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A biotic index using the seagrass *Posidonia oceanica* (BiPo), to evaluate ecological status of coastal waters

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ABSTRACT

The development of ecologically based indices that respond to disturbances in a predictable manner has been stressed by the EU Water Framework Directive. The seagrass *Posidonia oceanica*, given its ecological indicator characteristics, has been identified as one of the elements to determine ecological status under the EU Water Framework Directive. The purpose of this study is therefore to develop a biotic index based on *P. oceanica* (BiPo), focussing on: (i) the necessity of an index that may be applied over the largest geographical extent possible, (ii) the necessity of a tool for a baseline evaluation of *P. oceanica* status in the Mediterranean, (iii) the compliance with WFD requirements, (iv) the efficiency of the method in terms of reliability and cost. The BiPo index is developed on the basis of all *P. oceanica* monitoring data available in the western Mediterranean and on a standard assessment of anthropogenic pressures. The index metrics are selected and evaluated on the basis of this pressures assessment, and are subsequently integrated for the evaluation of ecological status. The index is then tested on 15 sites around Corsica (France). The results show that the BiPo well reflects meadow health status and ecological status. Furthermore it is reliable, standard and cost-effective, and can be applied to a wide array of management and conservation purposes.

1. Introduction

Aquatic ecosystems are increasingly subject to human-induced pressures, which significantly jeopardise ecosystem conservation. The use of ecological indicators is considered at present the most adequate tool to assess water status, as they supply information about the state of the ecosystem and, if chosen accurately, reflect changes in environmental quality (Blandin, 1986; Dauer, 1993). The main criteria that should be considered when selecting an ecological indicator are its sensitivity to disturbance, its ability to synthesise complex information in a reliable way, its easiness and simplicity of application, its applicability in extensive geographical areas, and its relevance to policy and management requirements (Blandin, 1986; Dale and Beyeler, 2001; Salas et al., 2006). Seagrass are increasingly used as ecological indicators, because of their wide distribution, their ecological role, and their sensitivity to disturbance (Pergent et al., 1995; Short and Wyllie-Echeverria, 1996; Krause-Jensen et al., 2005). The seagrass *P. oceanica* is widely distributed in Mediterranean coastal waters (Procaccini et al., 2003) and its specific responses to anthropogenic disturbance are acknowledged (Balestri et al., 2004; Ruiz and Romero, 2003; Leoni et al., 2006). However information and data remain heterogeneous, and are rarely synthesised or associated with a meadow quality assessment (Lopez y Royo et al., 2007).

The use of seagrass as tool for an ecological evaluation of coastal waters has recently been highlighted in European legislation by the Water Framework Directive (WFD) (EC, 2000). The Directive establishes the basis for monitoring, protection and enhancement of all aquatic ecosystems in European Member States (MS), by setting ecological quality objectives (i.e. "good water status" for all European waters by 2015) which require to assess water quality status using a combination of ecological indicators in priority. Angiosperms are one of the four biological quality elements required for the evaluation of ecological status. Given its policy relevance, it is therefore essential, for seagrass in Europe, to consider WFD requirements when developing an ecological index.

The WFD requires the establishment of assessment systems, based on pressures and impacts on the ecosystems and their relevant components, and sets precise criteria and a stepwise procedure for their development (EC, 2000). The main requirements being: (i) the use of specific biological elements (BQE) supported by physico-chemical elements, (ii) the development of the classification on the basis of the relationship between BQE and disturbance, (iii) the quantification of ecological status on the basis

