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A new ecological index for the status of mesophotic megabenthic assemblages in the Mediterranean based on ROV photography and video footage

Almudena Cánovas-Molina[a,*], Monica Montefalcone[a], Giorgio Bavestrello[a], Angelo Cau[b], Carlo Nike Bianchi[a], Carla Morri[a], Simonepietro Canese[c], Marzia Bo[a]

[a]*DiSTAV, Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università di Genova, Corso Europa 26, 16132 Genova, Italy*

[b]*DiSVA, Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, Via T. Fiorelli,* 09126 *Cagliari, Italy*

[c]*ISPRA, Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Via Vitaliano Brancati, Rome, Italy*

*E-mail address: almucanovass@gmail.com (Almudena Cánovas-Molina).

Abstract

A new index of ecological status, named Mesophotic Assemblages Ecological Status (MAES) index, was elaborated on the basis of ROV (Remotely Operated Vehicle) photography and video footage in order to assess the status of mesophotic megabenthic assemblages from hard bottom. The index was tested on seven sites located between 50 and 150 m depth in the Ligurian and Tyrrhenian seas (western Mediterranean Sea). The MAES index considers three main parameters: i) the community structure (number of megabenthic taxa, percent biotic cover in the basal layer, density of erect species); ii) the condition of the dominant erect species (average height, percent of colonies with epibiosis/necrosis); iii) the visible human impact (density of marine litter, including lost fishing gears). Two versions of the index have been elaborated, the complete version (MAES) and the quick version (*q*-MAES), which showed comparable results, therefore suggesting the possibility of fastening assessment times. The sensitivity of the MAES index was correlated with the putative human pressure acting upon the site (semi-quantitatively assessed considering fishing effort and coastal urbanisation). A standard working protocol related to the evaluation of the MAES index is here proposed with the intent to create an effective monitoring tool for the assessment of the ecological status of mesophotic assemblages on a large scale, as required by the EU Marine Strategy Framework Directive. MAES index will enhance the comprehension of the dynamics of mesophotic Mediterranean megabenthic assemblages with respect to human pressures and will also provide marine scientists and managers with a valuable tool specifically designed for the conservation of such vulnerable marine ecosystems.

Key-words: mesophotic assemblages, ecological status, Mediterranean Sea, MAES index, ROV.

1. Introduction

The requirement to assess the ecological status of marine waters is growing worldwide. Increasing human pressures can degrade these ecosystems if no mitigation occurs, consequently marine legislations on different countries (as Clean Water Act (CWA)or Oceans Act in USA, Australia or Canada; Water Framework Directive (WFD) or Marine Strategy Framework Directive (MSFD) in Europe, National Water Act in South Africa, etc.) report the urge of assessing ecological quality or integrity of ecosystems (Halpern et al., 2008). In particular, the European Marine Strategy Framework Directive (MSFD, 2008/56/EC) states that marine environment is a precious heritage that must be protected, preserved and, where practicable, restored with the ultimate aim of maintaining biodiversity and providing diverse and dynamic oceans and seas that are clean, healthy and productive. To do so, Member States shall take the necessary measures to achieve or maintain Good Environmental Status (GES) in the marine environment by the year 2020 at the latest. To guide progress towards achieving GES, a comprehensive set of environmental targets and associated indicators must be established taking into account the main pressures on the marine environment and on the basis of descriptors of seafloor integrity, biological diversity and presence of marine litter, among others.

When assessing the ecological status of a habitat, three aspects need to be taken into account (Borja et al., 2012): (i) human pressures must be assessed (quantitatively, whenever possible); (ii) sites with the absence of human pressures (or least disturbed locations) must be individuated to provide reference conditions; (iii) methods to assess the ecological status in biological elements and aquatic ecosystems must be validated against human pressures, to determine the management responses.

Following the requirements of the European Directives, several indices of ecological status have been developed for soft bottom habitats (Borja et al., 2000; Simboura and Zenetos, 2002; Rosenberg et al., 2004; Muxika et al., 2007), upper infralittoral algal belts (Orfanidis et al., 2001; Ballesteros et al., 2007), seagrass meadows (Romero et al., 2007; Gobert et al., 2009; Montefalcone, 2009), and shallow-water coralligenous assemblages (Deter et al., 2012; Cecchi et al., 2014; Gatti et al., 2015). No index has been proposed yet to assess the ecological status of hard bottom assemblages thriving in the mesophotic zone, an area extending into the lower portion of the photic zone.

The benthic assemblages thriving between 50 m depth down to the edge of the continental shelf (150-200 m depth) have been understudied, consequently their distribution, ecological roles, threats, and status remains poorly understood. Understanding these assemblages has particular importance, since it is hypothesized that moderate deep reefs may serve as refugia for their shallower counterparts (Glynn, 1996; Reigl and Piller, 2003). With the recent increased availability of equipment such as drop cameras, remotely operated vehicles, autonomous underwater vehicles, submersibles, and advancements in technical scuba diving practices, some focus has shifted to examine these deep formations (Menza et al., 2007). In particular, the use of Remotely Operated Vehicles (ROVs) provides information at fine resolutions over relatively large and contiguous extents of the seafloor and can be deployed beyond diver depths (Jones et al., 2007; Kahng et al, 2007; Menza et al., 2007). However, the use of ROVs has some limitations (e.g. cost of operation, requirement for trained operator, limitations in strong currents). In the Mediterranean, the use of ROVs has grown steadily in the last years (Freiwald et al., 2009; Orejas et al., 2009; Gili et al., 2011; Aguilar et al., 2014; Fourt and Goujard, 2014; Bo et al., 2015).

