
291 difference in the benthic OTU was found between the two assemblages considered (i.e., with and without bamboo coral).  
292 Based on these results, *I. elongata* seems to generate an important effect on the associated assemblage, more evident on  
293 the demersal than benthic fauna. Indeed, due to its candelabrum-like shape, the bamboo coral is one of the soft-bottom  
294 cold-water corals (CWCs) with a distinct frame-building ability to construct coral forests (Chimienti et al., 2019b) that  
295 also act as trophic areas where many species, such as the sharks *G. melastomus* and *E. spinax*, the teleost fishes *M.*  
296 *merluccius*, *M. poutassou*, *P. blennoides*, *H. dactylopterus*, *H. mediterraneus* and *Lepidorhombus boscii*, find preys  
297 swimming among the colonies or climbing on them (Mastrototaro et al., 2017; Carbonara et al., 2020; Cartes et al., 2022).  
298 Several studies on CWCs reveal a link between habitat complexity formed by corals and species diversity, particularly in  
299 bathyal zones (D’Onghia, 2019; Rueda et al., 2019; Cartes et al., 2022) where habitat-forming species variety is generally  
300 limited (Gage and Tyler, 1992). The presence of the bamboo coral, as a passive feeder, is often associated with the high  
301 abundance of zooplankton (Cartes et al., 2013). This biological feature, in turn, qualitatively and quantitatively influences  
302 IF-associated fauna in terms of demersal species within and around the colonies (Mastrototaro et al., 2017; Rueda et al.,  
303 2019; Carbonara et al., 2020). The large amount of zooplankton can produce a positive effect on several species in various  
304 food web positions, from the lower trophic levels to the higher of top predators (Rueda et al., 2019; Carbonara et al.,  
305 2020). For example, the presence of red shrimps in the IF (Cartes et al., 2013; Carbonara et al., 2020) seems to be linked  
306 with the presence of the Pandalidae species, which probably shares the same prey as bamboo coral (zooplankton), and, in  
307 turn, they are the favourite prey of both *A. foliacea* and *A. antennatus* (Kapiris, 2012). Moreover, the high concentration  
308 of pandalids and other crustaceans, including Aristeidae, attracts several bony fishes and ommastrephid cephalopods,  
309 producing a cascade of effects that could modify the fauna in the areas characterized by the presence of *I. elongata* and  
310 the energy flow through the food web (Eddy et al., 2021). Fishing is known to remove individuals at various trophic levels

311 from natural habitats, thus affecting the overall energy flow through the food web (Watling & Norse, 1998; Pauly &  
312 Palomares, 2005; Sieben et al., 2011; Carbonara et al., 2022), making the environment less efficient in supporting a certain  
313 level of biodiversity (Hooper et al., 2005; Thompson et al., 2012; Ullah et al., 2018). Finally, the typical candelabrum-  
314 like shape of *I. elongata* also plays a role in terms of refuge from predators (Mastrototaro et al., 2017) and spawning area  
315 for several elasmobranch species (e.g., *Galeus melastomus* and *Scyliorhinus canicula*) (Carbonara et al., 2020). This effect  
316 was already observed in other CWC habitats in both the Ionian and southern Adriatic Seas (D’Onghia et al., 2010, 2012,  
317 2016; Sion et al., 2019).

318 In this study, the time-series analysis also highlighted a temporal effect on biodiversity of assemblages with *I.*  
319 *elongata*, but with different results for the two indices. The richness index shows a significant negative trend over time  
320 in the assemblages with *I. elongata* whereas the Shannon-Wiener index exhibits no clear trend. The Margalef index has  
321 a good discriminatory ability towards species richness (Yeom & Kim, 2011; Magurran, 2013) that probably presents a  
322 faster response to the impact whereas the Shannon index is influenced by both species richness and species abundance  
323 distribution (evenness) that can exhibit a slower change in deep-sea communities. Moreover, the significant negative  
324 trend for the Margalef index could be linked to a decrease of the IF consistency over time (see Fig. 2,3). Indeed, the IF  
325 can play its role as a biodiversity hotspot (Cartes et al., 2013; Mytilineou et al., 2014; Mastrototaro et al., 2017) when it  
326 shows a certain level of integrity (Carbonara et al., 2020). When the IF is strongly impacted by fishery activity and consists  
327 of a few young and isolated colonies (Mastrototaro et al., 2017; Pierdomenico et al., 2018), it cannot fulfil its role as an  
328 attractive three-dimensional habitat (e.g., food riches, refuges) (Rueda et al., 2016).

329 Furthermore, the analyses carried out on the assemblages (with and without *I. elongata*) in the Otranto Channel  
330 showed significant differences in their specific compositions. The complete list of the OTUs found in the different years  
331 of the study is available in the supplementary material. In particular, among the most abundant species in the areas  
332 associated with IF, key species determine these differences more than others (Carbonara et al., 2020). Included in these  
333 key species are the osteichthyes *Hoplostethus mediterraneus mediterraneus* Cuvier, 1829, *Phycis blennoides* (Brünnich,  
334 1768) *Helicolenus dactylopterus* (Delaroche, 1809), and *Coelorinchus caelorhincus* (Risso, 1810); the elasmobranchs  
335 *Etmopterus spinax* (Linnaeus, 1758), *Dalatias licha* (Bonnaterre, 1788), and *Chimaera monstrosa* (Linnaeus, 1758); the  
336 crustaceans *Aristaeomorpha foliacea* (Risso, 1827), *Aristeus antennatus* (Risso, 1816), and *Paromola cuvieri* (Risso,  
337 1816); and the cephalopod *Todarodes sagittatus* (Lamarck, 1798). Several of these species are commercial (see  
338 supplementary material), in particular the red shrimps (*A. foliacea* and *A. antennatus*) that are the target of the trawlers  
339 fishing on the slope (deeper than 200 m) (Maiorano et al., 2010; Russo et al., 2017).

340 Mature colonies (i.e., larger colonies with higher density) of *I. elongata* exhibit a higher macrofaunal community  
341 diversity (Cartes et al., 2022), also highlighting the importance of the colony size in the species diversity. Basal diameter  
342 is correlated with the height and width of the colonies in *I. elongata* (Carbonara et al., 2020) and with the age of colonies  
343 in congeneric species (Roark et al., 2005; Andrews et al., 2009). In this study, we observed that the BDD was different  
344 among the three periods considered (2014, 2015–2017 and 2018–2020). In the first period (2014), the BDDs are  
345 dominated by larger and older colonies (basal diameters > 4 mm), while in the 2015–2017 period, the BDDs are  
346 characterized by two similar peaks (1.5–2 mm and 3.5–4 mm) with a lower peak at 5 mm. In the last period considered  
347 (2018–2020), the BDD is quite monomodal with a peak at 1.5–2 mm. Thus, in the time series considered, the colony  
348 dimension/age seems to shift from a non- or little-impacted population (2012–2014) to a highly impacted population  
349 (2018–2020). The records from the last period considered (2018–2020) are similar to those found by Carbonara et al.  
350 (2020) in a population that was highly impacted by fishing (Tyrrhenian Sea). The population dominated by colonies with  
351 BD greater than 4 mm, found in 2014, could be considered quite pristine or minimally impacted by fishing (Bo et al.,  
352 2015a, 2015b; Carbonara et al., 2020). Colonies with BD greater than 4 mm correspond to the overall size of colonies  
353 (Carbonara et al., 2020) in pristine areas (Bo et al., 2015a, 2015b) with a capacity for reproduction and the renewal of the  
354 population still untouched (Bo et al., 2015a, 2015b). These changes over time are also confirmed by the significant trend  
355 of a decrease in yearly average BD. In this case, as well as for the spatial analysis, the reason for this decrease (average  
356 BD) could be the increased pressure of deep-sea fishing (FAO, 2018; IUCN, 2019). Although the age and growth for the  
357 *I. elongata* data are missing, congeneric species have been found to have a slow growth rate (Roark et al., 2005; Andrews  
358 et al., 2009). In the Gulf of Alaska, it was estimated that the lifespan for *Isidella spp.* was 75 to 126 years, with a slow  
359 growth rate ranged between about 0.1–0.05 mm of radial basal diameter per year (Roark et al., 2005; Andrews et al.,  
360 2009). From the perspective of *I. elongata* VME conservation, such fast decreasing trend detected in few years together  
361 with the low growth of *Isidella sp.* (Andrews et al., 2009) could be a dangerous warning sign of a severe progressive  
362 impact on IF. This biological characteristic of *Isidella sp.* coral indicates that the recovery of areas impacted by fishing  
363 (Mastrototaro et al., 2017; Pierdomenico et al., 2018) can take a very long time, ranging from a few decades to a century  
364 (Roark et al., 2005). Furthermore, it should be emphasised that studies on the growth and age of *I. elongata* have not yet  
365 been carried out and should be implemented as soon as possible not only for the purpose of biological and ecological  
366 knowledge but also for the conservation of this species (Carbonara et al., 2020).

