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

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Abstract

A new index of ecological status, named Deep Assemblages Ecological Status (DAES) index, was elaborated on the basis of ROV (Remotely Operated Vehicle) photography and video footage in order to assess the status of deep circalittoral hard bottom megabenthic assemblages. The index was tested on seven sites located between 50 and 150 m depth in the Ligurian and Tyrrhenian seas (western Mediterranean Sea). The DAES 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 (DAES) and the quick version (*q*-DAES), which showed comparable results, therefore suggesting the possibility of fastening assessment times. The sensitivity of DAES 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 DAES index is here proposed with the intent to create an effective monitoring tool for the assessment of the ecological status of deep circalittoral assemblages on a large scale, as required by the EU Marine Strategy Framework Directive. DAES index will enhance the comprehension of the dynamics of deep circalittoral 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: deep circalittoral assemblages, ecological status, Mediterranean Sea, DAES index, ROV.

1. Introduction

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; Lopez y Royo et al., 2009; Montefalcone 2009), and shallow-water coralligenous assemblages (Deter et al., 2012; Gatti et al., 2012, 2015; Cecchi et al., 2014). No index has been proposed yet to assess the ecological status of hard bottom assemblages thriving in the deep circalittoral 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) are not accessible by means of SCUBA diving, and therefore the indices developed for shallow assemblages become inapplicable. The use of Remotely Operated Vehicles (ROVs), on the other hand, offers the possibility to survey benthic assemblages virtually without depth and time limits, therefore gathering large archives of photos and videos. For these reasons, the use of ROV in the Mediterranean Sea has grown steadily in the last years (Aguilar et al., 2009, 2014; Bo et al., 2009, 2011a, 2011b, 2012, 2013, 2014, 2015; Freiwald et al., 2009; Orejas et al., 2009; Gili et al., 2011; Gori et al., 2011, 2013; Fourt and Goujard, 2014).

In the Mediterranean Sea, deep circalittoral megabenthic assemblages along the continental platform are found either in deep coralligenous formations in clear waters or on offshore rocks 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).

Recently, a series of ROV-based indicators of fishing impact have been described for the main relevant structuring species of the deep circalittoral Mediterranean megabenthic assemblages (Bo et al., 2014). These indicators include the species richness of the assemblages, the number of lost gears and their typology, the vulnerability of certain species based on their shape and the nature of their skeleton, as well as on the susceptibility of damaged colonies to parasitic epibionts.

The aims of this study were, therefore, to integrate these findings into a comprehensive index (DAES or Deep Assemblages Ecological Status index), to propose it as a new methodology to assess the ecological status of deep circalittoral 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 providing a 10 cm scale for the measurement of the size of the organisms.

Data for each site were obtained from the analysis of one 500 m long linear video transect on rocky surface (with an average visual field of 2 m) and from the analysis of 20 random HQ photographs taken frontally on the seafloor.

Fig. 1. Geographical location of the sites surveyed.

Table 1. Main characteristics of the sites surveyed.

Site	Geographic coordinates	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. DAES metrics

The DAES index is proposed as a multimetric index to assess the ecological status of deep circalittoral megabenthic assemblages. The six metrics of the DAES index are based on the following three parameters:

i. *Community structure* - 1) number of megabenthic taxa (T): a list of megabenthic sessile algal and animal species observed in the video transect was filled in; 2) percent biotic cover in the basal layer (CB): percent cover of encrusting, or with limited vertical growth, living organisms was estimated from each photo, the average from the 20 photos of each site being the final value of the metric; 3) density of all erect megabenthic species (E): the number of colonies or individual m^{-2} encountered in the video transect was counted.

ii. *Dominant erect species condition* - 4) average height (H) of the most abundant erect species (within arborescent anthozoans): 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.

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 the incidence of litter types (Table 2).

Table 2. Scores given to each type of marine litter encountered along ROV video transects.

Marine litter	Scores
Plastic bags, glass bottles	0.3
Anchors, ropes	0.5
Long lines	0.8
Fishing nets	1
Entangled organisms	1.5

Given that no pristine conditions could be determined among the studied sites, we postulated the reference condition as the one corresponding to the best value recorded for each metric. Considering reference conditions, scores from 1 to 3 were given arbitrarily to each metric (Table 3).

