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Improvement of the Esca index for the evaluation of ecological quality of *coralligenous habitats* under the European framework directives

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Abstract

The ESCA (Ecological Status of Coralligenous Assemblages) index was recently developed to evaluate the ecological quality of coralligenous habitats. The study aims to improve the first index proposal through testing responses to different sources of anthropogenic pressures and optimizing the sampling effort. ESCA was calculated for 14 sites and tested against a gradient of human pressures. Moreover, the main scales of spatial variability of assemblages were evaluated and values of index obtained with different sampling designs were compared. Results showed that study sites resulted in high, good or moderate ecological status, according to an increasing gradient of anthropization level. Values of ESCA index obtained with different methods have been compared, and photographic methods provide EQR values lower than destructive methods. Spatial variability of assemblages was higher at large and small scales than at intermediate ones. Two locations for each study site and 20 replicated samples for each location may be consider the best sampling combination providing reliable values of ESCA index for the evaluation of ecological quality of coralligenous assemblages.

Keywords: Coralligenous habitat, Mediterranean Sea, biotic index, macroalgae, ecological quality, human pressures.

Introduction

The Water Framework Directive (WFD, EC, 2000) and the Marine Strategy Framework Directive (MSFD, EC, 2008) aim at maintaining and improving the ecological status of marine coastal waters and they consider the assessment of ecological status of coastal areas a primary step to plan management strategies suitable to prevent further environmental deterioration. In this context, the development of biotic indices to assess the ecological quality of marine coastal ecosystems is considered a fundamental tool for the implementation of European legislation (Borja *et al.,* 2010; Martinez-Crego *et al.,* 2010). Over the last years, many indices have been used to assess the ecological status of different coastal marine ecosystems (Birk *et al.,* 2012), but only a few of them have focused on the coralligenous habitat (Deter *et al.,* 2012; Gatti *et al.,* 2012; Cecchi *et al.,* 2014). Coralligenous habitats develop in the Mediterranean Sea on deep subtidal rocky bottoms, where it represents one of the most important habitat for extension, biodiversity and role in CO_2 dynamics (Laborel, 1961, 1987; Ballesteros, 2006; UNEP, 2007). It consists primarily of calcareous structures edified by Rhodophyta belonging to Corallinales and Peyssonneliales and secondarily by several sessile animals, mostly the Cnidaria, Polychaeta and Bryozoa (Ballesteros, 2006). The ecological importance of coralligenous habitat and its scientific and biodiversity interest are recognized by community legislation (Habitats Directive 92/43/CEE) and international conventions (Barcelona 1995); thus it can be considered one of the most relevant "special habitat types" that should be assessed under the Marine Strategy Framework Directive (EC, 2008) through accurate monitoring plans.

At the current state of knowledge, the Coralligenous Assemblage Index (CAI) (Deter *et al.,* 2012), the COralligenous Assessment by Reef Scape Estimate (COARSE) (Gatti *et al.,* 2015) and the Ecological Status of Coralligenous Assemblages (ESCA) (Cecchi *et al.,* 2014) are the only indices developed for coralligenous habitat. The ESCA index was recently proposed to evaluate the ecological quality of coralligenous habitats through the structural and functional analysis of the macroalgal assemblages (Cecchi *et al.,* 2014). Development of the ESCA index began with impact evaluation studies performed through destructive methods, which were used to select and test suitable descriptors for macroalgal coralligenous assemblages. Then, the photographic method for ESCA was developed by adjusting the metrics obtained from destructive methods to the visual census approach

(Cecchi *et al.,* 2014). The index was validated on an independent dataset and tested on a gradient of anthropogenic stressors (Cecchi *et al.,* 2014) at a regional scale in the Mediterranean Sea (the Tuscany study area). Among points to be addressed, a particular relevance should be focused on the optimization of sampling designs and the definition of more accurate boundaries among ecological classes. According to the Water Framework Directive, five ecological classes were defined for the ESCA index on the basis of the following ranges of Ecological Quality Ratio (EQR') values: 1.0-0.81 High; 0.80-0.66 Good; 0.65-0.51 Moderate; 0.50-0.36 Poor; 0.35-0 Bad (Cecchi *et al.,* 2014). Nevertheless, the definition of the ecological classes, especially related to the intermediate classes, is often problematic (Orfanidis *et al.,* 2001; Mangialajo *et al.,* 2007) and a large set of data is needed to properly fix the boundary classes (Borja & Dauer, 2008). Moreover, the optimization of sampling effort is considered a fundamental goal in order to obtain a cost-effective monitoring method (Panayotidis *et al.,* 2004).

The study aims to improve the ESCA index through testing responses to different sources of anthropogenic pressures and optimizing sampling effort. Moreover, better definitions of the ecological classes have been pursued. To achieve these objectives, the index was calculated at 14 sites and tested against a gradient of pressures. Moreover, the main scales of spatial variability of assemblages were evaluated and values of index obtained with different sampling designs were compared.