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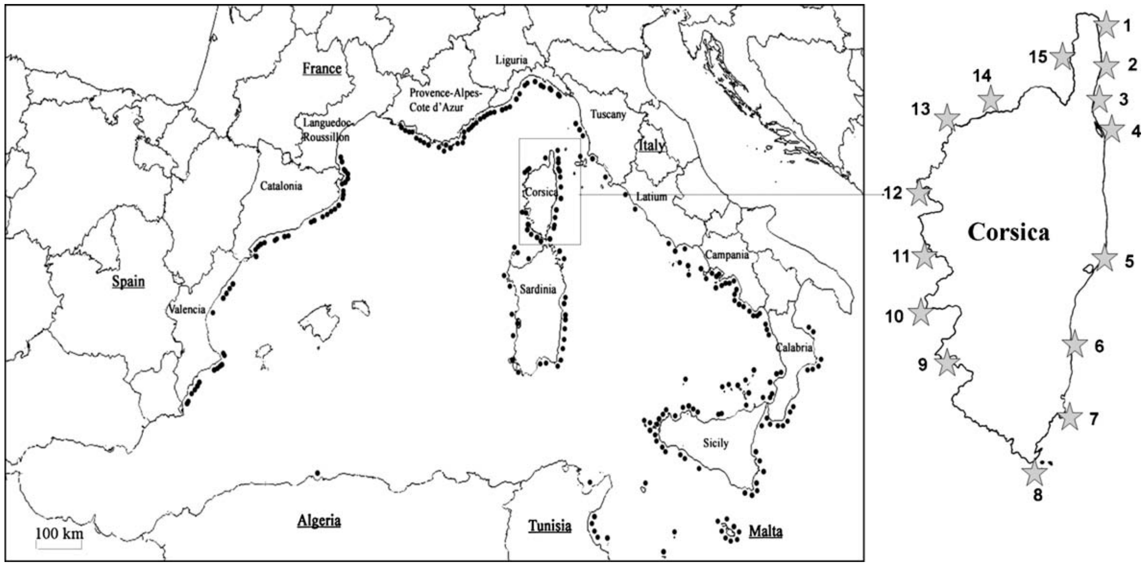

Fig. 1. Sites considered in this study ((●) data available in literature and used for the development of the index; (☆) sites sampled in this study for the application of the index—(1) Macinaggio, (2) Cap Sagro, (3) Toga, (4) Arinella, (5) Diana, (6) Secteur Est, (7) La Chiappa, (8) Lavezzi, (9) Porto Pollo, (10) La Parata, (11) Sagone, (12) Porto, (13) Calvi, (14) Ile Rousse, (15) Canari).

of the ratio between the actual condition and the reference condition of the BQE, (iv) the definition of 5 status classes (high, good, moderate, poor, bad) and their relative boundaries and, (v) the comparability of the different classification results across an ecoregion (EC, 2000). The WFD normative definitions for angiosperms ecological status specify that the assessment be based on two metrics: composition (presence/absence of disturbance-sensitive taxa) and abundance (EC, 2000—Annex V). Throughout the WFD implementation process in Mediterranean coastal waters, at one of the initial stages, i.e. the Intercalibration process (EC, 2000—Annex V), Member States' experts reached a common agreement concerning angiosperm classification. Given that all seagrass are disturbance-sensitive (Short and Wyllie-Echeverria, 1996) and moreover that most Mediterranean seagrass meadows are monospecific, only one of the two required metrics, namely abundance, will be considered, supported by a number of additional descriptors related to environmental quality (Med-GIG, 2007). Furthermore, *P. oceanica* was selected as representative species of the angiosperm quality element, for classification and intercalibration.

The aim of this paper is to develop a biotic index based on *P. oceanica* (BiPo), that may be a simple and effective indicator of seagrass ecological status, while considering the issues described above. The index will focus on: (i) the necessity of an index that may be applied over the largest geographical extent possible, (ii) the necessity of a tool for a baseline evaluation of *P. oceanica* status in the Mediterranean, (iii) the compliance with WFD requirement, (iv) the efficiency of the method in terms of reliability and cost.

2. Material and methods

2.1. Study area and data matrix

2.1.1. Study area

The area under study is the entire western Mediterranean basin (Fig. 1). The data collected belongs to most of the coastal regions in that area, in which *P. oceanica* is present (Procaccini et al., 2003).

2.1.2. Datasets

Two datasets are produced for the purpose of this study (Fig. 1). The first dataset is composed of the available data in *P. oceanica* monitoring programmes in the western Mediterranean, provided by national or regional administrations and researchers (Table 1). This dataset is used to develop the biotic index. The second dataset

Table 1
Monitoring programmes that were available for this study.