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

Metrics	Score 1	Score 2	Score 3	Reference conditions
1. Number of megabenthic taxa* (T)	<20	20≤T≤30	>30	38
2. Percent biotic cover in the basal layer* (C_B)	<30	30≤%≤60	>60	87
3. Density erect species (E)	<0.5	0.5≤E≤1.5	>1.5	3.5
4. Average height dominant erect species (H)				
Eunicella cavolini	<15	15≤H≤20	>20	22
Antipathella subpinnata	<30	30≤H≤40	>40	47
4b. Percent cover erect species* (C_E)	<15	15≤%≤30	>30	43
5. Percent of colonies with epibiosis/necrosis (NE)	>20	10≤%≤20	<10	0
6. Density of marine litter* (L)	>0.05	0.03≤L≤0.05	<0.03	0.01

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

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

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 (within arborescent anthozoans); 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.

In order to obtain a classification of the ecological status of deep circalittoral megabenthic assemblages, three classes were considered: bad, moderate, and good (Table 4).

Table 4. Final scores to define the ecological status of deep circalittoral megabenthic assemblages.

Ecological status	Scores DAES	Scores *q*-DAES
Bad	$6 \leq$ DAES ≤ 9	$4 \leq q$-DAES ≤ 6
Moderate	$10 \leq$ DAES ≤ 14	$7 \leq q$-DAES ≤ 9
Good	$15 \leq$ DAES ≤ 18	$10 \leq q$-DAES ≤ 12

2.3. Quick DAES (q-DAES)

A simpler version of the DAES index, named *q*-DAES (for quick DAES), 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 with the same sampling effort. Three metrics from DAES were considered in *q*-DAES: 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 photos at each site, of the percent cover of the erect species taken altogether. Reference conditions and scores are summarized in Table 3. The final value of the *q*-DAES index is obtained by summing up the score of each metric according to the following formula.

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

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 deep circalittoral megabenthic assemblages from this shorter version of the index, three classes were considered: bad, moderate, and good (Table 4).

2.4. Validation of the indices

To check if the DAES and the *q*-DAES indices are adequately sensitive to human pressures, we adopted a semi-quantitative assessment (ranging from 0, no pressure, to 5, high pressure) aiming at ranking the seven study sites in order of expected human impact. The assessment takes into consideration the main human pressures affecting deep circalittoral 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 first parameter was estimated on the basis of the number of professional fishing boats listed in the ports over an arc of coast with a radius of 18 km (approximately 10 NM) from the study site. Vessels equipped with trawling devices were not considered because they generally avoid rocky bottoms (a specific and independently developed index targeting soft bottom megabenthic assemblages subjected to trawling would be greatly welcome). 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). The second parameter was evaluated taking into consideration the occurrence, within an arc of coast with a radius of 9 km (approximately 5 NM) centred in the sampling site, of river mouths, commercial or industrial ports, and the total population. The overall human pressure over each site was assessed by summing up all the pertaining semi-quantitative scores (Table 5).

Table 5. Semi-quantitative assessment of human pressures affecting deep circalittoral assemblages. N° of fishing vessels in ports refers to: number of vessels using gillnets, trammel nets and longline gears in ports located <18 km from the studied sites. Information is freely available on EU Fleet Register (http://ec.europa.eu/fisheries/fleet/index.cfm?method=Download.Menu&country=ITA).

Fishing effort	
N° fishing vessels in ports (<18 km)	**0** (N°<50), **1** (50≤N°≤100), **2** (N°>100)
Urban pressure	
River mouths (<9 km)	**0** (absence), **1** (presence)
Population >100000 (<9 km)	**0** (absence), **1** (presence)
Commercial or industrial ports (<9 km)	**0** (absence), **1** (presence)
Level of pressure (Sum)	**0-5**

Finally, the correlation between the DAES and *q*-DAES indices and the level of human pressure was analysed.

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 6). In Campo Scogli, the species richness resulted far lower (N°=16) than in all other sites (Table 6). 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 6). In Favazzina there was also the highest cover of erect species (43%, used for the *q*-DAES) 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 6). 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 6).

