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Effectiveness of *Posidonia oceanica* biotic indices for assessing the ecological status of coastal waters in the Saronikos Gulf (Aegean Sea, Eastern Mediterranean)

V. GERAKARIS[1,2], P. PANAYOTIDIS[1], S. VIZZINI[3], A. NICOLAIDOU[2] and A. ECONOMOU-AMILLI[2]

[1] Institute of Oceanography, Hellenic Centre for Marine Research, 19013 Anavyssos, Greece
[2] Department of Ecology & Systematics, Faculty of Biology, University of Athens, 15784 Athens, Greece
[3] Department of Earth and Marine Sciences, University of Palermo, CoNISMa, Via Archirafi 18, 90123 Palermo, Italy

Corresponding author: vgerakaris@hcmr.gr

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Abstract

Biotic indices are considered key assessment tools in most national and European policies aimed at improving the quality of coastal waters. At present, several Water Framework Directive (WFD)-compliant biotic indices based on the marine angiosperm *Posidonia oceanica* have been developed and applied in the Mediterranean Sea. In this study, we investigated the effectiveness of four different *P. oceanica* indices (POMI, PREI, Valencian CS, and BiPo) in evaluating the ecological status of coastal waters in a case study area of Greece. The evaluation, comparison, and validation of the Ecological Status Class (ESC) assessments obtained by each index were based on a set of eight common sites that encompasses the maximum range of environmental quality in the study area. Four sampling sites separated by tens of km were chosen in each of the two water bodies (WBs) studied. The spatial variations of the features of *P. oceanica* meadows were examined according to a hierarchical sampling design across four spatial scales, ranging from metres to tens of km, using independent nested analysis of variance. Except for the BiPo index, the reference values for each metric/index were defined by the dataset available for the study area. All biotic indices classified the WBs of the study area in *Good* ESC category. Only three of the four indices (PREI, Valencian CS, and BiPo) showed high comparability in the assessment of ESC at study site level. It is assumed that the differences found in the remaining index (POMI) are due to the different type of metrics taken into consideration and the different weighting given to them. Our findings suggest that all indices can provide an overall view of the cumulative impact of multiple environmental stressors existing in the study area, and can thus help raise awareness of ecosystem degradation.

Keywords: Seagrass, Biotic index, Ecological status, Water Framework Directive, Eastern Mediterranean.

Introduction

The implementation of the European Water Framework Directive (WFD 2000/60/EC) has been a key driver for increasing research effort focused on the development of several biological or ecological indicators (Marbà *et al.,* 2012).

Seagrasses (i.e. marine angiosperms) are increasingly being used as ecological indicators for the assessment of overall environmental health status (Pergent *et al.*, 1995; Short and Wyllie-Echeverria, 1996; Hemminga & Duarte, 2000), due to their wide spatial distribution, essential ecological role, and high sensitivity to anthropogenic disturbances. The indicator value of seagrasses has been clearly outlined in the WFD, thus being included among the four Biological Quality Elements (BQEs) that need to be monitored for the ecological classification of coastal waters (EC, 2000). In fact, the use of BQEs along with detailed knowledge of anthropogenic pressures and their impact on the coastal marine environment is fully recommended by the WFD. The DPSIR (Driver, Pressure, State, Impact, Response) approach (IMPRESS, 2002) is the main framework used for determining pressures, impacts and responses under the WFD and is considered to provide an overall mechanism for analysing environmental problems (Borja *et al.,* 2006).

The implementation of the WFD has led to the development of several biotic indices aiming to assess the response of marine communities to anthropogenic impacts (Marbà *et al.,* 2012). The efficiency of biotic indices as classification and monitoring tools is mainly determined by their ability to identify the ecological quality objectives to be achieved and their ability to provide guidance to policy-makers and managers in planning adequate intervention policies to restore water quality (Bacci *et al.,* 2013).

Posidonia oceanica (L.) Delile is the most common and abundant seagrass in the Mediterranean Sea, forming extensive meadows and playing a key ecological, geological and economic role (Boudouresque *et al.,* 2012). Due to its high sensitivity to environmental degradation and its responses to specific human-induced disturbances, *P. oceanica* is widely considered as an effective ecological indicator (Pergent *et al.,* 1995; Ruiz & Romero 2001; Martínez-Crego *et al.,* 2008; Boudouresque *et al.,* 2012). Indeed, the indicator value of *P. oceanica* has been highlighted through intercalibration exercises performed by the Mediterranean Geographical Intercalibration Group

(MedGIG), where the species was selected as BQE representative of Mediterranean marine angiosperms for monitoring the ecological status of coastal waters (MedGIG, 2007). Several biotic indices have been proposed to assess the ecological quality of coastal waters using *P. oceanica* (POMI: Romero *et al.,* 2007; Valencian CS: Fernandez-Torquemada *et al.,* 2008; PREI: Gobert *et al.,* 2009; BiPo: Lopez y Royo *et al.,* 2010). Most of *P. oceanica* biotic indices are WFD-compliant, i.e. they meet a set of required criteria, including: (i) the expression of ecological status, called the Ecological Quality Ratio (EQR), as a numerical value between 0 and 1, (ii) the existence of a significant relationship between EQR and anthropogenic pressures, and (iii) the use of a common scale of five ecological status classes: *High, Good, Moderate, Poor* and *Bad*.

P. oceanica biotic indices has been used successfully mainly in the Western Mediterranean and the Adriatic Sea (central Mediterranean Sea) (Nicolic *et al*., 2009; Lopez y Royo *et al.,* 2011; Mascaró *et al.,* 2012; Costantino *et al*., 2015). In Greece, *P. oceanica* is the dominant seagrass species with a wide distribution along the coastlines of the Ionian and Aegean Seas (Telesca *et al.,* 2015). Indeed, *P. oceanica* meadows can be found from the very remote and pristine areas up to the most urbanized areas with significant human pressure. However, none of the proposed biotic indices has ever been applied in Greek seas or the wider area of the Eastern Mediterranean Sea.

In this context, the aim of this study was to evaluate the efficiency of *P. oceanica* biotic indices as classification and monitoring tools in a case study area in the Eastern Mediterranean Sea (Saronikos Gulf, Aegean Sea, Greece). To this end, the main objectives of our study include: (i) the application and comparison of four biotic indices (POMI, PREI, Valencian CS, and BiPo), (ii) the validation of the obtained results by correlating ESCs and anthropogenic pressures, and (iii) an overall evaluation of the performance of biotic indices and their usefulness as monitoring tools.

Materials and Methods

Study area

The Saronikos Gulf is a semi-enclosed embayment on the western coastline of the Aegean Sea in the Eastern Mediterranean (Fig. 1). The Gulf is characterised by sea surface temperatures (13-26 ^{o}C) and salinities (38-39 ‰) that are typical of the Eastern Mediterranean basin (Kontoyiannis *et al.,* 2005). The Gulf region exhibits very low rainfall rates throughout the year while the prevailing winds blow predominantly from the north (HNMS, 1999). Around the Gulf, there are ten river basins with one main river system (Kifissos River). However, these river basins have limited runoff to the coastal areas.

The main human activities that are considered to have major impacts on the marine environment of the study area include national and international shipping (the port of Piraeus is the third largest port in the Mediterranean in terms of container traffic), urbanization and extensive modification of the coastline (Athens metropolitan area: ca. 4 million inhabitants), industrial discharges, increas-

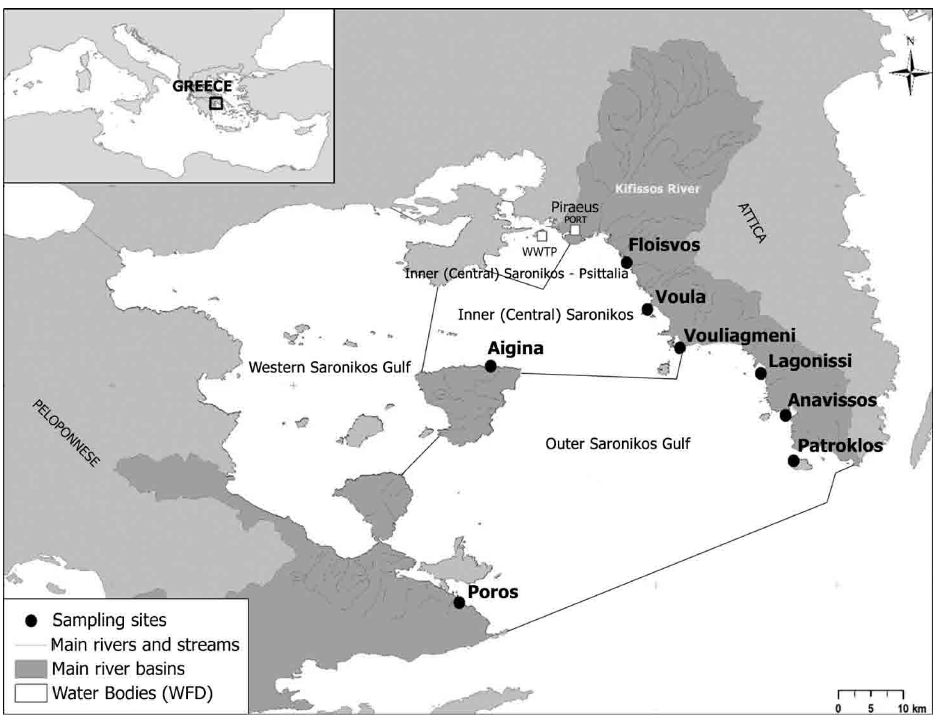

Fig. 1: Geographic location of the eight sampling sites along the coasts of Saronikos Gulf. The main river basins, main rivers and streams, and water bodies are also included on the map.

ing recreational and fishing activities, and urban sewage discharges (Psittalia's Waste Water Treatment Plant - WWTP).

