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Highlights

- Temperate mesophotic reefs host diverse animal assemblages, vulnerable to fishing activities.
- The evaluation and the monitoring of their status is of primary interest, since these habitats provide goods for the society.
- A multiparametric index (MACS), based on ROV video footages and characterized by an ecosystem-based approach, is herein proposed and tested on a large dataset.
- Results show five different ecological status levels for the investigated sites, providing a valuable tool to monitor the health status of mesophotic reefs.

Criteria to assess the environmental status of temperate mesophotic reefs

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Abstract

Temperate mesophotic reefs (circalittoral and offshore circalittoral hard/firm habitats), extending between 40 and 300 m depth and hosting complex three-dimensional animal forests, are vulnerable habitats protected by international agreements. In order to evaluate and monitor the status of these ecosystems, the multi-parametric index Mesophotic Assemblages Conservation Status (MACS) was developed, composed by two independent components, the Index of Status (I_s) and the Index of Impact (I_i). The I_s includes six metrics, targeting conspicuous species diversity, basal layer and canopy composition, whilst the I_i is composed by seven metrics targeting canopy condition, silting levels, and marine litter. Underwater video transects recorded with a Remotely Operated Vehicle (ROV) along the Ligurian Sea offered the chance to test the index on a large geographic scale, comprehensive of four macro-areas and 24 sites subjected to different human pressures. Most of the Ligurian mesophotic communities investigated exhibited an overall moderate ecological status, with local critical situations mainly related to high fishing pressure. The MACS index represents a valuable tool for monitoring the status of mesophotic reefs, with a high potentiality to use ROV footages, as required by the EU Marine Strategy Framework Directive, the Habitats Directive and the guidelines of the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast of the Barcelona Convention.

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Declaration of interest: none.

1. Introduction

The mesophotic environments, receiving less than 3% of the surface irradiance, embrace the continental shelf and the upper bathyal zone between 40 to 300 m depth and include both the "circalittoral" and the "offshore circalittoral" level 2 units of the marine component of the revised European EUNIS habitats classification (Evans et al., 2016). Explorations with Remotely Operated Vehicle (ROV) showed that they are extremely rich and diverse in terms of benthic ecosystems, hosting complex three-dimensional animal forests over biogenic and rocky reefs both in tropical environments (Lesser et al., 2009; Kahng et al., 2010) and at temperate latitudes (James et al., 2017; Bo et al., in press). The characterization of these ecosystems highlighted the importance of these animal-dominated habitats and their species, mainly represented by arborescent, structuring anthozoans, sponges and bryozoans (Lombardi et al., 2014; Gori et al., 2017; Maldonado et al., 2017). Some studies also identified the main sources of vulnerability for these habitats and these structuring taxa proved to be important indicators of stress conditions (Bo et al., 2014; Gori et al., 2017; Kaiser et al., 2018). Since these ecosystems provide goods and services for the society (Folkersen et al., 2018), understanding their functioning and assessing their health status are topics of primary interest.

The European Union Marine Strategy Framework Directive (European Commission, 2008) has been elaborated as a result of a growing international interest towards an ecosystem-based management of marine environments, including scientific-based reactive criteria. Eleven descriptors have been identified to describe the Good Environmental Status (European Commission, 2008; Van Hoey et al., 2010; Borja et al., 2011). Benthic ecosystems are crucial elements for many MSFD Descriptors (in particular D1 – Biological diversity, D6 – Sea floor integrity, D10 - Litter), for the reporting under Article 17 of the Habitats Directive (Council of the European Union, 1992) and for the IMAP guidelines (Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast) (UNEP-MAP, 2008) of the Barcelona Convention. In this regard, ecological indices are effective tools to evaluate and monitor over time the status of marine benthic ecosystems. In the last two decades many indices have been developed for the Mediterranean benthic ecosystems, including soft bottoms (Borja et al., 2000; Simboura and Zenetos, 2002; Rosenberg et al., 2004; Dauvin and Ruellet, 2007), seagrass meadows (Gobert et al., 2009; Montefalcone, 2009), underwater marine caves (Rastorgueff et al., 2015), as well as shallow-water hard bottoms and coralligenous concretions (Ballesteros et al., 2007; Cecchi and Piazzi, 2010; Deter et al., 2012; Cecchi et al., 2014; Gatti et al., 2015a; Ruitton et al., 2015; Paoli et al., 2016; Montefalcone et al., 2017; Sartoretto et al., 2017).

Some efforts have been made to define indices for mesophotic environments based on ROV footages, resulting in two seascape approaches, namely MAES (Mesophotic Assemblages Ecological Status) (Cánovas-Molina et al., 2016a) and CBQI (Coralligenous Bioconstructions Quality Index) (Ferrigno et al., 2017), both tested on a relatively low number of sites. The Italian MSFD protocol for monitoring coralligenous and rocky reefs from 40 to 100 m depth includes a standard sampling design conceived to gather various quantitative components, such as the occurrence and extent of the habitat (here biogenic and rocky reefs), the silting levels, and the abundance, status and population composition of structuring megabenthic species forming aggregations (here animal forests), as well as presence and typology of marine litter (MATTM-ISPRA, 2016). This methodology has been defined as the standard for the monitoring that will be continued in the years to come.

The possibility to use a large archive of ROV video-transects collected along the Ligurian Sea (NW Mediterranean) in 2015 and 2016 offered the opportunity to develop and test, on a large geographic extent, a new index that combines status and impact indicators following a DPSIR (Driving forces - Pressures - Status - Impacts - Response) approach (Elliot, 2002; Atkins et al., 2011). The Mesophotic Assemblages Conservation Status (MACS) multi-parametric index, integrates three major descriptors proposed by the MSFD to define the ecological quality of the marine environment, namely biological diversity, seafloor integrity, and marine litter (European Commission, 2008), and is in line with the IMAP guidelines of the Barcelona Convention (UNEP-MAP, 2008). The MACS index was thus applied on 24 temperate mesophotic reefs in the Ligurian Sea subjected to different levels of human pressures.

2. Materials and methods

2. 1 Study area

This study focused on the circalittoral zone of the Ligurian Sea, located in the North-western sector of the Mediterranean Sea (Fig. 1, Tables 1 and SM1). The Ligurian coastline extends for over 350 km, from Ventimiglia at the western border to La Spezia at the eastern one. The seabed topography differs remarkably between western and eastern coasts (Fanucci et al., 1989). The shelf in the western coast is deeper, steeper and narrower, whereas in the eastern coast is larger and with a gentler slope (Fig. 1).

About 76 rivers, predominantly torrential streams, flow through the Alpine and Apennine chains within the Ligurian basin (Fig. 1, Table SM2) (http://www.pianidibacino.ambienteinliguria.it). Due to the cyclonic circulation, the overall superficial current in the basin moves westward (Cattaneo-Vietti et al., 2010). Intermediate Levantine waters and deep waters entering the basin from the Tyrrhenian Sea through the Tuscan Archipelago and along the western coasts of Corse follow a similar cyclonic path (Taviani et al., 2015; Chimienti et al., in press).

The Ligurian coast is among the most urbanised of the Mediterranean Sea (Table SM2) with an estimated overall population of 1,262,000 residents (subjected to summer increase) (Fig. 1, Table SM2). Some of the Ligurian smaller towns do not have a sewage system; therefore black water outlets are occasionally present along the coast. Liguria hosts approximately 42 harbour structures, with those of La Spezia, Genova and Vado Ligure being the largest commercial ports (Fig. 1, Table SM2). Ligurian harbours count over 20,300 berths, of which approximately 750 are registered for professional fishing boats, mainly targeting coastal demersal *metiers* or deep trawling activities. Boats are mainly dislocated in the fishing harbours of La Spezia, Santa Margherita Ligure, Genova, Savona, Imperia, and Sanremo, each one allocating more than 50 fishing boats (Fig. 1, Table SM2) (http://ec.europa.eu/fisheries/fleet/index.cfm; https://www.tuttobarche.it/ricerca-rade-e-porti). No precise census is available for recreational fishermen, which however have been estimated to be more than 160,000 distributed along the entire territory (Regione Liguria, 2014).

Differently from other bathymetric zones, the circalittoral extending between 40 and 200 m depth has received far less attention mainly due to sampling difficulties; nevertheless, the occurrence of coralligenous and rocky reefs dominated by erect filter-feeding species forming dense aggregations in dim-light conditions is well known (Ballesteros, 2006; Diviacco & Coppo, 2006; Cattaneo-Vietti et al., 2010; Cánovas-Molina et al., 2016b). Despite the presence of three Marine Protected Areas and other three tutelary marine sites (Fig. 1), the Ligurian circalittoral zone is enormously exploited by recreational and professional fishermen contributing to the general degradation of the environment (Bavestrello et al., 1997; Cattaneo-Vietti et al., 2010; Bo et al., 2014; Cánovas-Molina et al., 2016b).

2. 2 Data acquisition

Following the methodological Italian protocol on MSFD Habitat 7 (MATTM-ISPRA, 2016) on coralligenous and rocky reef habitats, two oceanographic surveys on board of R/V *Astrea*, were conducted in August 2015 and September 2016 by ISPRA (Rome). Twenty-four sites were identified along the Ligurian region, divided into four macro-areas namely A1 (western sector), A2 (central-western sector), A3 (central-eastern sector) and A4 (eastern sector) respectively from West to East (Tables 1 and SM1; Fig. 1). For each site, from one to four 200 m long standard video-transects were collected, for a total of 70 transects, ranging from 30 to 140 m depth. Footages were obtained by means of the ROV Pollux III, equipped with a digital camera (Nikon D80, 10 megapixels), a strobe

(Nikon SB 400), a high definition video camera (Sony HDR-HC7), lights, and a 3-jaw grabber. The ROV hosted also an underwater acoustic positioning system (Ultra Short Baseline Linquest Tracklinq 1500 MA), a depth sensor, and a compass to obtain georeferenced tracks overlapped to multibeam maps. Two parallel laser beams provided an 8 cm scale for size reference.

