

## Infralittoral coralligenous reefs: a modified ESCA index for the ecological quality assessment

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### Abstract

Coralligenous reefs characterize the circalittoral zone of the Mediterranean Sea, but, in many areas, they are also distributed within the infralittoral zone. Despite their wide distribution, the infralittoral coralligenous reefs are less considered in monitoring programs and no specific methods have yet been developed for their assessment. This study aims at testing a modified ESCA index (I-ESCA) on the infralittoral coralligenous reefs in order to propose a new tool suitable to evaluate the ecological quality of this habitat. Thirteen sites were sampled in the western Mediterranean Sea and the relationship between I-ESCA and the Anthropization Index was tested with a regression model in order to evaluate the response of the index to human pressures. Infralittoral coralligenous assemblages were dominated by macroalgae, while the bryozoan *Myriapora truncata* and the cnidarian *Parazoanthus axinellae* were the most widespread animals. The I-ESCA index allowed to highlight the level of human pressures of the sites similarly to ESCA. Thus, results of this study suggested the possibility of applying the index also to the infralittoral habitat using an adequate calibration of the original method on the new spatial scale and reference conditions.

**Keywords:** infralittoral coralligenous reefs; ecological quality; Mediterranean Sea; spatial variability.

### Introduction

The recent European Directives (EU, 2000, 2008) aim at maintaining and improving the ecological status of marine ecosystems and recognize the monitoring and assessment of their ecological quality as main tools to plan conservation strategies (Borja *et al.*, 2010). In this context, the marine coastal habitats, being more vulnerable to anthropogenic pressures acting along the coastal strip, have been more thoroughly studied by scientific community and many biotic indices were developed and applied to assess their ecological quality status (Birk *et al.*, 2012; Bevilacqua *et al.*, 2020). Among the sensitive habitats protected by European legislation, coralligenous reefs are recognized as “special habitat types” (EU, 2008), whose ecological status should be assessed through accurate monitoring plans.

Coralligenous reefs are calcareous structures built mostly by encrusting calcareous algae (Ballesteros, 2006) and are one of the most important coastal ecosystems of the Mediterranean Sea in terms of distribution, biodiversity, productivity, and their role in CO<sub>2</sub> cycle (Thierry de Ville d’Avray *et al.*, 2019). Coralligenous reefs develop over a wide depth range, from 15 m to about 150 m (De Falco *et al.*, 2022), characterizing the circalittoral zone below the deeper limit of seagrass beds (Martin *et al.*,

2014) and thus forming a wide range of different assemblages and habitats (Pinna *et al.*, 2021; SPA/RAC-UN ENVIRONMENT/MAP, 2021). Circalittoral coralligenous reefs have been widely investigated over a large geographic scale (Casas-Guell *et al.*, 2015; Cinar *et al.*, 2020; Casoli *et al.*, 2024). The deep coralligenous reefs are usually surveyed through remotely operated vehicles (i.e., ROVs; Cánovas-Molina *et al.*, 2016; Ferrigno *et al.*, 2017; Enrichetti *et al.*, 2019), while shallow reefs (within 50 m depth) are monitored by SCUBA diving (Gatti *et al.*, 2015; UNEP, 2017; Piazz *et al.*, 2022a), although rebreather system is locally used to survey coralligenous reefs (Langlois *et al.*, 2021).

However, in many Mediterranean areas coralline algae constitute conspicuous calcareous structures also in the infralittoral bottoms, alternating with seagrass beds. In the past, infralittoral coralligenous reefs were considered a transitional habitat or included in the so called “pre-coralligenous reefs” (Peres & Picard, 1964;), while now they are recognized as a peculiar coralligenous habitat (Montefalcone *et al.*, 2021; SPA/RAC-UN ENVIRONMENT/MAP, 2021).

