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Application of a new multi-metric index with a comparative analysis for assessing the environmental status of *Posidonia oceanica* meadows (Algeria, Southern Mediterranean)

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Abstract

Several biotic indices have been developed over the last two decades to assess water and habitat quality in the Mediterranean Sea, with *Posidonia oceanica* serving as a Biological Quality Element (BQE). To contribute to this effort, we created a new Multi-metric Index for the Algerian coast based on *P. oceanica* (MIAPo), adapted from existing indices and calibrated at eight sites with varying human pressures. This study also intends to compare the performance of MIAPo and PREI (*P. oceanica* Rapid Easy Index) in assessing the ecological status of coastal Algerian waters. MIAPo was built using multivariate analyses that combined five metrics: lower limit depth, leaf area index, epiphytic load, leaf tannin cell density, and leaf nitrogen content. To accurately interpret MIAPo and PREI, a Pressure Index (PI) was calculated, taking into account urbanization, agriculture, coastal population, uncontrolled dumping, fishing activity, rivers, industrial activity, and urban effluents. According to the Water Framework Directive (WFD) classification, the indices MIAPo and PREI demonstrated high comparability in the ecological status assigned to the studied sites (62.5% agreement), which was validated using Spearman's correlation coefficient ($R = 0.88$; $p = 0.004$). In addition, MIAPo and PREI show a strong correlation with the PI ($R = -0.96$; $p = 0.0002$ and $R = -0.80$; $p = 0.02$, respectively). The proposed index, MIAPo, provides a more comprehensive measure than PREI for the assessment and monitoring of Algerian coastal waters. Nonetheless, more field data collection is needed to expand the use of this valuable tool on a broader geographical scale.

Keywords: *Posidonia oceanica*; Biological indicator; Pressure-impact relationship; Water quality; Ecological status.

Introduction

The biological indicator *Posidonia oceanica* is widely used as a Biological Quality Element (BQE) under the Water Framework Directive (WFD, 2000/60/EEC). Several multi-metric indices have been proposed to assess the ecological quality of coastal waters based on plant characteristics and/or meadow structure: the "PosSte" (Buia *et al.,* 2004), the "POMI" (Romero *et al.*, 2007), the "Valencian CS" (Fernández Torquemada *et al.*, 2008), the "PREI " (Gobert *et al.*, 2009), the "BiPo" (Lopez y Royo *et al.*, 2010a) and "the Vitality Index" (Pergent *et al.*, 2015) (Table 1). To comply with the WFD, these indices must meet a set of required criteria, including (i) a significant relationship between the Ecological Quality Ratio (EQR) and anthropogenic pressures and (ii) the use of a common scale of five ecological status classes: High, Good, Moderate, Poor and Bad.

Several studies analyzed and compared these multi-metric indices (including the number and type of metrics, survey depth, metric aggregation method, and reference condition definition) as well as their performance across pressure gradient (Table 1). BiPo and PREI appear to be the most widely adopted tools for quality water assessment due to their rapid field-based protocols, ease of use, and low cost (Table 1). On the other hand, the performance of multi-metric indices based on *P. oceanica* metrics to assess water quality appears to be related to the number of metric types used, as demonstrated by Bennett *et al.* (2011) and Mascaró *et al.* (2012).

Moreover, the intricate nature of seagrass habitats has led numerous studies to underscore the importance of employing multiple indices derived from various metrics to evaluate the ecological status of *P. oceanica* meadows (Montefalcone *et al.*, 2006; Martínez-Crego *et al.*, 2010; Lopez y Royo *et al.*, 2011; Güreşen *et al.*, 2020a; Manci-

Table 1. Multi-metric indices based on *P. oceanica* to assess the ecological status of Mediterranean coastal waters within the WFD classification (metrics used for the reduced versions POMI 9 and POMI 5 are superscripted by "9" and "5", respectively).

Indices	Regions of development and other application regions	Methods	Metrics and depths	Reference conditions	Pressures tested
PoSte system for coastal water classification using *P. oceanica*	**Italy** (Buia *et al.*, 2004) Catalonia (Spain) and Corsica (France) Ischia (Italy) (Lopez y Royo *et al.*, 2011)	Combination of 5 metrics in a data warehouse	<u>At intermediate depth (15 m)</u> Shoot density, intermediate leaf width, leaf production, rhizome production, rhizome elongation.	Existing pristine/undisturbed sites	/
POMI: *Posidonia oceanica* Multivariate Index	**Catalonia (Spain)** (Romero *et al.*, 2007) Catalonia (Spain) and Corsica (France) (Lopez y Royo *et al.*, 2011); Saronikos (Greece) (Gerakaris *et al.*, 2017) **POMI 9: Catalonia (Spain)** (Bennett *et al.*, 2011) **POMI 5: Catalonia, Balearic Islands and Croatia** (Mascaró *et al.*, 2012)	Combination of 14 metrics using PCA	<u>At intermediate depth (15 m)</u> Shoot density^{9}, meadow cover ^{9,5}, percentage of plagiotropic rhizomes, shoot leaf area ^{9,5}, percentage of foliar necrosis ^{9}, N and P rhizomes content, sucrose rhizomes content ^{9,5}, $\delta^{15}N$ ^{9,5} and δ ^{34}S ^{9} isotopic ratio in rhizomes, N epiphyte content ^{9,5}, Cu, Pb^{9} and Zn in rhizomes.	A composite site from existing values and expert judgment	Coastline constructions, beach regeneration, urban sewage, agricultural and urban soil use, tourism, fishing, recreational, fishing , commercial and industrial harbors
CS Valencian	**Valencia (Spain)** (Fernández Torquemada *et al.*, 2008) Saronikos Gulf (Greece) (Gerakaris *et al.*, 2017) Tremiti Islands (Italy) (Tursi *et al.*, 2022a)	Combination of 9 metrics using PCA	<u>At intermediate depth (14-17 m)</u> Shoot density, meadow cover, dead matte cover, percentage of plagiotropic rhizomes, rhizome baring/burial, shoot leaf area, percentage of foliar necrosis, herbivore pressure, leaf epiphyte biomass.	Virtual reference site from existing values and expert judgment	Coastal constructions, beach regeneration, urban and industrial sewage, rivers and channels, agricultural soil use

Continued

Table 1 continued

Indices	Regions of development and other application regions	Methods	Metrics and depths	Reference conditions	Pressures tested
BiPo: Biotic Index using the seagrass *Posidonia oceanica*	**Corsica (France)** (Lopez y Royo *et al.*, 2010a) Catalonia (Spain), Corsica (France), Ischia (Italy) (Lopez y Royo *et al.*, 2011) Corsica (France) (Pergent *et al.*, 2015) Tipaza, Algiers (Algeria) (Boumaza *et al.*, 2015) Mostaganem and Oran (Algeria) (Bentaallah, 2017) Saronikos Gulf (Greece) (Gerakaris *et al.*, 2017) Vasiliko (Cyprus) (Kletou *et al.*, 2020) Gökçeada (Turkey) (Güreşen *et al.*, 2020a) Gökçeada (Turkey) and Corsica (France) (Güreşen *et al.*, 2020b) Tremiti Islands (Italy) (Tursi *et al.*, 2022a)	Normalization and combination by averaging 4 metrics	<u>At the lower limit</u> Lower limit depth and type <u>At intermediate depth (15 m)</u> Shoot density, shoot length	Virtual reference sites from existing values	Land use, industrial activity, river discharges, port activities and coastal planning.
PREI: *Posidonia oceanica* Rapid Easy Index	**Corsica and PACA** (Gobert *et al.*, 2009) *Italia* (Rende *et al.*, 2011; Rigo *et al.*, 2019; Oprandi *et al.*, 2021) Tipaza, Algiers (Algeria) (Boumaza *et al.*, 2015) Saronikos Gulf (Greece) (Gerakaris *et al.*, 2017) Liguria (Italy) (Mancini *et al.*, 2020) Vasiliko (Cyprus) (Kletou *et al.*, 2020) Sicily(Bellissimo *et al.*, 2020)2000/60/EC Tremiti Islands (Italy) (Tursi *et al.*, 2022a)	Normalization and combination by averaging 5 metrics	<u>At the lower limit</u> Lower limit depth and type <u>At intermediate depth (15 m):</u> Shoot density, shoot leaf area, ratio between epiphytic and leaf biomass	A composite site from existing values and modeling	Fish farming, industrial development, agriculture, tourism, fishing, commercial ports and urbanization