ROV footages were recorded within sites previously mapped with a high-resolution Multibeam Echo Sounder (MBES, Kongsberg EM2040) and characterized by the occurrence of hard bottom and possibly homogeneous depth. Selection was driven also by the presence of hard-bottom structuring species, here intended as arborescent or massive megabenthic species (e.g. sponges, anthozoans, bryozoans), reaching elevated sizes (decimetric) and densities, and hence able to shape the environment and support a complex biocoenosis, *sensu* Buhl-Mortensen et al. (2010). In order to guarantee the best quality of videos footages, ROV moved along linear tracks, in continuous recording mode, at constant slow speed (<0.3 m/sec) and at a constant height from the bottom (<1.5 m), thus allowing for adequate illumination and facilitating the taxonomic identification of the megafauna.

Transects were positioned along full dive tracks by means of QGIS software editing. Transects were obtained from 43 ROV paths on average 870 m ± 53 long (from 370 to 1860 m). Each video transect was analysed through ROV-imaging technique with Apple Final Cut Software, using starting and end time of the transect track as reference. Visual census of megabenthic species was carried out along the complete extent of each 200 m long transect and within a 50 cm wide visual field, for a total of 100 m^2 of bottom surface covered per transect.

The ROV methodology allowed to collect the following parameters:

I. Extent of hard bottom calculated as percentage of total video time showing this type of substratum (rocky reefs and biogenic reefs) and expressed in m^2;

II. Species richness, considering only the conspicuous megabenthic sessile and sedentary species of hard bottom in the intermediate and canopy layers (*sensu* Gatti et al., 2015a) of the biogenic rocky reefs. Organisms were identified to the lowest taxonomic level and counted. Fishes and encrusting organisms have not been considered, as well as typical soft bottoms species. Some hard bottom species, especially cnidarians, can occasionally invade soft bottoms by settling on small hard debris dispersed in the sedimentary environment (Morri et al., 1991). For this reason, typical hard bottom species (e.g., *Eunicella verrucosa*) encountered on highly silted environments have been considered in the analysis;

III. Structuring species have been counted, measured (height expressed in cm) and the density of each structuring species was computed and referred to the hard bottom surface (as n° of colonies or individuals m^{-2});

IV. The percentage of colonies with signs of epibiosis, necrosis and directly entangled in lost fishing gears were calculated individually for all structuring anthozoans;

V. Marine litter has been identified in terms of typology and size, and counted. The final density (as n° of items m^{-2}) has been computed considering the entire transect (100 m^2).

Within each transect, 20 random high definition pictures targeting hard bottom were obtained, and for each of them four parameters have been estimated, following an ordinal scale. Modal values for each transect were calculated. Evaluated parameters include:

VI. Slope of the substrate: 0°, <30° (low), 30°-80° (medium), >80° (high);

VII. Basal bio-cover, estimated considering the percentage of hard bottom covered by organisms of the basal (encrusting species) and intermediate (erect species but smaller than 10 cm in height) layers: 0, 1 (<30%), 2 (30-60%), 3 (>60%);

VIII. Coralline algae cover (indirect indicator of biogenic reef), estimated considering the percentage of basal bio-cover represented by encrusting carbonatic algae: 0, 1 (sparse), 2 (abundant), 3 (very abundant);

IX. Sedimentation level, estimated considering the percentage of hard bottom covered by sediments: 0%, <30% (low), 30-60% (medium), >60% (high).

2. 3 Data analysis

2.3.1 Methodological issues

In order to test whether the size of the sampling units adopted (200 m in length) was representative of the whole megabenthic community of mesophotic reefs, seven complete video paths, ranging from 600 to 1250 m in length, were analysed. Each video track was divided into 50 m-long segments. Species accumulation curves (sample-based rarefaction curves) were then traced for each transect according to Bianchi et al. (2018). Species occurring with less than three individuals (or colonies) were not considered in the analysis. Additionally, the number of species found in each 200 m-long transect was compared to the total species richness of the dive track from which it was extrapolated.

In order to define the minimum percentage of hard bottom assuring the reliability of a 200 m-long path conducted in fragmented habitats, the correlation between species richness and percentage of hard bottom in each site was evaluated.

2.3.2 Index metrics

The MACS (Mesophotic Assemblages Conservation Status) multi-parametric index for the assessment of the ecological status of mesophotic reefs is composed by two independent units, the Index of Status (I_s) and the Index of Impact (I_i) (Fig. 2, Table 2), following the DPSIR approach (Elliot, 2002; Atkins et al., 2011). The I_s describes the general condition of the community, whereas the I_i identifies the impacts affecting reefs. A total of 13 metrics has been considered for the final index. All the metrics adopted are 1st order indices and belong to the list of the MSFD parameters (Borja et al., 2011), and are easily inferable from the video analysis previously described. I_s and I_i are composed by six and seven metrics respectively, and thus are 2nd order indices. The final MACS index is a 3rd order index, combining information from the status and the impact indices. For each metric, a score from 1 to 3 was assigned, following the ordinal ranks reported in Table 2, with absence scoring zero (Bianchi et al., 2004).

The I_s analyses the health status of the megabenthic communities by focusing on conspicuous species diversity, basal layer status and canopy condition (Table 2):

i) *species richness*. This indicator reflects the MSFD Descriptor "Biological diversity", and considers: 1) the mega benthic conspicuous species richness (SR) as described in paragraphs 2.2.II;

ii) *biogenic substrate*. This parameter reflects the MSFD Descriptor "Seafloor integrity". Here, the basal layer is investigated by ranking: 2) the basal bio-cover (BC); and 3) the coralline algae cover (CC) as mentioned in paragraphs 2.2.VII and 2.2.VIII.

iii) *canopy*. This parameter investigates the upper layer of the community and reflects the MSFD Descriptor "Seafloor integrity". The components involved are: 4) the dominance (DM) of the structuring species forming the canopy, classified as mono-, oligo- and polyspecific (Bianchi, 2001); 5) the density of all the structuring species (SSD); and 6) the mean height of the dominant structuring species (SSH) as mentioned in paragraph 2.2.III. The dominance of the canopy is evaluated considering the number of structuring species reaching abundance higher than the mean value per transect of the entire structuring assemblage. A lower score is given to monospecific communities with respect to oligospecific and polyspecific ones (Table 2). The maximum height for each species has been obtained from the literature (Table 3) and half of it has been considered has a reference condition for the populations (Linares et al., 2008; Sini et al., 2015). Scores were thus assigned considering one third and two thirds of the half of the maximum high (Table 3).

The I_i includes seven components and focuses on the silting level of the environment, the percent of damaged structuring anthozoans, and the occurrence of marine litter (Table 2):

iv) *sedimentation*. This parameter reflects the MSFD Descriptor "Seafloor integrity": 7) the percentage of hard bottoms covered by sediments (SD) is calculated as specified in paragraph 2.2.IX.

v) *damaged anthozoans*. This parameter reflects the MSFD Descriptor "Seafloor integrity". Due to their documented response to anthropogenic mechanical pressures (Bavestrello et al., 1997; Bo et al., 2014), structuring anthozoans were chosen to depict the health status of megabenthic communities. The investigated components included: 8) the percent of colonies directly entangled with marine litter or fishing gears (ENT); 9) the percent of colonies showing necrotic portions (NCR); and 10) the percent of colonies showing epibiotic portions (EPB), as specified in paragraph 2.2.IX.

vi) *marine litter*. This section refers to MSFD Descriptor "Litter" and considers three different aspects as mentioned in paragraph 2.2.V: 11) the density of litter items observed in each video-transect (LD); 12) the type of garbage (LT), distinguishing the occurrence of general litter (score 1) and lost fishing gears (score 2); and 13) the size of the items observed (LS). The lowest score is assigned to transects with only small items, identified as objects playing their influence only on a restricted area of one or few square meters (e.g. bottles, tyres, shoes), whereas intermediate score is assigned to large items, impacting a larger area (e. g. fishing nets, longlines). In both cases, the highest score is assigned when either typologies or sizes occur (Table 2).

2.3.3 Reference conditions definition

The identification of reference conditions is of fundamental importance when evaluating the ecological status of a habitat. Different methods are commonly used to this purpose, including: i) the identification of pristine conditions, ii) the comparison with historical information, and iii) the development of a model (Gatti et al., 2015b). Previous studies have highlighted the rarity of pristine habitats in the Italian circalittoral zone, persisting only where the occurrence of fortuitous obstacles limits fishing activity (Bo et al., 2014, 2015; Angiolillo et al., 2015). Historical data are not available for these ecosystems, so that only a conceptual model can be adopted. The Ecological Quality Ratio (EQR) has been commonly adopted in order to compare the observed situation with a hypothetical ‘ideal’ reference condition (Borja et al., 2010, 2011; Gatti et al., 2015a). Obviously, biological and environmental factors play a relevant role in defining the reference conditions. We can expect that, in the hypothetical reference condition, the parameters of the I_s reach the maximum value of 3 for each metric, corresponding to a total score of 18 (indicative of high biodiversity, high biotic cover in the basal layer, and well-developed canopy). Similarly, the metrics of the I_i are expected to have the zero score in the reference condition (hence, absence of silting, of damaged structuring anthozoans, and of marine litter). Thus, each metric is normalized, by dividing for three (the maximum value expected) and multiplying for 100 (the scale of EQR).

