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Impact assessment of fish cages on coralligenous reefs through the use of the STAR sampling procedure

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Abstract

The study aimed at contributing to the development of methods to assessing the effects of human disturbance on coralligenous reefs. The effects of the presence of a fish farm on coralligenous reefs were evaluated using the STAR (STAndaRdized coralligenous evaluation procedure) sampling procedure. An asymmetrical sampling design was used to compare the aquaculture site with two reference sites in areas unaffected by human pressure. The response of different ecological indices (ESCA, Ecological Status of Coralligenous Assemblages; ISLA, Integrated Sensitivity Level of coralligenous Assemblages; COARSE, COralligenous Assessment by Reef Scape Estimate) and descriptors (α-diversity, β-diversity and Sensitivity Level) of this kind of disturbance was compared. Results indicate that coralligenous reefs are vulnerable to aquaculture fish cages, and differences in the structure of coralligenous assemblages between the disturbed and the reference sites were mostly due to the decrease in β-diversity. On the contrary, no significant differences in the number of taxa/groups were highlighted. Encrusting Corallinales, erect Rhodophyta, Dictyotales, Fucales and *Halimeda tuna* were more abundant in reference sites than in disturbed site, while *Peyssonnelia* spp. and algal turfs had the opposite trend. Conversely, no significant differences between conditions were found in the abundance of sessile invertebrates. The study supports the suitability of the STAR approach to be employed in impact evaluation assessments, such as in monitoring programs. The present study is a first attempt to combine three different ecological indices (ESCA, ISLA and COARSE) within a unified approach, in order to assess the status of coralligenous reefs subjected to a moderate human-induced disturbance. The inconsistent response of the different indices highlights the advantage of applying different indices and descriptors to evaluate the variable human pressures on natural systems.

Keywords: aquaculture; coralligenous reefs; ecological indices; impact evaluation; standardized evaluation procedure.

Introduction

Marine coastal systems are threatened worldwide by overfishing, pollution, eutrophication, global warming, ocean acidification, and the spread of invasive species (Borja *et al*., 2008; Thrush *et al*., 2009). In this context, assessment of the sustainability of human activities in marine coastal systems represents a primary goal for ecologists and environmental managers. However, evaluation of human impact is not always obvious; it is necessary to employ methods and sampling designs suitable to separate human-caused effects from patterns of natural temporal and spatial variability (Underwood, 1993; Hewitt *et al*., 2001). On the other hand, methods of investigation should be easily achievable and cost-effective and allow comparison of results. Thus, the optimisation of the sampling effort becomes crucial as well as obtaining data suitable to detect a variety of possible impacts.

Among all sorts of human activities, aquaculture represents an important tool to support the demand for fish products as wild fisheries stocks decline worldwide (Zenetos *et al*., 2003; F.A.O., 2007), and to preserve natural systems, facilitate recovery of natural populations, and conserve endangered species. However, the impact of aquaculture on natural systems is an increasing cause of environmental concern (Holmer, 2010). In fact, light reduction and deposition of particulate carbon have been described to have profound effects on the water column (Islam, 2005; Sarà, 2007) and sediment quality (Holmer *et al*., 2003; Pusceddu *et al*., 2007) with detrimental consequences on the benthic communities (Tomassetti *et al*., 2009, 2016; Edgar *et al*., 2010; Sanchez-Jerez *et al*., 2018).