The term mesophotic, or twilight zone generally refers to the bathymetric belt extended from 40 to 150 m depth, in the lower portion of the photic zone (Lesser et al., 2009). In the Mediterranean Sea, megabenthic assemblages thriving in the hard bottoms of the mesophotic zone can be either deep coralligenous formations in clear waters or offshore rocks/*roche du large* in turbid waters (Pérès and Picard, 1964; Bo et al., 2012). They are mainly dominated by large arborescent anthozoans that create important three-dimensional underwater animal forests, often called coral gardens, which are able to attract a notable invertebrate and fish fauna (Cerrano et al., 2010; Bo et al., 2015). Due to the general longevity and slow growth rates of these species, and to their vulnerability towards the mechanical injuries inflicted by fishing gears (Bo et al., 2014; Angiolillo et al., 2015), coral gardens are regarded as vulnerable marine ecosystems (GFCG, 2009) and are now being considered for protection by numerous international conservation measures. In an enclosed, highly anthropized and over-exploited basin as the Mediterranean Sea (Bianchi et al., 2012), pristine deep-water assemblages are not longer expected to thrive, leading to a great reduction in the original abundance as well as geographic and bathymetric ranges of the animal forests (Bo et al., 2014, 2015).

The aims of this study were, therefore, to integrate these findings into a comprehensive index (MAES or Mesophotic Assemblages Ecological Status index), to propose it as a new methodology to assess the ecological status of mesophotic megabenthic assemblages (50-150 m depth) by means of ROV photography and video footage, and to test preliminarily its sensitivity to different levels of human disturbance over a wide geographic area.

2. Materials and methods

2.1. Sites surveyed

Surveys have been conducted on four shoals in the Ligurian Sea, namely Punta Faro, Corallone, Mantice and Bordighera (Liguria), and three shoals in the Tyrrhenian Sea, namely Campo Scogli (Gulf of Naples, Campania), Olbia Canyon (East Sardinia) and Favazzina (Calabria) (Fig. 1, Table 1). All the sites surveyed are located at a relatively short distance from the coast, with the farthest site, Olbia Canyon, located about 13 km offshore (Table 1).

Data were collected at 50 to 150 m depth through *Pollux*, a Remotely Operated Vehicle (ROV), during four cruises on board of the R/V *Astrea* between 2009 and 2013. *Pollux* was equipped with a digital camera (Nikon D80, 10 megapixels), a strobe (Nikon SB 400), a high definition video camera (Sony HDR-HC7), and three jaw grabbers; it also hosted a depth sensor, a compass, and two parallel laser beams to measure the size of the organisms.

Video transects were carried out during 7 different surveys and the ROV moving at 1 m height from the seabed (about 2 m of visual field). To uniform sampling effort with different video transect lengths per site, we analysed one 500 m long linear video transect on rocky surface, covering an explored area of 1000 m^2. 500 m was chosen because: (i) sampling times were feasible; (ii) beyond this length no additional information was gained; (iii) before this length information was lost due to the high heterogeneity common to hard bottom assemblages, where species are frequently found aggregated in small patches. The ROV took 20 random high resolution photographs (300 dpi) frontally on the seafloor. Data was extracted from the visualization of the whole video transect by the same operator by means of VLC program (http://www.videolan.org/vlc/) using time in the video and velocity of ROV to infer transect length. Additionally; photographs were analysed by means of Image J software (http://rsb- web.nih.gov/nih-image) by the same operator.

Fig. 1. Geographical location of the sites surveyed. **1.5 column size**

Table 1. Main characteristics of the sites surveyed.

Site	Geographic coordinates (WGS84)	Depth range (m)	Distance from the coast (km)	Main erect species
Punta Faro	44.287ºN, 9.221ºE	50-80	1.3	*Eunicella cavolini*
Corallone	44.226ºN, 8.460ºE	60-110	1.9	*Eunicella cavolini*
Mantice	44.271ºN, 8.523ºE	70-150	4.5	*Eunicella cavolini*
Bordighera	43.779ºN, 7.681ºE	60-80	0.3	*Eunicella cavolini*
Campo Scogli	40.768ºN, 14.269ºE	110-120	6	*Eunicella cavolini*
Olbia Canyon	41.070ºN, 9.798ºE	100-120	13	*Eunicella cavolini*
Favazzina	38.264ºN, 15.739ºE	50-100	1.1	*Antipathella subpinnata*