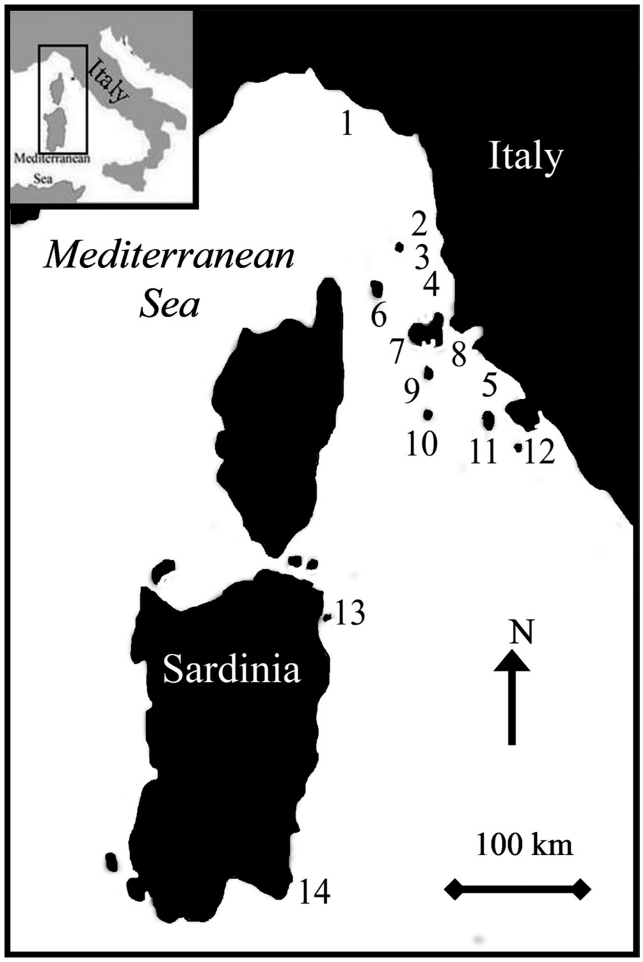

Fig. 1: Location of the study sites. 1: Portofino, 2: Meloria, 3: Livorno, 4: Vada, 5: Formiche, 6: Capraia, 7: Elba, 8; Piombino, 9: Pianosa, 10: Montecristo, 11: Giglio, 12: Argentario, 13: Tavolara, 14: Capo Carbonara.

Material and Methods

Coralligenous assemblages were sampled in 14 sites at the north-western Mediterranean Sea, seven islands and seven sites along the continental coasts (Fig. 1). To evaluate the human pressures affecting each study site, an indicator of the anthropogenic stress level was elaborated following an approach similar to that used by Gobert *et al.* (2009). The anthropization level was defined as the sum of eight impact factors affecting coralligenous assemblages: urbanization and urban waste, ports, tourism, industrial activities, sediment load, aquaculture, agricultural waste and fishing. Each impact factor was classified from 0 (no impact) to 3 (strong impact), according to presence and entity of human pressure, and distance of the sites from the source of impact. The final value of the anthropization level ranged from 0 to 17 (Table 1). In order to compare the structure of assemblages associated with areas with different ecological values, two different classes have been recognized based on values referred to protected areas: the low pressure class with values < 5 and the high pressure one with values > 5. At each site, two locations 100s of meters apart were randomly selected and in each location 30 photographic samples of 1875 cm^2 were collected on vertical rocky substrates between 30 and 35 m deep. Samples were analysed by Image J software (Cecchi *et al.,* 2014) and the percentage cover of the main macroalgal taxa and morphological groups (Balata *et al.,* 2011) were evaluated. According to the ESCA index methodology (Cecchi *et al.,* 2014), three assemblages descriptors were used: the sensitivity level (SL), the α diversity and the β diversity. The SL of each study site was calculated by adding all the values of SL obtained for each of the taxa/groups observed in each photographic sample α -diversity was defined as the mean number of the main taxa/groups obtained in each photographic sample; β -diversity was evaluated as the mean distance of the photographic samples from centroids calculated in the PERMDISP analysis (Primer 6 + PERMANOVA, Anderson, 2006; Anderson *et al.,* 2006). The ESCA index of each study site was expressed as Ecological Quality Ratio (EQR) calculated as the mean of three EQR_i obtained for the assemblage descriptors $((EQR_{SL} + EQR_{\alpha} + EQR_{\beta})\ /3)$. The EQR_i were calculated as the ratios between values of SL, α-diversity and β-diversity and values obtained for the same descriptors in the Reference Conditions (Montecristo Island, Tuscan Archipelago National Park) (Cecchi *et al.,* 2014). The index was

Table 1. Classification of the eight impact factors of each site studied, and the corresponding total values of anthropization level.