Despite their presence throughout the Mediterranean Sea and the ecological role similar to circalittoral reefs, the infralittoral coralligenous reefs are very little known and under-represented in monitoring programs and im-

pact evaluation studies (Piazzi *et al.*, 2023a). However, the assessment of the ecological quality of infralittoral coralligenous reefs is now necessary, especially to address the needs of the impact evaluations of human activities in coastal areas, and, in this contest, the identification of a suitable tool is mandatory.

In the last ten years, several biotic indices have been proposed to evaluate the ecological quality of coralligenous assemblages of circalittoral reefs (Kipson *et al.*, 2011; Deter *et al.*, 2012; Gatti *et al.*, 2015; Zapatá-Ramírez *et al.*, 2013; Montefalcone *et al.*, 2017; Ferrigno *et al.*, 2017; Sartoretto *et al.*, 2017, Enrichetti *et al.*, 2019; Piazzi *et al.*, 2021b, 2023b; Turicchia *et al.*, 2021), while no specific methods has yet been developed for the assessment of infralittoral coralligenous habitat.

The ESCA index was initially proposed to evaluate the ecological quality of shallow coralligenous reefs (above 50 m depth) through the analysis of macroalgal assemblages (Cecchi *et al.*, 2014), and later it was implemented through the use of sessile invertebrates (Piazzi *et al.*, 2017). As required by European directives, the index was tested on gradients of anthropogenic stressors (Piazzi *et al.*, 2015, 2021b) and employed in monitoring programs and impact evaluation studies (Penna *et al.*, 2017; Piazzi *et al.*, 2018, 2019a, 2021a). Although the index was developed to assess the ecological status of circalittoral assemblages growing on vertical bottoms (named “cliffs”), it is conceived to be theoretically used for any type of hard bottoms, as the ESCA descriptors are suitable to detect effects of human alterations independently of the considered habitat. However, the application of ESCA index to habitats with characteristics

of assemblages different from those used to develop the index requires the definition of different reference values and site-specific scales of sensitivity level. In fact, the infralittoral coralligenous reefs are normally characterized by a higher quantity of sciophilous soft algae and a lower number of invertebrate species compared to the circalittoral ones (Piazzi *et al.*, 2023a).

This study aims at testing the ESCA index on infralittoral coralligenous reefs in order to propose a new tool suitable to evaluate the ecological quality of this type of habitat. To achieve this goal, ESCA was applied over a large area of the Western Mediterranean Sea, selecting sites where infralittoral coralligenous assemblages were present. Firstly, sites subjected to different levels of human pressure were chosen to test the index along a gradient of environmental quality. Secondly, the reference values of the ecological descriptors used for circalittoral coralligenous assemblages were recalibrated on the new habitat. Finally, the response of the new ESCA to anthropization levels was compared to those obtained for circalittoral cliffs in the same sites.

## Methods

### Study sites

Thirteen sites were sampled in the western Mediterranean Sea at a depth ranging between 18 and 25 m, six on the western coast of mainland Italy, six in Sardinia and one in Sicily (Fig.1). In order to measure the level of human pressures affecting each study site, the anthropo-



**Fig 1:** Map of the study sites. The legend of acronyms is reported in Table 1.

zation index was calculated (Piazzi *et al.*, 2021b). The index was defined considering the nine main human pressures affecting coralligenous reefs: urbanization and urban waste, ports, tourism, industrial activities, sediment load, aquaculture, agricultural waste, fishing, and anchoring. Each pressure was classified as 0 (no pressure), 1 (low or medium pressure), or 2 (strong pressure), according to its presence and entity and the distance of the sites from the source (Piazzi *et al.*, 2021b). For each site, the anthropization index values, ranging from 0 to 18, were obtained as the sum of the values of each individual pressure considered for that site (Table 1).