Continued

Table 1 continued

Indices	Regions of development and other application regions	Methods	Metrics and depths	Reference conditions	Pressures tested
Vitality Index	**Corsica (France)** (Pergent *et al.*, 2015) Gökçeada (Turkey) and Corsica (France) (Güreşen *et al.*, 2020b) Tremiti Islands (Italy) (Tursi *et al.*, 2022a);	Averaging of 8 metrics	<u>At the lower limit</u> Lower limit depth and type, meadow cover, percentage of plagiotropic rhizomes, leaf production, rhizome elongation <u>At intermediate depth (15 m)</u> Shoot density, shoot leaf area	Exposed and non-exposed (or less exposed) sites from the study area	Distance to cities and harbors, trawling, urban and industrial discharges

ni *et al.*, 2020; Tursi *et al.*, 2022a). The study conducted by Boumaza *et al.* (2015) on *P. oceanica* meadows along the central Algerian coast indicates that the PREI most accurately represents the anticipated ecological status. In contrast, the BiPo exhibits a diminishing correlation with human pressures. Kletou *et al*. (2020) established a strong correlation between human pressures and both PREI and BiPo in Vasiliko Bay, Cyprus. Significant correlations between BiPo and anthropogenic pressures have been identified along the Western Algerian coast (Bentaallah, 2017) and the Turkish coasts (Güreşen *et al.*, 2020b).

Using multi-metric indices outside their intended regional range requires adaptation, calibration, and validation to ensure effectiveness (Martínez-Crego *et al.*, 2010; Pergent *et al.*, 2015; Dallas, 2021). The selection of appropriate multi-metric indices for assessing ecological status is thus critical for coastal water quality management and monitoring. The challenge of implementing a multi-metric index based on *P. oceanica*, which is shared throughout the Mediterranean basin, appears to be essential and requires the collaboration of all scientists, as highlighted by Di Camillo *et al.* (2023) in the case of coralligenous reefs. As part of ongoing efforts to harmonize monitoring tools across the Mediterranean region, the purpose of this work is to present a new multi-metric index called the Multi-metric Index for the Algerian coast based on *P. oceanica* (MIAPo), developed for the first time along the southern Mediterranean coasts. It incorporates tannin cells density as an additional descriptor alongside those commonly used. The sensitivity of this cellular indicator to anthropogenic pressures has been tested and demonstrated by Boumaza *et al.* (2022). Its inclusion aligns with current recommendations advocating for the integration of complementary metrics from the community and population levels to biochemical and cellular levels to achieve a more comprehensive ecosystem assessment (Martínez-Crego *et al.,* 2010; Martinez-Haro *et al.,* 2015) and rapid time responses (Ferrat *et al.*, 2003; Romero *et al.*, 2016). Notably, this parameter stands out from other metrics at a similar level due to its ease and rapid measurement, without the need for advanced equipment. The proposed index is based on previous multi-metric indices listed in Table 1 and attempts to assess the ecological status of Algerian coastal waters. It was compared to the previously existing multi-metric index PREI (Gobert *et al.*, 2009). We estimated the robustness of MIAPo and PREI by investigating their relationships with cumulative multiple human pressures represented by a Pressure Index (PI).

Materials and Methods

Metrics selection and P. oceanica *indices application*

The most important aspect of the multi-metric index development procedure is metric selection, which prevents an incorrect interpretation of the biological response to environmental degradation (Stoddard *et al.*, 2008; Hawkins *et al.*, 2010). MIAPo metrics are chosen based on our previous studies within the *P. oceanica* meadows in the studied geographic area (Boumaza *et al.*, 2012, 2014, 2015, 2022; Sengouga, 2017; Sengouga *et al.,* 2018, 2019). These studies helped us identify the most relevant metrics, particularly those that appeared to be sensitive to anthropogenic pressures. Five metrics were chosen (Table 2) based on recommendations from Martínez-Crego *et al.* (2010)and Romero *et al.* (2016). The researchers emphasized the importance of using metrics at the population and community levels to maintain ecological integrity and biomarkers to detect disturbances early. Each type of metric was represented by one metric on one side. This method is simple and avoids the problems that come with weighing one type more than another. On the other hand, the performance characteristics of the chosen metrics were related to their ability to distinguish between the least-disturbed and most-disturbed sites (Stoddard *et al.*, 2008). Furthermore, based on the 0.71 threshold established by Stoddard *et al.* (2008), a redundancy test was carried out using Spearman's correlation analysis and showed that the selected metrics were not redundant (Table 3). The PREI was elaborated according to Gobert *et al.* (2009), and it differed from MIAPo in the metrics and their types (Table 2).

Reference levels and reference conditions

According to Romero *et al.* (2007), reference levels, which referred to both the upper and lower limits of a metric's range, were used in the normalization process required to generate MIAPo. The "hypothetical reference site" was determined by averaging the two higher and two lower values for metrics that decreased and increased in response to anthropogenic disturbances, respectively (Table 2). The "hypothetical worst site" was established using the reverse procedure. In the case of the lower limit depth, the maximum and minimum values observed in the prospected geographical zone were used to elaborate on the previous "hypothetical sites". The reference condition for generating the PREI index was defined by Gobert *et al.* (2009). We adapted this approach to our studied area by averaging the three highest values (after discarding the maximum) for shoot density and leaf surface area, 0 dry weight for epiphytic leaf biomass E/L (considering that healthy seagrass has non-epiphyted leaves), and the maximum value for the lowest limit depth recorded in the studied sites.

Table 2. Metrics used for MIAPo and PREI indices, categorized by metric type and expected response to anthropogenic disturbance (based on previous studies).