SITE	Urbanization and urban waste	Ports	Tourism	Industrial activity	Sediment load	Acquaculture	Agriculture waste	Fishing	Total
Montecristo	0	0	0	0	0	0	0	0	0
Pianosa	0	0	0	0	0	0	0	0	0
Capraia	0	0	1	0	0	0	0	0	1
C. Carbonara	0	0	1	0	0	0	0	0	1
Tavolara	0	0	1	0	0	0	0	0	1
Giglio	0	0	1	0	0	0	0	1	2
Elba	0	0	1	0	1	0	0	1	3
Portofino	0	0	2	0	2	0	0	0	4
Formiche	0	0	1	0	2	0	2	1	6
Meloria	0	2	1	2	2	0	1	0	8
Vada	0	0	1	2	2	0	1	2	8
Argentario	0	0	2	0	2	1	1	2	8
Livorno	2	3	3	2	3	0	0	2	15
Piombino	2	2	1	3	3	1	3	2	17

classified according to new boundaries: 0-0.2 Bad, 0.21-0.4 Poor, 0.41-0.6 Moderate, 0.61-0.8 Good, and 0.81-1 High. A regression line was performed in order to test the relationship between values of ESCA index (expressed as EQR) obtained for the studied sites and level of pressures calculated for the same sites. The degree of correlation between EQR and level of pressures was calculated and reported as value of square correlation coefficient (determination coefficient, R^2). Significance of regression was tested by means of the appropriate test performed with the Statistica 10 software. To compare values of ESCA index obtained with different methods (destructive *vs*. photographic), five sites among the 14 studied were chosen and sampled through destructive techniques, following the same methods used to obtain the developed datasets of the ESCA index (Cecchi *et al.,* 2014). In each of these sites, five samples of 400 cm^2 were collected in each of the two locations by scraping the bottom with a hammer and a chisel; abundance of the same taxa/morphological groups used for the analysis of photographic samples was evaluated as a percentage of cover (Boudouresque, 1971). ESCA index was calculated for each site through methods previously described and compared with the values obtained from photographic samples.

To evaluate the effectiveness of sampling design in terms of best sampling spatial scale, six sites among the 14 studied were chosen (two sites for each of the high, good and moderate ecological classes) and two areas 10s of meters apart were sampled in each of two locations.

The pseudo-variance components (Primer v6 + PERMANOVA, Anderson, 2001) were calculated for each spatial scale: site, location, area and sample.

For evaluating importance of intermediate spatial scales (location and area), three different datasets obtained by the six chosen locations were compared: 1) two locations and two areas per locations (i.e. the complete design); 2) two locations and one area per location (i.e. the original sampling design); 3) one location with two areas. Moreover, to evaluate the effectiveness of the index using different numbers of replicates, values of ESCA obtained with 30, 20 and 10 samples per location were compared.

The SIMPER test was used to compare the suitability of different sampling designs in detecting differences among assemblages subjected to different ecological conditions. The Bray-Curtis dissimilarity index was used to evaluate dissimilarity among conditions.

Results

Macroalgal assemblages in sites subjected to low human-induced pressure (pressure level 5 or less) were characterized by a stratified structure with a dominance of erect species, such as corticated Rhodophyta and the Chlorophyta *Halimeda tuna* and *Flabellia petiolata*; both *Peyssonnelia* spp. and turf represented approximately 20% of the assemblages (Fig. 2). In sites subjected to

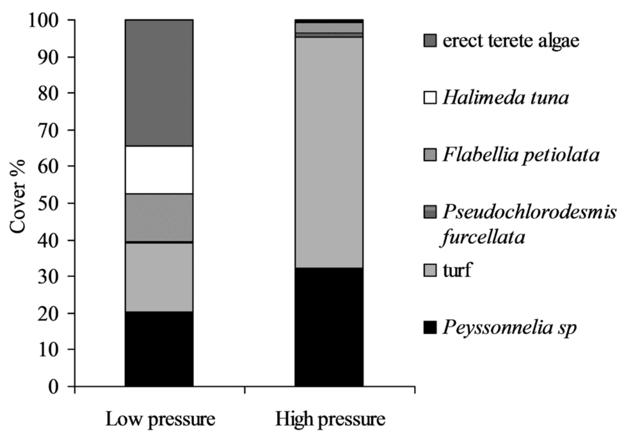

Fig. 2: Mean abundance (percentage of total cover) of the main taxa/morphological groups of macroalgae in sites subjected to high and low pressures.