2.2. MAES metrics

The MAES index is proposed as a multimetric index to assess the ecological status of mesophotic megabenthic assemblages. The metrics of the MAES index were chosen for their feasibility to be calculated from ROV photography/video footage and for the information they provide on community structure, dominant erect species condition and visible human impact. In total, 6 metrics were considered:

i. *Community structure* - 1) number of megabenthic taxa (T): a list of megabenthic sessile algal and animal species identified in the whole video transect was recorded; 2) percent biotic cover in the basal layer (CB): percent cover of encrusting, or with limited vertical growth, living organisms was visually estimated from each photograph, the average from the 20 photos of each site being the final value of the metric. The use of high resolution photographs is highly recommend but not indispensable; 3) density of all erect megabenthic species (E): the number of colonies or individual encountered in the whole video transect was counted and divided for the total area of the transect ($1000m^2$).

ii. *Dominant erect species condition* - 4) average height (H) of the most abundant erect species: the height (in cm) of all the measurable colonies was averaged; 5) percent of colonies with epibiosis/necrosis (EN) of the most abundant erect species: the number of colonies showing epibiosis or necrosis was counted along the transect and divided by the total number of colonies along the transect.

iii. *Visible human impact* - 6) density of litter (L): the number of anthropogenic debris m^{-1} recorded in the video transect was calculated. Each litter type recorded has been multiplied by a given score in order to differentiate their incidence on living organisms.

In accordance with Angiolillo et al. (2015) results, we gave the higher punctuation to the actual entangled organisms recorded in the video transect (1.5), followed by fishing nets and long lines (1 and 0.8 respectively) for their important potentially abrasive action. Anchors and ropes, plastic bags and glass bottles, were given a 0.5 and 0.3 score respectively, for their small size but still damaging capacity.

Given that no pristine conditions could be determined among the studied sites, we postulated the reference condition as "theoric optimal site", corresponding to the 'best' values of each metric noted, similarly as Romero et al. (2007) and Deter et al. (2012). Best values of the 7 metrics could be maximum or minimum depending on the nature of the metric, e.g. minimum for percent of colonies with epibiosis/necrosis, and maximum for number of megabenthic taxa. For the metric 'density of marine litter' we made an exception and 0 was assigned as reference condition. Considering reference condition (RC) and metric value (MV) score 1 was assigned when MV > 80% RC, score 2 when 50% RC ≤ MV ≤ 80% RC, score 3 when MV < 50% RC. For metrics NE and L, maximum values found were used to defined scores (Table 2).

Table 2. Metrics used in the MAES index with reference conditions (see Results) and threshold used to assign 1 to 3 scores. *Metric considered in the *q*-MAES index.

Metrics	**Score 1**	**Score 2**	**Score 3**	**Reference conditions**
1. Number of megabenthic taxa* (T)	<19	19≤T≤30	>30	38
2. Percent biotic cover in the basal layer* (C_B)	<43.5	43.5≤%≤69.6	>69.6	87
3. Density erect species (E)				
Eunicella cavolini	<1.75	1.75≤E≤2.8	>2.8	3.5
Antipathella subpinnata	<0.5	0.5≤E≤0.8	>0.8	1.02
4. Average height dominant erect species (H)				
Eunicella cavolini	<10.9	10.9≤H≤17.4	>17.4	21.8
Antipathella subpinnata	<23.3	23.3≤H≤37.3	>37.3	46.6
4b. Percent cover erect species* (C_E)	<21.5	21.5≤%≤30.4	>34.4	43
5. Percent of colonies with epibiosis/necrosis (NE)	>15.4	15.4≤%≤9.6	<9.6	0
6. Density of marine litter* (L)	>0.1	0.1≤L≤0.06	<0.06	0

The final value of the MAES index is obtained by summing up the score of each metric according to the following formula:

$$MAES = S_T + S_{CB} + S_E + S_H + S_{EN} + S_L$$

where: S_T = score of the number of megabenthic taxa; S_{CB} = score of the percent biotic cover in the basal layer; S_E = score of the density of all erect megabenthic species; S_H = score of the average height of the most abundant erect species; S_{EN} = score of the percent of colonies with epibiosis/necrosis of the most abundant erect species; S_L = score of the density of litter.

As MSFD does not give a defined classification of ecological status, we decided to give three classes of ecological status similarly as Gatti et al. (2015). Three classes were considered: bad, moderate, and good (Table 3).

Table 3. Final scores to define the ecological status of mesophotic megabenthic assemblages.