For two metrics, namely coralline algae cover and sedimentation, EQR is defined exceptionally on a case-basis due to the fact that natural decreasing trends related to environmental factors do not allow reaching maximum scores. Coralline algae in the Mediterranean coralligenous bioconstructions, for instance, are known to develop up to 120 m depth (Ballesteros, 2006), although their maximum abundance is expected to be found at around 40-60 m in optimal conditions. For this reason, EQR for coralline algae cover considers that the maximum score of 3 can be potentially obtained only for transects up to 60 m, the score of 2 for those up to 90 m and the score of 1 is the maximum value that we can assign at depths greater than 90 m.

Similarly, sedimentation on rocky reefs is strictly related to substratum inclination. As the maximum angle of repose for sediment is 45° (Al-Hashemi et al., 2018), we adopted this value as the maximum inclination to score 3 for sedimentation. For more accentuate inclinations (greater than 45°), the maximum score expected is 2.

2.3.4 Combined MACS index and quality status

After transforming the scores of the 13 metrics in EQR, the two indices were aggregated for each transect using the following formulae (see paragraph 2.3.2 and Fig. 2 for acronyms explanation):

$$I_s = (EQR_{SR} + EQR_{BC} + EQR_{CC} + EQR_{DM} + EQR_{SSD} + EQR_{SSH})/6$$

$$I_i = (EQR_{SD} + EQR_{ENT} + EQR_{NCR} + EQR_{EPB} + EQR_{LD} + EQR_{LT} + EQR_{LS})/7$$

The mean value of I_s and I_i for each site are then obtained by averaging the values of the three transects. Radar graphs were used to show the contribution of the various metrics in the resulting I_s and I_i indices in the sites of Spotorno, Diano Marina, Nervi and Bordighera, chosen as examples. A multivariate Correspondence Analysis has also been carried out by means of the software PAST (Hammer et al., 2001), to identify which metrics are more important in distinguishing sites.

The final MACS index has been calculated for each site, combining information of status and impact indices, using the following formula:

$$\text{MACS} = \frac{Is+(100-Ii)}{2}$$

The I_s, the I_i and the resulting index MACS are all numbers ranging from 0 to 100.

Categories of the ecological quality status (following the Water Framework Directive) are defined based on the following scheme:

Index of Status	***Index of Impact***	***MACS***
≤ 35 = Bad	≤ 35 = Very low	≤ 35 = Bad
36-45 = Poor	36-45 = Low	36-45 = Poor
46-55 = Medium	46-55 = Moderate	46-55 = Medium
56-65 = Good	56-65 = High	56-65 = Good
≥ 66 = High	≥ 66 = Very high	≥ 66 = High

2.3.5 Index validation

I_s and I_i for the 24 sites were correlated with two topographic features in order to evaluate natural patterns of variability.

In order to evaluate how the three indices (I_s, I_i, and MACS) respond to different levels of human pressures, the approach carried out by Cánovas-Molina et al. (2016a) was applied, considering the following anthropogenic pressures within a radius of 10 km from the centroid of each site: i) the number of inhabitants of the costal municipalities, obtained from the online database of the Italian national STATistical institute - ISTAT (http://dati.istat.it), ii) the number of berths for each port, obtained from the EU Fleet register (http://ec.europa.eu/fisheries/fleet), iii) the number of fishing boats listed in each harbour, found at the on-line pilot's book for the Mediterranean Sea, (https://www.tuttobarche.it/ricerca-rade-e-porti); and iv) the cumulative surface of the catchment areas of the rivers, available in the Ligurian Region official web-site (http://www.pianidibacino.ambienteinliguria.it) (Table SM2). The robustness of each index to each pressure has been assessed using linear regression.

3. Results

3.1 General description of sites and metrics

The four macro-areas investigated are well distinguished based on their general topographic characteristics (Table 1, SM1). In terms of bathymetric location, the mesophotic reefs of the Ligurian

Sea are mainly located within 40-70 m depth, with those in the central-western sector (A2) reaching the greatest depths (60-70 m). The average slope of the sites indicates a more gentle slope for the eastern sectors (30°-40°) with respect to the western ones (40°-50°), the latter including numerous sub-vertical substrates represented by rocky cliffs or large boulders. Mesophotic reefs in the Ligurian Sea are highly fragmented, in particular in the eastern Ligurian Sea (A4), with 40-50% of the transects covered by rocky or biogenic reefs. A1 is characterized by the widest extension of hard bottom, being most of the transects covered by 70% with rock.

Megabenthic species richness varies from a minimum of 7 species (NE3) to a maximum of 55 (SP1) and it is lower in the eastern areas respect to the western ones (SM3 and SM4). The basal bio-cover is similar in all four areas, with a slight increase moving from East to West. The occurrence of coralline algae is generally low in all sites, with the lowest values found in A4 and the highest values in the Portofino Marine Protected Area.

A total of 13 structuring species (with over 16,000 individuals or colonies counted) has been recorded in the video-transects: three sponges (*Axinella polypoides*, *Spongia (Spongia) lamella*, *Sarcotragus foetidus*), one scleractinian (*Dendrophyllia cornigera*), one antipatharian (*Antipathella subpinnata*), six gorgonians (*Corallium rubrum*, *Eunicella cavolini*, *Eunicella verrucosa*, *Eunicella singularis*, *Paramuricea clavata*, *Leptogorgia sarmentosa*), and two bryozoans (*Myriapora truncata*, *Pentapora fascialis*) (Table 3). Canopies are generally monospecific, with *P. clavata* and *E. verrucosa* as the most commonly recorded species. Excluding *A. polypoides*, *S. lamella*, *D. cornigera*, and *A. subpinnata*, all the other structuring species have been included in the group of the dominant species. Various site-specific differences have been found in the metrics regarding the density and the height of the canopies (SM3 and SM4).

Silting levels are generally high in all areas, but in A1 (SM3 and SM4). Anthozoan colonies entangled by demersal fishing gears appear more abundant in the western sector. The highest values of necrosis are observed in area A2, whereas epibiosys is higher in A1. There are no sites completely free of marine litter. Litter density ranges from 0.02 (CM1) to 0.51 (SP1) items per 100 m^2 and all four areas show a similar average density (around 0.14 items per 100 m^2) (SM3 and SM4). Litter includes lost fishing gears (mainly artisanal trammel nets, longlines, ropes and pots) and general litter (including plastic bottles and bags, tires, glass and rubber objects, textile robe, metal objects, barrels and anchors), mostly deriving from ships. 100% of the sites show traces of lost fishing gear and 30% show also general litter.

3.2 Minimum length of the transect

The cumulative richness analysis of the seven complete ROV paths showed that, on average, 55±3.5% (SE) of the species are found in the first 200 m of the video transect (Fig. 3A). When the 200 m-long tracks are arranged in concomitance of coral forests, as required by the protocol, they contain on average 30±2 species, representing 60±2% of the total richness of the entire dives from which the transect has been extrapolated.

Most of the transects showed highly fragmented, with hard bottom covering from 7% to 100% of the transect length (SM1); however, no significant correlation was found between the number of conspicuous species and the percentage of hard bottom in the 24 studied sites (Fig. 3B), which implies that only a limited amount of hard bottom within the transect (e.g., 20%) would be enough.

3.3 Index of status and index of impact

I_s ranges from 43 to 74, while I_i varies from 48 to 73 (SM4, Fig. 4, Fig. 5). The site with the highest ecological status is Diano Marina (in A1), whereas the site showing the lowest ecological status is Nervi (in A3), followed by Bordighera (in A1). Bordighera is also the site having the highest level of human impact, together with Spotorno (in A2). Low degrees of human impact have been observed in the sites of Isuela and Cala degli Inglesi (both located inside the Portofino Marine Protected Area, A3) as well as in the sites of Nervi and Sori (A3).

On average, the Ligurian mesophotic reefs display a good ecological status notwithstanding the high levels of impact. No correlation between ecological status and impact level has thus been found (R^2 = 0.0035), being the conditions of the 24 sites idiosyncratic (Fig. 6). A slight correlation has been observed ($R^2 = 0.2241$, $p < 0.05$) when the sites of Nervi, Bogliasco, Sori and Spotorno (encircled in Fig. 6) are excluded from the analysis, since they represent extreme situations.

In the sites characterized by high ecological status and low impact, diversity, structure of the canopy, and basal bio-cover represent the driving factors (Fig. 7). Absence of evident signs of damage on the canopy, low levels of sedimentation and moderate litter are indicative of low impact. On the contrary, in sites with low ecological status and high impact, the environment is characterized by a poorly structured canopy coupled to high occurrence of litter, silting and entanglements. A diverse community with high densities of structuring species, but also a high density of large litter entangling and damaging the canopy, characterizes the site of Spotorno. The site of Nervi is extremely poor in terms of diversity and canopy, despite the low density of litter.

In the Correspondence Analysis (Fig. 8), the first axis, related to the presence of coralline algae according to depths, explains 35% of the variance, whereas the second axis explains 20% and is mainly related to entanglement, necrosis and epibiosis.

The integrated MACS index (Fig. 4, SM4) depicts an overall moderate ecological status of the Ligurian mesophotic reefs. Values of the MACS index range between 36 (Bordighera) to 59 (Isuela), with three sites showing a good ecological status, 15 sites a moderate ecological status and 6 sites a poor status, the latter mainly located in A2 and A4.