Types of metrics	Metrics	MIAPo	PREI	Responses
Community	Epiphytic leaf biomass (E/L) ($mg.mg^{-1}$)	+	+	Increase
	Epiphytic load ($mg.cm^{-2}$)			Increase
Population	Shoot density ($shoot.m^{-2}$)		+	Decrease
	Leaf area index ($m^2.m^{-2}$)	+		Decrease
Lower limit	Type of lower limit		+	Typology modification
	Depth of lower limit (m)	+	+	Decrease
Individual	Shoot leaf surface (cm^2/shoot)		+	Decrease
Cellular	Leaf tannin cell density ($cells.mm^{-2}$)	+		Increase
Biochemical	Leaf nitrogen content (% DW)	+		Increase

Table 3. Redundancy test using Spearman's correlation analysis for the MIAPo selected metrics. Spearman correlation coefficients (R) are in bold; p-values are in regular font.

p-value \ Spearman correlation R	Epiphytic load	Leaf area index	Depth of lower limit	Leaf nitrogen content	Leaf tannin cell density
Epiphytic load		**-0.52**	**-0.19**	**0.50**	**0.45**
Leaf area index	0.1827		**0.49**	**-0.12**	**-0.02**
Depth of lower limit	0.6494	0.2166		**-0.37**	**-0.44**
Leaf nitrogen content	0.207	0.7789	0.3652		**0.43**
Leaf tannin cell density	0.2604	0.9554	0.2715	0.2894	

Ecological Quality Ratio

To ensure comparable values of MIAPo and PREI and highlight concordant or discordant responses, MIAPo's EQR was estimated by normalizing data between 0 and 1 (PREI already represents the EQR value). An arbitrary value of 0.1 was assigned to the boundary of the "bad" status and the remaining interval from 0.1 to 1 was divided into four equal intervals as required by the WFD (Romero *et al.*, 2007; Gobert *et al.*, 2009). This EQR was calculated as described by Romero *et al.* (2007), Oliva *et al.* (2012) and García-Marín *et al.* (2013)for their indices. Thus, we combined the MIAPo metrics into a single scale through a principal component analysis (PCA) based on the correlation matrix to account for differences in measurement scales (Legendre & Legendre, 1998). The two hypothetical sites corresponding to the extreme conditions (reference and worst) were included as supplementary objects. The EQR was calculated as follows:

$$EQR'_x=(Cl_x - Cl_{worst})/(Cl_{Reference} - Cl_{worst}) \quad (1)$$

$$EQR_x=(EQR'_x+0.11)/(1+0.10) \quad (2)$$

where, CI is the PCA's first component scores; EQR_x is the ecological quality ratio of the site $\varkappa$; $CI_\varkappa$ is the score of the site $\varkappa$; $CI_{reference}$ is the score of the hypothetical "reference" site; CI_{worst} is the score of the hypothetical "worst" site.

Study area, sampling collection and metrics measurements

This study was carried out along the central Algerian coast (Fig. 1). Eight *P. oceanica* meadows from two provinces were analyzed, each with different levels and types of anthropogenic pressure. Four sites in the western part of the study area are in Tipaza province (Chenoua, Kouali, Berrard, and Bou Ismail), while four sites in the eastern part are in Algiers province (El Djamila, Ain Benian, Rais Hamidou, and Aguelli). The available state data on the various human activities in these two provinces, obtained from publicly available and several studies on the region (PNUE/PAM/CAR PAP, 2006; Bakalem *et al.*, 2009; Mangos & Claudot, 2013; Haouchine *et al.*, 2015), indicate that Tipaza is renowned for its significant agricultural and forestry potential as well as tourism and fishing vocations. Algiers, the country's capital, is the most densely populated area, with the highest concentration of services, facilities, infrastructure, industry, and major urban developments.

For the assessment of MIAPo and PREI metrics, the following data were collected in the field between July and August 2018 at approximately 12 m depth (excluding the meadow lower limit depth and typology) to avoid the masking effects of depth and seasonal variability. This intermediate depth was selected considering the lower limits of *P. oceanica* meadows in the geographical area, which range from 14 to 20 m and mostly coincide with the lower limit of the rocky substrate. The WFD recommends using the protocols at shallower depths when meadows do not reach the standard depth of 15 m (Blouet *et al.*,

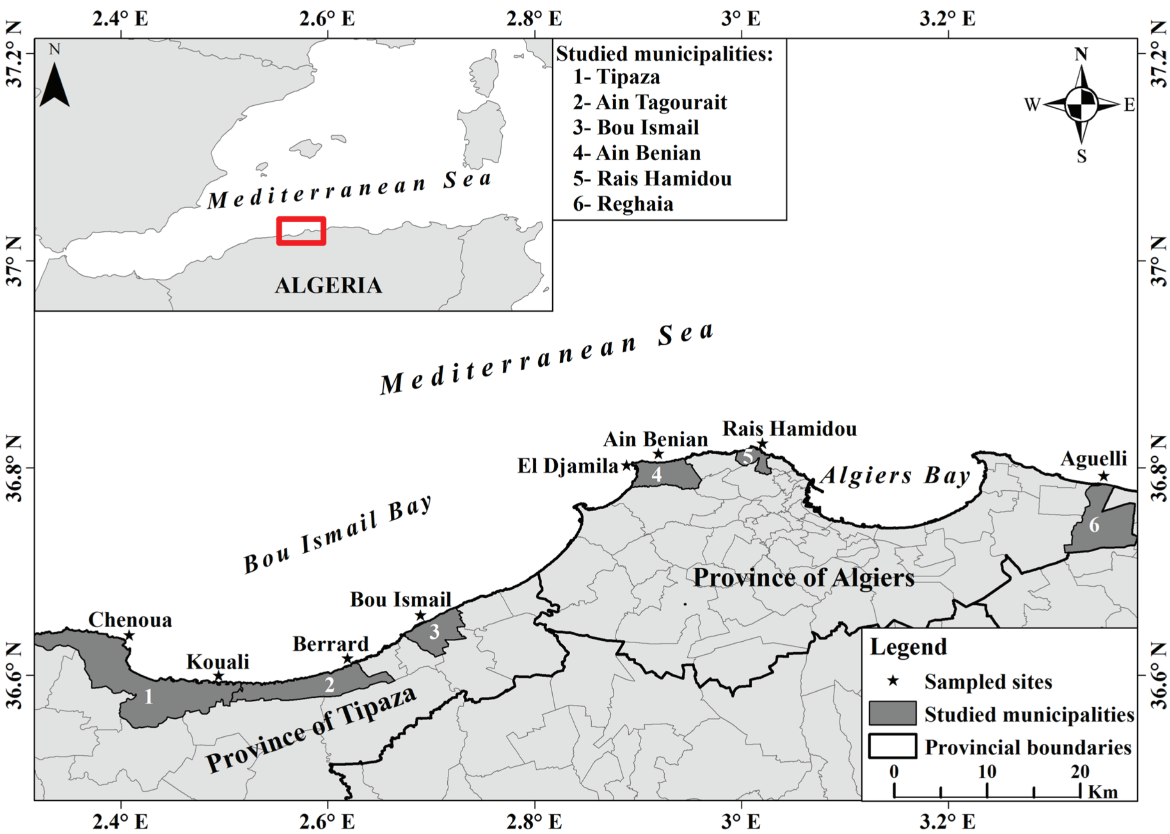

Fig. 1: Map of the study area with studied municipalities and location of the 8 *P. oceanica* meadows investigated.

2011), and where the conditions are optimal for meadow development (PNUE/PAM CAR/ASP, 2019):

Metrics 1-2: The lower limit depth was recorded *in situ* and classified according to Meinesz & Laurent's (1978) typology.

Metric 3: Shoot density (shoots. m^{-2}) was estimated using 10 randomly located 0.16 m² quadrats. This sampling effort provides the minimum standard error required for an accurate estimation of mean seagrass density (Panayotidis *et al.*, 1981; Pergent *et al.*, 1995; Pergent-Martini *et al.*, 2005; Lopez Y Royo *et al.*, 2010b).