Ecological status	**Scores MAES**	**Scores *q*-MAES**
Bad	$6 \leq MAES \leq 9$	$4 \leq q\text{-}MAES \leq 6$
Moderate	$10 \leq MAES \leq 14$	$7 \leq q\text{-}MAES \leq 9$
Good	$15 \leq MAES \leq 18$	$10 \leq q\text{-}MAES \leq 12$

2.3. Quick MAES (q-MAES)

A simpler version of the MAES index, named *q*-MAES (for quick MAES), was also created to test whether a reduced number of metrics could be sensitive enough with respect to the complete set of metrics, thus making it possible to reduce the working time. Saving time would allow carrying out the assessment of a larger number of sites. Three metrics from MAES were considered in *q*-MAES: 1) number of megabenthic taxa (T), 2) percent biotic cover in the basal layer (CB), and 3) density of marine litter (L). A fourth metric, 4) the percent cover of erect species (CE), was added and obtained as the average, from the 20 photographs at each site, of the percent cover of the erect species taken altogether. Reference conditions and scores are summarized in Table 2. The final value of the *q*-MAES index is obtained by summing up the score of each metric according to the following formula:

$$q\text{-MAES} = S_T + S_{CB} + S_{CE} + S_L$$

where: S_T = score of the number of megabenthic taxa; S_{CB} = score of the percent biotic cover in the basal layer; S_{CE} = score of the percent cover of erect species; S_L = score of the density of marine litter.

In order to obtain a classification of the ecological status of mesophotic megabenthic assemblages from this shorter version of the index, three classes were considered: bad, moderate, and good (Table 3).

2.4. Validation of the indices

To check if the MAES and the *q*-MAES indices are adequately sensitive to human pressures, we adopted a semi-quantitative assessment aiming at ranking the seven study sites according to the expected human impact. The assessment takes into consideration the main human drivers affecting mesophotic assemblages: these were identified as fishing effort and coastal urbanisation (in terms of water pollution and silting), as suggested by previous studies (Airoldi, 2003; Balata et al., 2007; Bo et al., 2014, 2015; Gatti et al., 2015). The assessment included a score, from 0 (no impact) to 5 (dramatic pressure), similarly as done by Deter et al. (2012), for four human pressures: number of professional fishing boats listed in the ports within a radius of 18 km (approximately 10 NM) from the study site, distance to the nearest river, population within a radius of 10 km, and distance to the nearest industrial or commercial port.

For the first pressure we considered vessels using gillnets, trammel nets and longline gears, these type of vessels generally do not go beyond 10 NM from the port. Vessels equipped with trawling devices were not considered because they generally avoid rocky bottoms. Scallop dredging, another towed demersal fishing gear, targets the margins of rocky reefs (Seehan et al., 2013) but was not found in the studied sites. No quantification of recreational fishing boats was possible for the studied areas, even if they may contribute significantly to the fishing effort both in coastal sites and offshore locations (Bo et al., 2014). Distance of 10 km for the population was chosen following the methodology in Lopez y Royo et al. (2009) for type of land use. Scores for each pressure were estimated from freely available information on EU Fleet Register (http://ec.europa.eu/fisheries/fleet/index.cfm?method=Download.Menu&country=ITA), Google-Earth (http://earth.google.com) and Wikipedia (https://www.wikipedia.org/). The overall human pressure over each site was assessed by summing up all the four human pressures affecting mesophotic assemblages. Finally, the robustness of MAES indices was tested with a comparison to level of human pressure using a linear regression.

3. Results

3.1. Characterization of the study sites

The megabenthic assemblages of the studied sites comprised a wide range of taxa, including algae, encrusting and massive sponges, hydrozoans, anthozoans, polychaetes, ascidians, and bryozoans (Fig. 2A-C). The dominant assemblage found was represented by gorgonian forests of *Eunicella cavolini*, seen in all the Ligurian sites (Punta Faro, Bordighera, Corallone, Mantice) and, to a lesser extent, in Campo Scogli (Campania) and Olbia Canyon (Sardinia). In the Ligurian sites, the benthic assemblages included also other gorgonian species (such as *Paramuricea clavata*, *Eunicella verrucosa* and *Corallium rubrum*) and numerous massive sponges. In particular, at Punta Faro, the average density of gorgonians (*Eunicella cavolini*, *Paramuricea clavata*, *Eunicella verrucosa*) and black corals (*Antipathella subpinnata*) reached 3.5 colonies m^{-2} (Table 4). In Campo Scogli, the species richness resulted far lower (N = 16) than in all other sites (Table 4). The site of Favazzina was the only shoal where evident algal coverage by *Mesophyllum expansum* thrived between 50-100 m depth together with the black coral *Antipathella subpinnata* as dominant erect species. The community of Olbia Canyon showed the highest number of erect anthozoan species, including gorgonians, alcyonaceans, scleractinians, zoanthids and antipatharians. This site, together with Favazzina, showed the highest percent biotic cover in the basal layer (87% and 64%, respectively) due to a dense coverage of encrusting organisms and virtually no silting, as opposed to what observed for Campo Scogli (23%) (Table 4). In Favazzina there was also the highest cover of erect species (43%) due to the dense population of the black coral *A. subpinnata*. Favazzina and Corallone showed the highest values for the average height of their dominant erect species *E. cavolini* and *A. subpinnata*, respectively (Table 4). The metrics characterizing the visible human impact showed the highest density of marine litter on the Corallone shoal (0.13 debris m^{-1}) and the highest percent of epibionted colonies on the Mantice shoal, where about 20% of the colonies were overgrown by the parasitic anthozoan *Alcyonium coralloides* (Table 4).