3. 4 Indices validation

A slight significant correlation was depicted only between depth and I_i (R^2 = 0.195, $p < 0.05$), indicative of the effect of depth on the entity of damages on canopies due to an increased density of lost litter.

The distribution of anthropogenic pressures is different along the coast: inhabitants show a peak in close proximity to the sites eastward of Genoa (Sori, Bogliasco and Nervi), while a minimum is observed in the easternmost sites (SM2, Fig. 9). These latter sites are also those with a reduced number of boats and fishing vessel; these two pressures, however, are largely distributed in the proximity of all sites, peaking in A2 and A3. River inflows are largely present in the most extreme parts of the Ligurian coast, especially near Monterosso (under the influence of Magra river) and near Bordighera and Capo Mortola (under the influence of Roja river). Negative correlations have been found between I_s and the number of inhabitants (R^2 = 0.213, $p < 0.05$), between I_s and the area of rivers (R^2 = 0.232, $p < 0.01$), while a positive correlation has been found between I_i and the catchment area of rivers (R^2 = 0.429, $p <$ 0.01).

4. Discussion

This study describes the use of a multi-parametric index (MACS) applied for the first time on a large-extent assessment of mesophotic temperate reefs. This index takes into consideration three descriptors listed by the Marine Strategy Framework Directive, i.e. biodiversity, seafloor integrity and marine litter (European Commission, 2008), and is composed of a number of metrics obtained from ROV surveys. The MSFD represents the most recent European legislation targeting the protection and restoration of the ecological quality and the integrity of marine ecosystems (Borja et al., 2008). The final goal of MSFD is to reach and maintain a Good Environmental Status for marine waters, habitats and resources, within a holistic functional ecosystem-based approach (Browman et al., 2004; Borja et al., 2010).

ROV has been largely employed in the last 10 years as a technological tool for deep Mediterranean exploration (Bo et al., 2009, 2015; Mastrototaro et al., 2010; Grinyó et al., 2018), but only limited experiences used the ROV footage to elaborate ecological indicators (Cánovas-Molina et al., 2016a; Ferrigno et al., 2017). ROV offers various advantages including a seascape approach, a wide geographic coverage, and no bathymetric limits (within the depth range of interest). The ROV MSFD methodology focuses on conspicuous species that are relatively easy to identify, therefore minimizing the observer effect and the specialist-oriented analysis, and maximizing the taxonomic resolution. The natural history of the target species is usually well known, which allows for an easier identification of the responses to various natural and anthropic pressures such as fishing, sedimentation, and mass mortalities. Disadvantages related to the employment of this technique include relatively high operative costs, low taxonomic resolution in the basal layer species identification, and influence of the quality of the ROV footage on the quality of the output.

With respect to existing ROV-based indices developed for assessing the quality of circalittoral Mediterranean rocky reefs, i.e. the MAES index (Cánovas-Molina et al., 2016a) and the CQBI index (Ferrigno et al., 2017), the MACS index here proposed integrates a larger number of metrics that better delineate the environmental and anthropic variability. This implementation is based on video-imaging showing a large coverage of the substrate and is executable both by ROV and classic scuba video-operators (for the shallowest depth).

Its DPSIR approach (Elliot, 2002; Atkins et al., 2011), coupling status and impact indicators, reflects more efficiently site-specific situations of each area. From a general point of view, Ligurian mesophotic reefs seem to show a moderate ecological status; following the MSFD this means that during the first two years of sampling, only 12.5% of the investigated sites fully achieves GES. Considering that hard-bottom structuring species are a focal point in the MACS index, it is necessary to find additional indexes to assess sites with no canopies. Similarly, it is necessary to give a more critical interpretation to those sites, such as Sori, Nervi and Bogliasco, which are close to the chief town of Genoa and where reduced water quality (Montefalcone et al., 2007), rather direct local pressures, may be the cause of the low values observed.

Natural patterns of communities related to topographic features are occasionally difficult to be discriminated from human-induced ones. Correlations were depicted only between depth and the I_i, supporting a positive effect of depth on the entity of damages on the canopies, as well an increasing effect of lost litter. This may be supported by the highest concentration of coastal fishing activities in the mesophotic zone where hard bottom occurs. Significant negative correlations have also been shown between I_s and the number of inhabitants, as well as between I_s and the impact and catchment area of rivers. Large coastal urbanisations (as in the proximity of the city of Genoa), as well as large river outflows (as in the most extreme macro-areas) are associated to high silting levels and poor canopies of the structuring species. Deep reefs within or nearby Marine Protected Areas (such as Portofino) support well-structured canopies and comparatively lower impacts.

Focusing on the canopy-forming species is not related just to methodological taxonomic issues, but also to ecological issues. The selected metrics involving structuring, long-living species (such as carbonatic organisms and arborescent gorgonians and antipatharians) are effective indicators of disturbance and recovery ability (Bianchi et al., 2017), in particular the species abundances and changes in size (Kaiser et al., 2018). With regards to the changes in size, structuring anthozoans were chosen to depict the health status of megabenthic communities due to their documented response to anthropogenic mechanical pressures (Bavestrello et al., 1997; Bo et al., 2014). This approach only partially works for sponges and bryozoans, whose response includes a more active reaction to lost fishing gears (keratose sponges generally incorporate lines and branched bryozoans generally break down).

The 200 m-long transect are representative of the overall diversity of the studied environments, and the survey proven to be efficient also in areas with highly fragmented hard bottom. These results support the adaptability of the method to a wide array of habitat situations.

Benthic indicators are fundamental tools to implement MSFD requirements as they allow an effective and representative picture of ecologically complex situations so to give the synthetic information essential to identify the areas where specific management measures must be undertaken to allow the GES achievement. The MACS index is here proposed as a comprehensive, seascape-approached, monitoring tool with the potential to be employed by a wide array of marine technicians over hard-to-reach environments. Results represent valuable information to be employed to evaluate improvements of the ecological status and thus the effectiveness of the measures that will must be undertaken in the monitoring sites and for future coastal planning and definition of protection zones.

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Figures legends

Fig. 1 A) Map of the study area with indication of the four macro-areas, the 24 sites (black dots) and location of the Ligurian Sea within the Mediterranean basin (up-left rectangle). The main canyons, river outflows, urban centres, Italian National Marine Protected Areas (in blue), Ligurian Regional Marine Protected Area (in orange) and dominant currents are also indicated. Close-up view of the four investigated macro-areas (transects are represented by black dots): **B)** A1, located in the western sector of the Ligurian Sea; **C)** A2, in the central-western sector; **D)** A3 corresponding to the central-eastern sector; and **E)** A4 in the easternmost sector.

Fig. 2 Scheme of the MACS index composed by two independent units, the Index of Status (I_s) and the Index of Impact (I_i). The thirteen metrics composing the two indices are reported in light gray. The three rectangles at the base of the figure represent the three MSFD descriptors and the relative indicators (dark gray). SR, species richness; BC, basal bio-cover; CC, coralline algae cover; DM, dominance; SSD, structuring species density; SSH, structuring species height; SD, sedimentation; ENT, entanglement; NCR, necrosis; EPB, epibiosys; LD, litter density; LT, litter type; LS, litter size.

Fig. 3 A) Species accumulation curves for seven full-length ROV dive paths: ME, Punta Mesco; MA, Punta Manara; FA, Punta del Faro; CL, Celle Ligure; SP, Spotorno; SS, Santo Stefano; CM, Capo Mortola. **B)** Correlation between species richness and percentage of hard bottom.

Fig. 4 Results of I_s and I_i for the 24 investigated sites.

Fig. 5 Mean value (±SD) of EQR-transformed results for I_s and I_i for each investigated site. Refer to Table 1 for the code of each site.

Fig. 6 Correlation between averaged EQR-transformed results for I_s and Ii for each investigated site. Shaded areas indicate four relevant sites.

Fig. 7 Radar graphs for the 13 MACS metrics in four selected sites. Graphs on the left side correspond to I_s, whereas on the right side to I_i. **A)** The case of Spotorno (SP), located in the central western area, identifies a site with high status and high impact. **B)** In the western area, Diano Marina (DM) is characterized by high status and low impact. **C)** Nervi (NE), from the central eastern area, exhibits low status and low impact. **D)** The site of Bordighera (BG) has high impact and low status. See text for more details.

Fig. 8 Multivariate Correspondence Analysis of the 13 MACS metrics in the 24 investigated sites.

Fig. 9 Anthropogenic pressures in a 10-km radius around each investigated site.