Metric 4-5: Leaf area per shoot (cm²) was estimated from leaf morphometrics of 15 shoots collected and leaf area index (LAI) from shoot leaf area and shoot density ($m^2.m^{-2}$) (Buia *et al.*, 2004; Pergent *et al.*, 1995). To minimize the impact of destructive sampling methods (Buia *et al.*, 2004; Rotini *et al.*, 2013; PNUE/PAM CAR/ASP, 2019), we adopted a relatively limited sampling effort of 15 shoots, which is considered sufficient for this purpose (Pergent *et al.*, 1995; Pergent-Martini *et al.*, 2005). Furthermore, the PREI assessment in this work is based on a previous multi-year monitoring (Boumaza *et al.*, 2015; Sengouga, 2017; Sengouga *et al.*, 2019) that showed consistent PREI-anthropogenic pressure trends when using 20 shoots in 2011 and 15 shoots in both 2015 and 2018.

Metrics 6-7: Epiphyte biomass separated from each shoot (15 shoots) was measured after drying (70°C for 72 h) and standardized to leaf dry mass ($mg.mg^{-1}$DW) (E/L) (Terrados & Pons, 2008) and to leaf area (mg $DW.cm^{-2}$) (Boumaza *et al.*, 2014) to obtain epiphytic load.

Metric 8: Leaf tannin cell density (cells. mm^{-2}) was carried out under a microscope, over cross-sections at the basal, the middle and the apical region of all adult and intermediate leaves (*sensu* Giraud, 1979) of 5 collected shoots, as described by Boumaza *et al*. (2022).

Metric 9: Leaf nitrogen content was analyzed in all adult and intermediate leaves from 9 collected shoots, which were deemed sufficient based on sample sizes used in previous studies (Delgado *et al.*, 1999; Fourqurean *et al.*, 2007; Leoni *et al.*, 2007; Scartazza *et al.*, 2017). The epiphyte-free leaves were lyophilized and ground into powder. The nitrogen content was determined using the Kjeldahl method with three or two subsamples (0.2 g) from each aliquot of ground material and expressed as %DW. This method has already been used with seagrasses (Duarte, 1990; Dawson & Dennison, 1996; Udy & Dennison, 1997; Walker *et al.*, 2004; Zhang *et al.*, 2011). Ammonium chloride was used as a reference material and was analyzed in each series of six samples. The tests produced a Root Mean Square Error of 0.02% DW.

Pressure assessment

To relate the status of *P. oceanica* meadows to human activities, we quantified the key pressures in the studied geographical area using publicly available and satellite data, focusing on those that affect water quality. Eight quantitative data-driven pressures were considered: urbanization (% of urbanized area to municipality area), coastal population (% of municipality demography to province demography), agriculture (% of agricultural area to municipality area), fishing (number of fishing vessels per harbor), urban effluents (number of discharge points per municipality), uncontrolled dumping (% of wastewater volume not connected to the sewer system per municipality), rivers and industry (distance from the nearest river or industrial zone). In the case of industrial activities, river and fishing harbors, a distance of 10 km was considered significant (Comeleo *et al.*, 1996; Rodriguez *et al.*, 2007; Lopez y Royo *et al.*, 2009a; Parravicini *et al.*, 2012), implying that river mouths, industrial zones, and harbors located more than 10 km away from a site are not considered significant pressures.

PI was calculated using a rapid approach inspired by prior research on the vulnerability of coastal areas and rivers (Leopold, 1969; Bodéré *et al.*, 1991; Hallegouët *et al.*, 1997; Larid, 2002). It represents a cumulative of selected key pressures. It entails assigning scores ranging from 1 (no effect) to 5 (high effect) to various anthropogenic pressure values (Table 4) to categorize the pressures within this scale in two steps:

(i) definition of "high effect" and "no effect" conditions. In fact, for percentage-based pressures (urbanization, coastal population, agriculture, and uncontrolled dumping), "high effect" conditions correspond to 100%, while "no effect" conditions correspond to an arbitrary value of less than 5%, as assigned by (Larid, 2002). For pressures represented as distances (industrial activity and rivers), "high effect" conditions correspond to 0 km and "no effect" conditions correspond to distances greater than 10 km, as predicted by Comeleo *et al.* (1996), Rodriguez *et al.*(2007), Lopez y Royo *et al.*(2009a) and Parravicini *et al.* (2012). For pressures represented as activity counts (fishing activity and urban effluents), the lowest recorded values

Table 4. Definition of "high effect" and "no effect" conditions of the selected key pressures.

Units	Percentages (%)				Distances (km)		Numbers	
Pressures	Urbanization	Coastal population	Agriculture	Uncontrolled dumping	Industrial activity	Rivers	Fishing activity	Urban effluents
No effect	< 5 %				> 10 km		0	0
High effect	100 %				0 km		339	11

represent "no effect" conditions, while the highest recorded values represent "high effect" conditions.

(ii) pressure categorization (Table 5) is achieved by dividing the interval between "no effect" condition values and "high effect" condition values into 5 intervals of equal amplitude.

PI, which ranges from 0 (no human impact) to 1 (high human impact), is calculated as follows:

$$PI = \frac{\sum_1^N S_n}{5.\sum_1^N P_n} \quad (3)$$

where, S_n is the rate for each pressure, varying from 1 to 5, and P_n is the number of the considered pressures.

Statistical analysis

Statistical analyses were carried out in the R4.2.0 environment, with all tests performed at the 95% significance level. When the parametric assumptions were verified, one-way ANOVA was used to test differences between sampling sites. Otherwise, a non-parametric Kruskal-Wallis test was used. The Shapiro-Wilk and Bartlett tests were used to prove distribution normality and variance homogeneity, respectively. Metrics used to construct MIAPo were combined using PCA. Then, hierarchical clustering was used to determine whether the ordination of the studied sites along the first PCA axis was consistent with meadow quality. The number of clusters was decided using the number of ecological statuses obtained from the MIAPo. The relationship between MIAPo and PREI was investigated using Spearman's rank correlation, and an absolute average class difference (AACD) analysis was carried out to assess the agreement between these two indices, as described by Lopez y Royo *et al.* (2011). The Spearman's rank correlation was also used to determine how well MIAPo, PREI, and their metrics reflect human pressures. As the pressure data were evaluated at the municipality level, we averaged the EQRs and metrics of sites within the same municipality to allow for correlation estimation.

Results

Ecological status class evaluation using MIAPo

Table 6 summarizes the mean (± SE) values per site for each metric used in the MIAPo calculation. Statistically significant differences among sites for all the metrics can be noted, allowing their use for the MIAPo concept, as indicated by Stoddard *et al.* (2008).

Table 5. Anthropogenic pressure (Pn), data sources, and scores (Sn) by value interval.