Country	Region	Programme	Reference
Algeria		Monitoring set-up	Boumaza and Semroud (2000)
France	Corsica	Regional monitoring	Pergent et al. (2005)
	Provence-Alpes-Côte-d'Azur	Regional monitoring	Cadiou et al. (2004)
	Languedoc Roussillon (LR)	Site monitoring	Ballesta et al. (2000)
	PACA and LR	WFD monitoring	Gobert et al. (2007)
Italy	All coastal regions	National monitoring	MATT (2001a)
	Liguria	Regional monitoring	ARPAL (2005)
	Liguria, Tuscany, Latium	Mapping follow up	RIPO (2002)
	Calabria, Campania	*P. oceanica* mapping	MATT (2004)
	Sardinia	*P. oceanica* mapping	MATT (2001b)
	Sicily	*P. oceanica* mapping	SINPOS (2001)
Malta	All	Baseline Survey	MEPA (2002)
Spain	Catalonia	WFD monitoring	Romero et al. (2005)
	Valencian Community	WFD monitoring	Ramos Esplà et al. (2005)
Tunisia		Different studies	Vela (2006), Sghaier (2006)

is composed of data collected in the field (Section 2.3) in 15 sites along the Corsican coastline (Fig. 1), and it is used to test the index developed.

The first dataset, for the development of the biotic index, has been previously screened for data comparability issues; these issues have been resolved prior to their use in this study (Lopez y Royo et al., 2007). The dataset is composed of the descriptors that are most commonly used in *P. oceanica* monitoring in western Mediterranean (Pergent-Martini et al., 2005; Lopez y Royo et al., 2007); i.e. lower limit depth, limit type, shoot density, leaf cover, plagiotropic rhizome growth, rhizome baring, number of leaves, coefficient *A* (i.e. the percentage of leaves with broken apices), shoot leaf surface, leaf production, rhizome elongation, rhizome production, epiphytes biomass.

2.2. Development of the biotic index, BiPo

The WFD requires the establishment of assessment systems that are based on pressures and impacts on the ecosystems and their relevant components (EC, 2000). In this perspective anthropogenic pressures are assessed prior to the development of the index and are used as the basis for the following analysis. The development of the biotic index BiPo follows 4 different steps: depth selection, metrics selection, metrics evaluation, and evaluation of ecological status.

2.2.1. Evaluation of anthropogenic pressures

Human-induced pressures in the sites under study are assessed according to the method described in Lopez y Royo et al. (2009). This method is based on satellite images and public census data. The main types of pressures adopted as indicators for the assessment are: landuse, industrial activity, river discharges, port activities and coastal planning. The method identifies anthropogenic pressures and distributes their relevance along a gradient, through a standard process, that enables to distinguish, in a homogeneous and objective way, between sites that are subject to significant pressures and sites that are subject to non-significant pressures, *sensu* WFD (EC, 2000—Annex II). For clarity, hereafter, sites assessed for pressures will be indicated as SP (sites subject to significant pressures) and NSP (sites subject to non-significant pressures).

2.2.2. Depth selection

The *P. oceanica* monitoring data are spread over most of the seagrass' habitat depth range (0–45 m). As most of the *P. oceanica* descriptors considered are depth-dependent (Pergent-Martini et al., 1994; Buia et al., 2004), the selection of a specific depth is necessary. The reference depth has to be the least subject to natural variability and the most sensitive to environmental disturbance.

Natural sources of variability of *P. oceanica*, at a same depth or isobath, are particularly high in shallow meadows (Alcoverro et al., 1995; Marbà and Duarte, 1997; Balestri et al., 2003), whereas variability is more clearly linked to environmental quality in deep meadows (Alcoverro et al., 1995; Marbà and Duarte, 1997). The lower limit depth of the meadow moreover, being at photosynthetic compensation depth, is particularly sensitive and is therefore selected as reference depth for quality assessment. However the bathymetric depth of lower limits varies between meadows (Pergent et al., 1995; Pasqualini et al., 1998) and its selection does not resolve the issue of depth-dependence of descriptors. The selection of an additional homogeneous depth (same isobath) is therefore necessary: an intermediate depth (15 ± 1 m) is the most appropriate, as identified in the dataset, it is the optimal combination between maximum depth and maximum availability in meadows across the Mediterranean. This intermediate depth (15 ± 1 m) has also been adopted by other *P. oceanica* indices of ecological status (Buia et al., 2005; Romero et al., 2007). Therefore both, the lower limit depth and the intermediate depth of 15 ± 1 m, are selected as reference depths for the development of the index.

2.2.3. Selection of metrics

The *P. oceanica* descriptors, considered for potential selection as index metrics, are those included in the first dataset described (Section 2.1.2) and are reported in Table 2. Descriptors are selected as metrics on the basis of the pressure analysis (Section 2.2.1). Each of these descriptors is tested for significant differences between SP and NSP sites, on both the selected depths (lower limit depth and 15 ± 1 m). The descriptors that differ significantly are then selected as individual metrics of the *P. oceanica* classification index, for the depth in which they differ (lower limit, or 15 m, or both). Moreover, the metrics selected will have to respond to pressures as predicted and acknowledged by literature, in order that all metrics be effective and unequivocal indicators (Dale and Beyeler, 2001) of *P. oceanica* status (i.e. if their response is not in agreement with literature they will be discarded).