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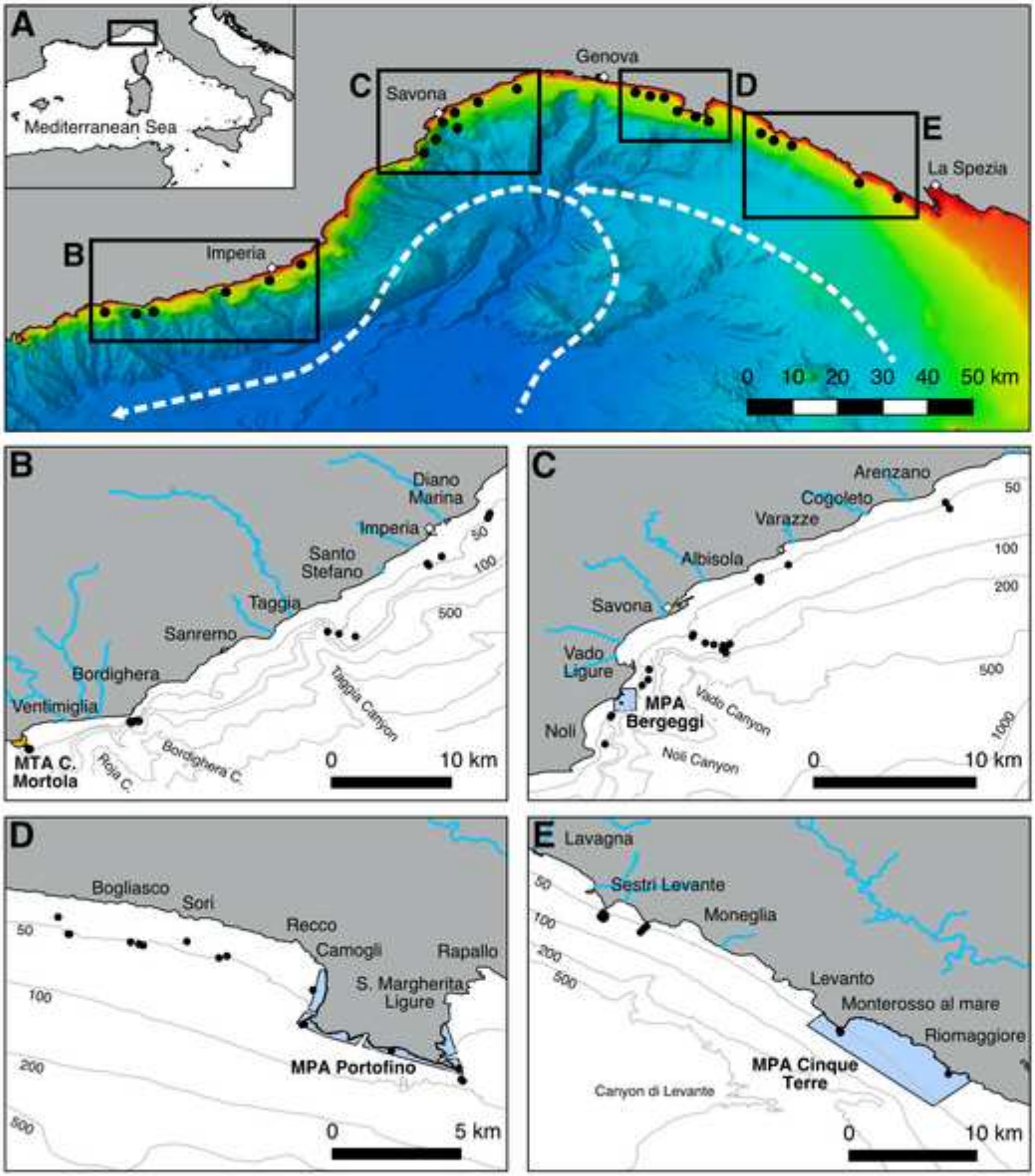

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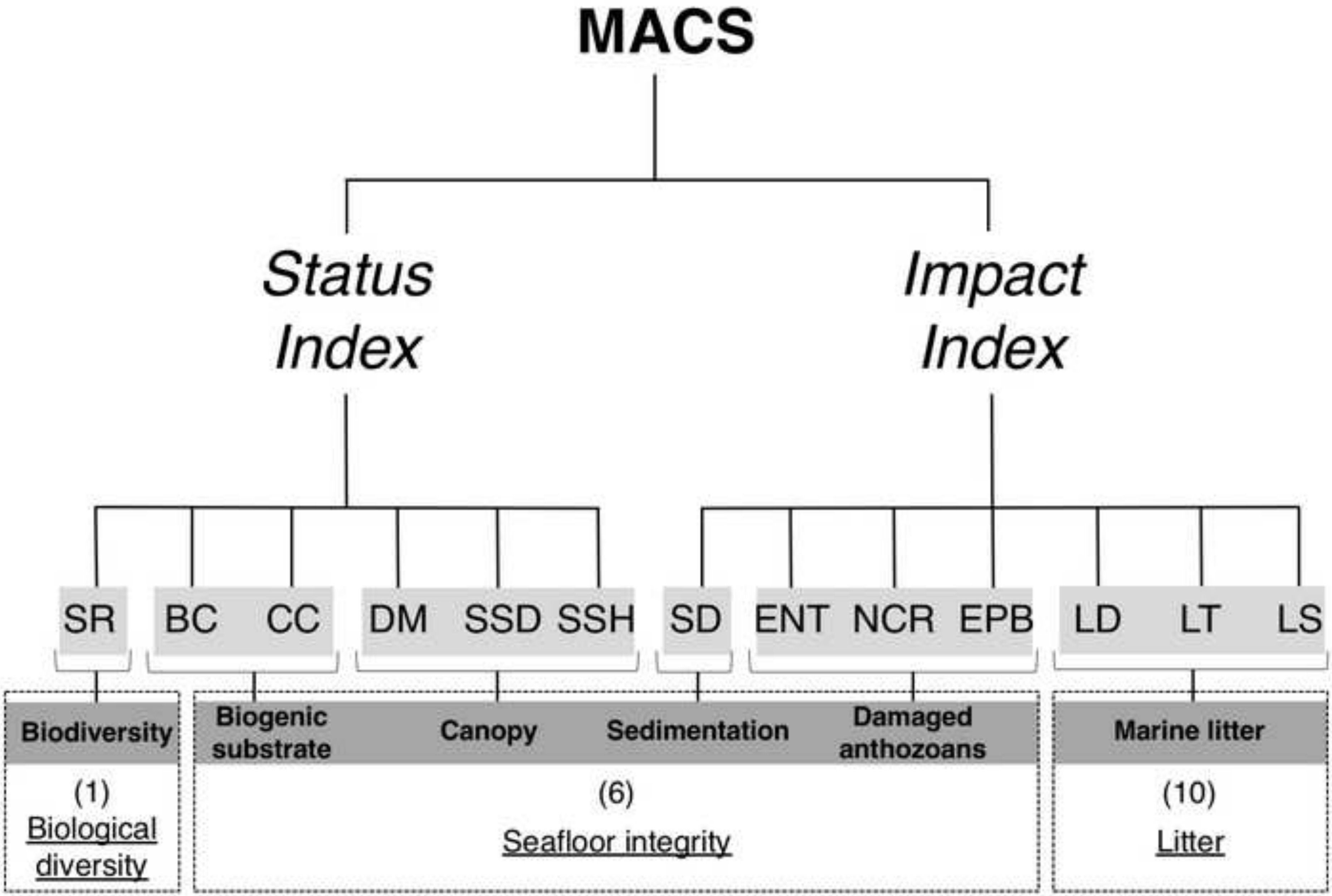

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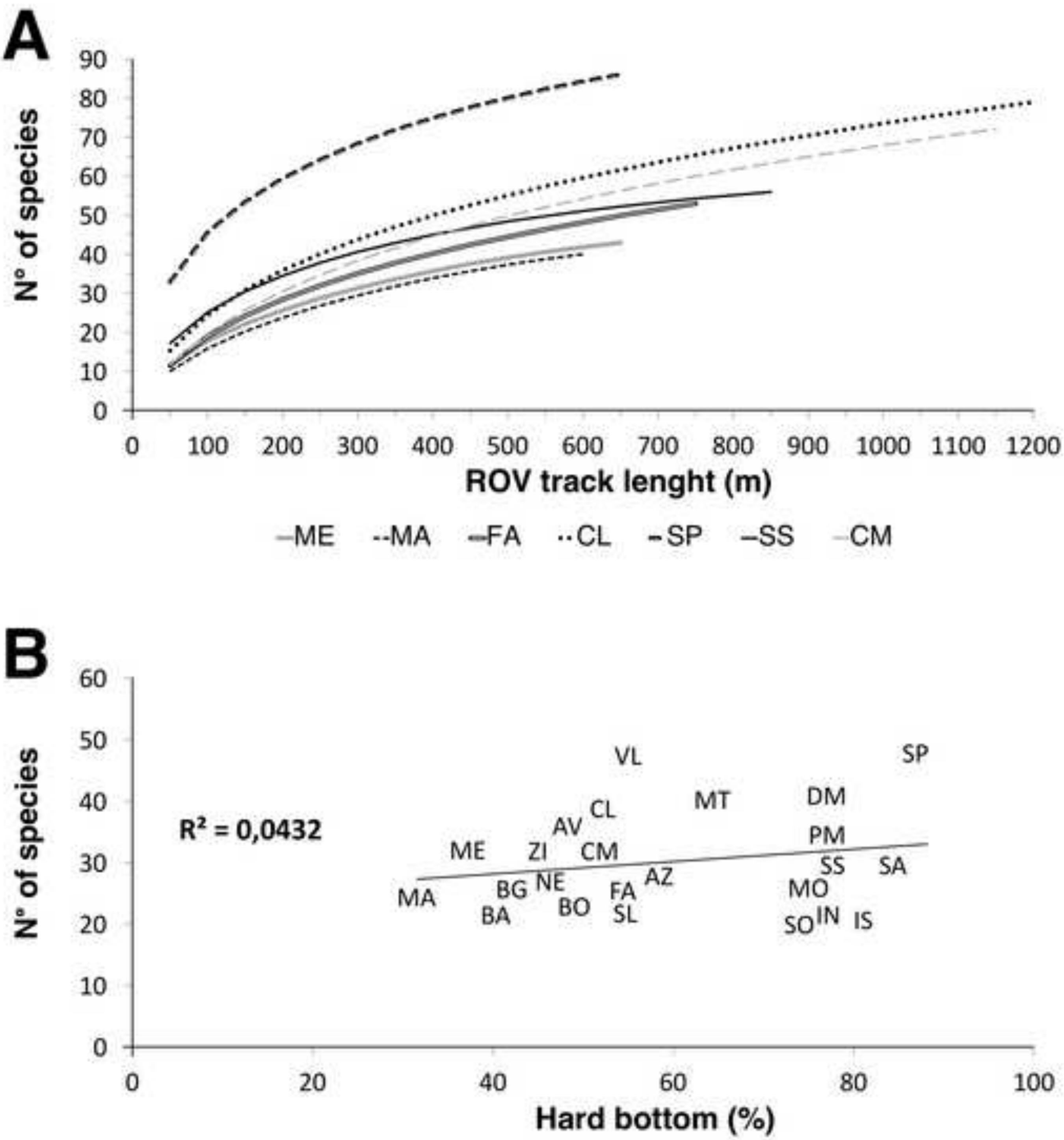