### 367 **Concluding remarks**

368 In conclusion, the consistent presence of an *I. elongata* VME was confirmed in the Otranto Channel in the southern  
369 Adriatic Sea (Carbonara et al., 2020). Over a period of nine years, this VME seems to have a constant extension, but with

370 a significant decrease in biomass and changes in demographic structure. The overlap between the spatial distribution of  
371 the bamboo coral and that of the fishing effort, indicates a severe impact clearly highlighted by the results of this study.  
372 Furthermore, other signs of the increasing impact of anthropogenic activities, such as fishing, are the changes in BDD  
373 (skewed towards smaller colonies in the last period from 2018 to 2020, unlike in 2014, where it skewed more towards  
374 large sizes) and the significant decrease in BD over time (from 2014–2020). It is also important to emphasise that IF  
375 represents biodiversity hotspots for deep environments in the study area (Cartes et al., 2009; Chimienti et al., 2019a,  
376 2019b) that could be compromised by fisheries (Cartes et al., 2013). Until now, no fishing restrictions have been  
377 implemented in the area where the presence of IF (Otranto Channel) has been detected; indeed, as shown by our analysis,  
378 this area is a fishing ground for deep trawlers. Thus, it was recently proposed, in the context of the GFCM Subregional  
379 Committee for the Adriatic Sea, that the process to establish a fisheries restricted area (FRA) at the Otranto Channel  
380 (GFCM, 2021) must be started in order to help the conservation of *I. elongata* in the southern Adriatic Sea, achieving the  
381 goal of protecting VMEs set by various international organizations/conventions (FAO, 2009; UNEP-MAP-RAC-SPA,  
382 2015).

383

#### 384 **Statements and Declarations**

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#### 390 **Data availability**

391 All data generated or analysed during this study are included in this published article and its supplementary information  
392 files, further inquiries can be directed to the corresponding author.

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646 **Captions for figures**

647 **Figure 1.** The study area is the southern Adriatic Sea (Geographical sub-area GSA 18 *sensu* GFCM-FAO). The  
648 geographic allocation of the hauls explored during the MEDITS trawl surveys in GSA 18 are indicated by black points.

649 **Figure 2.** The bidimensional splines of the Gaussian GAM models used to describe the spatial distribution of *Isidella*  
650 *elongata* biomass (kg/km<sup>2</sup>) in (a) 2012–2014, (b) 2015–2017, and (c) 2018–2020. X: Longitude, Y: Latitude.

651 **Figure 3.** The spatial distribution of *Isidella elongata* biomass (kg/km<sup>2</sup>) in the three considered periods: (a) 2012–2014,  
652 (b) 2015–2017, and (c) 2018–2020. The green line borders the 75th percentile of the estimated biomass distribution.

653 **Figure 4.** *Isidella elongata* biomass index (kg/km<sup>2</sup>) (blue trend in macrostratum at depths of 200–800 m). The Spearman's  
654 rho correlation test showed a significant decrease ( $p < 0.05$ ). The solid line represents the biomass index, while the dashed  
655 lines represent the standard deviation.

656 **Figure 5.** Benthos OTU box plots of the (a) Margalef (D) and (b) Shannon (H') indexes of assemblages with (associated)  
657 and without (not associated) *Isidella elongata* per year. The solid line of each boxplot indicates the median, and the boxes  
658 on either side represent the quartiles, with the whiskers covering 95% of the values. Outlier values are represented by  
659 black dots. Statistics reported in the figure are based on Wilcoxon–Mann–Whitney tests carried out within each period  
660 (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; NS = not significant). The Spearman's rho correlation test was not significant ( $p > 0.05$ ) for  
661 both assemblages (associated and not associated) and both indices (Margalef and Shannon).

662 **Figure 6.** Demersal OTU box plots of the (a) Margalef (D) and (b) Shannon (H') indexes of assemblages with (associated)  
663 and without (not associated) *Isidella elongata* per year. The solid line of each boxplot indicates the median, and the boxes  
664 on either side represent the quartiles, with the whiskers covering 95% of the values. Outlier values are represented by  
665 black dots. Statistics reported in the figure are based on Wilcoxon–Mann–Whitney tests carried out within each period  
666 (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; NS (not significant):  $p > 0.05$ ). The Spearman's rho correlation test was not significant ( $p >$   
667  $0.05$ ) for both assemblages (associated and not associated) for Shannon's index. For the Margalef's index the “associated”  
668 assemblage shows a significant decreasing trend (Spearman's rho correlation test  $p < 0.05$ ), while that of the “not  
669 associated” assemblage was not significant ( $p > 0.05$ ).