Differences between sites are tested using a *t*-test for independent samples when conditions are met (normality and homogeneity of distribution), or the non-parametric Mann–Whitney *U* test when they are not. For qualitative data, difference between sites is tested using a Chi square ($2 \times N$) test. Season-dependent parameters are tested using a Factorial ANOVA, for which the "pressure*month" effect is investigated. Normality of

Table 2
Statistical tests and results, for differences between SP and NSP sites, for the most commonly used descriptors (M–W *U*: Mann–Whitney *U* test; Test value for M–W U is the *U* value, for *t*-test it is the *t* value, for χ^2 it is the Chi square value, for the factorial ANOVA it is the *F* value for the "pressure*month" effect).

Descriptor	Lower limit			15 m		
	Test	Value	*p*-Value	Test	Value	*p*-Value
Lower limit depth	M–W *U*	806.50	0.00*	n/a		
Limit type	χ^2	35.6	0.00*	n/a		
Shoot density	*t*-Test	1.72	0.09	M–W *U*	302.0	0.00*
Leaf Cover	ANOVA	0.52	0.72	ANOVA	0.83	0.51
Percentage of plagiotropes	*t*-Test	0.41	0.68	M–W *U*	73.5	0.00*
Shoot baring	*t*-Test	−0.77	0.45	*t*-Test	−0.76	0.46
Number of leaves	ANOVA	1.09	0.35	ANOVA	0.41	0.66
Coefficient *A*	*t*-Test	0.36	0.72	*t*-Test	0.10	0.91
Shoot leaf surface	ANOVA	0.31	0.87	ANOVA	3.17	0.01*
Leaf production	M–W *U*	86.00	0.41	*t*-Test	0.17	0.86
Rhizome elongation	M–W *U*	91.50	0.56	*t*-Test	0.11	0.92
Rhizome production	*t*-Test	0.26	0.80	*t*-Test	−0.74	0.46
Epiphytes biomass		Not sufficient data		ANOVA	0.88	0.42

* Significant values at $p < 0.05$.

distribution is investigated using the Kolmogorov–Smirnov normality test and homogeneity of variance using the Levene's test. Significant levels are tested at $p < 0.05$.

2.2.4. Evaluation of metrics

The descriptors selected as metrics of the classification index are assessed individually, according to the EU-WFD requirements.

Reference conditions describe the characteristics of the quality element in undisturbed conditions (EC, 2000), and can be defined using different options (EC, 2000—Annex II; CIS-WFD, 2003). In this index, reference conditions are set for individual metrics on the basis of spatial data (i.e. existing sites in undisturbed conditions). The three highest values are averaged in order to buffer possible outliers. Sites to which these highest values belong to are cross-checked with satellite images, in order to ensure that they refer to undisturbed areas.

An evaluation scale is developed for each selected metric. The WFD requires the definition of five status classes on the basis of the relationship to disturbance, i.e. from no disturbance (high ecological status) to severe disturbance (bad status) (EC, 2000; CIS-WFD, 2003). In this index, the bad status class corresponds to the observed value zero for all metrics, as it represents the recent disappearance of a *P. oceanica* meadow (see Section 2.2.5). The remaining three class boundaries are set for "real values" of individual metrics selected, on the basis of the significant statistical differences that exist between SP and NSP sites, which have enabled to identify a discontinuity in the relationship of the metric to pressures. This discontinuity will correspond to the class boundary (Pollard and van de Bund, 2005) between Good and Moderate. Thus, the Good/Moderate boundary is set by the central value between confidence intervals (0.95) of the two groups of data, whereas High/Good and Moderate/Poor boundaries are set by upper or lower confidence intervals of the relevant group of data (Fig. 2). For season-dependent descriptors, this procedure is applied once the effects of season and pressure have been assessed, and if necessary by selecting the most appropriate season. Effect of months is determined by one-way ANOVA and categorised box plots, separate *t*-tests are performed on significantly different months. For qualitative data, classes are attributed according to frequency distribution of data in the two groups, with the support of literature.