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

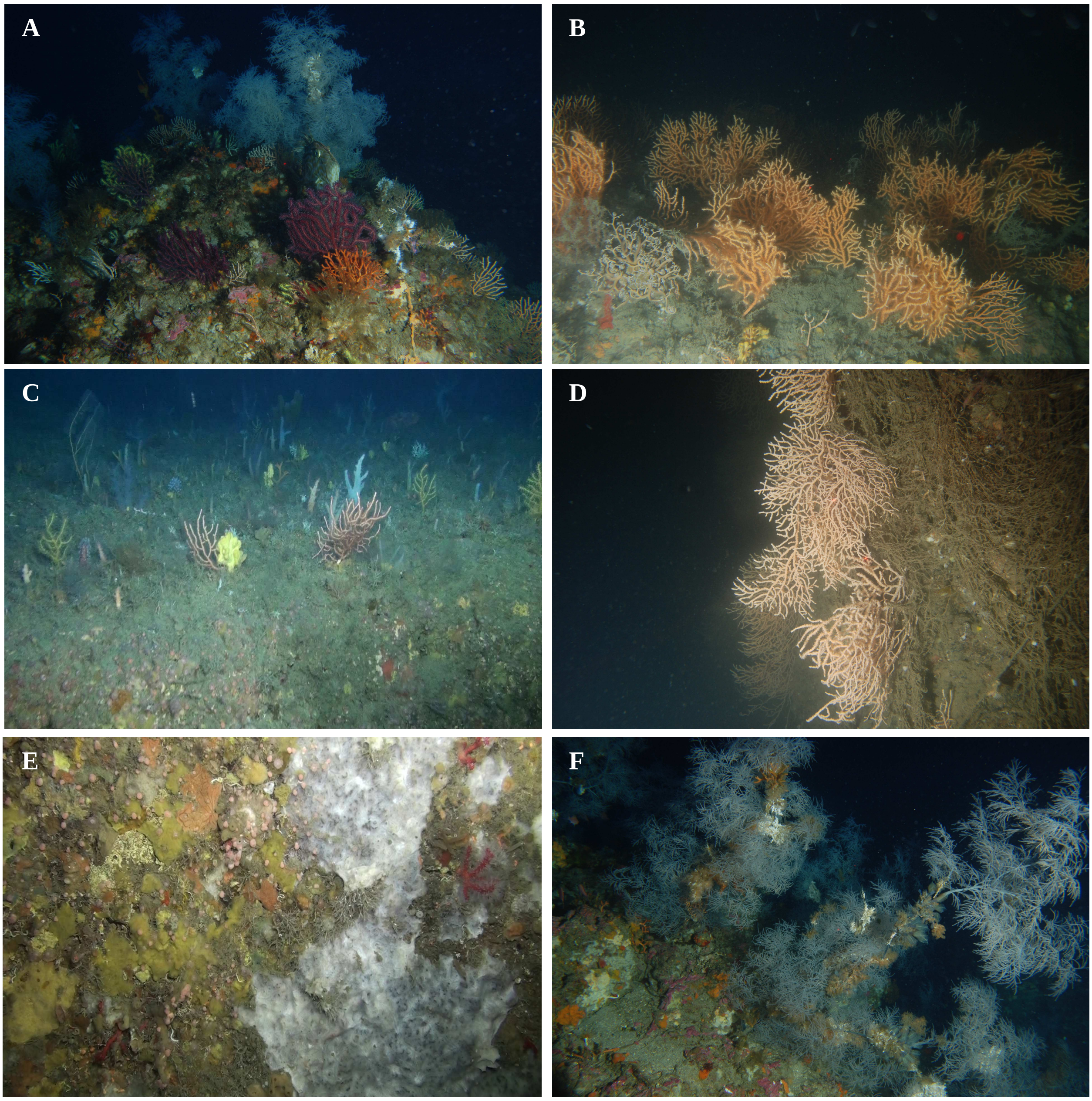


Fig. 2. Deep circalittoral assemblages of the shoals surveyed. A) Deep circalittoral assemblages of Favazzina at 70 m depth with *Mesophyllum expansum* as calcareous encrusting algae and *Antipathella subpinnata*, *Paramuricea clavata* and *Eunicella cavolini* as coral erect species. **B)** Offshore rocky bottoms of Punta Faro at 60 m depth with *Eunicela 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 a fishing net, Campo scogli at 121 m depth. **E)** Detail of basal biocover, Olbia Canyon 115 m depth. **F)** Epibiosis on colonies of *Antipathella subpinnata*, Favazzina at 60 m depth.

