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Ecological status of coralligenous assemblages: Ten years of application of the ESCA index from local to wide scale validation

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Abstract :

This paper aims at collating and reviewing all data collected using the ESCA (Ecological Status of Coralligenous Assemblages) index from 2009 to 2018 during different local applications, in order to evaluate at large spatial scale its effectiveness and temporal variability. To this scope, the large-scale response of ESCA to anthropogenic disturbance was tested comparing ESCA values calculated at 42 sites of the Western Mediterranean Sea with the anthropization index. Moreover, the sensitivity of ESCA to punctual human disturbance and the robustness of the index across the natural space and time variability were evaluated. The large spatial scale study showed significant correlation between ESCA and the anthropization index, while very low correlation was detected when descriptors of ESCA (i.e., sensitivity levels, α-diversity, and β-diversity) were considered separately. The three impact evaluation studies highlighted significantly lower values of the ESCA index in disturbed conditions than in the control ones. The coastal monitoring study confirmed the robustness of the index which showed a high ecological quality of coralligenous reefs in reference conditions compared to more anthropized sites, and this pattern was maintained throughout the ten years study period. Application of the ESCA index to different situations tested positively its sensibility to different levels and type of human disturbance and its stability with respect to regional spatio-temporal variability. This confirm the reliability of the ESCA index already tested on the local and annual scale, thus broadening its range of application and validating it on a wider space–time scale.

Highlights

► Data of ESCA index from 2009 to 2018 were collated and reviewed. ► ESCA values of 42 sites were compared with an anthropization index. ► ESCA detected differences among sites subjected to different levels of pressure. ► ESCA discriminated disturbed and control sites in three impact evaluation studies. ► ESCA was stable respect to the regional spatio-temporal variability.

Keywords : Coralligenous reefs, Ecological index, Impact evaluation, Mediterranean Sea, Monitoring

1.Introduction

European legislation promotes the conservation of ecological systems and the development of management strategies for the sustainable use of natural resources. In particular, the Water Framework Directive (EC, 2000) and the Marine Strategy Framework Directive (EC, 2008) include among their goals the maintenance and improvement of the ecological status of marine coastal ecosystems to prevent environmental deterioration.

In this context, many biotic indices have been developed and used to assess the ecological status of the major marine coastal ecosystems (Birk et al., 2012). Coralligenous reefs are considered relevant habitats to be used as an indicator of biodiversity maintenance, and they are included among the "special habitat types" of the Marine Strategy Framework Directive. Thus, their ecological status should be assessed through appropriate monitoring plans (Bavestrello et al., 2016).

Coralligenous reefs are one of the most important coastal ecosystems of the Mediterranean Sea in terms of distribution, biodiversity, productivity, and their role in the CO_2 cycle (Martin et al., 2014; Casas-Güell et al., 2015). They are characterized by a basal layer mostly formed by calcareous red algae belonging to the Corallinales and Peyssonneliales. These bioconstructions develop over a large range of depths (Cánovas-Molina et al., 2016b; Ferrigno et al., 2017) and exhibit high structural and functional complexity (Ballesteros, 2006). The two most common geomorphotypes of coralligenous reefs are cliffs (i.e. vertical or near-vertical walls from a steep littoral rock face) and banks (isolated outcrops surrounded by sand or biodetritic sediments) (Ballesteros, 2006), which can develop at different depths depending on environmental conditions. However, cliffs usually characterize shallower coastal rocky bottoms, whereas banks mainly occur on deeper continental shelves (Ballesteros, 2006; Cánovas-Molina et al., 2016b).

Coralligenous reefs are particularly sensitive to human disturbance (Balata et al., 2005; Piazzi et al., 2011; Gatti et al., 2015b; Canessa et al., 2017). In particular, the shallower reefs are highly exposed to those human disturbances that have the most serious impact, which are concentrated along coastal areas (Aguado-Gimenez and Ruiz-Fernandez, 2012; Piazzi et al., 2012; Ferrigno et al., 2017, 2018).

Shallow reefs (within 50 m depth) can be effectively surveyed by SCUBA diving, thus obtaining direct information about descriptors that cannot be evaluated or measured through other instrumental methods, such as acoustic devices of remotely operated vehicles (Gatti et al., 2012, 2015a; Piazzi et al., 2018b). In contrast, unmanned vehicles are suitable for surveys of deep coralligenous reefs (Cánovas-Molina et al., 2016a; UNEP, 2017; Ferrigno et al., 2018; Enrichetti et al., 2019). Since biological monitoring techniques and ecological indices are generally habitat-specific, assessment of the ecological status of marine coastal environments should be carried out by applying multiple approaches depending on the habitat types occurring in the investigated area (Borja et al., 2010).