2.2.5. Evaluation of ecological status: integration of metrics

In order to evaluate ecological status of *P. oceanica* according to the WFD, individual metrics need to be integrated and translated

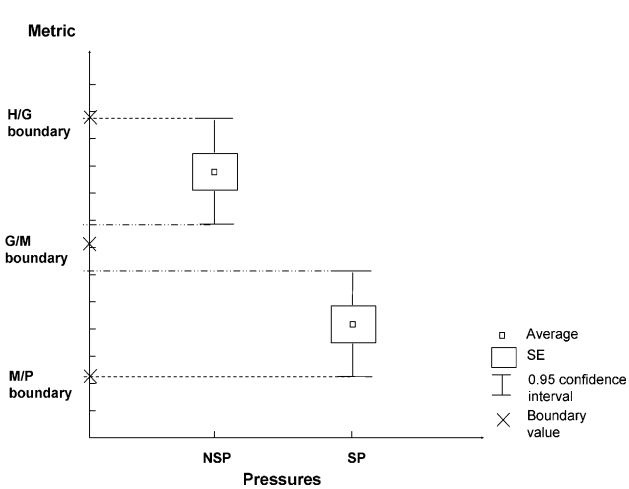

Fig. 2. Boundary setting procedure for individual metrics (NSP: sites subject to non-significant pressure; SP: sites subject to significant pressures; H/G: High/Good: G/M: Good/Moderate; M/P: Moderate/Poor).

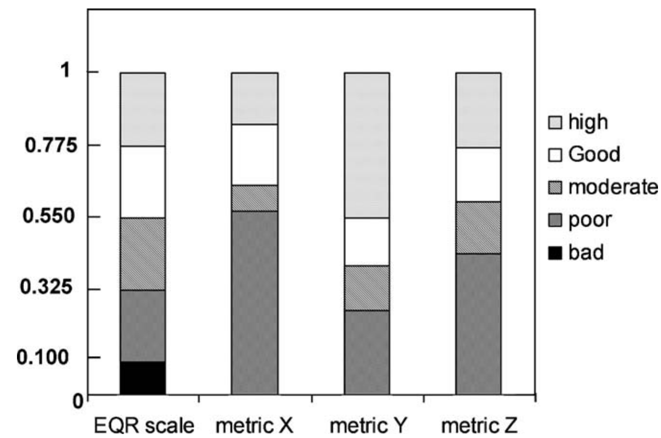

Fig. 3. EQR scale of the BiPo index and harmonisation of individual metrics: example of possible differences in the deviation from reference conditions of class boundaries.

into a single scale. Ecological status has to be quantified upon the degree of alteration or deviation of observed values from reference conditions, and expressed as a numerical value, the ecological quality ratio (EQR), comprised between 1 (in reference condition) and 0 (in the worst condition).

Reference conditions for the *P. oceanica* quality element are set by combining the reference values of individual metrics. These reference conditions refer to an "optimal site" composed of existing values, but which do not necessarily belong to a single existing site.

In terms of the integrated EQR scale, as seagrass are recognised to be highly sensitive to human disturbance (Short and Wyllie-Echeverria, 1996), and *P. oceanica* cannot survive in extremely degraded environments (Boudouresque et al., 2006; Romero et al., 2007), the presence of *P. oceanica* indicates that the ecological status of water quality is above bad (Romero et al., 2007). Bad ecological status in *P. oceanica* will therefore correspond to a recorded recent die-off of the meadow (<5 years) (Med-GIG, 2007). This is in agreement with normative definitions, in which the bad status is defined also by the absence of large portions of the relevant communities (EC, 2000). The bad ecological status class is therefore arbitrarily set at <0.1, on the EQR scale. The other four EQR class boundaries are established by the individual metrics.

Individual metrics have their own evaluation scale, and although class boundaries are set according to the same methodology, their degree of alteration from reference conditions may differ according to the results of the statistical analysis of individual metrics (Fig. 3). To avoid unequal weighting of each metric on status classes, the evaluation scales and class boundaries are normalised on a fixed EQR scale. The EQR class boundaries are therefore established by dividing the remaining scale (0.1–1) into four equal classes (Fig. 3). Harmonisation of individual metrics is performed on the basis of this scale, according to the following formula:

$$\mathrm{EQR}'_{\mathrm{metric}} = \left(\left(\frac{X - \mathrm{LB}}{\mathrm{HB} - \mathrm{LB}}\right) \times 0.225\right) + \mathrm{LB} \qquad (1)$$

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 corresponds to the width of a class on the EQR scale in Fig. 3.