The impact of fish cages on coral reefs (*e.g.* Hedberg *et al*., 2017), maërl beds (Wilson *et al*., 2004), and seagrass meadows (Delgado *et al*., 1999; Ruiz *et al*., 2001;

Pergent-Martini *et al.,* 2006) has been investigated (Holmer, 2010) with the aim of reducing the risk of habitat degradation and maintaining resistance and resilience of sensitive marine habitats. The erosion of biodiversity, combined with evidence of progressive habitat degradation in surrounding areas even after cessation of fish-farming activity (Aguado-Giménez *et al*., 2012), has been highlighted, encouraging development of further investigations of aquaculture impacts. However, no efforts have been made to assess the vulnerability of the Mediterranean coralligenous reefs to aquaculture activities yet. These reefs biogenic structures mostly edified by calcareous red algae belonging to Corallinales and various sessile animals such as Cnidaria, Polychaeta, and Bryozoa; overall they are characterised by high structural and functional complexity (Ballesteros, 2006; Paoli *et al*., 2016). In the Mediterranean basin, coralligenous reefs represent one of the most important coastal ecosystems due to their extensive distribution, biodiversity, biomass, and role in the carbon cycle (Laubier, 1966; Ballesteros, 2006; Sini *et al*., 2019), and they provide multifarious ecosystem services to humans (Paoli *et al*., 2017). Moreover, coralligenous reefs are vulnerable to global and local impacts (Piazzi *et al*., 2012; Gatti *et al*., 2015b; Montefalcone *et al*., 2017). Thus, coralligenous reefs are included in the habitats that should be assessed under the Marine Strategy Framework Directive (EU, 2008). This highlights the need for accurate monitoring plans and impact assessment studies (Ballesteros, 2008).

In this context, several methods have been proposed to evaluate the ecological quality of coralligenous assemblages (Kipson *et al*., 2011; Deter *et al.,* 2012; Gatti *et al*., 2012, 2015a; Zapata-Ramírez *et al*., 2013; Cecchi *et al*., 2014; Féral *et al*., 2014; Ruitton *et al*., 2014; Teixidó *et al*., 2014; Montefalcone *et al*., 2017; Piazzi *et al*., 2017a; Sartoretto *et al*., 2017). These methods developed different approaches and adopted distinct ecological descriptors and sampling techniques. Recently, a standardised monitoring protocol, named STAR (STAndaRdized coralligenous evaluation procedure, Piazzi *et al*., 2018b), was proposed for the assessment of the ecological quality of coralligenous reefs. The protocol aims at optimising the sampling effort and collecting information deemed useful by existing ecological indices, and to make easier and more effective the comparison of data obtained throughout the Mediterranean Sea through the adoption of different sampling procedures (Piazzi *et al*., 2018b).

The study aimed at contributing to the development of methods for assessing the effects of human disturbance on coralligenous reefs. The effects of the presence of a fish farm on coralligenous reefs were evaluated using the STAR sampling procedure. An asymmetrical sampling design (ACI, After-Control/Impact, Underwood 1997), similar to Chapman *et al*. (1995), Guidetti *et al*. (2002), and Penna *et al*. (2017), was used to compare the aquaculture site with two reference sites in areas unaffected by human pressure. The hypothesis was that at the disturbed site the structure of the assemblage would change, with a decrease in abundance of sensitive taxa and an increase of opportunistic taxa, as compared to the undisturbed sites. Evidently, mechanisms of impact cannot be highlighted without appropriate manipulative experiments and thus any speculative insights about them have been purposefully avoided. Aiming to identify the best tool to use in fish farm impact evaluation on coralligenous reefs, results obtained by different ecological indices and descriptors (Gatti *et al*., 2015a; Piazzi *et al*., 2016, 2017b; 2018a; Penna *et al*., 2017) were compared.

Material and Methods

Study site

The study was done at Gorgona Island (about 2 km^2) in the Tuscan Archipelago National Park (North-Western Mediterranean Sea, Italy). The island is far from sources of human pressure (only about 200 inhabitants), as human activities are concentrated in a limited area of the island. In this area, a small fish farm consisting of eight cages (total volume of 4,130 m^3) with a mean annual production around 30 tons of *Sparus aurata* Linnaeus, 1758 was placed for 17 years (from 2001 to 2017) on a 20 m deep rocky bottom. During fish farm activity, both the sediment deposition rate (SDR) and the total organic carbon (TOC) of sediments were significantly higher at the disturbed site (720±67 g/month 0.76±0.12%, of SDR and TOC, respectively) compared to the references (287±55 g/month and 0.31±0.08%, according to De Biasi *et al*., 2016). These changed conditions had altered the structure of the seagrass community up to 300 m of distance from the cages (De Biasi *et al*., 2016).