### Sampling methods and image analysis

The sample design followed the STAndaRdized coralligenous evaluation procedure (STAR, Piazzi *et al.*, 2019b; Gennaro *et al.*, 2020), appropriately recalibrated on the morphology and natural variability of the investigated habitat. In fact, the STAR protocol was specifically developed for coralligenous cliffs, so its sampling design was calibrated on the high spatial variability at small scales typical of vertical bottoms assemblages. The infralittoral coralligenous reefs are instead characterized by horizontal/sub-horizontal substrate emerging from the bottom as outcrops of variable size scattered among seagrass beds. Thus, infralittoral coralligenous assemblages require larger sampling areas to intercept variability of this scattered habitat. Following this approach, size of the original 4 m<sup>2</sup> sampling surface was increased and three plots of about 10 m<sup>2</sup>, tens of metres apart, were randomly selected for each site on horizontal or sub-horizontal bottoms between 15 and 25 m. In each plot, 10 photographic

samples of 0.2 m<sup>2</sup> were collected.

Photographic samples were analysed using ImageJ software to evaluate the percentage cover of the main taxa or morphological groups (Piazzi *et al.*, 2021b). Organisms easily detected by photographic samples were identified to the lowest possible taxonomic level, while thenot easily recognizable ones were identified according to morphological groups (Gennaro *et al.*, 2020).

### Index calculation and data analysis

The Infralittoral ESCA index (I-ESCA) was calculated as the mean of the contribution of the three components (i.e.,  $\alpha$ -diversity,  $\beta$ -diversity, and Sensitivity Levels) using the formula  $I\text{-ESCA} = ((EQR_{\alpha} + EQR_{\beta} + EQR_{SL})/3)$ .  $\alpha$ ,  $\beta$  and SL individual EQRs were calculated as the ratio of the values of the three descriptors obtained under the investigated conditions and the values obtained for the same descriptors in the Reference Conditions (RC), identified in the Marine Protected Areas of the North Sardinia where there were no or very low human pressures and the infralittoral coralligenous outcrops were particularly developed (Pinna *et al.*, 2021). In fact, the reference values of Montecristo Island, normally used for ESCA (Piazzi *et al.*, 2021b), cannot be used for the infralittoral coralligenous assemblages as this habitat is not present at Montecristo Island. Thus, following the European directives approach, the new RCs were referred to the infralittoral coralligenous assemblages observed in undisturbed conditions (EC, 2000).

The  $\alpha$ -diversity was calculated as the mean number of taxa/ morphological groups per photographic sample. The  $\beta$ -diversity, considered as variability of species/

**Table 1.** Anthropization Index of the study sites.

Sites	Acronym	Depth	Urbaniz., urban waste	Ports	Tourism	Industr. activity	Sedim. load	Acquacult.	Agricul. waste	Fishing	Anchor.	Total
Tavolara	TAV	23	0	0	2	0	0	0	0	0	0	2
Santa Teresa	S.TER	19	0	0	2	0	0	0	0	0	0	2
Alghero	ALG	23	0	0	2	0	0	0	0	0	0	2
Asinara	ASI	23	0	0	2	0	0	0	0	0	0	2
Capo Carbonara	C.CAR	24	0	0	2	0	0	0	0	0	0	2
Capo Murro	C.MUR	24	0	0	2	0	1	0	1	0	0	4
Costa Paradiso	C.PAR	19	1	0	2	0	0	0	0	2	1	6
Meloria	MEL	18	1	1	1	0	1	0	1	1	1	6
Vada	VAD	25	0	0	1	1	1	0	1	1	1	6
Livorno	LIV	19	1	1	2	1	2	0	0	2	2	11
CivitavecchiaN	CIVn	23	2	1	1	1	2	0	2	2	1	12
CivitavecchiaS	CIVs	24	2	1	1	1	2	0	2	2	1	12
CivitavecchiaC	CIVc	22	2	2	1	2	2	0	1	2	1	13

abundance composition among sampling units (heterogeneity of assemblages), was measured in terms of multivariate dispersion calculated on the basis of distance from centroids (Anderson *et al.*, 2006) through permutational dispersion multivariate analysis (PERMDISP; Anderson, 2006).