Sewage discharge from Psittalia's WWTP outfall, in particular, is considered as the main source of stress exerted on the marine ecosystem of the Gulf, and the effects have been monitored regularly for more than two decades (Siokou *et al.,* 1999; Simboura *et al.,* 1995; 2005). Integrative studies on different aspects of the marine environment (benthic communities, macroalgae, chemical compounds) indicate the presence of a clear environmental gradient along the coasts of the Saronikos Gulf (Simboura *et al.,* 2005; 2014; Tsiamis *et al.,* 2013). More specifically, earlier studies on the Ecological Quality Status (EQS) of the Saronikos Gulf indicated that water quality –especially in the inner part of the Gulf– presents a clear gradient from *Poor* to *Good* status, depending on the distance from Psittalia's WWTP outfall (Simboura *et al.,* 2005; 2014; Tsiamis *et al.,* 2013).

Under the WFD monitoring plan, four distinct coastal water bodies (WB) have been designated in the study area: "Inner (Central) Saronikos Gulf", and "Inner (Central) Saronikos-Psittalia" as operational WB, i.e. WBs identified as being at risk of failing to meet their environmental objectives, and "Western Saronikos Gulf", and "Outer Saronikos Gulf" as surveillance WB, i.e. WBs assessed for the likelihood of failing to meet their environmental objectives (Fig. 1) (HCMR, 2008). Definition of the boundaries of each WB was based on hydrological and geomorphological features (Coachman *et al.,* 1976), as well as on the distribution of anthropogenic pressures along the coastal zone of the study area (HCMR, 2008). However, the division of each WB was carried out irrespective of the extent or ecological status of *P. oceanica* meadows within the study area.

P. oceanica meadows are absent in two of the four WBs ("Western Saronikos Gulf" and "Inner (Central) Saronikos-Psittalia") (Panayotidis & Simboura, 1989; authors' personal observations). Therefore, the assessment of ESC was conducted only in the other two WBs ("Inner (Central) Saronikos Gulf" and "Outer Saronikos Gulf"). In the inner Saronikos Gulf, *P. oceanica* meadows colonize mostly sandy bottom with mild slopes, whereas, in the outer Saronikos Gulf, meadows extend both on sandy and rocky bottoms with moderate to high slopes.

Posidonia oceanica biotic indices applied

Four biotic indices (POMI, PREI, Valencian CS and BiPo) were applied. Three of them (POMI, PREI, and Valencian CS) have been successfully intercalibrated through MedGIG (Mediterranean Geographical Intercalibration Group) exercises (MedGIG, 2011). The biotic indices differed in two aspects: (i) the different set of metrics used (Table 1) and (ii) how the different metrics were aggregated or combined to produce values on the Ecological Quality Ratio (EQR) 0 - 1 scale (Table 2).

For the application of each index, a specific dataset of various metrics is required (Table 1). The individual metrics selected for each index were gathered from several levels of biological organization, ranging from biochemical to community level and thus, encompassing different time responses to stress and different specificity to stressors (Romero *et al*., 2016). All indices incorporate

Table 1. The different metrics used by each biotic index.

Biotic Level	Metric	POMI	Valencian CS	PREI	BiPo
Community	Herbivore pressure (%)		+		
	Epiphytic biomass (mg/cm^2)		+		
	Epiphytic/Leave Biomass (E/L)			+	
	N content in epiphytes (% dw)	+			
Population	Type of lower limit			+	+
	Depth lower limit (m)			+	+
	Meadow cover (%)	+	+		
	Dead matte cover (%)		+		
	Shoot density (shoots/m^2)	+	+	+	+
	Plagiotropic rhizomes (%)	+	+		
Individual	Rhizome baring/burial (cm)		+		
	Shoot leaf surface (cm^2/shoot)	+	+	+	
	Shoot length (mm/shoot)				+
	Leaf necrosis (% leaves/shoot)	+	+		
Physiological - Biochemical	N content in rhizomes (% dw)	+			
	P content in rhizomes (% dw)	+			
	Total n-s carbohydrates (% dw)	+			
	δ^{15}N ratio in rhizomes (‰)	+			
	δ^{34}S ratio in rhizomes (‰)	+			
	Cu content in rhizomes (μg/g)	+			
	Pb content in rhizomes (μg/g)	+			
	Zn content in rhizomes (μg/g)	+			

Table 2. Class boundaries and colour code for the different levels of ecological status.

EQR	ESC	Colour Code
1 - 0.075	High	Blue
0.774 - 0.550	Good	Green
0.549 - 0.325	Moderate	Yellow
0.324 - 0.100	Poor	Orange
< 0.100	Bad	Red

metrics from individual, population and community levels while POMI also embodies metrics from biochemical and physiological levels (Table 1).

Concerning the integration or combination of metrics into a single index, two different approaches (classification methods) have been used. The BiPo and PREI indices are multimetric and thus the integration of their metrics into a single index is based on averaging the scores of the chosen individual metrics (Gobert *et al.,* 2009; Lopez y Royo *et al.,* 2010), while POMI and Valencian CS are considered as multivariate indices, since multivariate analysis methods (e.g. Principal Component Analysis-PCA) are used for the aggregation of the metrics (Romero *et al*., 2007; Fernandez-Torquemada *et al*., 2008).

Regarding the POMI index, the isotopic ratio in rhizomes $\delta^{34}S$ was not included in the current evaluation due to technical and logistic constraints. Still, according to Bennett *et al.* (2011), the classification precision of POMI is resilient enough in a reduced version as long as the different levels of organization are still represented. Reduced versions of POMI (POMI 9 and POMI 5) have been previously applied and revealed consistent results compared to those of the initial POMI version (POMI 14) (Bennett *et al.,* 2011; Mascaró *et al.,* 2012).

Reference conditions

Since all indices were initially developed in the Western Mediterranean basin, Reference Condition (RC) values have been modified from their original values and were defined by the available dataset in the spatial extent of the case study area. With an exception in the case of BiPo index whose RC values have already been determined for the whole Mediterranean Sea based, on a western Mediterranean dataset, the setting of RC values for the remaining indices (POMI, Valencian CS, and PREI) was based on the formation of a "virtual" site, serving as a reference site. In each case, this hypothetical site was constructed based on the best values observed for each of the metrics, under the assumption that this "virtual" site has ecologically ideal conditions in relation to each of the metrics (Romero *et al.,* 2007; Fernandez-Torquemada *et al.,* 2008; MedGIG, 2011). In all cases, the optimum (i.e. reference) value of each metric for the "virtual" reference site (i.e. "best" site) was derived as follows: the three best values recorded for each metric were chosen when all sites were included, and the highest value excluded; the final reference value was calculated as the mean of the remaining two values. A "worst" site was also calculated for the POMI and Valencian CS indices, following the same procedure and using the worst values for each metric. The reference values used for each biotic index are summarized in Table 3.

Table 3. Reference values for each metric in the Saronikos Gulf.