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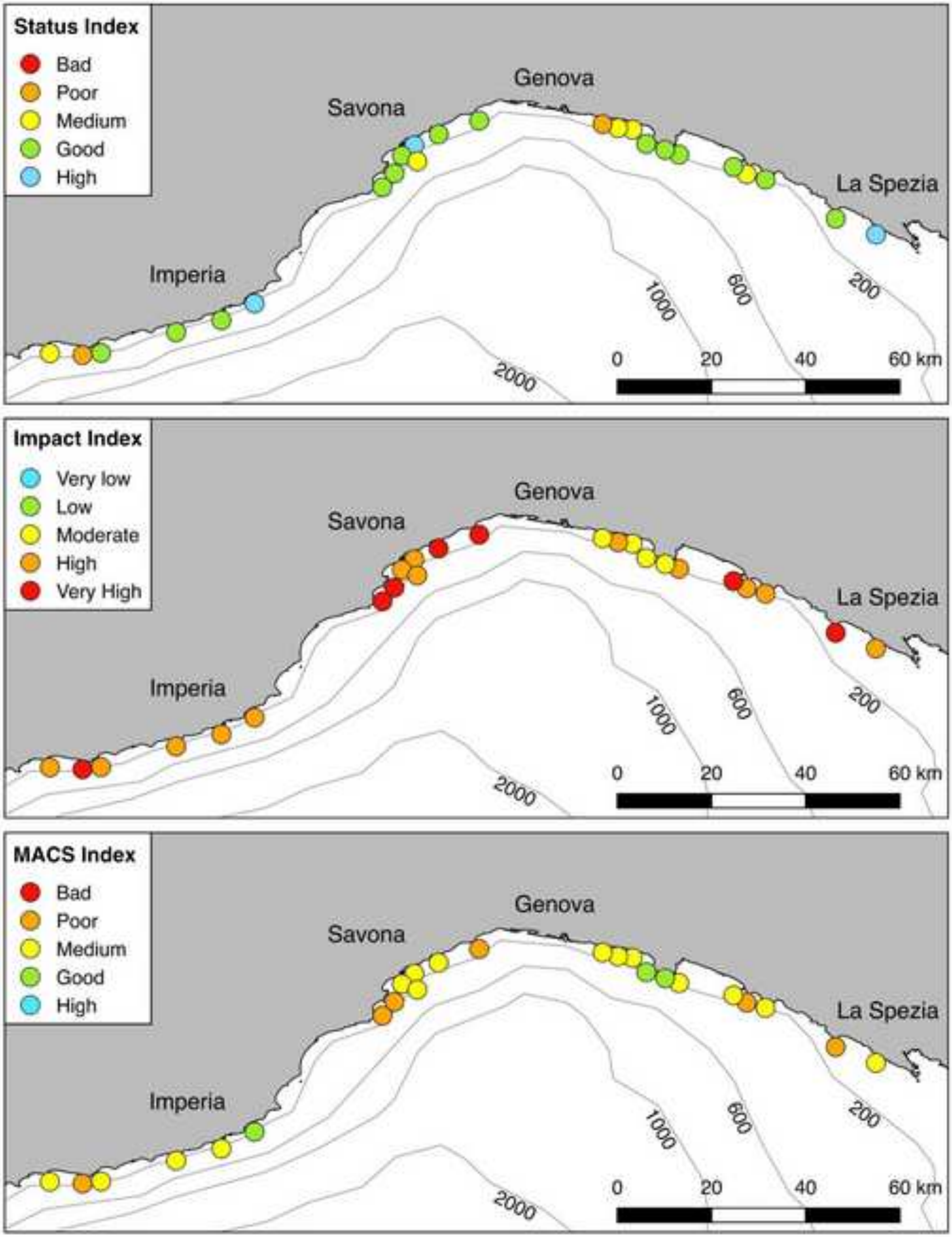

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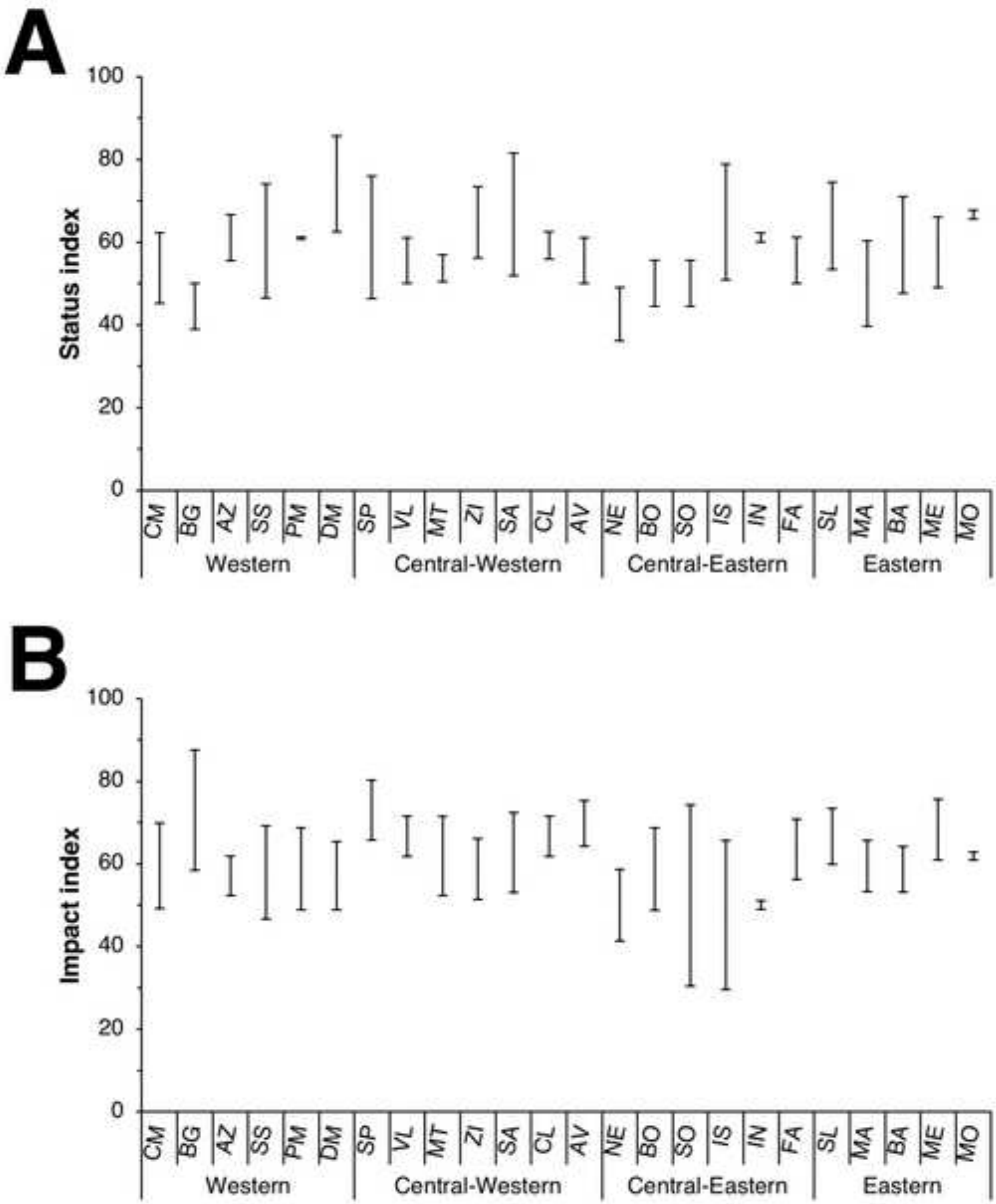