Table 6. DAES metrics' values for each surveyed site. * metrics used in the *q*-DAES index. ^best value recorded for the corresponding metric.

	Punta Faro	Corallone	Mantice	Bordighera	Campo Scogli	Olbia Canyon	Favazzina

1. Number of megabenthic taxa* (T)	36	27	19	27	16	**37^**	31
2. Percent biotic cover in the basal layer* (C_B)	40	48	31	38	23	**87^**	64
3. Density of erect species (E)	**3.5**	1.7	1.4	0.25	0.3	0.6	1.02
4. Average height (±sd) of the dominant erect species (H)	20.6 (±0.2)	**21.8 (±0.3)^**	21.7 (±0.2)	16.8 (±0.7)	16.8 (±0.5)	15.9 (±0.9)^	**46.6 (±1)^**
4b. Percent cover erect species* (C_E)	38	34	24	8	12	16	**43^**
5. Percent of colonies with epibiosis/necrosis (EN)	3.3	0.8	19.2	15.9	11.3	**0.0^**	16.0
6. Density of marine litter* (L)	0.10	0.13	0.09	0.05	0.06	**0.01^**	0.02

3.2. DAES vs. q-DAES

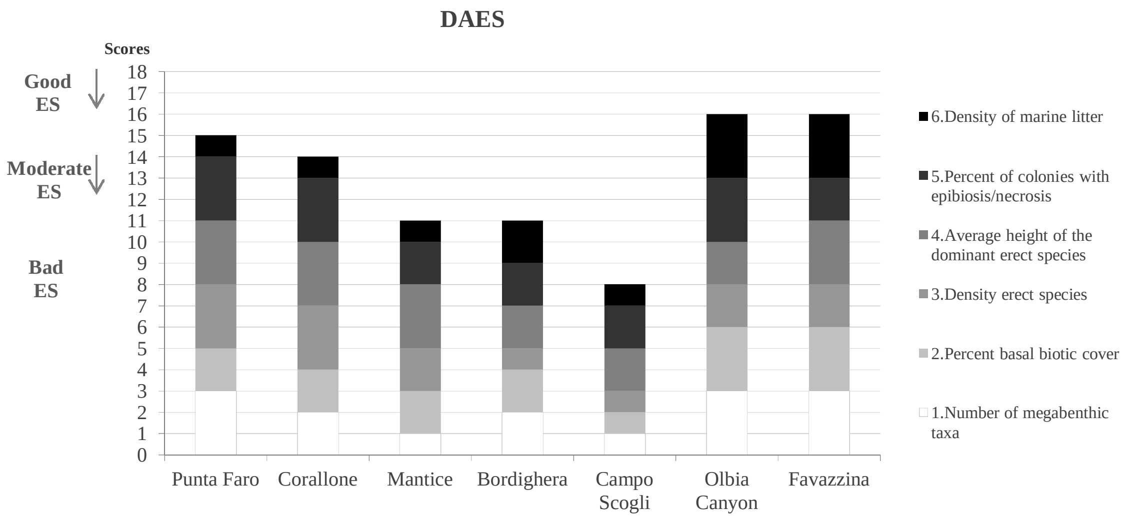
The *q*-DAES index has been proposed as a quicker tool for the assessment of the ecological status of deep circalittoral assemblages. In fact, working time is reduced to less than half (Table 7).

Table 7. Working time (min) of DAES versus *q*-DAES index for each site surveyed.

	DAES	***q*-DAES**
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’

Both DAES and *q*-DAES classified two sites in a “good ecological status” (Favazzina and Olbia Canyon), Bordighera and Corallone in a “moderate ecological status” and Campo Scogli in a “bad ecological status”. The two indices disagreed about the ecological status of

Punta Faro and Mantice: *q*-DAES caused the former to shift from "good" to "moderate", and the latter from "moderate" to "bad" (Fig. 3). No relationship was found between the indices (DAES, *q*-DAES) and the distance of the site from the coast (Fig. 4), as 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.