A value of Sensitivity Level (SL, Piazzi *et al.*, 2021b) has been assigned to each taxon/morphological group, with SL values varying within a numerical scale, where low values correspond to the most tolerant organisms and high values to the most sensitive ones. The mean coverage of each taxon/ group was converted into one of the eight established abundance classes (1:  $0 < \% < 0.01$ ; 2:  $0.01 < \% < 0.1$ ; 3:  $0.1 < \% < 1$ ; 4:  $1 < \% < 5$ ; 5:  $5 < \% < 25$ ; 6:  $25 < \% < 50$ ; 7:  $50 < \% < 75$ ; 8:  $75 < \% < 100$ ) and these classes were then associated with the corresponding sensitivity level. The SL of each photographic sample was calculated as the sum of the values obtained by multiplying the sensitivity value of each taxon/group by its class of abundance. The SL of each study site was calculated as the mean of the SL values of all samples.

According to the recent revision on the STAR method, the encrusting calcified Rhodophyta were excluded from the calculation of alpha and beta diversity and sensitivity levels (Piazzi *et al.*, 2025).

Five ecological status classes were defined by dividing the entire range of ESCA values into five equal parts (Piazzi *et al.*, 2021b): Bad = [0-0.2); Poor = [0.2-0.4); Moderate = [0.4-0.6); Good = [0.6-0.8); High = [0.8-1.0].

A canonical principal co-ordinate analysis (CAP) was performed on the untransformed Bray-Curtis similarity matrix (Anderson & Willis, 2003) to discriminate the main categories (i.e., taxa/morphological groups) contributing to dissimilarities between sites.

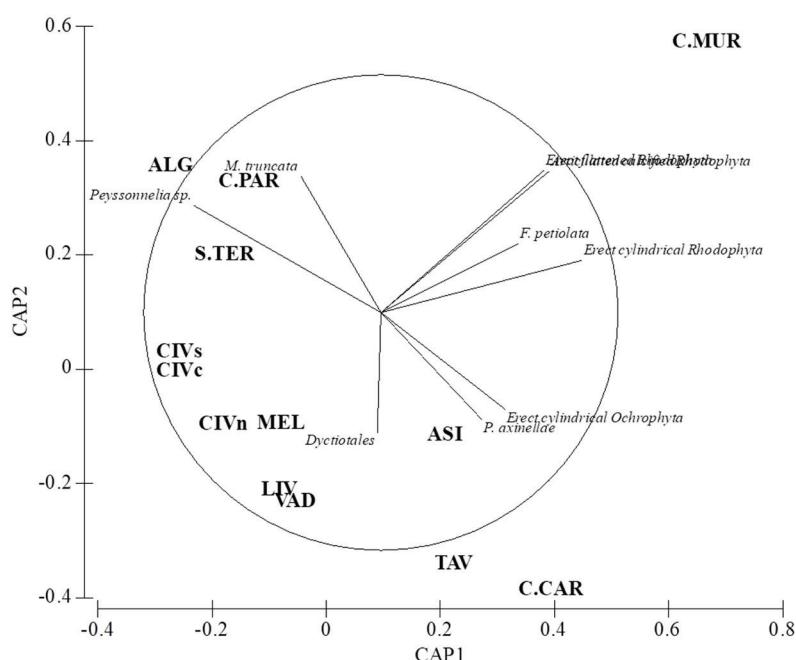
A linear regression was performed in order to test the relationships between I-ESCA and the Anthropization

Index (Piazzi *et al.*, 2021b). Values of the ESCA index calculated on circalittoral coralligenous reef in the same sites were also related to the Anthropization Index. The degree of correlation was calculated and reported as the value of the square correlation coefficient (determination coefficient,  $R^2$ ). Significance of regression was tested by means of the Fisher-Snedecor test performed by the Statistica 10 software. Before running the analysis, the main assumptions underlying the linear regression were verified using the appropriate tests (Pearson correlation, Shapiro-Wilk and Breusch-Pagan tests) and, when they were not met, more robust models were tested.