At about 30 m far from the cages, coralligenous reefs are present on the cliffs sloping down from 30 to 40 m of depth. At reference sites similar rocky cliffs with the same orientation were chosen.

Sampling design and data collection

An asymmetrical design was used to compare the disturbed site (D) versus two reference sites sited at about 1000 m far from the cages in two opposite directions (R1 and R2, Fig. 1).

During July 2018, in each site, three areas of about 4 m^2 and 10s of meters apart were randomly selected on vertical bottoms at 35 m of depth and were sampled using the STAR procedure (Table S1, Piazzi *et al*., 2018b): in each area, 10 photographic samples of 0.2 m^2 each were collected; the thickness of the calcareous layer was measured through a hand-held penetrometer with six replicated measures per area; moreover, the size (mean height), the percentage of necrosis and epibiosis of erect anthozoans and the percent cover of sediment were assessed through an RVA (Rapid Visual Assessment) approach (Gatti *et al*., 2015a).

Images were analysed at the laboratory; organisms easily detected by photographic samples were assessed as distinct taxa, while those organisms displaying similar morphological features were assembled into morpholog-

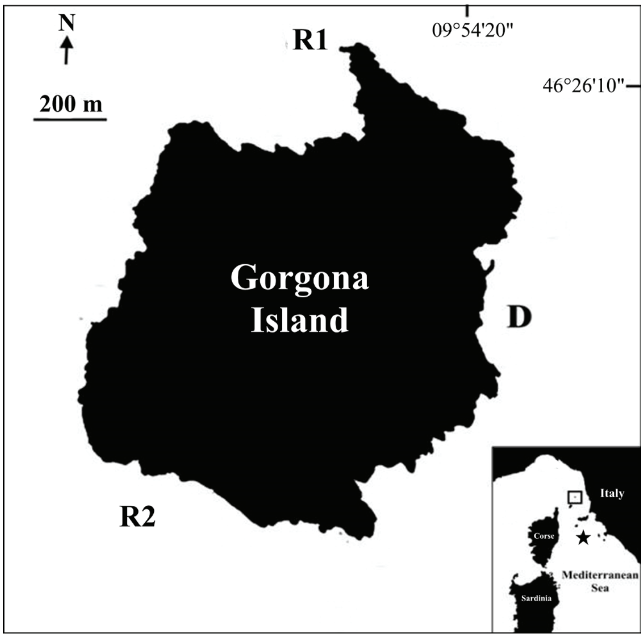

Fig. 1: Map of the study area: D = Disturbed site, R1 and R2 = Reference sites. The square indicates Gorgona Island and the star indicates Montecristo Island.

ical groups (Table S2, Piazzi *et al*., 2017a). The percentage cover of the main taxa/morphological groups was evaluated by a manual contouring technique through the ImageJ software (Cecchi *et al*., 2014).