The Olbia Canyon site concentrated 3 out of the 6 best values for the MAES index metrics and therefore provided reference conditions for: number of megabenthic taxa, percent biotic cover in the basal layer (Fig. 2E) and percent of colonies with epibiosis/necrosis (Fig. 2F) (Table 4).

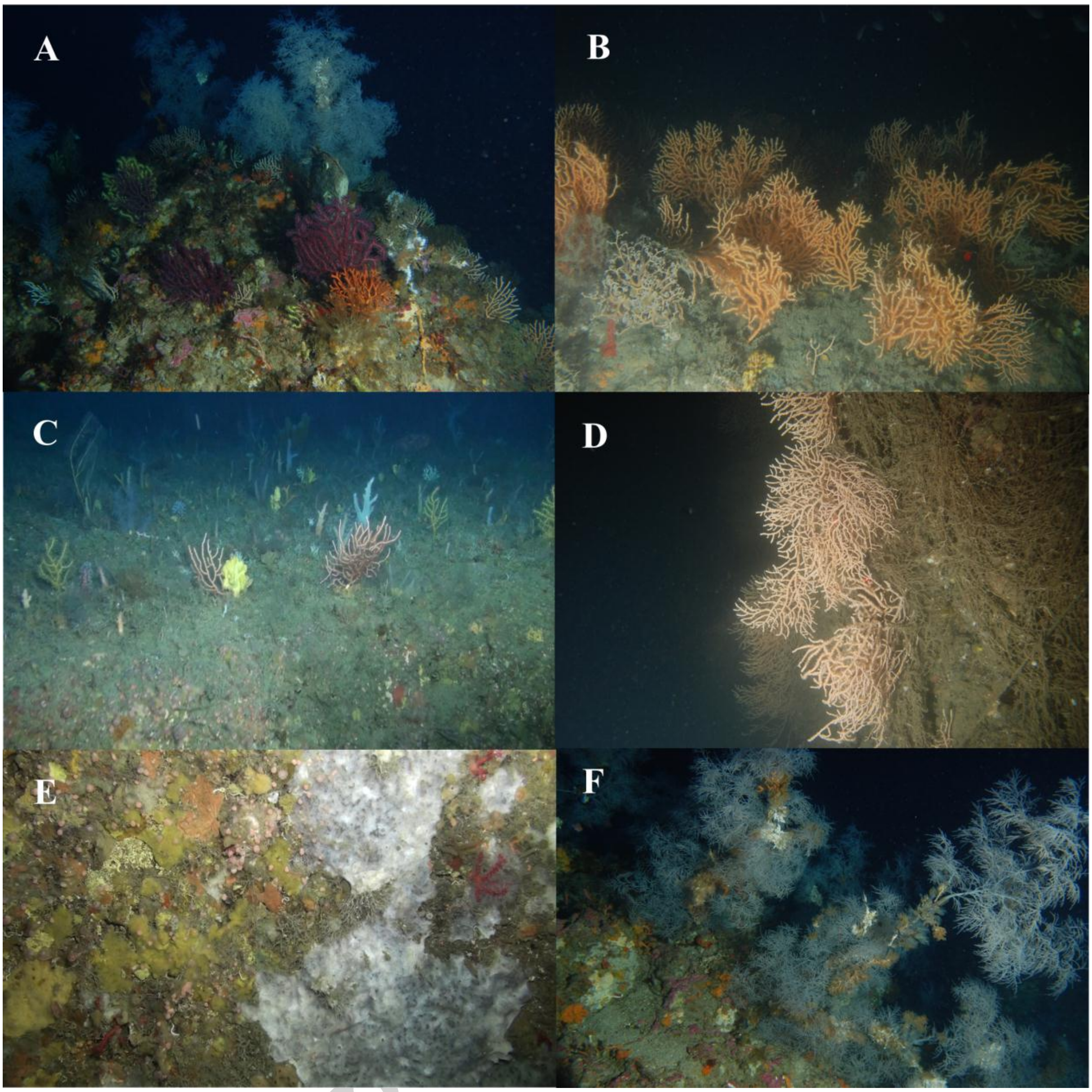

Fig. 2. Mesophotic assemblages of the shoals surveyed. A) Mesophotic assemblages of Favazzina at 70 m depth with *Mesophyllum expansum* as calcareous encrusting algae and *Antipathella subpinnata*, *Paramuricea clavata* and *Eunicella cavolini* as coral erect species. **B)** Offshore rocky bottoms of Punta Faro at 60 m depth with *Eunicella cavolini*. **C)** Offshore rocky bottoms of Olbia Canyon at 110 m depth with *Alcyonium acaule* and *Eunicella cavolini* as coral erect species. **D)** Colony of *Eunicella cavolini* entangled in fishing net, Campo Scogli at 121 m depth. **E)** Detail of biotic cover in the basal layer, Olbia Canyon 115 m depth. **F)** Epibiosis on colonies of *Antipathella subpinnata*, Favazzina at 60 m depth. **2 column size**

Table 4. MAES metrics' values for each surveyed site. (T): number of megabenthic taxa, (C_B): percent biotic cover in the basal layer, (E): density of all erect megabenthic species, (H): average height of the most abundant erect species, (C_E): percent cover erect species, (EN): percent of colonies with epibiosis/necrosis of the most abundant erect species, (L): density of litter. ^best value recorded for the corresponding metric.