Recently, several methods have been proposed to evaluate the ecological quality of both shallow and deep coralligenous reefs (Kipson et al., 2011; Deter et al., 2012; Gatti et al., 2015a; Teixidó, et al., 2013; Zapata-Ramírez et al., 2013; Ferrigno et al., 2017; Montefalcone et al., 2017; Sartoretto et al., 2017; Enrichetti et al., 2019). The ESCA index (Ecological Status of Coralligenous Assemblages; Cecchi et al., 2014; Piazzi et al., 2017b) evaluates the ecological quality of shallow coralligenous reefs through a community approach; it was initially proposed for macroalgal assemblages (Cecchi et al., 2014) and later implemented with the inclusion of sessile invertebrates (Piazzi et al., 2017b). The ESCA index was tested on gradients of anthropogenic pressure (Piazzi et al., 2015, 2017a, 2017b) and applied at local scale in many different conditions and sites during monitoring surveys and impact evaluation studies (Penna et al., 2017; Piazzi et al., 2018a, 2019). In the ten years since its first adoption in the field the data collected cover a wide geographical area of the North-Western Mediterranean Sea.

This paper aims to collate and review all the data collected using the ESCA index for different local applications from 2009 to 2018, in order to evaluate at large spatial scale its effectiveness and temporal variability. For this purpose, three important aspects of the index were tested: i) the large-scale response of ESCA to anthropogenic disturbance; ii) the sensitivity of ESCA to specific human disturbances; and iii) the robustness of the index across natural space and temporal variability.

2.Material and methods

2.1.Sampling methods

In each study site, three areas of 4m², 10 meters apart, were randomly selected on vertical rocky bottoms, at about 35 m depth. In each area, 10 photographic samples of 0.2 m^2 were collected. Photographic samples were analysed using ImageJ software (Cecchi et al., 2014) to evaluate the percentage cover of the main taxa or morphological groups (Bianchi et al., 2004; Parravicini et al., 2009; Balata et al., 2011). Organisms easily detected by photographic samples were identified to the lowest possible taxonomic level, while those not easily recognizable were identified according to morphological groups (Piazzi et al., 2015, 2017b). Three assemblage descriptors were used according to Cecchi et al. (2014): i) "sensitivity level" (SL), based on the cover of different sensitive taxa; ii) diversity of assemblages expressed as "α-diversity"; and iii) heterogeneity of assemblages, expressed as "β-diversity". To calculate the SL of study sites, each taxon was associated with a sensitivity value (from 1 to 10, with minimum values corresponding to the most tolerant organisms and maximum values to the most sensitive ones, Table S1) and with one of eight classes of abundance (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: $75 < \% < 100$) according to Piazzi et al. (2017b). The SL of each photographic sample was calculated as the sum of the values obtained by multiplying the sensitivity value of each taxon/group values by its class of abundance. The SL of each study site was calculated as the sum of the SL values of all samples. α-diversity was defined as the mean number of taxa/groups calculated for the 30 photographic samples. β-diversity was evaluated as the mean distance of all photographic samples from centroids calculated in the PERMDISP analysis (Primer 6 + PERMANOVA; Anderson et al., 2006). ESCA was expressed as Ecological Quality Ratio (EQR), calculated as the mean of the three EQR_S obtained for the assemblage descriptors: $EQR = ((EQR_{SL} + EQR_{\alpha} + EQR_{\beta}) \times 3^{-1})$. Individual EQRs were calculated as the ratios between the values of SL, α-diversity and β-diversity, respectively, and the values obtained for the same descriptor in the Reference Conditions (RC) (i.e. 550, 15 and 20 respectively calculated for Montecristo Island, Tuscan Archipelago National Park). Montecristo Island was chosen as RC for the considered geographic region because it is a protected nature reserve subjected to very stringent protection restrictions which reduce the human impact almost to zero. For this reason, the ecological status of Montecristo coralligenous assemblages is suitable to represent "true" RC, in accordance with the requirements of European Directives (Cecchi et al., 2014). The reference values of the three index metrics were the highest values obtained during four years monitoring studies carried out in the natural reserve. The ecological quality of coralligenous reefs was then classified according to the following five classes (Piazzi et al., 2015): i) high ($EQR \geq 0.8$); ii) good ($0.6 \leq EQR < 0.8$); iii) moderate ($0.4 \leq EQR < 0.6$); iv) poor ($0.2 \leq EQR < 0.4$); and v) bad ($EQR < 0.2$).

2.2.Large-scale response of ESCA to anthropogenic disturbance

Coralligenous reefs were sampled at 42 sites of the NW Mediterranean Sea (Figure 1). In order to measure the degree of human disturbances affecting each study site, the anthropization index was computed following an approach similar to that used by Gobert et al. (2009). The index was defined considering the main anthropogenic activities that may affect negatively coralligenous reefs in the investigated sites (disturbance factors or drivers), urbanization, ports, industry, aquaculture, agriculture, sources of terrigenous sediment, diving activities, artisanal fishing and anchoring (Table 1), and the resulting pressures (polluting discharges, biological invasions, hypersedimentation and mechanical aggression) (EC, 2003). Each disturbance factor was classified from 0 (no or very low pressure) to 2 (high pressure), according to its presence and entity and distance of the investigated sites from the source of disturbance (Piazzi et al., 2015, 2017b). For each site, the anthropization index, ranging from 0 to 16, is the sum of the values of each single disturbance factor.