<table>
<tr><th rowspan="2">Pressures (Pn)</th><th rowspan="2">Data sources</th><th colspan="5">Scores (Sn)</th></tr>
<tr><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th></tr>
<tr><td>Urbanization (%)</td><td>• Cartography using Google Earth satellite imagery</td><td rowspan="4">< 5</td><td rowspan="4">5-28.7</td><td rowspan="4">28.8-52.5</td><td rowspan="4">52.6-76.2</td><td rowspan="4">76.3-100</td></tr>
<tr><td>Coastal population (%)</td><td>• Website of the Province of Tipaza
• Statistical yearbook of the province of Algiers</td></tr>
<tr><td>Agriculture (%)</td><td>• Cartography using Google Earth satellite imagery</td></tr>
<tr><td>Uncontrolled dumping (%)</td><td>• Website of the Province of Tipaza
• Statistical yearbook of the province of Algiers</td></tr>
<tr><td>Fishing activity (number)</td><td>• Website of the Province of Tipaza
• The Directorate of Fisheries and Fisheries Production of the province of Algiers</td><td>< 84</td><td>84-168</td><td>169-253</td><td>254-338</td><td>≥ 339</td></tr>
<tr><td>Urban effluents (number)</td><td>• National Coastal Commission of Tipaza
• Public establishment for urban hygiene and environmental protection of the province of Algiers</td><td>< 3</td><td>3-5</td><td>6-8</td><td>9-10</td><td>≥ 11</td></tr>
<tr><td>Industrial activity (Km)</td><td>• Ministry of Industry and pharmaceutical production, and cartography</td><td rowspan="2">> 10</td><td rowspan="2">10-7.5</td><td rowspan="2">7.6-5</td><td rowspan="2">5.1-2.4</td><td rowspan="2">≤ 2.5</td></tr>
<tr><td>Rivers (Km)</td><td>• Cartography using Google Earth satellite imagery</td></tr>
</table>

Two axes, PCI and PCII, of 49.5% and 21%, respectively, were extracted using PCA, explaining 70.5% of the variance in *P. oceanica* metrics (Fig. 2). PCI was significantly correlated with most of the metrics, indicating that this axis is effective at indicating the state of meadow health. Lower limit depth and leaf area index were the most correlated with PCI, with the highest negative loading scores (-0.83 and -0.81, respectively), indicating a good status of meadows. In contrast, epiphytic load, leaf tannin cell density, and leaf nitrogen content were positively correlated with PCI, indicating a poor status of meadow health (Fig. 2).

The PCA applied to the studied sites illustrates their logical ordination along the first axis, following a quality gradient from the 'optimal' site to the 'worst' site (Fig. 3). The sampling sites were grouped into three statistically significant clusters (Fig. 3).

(i) Cluster 1 (high status) consists of the Kouali and Berrard sites in Tipaza Province. These sites are distinguished by the lowest leaf nitrogen content, the highest lower limit depth, and the leaf area index (the latter only in Kouali) (Table 6).

(ii) Cluster 3, which corresponds to the moderate status at the El Djamila site (Algiers Province), has the

Table 6. Mean values of the selected metrics for MIAPo calculation (Mean values ±SE) and the P-values from One-Way ANOVA and Kruskal-Wallis (K-W) tests applied to *P. oceanica* metrics for the 8 studied sites.

Sites	Tannin cell density (cells.mm^{-2})	Leaf nitrogen content (%DW)	Epiphytic load (mg DW .cm^{-2})	Leaf area index (m^2.m^{-2})	Lower limit depth (m)
Chenoua	44.54 ± 8.10	2.29 ± 0.04	1.32 ± 0.10	4.60 ± 0.43	17
Kouali	17.12 ± 2.91	1.83 ± 0.04	1.23 ± 0.11	7.39 ± 0.57	20
Berrard	14.94 ± 3.66	1.71 ± 0.03	0.71 ± 0.10	4.97 ± 0.33	19
Bou Ismail	22.03 ± 1.97	2.55 ± 0.02	1.83 ± 0.18	4.64 ± 0.47	18
El Djamila	33.17 ± 6.47	1.91 ± 0.09	1.69 ± 0.20	2.52 ± 0.26	14
Ain Benian	11.51 ± 1.84	2.18 ± 0.02	1.53 ± 0.12	3.53 ± 0.34	18
Rais Hamidou	19.58 ± 1.94	2.28 ± 0.07	0.81 ± 0.10	5.91 ± 0.43	17
Aguelli	11.46 ± 2.65	2.17 ± 0.02	1.15 ± 0.11	4.45 ± 0.29	17
Optimal	5.99	1.69	0.07	6.65	20
worst	68.68	2.57	3.20	2.25	14
p-value	2.0 E-04	2.3 E-08	3.9 E-07	2.3 E-12	/
Test	K-W	ANOVA	K-W	ANOVA	/

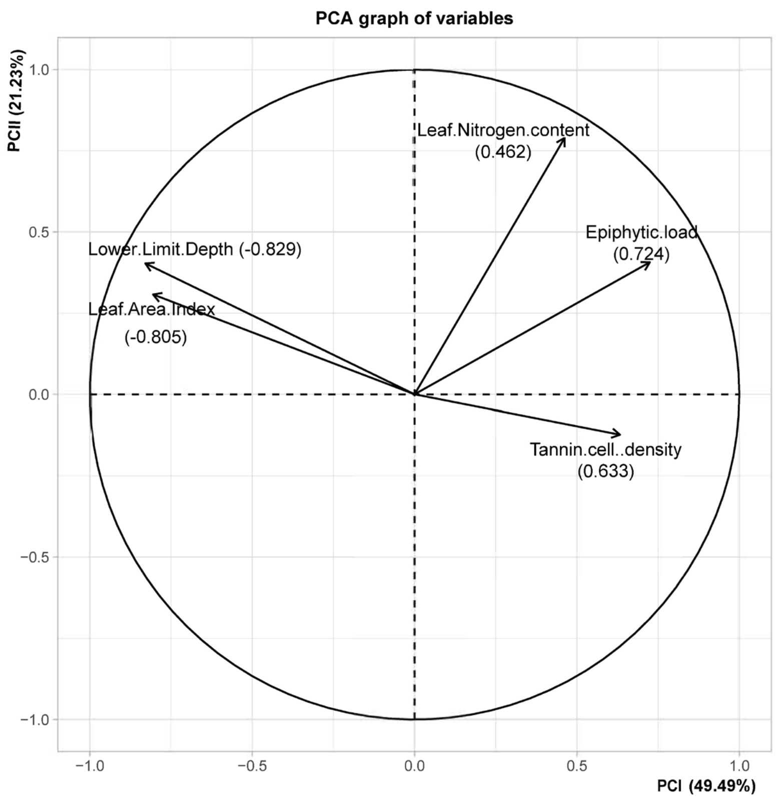

Fig. 2: Principal Component Analysis plot of *P. oceanica* metrics and their loadings on the first axis (in brackets).