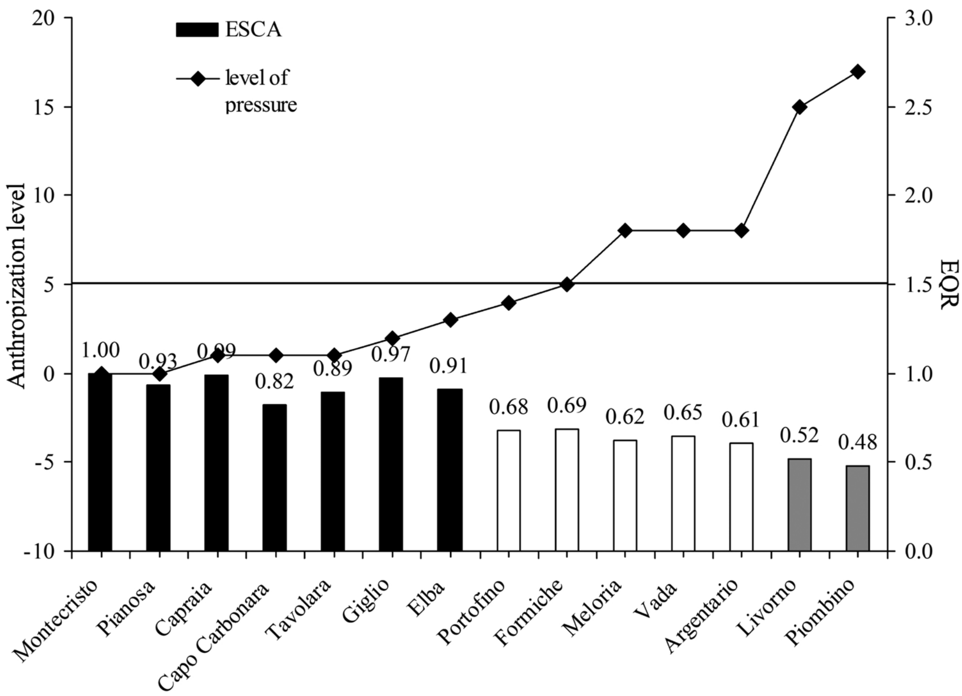

Fig. 3: Values of the ESCA index and anthropization level in the studied sites. Black bars: high ecological status, white bars: good ecological status, grey bars: moderate ecological status.

high pressure (pressure level greater than 5), the turf was dominant while the erect species showed low abundance (Fig. 2). Values of ESCA index ranged from 1.00 in reference conditions to 0.48 at Piombino, showing a correspondence with total levels of human pressures (Fig. 3). The regression line showed a significant negative correlation ($F<0.000$) between EQR values of ESCA and anthropization level (Fig. 4). The comparison between ESCA values obtained at the same sampled site with two different techniques (photographic and destructive methods) showed that photographic method provided lower values of EQR; moreover, this difference in the EQR values between the two methods increased with the values of index (Fig. 5). Pseudo-variance components showed high variability at large (among sites) and small (among samples) spatial scales, while intermediate spatial scales (location and area) had low values of variability; the lowest spatial variability was detected among areas 10s of meters apart (Fig. 6).

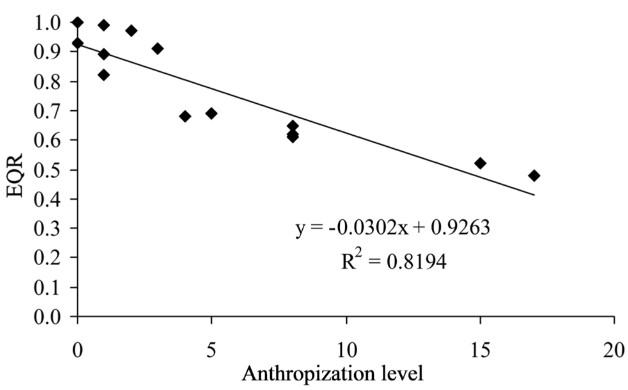

Fig. 4: Relationship between values of the ESCA index and values of anthropization level obtained in the sites studied.

The comparison between values of ESCA index calculated with different sampling designs showed that differences from the complete design (two locations, two areas) were greater if only one location was considered, while values obtained with only one area (the original sampling design) were not far from those obtained with the complete design (Table 2, Fig. 7). The comparison between values of ESCA index calculated with a different number of replicates showed that differences between 20 and 30 replicates were very low while differences increased with 10 replicates, and one site (Meloria) changed its quality status from good to moderate (Table 2, Fig. 8).