A linear regression was performed in order to test the relationships between ESCA and its three descriptors (i.e., SL, α-diversity, and β-diversity) with the anthropization index. The degree of correlation was calculated and reported as the value of 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.

2.3.The sensitivity of ESCA to punctual human disturbance

The ESCA index was employed in three independent impact evaluation studies to assess the effects of three different environmental conditions directly or indirectly related to punctual human disturbance. In each study, the disturbed site was compared with two reference sites with similar morphological characteristics and exposition, in agreement with an ACI design (After-Control-Impact; Underwood, 1993, Chapman et al., 1995). The disturbance factors subjected to the impact assessment were: 1) the mucilage occurred in the Marine Protected Area of Capo Carbonara (SE Sardinia, Italy) in 2017 (Piazzi et al., 2018a); 2) the underwater works to remove a shipwreck at Giglio Island (Tuscan Archipelago, Italy) (Penna et al., 2017), and 3) the fish-farmig activities at Gorgona Island (Tuscan Archipelago, Italy) (De Biasi et al., 2016; Piazzi et al., 2019). ESCA values obtained in the disturbed and the control sites in the three impact studies were analysed together through an asymmetrical PERMANOVA: a 2-way model was used with the factor Condition (disturbed *vs* control) as fixed and the factor Site (3 levels) as random nested in Condition, with three replicated areas in each site. The mean square of the factor Condition was partitioned into disturbed *vs* control and among control sites (Terlizzi et al., 2005).

2.4.The robustness of the index across the natural space and time variability

The ESCA index was applied in coastal monitoring studies, from 2009 to 2018 in 3 sites (Montecristo, Livorno, and Argentario) of Tuscany (Italy, NW Mediterranean Sea). Each site was sampled every three years, for a total of four monitoring times (T1-T4) within a ten-years period.

The ecological quality of coralligenous reefs in each site was compared through a 2-way PERMANOVA analysis, with the factors Year (4 levels, fixed) and Site (3 levels, random and orthogonal to Year). The Euclidean distance was calculated before analysis and PERMDISP analysis was used to check for homogeneity of variance (Anderson, 2001).

3.Results

3.1.Large-scale response of ESCA to anthropogenic disturbance

Values of ESCA varied from 0.46 at S. Agostino to 0.98 at the reference site of Montecristo (Fig. 2), while values of the anthropization index ranged from 0 (at Montecristo) to 13 (at S. Agostino and Civitavecchia) (Table 1 and Fig. 2). The high quality sites were characterized by different assemblages, as some of them were dominated by invertebrates, mostly erect anthozoans and bryozoans (French sites, Giannutri, Giglio, Tavolara), while other sites were dominated by macroalgae, such as Fucales, Udoteaceae or erect Rhodophyta (Asinara, Catalano, Capraia and Pianosa). The linear regression showed a linear association and a significant negative correlation ($F < 0.001$) between ESCA and the anthropization index (Fig. 3). The linear regressions between the anthropization index and the three descriptors of ESCA (i.e., SL, α-diversity, and β-diversity) showed lower values of the squared correlation coefficient compared to that of ESCA (Fig. 3).

3.2.The sensitivity of ESCA to punctual human disturbance

The three impact evaluation studies showed a moderate ecological quality of coralligenous reefs in all the disturbed sites and high or good qualities of coralligenous reefs in the control sites (Fig. 4). The number of taxa/groups per sample was 7.7±1.5 (mean±SE, n=3) in the disturbed sites and 10.4±0.3 (mean±SE, n=6) in the control sites. The main differences between conditions were mostly related to a higher abundance of erect bryozoans, *Halimeda tuna*, and erect Rhodophyta at the control sites and a higher abundance of algal turf at the disturbed sites. ESCA significantly differed between disturbed and control condition, while differences among controls were not significant (Table 2). Variability at the site level was also significant.

3.3.The robustness of the index across the natural space and time variability

ESCA indicated a high ecological quality of the coralligenous reefs at Montecristo, a good quality at Argentario and a moderate quality at Livorno. This pattern was maintained throughout the ten years study period (Fig. 5). PERMANOVA showed a significant difference among sites but not among the sampling times; the interaction Site x Year was not significant either (Table 3).

4.Discussion

The results of the large spatial scale study showed the effectiveness of the ESCA index to detect differences among sites subjected to different levels of human disturbance. ESCA was also able to discriminate between disturbed and control sites in all the three impact evaluation studies, regardless of the kind of disturbance. Finally, the results of the coastal monitoring studies confirm the stability of the index with respect to regional spatio-temporal variability.