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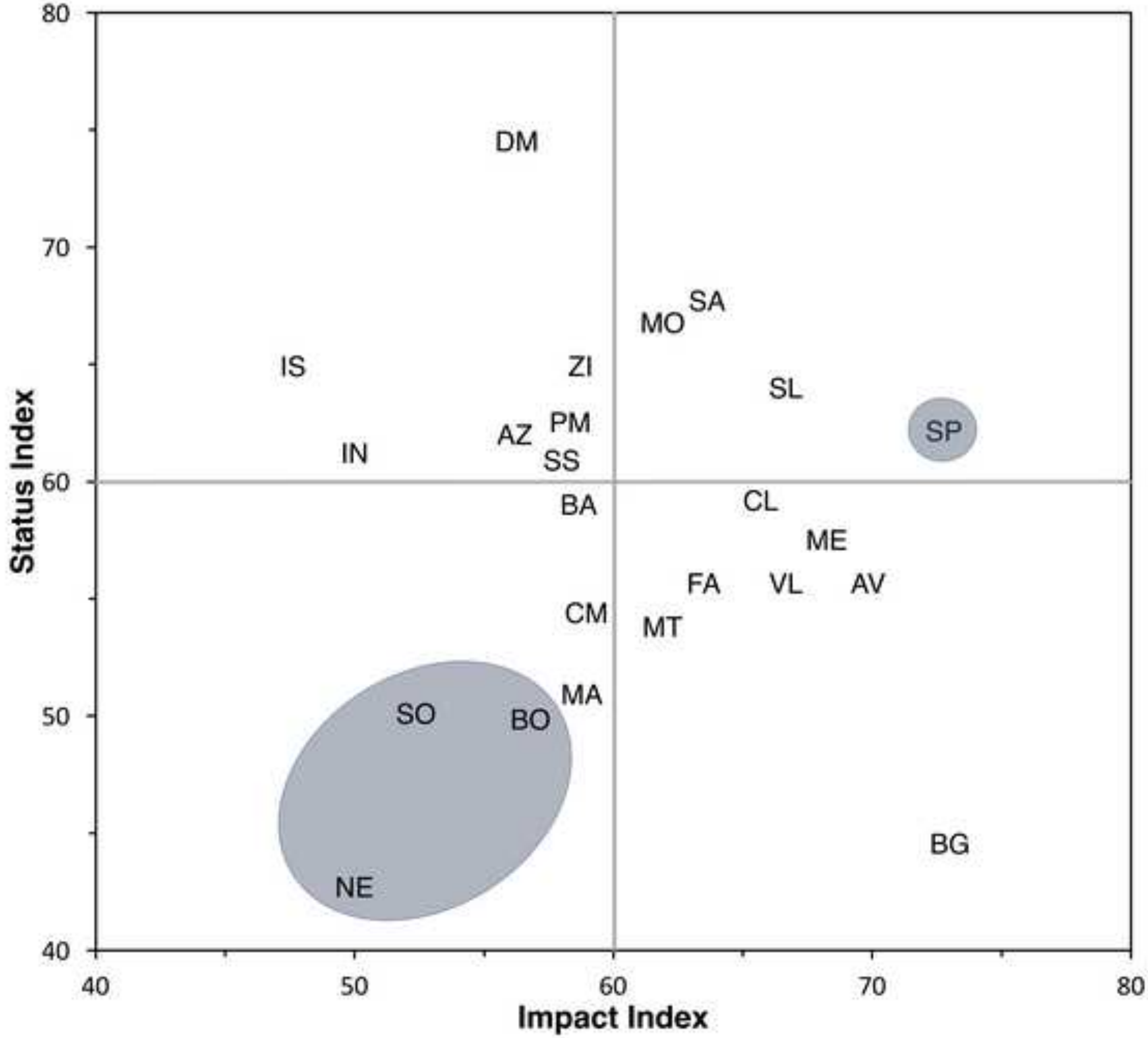

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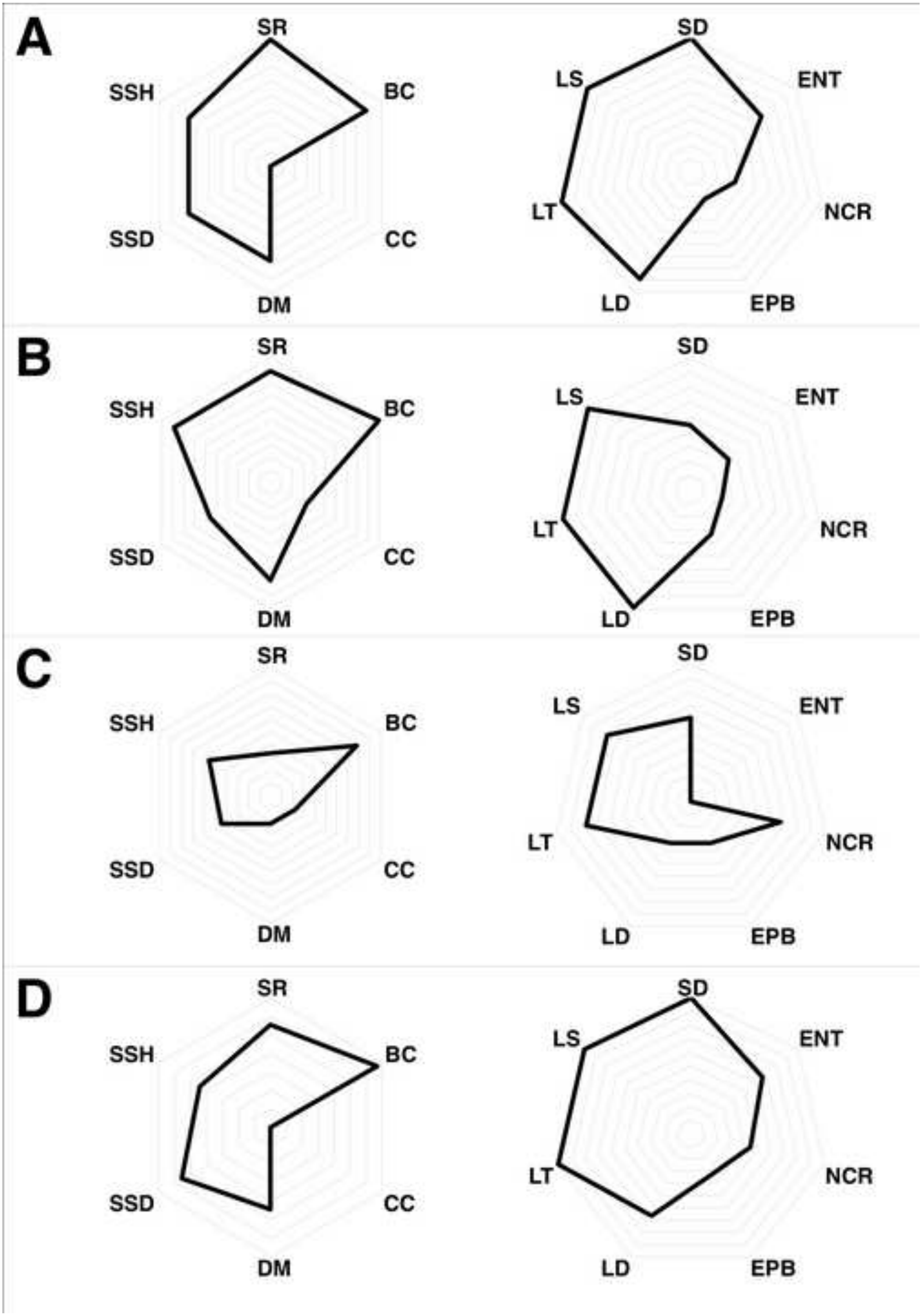

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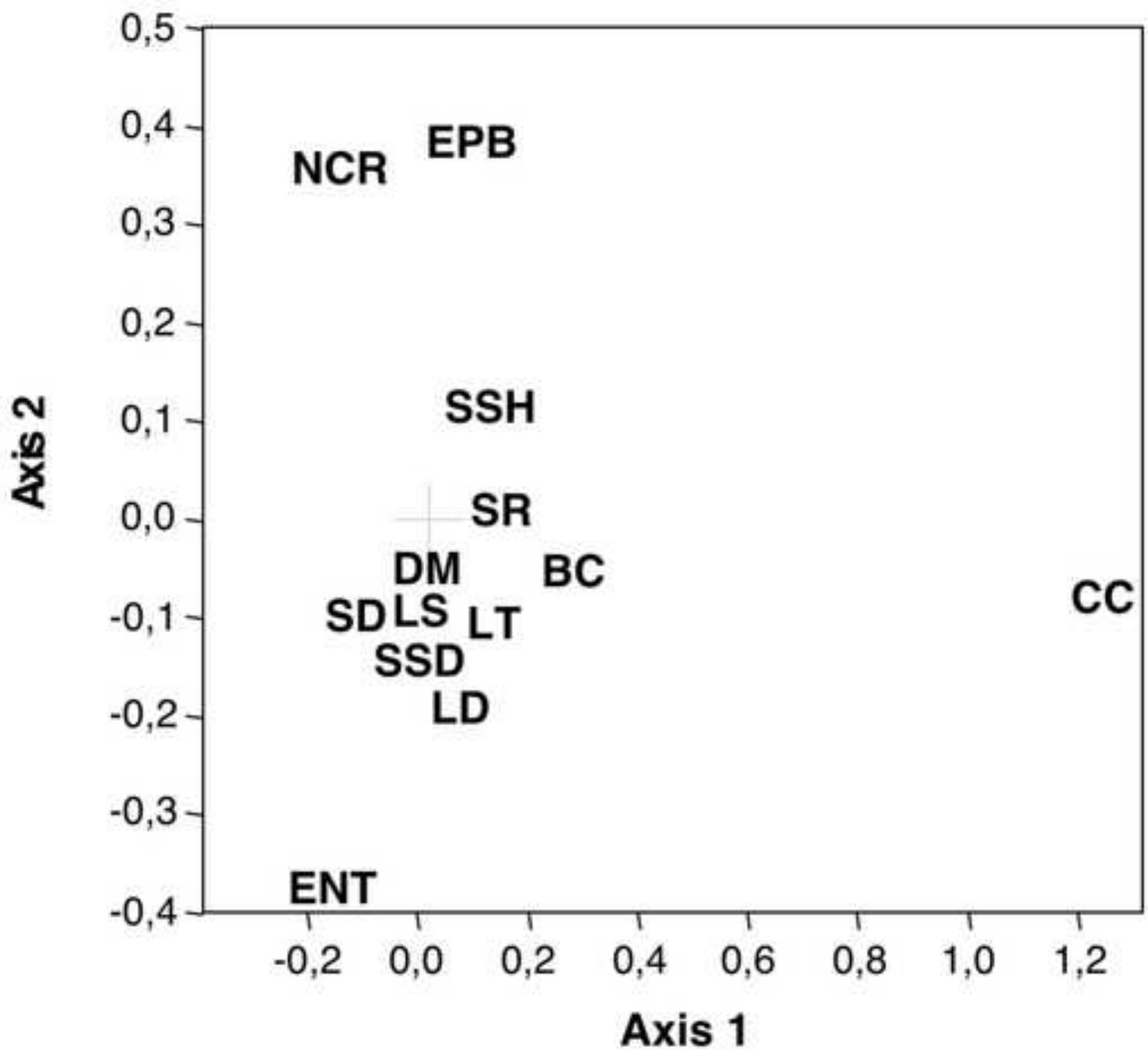