Indices calculation

The ESCA index (Ecological Status of Coralligenous Assemblages; Cecchi *et al*., 2014; Piazzi *et al*., 2017a) was calculated using the formula $EQR=((EQR_{SL}+EQR_{\alpha}+EQR_{\beta})\times 3^{-1})$, where SL α, and β, correspond to Sensitivity Level, α-diversity, and β-diversity, respectively (Table S3). The percent cover of the main taxa/morphological groups in each photographic sample was classified according to 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) $75<\%<100$. The total Sensitivity Level of each photographic sample (SL_{sa}) was calculated by multiplying the sensitivity value of each taxa/groups (Table S2, Piazzi *et al*., 2017a) for its class of abundance (from 1 to 8) and adding values of all taxa/groups present in the sample. The α-diversity of the assemblages was evaluated as the number of taxa/morphological group per sample; the β-diversity was evaluated based on the spatial heterogeneity of assemblages as calculated by PERMDISP analysis (Primer 6+ PERMANOVA; Anderson, 2006); i.e. taking the mean distance of the centroids of photographic samples as a measure of the β-diversity of the system (Anderson *et al*., 2006). Individual EQRs were calculated as the ratios of the values of the three descriptors to the values of the same descriptors of the reference location for the North-Western Mediterranean Sea (Montecristo Island, Tuscan Archipelago National Park, a protected inhabited island selected as Reference Location in common benchmarking setting for the Mediterranean intercalibration within the European Directives, GIG, 2013; Cecchi *et al*., 2014).

The ISLA index (Integrated Sensitivity Level of coralligenous Assemblages, Montefalcone *et al*., 2017) was calculated by multiplying the abundance (expressed with the same classes used for ESCA) of each taxon/group for the corresponding score of Integrated Sensitivity Level (ISL) (Table S4), and summing all values of each sample. The EQR was calculated as the ratio between the values of ISL and the value of the index obtained in the Montecristo reference condition (Montefalcone *et al*., 2017).

The COARSE index (COralligenous Assessment by Reef Scape Estimate; Gatti *et al*., 2012, 2015a) was calculated for the three distinct layers characterizing coralligenous reefs: 1) basal layer, consisting of encrustations or organisms with limited (<1 cm) vertical growth; 2) intermediate layer, composed of organisms with moderate (1-10 cm) vertical growth; and 3) upper layer, characterized by organisms with considerable (>10 cm) vertical growth. In the basal layer, three descriptors were measured: i) percentage cover of encrusting calcified Rhodophyta, non-calcified encrusting algae, encrusting animals, turf-forming algae and sediment; ii) signs of borer species; and iii) thickness and consistency of calcareous concretions. In the intermediate layer three descriptors were assessed: i) species richness; ii) number of erect calcified organisms; and iii) sensitivity of bryozoans (Gatti *et al*., 2015a). For the upper layer, the three descriptors were: i) percentage cover of each species; ii) necrosis percentage of each population (even if covered by epibionts); and iii) maximum height of the largest sessile organism. Each of the nine descriptors was scored according to Gatti *et al.* (2015a) and the mean value among replicate scores gave the final score for each descriptor (Table S5). To obtain the quality score for each layer (Q_L) from the descriptor scores, the following formula was applied: $Q_L=(X_L\times Y_L\times Z_L)\times k^{(1-n)}$, where X_L, Y_L and Z_L are the quality scores assigned to the three descriptors, k is the maximum value of the scores (three in this case, as it is the maximum value attributed to each descriptor), and n is the number of descriptors considered. For each site, the mean of the three Q_Ls was then used to calculate an overall quality score of the coralligenous reef (Q_O).

Analyses of data

Spatial differences in the structure of assemblages were analysed by a permutational analysis of variance (Primer6 + PERMANOVA, Anderson, 2001) based on Bray-Curtis resemblance matrix of untransformed data. Data were not transformed in order to stress the importance of the abundance of taxa/groups in determining the differences among conditions. An asymmetric two-way design was used with Site (one disturbed and two reference sites, as fixed factor), and Area (three areas of photoquadrat collection per site, as a random factor nested in Site). The mean square of the factor Site was partitioned into two components: i) the contrast Impact vs Reference sites (I vs Rs) and ii) variability between Reference sites (Terlizzi *et al*., 2005).

A canonical analysis of principal coordinates (CAP) on untransformed Bray-Curtis resemblance matrix (Anderson & Robinson 2003) was performed in order to discriminate the differences in assemblages structure among conditions. Values of squared canonical correlation of the first and second axis were 0.606 and 0.296, respectively; the variations in the original similarities matrix explained by the axes achieving the highest allocation success was 0.989.