The pressures response test carried out on the 42 sites demonstrated the higher consistency between ESCA and the anthropization index when compared to the use of single descriptors of the index individually (i.e., SL, α-diversity, and β-diversity), confirming the effectiveness of synthetic tools compared to single descriptors (Simboura et al., 2005; Borja and Dauer, 2008; Piazzi et al., 2017a). The integrated use of multiple bioindicators with complementary strengths is recommended in order to minimize the variability of ecosystems' response to different stressors (Martinez-Crego et al., 2010; Borja et al., 2009a, 2009b). At the same time, the test also highlighted the effectiveness of the index in detecting different levels of anthropogenic disturbance at large scale as well as at smaller scale, since the sites investigated covered a wide area of the western Mediterranean.

The ability of ESCA to determine the ecological quality of coralligenous reefs under different environmental conditions (including reference sites) confirms the value of the index in impact evaluation studies (Penna et al., 2017; Piazzi et al., 2018a, 2019) and its sensitivity to human disturbance of different origins. This aspect is particularly important as ecological indices supporting management actions should be able to distinguish among different forms of human impact in order to appropriately address corrective or restoration actions. Moreover, the application of the index throughout a ten-year period in Tuscany showed similar values among the sampling periods, thus suggesting the index is stable across time in the absence of regular disturbances. The robustness of an index is based on the assertion that it must not be influenced by patterns of natural spatial and temporal variability of assemblages but only by changes related to alteration in the ecological quality of the environment (Borja and Dauer, 2008; Martinez-Crego et al., 2010). Coralligenous assemblages may vary at different spatial scales (Casas-Güell et al., 2015, 2016; Doxa et al., 2016; Piazzi et al., 2016) and different assemblages may characterize sites with similar ecological quality (Casas-Güell et al., 2015, 2016). At the same depth, structural changes may be related to natural environmental drivers, such as hydrodynamic conditions, substrate mineralogy and morphology, biogeographical gradients (Virgilio et al., 2006; Falace et al., 2015; Fava et al., 2016; Ferrigno et al., 2017; Canessa et al., 2020), and biotic interaction among sessile organisms (Ponti et al., 2014, 2018). The ESCA index is independent of the species composition of assemblages and considers that different organisms may have similar sensitivity to disturbance; high ecological quality, for example, may be indifferently obtained by assemblages dominated by erect anthozoans, bryozoans, Fucales, Udoteaceae, or erect Rhodophyta (Piazzi et al., 2017b). ESCA can thus be employed beyond biogeographical or local patterns, at least in the Western Mediterranean Sea.

ESCA should be tested in other Mediterranean areas, such as the Adriatic Sea or the southern and eastern Mediterranean basins, where characteristics of coralligenous assemblages might be different from those used to develop the index (Çinar et al., 2014; Falace et al., 2015; Sini et al., 2019). Most of the organisms found in the high quality coralligenous assemblages in the western Mediterranean Sea (Linares et al., 2008; Casoli et al., 2017; Piazzi et al., 2018a) are absent from other areas, such as the northern Adriatic Sea, where coralligenous assemblages are dominated by ascidians or sponges, although also in high quality conditions (Ponti et al., 2011). The definition of different reference values, as well as site-specific scales of sensitivity level, is likely to be necessary to apply the index in other Mediterranean areas.

5.Conclusions

The objective of management is to preserve sensitive coastal habitats in good environmental and ecological status or return them to this status (Borja, 2005). Indices are a scientific response that helps to satisfy management needs for accurate and reliable information about the condition of biological ecosystem elements (Borja et al., 2009b) and their validation in space and time represents a key step in their development (Borja and Dauer, 2008; Borja et al., 2009b). The results of this study confirm the reliability of the ESCA index, which had already been tested on a local and annual scale, thus broadening its range of application and validating it on a wider space-time scale. Ten years of studies allowed a validation dataset to be built that covers a wide spatial scale, from southern France to central Italy (Piazzi et al., 2017a), and the entire range of conditions (from highly degraded to non-degraded or pristine situations), and environmental alterations of different origins were also considered. The dataset also includes data from ten years of monitoring carried out at a regional scale, which demonstrated the stability of the index across time. ESCA has responded positively to the validation tests, proving to be an effective and sensitive tool that is also robust in space and time, thus facilitating communication with environmental managers.