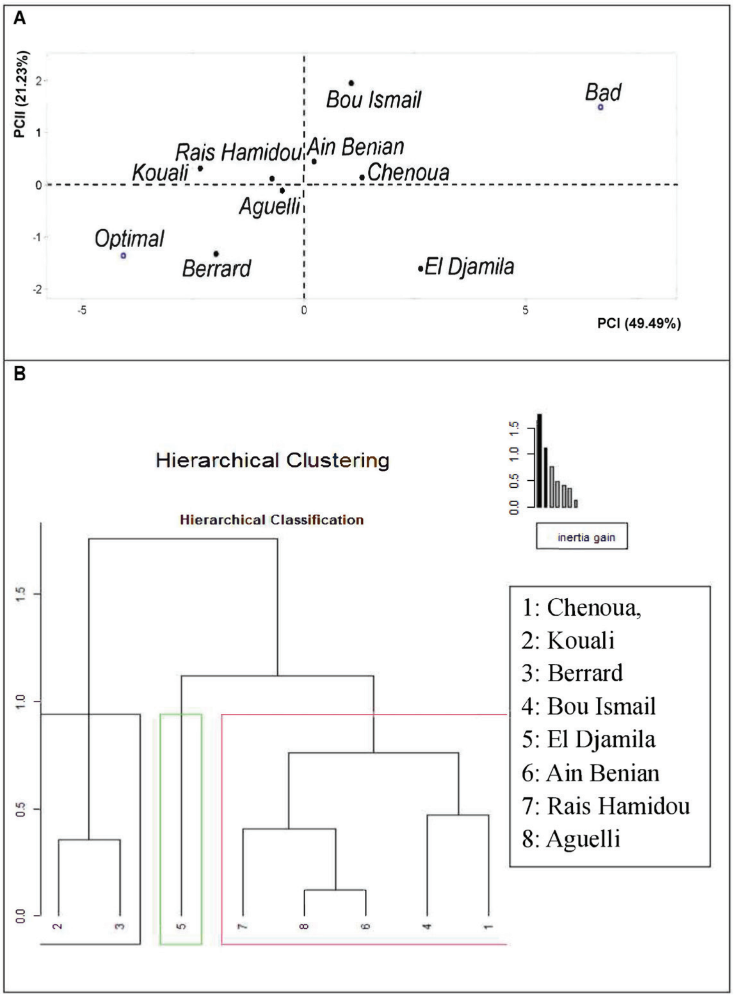

Fig. 3: (A) Principal Component Analysis plot with site scores. (B) Hierarchical clustering dendrogram, highlighting the 3 main clusters corresponding to the 3 types of ecological statuses obtained from MIAPo.

lowest values for the leaf area index and lower limit depth.

(iii) Cluster 2 represents a good status and includes the remaining sites.

The EQR calculation classified the studied sites into three levels of status: high, good, and moderate, with values ranging from 0.44 to 0.86 (Table 7). The Algiers province meadows had a lower mean EQR than the Tipaza province meadows (0.62 ± 0.13 and 0.71 ± 0.16, respectively). Only one meadow was designated as moderate (El Djamila), five as good (Chenoua, Bou Ismail, Ain Benian, Rais Hamidou, and Aguelli), and two as high (Kouali and Berrard).

Ecological status class evaluation using PREI and comparability with MIAPo

Table 8 summarizes the mean values per site (± SE) for each metric used in the PREI calculation. The data analysis revealed statistically significant differences across sites for all metrics.

The AACD shows that MIAPo and PREI are highly comparable (62.5% agreement). Only three sites, Berrard, Bou Ismail, and El Djamila, changed their MIAPo status from “high, good, and moderate” to “good, moderate, and poor” by the PREI (Fig. 4). Furthermore, a highly significant correlation was found between MIAPo and PREI (R = 0.88, p = 0.004; Fig. 5), while MIAPo consistently produced higher values than PREI across all sites (EQR mean values: 0.665 and 0.605 respectively), with the exception of the Chenoua site (Fig. 4).

Evaluation of anthropogenic pressures

Table 9 lists the results of each site’s human pressure evaluation. The PI values revealed that sites in Algiers province experience higher pressures than sites in Tipaza province.

The Spearman correlations between the PREI EQR and MIAPo EQR values and the PI are statistically significant (R = -0.80; p = 0.02 and R = -0.96; p = 0.0002, respectively; Fig. 6).

Responses of metrics to human pressures

Correlations between *P. oceanica* metrics and the PI are statistically significant for population and community

Table 7. The scores on the first Principal Component Analysis axis and the resulting EQR values from each sampling site for the MIAPo.

Sites	MIAPo		
	CI	EQR	Status
Chenoua	1.042	0.555	Good
Kouali	-2.143	**0.863**	High
Berrard	-1.94	0.832	High
Bou Ismail	0.944	0.575	Good
El Djamila	2.961	**0.443**	Moderate
Ain Benian	0.087	0.646	Good
Rais Hamidou	-0.679	0.726	Good
Aguelli	-0.272	0.684	Good
Optimal	-4.069	1.000	
Worst	6.692	0.099	

Table 8. Mean values of the metrics used for PREI calculation (Mean values ± SE), P- values from One-Way ANOVA and Kruskal-Wallis (K-W) tests applied to *P. oceanica* metrics and PREI EQR in the 8 studied sites.

Sites	Shoot density (shoots. m^{-2})	Leaf surface area (cm^2. $Shoot^{-1}$)	E/L ($mg.mg^{-1}$ DW)	Lower limit depth (m) and type		PREI EQR	Status
Chenoua	169.38 ± 8.2	271.68 ± 25.5	0.31 ± 0.03	17	Stable	0.616	Good
Kouali	211.88 ± 20.2	348.73 ± 26.8	0.32 ± 0.02	20	Stable	0.826	High
Berrard	195.00 ± 13.2	254.99 ± 16.8	0.17 ± 0.02	19	Stable	0.734	Good
Bou Ismail	217.50 ± 21.9	213.42 ± 21.6	0.54 ± 0.04	18	Regressive	0.509	Moderate
El Djamila	119.38 ± 7.2	211.26 ± 21.8	0.39 ± 0.05	14	Regressive	0.270	Poor
Ain Benian	151.88 ± 8.5	232.58 ± 22.5	0.38 ± 0.03	18	Stable	0.622	Good
Rais Hamidou	187.50 ± 21.6	315.27 ± 22.9	0.23 ± 0.03	17	Stable	0.662	Good
Aguelli	155.00 ± 13.1	287.37 ± 18.4	0.29 ± 0.03	17	Stable	0.616	Good
Optimal	300	467.76	0	20			
Worst	0	0	1	14			
p-value	2.4 E-04	8.7 E-05	3.8 E-08	/			
Test	K-W	ANOVA	K-W	/			

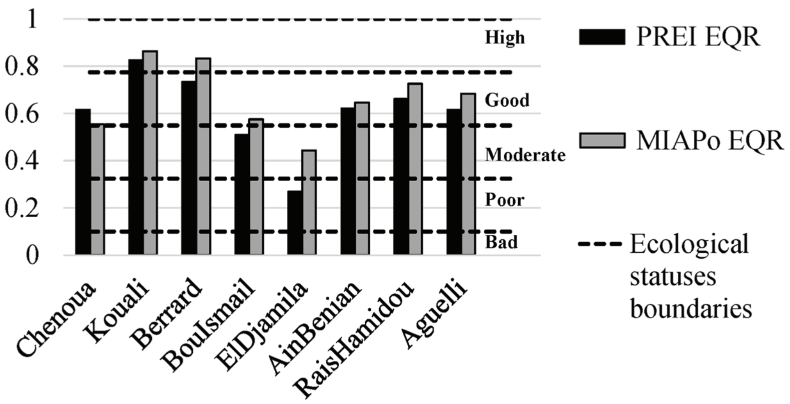

Fig. 4: EQR values and ecological status classification of sites according to MIAPo and PREI indices.

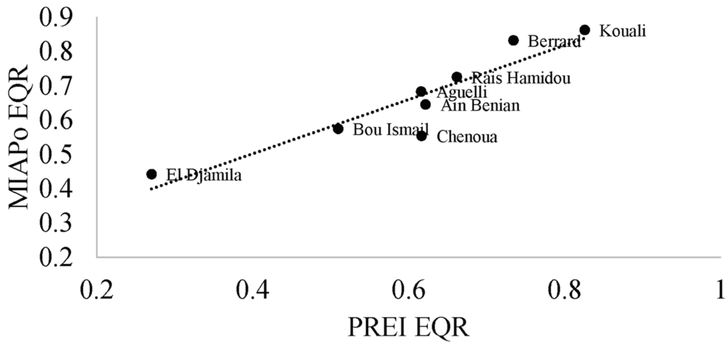

Fig. 5: Significant positive correlation between the PREI EQR and the MIAPo EQR (R = 0.88, *p* = 0.004).