Sites surveyed	(T)	(C_B)	(E)	(H)	(C_E)	(EN)	(L)

Punta Faro	36	40	**3.5^**	20.6 (±0.2)	38	3.3	0.10
Corallone	27	48	1.7	**21.8(±0.3)^**	34	0.8	0.13
Mantice	19	31	1.4	21.7 (±0.2)	24	19.2	0.09
Bordighera	27	38	0.25	16.8 (±0.7)	8	15.9	0.05
Campo Scogli	16	23	0.3	16.8 (±0.5)	12	11.3	0.06
Olbia Canyon	**37^**	**87^**	0.6	15.9 (±0.9)	16	**0^**	0.01
Favazzina	31	64	1.02	**46.6 (±1)^**	**43^**	16.0	0.02
MAES	37	87	3.5	21.8(±0.3) 46.6 (±1)		0	0
q-MAES	37	87			43		0

3.2. MAES vs. q-MAES

The *q*-MAES index has been proposed as a quicker tool for the assessment of the ecological status of mesophotic assemblages. In fact, working time is reduced to less than half (Table 5).

Table 5. Working time (min) of MAES versus *q*-MAES index for each site surveyed.

	MAES	***q*-MAES**
Punta Faro	340’	100’
Corallone	230’	75’
Mantice	270’	120’
Bordighera	130’	60’
Campo Scogli	130’	70’
Olbia Canyon	210’	80’
Favazzina	260’	80’
average	224’	83’

MAES indices agreed with the ecological status of all 7 studied sites. Both MAES and *q*-MAES classified two sites in a “good ecological status” (Favazzina and Olbia Canyon), Punta Faro, Corallone, Mantice and Bordighera in a “moderate ecological status” and Campo Scogli in a "bad ecological status" (Fig. 3). No relationship was found between the indices (MAES, *q*-MAES) and the distance from the coast (Fig. 4). The two sites classified in a "good ecological status" (Favazzina and Olbia Canyon) were located at 1.1 and 13 km from the coast, respectively.