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Figure legends

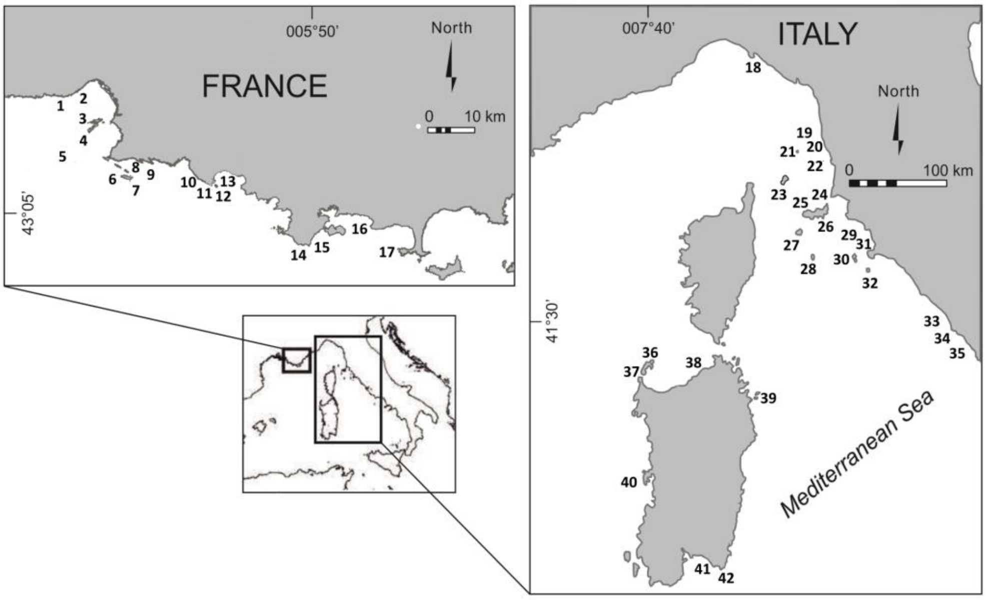
Figure 1. Map of the 42 sites in the NW Mediterranean where the ESCA index was applied. Numbers refer to the sites listed in Table 1.

Figure 2. Values of the anthropization index (black line) and ESCA (bars) in each of the 42 sites. White bars correspond to a moderate ecological quality, grey bars to a good ecological quality, and back bars to a high ecological quality.

Figure 3. Linear regressions between the anthropization index and the ESCA index, the α-diversity, the β-diversity, and the sensitivity level.

Figure 4. Mean values (+ S.E.) of ESCA detected in three different impact evaluation studies. D = disturbed sites, C = control sites; me = mucilage event, uw = underwater works to remove a shipwreck, ap = aquaculture plant. White bars correspond to moderate ecological quality, grey bars to good ecological quality, and back bars to high ecological quality.

Figure 5. Values of ESCA in the three sites of Tuscany during the four monitoring times (T1-T4) between 2009 and 2018. Colors of the bars corresponds to sites investigated (as reported in the figure label), as well as to the coralligenous ecological quality: white correspond to moderate ecological quality, grey to good ecological quality, and back to high ecological quality.


FRANCE
005°50'
North
0
10 km
43°05'
1
2
3
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12
13
14
15
16
17
ITALY
007°40'
North
0
100 km
41°30'
18
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41
42
Mediterranean Sea

Figure 1.