Discussion

In sites subjected to low human-induced pressures, macroalgal assemblages were characterized by a stratified structure with abundant erect taxa, showing the typical structure of coralligenous assemblages at the studied depth range (Piazzi *et al.,* 2004; Piazzi & Balata, 2011). In sites subjected to higher pressure, the sensitive taxa, such as *Halimeda tuna* and erect Rhodophyta, decreased while the filamentous species became dominant. Thus, data obtained by this large-scale investigation confirmed a pattern already highlighted by local correlative and experimental studies (Balata *et al.,* 2005, 2007a, b, 2011; Gennaro & Piazzi, 2011, 2013; Piazzi *et al.,* 2011, 2012). Turf-forming species may be at an advantage under stressed condition either directly, by improving their

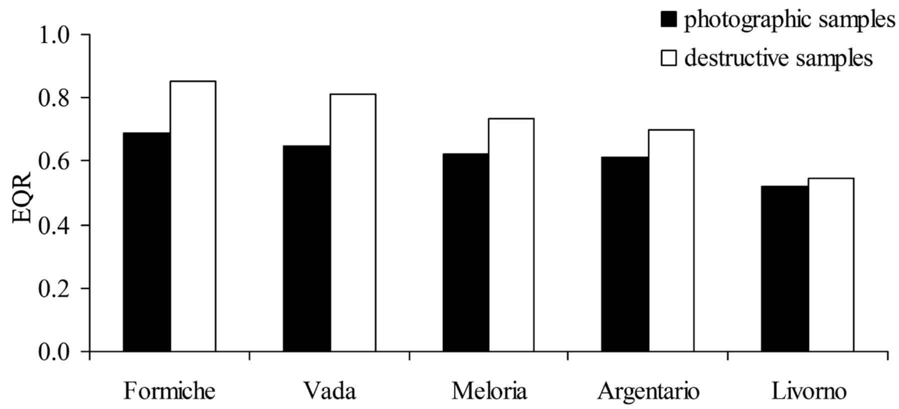

Fig. 5: Comparison between ESCA index values obtained with photographic and destructive methods applied at the same sampled sites.

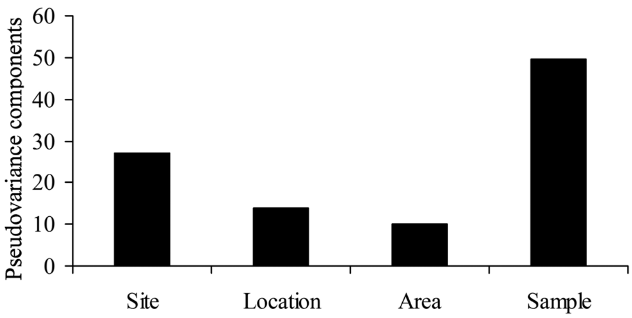

Fig. 6: Pseudo-variance components calculated for each spatial scale.

nutrient uptake in eutrophic conditions (Taylor *et al.,* 1998) or indirectly, by increasing their competitiveness compared to erect species due to their ability to recover quickly after disturbance (Airoldi, 1998, 2000). Values of the index responded well to pressure levels, since all sites characterized by low pressures, except Portofino, resulted in high quality class of EQR, while the other sites resulted in good or moderate classes, according to an increasing gradient of the anthropization level. This result confirms the positive response of the index to environmental stressors highlighted in the previous study (Cecchi *et al.,* 2014) and its sensitivity towards different levels of anthropogenic pressures. The choice of boundary values between good and moderate classes represents a critical point already highlighted with other indices (Orfanidis *et al.,* 2001; Mangialajo *et al.,* 2007). By analyzing results of studies on the responses of assemblages to experimental stress obtained through destructive sampling, this boundary was fixed at 0.65 (Cecchi *et al.,* 2014). However, in the present study the sites subjected to relatively low pressure showed values of EQR lower than 0.65. Moreover, the lower values of the index obtained with the photographic methods compared to the destructive one confirmed that analysis of destructive samples allows the identification of a higher number of taxa, clearly influencing the values of index. Thus, we propose a new scale of values more fitted the photographic sampling method of ESCA and that takes into account results of this large-scale study; the new boundary value between good and moderate classes was fixed at 0.6. Patterns of spatial variability of assemblages agree with those described by previous studies (Piazzi *et al.,* 2004; Balata *et al.,* 2005), showing high variability at large (among sites) and small (among samples) scales, while variability at intermediate scales (between locations and areas) was low. The comparison between values of the index obtained with different sampling designs showing the greatest differences were obtained by eliminating one location from the complete design and not eliminating one area (i.e. the original design adopted). Concerning the number of replicates, previous studies suggested that a number of replicates higher than 10 for each location should be made to describe macroalgal coralligenous assemblages well (Piazzi *et al.,* 2014a). The present paper confirms this finding, highlighting that 20 and 30 replicates allowed correctly classification of the study sites, with small differences of EQR values obtained with the two sets of replicates, while 10 replicates were not sufficient to calculate the ESCA index.