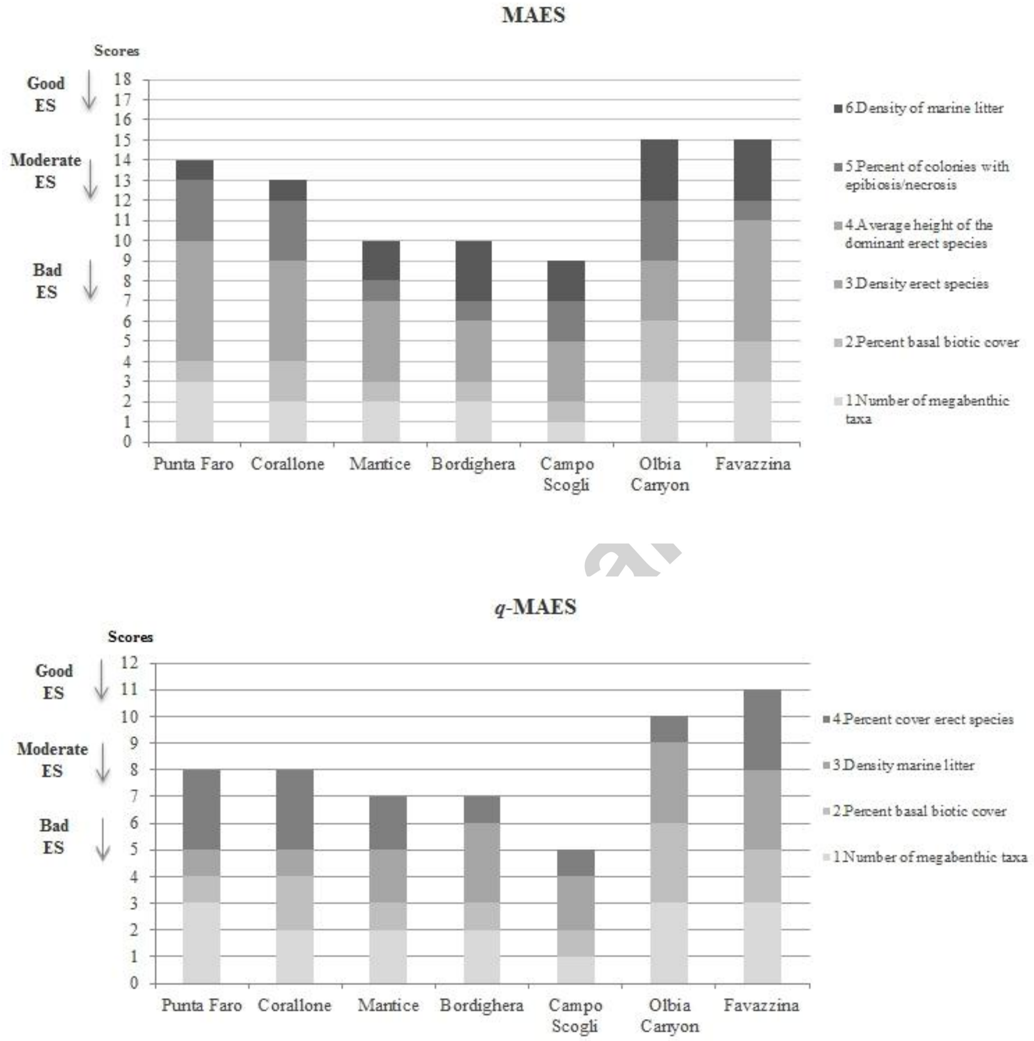

Fig. 3. Metrics' scores for MAES and *q*-MAES indices for each site. Final scores of both indices are grouped according to the ecological status (ES). **1.5 column size**

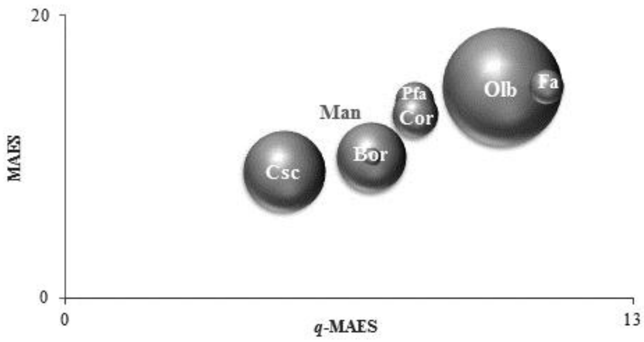

Fig. 4. MAES and *q*-MAES values for the studied sites. Area of the bubbles is proportional to the distance from the coast (in NM). Fa: Favazzina, Olb: Olbia Canyon, Pfa: Punta Faro, Cor: Corallone, Bor: Bordighera, Man: Mantice, Csc: Campo Scogli. **Single columnn size**

3.3. Human pressure

The ecological status of mesophotic megabenthic assemblages assessed through MAES and *q*-MAES was negatively and significantly correlated with the level of human pressure assessed semi-quantitatively (Pearson's r = -0.81 (MAES), Pearson's r = -0.88 (*q*-MAES), Fig. 5). Campo Scogli was the site with the highest level of pressure (Table 6), and was consistently classified in a "bad ecological status". On the contrary, Favazzina and Olbia Canyon, which exhibited "good ecological status", were the sites less subjected to human pressures.

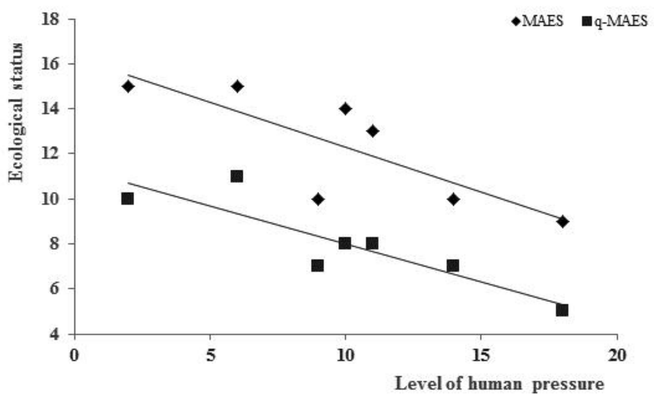

Fig. 5. Linear regression between the level of human pressure and the ecological status obtained by MAES and *q*-MAES. N= 7 sites, r= -0.81 (MAES), r= -0.88 (*q*-MAES), p<0.01. **1.5 columnn size**

Table 6. Mesophotic Assemblages Ecological Status (MAES) indices and level of human pressures values for the 7 studied sites.

	MAES	*q*-MAES	Ecological status	Human pressure level
Punta Faro	14	8	Moderate	10
Corallone	13	8	Moderate	11
Mantice	10	7	Moderate	14
Bordighera	10	7	Moderate	9
Campo Scogli	9	5	Bad	18
Olbia Canyon	15	10	Good	2
Favazzina	15	11	Good	6

4. Discussion

Since the idea of biotic indices gained acceptance, there has been a proliferation of applications, especially to benthic communities, which summarize complex biological monitoring information in a form that is easily communicated to managers (Diaz et al., 2004). On the other hand, managers can find a situation of multiple indices validated in a specific region for a type of biota and therefore a confused situation with various, and often conflicting, answers for a single water body (Borja et al., 2009).

In the present work, the two versions of the MAES index have been tested in different geographical contests (Ligurian and Tyrrhenian seas) and on various types of mesophotic habitats between 50 and 150 m depth, contributing to the desirable trend of applicability of indices across regions and depths. Scientific approaches should also be simplified to help managers use this information effectively and efficiently (Borja et al., 2009). Following this necessity, MAES indices gave an easy understandable final result of “good”, “moderate” or “bad” ecological status, reducing complex information from multiple ecosystem elements to a single word. Moreover, we proposed the *q*-MAES index as a simplified version of the MAES index to allow faster assessments with comparable results (Table 6). The *q*-MAES index could therefore be the preferred tool when large amounts of data need to be analyzed effectively and efficiently in a short period of time.