Metrics	Reference values				"worst values"
	POMI	Valencian CS	PREI	BiPo	
Depth lower limit (m)	-	-	30 *+	38	14
Meadow cover (%)	100	100	-	-	25
Dead matte cover (%)	-	0	-	-	27.5
Shoot density (shoots/m^2)	615	615	615*+	599	50
Plagiotropic rhizomes (%)	0	0	-	-	77.5
Rhizome baring/burial (cm)	-	10	-	-	-2
Shoot leaf surface (cm^2/shoot)	442	442	442*+	-	91
Shoot length (mm/shoot)	-	-	-	978	358
Leaf necrosis (% leaves/shoot)	33	33	-	-	100
Herbivore pressure (%)	-	0	-	-	50
Epiphytic biomass (mg/cm^2)	-	0	-	-	1.57
Epiphytic/Leave Biomass (E/L)	-	-	0 *+	-	0.43
N content in rhizomes (% dw)	0	-	-	-	2.2
P content in rhizomes (% dw)	0	-	-	-	0.2
Total n-s carbohydrates (% dw)	44	-	-	-	2
$\delta^{15}N$ ratio in rhizomes (%)	2	-	-	-	7.3
N content in epiphytes (% dw)	0	-	-	-	1.9
Cu content in rhizomes (μg/g)	0	-	-	-	20
Pb content in rhizomes (μg/g)	0	-	-	-	5
Zn content in rhizomes (μg/g)	0	-	-	-	151

* RC values used in PACA region (France) by Gobert *et al.* (2009) were: Depth lower limit (m)=34; Shoot density (shoots/m^2) = 675; Shoot leaf surface (cm^2/shoot) = 465; E/L = 0

\+ RC values used in Italy by Bacci *et al.* (2013) were: Depth lower limit (m)=38; Shoot density (shoots/m^2) = 599; Shoot leaf surface (cm^2/shoot) = 310; E/L = 0

Sampling design and data acquisition

Eight sampling sites were chosen encompassing the maximum range of environmental quality in the study area. Four sites (Floisvos, Voula, Vouliagmeni, Aigina) located in the inner Saronikos Gulf and four sites (Lagonissi, Anavyssos, Patroklos, Poros) in the outer Saronikos Gulf (Fig. 1). The study was conducted in August 2013, thus avoiding the possible masking effects of seasonal variability (Alcoverro *et al.,* 1995; Vizzini *et al.,* 2003).

The data required for the application of the indices was collected using a sampling protocol resulting from the combination of sampling methods used for the four indices.

To take into account the spatial variability existing in the different *P. oceanica* metrics (Balestri *et al.,* 2003; Gobert *et al.,* 2003), a hierarchical sampling design was selected. This design focused on variability among meadows on four spatial scales, ranging from meters to tens of kilometres. Four sites separated by tens of km were chosen in each water body (WB). At each site, two 300m^2 zones, 25m apart, were randomly selected, while in each zone three linear 10m transects were randomly selected for the measurements of meadow cover and dead matte cover. In each zone, ten 20×20cm quadrats, separated by at least 1m, were randomly chosen for the measurements of shoot density, plagiotropic rhizomes, and rhizome baring (Fig. 2). In each quadrat, one orthotropic shoot of *P. oceanica* was randomly measured for its shoot length and rhizome baring and then taken as a sample.

To partially minimize spatial variability that may exist among the different meadows on the larger scale (tens of km), factors such as wave exposure, substrate type, and slope of the seabed were taken into account for the selection of sampling sites (meadows) (Balestri *et al.,* 2003). Specifically, all sampling sites were selected in meadows growing on sandy substrate with low or medium bottom slopes and under the same wave exposure regime.

At each study site, data collection and sampling were performed by SCUBA diving at two depths: the intermediate depth of 15m (MedGIG, 2007) and the lower limit of each meadow. The methods used to obtain the data for the application of all the metrics studied are summarized in Table 1 of the Appendix. Further details on laboratory analyses can be found in the respective references.

Assessment of Ecological Status Classes

The ESC for each sampling site was determined by each of the four indices. For all indices, ecological status was classified into one of five ESCs from "High" to "Bad", set within the EQR scale (Table 2) (MedGIG, 2007). The overall status of each WB was determined by averaging the EQR values of the respective four sampling sites.

Assessment of spatial variability of **P. oceanica** *metrics*

The total variance and variance components associated with each spatial factor were estimated using nested analyses of variance (ANOVA) on untransformed data with quadrats nested within zones, zones nested within sites and sites nested within WB. All spatial factors were treated as random. The homogeneity of variance was tested by Levene's test. When the test was significant ($p < 0.05$), a more stringent criterion of α=0.01 was applied to avoid Type I errors (Underwood, 1997).

Assessment of anthropogenic pressures

The anthropogenic pressures were evaluated at each of the studied sites using two simple, time and cost-effective methods, which cumulatively consider all the potential sources of impact: the satellite image method of Lopez y Royo *et al.* (2009) and the Land Uses Simplified Index (LUSI) (Flo *et al.,* 2011). Both methods provide a qualitative visual assessment of human-induced pressures through the analysis of satellite images (Google

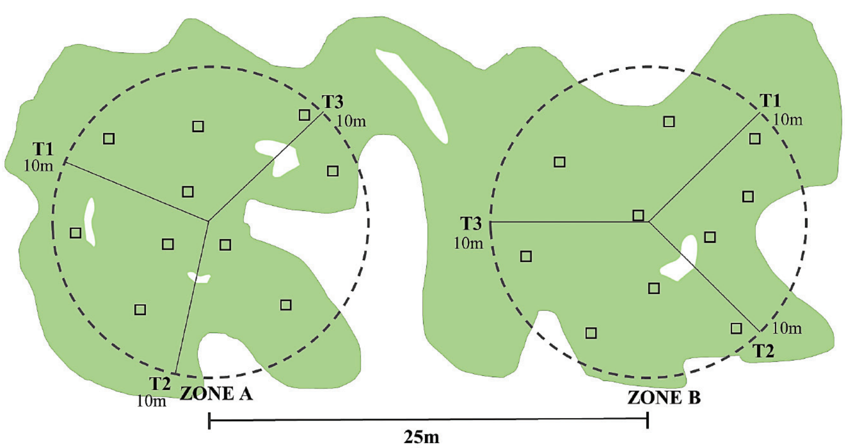

Fig. 2: Sampling design to test the spatial variability within each sampling site. Two circular zones (A, B) of ca. 300 m^2 were marked 25m apart. Shoot density was measured in 10 random quadrats of 20×20 cm in each zone. Meadow cover was estimated along 3 linear 10m transects (T1, T2, T3) in each zone. One orthotropic shoot was randomly sampled from each quadrat. The green polygon is a part of a hypothetical meadow.

Earth, 2013) or land use map data (e.g. CORINE Land Cover 2000 database). The main types of pressure that have been taken into consideration in the method of Lopez y Royo *et al.* (2009) were: land use, industrial activity, river discharges, port activities and artificial structures. Regarding the application of the LUSI index, we selected the LUSIsg version that takes into consideration indirect (land-based: urban, commercial and industrial, agriculture) and direct (sea-based: mariculture, sewage outfall, harbour) anthropogenic pressures within a 3 km radius (MedGIG, 2011).

Biotic indices comparability

The results obtained by each index were compared pairwise both qualitatively and quantitatively. The qualitative comparison was performed using the absolute average class difference (AACD), an indicator recommended by the WFD-GIG (EC*,* 2011). The criterion proposed to define sufficient comparability between classification systems is that of less than a half class (0.5) difference (EC*,* 2011). The quantitative comparison was carried out using non-parametric Spearman's rank correlation analysis between the EQR values obtained by each method. The ability of each biotic index to reflect human-induced pressures was also demonstrated using Spearman's rank correlation analysis. Correlation analyses were conducted using sampling sites as replicates. All statistical analyses were performed using IBM SPSS Statistics 20 (IBM Corp., 2011).

Results

Evaluation of Ecological Status Class

Three of the four indices, specifically PREI, Valencian CS and BiPo, classified seven sites as *Good* ESC and one as *Moderate* (Tables 4, 5, 6). In the case of the POMI index, five sites were classified as *Good* ESC and three as *Moderate* (Table 7).

The ESC of the "Inner (Central) Saronikos" and "Outer Saronikos" WBs was *Good* according to all the applied indices (Tables 4-7).

Evaluation of spatial variability components

Fifteen out of 21 metrics studied showed statistically significant differences of their mean values for at least one of the spatial scales investigated, with most differences observed at site scale (Table 2, Appendix). Components of variation calculated on each of the spatial scales indicate that the spatial scales <10m (among quadrats within-zones) and the scale at 10km (among meadows within-WB) were the most important in explaining total variances (11-77%, mean value=28.2% and 12-85%, mean value=52% of total variance, respectively) (Fig. 1, in the Appendix). The tens of m scale (i.e. among zones within-meadow), on the other hand, was the least important source of variation for all metrics examined (<25% of total variance, mean value= 7.5%).

Table 4. Evaluation of sites and water bodies using the PREI index. Reported values are means ± standard errors. Maximum values are in bold; minimum values are in italic. N score estimated for each metric at each site using the corresponding equations (Table 3, Appendix).