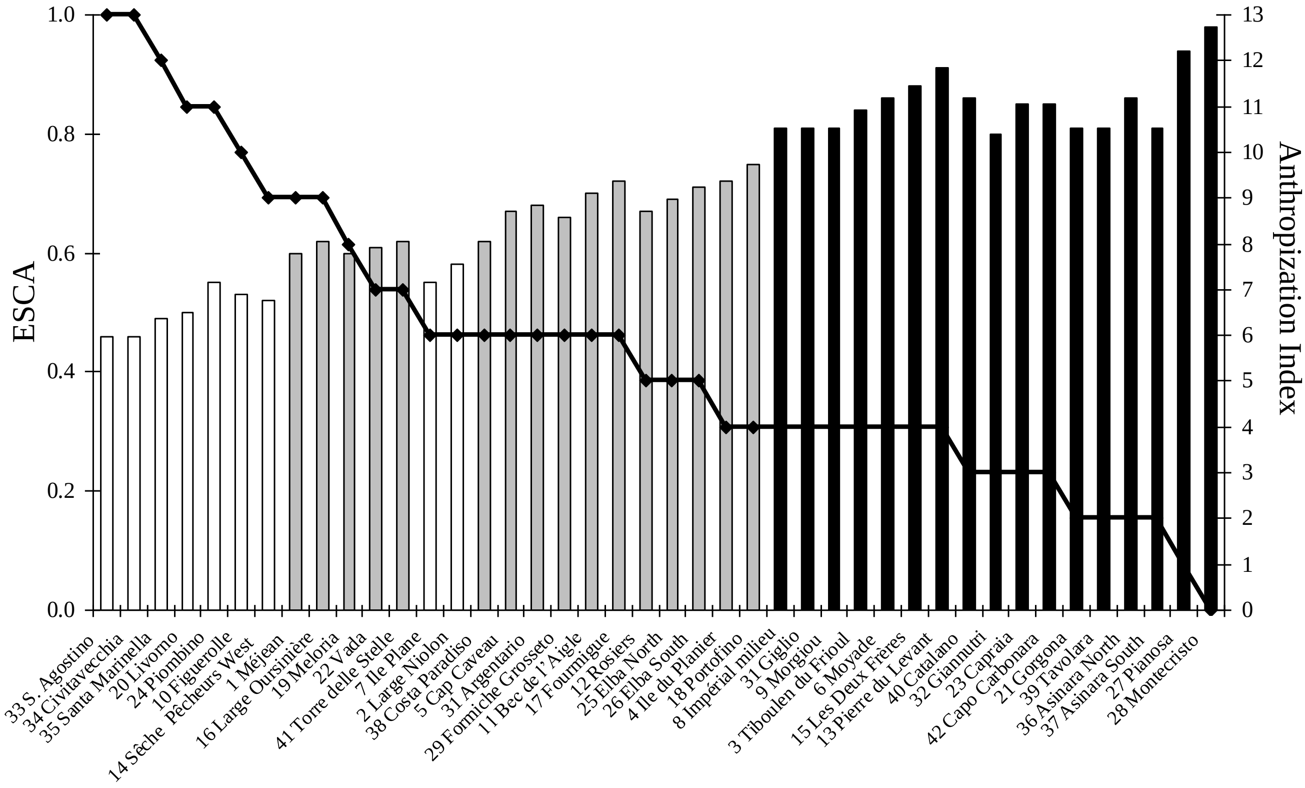

ESCA
Anthropization Index
1.0
0.8
0.6
0.4
0.2
0.0
13
12
11
10
9
8
7
6
5
4
3
2
1
0
33 S. Agostino
34 Civitavecchia
35 Santa Marinella
20 Livorno
24 Piombino
10 Figuerolle
14 Sêche Pêcheurs West
1 Méjean
16 Large Oursinière
19 Meloria
22 Vada
41 Torre delle Stelle
7 Ile Plane
2 Large Niolon
38 Costa Paradiso
5 Cap Caveau
31 Argentario
29 Formiche Grosseto
11 Bec de l'Aigle
17 Fourmigue
12 Rosiers
25 Elba North
26 Elba South
4 Ile du Planier
18 Portofino
8 Impérial milieu
31 Giglio
9 Morgiou
3 Tiboulen du Frioul
6 Moyade
15 Les Deux Frères
13 Pierre du Levant
40 Catalano
32 Giannutri
23 Capraia
42 Capo Carbonara
21 Gorgona
39 Tavolara
36 Asinara North
37 Asinara South
27 Pianosa
28 Montecristo