MAES indices can be applied abroad Mediterranean taking into consideration that: (i) reference conditions need to be defined for each region based on the best values found, except for marine litter that remains 0; (ii) other possible pressures should be considered, different from those found in our study (e.g. scallop dredging) and different from human caused (e.g. infectious diseases); (ii) for soft bottom megabenthic assemblages indices should pressures assessment should take into account trawling effects.

Both MAES indices were sensitive to the level of anthropogenic pressure but some results were different from the expected. As an example, density of erect species was found low in Olbia Canyon, where no human pressures were identified. Lots of other factors as depth, slope, geomorphology, cold-water intrusion, descended dense water masses, currents rich in suspended matter, catastrophic sedimentation, other oceanographic factors, infectious disease or some combination of these (Menza et al., 2007; Locker et al., 2010; Bo et al., 2011; McClain et al., 2015) could be affecting mesophotic assemblages and were not included among the factors considered.

However, our results suggest that, from the factors considered in the present study, the ecological status of mesophotic assemblages seems to be mostly related to the degree of coastal urbanisation, as Deter et al. (2012) and Gatti et al. (2015) found for coralligenous assemblages in France. MAES indices classified Campo Scogli under a "bad ecological status". The proximity of this shoal to the highly populated city of Naples, as well as to its fishing, commercial and industrial port, explained the high level of pressure affecting Campo Scogli. On the contrary, Favazzina (1 km offshore) and Olbia Canyon (13 km offshore) were both located in front of little urbanized areas. In the proximity of Favazzina shoal are found only Favazzina, Bagnara Calabra and Scilla, small coastal towns of 15,000 inhabitants in total. Golfo Aranci is the closest (15 km) urbanized area (2300 inhabitants) in the proximity of the Olbia Canyon.

Both MAES indices showed to be sensitive to the level of pressure therefore proved to be efficient tools for the assessment of the ecological status of Mediterranean mesophotic assemblages, which are currently without any formal protection and are not even considered in the European Nature Information System (EUNIS) habitat classification (Davies et al., 2004). While deep cold-water coral reefs are now included within the EUNIS habitat classification and the UN Resolution attempting to protect vulnerable marine ecosystems (VMEs) from destructive fishing practices in international waters (A/RES/61/105), no special regional, national or international legislation or resolution manifestly asks for the protection of deep circalittoral assemblages, even if they are considered to have an extraordinary role in controlling benthic biodiversity of the circalittoral Mediterranean Sea at levels and spatial scales comparable to those identified for deep water corals (Bo et al., 2012). Protection is urgently needed for the animal forests that lie between two protected shallower (coralligenous formations) and deeper (deep cold-water coral reefs) habitats. While the control of land-based pollution should be pursued as a general goal, the application of a buffer zone around the coral forests to reduce the indirect impacts of fishing should be accomplished as a precautionary measure to maintain the “good ecological status” of benthic assemblages and to ensure compliance with the European Council Regulation (EC) Nº 1967/2006 (Council of European Communities, 2006).

In an international context, the status assessment of mesophotic assemblages through MAES indices could contribute to the knowledge of these understudied and still subjected to coral loss ecosystems.

5. Conclusions

We proposed MAES and *q*-MAES as multimetric indices that use what the MSFD listed as descriptors of GES: (i) biological diversity (ii) seafloor integrity and (iii) marine litter. Reference conditions are optimally defined from data (i) acquired from multiple sites with similar physical characteristics, (ii) that ideally represent undisturbed conditions, and (iii) that provide an estimate of the variability in biological communities due to natural factors (Borja et al., 2012).

MAES indices provide environmental targets contributing to guide progress towards achieving GES by 2020, as requested by the MSFD. In the present era of lost "intact marine ecosystems" (Stachowitsch, 2003), we adopted as references conditions those corresponding to the best values recorded for each metric in our study. The application of the theoretical maxima as best values attainable for each metric, using for example specialist literature, could lead to unrealistic results depressing the MAES indices of real sites. Therefore we defined reference conditions as the best value obtained from real sites. A future improvement of the two MAES indices will be their application in multiple Mediterranean sites and in a larger geographic and bathymetric range in order to give a wider overview of the variability of mesophotic assemblages due to natural factors and a better definition of reference conditions and scores. Already tested in two Mediterranean seas (Ligurian and Tyrrhenian seas) and in a wide bathymetric range (50-150 m depth), MAES indices should be applied across other seas abroad Mediterranean for the assessment of ecological status of benthic ecosystems by exploiting existing archives of ROV photography and video footage with particular interest on mesophotic reefs.

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Highlights

- We proposed MAES index to assess the ecological status of mesophotic assemblages.
- Sampling was conducted by means of ROV photography and video footage.
- The index was tested in western Mediterranean Sea.
- MAES index showed to be sensitive to the level of pressure.