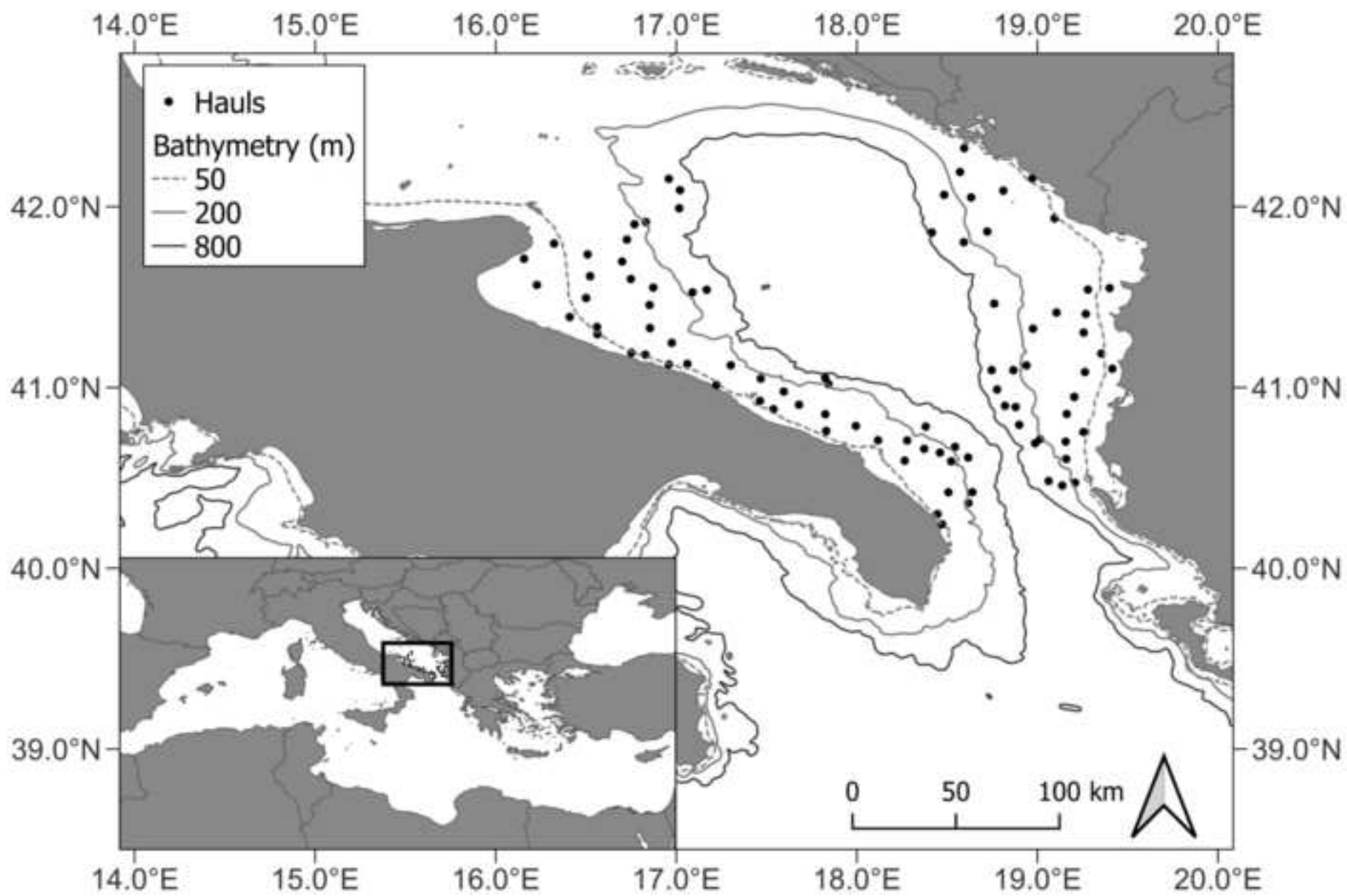
670 **Figure 7.** Fishing effort in the southern Adriatic Sea in (A) 2015–2017 and (B) 2018–2020, estimated using the aggregated  
671 AIS data provided by Global Fishing Watch (<https://globalfishingwatch.org/>). The hotspot of fishing activities was  
672 calculated as the 75th percentile of effort data distribution. Moreover, the spatial and percentage overlap between the  
673 hotspot of *Isidella elongata* presence and fishing efforts is also shown.

674 **Figure 8.** Basal diameter (mm) distribution of *Isidella elongata* colonies in **(a)** 2014, **(b)** 2015–2017, and **(c)** 2018–2020.

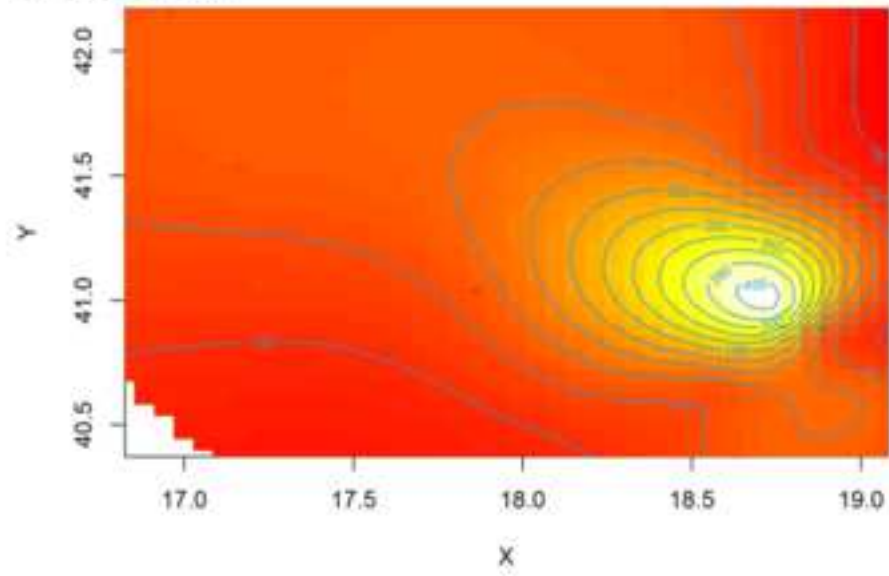
675 **Figure 9.** Box plots of the colony basal diameter (mm) measured from 2014 to 2020 in the GSA 18. For boxplot  
676 representation, the central line of each boxplot indicates the median, and the boxes on either side represent the quartiles,  
677 with the whiskers covering 95% of the values. Outlier values are represented by black dots. Different letters indicate  
678 significant differences among groups between the years (Kruskal–Wallis and Nemenyi post-hoc tests,  $p < 0.05$ ).

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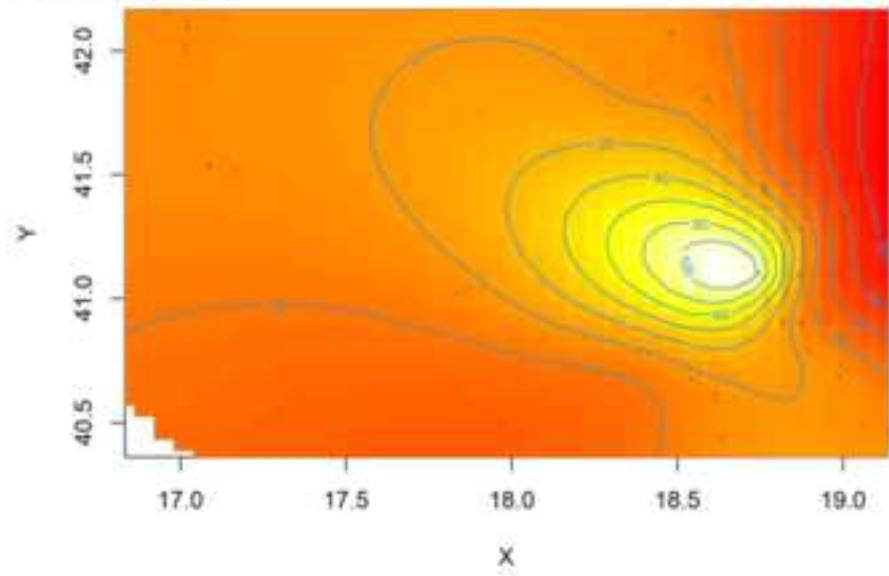