Summarizing the main results of the study, a sampling design that considers two locations 100s of meters far from each other for each study site and at last 20 replicate samples for each location may be considered sufficient to evaluate the ecological quality of coralligenous habitats through the ESCA index. The total sampling surface needed to investigate each study site was approximately 3.7 m^2 if 20 replicates were taken and 5.6 m^2 when 30 replicates were collected. This latter surface area agrees with the total surface area used by other methods to assess the quality status of coralligenous habitats (Kipson *et al.,* 2011; Deter *et al.,* 2012; Gatti *et al.,* 2012, 2015). The use of sessile animals as indicators needs a larger sampling surface (Bianchi *et al.,* 2004) due to the size and the distribution patterns of these while, if only macroalgal assemblages are considered, a smaller total surface may be enough. Although the index has been tested on a data set larger than that used to develop the method, the study is to be considered as preliminary for the evalu-

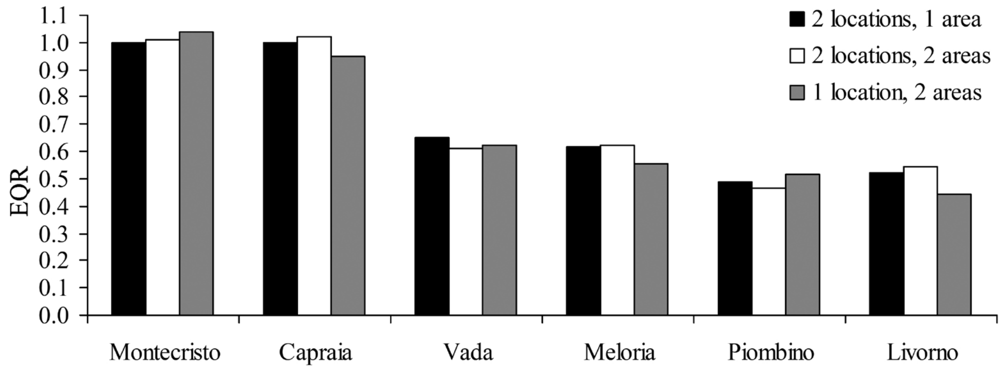

Fig. 7: Comparison between values of ESCA index calculated with different sampling designs.

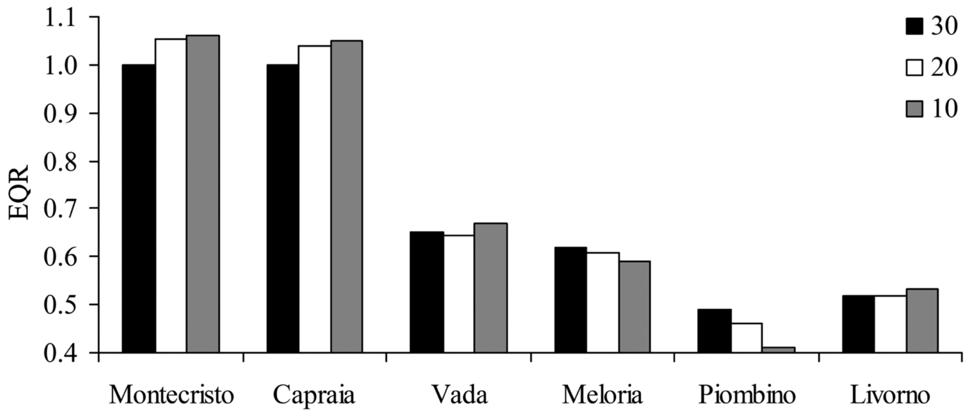

Fig. 8: Comparison between values of ESCA index calculated with different number of replicates (10, 20 and 30).

Table 2. Results of the SIMPER test showing values of Bray-Curtis dissimilarity index.

	2 locations, 2 areas, 30 replicates	1 location, 2 areas, 30 replicates	2 locations, 1 area, 30 replicates	2 locations, 1 area, 20 replicates	2 locations, 1 area, 10 replicates
High-Good	83.35	79.06	79.05	82.63	81.13
Good-Moderate	69.07	66.64	68.33	67.82	69.26
High-Moderate	84.44	79.54	80.15	83.50	81.54

ation of the efficiency of the index on a large scale. In fact, it was aimed at improving the existing index and defining the best sampling design, all necessary steps to test the index on a larger geographical area and within a higher number of different conditions. In this regard, particular attention should be paid to the environmental and ecological conditions (gradients of anthropogenic stress) that may occur at different depths and mesological situations (geomorphology, orientation, slope, etc.), so as to test in really effective way the robustness of the index.

ESCA has been compared with other indices and methods to assess ecological quality of coralligenous assemblages highlighting similar patterns but also interesting complementary aspects (Montefalcone *et al.,* 2014; Piazzi *et al.,* 2014b). These studies suggest that coupling of data obtained through different sampling techniques represent an aspect to be developed to give a more complete picture about ecological quality of coralligenous habitat. In this context, the application of ESCA index also using sessile animals may represent a topic of further investigations. Moreover, the distribution of sampling surface may be an interesting aspect to be studied (Parravicini *et al.,* 2009), given that the surface of a single replicate and the number of replicates are strictly related.