## Results

The results of the CAP analysis showed a highly significant model explaining 83.7% of the data variability, which is represented by 72.7% in the two-dimensional space of the CAP1 (40.1%) and CAP2 (32.6%) axes. The assemblages were dominated by macroalgae, with a spatial pattern characterized by four main groups apparently not related to geographical distance: among Sardinian sites, Alghero, Costa Paradiso and Santa Teresa segregated from Asinara, Tavolara and Capo Carbonara; continental sites were closed together and separated from Capo Murro (Fig. 2). The continental assemblages were mostly characterized by Dictyotales, Capo Murro by *Flabellia petiolata*, erect cylindrical, flattened and articulated calcified Rhodophyta; in Sardinian assemblages, erect cylindrical Ochrophyta and *Parazoanthus axinellae* were abundant (Fig. 2, Table 2). *Peyssonnelia* spp. and *Myriapora truncata* were common everywhere with variable cover; *Halimeda tuna*, although widespread, showed high abundance only in some Sardinian sites (Table 2).

The high values of the three descriptors detected in



**Fig. 2:** CAP analysis on Infralittoral coralligenous assemblages. The legend of acronyms is reported in Table 1. The overlapping taxa/groups are the erect flattened and articulated calcified Rhodophyta.

**Table 2.** Values of sensitivity levels (SL) and mean abundance of taxa/morphological groups in the study sites. The legend of acronyms is reported in Table 1.

SL	Taxa/morphological groups	C.CAR	TAV	S.TER	ASI	ALG	C.PAR	MEL	C.MUR	VAD	LIV	CIVn	CIVc	CIVs
<b>Macroalgae</b>														
1	Algal turf	11.52	14.64	1.82	15.66	0.02	5.00	30.32	21.02	29.45	22.84	37.50	45.90	36.50
0	Alien species	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.67	1.40
2	Siphonal Chlorophyta with separate filaments	0.00	0.17	0.00	0.01	0.00	0.07	0.00	0.32	0.19	0.89	0.70	0.17	0.00
4	Siphonal Chlorophyta with vesicle thallus	0.09	0.04	0.02	0.50	0.42	0.10	0.00	0.03	0.16	0.32	0.00	0.00	0.00
6	<i>Flabellia petiolata</i>	0.28	5.45	2.79	1.30	0.54	3.13	4.58	13.67	5.62	10.39	2.77	0.70	0.13
10	<i>Halimeda tuna</i>	20.01	19.74	6.35	5.03	4.01	12.79	0.67	0.08	0.47	0.20	0.00	0.00	0.00
7	<i>Palmophyllum crassum</i>	0.16	0.74	2.15	0.70	5.91	0.63	0.00	0.06	0.39	0.00	0.00	0.00	0.00
3	Dictyotales	0.21	3.52	0.00	1.28	0.02	0.00	4.07	0.00	3.59	19.08	0.00	0.00	0.37
6	Erect cylindrical Ochrophyta	9.22	8.07	0.07	12.31	0.19	3.68	0.00	0.61	0.00	1.36	0.00	0.00	0.00
6	Encrusting Ochrophyta	0.03	0.00	0.02	0.02	0.30	0.00	0.00	1.11	7.49	3.52	0.00	0.00	0.43
10	Fucales	0.00	0.00	0.00	1.73	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00
4	Encrusting calcified Rhodophyta	13.66	9.94	43.93	12.71	37.33	17.02	9.56	9.44	23.32	10.11	14.50	6.87	7.83
4	Articulated calcified Rhodophyta	0.00	0.04	0.08	0.96	0.03	0.33	5.37	14.00	2.20	7.78	0.00	0.00	0.00
4	<i>Peyssonnelia</i> spp.	11.53	18.67	28.26	22.75	37.25	34.41	30.63	17.29	21.57	6.22	28.17	36.77	39.33
9	Erect cylindrical Rhodophyta	6.91	2.54	0.20	6.59	0.27	2.54	7.48	13.46	2.36	11.02	1.17	1.65	0.47
10	Erect flattened Rhodophyta	0.00	0.00	0.00	0.00	0.05	0.00	0.00	23.61	0.00	4.70	0.10	0.03	0.60
<b>Invertebrates</b>														
3	Encrusting sponges	15.33	5.51	7.98	5.30	4.44	4.82	1.99	0.62	2.09	0.31	2.20	1.60	3.67
5	Prostrate/hemispherical sponges	0.01	0.03	1.51	0.00	0.23	0.04	0.11	0.28	0.12	0.10	1.50	0.57	0.47
8	Bushy sponges	0.03	0.14	0.07	1.65	0.44	0.09	0.00	0.00	0.00	0.00	0.37	0.25	0.13
2	Hydrozoans	0.29	0.10	0.12	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
5	<i>Parazoanthus axinellae</i>	5.35	3.69	1.08	1.32	5.28	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	Azooxantellate individual scleractinians	0.28	0.01	0.03	0.09	0.01	0.05	0.07	0.00	0.43	0.00	0.00	0.00	0.00
10	<i>Eunicella</i> spp.	0.23	1.45	0.35	0.38	0.01	0.02	0.00	0.00	0.00	1.06	2.40	0.23	0.00
5	Large serpulids	0.09	0.08	0.02	0.01	0.11	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00
3	Encrusting bryozoans	1.00	0.96	0.05	4.42	0.31	3.40	0.00	0.00	0.00	0.00	0.15	0.03	0.00
6	Ramified bryozoans	0.16	0.17	0.45	0.45	0.20	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	<i>Myriapora truncata</i>	0.00	0.07	0.76	0.26	2.43	1.90	0.00	0.22	0.54	0.10	0.18	0.03	0.00
9	<i>Pentapora fascialis</i>	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	<i>Adeonella calvetti</i> , <i>Smittina cervicornis</i>	0.03	0.09	0.66	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	Erect ascidians	0.57	1.25	0.17	0.07	0.04	0.01	0.00	0.00	0.00	0.00	0.03	0.17	