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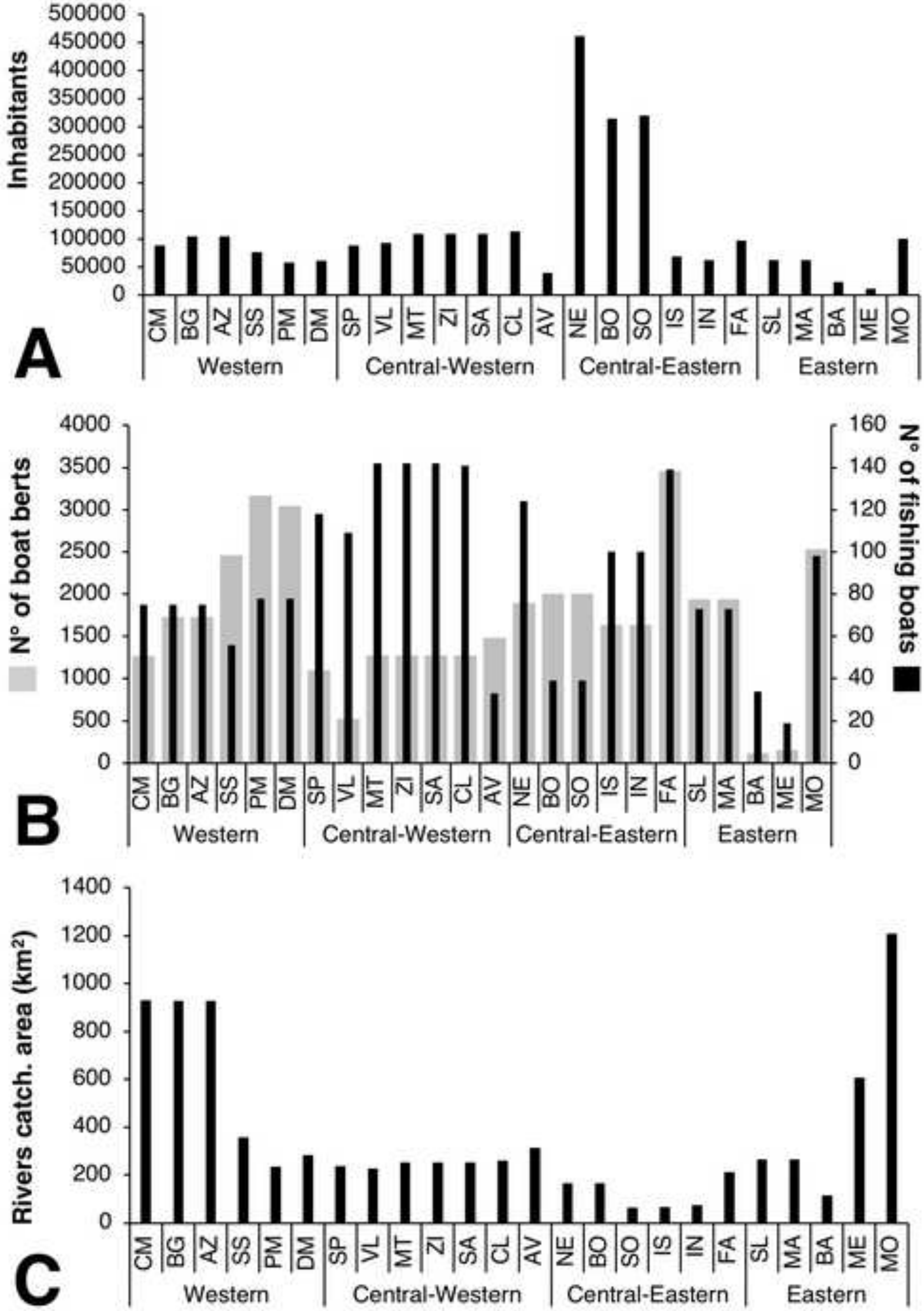

Table

Table 1. General information on the 24 sites investigated in this study.

Macro-area	Site	ID Code	Depth range (m)	Average slope (°)	Average hard bottom (m^2)
A1_Western sector	S1_Capo Mortola	CM	26-45	37	52
	S2_Bordighera	BG	47-101	56	43
	S3_Arziglia	AZ	46-70	35	56
	S4_Santo Stefano	SS	52-92	53	78
	S5_Porto Maurizio	PM	34-53	36	77
	S6_Diano Marina	DM	50-53	36	77
A2_Central/Western sector	S1_Spotorno	SP	54-143	64	88
	S2_Vado Ligure	VL	48-67	48	55
	S3_Mantice Shoal	MT	77-93	41	64
	S4_Zinola	ZI	45-56	38	45
	S5_Savona	SA	56-84	60	81
	S6_Celle Ligure	CL	39-52	25	53
	S7_Arenzano	AV	36-58	21	50
A3_Central/Eastern sector	S1_Nervi	NE	32-56	46	38
	S2_Bogliasco	BO	53-56	22	52
	S3_Sori	SO	34-53	34	77
	S4_Punta Chiappa	IS	32-54	49	77
	S5_Cala degli Inglesi	IN	33-44	59	77
	S6_Punta del Faro	FA	55-83	36	56
A4_Eastern sector	S1_Sestri Levante	SL	43-68	23	54
	S2_Punta Manara	MA	55-71	29	31
	S3_Punta Baffe	BA	37-70	30	41
	S4_Punta Mesco	ME	36-58	53	42
	S5_Punta Montenero	MO	28-32	25	77

Table

Table 2. Reference scores for the 13 components used in the MACS Index. See the text for more details. GL = general litter; LFG = lost fishing gears; SL = small items; LI = large items. Score 0 is acceptable.

Components	Score 1	Score 2	Score 3
Index of Status			
1. Species richness (SR)	≤20	>20 ≤40	>40
2. Basal bio-cover (BC)	<1.5	≥1.5 <2.5	≥2.5
3. Coralline algae cover (CC)	<1.5	≥1.5 <2.5	≥2.5
4. Dominance of structuring species (DM)	1 sp.	2 spp.	≥3 spp.
5. Density of all structuring species (SSD)	≤2	>2 ≤5	>5
6. Mean height of dominant structuring species (SSH)	≤1/3 of ½ max H	>1/3 ≤2/3 of ½ max H	>2/3 of ½ max H
Index of Impact			
7. Sedimentation (SD)	<1.5	≥1.5 <2.5	≥2.5
8. Percent of entangled colonies (ENT)	≤20	>20≤40	>40
9. Percent of necrotic colonies (NCR)	≤20	>20≤40	>40
10. Percent of epibionted colonies (EPB)	≤20	>20≤40	>40
11. Density of litter items (LD)	≤0.1	>0.1≤.0.2	>0.2
12. Litter typology (LT)	Presence of GL	Presence of LFG	GL + LFG
13. Litter size (LS)	Presence of SI	Presence LI	SI + LI

Table

Table 3. Size parameters for the 13 structuring species encountered in this study. Values are expressed in cm.

Species	Score 1	Score 2	Score 3	Max height	Reference
Axinella polypoides Schmidt, 1862	≤13	>13 <25	≥25	70	Topsent, 1934
Spongia (Spongia) lamella (Schulze, 1879)	≤10	>10 <20	≥20	60	Pronzato 2018, pers. comm.
Sarcotragus foetidus Schmidt, 1862	≤8	>8 <15	≥15	50	Pansini et al., 2011
Dendrophyllia cornigera (Lamarck, 1816)	≤8	>8 <15	≥15	40	Fourt et al., 2017
Antipathella subpinnata (Ellis & Solander, 1786)	≤25	>25 <50	≥50	150	Bo et al., 2009
Corallium rubrum (Linnaeus, 1758)	≤8	>8 <15	≥15	50	Fourt et al., 2017
Eunicella cavolini (Koch, 1887)	≤10	>10 <20	≥20	60	Sini et al., 2015
Eunicella singularis (Esper, 1791)	≤13	>13 <25	≥25	70	Linares et al., 2008
Eunicella verrucosa (Pallas, 1766)	≤13	>13 <25	≥25	80	Fourt et al., 2017
Paramuricea clavata (Risso, 1826)	≤18	>18 <35	≥35	100	Linares et al., 2008
Leptogorgia sarmentosa (Esper, 1789)	≤18	>18 <35	≥35	100	Weinberg, 1976
Myriapora truncata (Pallas, 1766)	≤2.5	>2.5 <5	≥5	15	de la Nuez-Hernández et al., 2014
Pentapora fascialis (Pallas, 1766)	≤5	>5 <10	≥10	30	Cocito et al., 1998

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