A SIMPER analysis (Clarke, 1993) was performed to identify percentage contribution of each taxon/group to the Bray-Curtis similarity among conditions.

Spatial differences in the three indices (ESCA, COARSE, and ISLA) and the three descriptors of ESCA index (α-diversity, β-diversity and sensitivity level) were analysed by one-way asymmetrical ANOVAs, with the factor Site as fixed and the sampling areas as replicates. Cochran's *C*-test was used before each analysis to check for homogeneity of variance and the data was transformed when necessary (Underwood, 1997).

Results

The assemblages were characterized by a stratified structure, with the basal layer mostly constituted by Corallinales and Peyssonneliaceae and secondarily by encrusting sponges and bryozoans. The most abundant organisms in the intermediate layer were the seaweeds *Flabellia petiolata*, *Halimeda tuna*, several erect Rhodophyta, and the bryozoans *Myriapora truncata*, *Reteporella* sp. and *Smittina cervicornis*. The erect layer was mainly characterized by *Eunicella cavolinii*.

Significant spatial differences in the structure of the assemblages were found at the scale of Site and Area: particularly, relevant differences were found (for both scales) between the disturbed assemblages and the references, but not between references (Table 1). Similarly, a graphical separation between D and Rs samples was found by the CAP analysis (Fig. 2). Both the CAP analysis and the SIMPER test indicated that encrusting Corallinales, erect Rhodophyta, Dictyotales, Fucales and *H. tuna* were more abundant in Rs than in D site, while *Peyssonnelia* spp. and algal turfs had the opposite trend (Fig. 2, Table 2). Noteworthy, invertebrates do not contribute greatly to differences in assemblage structure. No differences were highlighted among sites for the percent cover of sediment and the thickness of the calcareous layer. The necrosis of gorgonians was negligible everywhere.

ESCA and ISLA indices, β-diversity and SL were significantly higher in the references than in the disturbed site, while no significant differences were found using COARSE index and the α-diversity (Figs 3 and 4, Table 3 and 4). Moreover, values of COARSE and β-diversity also varied between references.

Table 1. Results of PERMANOVA on the structure of coralligenous assemblages. D = Disturbed site, Rs = Reference sites).

	Df	MS	Pseudo-F	P(perm)
Site = S	2	8914	3.03	**0.008**
D vs Rs	1	9986	3.14	**0.036**
among Rs	1	7842	3.48	0.101
Area(S) = A(S)	6	2938	2.30	**0.013**
A(D vs Rs)	4	3404	2.48	**0.013**
A(Rs)	4	2249	1.44	0.146
Residual	81	1274		

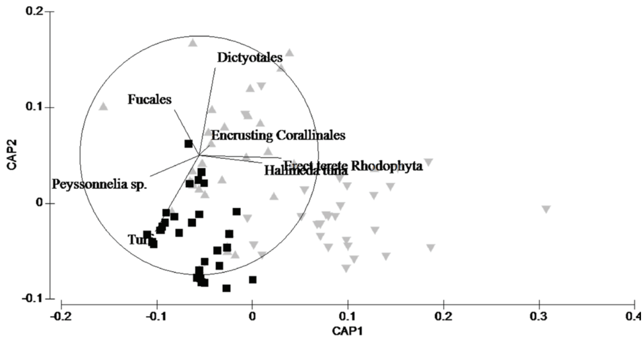

Fig. 2: Canonical analysis of principal coordinates (CAP) on coralligenous assemblages. Black squares = Disturbed site; grey up and down triangles = Reference sites.

Table 2. Results of SIMPER test showing the main taxa/groups influencing differences between Disturbed (D) and Reference (R1 and R2) sites.