References

Airoldi, L., 1998. Roles of disturbance, sediment stress and substratum retention on spatial dominance in algal turf. *Ecology*, 79, 2759-2770.

Airoldi, L., 2000. Effects of disturbance, life-history and overgrowth on coexistence of algal crusts and turfs. *Ecology*, 8, 798-814.

Anderson, M.J., 2001. A new method for a non-parametric multivariate analysis of variance. *Australian Ecology*, 26, 32-46.

Anderson, M.J., 2006. Distance-based test for homogeneity of multivariate dispersions. *Biometrics*, 62, 245-253.

Anderson, M.J., Ellingsen, K.E., McArdle, B.H., 2006. Multivariate dispersion as a measure of beta diversity. *Ecology Letter,* 9, 683-693.

Balata, D., Piazzi, L., Cecchi, E., Cinelli, F., 2005. Variability of Mediterranean coralligenous assemblages subject to local variation in sediment deposits. *Marine Environmental Research*, 60, 403-421.

Balata, D., Piazzi, L., Benedetti-Cecchi, L., 2007a. Sediment disturbance and loss of Beta Diversity on subtidal rocky reefs. *Ecology*, 88, 2455-2461.

Balata, D., Piazzi, L., Cinelli, F., 2007b. Increase of sedimentation in a subtidal system: effects on the structure and diversity of macroalgal assemblages. *Journal of Experimental Marine Biology and Ecology*, 351, 73-82.

Balata, D., Piazzi, L., Rindi, F., 2011. Testing a new classification of morphological functional groups of marine macroalgae for the detection or responses to disturbance. *Marine Biology*, 158, 2459-2469.

Ballesteros, E., 2006. Mediterranean coralligenous assemblages: a synthesis of present knowledge. *Oceanography and Marine Biology Annual Review*, 44, 123-195.

Bianchi, C.N., Pronzato, R., Cattaneo-Vietti, R., Benedetti-Cecchi, L., Morri, C. *et al.*, 2004. Mediterranean marine benthos: a manual of methods for its sampling and study. 6: Hard bottoms. *Biologia Marina Mediterranea*, 11, 185-215.

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A. *et al.,* 2012. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. *Ecological Indicators*, 18, 31-41.

Borja, A., Dauer, D.M., 2008. Assessing the environmental quality status in estuarine and coastal systems: Comparing methodologies and indices. *Ecological Indicators*, 8, 331-337.

Borja, A., Elliott, M., Carstensen, J., Heiskanen, A.S., van de Bund, W., 2010. Marine management - towards an integrated implementation of the European Marine Strategy Framework and the Water Framework Directives. *Marine Pollution Bulletin*, 60, 2175-2186.

Boudouresque, C.F., 1971. Méthodes d'étude qualitative et quantitative du benthos (en particulier du phytobenthos). *Tethys*, 3, 79-104.

Cecchi, E., Gennaro, P, Piazzi, L., Ricevuto, E., Serena, F., 2014. Development of a new biotic index for ecological stat.us assessment of Italian coastal waters based on coralligenous macroalgal assemblages. *European Journal of Phycology*, 49 (3), 298-312.

Deter, J., Descamp, P. Ballesta, L., Boissery, P., Holon F., 2012. A preliminary study toward an index based on coralligenous assemblages for the ecological status assessment of Mediterranean French coastal waters. *Ecological Indicators*, 20, 345-352.

EC, 2000. DIRECTIVE 2000/60/EC of the European parliament and of the council, of 23 October 2000, establishing a framework for Community action in the field of water policy. Official Journal of the European Communities, G.U.C.E. 22/12/2000, L 327.

EC, 2008. DIRECTIVE 2008/56/EC of the European Parliament and of the Council, of 17 June 2008, establishing a framework for Community action in the field of marine environmental policy (Marine Strategy Framework Directive). Official Journal of the European Commission, G.U.C.E. 25/6/2008, L 164/19.

Gatti, G., Bianchi, C.N., Morri, C., Montefalcone, M., Sartoretto, S., 2015. Coralligenous reefs state along anthropized coasts: application and validation of the COARSE index, based on a Rapid Visual Assessment (RVA) approach. *Ecological Indicators*, 52, 567-576.

Gatti, G., Montefalcone, M., Rovere, A., Parravicini, V., Morri, C. *et al.*, 2012. Seafloor integrity down the harbor waterfront: the coralligenous shoals off Vado Ligure (NW Mediterranean). *Advances in Limnology*, 3, 51-67.

Gennaro, P., Piazzi, L., 2011. Synergism between the invasion of macroalga *Caulerpa racemosa* var. *cylindracea* and seawater nutrient enrichment. *Marine Ecology Progress Series*, 427, 59-70.