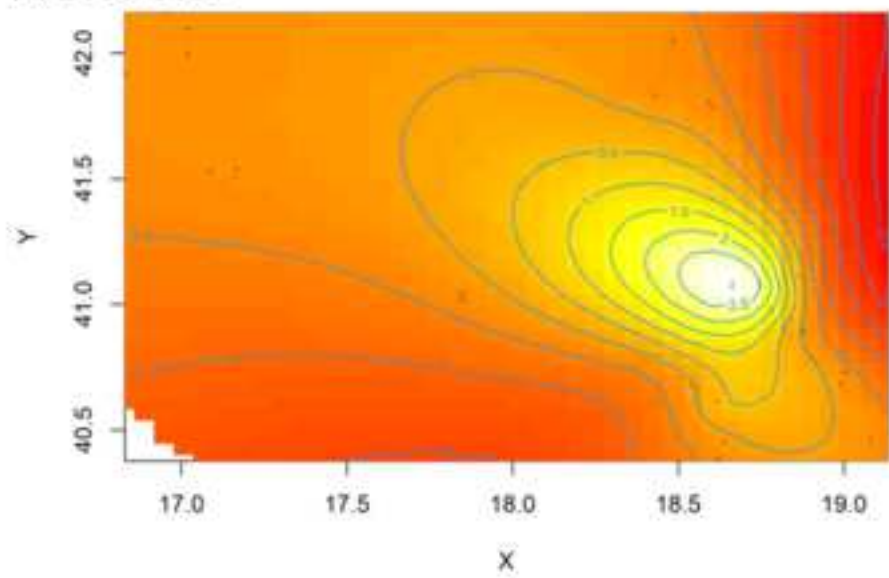
(a) 2012-2014

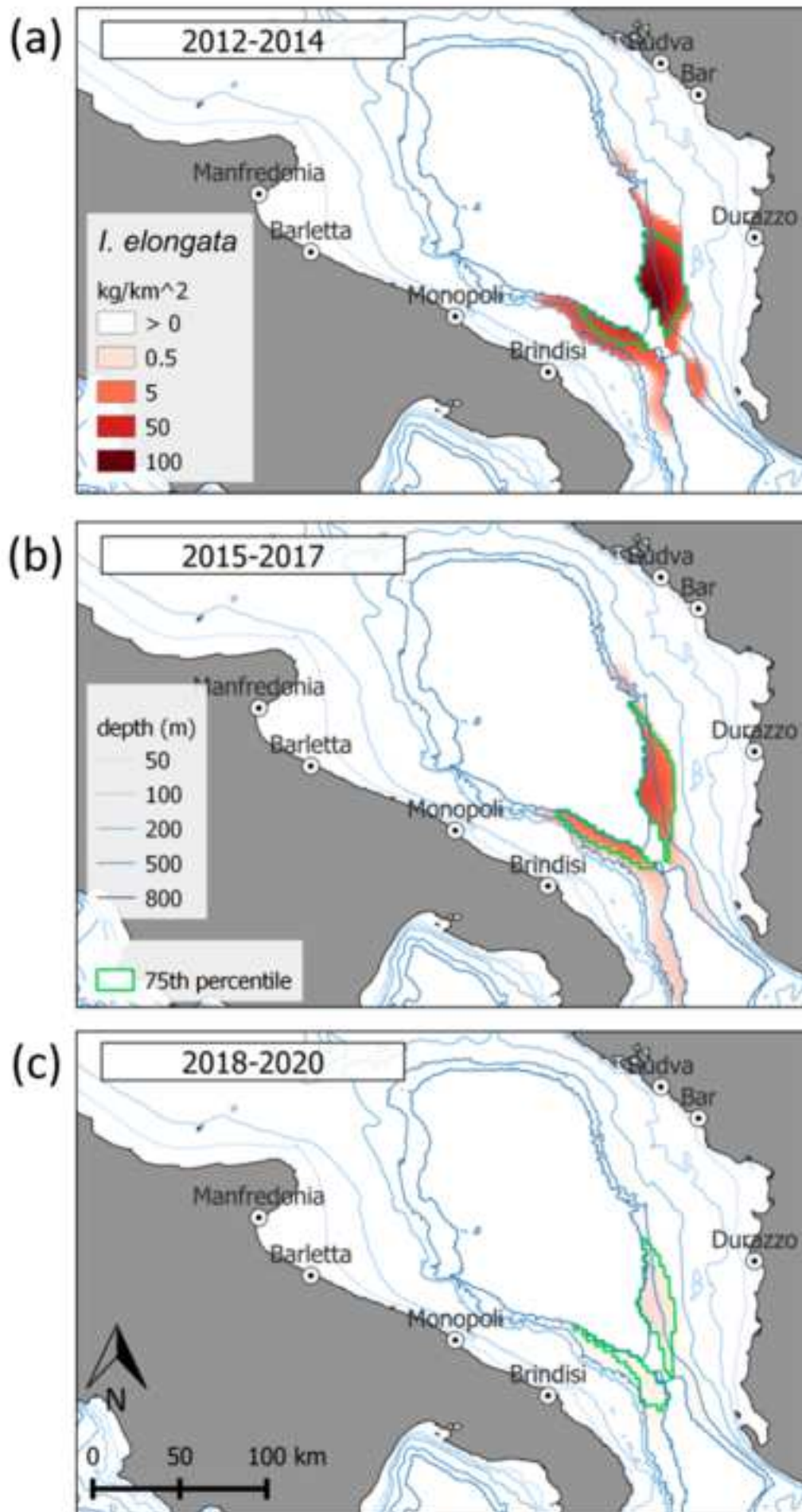


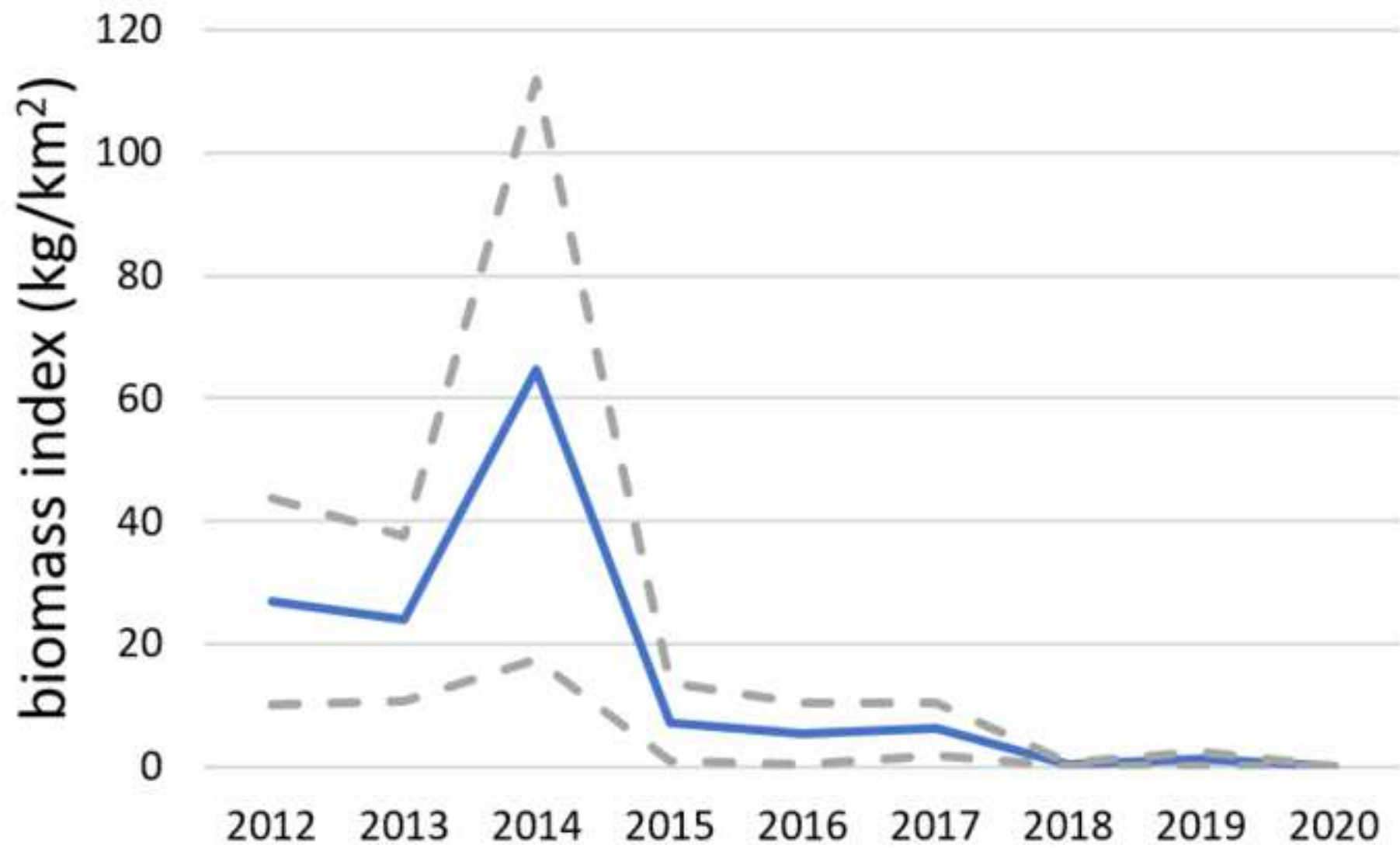