	Percent cover	**Percent cover**	**Contr.%**
	R1	**D**	Diss. 54.81
Peyssonnelia sp.	52.35	66.14	36.47
Encrusting Corallinales	21.19	16.60	25.45
Turf	2.08	8.72	8.26
Fucales	6.71	0	7.20
Dictyotales	5.94	0.94	6.42
Erect terete Rhodophyta	4.59	1.19	5.20
	R2	**D**	Diss. 51.09
Peyssonnelia sp.	41.23	66.14	36.14
Encrusting Corallinales	23.08	16.60	23.13
Erect terete Rhodophyta	16.57	1.19	15.17
Turf	1.84	8.72	7.40
Halimeda tuna	5.85	0.25	5.58

Discussion

Results of the study showed that coralligenous reefs were affected by 17 continuous years of fish caging aquaculture as these assemblages still exhibit signs of impact after one year of disturbance cessation.

Differences observed in the structure of coralligе-

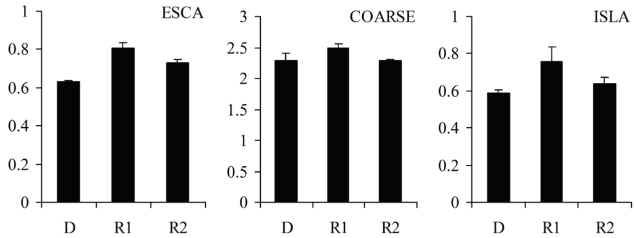

Fig. 3: Mean (+SE) values of ESCA, COARSE and ISLA indices (D = Disturbed site, Rs = Reference sites).`

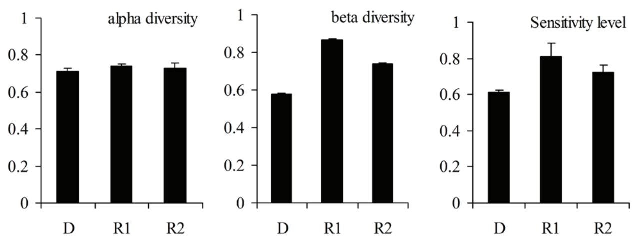

Fig. 4: Mean (+SE) values of the ESCA descriptors: α-diversity, β-diversity and sensitivity level (D = Disturbed site, Rs = Reference sites).

nous assemblages between the disturbed site and the reference sites were mostly due to an increase in tolerant taxa/groups and a decrease in sensitive species and β-diversity in the disturbed site. The decrease of sensitive algae, such as erect Rhodophyta and *H. tuna*, in the disturbed site confirms the role of these organisms as ecological indicators (Balata *et al*., 2007b, 2011). Their reduction may be related to the direct effect of pollutants and sedimentation, and to the indirect effects caused by competition with more opportunistic species (Piazzi *et al*., 2012). Turfs and Peyssonneliaceae may be enhanced by eutrophic conditions due to their life-strategy—algal species with a higher surface area/volume ratio tend to grow faster, requiring more nutrients and to have higher nutrient uptake rates than thicker algae with a lower surface/volume ratio (Taylor *et al*., 1998; Bokn *et al*., 2003; Karez *et al*., 2004). Moreover, turfs are mostly comprised of filamentous species that reproduce asexually and are well adapted to stressful environmental conditions, such as high sedimentation rates, thanks to their ability to quickly recover after disturbance (Airoldi, 1998; Balata *et al*., 2007a, 2011). The rapid spread of opportunistic species also reproduce asexually and may outcompete sensitive species that need available substrate for spore settling (Airoldi, 1998; Piazzi & Cinelli, 2001).

Although several sessile invertebrates, such as erect bryozoans and gorgonians, are considered valuable indicators of disturbance (Linares *et al*., 2010; Gatti *et al*., 2015a), no significant differences in abundance or in the proportion of individuals displaying necrosis were found in the present study. The sensitivity of organisms may change in relation to different pressures (Montefalcone *et al*., 2017), and macroalgae seem more suitable to highlight moderate changes in water quality compared to animals normally employed to evaluate mechanical impacts (Bavestrello *et al*., 1997; de la Nuez-Hernández *et al*., 2014).