Table 9. The rates assigned for each pressure and the results of the pressure index (PI) calculated by municipality.

Provinces	Tipaza			Algiers		
Municipalities	Tipaza	Ain Tagourait	Bou Ismail	Ain Benian	Rais Hamidou	Reghaia
Sites	Chenoua/ Kouali	Berrard	Bou Ismail	El Djamila / Ain Benian	Rais Hamidou	Aguelli
			Rates (Rn)			
Urbanization	2	2	5	5	5	4
Agriculture	2	3	1	4	1	4
Coastal population	1	1	2	1	1	1
Uncontrolled dumping	2	2	2	1	1	1
Fishing activity	2	2	2	5	4	2
Rivers*	3	1	1	4	1	4
Industrial activity*	1	2	4	4	5	3
Urban effluents	5	3	4	2	2	1
PI	**0.45**	**0.38**	**0.50**	**0.60**	**0.48**	**0.48**

* Despite the fact that the distances between sites within the same municipality vary, the outcome scores remain consistent.

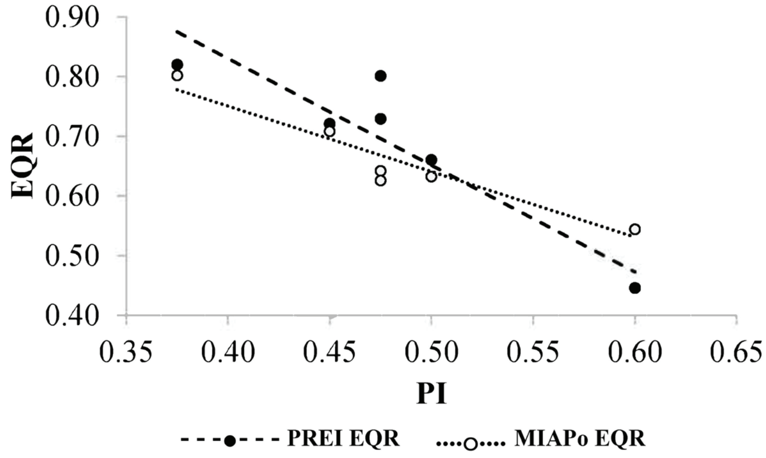

Fig. 6: Significative negative Spearman correlation between the pressure index (PI) and both PREI EQR and MIAPo EQR.

types, with the exception of shoot density (Table 10).

Discussion

The simultaneous use of two multi-metric indices, MIAPo and PREI, provides comparable results in the classification of the water quality of Algerian central coastal waters despite the differences they present. Metrics were chosen and combined using an average for the PREI and a PCA for the MIAPo. In fact, different studies discovered similarities when comparing such disparate indices (Gerakaris *et al.*, 2017; Kletou *et al.*, 2020; Tursi *et al.*, 2022a). Another notable difference between MIAPo and PREI lies in how they define reference conditions. In fact, while the worst condition of the PREI corresponds to extreme values for a recorded recent meadow die-off (*e.g.,* 0 shoot.m^{-2} for shoot density and 1 mg.mg^{-1} DW for epiphytic load), the MIAPo uses only values of field data from local scale to define both optimal and worst conditions (*e.g.,* 2.25 m^2.m^{-2} for leaf area index). Defining reference conditions can impact the precision and robustness of biotic indices (Martínez-Crego *et al.*, 2010; Romero *et al.*, 2016) and lead to incorrect interpretation of results (Borja *et al.*, 2012).

The proposed MIAPo has the potential to be a useful tool for assessing the ecological status of Algerian coastal waters for the following reasons: (i) The MIAPo is sensitive to human pressures (R = -0.96) and can accurately reflect changes in ecological stress. (ii) Using hierarchical classification, the studied sites were arranged into clusters that corresponded exactly to their ecological status. This implies that the spatial variability of the selected metrics within the study area is clearly due to differences in meadow quality. (iii) It integrates a small number of metrics combining insights provided by the maximum variety of metric types. (iv) Notably, it integrates leaf tannin cell density as an easily applicable cellular indicator, adding it to the commonly used descriptors, thereby improving both cost-effectiveness and early detection. (v) To date, it remains the only index developed within the southern Mediterranean coast. (vi) The MIAPo has high quantitative (R = 0.88) and qualitative (62.5%) agreement with the PREI, which has already been used and verified in

Table 10. Spearman's rank correlation between the pressure index (PI) and the studied metrics (MIAPo and PREI metrics are superscripted by 'M' and 'P', respectively). Significative correlations are in bold.

Types of metrics	Metrics	Spearman's rank correlation with PI	
		R	*p*-value
Community	Epiphytic leaf biomass ^{P}	**0.7239**	**0.04**
	Epiphytic load ^{M}	**0.7239**	**0.04**
Population	Shoot density ^{P}	-0.6258	0.1
	Leaf area index ^{M}	**-0.7976**	**0.02**
Lower limit	Depth of lower limit ^{P,M}	**-0.9203**	**0.001**
Individual	Shoot leaf surface ^{P}	-0.5767	0.13
Cellular	Leaf tannin cell density ^{M}	0.0491	0.91
Biochemical	Leaf nitrogen content ^{M}	0.135	0.75

the same region (Boumaza *et al.*, 2015; Sengouga, 2017; Sengouga *et al.*, 2019).

In terms of the sensitivity of the two applied indices to human pressure, the results show a stronger correlation between the MIAPo and the PI than the PREI (R = -0.96 and -0.80, respectively). This relative difference is most likely due to differences in metrics and their types (Lopez y Royo *et al.*, 2011; Gerakaris *et al.*, 2017; Mancini *et al.*, 2020). In fact, we have included additional metric types to supplement those already included in the PREI: cellular and biochemical metrics (leaf nitrogen content and leaf tannin cell density). Martínez-Crego *et al.* (2010) and Roca *et al.* (2015)suggested that incorporating these types of metrics can improve stress index sensitivity and early detection efficacy. Furthermore, several studies have supported the use of these indicators in monitoring programs (Dumay *et al.*, 2004; Pérez *et al.*, 2008; Boumaza *et al.*, 2012, 2014, 2022; Jones *et al.*, 2018; Helber *et al.*, 2021; Kerninon *et al.*, 2021; Jiménez-Casero *et al.*, 2023).

In terms of metric adequacy, the population and community metrics (lower limit depth, leaf area index, and epiphytic load) had strong loadings (higher than 0.72; Fig. 2) on the PCA first axis, which was supported by their high relationship with the PI. Gerakaris *et al.* (2017) highlighted the strongest relationships between population and community metrics and human pressures in their research. These types of metrics are more useful for assessing ecosystem conditions because they have longer response times and are more integrative (Roca *et al.*, 2015; Romero *et al.*, 2016). As for monitoring *P. oceanica* meadows, leaf area index (Pergent *et al.*, 1995; Pergent-Martini *et al.*, 2005; Koçak *et al.*, 2011; Dural *et al.*, 2012; Tursi *et al.*, 2022b), lower limit depth (Meinesz & Laurent, 1978; Pergent *et al.*, 1995; Pergent-Martini *et al.*, 2005; Boudouresque *et al.*, 2009; Descamp *et al.*, 2011; Tursi *et al.*, 2022b) and the epiphytic load (Pergent *et al.*, 1995; Delgado *et al.*, 1999; Dimech *et al.*, 2002; Cancemi *et al.*, 2003; Pergent-Martini *et al.*, 2005) are commonly used to reflect the light condition, water transparency and nutrient loading. In fact, the site with the lowest EQR, El Djamila, is characterized by a set of human activities that are the primary cause of water turbidity and enrichment: a highly urbanized coastal line, proximity to an important river, and proximity to a fishing and pleasure port with the largest number of vessels in the area.