this study and used as reference values were 15 for the number of taxa/groups per sample, 40 for the mean distance from centroids and 450 for the sensitivity levels.

The Anthropization Index varied between 2 and 13 (Table 1). I-ESCA ranged between 0.47 and 0.97 (Fig. 3, Table 3). Most sites were in good ecological quality status, four Sardinian protected sites (Capo Carbonara, Tavolara, Santa Teresa and Alghero) showed high quality status and Central and South Civitavecchia sites were in moderate status (Fig. 3).

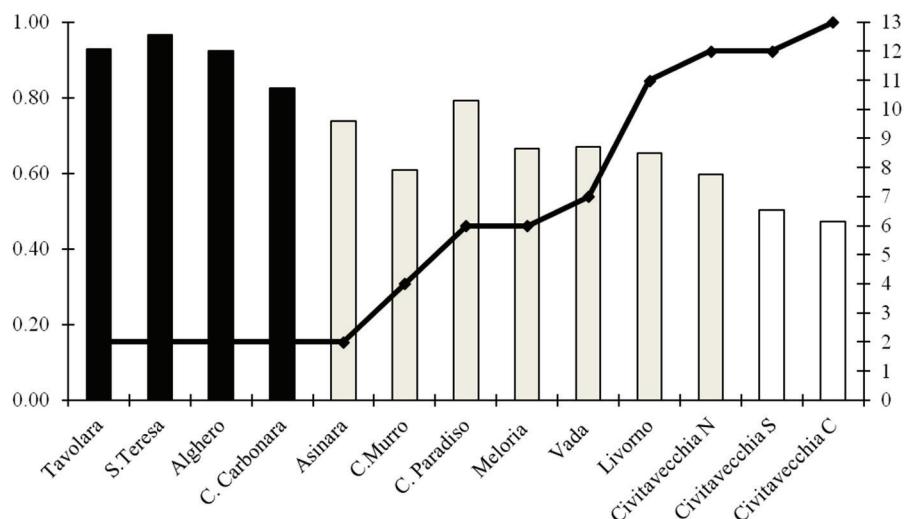
Testing the assumptions of the regression model confirmed a highly significant linear correlation between I-ESCA/ESCA and the Anthropization Index for both infralittoral and circalittoral relationships ( $p<0.0001$  and  $p<0.001$ , respectively), and a normal distribution of residuals was indicated by the Shapiro-Wilk test ( $p>0.05$ ). Homogeneity of variances was confirmed for the infralittoral relationship ( $p>0.05$ ), while the Breusch-Pagan test for the circalittoral regression ( $p<0.05$ ) highlighted a possible heteroskedasticity of the linear model, however, the