Figure 2

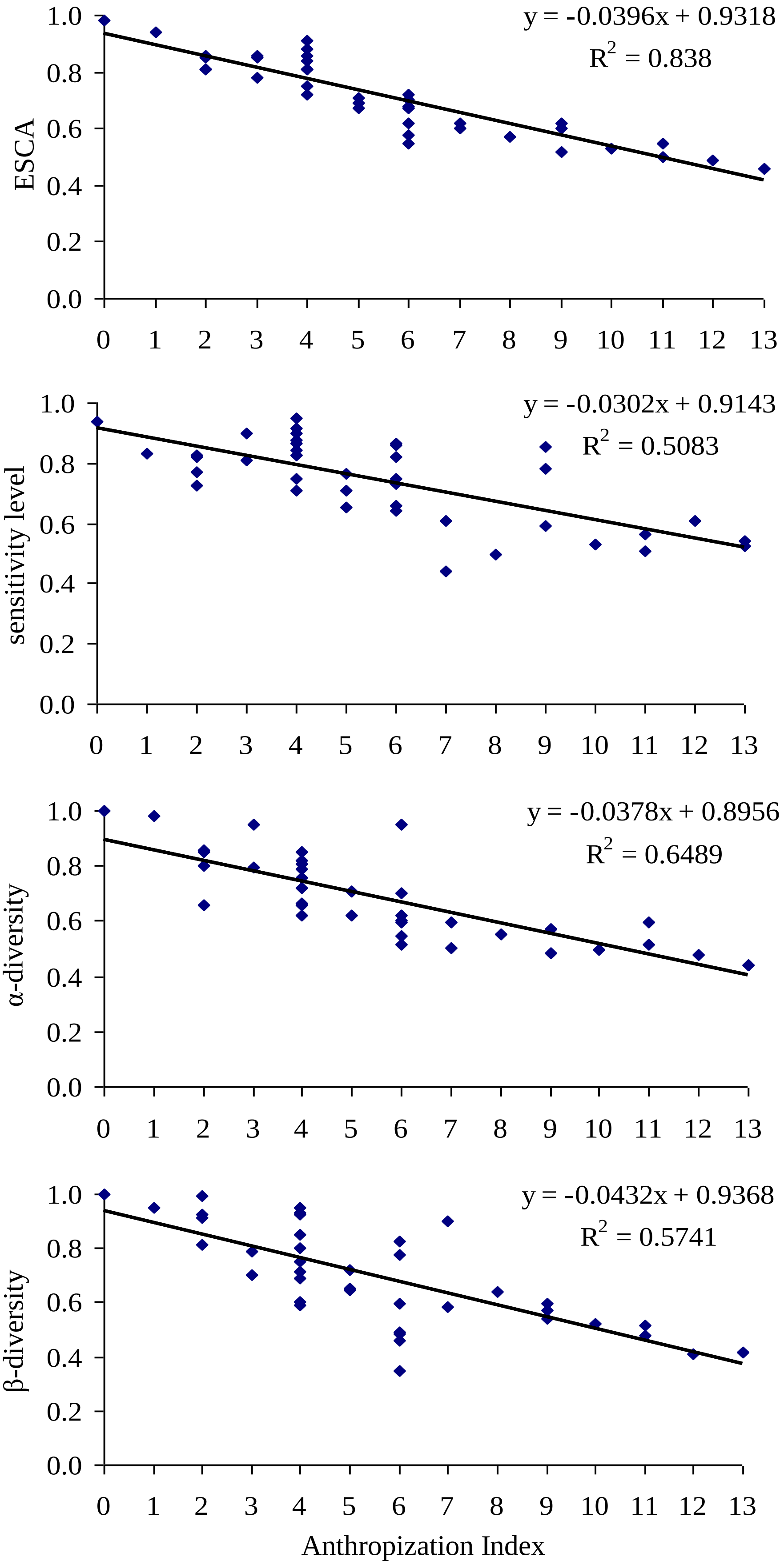

y = -0.0396x + 0.9318
R2 = 0.838
ESCA
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
y = -0.0302x + 0.9143
R2 = 0.5083
sensitivity level
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
y = -0.0378x + 0.8956
R2 = 0.6489
α-diversity
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
y = -0.0432x + 0.9368
R2 = 0.5741
β-diversity
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Anthropization Index

Figure 3.

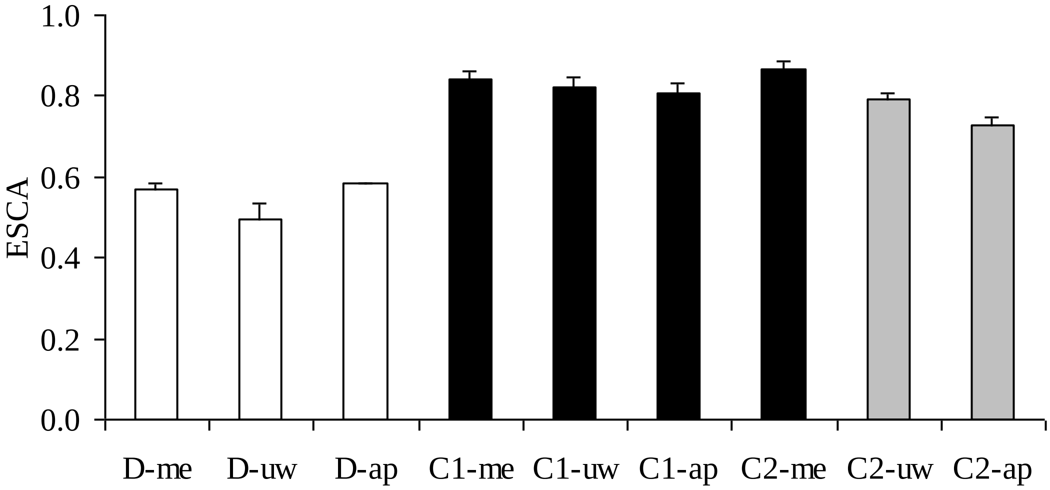
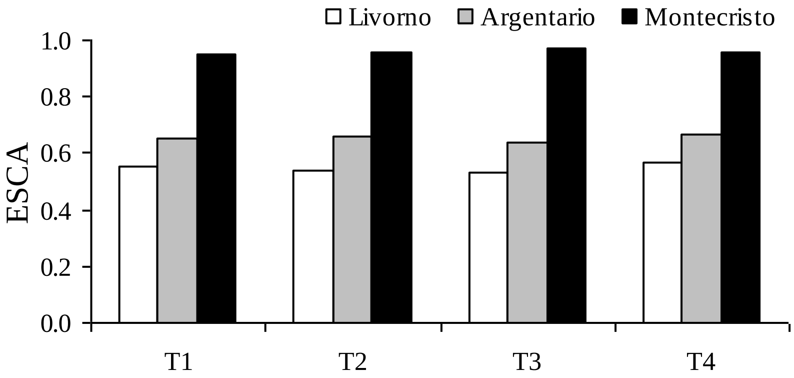


Figure 4.