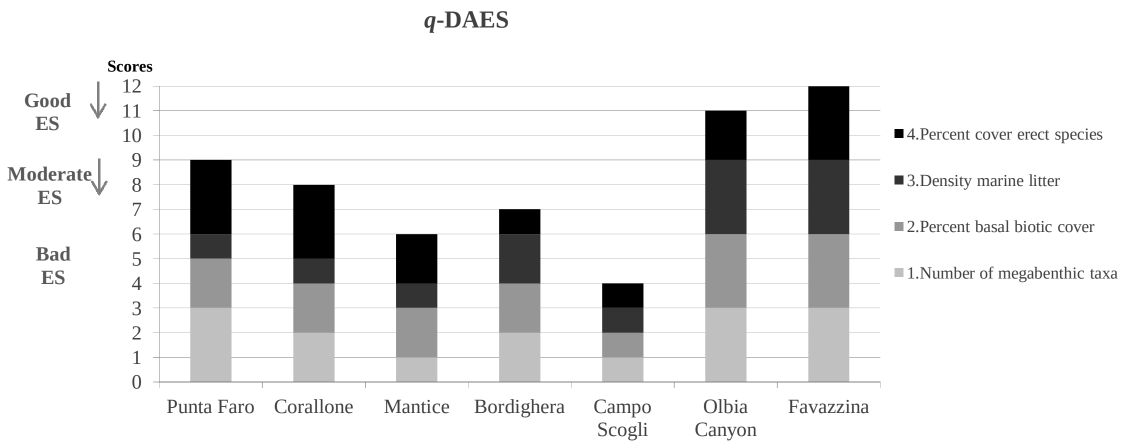


Fig. 3. Metrics' scores for DAES and *q*-DAES indices for each site. Final scores of both indices are grouped according to the ecological status (ES).

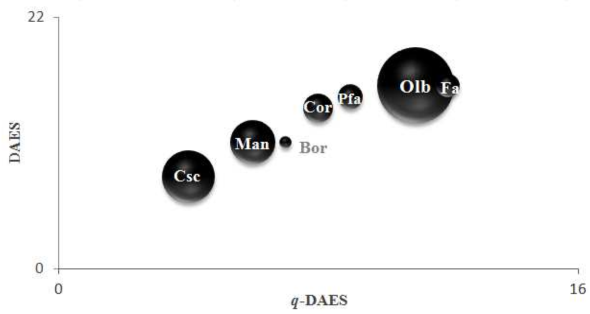


Fig. 4. DAES and *q*-DAES values for the studied sites. Area of the bubbles is proportional to the distance from the coast (in NM). Fa: Favazzina, Olb: Olbia Canyon, Pfa: Punta Faro, Cor: Corallone, Bor: Bordighera, Man: Mantice, Csc: Campo Scogli.

3.3. Human pressure

The ecological status of deep circalittoral megabenthic assemblages assessed through DAES and *q*-DAES was negatively and significantly correlated with the level of human pressure assessed semi-quantitatively (r = -0.9, Fig. 5). Campo Scogli was the site with the highest level of pressure (Table 8), and was consistently classified in a "bad ecological status" by both DAES and *q*-DAES. On the contrary, Favazzina and Olbia Canyon, which exhibited "good ecological status" according to both indices, were the sites less subjected to human pressures.

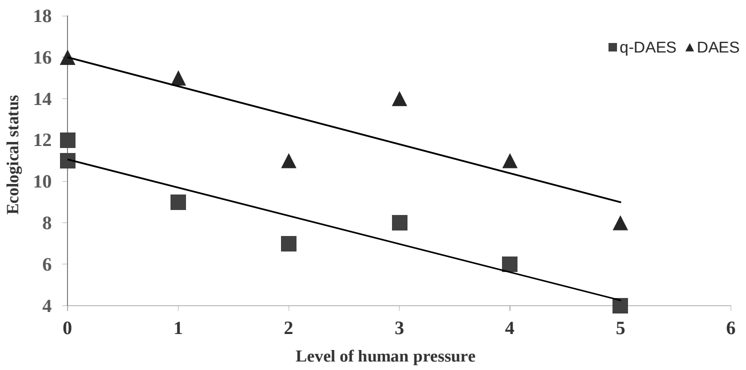


Fig. 5. Linear regression between the level of human pressure and the ecological status obtained by DAES and *q*-DAES. N= 7 sites, R^2= 0.91 (*q*-DAES), R^2= 0.8 (DAES).