PREI	Sites / Water Body															
	Inner - Central Saronikos Gulf								Outer Saronikos Gulf							
	Floisvos		Voula		Vouliagmeni		Aigina		Lagonissi		Anavyssos		Patroklos		Poros	
Metrics	Mean value	N score	Mean value	N score	Mean value	N score	Mean value	N score	Mean value	N score	Mean value	N score	Mean value	N score	Mean value	N score
Depth lower limit (m)	*15*	-0.214	25	0.500	25	0.714	25	0.714	26	0.786	27	0.857	29	1.214	**29**	1.214
Type of lower limit	Regressive		Regressive		Stable		Stable		Stable		Stable		Progressive		Progressive	
Shoot density (shoots/m²)	*231.3 ± 31.46*	0.321	281.3 ± 30.79	0.409	317.2 ± 21.50	0.473	318.8 ± 28.39	0.476	275.0 ± 27.14	0.398	318.8 ± 16.81	0.476	357.0 ± 33.07	0.543	**406.3 ± 42.21**	0.631
Shoot leaf surface (cm²/shoot)	**321.0 ± 15.55**	0.655	264.7 ± 10.02	0.495	*145.7 ± 13.75*	0.156	221.5 ± 14.00	0.371	199.1 ± 9.29	0.308	318.6 ± 9.29	0.648	207.5 ± 7.50	0.332	180.8 ± 11.18	0.256
Epiphytic/Leave Biomass (E/L)	**0.2 ± 0.02**	0.376	0.0 ± 0.00	0.496	0.2 ± 0.02	0.419	0.0 ± 0.00	0.491	0.1 ± 0.01	0.448	0.1 ± 0.01	0.459	0.1 ± 0.01	0.440	0.0 ± 0.00	0.482
EQR_Site	0.395		0.593		0.558		0.707		0.604		0.734		0.757		0.771	
Status_Site	Moderate		Good		Good		Good		Good		Good		Good		Good	
EQR_WB	0.563								0.717							
Status_WB	Good								Good							

Table 5. Evaluation of sites and water bodies using the Valencian CS index. Reported values are means ± standard errors. Maximum values are in bold; minimum values are in italic. Scores on the first axis (PC 1) of the PCA (not shown).

Valencian CS	Sites / Water Body							
	Inner - Central Saronikos Gulf				Outer Saronikos Gulf			
Metrics	Floisvos	Voula	Vouliagmeni	Aigina	Lagonissi	Anavyssos	Patroklos	Poros
Meadow cover (%)	*58.8 ± 5.91*	76.3 ± 2.39	81.3 ± 3.15	100.0 ± 0.00	75.0 ± 2.04	80.0 ± 2.04	**100.0 ± 0.00**	97.5 ± 1.04
Dead matte cover (%)	**27.5 ± 3.23**	18.8 ± 3.15	17.5 ± 3.23	0.0 ± 0.00	15.0 ± 2.38	11.3 ± 2.38	*0.0 ± 0.00*	2.5 ± 1.04
Shoot density (shoots/m²)	*231.3 ± 31.46*	281.3 ± 30.79	317.2 ± 21.50	318.8 ± 28.39	275.0 ± 27.14	318.8 ± 16.81	357.0 ± 33.07	**406.3 ± 42.21**
Plagiotropic rhizomes (%)	**38.3 ± 5.74**	22.1 ± 5.86	28.5 ± 3.85	16.3 ± 3.19	28.3 ± 4.77	*14.7 ± 4.77*	20.2 ± 2.70	22.3 ± 2.70
Rhizome baring (cm)	*3.3 ± 0.52*	6.3 ± 0.33	3.3 ± 0.53	5.1 ± 0.28	**8.5 ± 0.65**	6.3 ± 0.65	8.0 ± 0.83	5.2 ± 0.22
Shoot leaf surface cm²/shoot)	**321.0 ± 15.55**	264.7 ± 10.02	*145.7 ± 13.75*	221.5 ± 14.00	199.1 ± 9.29	318.6 ± 9.29	207.5 ± 7.50	180.8 ± 11.18
Leaf necrosis (% leaves/shoot)	74.6 ± 5.17	*61.9 ± 3.78*	65.5 ± 2.98	**78.4 ± 4.09**	67.1 ± 2.91	69.1 ± 2.91	78.8 ± 2.49	74.6 ± 3.49
Herbivore pressure (%)	15.7 ± 3.32	1.0 ± 1.00	0.0 ± 0.00	**20.3 ± 5.88**	1.0 ± 1.00	*0.8 ± 1.00*	9.5 ± 3.24	5.2 ± 3.02
Epiphytic biomass (mg/cm²)	**0.928 ± 0.096**	*0.034 ± 0.009*	0.689 ± 0.108	0.072 ± 0.014	0.429 ± 0.024	0.327 ± 0.026	0.533 ± 0.041	0.155 ± 0.019
Score on PC 1 *	-1.660	0.619	-0.494	0.662	0.061	0.889	0.602	0.953
EQR_Site	0.480	0.682	0.584	0.686	0.633	0.705	0.681	0.706
Status_Site	Moderate	Good	Good	Good	Good	Good	Good	Good
EQR_WB	0.608				0.681			
Status_WB	Good				Good			

* Score on PC 1 for "optimal" and "worst" site is 4.340 and -0.597, respectively.

Table 6. Evaluation of sites and water bodies using the BiPo index. Reported values are means ± standard errors. Maximum values are in bold; minimum values are in italic. EQR estimated using the corresponding equation (Table 3, Appendix).

BiPo	Sites / Water Body															
	Inner - Central Saronikos Gulf								Outer Saronikos Gulf							
	Floisvos		Voula		Vouliagmeni		Aigina		Lagonissi		Anavyssos		Patroklos		Poros	
Metrics	Mean value	EQR'	Mean value	EQR'	Mean value	EQR'	Mean value	EQR'	Mean value	EQR'	Mean value	EQR'	Mean value	EQR'	Mean value	EQR'
Depth lower limit (m)	*15*	0.278	25	0.550	25	0.550	25	0.550	26	0.588	27	0.625	29	0.700	**29**	0.700
Type of lower limit	Regressive (0,21)	0.210	Sparse (0,44)	0.440	Sharp (0,66)	0.660	Sharp (0,66)	0.660	Sharp (0,66)	0.660	Sharp (0,66)	0.660	Progressive (0.89)	0.890	Progressive (0.89)	0.890
Shoot density (shoots/m²)	*231.3 ± 31.46*	0.524	281.3 ± 30.79	0.645	317.2 ± 21.50	0.726	318.8 ± 28.39	0.729	275.0 ± 27.14	0.631	318.8 ± 16.81	0.729	357.0 ± 33.07	0.791	**406.3 ± 42.21**	0.833
Shoot length (mm/shoot)	**981 ± 24.05**	1.003	787 ± 32.92	0.740	564 ± 29.75	0.435	823 ± 19.62	0.790	586 ± 22.03	0.464	794 ± 22.03	0.750	*561 ± 14.58*	0.358	619 ± 18.54	0.508
EQR_Site	0.503		0.594		0.593		0.682		0.586		0.691		0.685		0.733	
Status_Site	Moderate		Good		Good		Good		Good		Good		Good		Good	
EQR_WB	0.593								0.674							
Status_WB	Good								Good							

Table 7. Evaluation of sites and water bodies using the POMI index. Reported values are means ± standard errors. Maximum values are in bold; Minimum values are in italic. Scores on the first axis (PC 1) of the PCA (not shown).