comparison with more robust models (Weighted Least Squares, WLS and Robust Linear Model, RLM) suggested that it did not affect the regression results. The determination coefficient of the relation between the anthropization index and I-ESCA was 0.7028, slightly lower than that obtained using ESCA (Fig. 4).

## Discussion

The assemblages which developed on infralittoral biogenic outcrops in the study area were characterized by high biodiversity and appeared well structured. The well-developed bioconstruction characterizing the studied infralittoral coralligenous reefs allows the development of high diversified assemblages; in fact, the encrusting calcified Rhodophyta increase the heterogeneity of the substratum enhancing the biodiversity, as already described for deeper assemblages (Cocito, 2024).

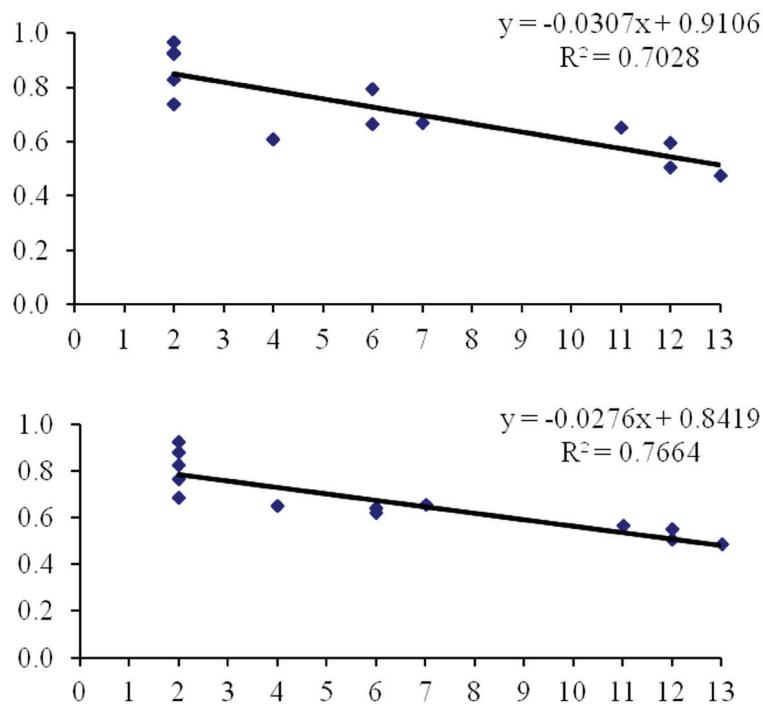
Macroalgae were dominant in the studied assem-



**Fig. 3:** I-ESCA values and anthropization index of the study sites. Black: high ecological quality, grey: good, white: moderate. The legend of acronyms is reported in Table 1.

**Table 3.** Values of I-ESCA metric and of the index for each studied site.

Sites	$\alpha$ -diversity	$\beta$ -diversity	Sensitivity Levels	I-ESCA
Tavolara	1.00	0.79	0.99	0.93
Santa Teresa	0.94	0.96	1.00	0.97
Alghero	0.74	0.60	0.88	0.74
Asinara	0.70	0.90	0.88	0.83
Capo Carbonara	0.96	0.79	1.00	0.92
Capo Murro	0.64	0.57	0.62	0.61
Costa Paradiso	0.80	0.71	0.86	0.79
Meloria	0.60	0.74	0.65	0.66
Vada	0.56	0.78	0.67	0.67
Livorno	0.71	0.67	0.58	0.65
Civitavecchia N	0.50	0.65	0.64	0.60
Civitavecchia S	0.39	0.55	0.57	0.50
Civitavecchia C	0.40	0.47	0.54	0.47