(b) 2015-2017

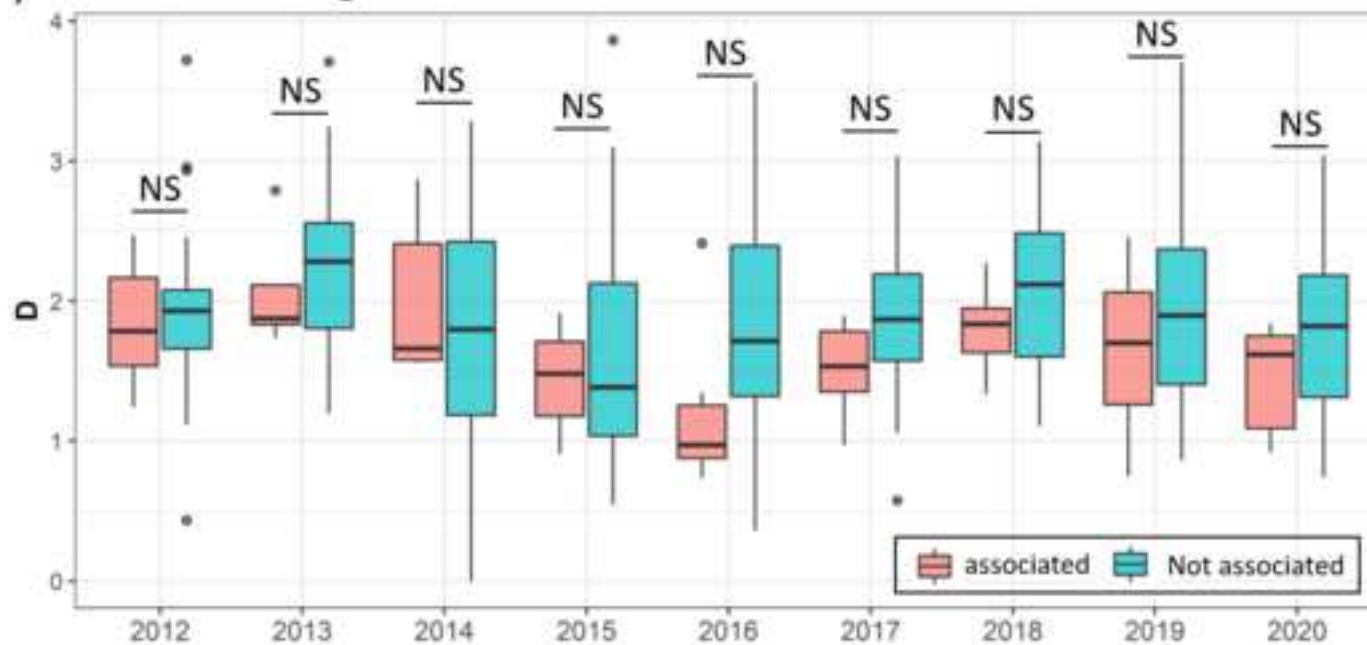
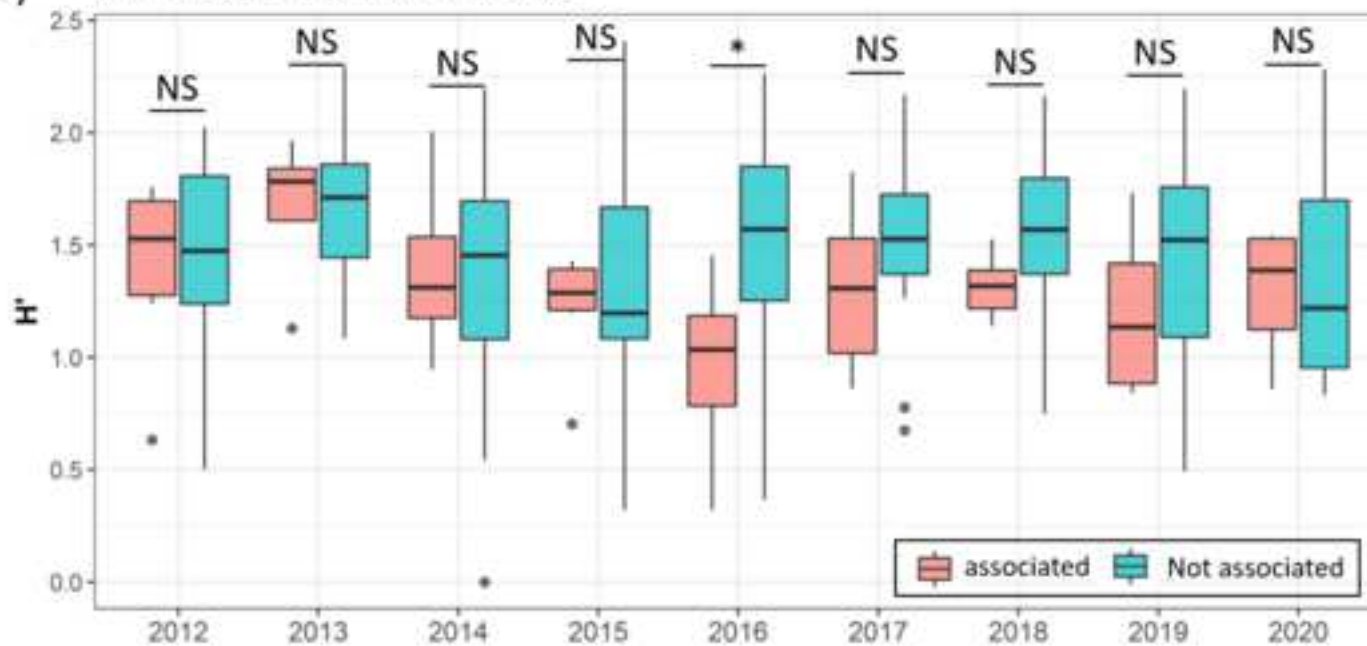