POMI 13	Sites / Water Body							
	Inner - Central Saronikos Gulf				Outer Saronikos Gulf			
Metrics	Floisvos	Voula	Vouliagmeni	Aigina	Lagonissi	Anavyssos	Patroklos	Poros
Meadow cover (%)	*58.8 ± 5.91*	76.3 ± 2.39	81.3 ± 3.15	100.0 ± 0.00	75.0 ± 2.04	80.0 ± 2.04	**100.0 ± 0.00**	97.5 ± 1.04
Shoot density (shoots/m²)	*231.3 ± 31.46*	281.3 ± 30.79	317.2 ± 21.50	318.8 ± 28.39	275.0 ± 27.14	318.8 ± 16.81	357.0 ± 33.07	**406.3 ± 42.21**
Plagiotropic rhizomes (%)	**38.3 ± 5.74**	22.1 ± 5.86	28.5 ± 3.85	16.3 ± 3.19	28.3 ± 4.77	*14.7 ± 4.77*	20.2 ± 2.70	22.3 ± 2.70
Shoot leaf surface (cm²/shoot)	**321.0 ± 15.55**	264.7 ± 10.02	*145.7 ± 13.75*	221.5 ± 14.00	199.1 ± 9.29	318.6 ± 9.29	207.5 ± 7.50	180.8 ± 11.18
Leaf necrosis (% leaves/shoot)	74.6 ± 5.17	*61.9 ± 3.78*	65.5 ± 2.98	78.4 ± 4.09	67.1 ± 2.91	69.1 ± 2.91	**78.8 ± 2.49**	74.6 ± 3.49
N content in rhizomes (% dw)	1.24 ± 0.06	1.60 ± 0.33	*0.92 ± 0.12*	1.50 ± 0.22	1.35 ± 0.08	1.08 ± 0.08	1.52 ± 0.14	**1.89 ± 0.20**
P content in rhizomes (% dw)	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	**0.19 ± 0.01**	0.09 ± 0.02	0.07 ± 0.01
Total n-s carbohydrates (% dw)	25.2 ± 1.75	30.1 ± 1.11	4.2 ± 0.27	*2.0 ± 0.22*	35.7 ± 1.29	15.0 ± 1.29	19.4 ± 0.92	**44.5 ± 1.07**
$\delta^{15}N$ ratio in rhizomes (‰)	**7.1 ± 0.44**	5.5 ± 1.02	5.4 ± 0.39	5.3 ± 0.60	2.8 ± 0.20	5.2 ± 0.20	*2.7 ± 0.84*	3.1 ± 0.38
Epiphyte N content (% dw)	0.4 ± 0.01	*0.0 ± 0.00*	0.6 ± 0.05	1.1 ± 0.00	1.1 ± 0.00	**1.9 ± 0.02**	1.5 ± 0.16	0.9 ± 0.00
[Cu] in rhizomes (μg/g)	12.5 ± 0.58	9.8 ± 0.89	13.6 ± 0.56	*6.6 ± 1.40*	14.3 ± 1.90	**18.8 ± 0.85**	10.1 ± 0.99	16.4 ± 1.40
[Pb] in rhizomes (μg/g)	3.5 ± 0.86	**4.1 ± 0.37**	3.0 ± 0.30	2.5 ± 0.80	2.6 ± 0.27	4.0 ± 1.13	*0.6 ± 0.12*	3.6 ± 0.60
[Zn] in rhizomes (μg/g)	67.0 ± 8.55	105.8 ± 5.64	*66.0 ± 10.67*	**146.5 ± 14.00**	75.3 ± 6.84	91.8 ± 4.77	92.4 ± 14.10	67.6 ± 5.60
Score on PC 1	0.402	-0.252	-0.003	0.225	-0.311	0.997	-0.753	-0.485
EQR_Site	0.531	0.581	0.562	0.544	0.585	0.485	0.619	0.599
Status_Site	Moderate	Good	Good	Moderate	Good	Moderate	Good	Good
EQR_WB	0.555				0.572			
Status_WB	Good				Good			

* Score on PC 1 for "optimal" and "worst" site is -5.840 and 6.020, respectively.

Evaluation of anthropogenic pressures

The results of the evaluation of human pressures at each study site are summarized in Tables 8 and 9. Both methods produced the same results and reached total agreement. The results indicate that one site was classified as subject to high pressures, five to moderate, and two to low pressures. Therefore, six out of the eight sites are subject to significant human-induced pressures (high-moderate).

Spearman's correlation coefficients revealed that the ESCs estimated by all indices were negatively correlated and thus, tended to decrease with the evaluation of anthropogenic pressures (Table 10). Specifically, two indices, PREI and BiPo, presented significantly high correlations with human pressures (Table 10).

Response of metrics to anthropogenic pressures

Correlations between metrics and anthropogenic pressures proved statistically significant for most of the metrics (15 out of 21). In particular, significantly high correlations were primarily observed in metrics belonging to the population and individual level, while most of the metrics for the physiological and biochemical level (e.g. Cu, Pb, Zn, P and total n-s carbohydrates in rhizomes) did not show significant correlation (Table 11).

Comparability of biotic indices

The absolute average class difference (AACD) results exhibit high comparability between the PREI, Valencian CS and BiPo indices (100% agreement) (Fig. 3). Sufficient comparability was also observed between these three indices and POMI: all pairwise comparisons showed an AACD <0.5 below the proposed criterion (75% agreement) (Table 12).

Spearman's correlation coefficients revealed that EQR values resulting from the application of each index were all positively correlated (Table 13). However, high significant correlation occurred only for the EQR values obtained by the application of the PREI, Valencian CS and BiPo indices ($p < 0.05$) (Table 13).

Discussion

The seagrass *P. oceanica*, being sensitive to environmental deterioration, has proven to be a useful indicator for the assessment of coastal ecosystems in the Mediterranean Sea. The *Posidonia oceanica* biotic indices applied

Table 8. Assessment of pressures for each type of pressure and overall evaluation for each site, using the method of Lopez y Royo *et al.* (2009). For rivers, industries and ports the distances from the site in km (within an area of 15 km radius), "No" when absent from the considered range. Agricult. Agricultural; Ind. Industrial; Com. Commercial; Recr. Recreational.

Sites	Landuse (%)			River (km)	Industry (km)	Ports (km)			Artificial Structures	Pressure Score	Pressure Evaluation
	Urban	Agricult.	Natural			Ind.	Com.	Recr.			
Floisvos	100	0	0	5	5	11	9	1	Yes	3	High
Voula	85	0	0	11	12	No	No	2	Yes	2	Moderate
Vouliagmeni	44	7	48	No	No	No	No	1	Yes	2	Moderate
Aigina	27	38	33	No	No	No	No	1	Yes	2	Moderate
Lagonissi	85	0	6	No	No	No	No	9	Yes	2	Moderate
Anavyssos	22	25	51	No	No	No	No	2	Yes	2	Moderate
Patroklos	12	20	68	No	No	No	No	7	Yes	1	Low
Poros	8	48	42	No	No	No	No	3	Yes	1	Low

Table 9. Assessment of pressures for each type of pressure and overall evaluation for each site, using the Land Uses Simplified Index (LUSI). Pressures evaluated within an area of 3 km radius, "No" when absent from the considered range. Confinement: refer to different types of coastline (correction numbers: Convex 0.75; Straight 1; Concave 1.25). Ind. Industrial; Artif. Artificial; Com. Commercial;

Sites	Landuse (%)				Sea-based				Confine-ment	Pressure Score	Pressure Evaluation
	Urban	Ind.-Artif.	Agri-cult.	Natu-ral	Sewage outfall	Aqua-cult.	Freshwa-ter input	Com. port			
Floisvos	88	12	0	0	Yes	No	Yes	Yes	Straight	7	High
Voula	66	34	0	0	No	No	No	No	Straight	3	Moderate
Vouliag-meni	30	29	5	36	No	No	No	No	Concave	3.75	Moderate
Aigina	0	0	75	25	No	No	No	No	Straight	3	Moderate
Lagonissi	34	4	18	43	No	No	No	No	Straight	3	Moderate
Anavyssos	19	0	44	36	No	No	No	No	Straight	3	Moderate
Patroklos	0	0	17	83	No	Yes	No	No	Convex	1.5	Low
Poros	8	0	42	50	No	No	No	No	Convex	1.5	Low

Table 10. Spearman's correlation coefficients (r) between the EQR values and pressures. LR: Lopez y Royo et al. (2009) method.

		PREI	BiPo	Valencian CS	POMI
LR *	Correlation Coef.	-0.913	-0.913	-0.730	-0.391
	p-value (two tailed)	0.002	0.002	0.040	0.338
	N	8	8	8	8
LUSI	Correlation Coef.	-0.932	-0.792	-0.702	-0.715
	p-value (two tailed)	0.001	0.019	0.052	0.046
	N	8	8	8	8

in this study allowed integration of relevant ecological information into an overall expression of ecological integrity (Martinez-Crego *et al.*, 2010).

Prior to the assessment of the ESC of the study area, the magnitude of potential spatial variability existing in *P. oceanica* meadows was investigated. According to our results, *P. oceanica* meadows exhibited a within-meadow heterogeneity that mainly exists on a smaller than 10m spatial scale. In particular, the high variability that was observed among quadrats within-zones (mean value=28.2%), along with the low variability detected among zones (mean value=7.5%), indicates that the recorded within-meadow heterogeneity may be caused by patchiness on the smaller spatial scales (Panayotidis *et al.,* 1981; Balestri *et al.,* 2003). According to Bennett *et al.* (2011), the above findings suggest that the spatial replication design (more than one spatial scale and multiple zones at each study site) that was employed in this study can be considered adequate to capture within-meadow heterogeneity. Thus, the estimates of the mean values of the different metrics can be considered good enough to provide an unbiased assessment of ESC at each site (Balestri *et al.,* 2003; Romero *et al.,* 2007).