In contrast, biochemical and cellular metrics are less effective than population and community metrics in reflecting human pressure ($p > 0.05$), as also reported by Gerakaris *et al.* (2017). However, our PCAfindings show that these indicators can provide insight into the variability of meadow health (Fig. 2). The effectiveness of leaf tannin cell density and leaf nitrogen content as early indicators was perceived at Chenoua, the only site where the EQR assessed by the PREI was higher than that assessed by the MIAPo. While the three commonly used metrics had moderate values at Chenoua site, these two additional indicators showed signs of disturbance. This finding supports previous recommendations to include more sensitive and complex indicators, particularly those of biochemical and cellular types, for earlier detection of environmental stress (Ferrat *et al.*, 2003). However, this pattern should be confirmed across a large number of sites and supplemented with *in situ* measurements of abiotic parameters. In fact, Martínez-Crego *et al.* (2010) highlighted the limitation of indices based solely on these types of metrics to assess the ecological integrity of the habitat and recommended a combination of metrics from the biochemical to the community level to offer a good diagnostic tool in the context of bioindication (Martínez-Crego *et al.*, 2010; Personnic *et al.*, 2014; Martinez-Haro *et al.*, 2015; Romero *et al.*, 2016). As a result, one of the benefits of the MIAPo is that it ensures the complementarity of metrics from community, population, biochemical, and cellular types to provide a comprehensive assessment of the ecosystem.

In terms of study area classification, the results were expected, and the sites of the capital, Algiers, were classified as the most disturbed zone, with the highest values of PI and the lowest mean EQRs with both PREI (Algiers: 0.54; Tipaza: 0.67) and MIAPo (Algiers: 0.62; Tipaza: 0.71), which is obviously due to the concentration of human activities in this area. To ensure the survival of *P. oceanica* meadows, it is strongly advised that measures be taken to reduce the impact of human activity in this area. Similarly, several studies using BiPo (Lopez y Royo *et al.*, 2010a; Boumaza *et al.*, 2015; Pergent *et al.*, 2015; Güreşen *et al.*, 2020b) and PREI (Gobert *et al.*, 2009; Boumaza *et al.*, 2015; Gerakaris *et al.*, 2017; Rigo *et al.*, 2019)found a decrease in EQR values in urbanized and densely populated sites.

It is also important to consider sites with EQR values near the good/moderate boundary (EQR = 0.550), as recommended by Lopez y Royo *et al.* (2009b) and Romero *et al.* (2016). It is the case of the Bou Ismail site, which improves from moderate (with PREI) to good (with MIAPo), and the Chenoua site, which has an MIAPo EQR of 0.555. If these sites are to be included in monitoring programs, they must be given special attention, and pressures must be precisely identified. The best EQR value obtained using both indices at the Kouali site corresponds to an undisturbed zone with low human pressure. The Kouali meadow has previously been examined and classified as having high primary productivity and vitality (Boumaza & Semroud, 2000; Boumaza *et al.*, 2014). It was classified as having good/high ecological status when using *P. oceanica* meadow as a BQE (Boumaza *et al.*, 2015; Sengouga, 2017; Sengouga *et al.*, 2019) or the macroalgae community as a BQE for the rocky bottom quality (CFR) index (Anteur *et al.*, 2024). This fact makes the site interesting for classification and protection (PNUE/PAM/CAR PAP, 2006; Mangos & Claudot, 2013; UNEP/MAP-SPA/RAC, 2021).

The results discussed above demonstrate that indices such as MIAPo and PREI, which use a smaller number of metrics, can be valuable and quick tools for monitoring and managing coastal ecosystems, providing critical insights for informed decision-making. However, in this study, some shortcomings were unavoidable. (i) The method for assessing human-induced pressure does provide a quick assessment of human impact, but the level of data aggregation is less precise than that used to collect meadow vitality data. To ensure its effectiveness, it is recommended that it be combined with other precise methods for assessing human pressure. (ii) The relatively small number of sites included in the study may have an impact on the robustness of our findings, especially when establishing the reference conditions. In fact, despite the varied environmental conditions that characterize the study area, no site has a bad ecological status. It appears that the various anthropogenic pressures recorded at these sites had no significant impact on the studied *P. oceanica* meadows.

To confirm the accuracy and robustness of MIAPo in reflecting the ecological status of Algerian water bodies, we aim to apply it to the entire Algerian coastline in sites with diverse environmental conditions (Martínez-Crego *et al.*, 2010). Finally, the *P. oceanica* meadows must be protected in accordance with national (Law 02-2002; Law 2012: Executive decree no. 12-03) and regional legislation (Barcelona Convention 1976, amended 1995). Thus, non-destructive techniques are recommended to reduce the negative impact of sampling methods on *P. oceanica* meadows (Montefalcone, 2009). The Non-Destructive Sampling Methods (NDSM) developed by Gobert *et al.* (2020)have already been tested and approved for the PREI index. Future MIAPo applications will require a similar test because the selected metrics do not require rhizome sampling.

Conclusion

The multi-metric index MIAPo offers several advantages. It was built from different types of metrics, and it relates to human pressure and encompasses various time responses to stress. It is simple, quick, effective, and low-cost, meeting managers' and decision-makers needs and inter-calibration requirements. Its inter-calibration with the PREI index yields comparable results with enhanced reliability to human stressors by integrating biochemical and cellular metrics (Martínez-Crego *et al.*, 2010), and it can be assessed using NDSM. However, further investigation is required for the index adoption: (i) a refinement of the approach used to define reference conditions to avoid eventual loss of precision in the classification of the ecological status; (ii) other population-type metrics should also be considered because they provide a more accurate picture of meadow health. Furthermore, it is recommended that the 'lower limit' indicator be calibrated, because most of our meadows exhibit lower limits associated with rocky substrates, controlled by hydrodynamic conditions, as described by Clabaut *et al.* (2010) in Corsica (France), the northern Gulf of Tunis (Tunisia), and El Kala National Park (Algeria); (iii) integration of environmental parameters measurement when assessing the pressure index, specifically the nitrogen content in interstitial water, in order to establish a relationship with the nitrogen content in tissues (Leoni *et al.*, 2008). In this context, we also propose testing Specific Leaf Area (SLA) as a nutrient enrichment indicator in *P. oceanica* meadows, as it has previously been associated with light attenuation and leaf photosynthetic rates (Olesen *et al.*, 2002; Enríquez *et al.*, 2004; Nicastro *et al.*, 2015)and leaf nutrient variation in other magnoliophyte (Ainley *et al.*, 2016) ; (iv) the tannin cell density in leaves should be compared to other parts of the plant, such as rhizomes, given their tendency to exhibit minor fluctuations in phenolic compounds and a notable response to effluent discharge (Migliore *et al.*, 2007); (v) MIAPo should be applied on a larger scale for comparability and inter-calibration purposes, and its relationship with several human pressure assessment methods should be investigated. Additionally, a comparison with other indices that utilize a greater number of descriptors, such as the POMI or the CS Valencian, is necessary to enhance understanding of its efficacy and facilitate the harmonization of these indices.

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