The β-diversity showed lower values in the disturbed site as compared to the reference sites, while no significant differences in the number of taxa/groups were observed. This pattern agrees with that described where nutrient enrichment was manipulated in some coralligenous

Table 3. Results of ANOVA on ESCA, COARSE and ISLA index values. (D = Disturbed site, Rs = Reference sites). F values in bold correspond to p<0.05.

		ESCA		COARSE		ISLA		
Source	**df**	**MS**	**F**	**MS**	**F**	**MS**	**F**	**denominator**
Site	2	0.0222	**23.94**	**0.0408**	3.22	0.0229	4.02	residual
D vs Rs	1	0.0357	**714.00**	**0.0184**	0.68	0.0232	**58.00**	residual D vs Rs
among Rs	1	0.0087	6.32	0.0632	**11.19**	0.0225	2.70	residual among Rs
Residual	6	0.0009		0.0127		0.0057		
D vs Rs	2	$5*10^{-5}$		0.0267		0.0004		
among Rs	4	0.0014		0.0056		0.0083		
		C = 0.712 (ns)		C = 0.811 (ns)		C = 0.811 (ns)		

Table 4. Results of ANOVA on the descriptors of ESCA index (D = Disturbed site, Rs = Reference sites).

		α-diversity			β-diversity			Sensitivity levels		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Site	2	0.32	3.04	0.122	127.6	784.6	**0**	7168	4.76	0.057
D vs Rs	1	0.16	0.98	0.426	214.2	946.4	**0.001**	12133	20.67	**0.045**
among Rs	1	0.48	6.28	0.066	41.0	313.8	**0.001**	2204	1.12	0.34
Residual	6	0.10			0.2			1505		
		C = 0.5158 (ns)			C = 0.4899 (ns)			C = 0.7404 (ns)		

reefs (Piazzi *et al*., 2011). Thus, changes in the structure of coralligenous assemblages due to a moderate increase in sedimentation rate and nutrient concentration seem to involve the relative importance of taxa and their spatial arrangement and not the α-diversity of the system. Sedimentation and eutrophication can alter natural competitive mechanisms controlling spatial distribution of sessile organisms (Balata *et al.*, 2005, 2007).

An increase in abundance of tolerant species at the expense of more sensitive ones as a consequence of nutrient and sedimentation increase can lead to wide biotic homogenisation of the system (sensu McKinney & Lockwood, 1999), with a decrease in β-diversity (Piazzi *et al*., 2011, 2012). Therefore, the species spatial arrangement seems a very sensitive feature of coralligenous reefs where assemblages are generally characterised by high spatial heterogeneity (Ferdeghini *et al*., 2000; Balata *et al*., 2007a; Piazzi *et al*., 2016). Thus, β-diversity may represent a valuable ecological descriptor to be considered, especially when the stressors' effects are sub-lethal and do not cause evident degradation of the assemblages (Piazzi & Balata, 2011; Piazzi *et al*., 2018a).

Although the impacts due to aquaculture have been widely investigated (Gao *et al*., 2005; Kalantzi & Karakassis, 2006; Apostolaki *et al*., 2007; Tomassetti *et al*., 2016), little is known about the recovery of benthic assemblages after fish farming cessation; some data indicate inconsistent patterns of recovery for soft bottom assemblages (Aguado-Giménez *et al*., 2012). Although the lack of data during the fish farm activity of Gorgona Island does not allow evaluation of the actual recovery of coralligenous reefs, the differences between disturbed and reference sites one year after cessation of farming suggest the persistence of impact and the necessity for long-term monitoring periods.