Overall, our results on the ESC of the Saronikos Gulf seem to be quite close to the general ecological quality gradient that has been recorded along the coasts of the Gulf in the context of previous integrative studies on different aspects of the marine environment (Simboura *et al.*, 2005; 2014; Tsiamis *et al.,* 2013). Indeed, given that qualitative agreement of all indices is high (75%) as regards ESC assessment, they classified the study site located in the proximity of Athens metropolitan area and within the impact zone of the WWTP as *Moderate* ESC. In contrast, with the exception of the POMI index whose ESC assessment was partially differentiated, the remaining three indices (PREI, Valencian CS and BiPo) classified the sites located in the outer part of the Gulf as *Good* ESC.

Table 11. Spearman's correlation coefficients (r) between metrics and anthropogenic pressures. LR: Lopez y Royo et al. (2009) method.

Metrics		LUSI	LR *	Metrics		LUSI	LR *
Depth lower limit (m)	Correlation Coef.	-0.895	-0.893	Epiphytic biomass (mg/cm^2)	Correlation Coef.	0.341	0.22
	p-value (two tailed)	0.003	0.003		p-value (two tailed)	0.000	0.005
	N	8	8		N	160	160
Meadow cover (%)	Correlation Coef.	-0.676	-0.723	Epiphytic/Leave Biomass (E/L)	Correlation Coef.	0.38	0.266
	p-value (two tailed)	0.000	0.000		p-value (two tailed)	0.000	0.001
	N	48	48		N	160	160
Dead matte cover (%)	Correlation Coef.	0.708	0.676	N content in rhizomes (% dw)	Correlation Coef.	-0.532	-0.429
	p-value (two tailed)	0.000	0.000		p-value (two tailed)	0.002	0.014
	N	48	48		N	32	32
Shoot density (shoots/m2)	Correlation Coef.	-0.289	-0.333	P content in rhizomes (% dw)	Correlation Coef.	0.142	0.193
	p-value (two tailed)	0.001	0.000		p-value (two tailed)	0.438	0.291
	N	160	160		N	32	32
Plagiotropic rhizomes (%)	Correlation Coef.	0.262	0.208	Total n-s carbohydrates (% dw)	Correlation Coef.	-0.347	-0.252
	p-value (two tailed)	0.003	0.021		p-value (two tailed)	0.052	0.165
	N	160	160		N	32	32
Rhizome baring/burial (cm)	Correlation Coef.	-0.359	-0.24	$\delta^{15}N$ ratio in rhizomes (‰)	Correlation Coef.	0.67	0.67
	p-value (two tailed)	0.000	0.011		p-value (two tailed)	0.000	0.000
	N	160	160		N	32	32
Shoot leaf surface (cm^2/shoot)	Correlation Coef.	0.232	0.412	N content in epiphytes (% dw)	Correlation Coef.	-0.482	-0.451
	p-value (two tailed)	0.003	0.000		p-value (two tailed)	0.013	0.021
	N	160	160		N	32	32
Shoot length (mm/shoot)	Correlation Coef.	0.412	0.579	Cu content in rhizomes (µg/g)	Correlation Coef.	-0.348	-0.418
	p-value (two tailed)	0.000	0.000		p-value (two tailed)	0.051	0.017
	N	160	160		N	32	32
Leaf necrosis (% leaves/shoot)	Correlation Coef.	-0.116	-0.098	Pb content in rhizomes (µg/g)	Correlation Coef.	-0.191	-0.191
	p-value (two tailed)	0.144	0.219		p-value (two tailed)	0.295	0.295
	N	160	160		N	32	32

Coupled with the total qualitative agreement (100%) of the PREI, Valencian CS and BiPo indices, the significantly high correlation (r >0.70) of their EQR values with human pressures [as evaluated following the two methods of Flo *et al.* (2011) and Lopez y Royo *et al.* (2009)] suggest that these three indices respond sufficiently to environmental impairment of the study area, and thus present adequate broad-scale applicability. Nevertheless, the usefulness of the applied indices for management purposes is primarily determined by a set of key properties such as sensitivity to environmental alteration, specificity to stressors, relevance to ecological integrity and early-detection capacity (Martinez-Crego *et al.*, 2010).

According to Roca *et al.* (2015), the properties mentioned above are defined by the various types of metrics that each index takes into consideration. Since all indices applied in this study are based, at least, on the structural or functional attributes of the individual, population or community level, they all have the ability to provide an integrative view of the ecological integrity of the marine environment (Martinez-Crego *et al.*, 2010). This fact is also enhanced by the results of correlation analysis, where the respective individual, population and community metrics showed the highest correlations with human pressures. However, as highlighted by Martínez-Haro *et al.* (2015) among others, it is expected that biotic indices whose metrics refer only to the previously mentioned biotic levels –as in the case of PREI, Valencian CS and BiPo– lack specificity to stressors and early-detection capacity. By contrast, the POMI index also integrates biochemical and physiological metrics that potentially increase its sensitivity to stressors and early-detection efficiency (Martinez-Crego *et al.,* 2010). Still, most of these metrics (e.g. Cu, Pb, Zn, P concentration and total ns carbohydrates in rhi-

Table 12. The results of qualitative comparison using the Absolute Average Class Difference (AACD).

	AACD	Agreement (%)	1 Class Difference (%)
PREI - BiPo	0	100	0
PREI - Valencian CS	0	100	0
PREI - POMI	0.25	75	25
BiPo - Valencian CS	0	100	0
BiPo - POMI	0.25	75	25
POMI - Valencian CS	0.25	75	25

zomes) did not show significant statistical correlations with human pressures. Therefore, it is apparent that in this study area all indices can provide only a broad assessment of the EQS and thus, cannot significantly help to identify and discriminate the multiple stress factors.

The level of qualitative disagreement (25%) observed in the classification of the ESC between the POMI and the remaining three indices (PREI, Valencian CS and BiPo), along with the low level of quantitative agreement of their EQR values, may indicate the existence of essential differences due to the definition of the reference conditions, and/or different types of metrics used, and/or different weighting of each metric.

Table 13. Spearman's correlation coefficients (r) between the EQR values obtained by the four indices.

		PREI	BiPo	Valencian CS	POMI
PREI	Correlation Coef.	1	0.905	0.810	0.452
	p-value (two tailed)		0.002	0.015	0.260
	N	8	8	8	8
BiPo	Correlation Coef.	0.905	1	0.905	0.286
	p-value (two tailed)	0.002	.	0.002	0.493
	N	8	8	8	8
Valencian CS	Correlation Coef.	0.810	0.905	1	0.143
	p-value (two tailed)	0.015	0.002	.	0.736
	N	8	8	8	8
POMI	Correlation Coef.	0.452	0.286	0.143	1
	p-value (two tailed)	0.260	0.493	0.736	.
	N	8	8	8	8

Regarding the definition of RC in relation to human pressures, the POMI index shares the same method with Valencian CS. Hence, its different ESC assessment can probably be explained by the different types of metrics and/or the different weighting given to each metric that it takes into consideration. It is likely that the physiological metrics of the POMI index (e.g. Zn, Cu, Pb, or P concentrations in rhizomes) play a determinant role in the final ESC assessment because of their different weighting in the application of the index. Indeed, since reference values under the POMI index were defined by the dataset available for the case study area, the weighting given to each metric was determined by the optimal and worst values taken into account in the respective spatial extent. Nevertheless, the Zn, Cu, Pb, or P concentrations reported in this study are not indicative of the existence of environmental stress (Campanella *et al.*, 2001). Thus, the resulting assessment of ESCs provided by the POMI index was probably misleading (Fig. 3, Table 7).