Gennaro, P., Piazzi, L., 2014. The indirect role of nutrients in enhancing the invasion of *Caulerpa racemosa* var *cylindracea*. *Biological Invasion*, 16, 1709-1717.

Gobert, S., Sartoretto, S., Rico-Raimondino, V., Andral, B., Chery, A. *et al.,* 2009. Assessment of the ecological status of Mediterranean French coastal waters as required by the Water Framework Directive using the *Posidonia oceanica* Rapid Easy Index: PREI. *Marine Pollution Bulletin*, 58, 1727-1733.

Kipson, S., Fourt, M., Teixido, N., Cebrian, E., Casas, E. *et al.,* 2011. Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops. *PLoS ONE* 6(11), e27103. doi:10.1371/journal.pone.0027103.

Laborel, J., 1961. Le concrétionnement algal "coralligene" et son importance geomorphologique en Méditerranée. *Recueil des Traveaux Scientifiques de la Statione Maritime d'Endoume*, 23, 37-60.

Laborel, J., 1987. Marine biogenic constructions in the Mediterranean, a review. *Scientific Reports of Port-Cross National Park*, 13, 97-127.

Mangialajo, L., Ruggieri, N., Asnaghi, V., Chiantore, M., Povero, P. *et al.,* 2007. Ecological status in the Ligurian Sea: The effect of coastline urbanisation and the importance of proper reference sites. *Marine Pollution Bulletin*, 55, 30-41.

Martinez-Crego, B., Alcoverro, T., Romero, J., 2010. Monitoring the quality of coastal waters at a large scale: bioindicator strengths and weaknesses. *Journal of Environmental Monitoring*, 12, 1013-1028.

Montefalcone, M., Cánovas Molina, A., Cecchi, E., Guala, I., Morri, C. *et al.,* 2014. Confronto tra due metodologie per la determinazione della qualità ecologica dei popolamenti coralligeni. *Biologia Marina Mediterranea*, 21, 210-211.

Orfanidis, S., Panayotidis, P., Stamatis, N., 2001. Ecological evaluation of transitional and coastal waters: A marine benthic macrophytes-based model. *Mediterranean Marine Science*, 2, 45-65.

Panayotidis, P., Montesanto, B., Orfanidis, S., 2004. Use of low budget monitoring of macroalgae to implement the European Water Framework Directive. *Journal of Applied Phycology*, 16, 49-59.

Parravicini, V., Morri, C., Ciribilli, G., Montefalcone, M., Albertelli, G. *et al.,* 2009. Size matters more than method: visual quadrats vs photography in measuring human impact on Mediterranean rocky reef communities. *Estuarine Coastal and Shelf Science*, 81, 358-367.

Piazzi, L., Balata, D., 2011. Coralligenous habitat: patterns of vertical distribution of macroalgal assemblages. *Scientia Marina*, 75, 399-406.

Piazzi, L., Balata, D., Pertusati, M., Cinelli, F., 2004. Spatial and temporal variability of Mediterranean macroalgal coralligenous assemblages in relation to habitat and substrate inclination. *Botanica Marina*, 47, 105-115.

Piazzi, L., Gennaro, P., Balata, D., 2011. Effects of nutrient enrichment on macroalgal coralligenous assemblages. *Marine Pollution Bulletin*, 62, 1830-1835.

Piazzi, L., Gennaro, P., Balata, D., 2012. Threats to macroalgal coralligenous assemblages in the Mediterranean Sea. *Marine Pollution Bulletin*, 64**,** 2623-2629.

Piazzi L., Balata D., Cecchi E., Gennaro P., Serena F., 2014a. Effectiveness of different investigation procedures in detecting anthropogenic impacts on coralligenous assemblages. *Scientia Marina*, 78, 319-328.

Piazzi, L., Cecchi, E., Serena, F., Guala, I., Canovas Molina, A. *et al.,* 2014b. Visual *vs*. photographic methods to estimate the quality of coralligenous reefs under different human pressures. p. 135-140. In: *Proceedings of the second Mediterranean Symposium on the conservation of Coralligenous and other Calcareous Bio-Concretions.* Bouafif, C., Langar, H., Ouerghi, A. (Eds). UNEP/MAP – RAC/SPA, RAC/SPA publ., Tunis.

Taylor, R.B., Peek, J.T.A., Rees, T.A.V., 1998. Scaling of ammonium uptake by seaweeds to surface area: volume ratio: geographical variation and the role of uptake by passive diffusion. *Marine Ecology Progress Series*, 169, 143-148.

UNEP, 2007. Draft decision on the "Action plan for the protection of the coralligenous and other calcareous bio-concretions in the Mediterranean". UNEP (DEPI)/MED WG.320/20, 2 pp + Annex.