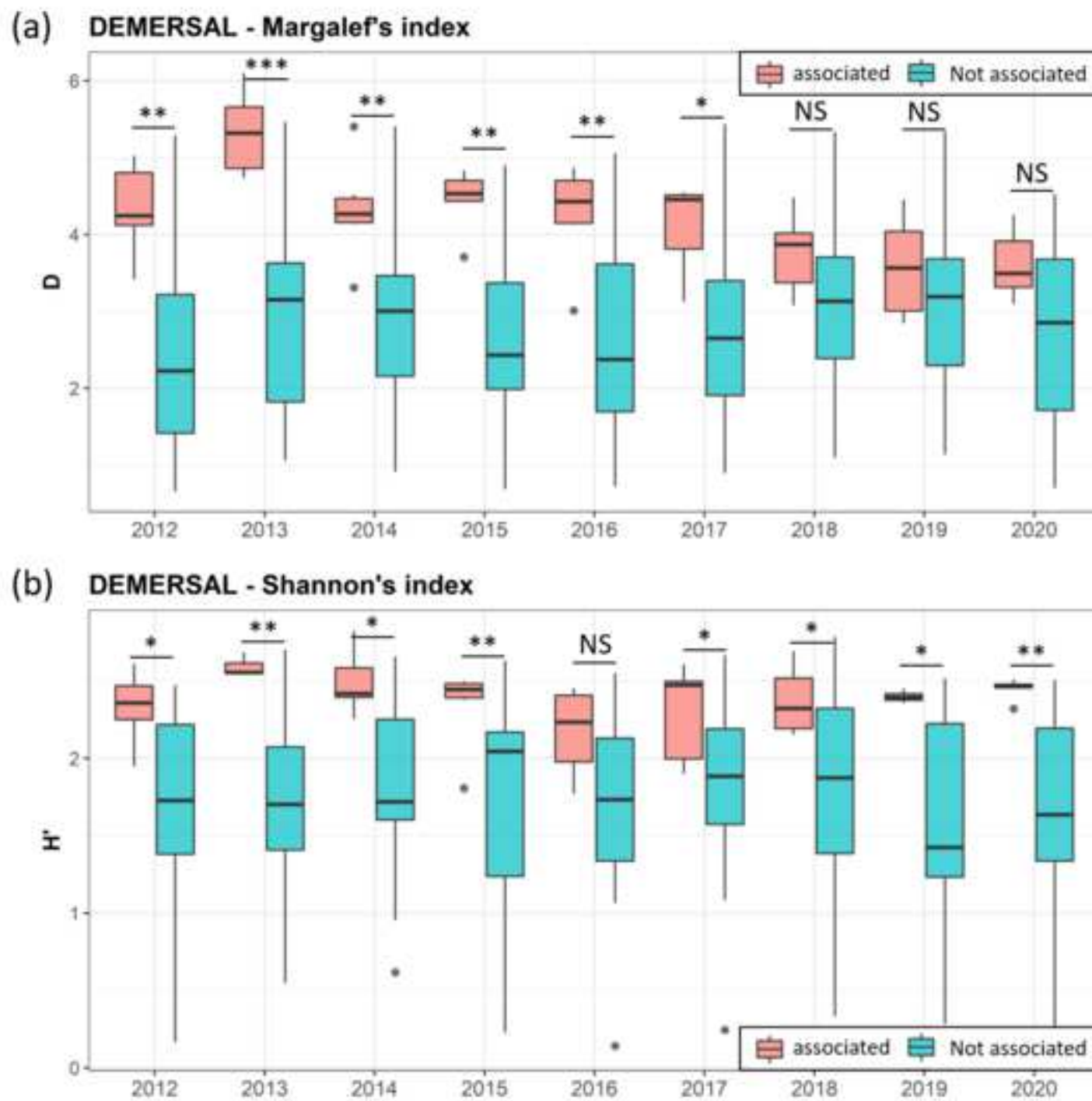
(c) 2018-2020

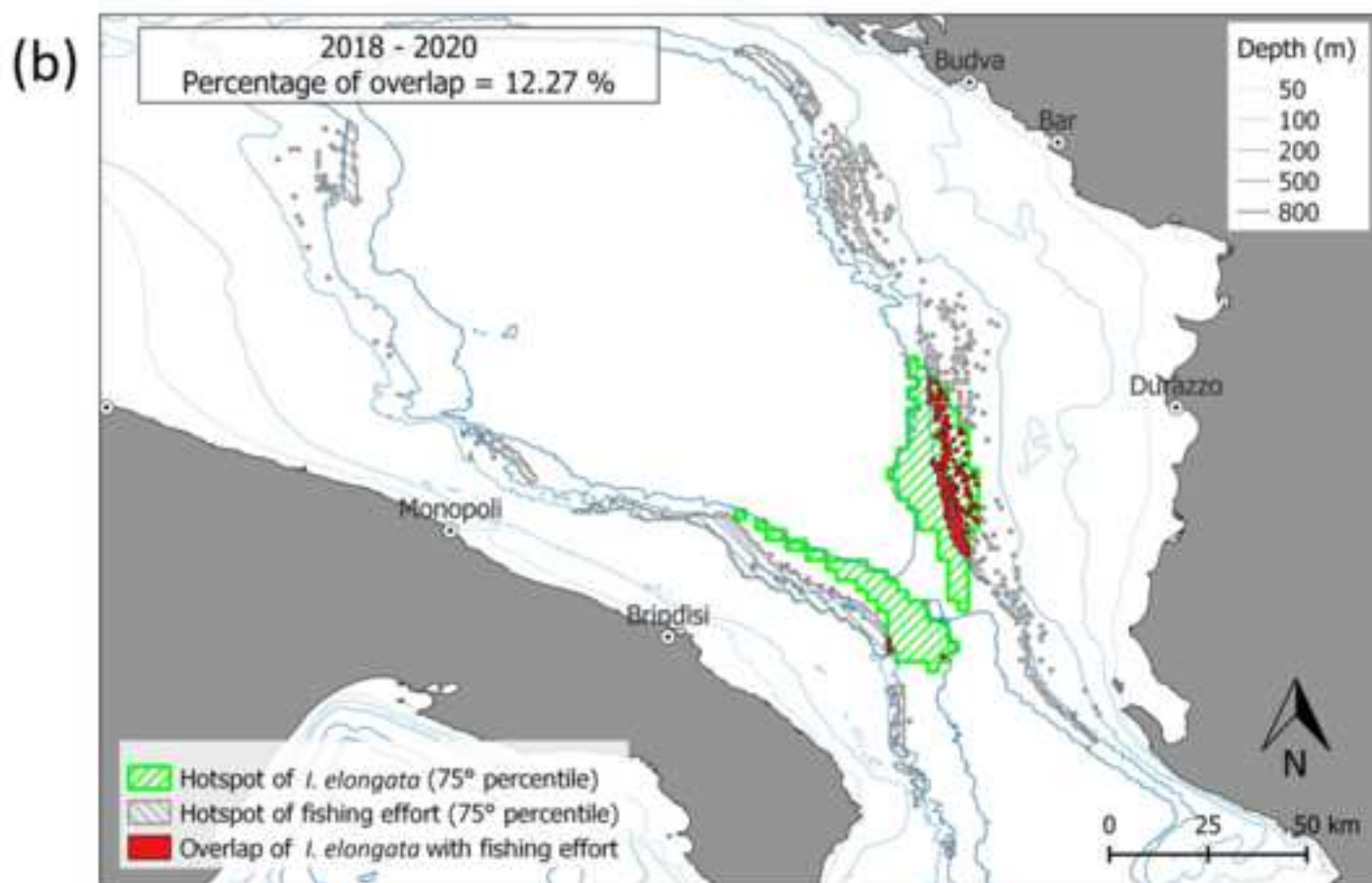
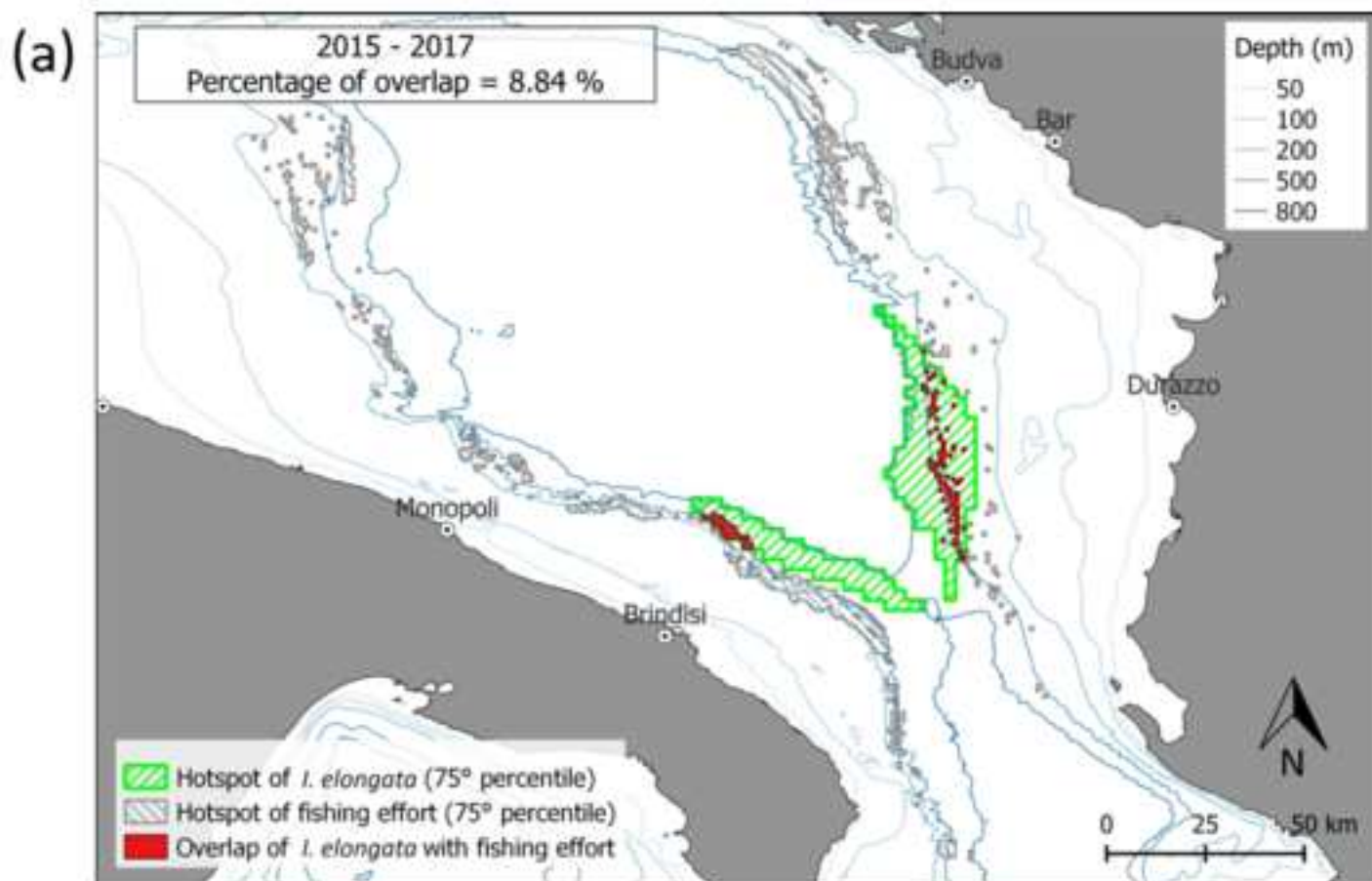