**Fig. 4:** Linear regressions between the anthropization index and ESCA and I-ESCA.

blages, confirming an already described pattern (Piazzi *et al.*, 2023a). The green alga *Halimeda tuna* is considered typical of this sublittoral zone (Ballesteros, 1991, 2006), while in the present study this species, although widespread, characterized only some Sardinian sites. The studied assemblages included both photophilous taxa such as Dictyotales, and sciaphilous ones, such as *Flabellia petiolata* and *Peyssonnelia* spp., confirming a pattern already described for the Tuscany coasts (Piazzi *et al.*, 2023a). Sessile invertebrates, although less abundant, were present everywhere, including iconic taxa such as gorgonians and bryozoans. *Myriapora truncata* was the most widespread bryozoan, as its broad ecological tolerance allows the species to be distributed over a wide bathymetrical range (Casoli *et al.*, 2020). *Eunicella singularis* and *E. cavolini* were also described as having shallower distribution limits than other gorgonians, due to their higher tolerance to a wide range of environmental conditions (Linares *et al.*, 2008; Sini *et al.*, 2019).

Patterns of spatial variability showed the occurrence of assemblages with different structure apparently not correlated with their relative distance. The study does not allow to attribute the observed pattern to geographical or ecological drivers, but the presence of different types of assemblages in the studied habitat should be considered. In fact, as for the circalittoral zone, the ecological quality is not related to a specific type of assemblage, since different taxa/morphological groups may have the same sensitivity level: therefore, high values of the index may be obtained with very different assemblages (Gennaro *et al.*, 2020).

The I-ESCA index allowed to highlight the sites' anthropization level similarly to ESCA. Thus, results of this study suggested the possibility of applying the index also to infralittoral habitat using an adequate calibration of the original method on the new spatial scale and reference conditions. The study also identified the new references

conditions for the geographic area considered in the oligotrophic sites of the Marine Protected Areas of the northern Sardinia characterized by well-developed infralittoral coralligenous outcrops (Pinna *et al.*, 2021). However, the dataset should be extended to other areas of the Mediterranean Sea in order to validate the proposed references condition values or possibly adjust them to the specific conditions of the selected areas.

The I-ESCA index tested in this study represents the first approach to the assessment of infralittoral coralligenous assemblages and provides a new tool suitable for monitoring this valuable habitat. This aspect is very important, since infralittoral coralligenous reefs consists everywhere of large biogenic structures and host a peculiar assemblages characterized by patterns of biodiversity comparable to those of circalittoral coralligenous reefs. Thus, they represent a key habitat in the coastal Mediterranean system and, at the same time, particularly sensitive to human-induced environmental alterations, such as sedimentation, pollution, mechanical destruction and invasion of alien species, due to the distribution usually close to the coasts (Piazzi *et al.*, 2023a). Moreover, the relatively shallow depth where the habitat develops, makes it more subject to climatic changes and extreme climatic events, such as storms, rainfalls and heatwaves (Teixidó *et al.*, 2013; Ceccherelli *et al.*, 2020; Piazzi *et al.*, 2021a). For these reasons, the assessment of the ecological status of infralittoral coralligenous reefs is very important and should be included in monitoring plans of coastal marine systems. Until now, the different characteristics of assemblages have prevented the use of the approaches adopted for the circalittoral coralligenous in shallower rocky bottoms. In this contest, the employing of I-ESCA opens new possibilities within monitoring network and impact evaluation studies where, coupled with the indices currently applied in the other type of habitats

(e.g., PREI for *Posidonia oceanica* meadows, Gobert *et al.*, 2009, M-AMBI for soft bottoms, Borja *et al.*, 2008), can allow a more complete evaluation of the environmental effects of human activities, better addressing the consequent management programs.

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