Livorno
Argentario
Montecristo
ESCA
1.0
0.8
0.6
0.4
0.2
0.0
T1
T2
T3
T4

Figure 5.

Table 1. Anthropization index in each of the 42 study sites. The numbers beside each site refer to their geographical location as showed in figure 1.

code	**protection**	**Sites**	**Urbanization**	**Ports**	**Industry**	**Acquaculture**	**Agriculture**	**Sources of terrigenous sediments**	**Diving activities**	**Artisanal fishing**	**Anchoring**	**Anthropization index**
28	yes	Montecristo	0	0	0	0	0	0	0	0	0	0
27	yes	Pianosa	0	0	0	0	0	0	1	0	0	1
21	yes	Gorgona	0	0	0	1	0	0	0	1	0	2
39	yes	Tavolara	0	0	0	0	0	0	2	0	0	2
36	yes	Asinara North	0	0	0	0	0	0	1	1	0	2
37	yes	Asinara South	0	0	0	0	0	0	1	1	0	2
42	yes	Capo Carbonara	0	0	0	0	0	0	2	1	0	3
23	yes	Capraia	0	0	0	0	0	0	2	0	1	3
32	yes	Giannutri	0	0	0	0	0	0	2	0	1	3
40	yes	Catalano	0	0	0	0	0	0	0	2	1	3
31	no	Giglio	0	0	0	0	0	0	2	1	1	4
18	yes	Portofino	0	0	0	0	0	2	2	0	0	4
4	no	Ile du Planier	0	0	0	0	0	0	2	0	2	4
6	no	Moyade	0	0	0	0	0	0	2	0	2	4
8	no	Impérial milieu	0	0	0	0	0	0	2	0	2	4
9	no	Morgiou	0	0	0	0	0	1	2	1	0	4
13	no	Pierre du Levant	0	0	0	0	0	0	1	2	1	4
3	no	Tiboulen du Frioul	0	0	0	0	0	1	1	1	1	4
15	no	Les Deux Frères	0	0	0	0	0	1	1	1	1	4
25	no	Elba-North	0	0	0	0	1	0	2	2	0	5
26	no	Elba-South	0	0	0	0	1	0	2	2	0	5
12	no	Les Rosiers	0	0	0	0	0	0	2	2	1	5
11	no	Bec de l’Aigle	0	1	0	0	0	1	1	2	1	6
2	no	Large Niolon	0	0	0	0	0	2	1	2	1	6
5	no	Cap Caveau	0	0	0	0	0	1	2	1	2	6
7	no	Ile Plane	2	0	0	0	0	2	1	0	1	6
38	no	Costa Paradiso	1	0	0	0	0	0	1	2	2	6
31	no	Argentario	0	0	0	0	1	2	1	1	1	6
17	no	Formigue	0	0	0	0	0	1	1	2	2	6
41	no	Torre delle Stelle	1	0	0	0	0	0	2	2	2	7
22	no	Vada	0	0	1	0	1	1	1	2	1	7
29	no	Formiche	0	0	0	0	1	2	2	1	1	7
19	no	Meloria	1	1	0	0	1	1	1	2	1	8
16	no	Large Oursinière	2	1	0	0	0	2	1	2	1	9
1	no	Méjean	0	1	0	0	0	2	2	2	2	9
14	no	Sêche des Pêcheurs West	2	0	0	0	0	2	2	2	1	9
10	no	Figuerolle	2	0	0	0	0	2	2	2	2	10
20	no	Livorno	1	1	1	0	0	2	2	2	2	11
24	no	Piombino	1	2	1	1	1	1	1	2	1	11
35	no	Santa Marinella	2	1	1	0	1	2	1	2	2	12
33	no	S. Agostino	1	1	2	1	1	2	1	2	2	13
34	no	Civitavecchia	2	2	2	0	0	2	1	2	2	13

Table 2. PERMANOVA analysis on ESCA in the three different impact evaluation studies. Significant values are in bold.

Source	**df**	**MS**	**Pseudo-F**	**P(perm)**
Condition = C	2	0.205	27.65	**0.031**
impact *vs* controls	1	0.408	88.31	**0.002**
among controls	1	0.003	0.41	0.611
Site (C)	6	0.007	7.29	**0.002**
impact *vs* controls	3	0.004	46.21	**0.001**
among controls	4	0.007	8.05	**0.005**
Residual	18	0.001		
impact *vs* controls	6	0.001		
among controls	12	0.001		

Table 3. PERMANOVA analysis on ESCA in the three sites of Tuscany monitored between 2009 and 2018. Significant values are in bold.

Source	df	MS	Pseudo-F	P(perm)
Site = S	2	0.5494	1373.7	**0.001**
Year = Y	3	0.0006	1.7	0.184
S×Y	6	0.0004	1.1	0.385
Residual	24	0.0004		