Table 8. Semi-quantitative assessment of the level of human pressure affecting the seven sites surveyed.

	Punta Faro	Corallone	Mantice	Bordighera	Campo Scogli	Olbia Canyon	Favazzina
Fishing effort							
Nº fishing vessels in ports (<18 km)	0	1	1	0	2	0	0
Urban pressure							
River mouths (<9 km)	1	1	1	1	1	0	0
Population >100000 (<9 km)	0	1	1	1	1	0	0
Commercial or industrial ports (<9 km)	0	0	1	0	1	0	0
Level of pressure (Sum)	**1**	**3**	**4**	**2**	**5**	**0**	**0**

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 DAES index have been tested in different geographical contests (Ligurian and Tyrrhenian seas) and on various types of deep circalittoral 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, DAES 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*-DAES index as a simplified version of the DAES index to allow faster assessments with comparable results. In fact, whilst minor changes were recorded in the moderate class, the extreme situations remained clear and evident. The *q*-DAES 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.

Both DAES indices were sensitive to the level of anthropogenic pressure (Fig. 5) but some results were different from the expected. Density of erect species was found low in Favazzina and Olbia Canyon, where no human pressures were identified. Although the clear waters at both shoals allow high cover values of the organisms in the basal layer, the low nutrient input could reduce food availability and therefore the density of erect suspension feeders. In Corallone, an intermediate level of human pressure, in terms of pollution, could increase food availability and have a positive effect on the density of erect suspension feeders. When human pressure gets higher, the density of erect species falls (Campo Scogli).

Our results suggest that the ecological status of deep circalittoral assemblages is mostly related to the degree of coastal urbanisation, as Deter et al. (2012) and Gatti et al. (2015) found for coralligenous assemblages in France. DAES 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 degree 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 15000 inhabitants in total. Golfo Aranci is the closest (15 km) urbanized area (2300 inhabitants) in the proximity of the Olbia Canyon. Mantice was classified as in “bad ecological status” only by *q*-DAES. This result could point out to the high probability for this site to undergo the same status as Campo Scogli if pressures keep going on or rise. In this sense, DAES indices provide marine scientists and managers with a valuable tool to anticipate ecological degradation in deep circalittoral assemblages and adjacent areas.

Both DAES indices showed to be sensitive to the level of pressure and so proved to be efficient tools for the assessment of the ecological status of Mediterranean deep circalittoral 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 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).

5. Conclusions

We proposed DAES and *q*-DAES 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).

DAES indices provide environmental targets contributing to guide progress towards achieving GES by 2020, as requested by the MSFD. However, further application of the two DAES indices are needed in order to give a wider overview of the variability of deep circalittoral assemblages due to natural factors and so better adjust reference conditions and scores.

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. A future improvement of the two DAES indices will be the definition of reference conditions as those to be expected in a “theoretical optimal site”. These may correspond to the best values attainable for each metric, against which to compute the Ecological Quality Ratio (EQR). Theoretical maxima could be easily envisaged for parameters such as biotic cover (that can reach 100 % at maximum by definition), maximum height of erect species (that can be taken from the specialist literature), maximum health of colonies (that might imply no epibiosis/necrosis at all), and even human pressure (that should be 0). The theoretical maximum number of megabenthic taxa might be estimated through the regression of the reciprocals of species accumulation curves (Bianchi et al., 2011) over a large number of sites, which are not available at the moment. Similarly, the maximal value of the density of all erect megabenthic species might be obtained from an extensive survey of a variety of localities or from space-occupation models knowing the maximal breadth of the organisms concerned (from the literature). The values thus obtained, however, might prove unrealistic, to the risk of depressing the DAES indices of real sites. Only the application of DAES indices in multiple Mediterranean sites and in a larger geographic and bathymetric range, could give a wider overview of the variability of deep circalittoral 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), DAES indices should be applied across other regions and depths for the assessment of ecological status of benthic ecosystems by exploiting existing archives of ROV photography and video footage.

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