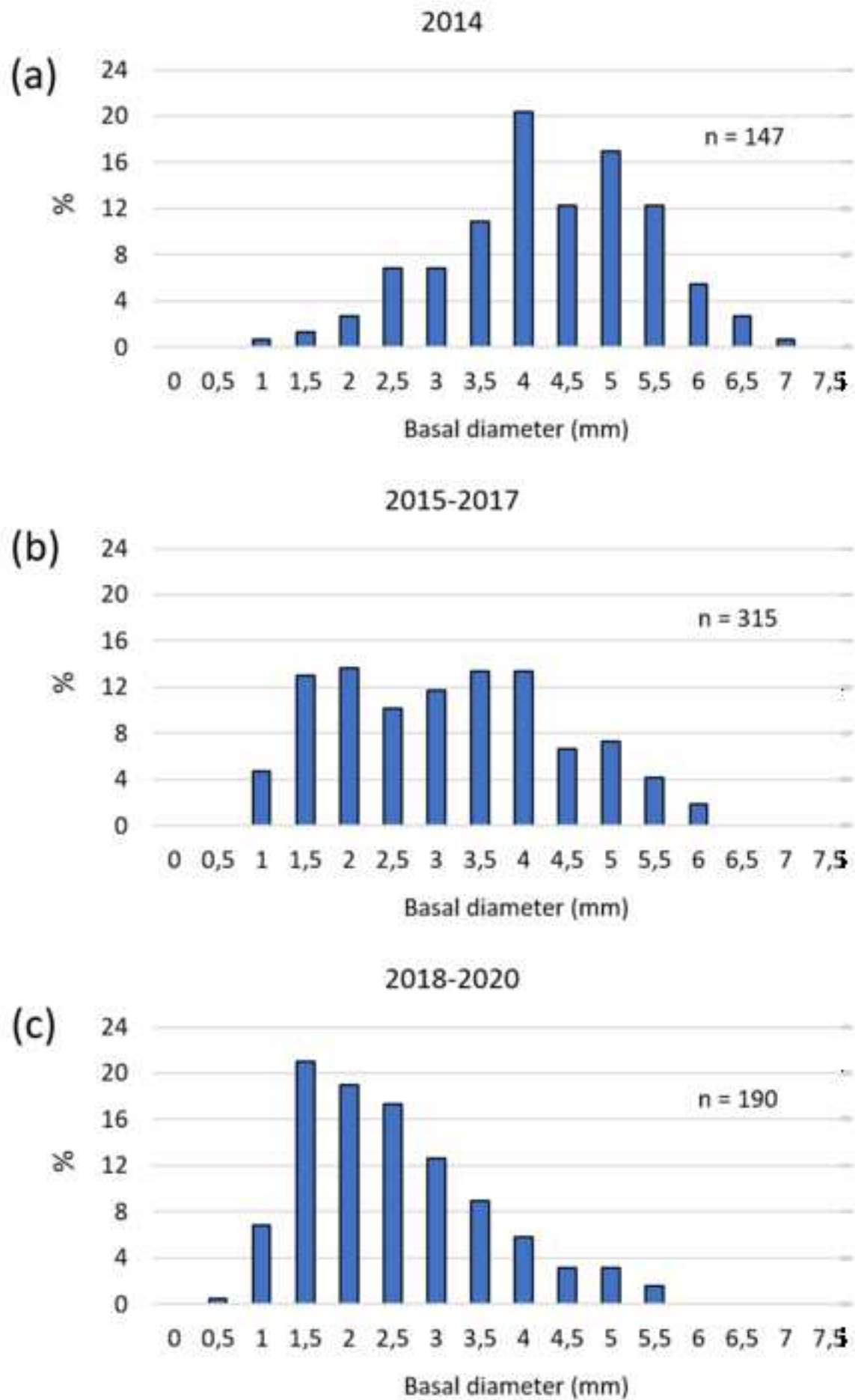




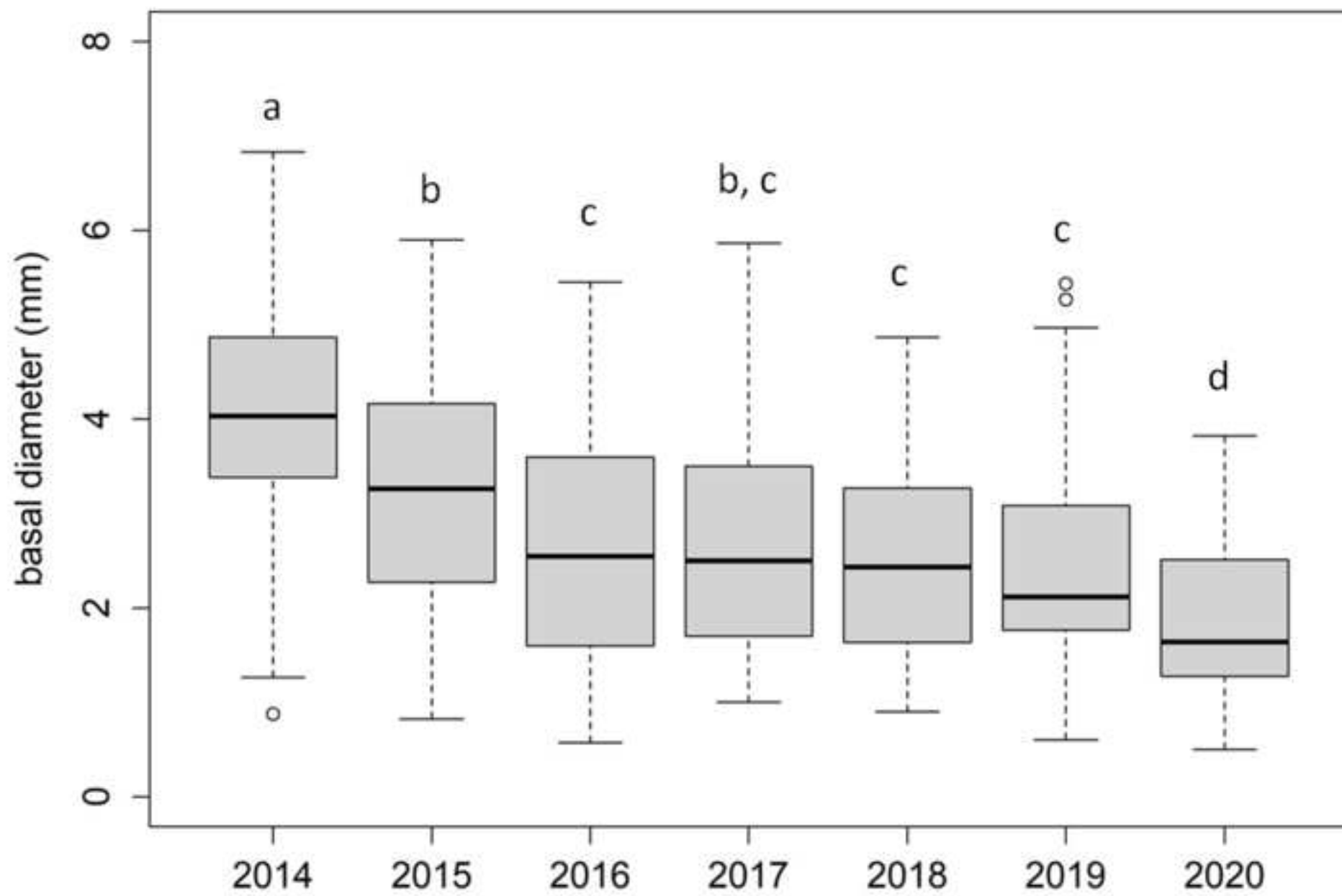
**(a) BENTHOS - Margalef's index****(b) BENTHOS - Shannon's index**











Dear Editor,

We are delighted of positive feedback from reviewers about our revised manuscript. We answered all minor comments of reviewer 2 below and corrected the references list as suggested.

We hope you will find this version of our manuscript suitable for publication in Hydrobiologia,

All the best

Sébastien Alfonso

Also on behalf of the coauthors

**Reviewer #2:** This review represents a third review of the submitted manuscript.

The manuscript is greatly improved and I would accept it for publication with some very minor corrections below and please check the references, they are still not fully elaborated, particularly the on-line ones.

We are delighted of the positive feedback from the reviewer concerning our revision. We thank him/her very much for providing us detailed feedback to fix last points before publication. Below, we answered the comments and did modify the manuscript and the reference list accordingly.

Line 210: change "On the contrary" to "In contrast..."

This has been corrected (L210).

Line 247 I am not quite sure of the syntax for "as well the results of GAM model also show" do you mean "as the results of the GAM model indicate"

The sentence has been corrected (L247).

Line 267 change to "...of the IF in the southern..."

Corrected (L267).

Line 268 change to "...at least in the area..."

This has been modified (L268).

Line 271 delete "more recently" to change to "...Lembo, 2011), in recent years, there has...."

This has been corrected as suggested (L271).

Line 277 should it be "...how the number of fishing vessels dedicated to..."

This has been corrected (L277).

Line 284 delete the space between 38.8 and %

Corrected.

Line 294 can you define CWC in this first use (it is shown a few lines down, just move it up)  
Thank you for pointing that. This has been corrected (L294).

Line 320 change to "whereas the Shannon-Wiener..." and then delete the second full stop a few words later.  
This has been corrected (L320).

Line 321 change to "...good discriminatory ability towards species richness..."  
This has been corrected (L321).

Line 323 change to "... that can exhibit a slower change in deep-sea..."  
This has been corrected (L323).

Line 337 change to "...and the cephalopod..." (singular)  
This has been corrected (L337).

Line 340 it is not clear what you mean "...are to show higher diversity of the macrofaunal community associated with *I. elongata* (Cartes..." do you mean "...exhibit a higher macrofaunal community diversity (Cartes..."