Given the correlation results of the EQR values and individual metrics with the estimated anthropogenic pressures, it is apparent that both methods used for the assessment of human pressures could be considered relatively conservative and may not always be sufficient for the identification of the pressure sources. In other words, although visual assessment of pressures based on satellite images

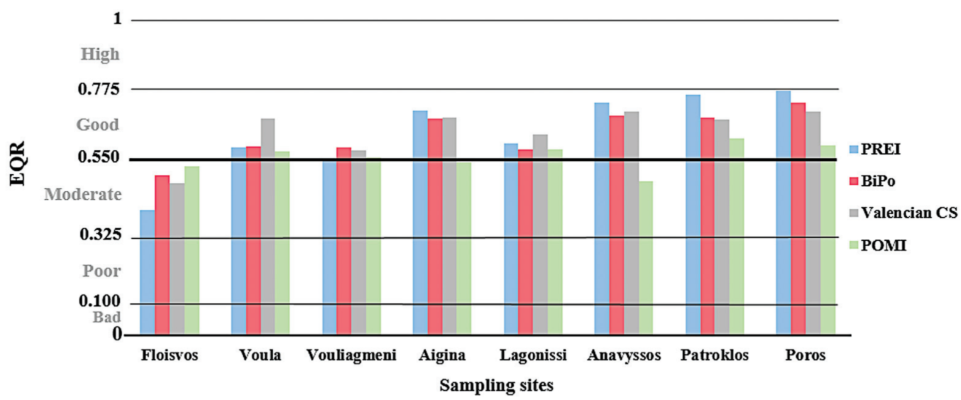

Fig. 3: EQR values and classification of sampling sites according to the four indices under study. The differences in EQR values among PREI, Valencian CS and BiPo were on average of 0.043±0.005, whereas their comparisons with the POMI index indicate average differences of 0.122±0.017.

or land uses is considered adequate and evaluates human-induced pressures on the coastal environment reliably, not all sources of pressure or special conditions that may occur only at local scale were taken into consideration. Both methods used could be considered reliable only in terms of broad assessment (Lopez y Royo *et al.,* 2009; Bacci *et al*., 2013). The use of an enriched with water and sediment key abiotic factors dataset may thus be useful for both an accurate identification of all sources of impact that can affect the meadows of the study area, and discrimination between pressures (Lopez y Royo *et al.,* 2009; Bacci *et al*., 2013).

Additionally, Lopez y Royo *et al.* (2009) noted the need for more precise identification of pressures in the case of classifications where EQR values are close to the boundary of *Good*/*Moderate* ecological status (EQR=0.550), as observed at the sites of Vouliagmeni and Aigina when classified by the POMI index. In such cases, the risk of misclassification is significantly high (up to 50%) (Bennett *et al.,* 2011); hence, it is possible that they have been subjected to pressures shifting their ESC from *Good* to *Moderate* (Lopez y Royo *et al.*, 2009).

Our results on the classification of WBs (*Good* status for both WBs: "Inner-Central Saronikos Gulf" and "Outer Saronikos Gulf") are not completely consistent with a previous integrative classification of the Gulf by Simboura *et al.* (2014). Specifically, the classification of the "Inner-Central Saronikos Gulf" WB as *Good* ESC by all indices proved less conservative than the classification given by other BQEs (macroalgae, macroinvertebrates), which classified the WB as *Moderate* ESC.

The fact that the WBs of the study area are subject to several sources of anthropogenic pressures indicates that the ESC assessment based on *P. oceanica* biotic indices should be considered accordingly (Mascaro *et al.,* 2012). In such cases, it is possible that the effects of human pressures are unevenly distributed among the different *P. oceanica* meadows in each WB. This widens the natural variability among meadows within a WB, and thus potentially increases the level of uncertainty of the ESC classification of *P. oceanica* meadows (Mascaro *et al.,* 2012).

As also noted by Mascaro *et al.* (2012), such high levels of variability among meadows within a WB might be due to either a high natural heterogeneity of meadows or a possible inadequate definition of the spatial extent and number of WBs in the study area. Indeed, the spatial replication design used in this study revealed the existence of spatial heterogeneity on the larger spatial scale (tens of km: among sites (meadows) within a WB). It is possible that an adequate spatial replication design could capture the extra variability caused by anthropogenic pressures. However, the differences in mean EQR values among different meadows of the same WB were high (e.g. PREI: 0.395 - 0.707 in WB "Inner Central Saronikos"), and a greater replication effort will not be able to reduce the uncertainty associated with the classification system (Mascaro *et al.,* 2012). A possible redefinition of the spatial extent and the number of WBs in the study area may therefore be needed to ensure that the classification of the spatial extent of coastal WBs adequately reflects their water quality and the human pressures to which the coastline is exposed (EC, 2000; Mascaro *et al.,* 2012). Nevertheless, the EQR values obtained by all indices for the "Inner-Central Saronikos Gulf" WB are close to the boundary of *Good*/*Moderate* ecological status (EQR=0.550). This different classification of *P.oceanica* biotic indices may be due to the significantly high (up to 50%) risk of misclassification that exists in such cases (Bennett *et al.,* 2011).

In conclusion, the *P. oceanica* biotic indices examined in this study provided a broad assessment of the ecological quality status of the Saronikos Gulf's coastal waters. Our findings suggest that only three out of the four indices (PREI, Valencian CS and BiPo) can provide an overall view of the cumulative impact of multiple environmental stressors existing at two different spatial scales (site, WB) in the study area, and can thus help raise awareness of ecosystem degradation. In comparison, the fourth index (POMI) provided a corresponding assessment of environmental impairment only at WB spatial scale.

Moreover, it is apparent that our results regarding the broad assessment of ecological quality status and human-induced pressures in the study area cannot adequately support the determination of the appropriate remedial actions to be implemented by decision-makers and managers. Hence, precise estimation of human-induced pressures and identification of their sources proved to be a critical point for validating the effectiveness of the indices for both ESC assessment and decision-making.

Still, it should be noted that our findings are based on a single case study, where the actual dataset on *P. oceanica* used for the evaluation of biotic indices is rather limited, particularly regarding the availability of data from critically degraded meadows (belonging to the *Poor* ESC). Therefore, it is evident that further research on the application of *P. oceanica* biotic indices in different case studies with known environmental gradients in the Aegean and Ionian Seas could contribute significantly to drawing conclusions on the larger scale of the Eastern Mediterranean basin.

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APPENDIX

Table 1. Methods and sampling protocols used to assess the population, individual and community metrics.

Metric (unit)	Sample size per zone (site)	Method
Lower depth limit (m)	-	Noted in situ by scuba diver
Type of lower limit	-	Noted in situ by scuba diver according to both classifications of Meinesz and Laurent (1978) for PREI and Pergent *et al.* (2015) for BiPo.
Meadow cover (%)	3 (6) in situ measurements	Estimated using the Line Intercept Transect (LIT) methodology (Montefalcone *et al.,* 2007b) along three transect lines of 10 m placed in three random directions.
Dead matte cover (%)	3 (6) in situ measurements	Estimated using the Line Intercept Transect (LIT) methodology (Montefalcone *et al.,* 2007b) along three transect lines of 10 m placed in three random directions.
Shoot density (shoots m^{-2})	10 (20) in situ measurements	Shoot number was counted in 10 (20×20 cm) quadrats randomly set along the three replicate line transects.
Plagiotropic rhizomes (%)	10 (20) in situ measurements	In situ observation of plagiotropic rhizomes in the same 10 replicate quadrats (20×20 cm) used for shoot density
Rhizome baring (cm)	10 (20) in situ measurements	In situ measurement of the distance between the sediment surface and leaf ligula (base of the leaves) minus 2 cm using a ruler (Boudouresque *et al.,* 1980) in 10 randomly selected orthotropic rhizomes.
Shoot leaf surface (cm^2/shoot)	10 (20) shoots	Measurement of total leaf surface per shoot according to the methodology of Giraud (1979)
Shoot length (mm/shoot)	10 (20) shoots	In situ measurement of the longest leaf (distance between the sediment surface and leaf tip) in mm using a tape measure.
Leaf necrosis (% leaves/shoot)	10 (20) shoots	Calculated as the percentage of leaves with necrosis marks per shoot.
Herbivore pressure (%)	10 (20) shoots	Calculated as the percentage of leaves with bite marks per shoot.
Epiphytic biomass (mg/cm^2)	10 (20) shoots	Measurement of dry weight of epiphytes per shoot. Epiphytes removed with a razor blade and epiphytes dried at 60°C for 48 h (Dauby and Poulicek, 1995)
Epiphytic/Leave biomass (E/L)	10 (20) shoots	Measurement of dry weights of epiphytes and leaves per shoot. Both epiphytes and leaves dried at 60°C for 48 h.

Table 2. Summary of comparisons between WBs, sites, zones and quadrats for all metrics using nested ANOVA.