Concerning the ecological quality of coralligenous reefs, ESCA and ISLA indices showed very similar patterns while contrasting results were obtained using the COARSE index. In fact, the ESCA index, summarising more descriptors, was able to highlight changes in the ecological quality of the reefs between disturbed and reference sites. This outcome confirms the suitability of a multi-descriptors approach to detect impacts of moderate magnitude (Borja *et al*., 2009c; Edgar *et al*., 2010) and supports the successful employment in monitoring programs and impact evaluation studies. Also, the ISLA index, based on the sensitivity of organisms to disturbance and stress, was able to discriminate the disturbed site from the others. Indeed, eutrophication may influence the overall sensitivity level of the assemblages causing a reduction of values of this index due to the enhancement of opportunistic species (Montefalcone *et al*., 2017). The descriptive information provided by the COARSE index of "no impact" of fish cages is likely due to the fact that this index was developed to assess the quality of the coralligenous habitat regardless of human pressures; in fact it was based on a seascape approach to provide information on the seafloor integrity considered as the characteristics (physical, chemical, biological) of the seabed (Gatti *et al.,* 2015a). Thus, COARSE may not be suitable to detect changes in environmental quality when these changes do not cause severe deterioration of the coralligenous structure (Piazzi *et al*., 2017b).

In conclusion, although the low level of replication used in this study needs additional investigation to address and evaluate the direct and indirect impacts of aquaculture on coralligenous formations, the results provide evidence to support the sensitivity of coralligenous assemblages to human pressures and their suitability as an ecological indicator, since they offer early signs for detection of impact caused by stressors (Hong, 1983; Balata *et al*., 2007b; Piazzi *et al*., 2012, 2018a). Moreover, the study highlighted the suitability of the STAR approach to be employed in impact evaluation assessments, such as in monitoring programs. In fact, different indices require diverse information, sampling methods, and designs, so that concurrent deployment in environmental investigations becomes not obvious. The STAR procedure can be a profitable solution for obtaining the data needed to calculate different ecological indices in a single sampling effort. Overall, although each descriptor is reliable to different human impacts (Gatti *et al*., 2015a; Montefalcone *et al*., 2017; Penna *et al*., 2017; Piazzi *et al*., 2018a), the challenge to identify early signals of stress and effects due to low-magnitude disturbance would be the most useful goal of conservation efforts. In this context, the fact that the three indices did not give homogeneous responses to moderate disturbances emphasises the need for a multi-descriptor approach to evaluate human pressure on natural systems, as already investigated for other marine systems (Borja *et al*., 2009a, 2009b; Martinez-Crego *et al*., 2010; Bedini & Piazzi, 2012). At the same time, ESCA and ISLA indices gave similar results suggesting that their redundancy should be accurately tested. The present study represents a first attempt to compare three different ecological indices in coralligenous reefs and the results suggest that a wide number of case studies are needed to determine the concurrent use of different indices and descriptors in impact evaluations and monitoring programs.

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The following supplementary information is available for the article:

Table S1. The main steps used to apply STAR procedure.

Table S2. Sensitivity Level (SL) of the main taxa/morphological groups in the coralligenous assemblages for ESCA index (from Piazzi et al., 2017a).

Table S3. Descriptors used to calculate ESCA index EQR=((EQRSL+EQRα+EQRβ)×3-1). Individual EQRs were calculated as the ratios of the values of the three descriptors to the values of the same descriptors of the reference location for the north-western Mediterranean Sea.

Table S4. Scores of the Integrated Sensitivity Level (ISL) for the main taxa/morphological groups in the coralligenous assemblages, as obtained combining the values of sensitivity to disturbance (DSL) and of sensitivity to stress (SSL) (Montefalcone et al., 2017). In the case of alien species, the ISL score is put to -1 a priori.

Table S5. Criteria for the assignment of quality scores to each descriptor for each replicate in COARSE index. ECR= encrusting coralline algae, NCEA= non calcified encrusting algae, EA= encrusting animals. L = the maximum height found in literature for each species. The necrosis is evaluated as the mean percentage of necrosis of each individual colony.