Indeed we aimed to say that "Mature colonies (i.e., larger colonies with higher density) of *I. elongata* exhibit a higher macrofaunal community diversity" as the reviewer pointed out. This has been now corrected in the revised version (L340).

Line 342 change to "Basal diameter is correlated...."  
Corrected as suggested (L341).

Line 349 you seem to talk about your data, but then quote Bo and Carbonara refs. You need to break the sentence to show that you showed a reduction in size over the periods and that this could be probably attributed to fishing impact delete the refs here because you cover this in the next sentence with the Carbonara ref and the following one with both the Bo and Carbonara refs.  
Indeed, thank you for pointing that. We removed the references in the sentences.

Line 371 change to "...a significant decrease in biomass...."  
This has been corrected (L370).

Refs still not quite there: Cartes 2020 missing the Journal detail and missing italic *I* on the *Isidella* Cartes, J.E., Díaz-Viñolas, D., González-Irusta, J.M. et al. The macrofauna associated to the bamboo coral *Isidella elongata*: to what extent the impact on isideidae affects diversification of deep-sea

fauna. Coral Reefs 41, 1273-1284 (2022). <https://doi.org/10.1007/s00338-022-02243-w>

This missing information has been added (L430).

Line 433 italics for second *Isidella*

This has been corrected (L433).

Line 448 - I found a bit more on this reference citation

Chimienti, Giovanni, Mastrototaro, Francesco, D'Onghia, Gianfranco. "Mesophotic and Deep-Sea Vulnerable Coral Habitats of the Mediterranean Sea: Overview and Conservation Perspectives" In *Advances in the Studies of the Benthic Zone*, edited by Luis Soto. London: IntechOpen, 2019.

10.5772/intechopen.90024

This has been corrected.

Line 449 Coll ref in full

Coll M, Piroddi C, Steenbeek J, Kaschner K, Ben Rais Lasram F, et al. (2010) The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. *PLoS ONE* 5(8): e11842.

doi:10.1371/journal.pone.0011842

This has been corrected (L456).

There's a few others too, please check them again, they should be cited fuller.....D'Onghia 2012, Danovaro 2020, EUMOFA 2019,... please try and get them all, particularly the e-journals *Plos1*, *FMARS*, *Intech*.

These references have been corrected. We also checked the other references. Please see the changes in the revised list of references.



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**Supplementary Material**

[Supplementary\\_Dataset\\_Hydrobiologia\\_rev.xlsx](#)