Source of Variation	Meadow cover (%)				Dead matte cover (%)				Rhizome baring/burial (cm)				Shoot leaf surface (cm²/shoot)			
	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
WB	1	854.297	0.76	0.418	1	875.521	1.57	0.257	1	93.006	3.50	0.110	1	5476.770	0.06	0.817
Sites (WB)	6	1130.859	31.13	0.000	6	558.594	24.10	0.000	6	28.170	9.66	0.002	6	93564.617	9.77	0.003
Zones (Sites (WB))	8	36.328	1.66	0.147	8	23.177	1.49	0.200	8	2.892	0.81	0.599	8	9576.361	2.27	0.026
Error	32	21.891			32	15.552			144	3.586			144	4220.111		

Source of Variation	Shoot length (mm/shoot)				Leaf necrosis (% leaves/shoot)				Herbivore pressure (%)				Epiphytic biomass (mg/cm²)			
	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
WB	1	883263.540	2.18	0.191	1	205.436	0.24	0.644	1	1048.593	0.86	0.390	1	0.195	0.09	0.779
Sites (WB)	6	405719.422	14.34	0.001	6	868.953	1.44	0.309	6	1223.145	22.88	0.000	6	2.261	11.49	0.001
Zones (Sites (WB))	8	28297.120	2.60	0.011	8	603.814	2.58	0.012	8	53.455	0.30	0.964	8	0.197	3.69	0.001
Error	144	4220.111			144	233.922			144	176.342			144	0.053		

Source of Variation	Epiphytic/Leave Biomass (E/L)				N content in rhizomes (% dw)				P content in rhizomes (% dw)				Total n-s carbohydrates (% dw)			
	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
WB	1	0.024	0.16	0.703	1	0.143	0.35	0.577	1	0.000	0.07	0.795	1	1448.029	1.78	0.231
Sites (WB)	6	0.148	17.32	0.000	6	0.413	2.28	0.139	6	0.006	8.18	0.004	6	818.353	139.54	0.000
Zones (Sites (WB))	8	0.009	2.67	0.009	8	0.181	2.09	0.093	8	0.001	1.19	0.357	8	5.873	1.87	0.130
Error	144	0.003			18	0.087			18	0.001			18	3.148		

Source of Variation	$\delta^{15}N$ ratio in rhizomes (‰)				N content in epiphytes (% dw)				Cu content in rhizomes (µg/g)				Pb content in rhizomes (µg/g)			
	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
WB	1	45.136	9.99	0.019	1	3.833	7.25	0.036	1	444.329	4.29	0.084	1	0.598	0.06	0.822
Sites (WB)	6	4.541	2.29	0.138	6	0.608	15.85	0.002	6	104.072	21.37	0.000	6	10.844	15.20	0.001
Zones (Sites (WB))	8	1.990	1.93	0.118	8	0.045	10.20	0.000	8	4.870	1.00	0.470	8	0.711	0.48	0.852
Error	18	1.031			18	0.004			18	4.881			18	1.471		

Source of Variation	Zn content in rhizomes (µg/g)				Shoot density (shoots/m²)				Plagiotropic rhizomes (%)			
	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
WB	1	91.571	0.01	0.919	1	93847.656	1.689	0.241	1	2013.703	1.73	0.237
Sites (WB)	6	8241.565	22.98	0.000	6	55576.823	1.099	0.438	6	1164.993	2.41	0.124
Zones (Sites (WB))	8	358.823	1.12	0.399	8	50582.031	4.329	0.000	8	483.075	1.99	0.052
Error	18	321.687			144				144			

Table 3. Equations used by each index for the calculation of EQR.

Biotic index	EQR' equations	Overall EQR equations
BiPo (Lopez y Royo *et al.,* 2009)	$EQR' = \left(\left(\frac{X-LB}{HB-LB}\right) \times 0{,}225\right) + LB$ X = value measured, LB = lower boundary value of class to which X corresponds, HB = higher boundary value of class to which X corresponds, 0.225 is the width of a class on the EQR scale.	$EQR = \frac{EQR'_{LL\ depth} + EQR'_{LLtype} + EQR'_{density} + EQR'_{shoot\ length}}{4}$
PREI (Gobert *et al.,* 2009)	$EQR' = \frac{N_{density} + N_{leaf\ surface\ area} + N\left(\frac{E}{L}\right) + N_{lower\ limit}}{3{,}5}$ $N_{density}$ = value measured/reference value; $N_{shoot\ leaf\ surface\ area}$ = value measured/ reference value; $N_{E/L}$ = [1-(E/L)]*0.5 $N_{lower\ limit}$ = (N'-worst value)/(reference value–worst value). N' = depth noted on the field + k, where k = 0 (stable limit), k =3 (progressive limit) or k = -3 (regressive limit).	$EQR = \frac{EQR' + 0{,}11}{1 + 0{,}10}$
POMI (Romero *et al.,* 2007) – **Valencian CS** (Fernandez-Torquemada *et al.,* 2008)	$EQR' = \frac{CL_X - Clworst}{Cl_{optimal} - Clworst}$ EQR'_x is the ecological quality of the site x; CI_x is the score of the site x on the 1st component; $CI_{optimal}$ is the score of the 'optimal' site (reference site) on the 1st component; CI_{worst} is the score of the 'worst' site on the 1st component.	$EQR = \frac{EQR' + 0{,}11}{1 + 0{,}10}$

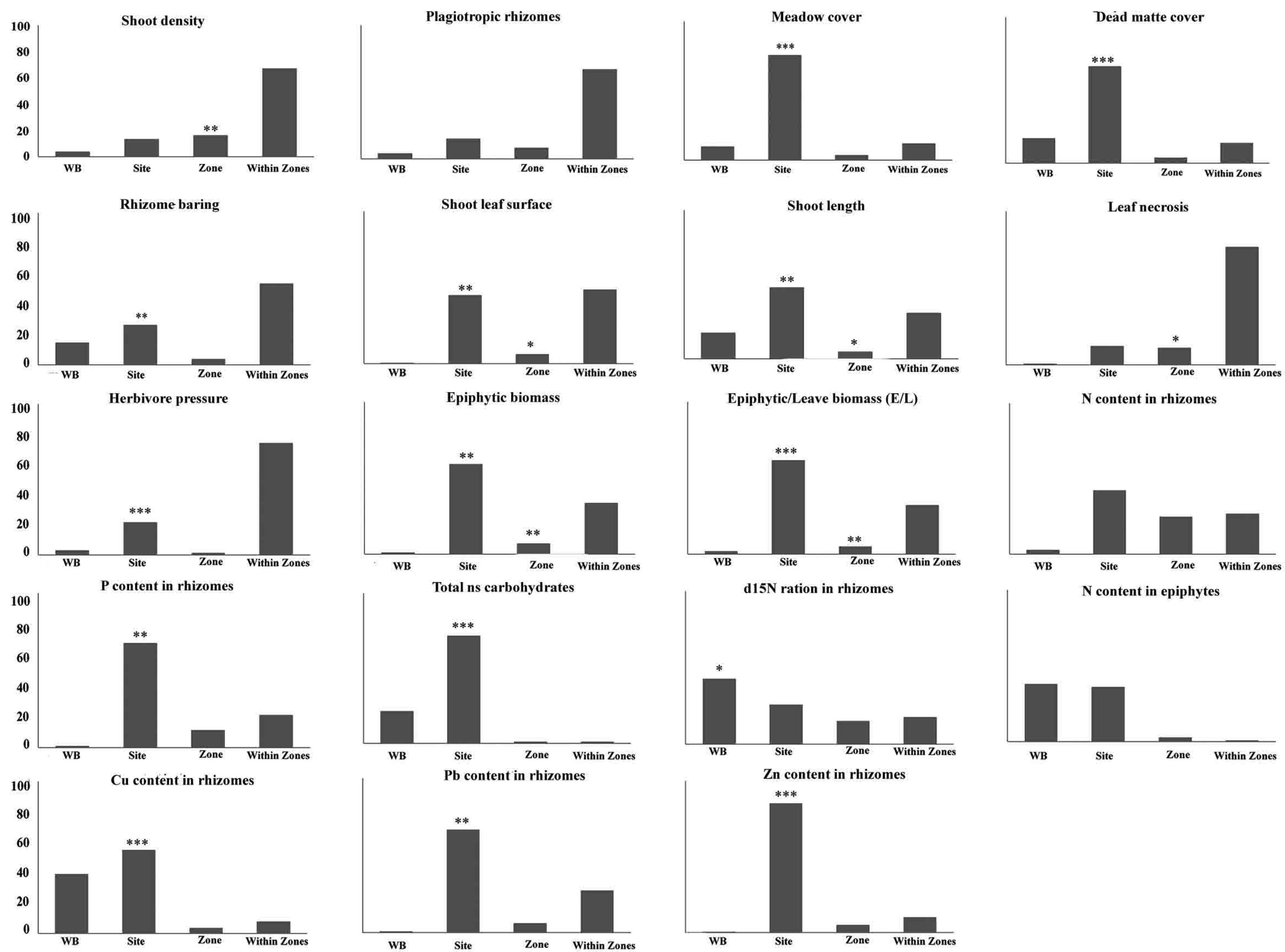

Fig. 1: Components of variation at different spatial scales for all metrics studied. Overall variation is partitioned among spatial scales and expressed as percentage of the total. Asterisks indicate the spatial scales where significant differences were recorded among water bodies, sites, zones, within zones (i.e. among quadrats).*p<0.05, ** p<0.01, ***p<0.001.