The overall EQR, quantifying *P. oceanica* ecological status, is then determined by averaging scores.

Table 3
Evaluation of individual metrics and definition of their class boundary values. (Metric lower limit depth; metric lower limit type; metric shoot density; metric shoot leaf surface or shoot length relative to measures in late summer August–September.).

Status class	High	Good	Moderate	Poor
Lower limit depth (m)	>31	31–25	25–19	<19
Type of limit	Progressive and erosive	Sharp	Sparse	Regressive
Shoot density (shoots m^{-2})	>339	339–239	239–172	<172
Shoot leaf surface (cm^2 $shoot^{-1}$)	>200	200–152	152–119	<119
Or shoot length (mm $shoot^{-1}$)	>812	812–651	651–481	<481

2.3. Case study: application to the Corsican coastline

Sites were sampled between June and July 2007 in 15 sites located around Corsica (Fig. 1). *P. oceanica* sampling is performed, on each site, at 15 ± 1 m and on the lower limit depth of the meadow. At both depths, shoot density, meadow cover, percent plagiotropic rhizomes, baring and shoot length are measured in the field by scuba diving; on the lower limit, depth and type of limit are also recorded. Additionally 20 shoots of *P. oceanica* are collected for laboratory analyses.

Leaves are sorted according to the protocol of Giraud (1979) and the phenological characteristics (leaf length, leaf width, shoot foliar surface area, coefficient *A*) determined. Lepidochronology is performed (Pergent, 1990), in order to determine mean rhizome growth and leaf production. Epiphytes are removed from the leaves using a glass strip, they are then dried and weighed.

The EQR and ecological status of each site is then determined according to the BiPo classification system developed. Pressures are assessed on the same sites, by attributing indicator scores, according to the method developed by Lopez y Royo et al. (2009). The relationship of the BiPo with pressures is verified by performing a linear regression between EQR results and results of the pressure assessment.

3. Results

3.1. Development of the biotic index based on Posidonia oceanica (BiPo)

3.1.1. Selection of metrics

The results show that the descriptors that are significantly different, in SP and NSP sites, are: depth and type on the lower limit, and shoot density, plagiotropic growth and shoot leaf surface at the intermediate depth of 15 ± 1 m (Table 2).

The sensitivity of these descriptors to human-induced pressures and their dependence on environmental quality is strongly supported and acknowledged by literature (Meinesz and Laurent, 1978; Duarte, 1991; Pergent et al., 1995; Ruiz and Romero, 2003; Leoni et al., 2006). The only descriptor for which response to pressure is controversial is the percent plagiotropic rhizomes at intermediate depth. A high percentage of plagiotropic rhizomes at an intermediate depth may be due to the natural patchiness of the meadow (Panayotidis et al., 1981) or to physical disturbances such as anchoring or trawling (Francour et al., 1999). Therefore it seems that (i) plagiotropic rhizomes at intermediate depths increase following anthropogenic disturbance (Francour et al., 1999; Romero et al., 2007), (ii) however a recolonisation of the patch created by the mechanical damage is indicative of a certain vitality or health of the meadow. Thus, due to its controversial relationship to ecosystem status, this descriptor will not be considered.

Four descriptors are therefore selected as metrics for the *P. oceanica* index of ecological status and water quality: lower limit depth, lower limit type, shoot density (at 15 ± 1 m) and shoot leaf surface (at 15 ± 1 m).

3.1.2. Evaluation of the metric lower limit depth

The results of the statistical analysis for the lower limit depth show that there are significant differences between SP and NSP sites. The class boundaries for the lower limit depth are set straightforwardly and are reported in Table 3.

3.1.3. Evaluation of the metric lower limit type

Five types of lower limits of the *P. oceanica* meadows have been described: progressive, erosive, sharp, sparse and regressive limits (Meinesz and Laurent, 1978; Pergent et al., 2005). The Chi square test shows that there are significant differences between types of limit in SP and NSP sites. Frequency of distribution of lower limit types shows that erosive, progressive and sharp limits are mainly present in NSP sites, whereas sparse and regressive limits are mainly present in SP sites (Table 4).

According to this analysis, it is possible to assign lower limit types to four status classes, in agreement with literature (Meinesz and Laurent, 1978; Pergent et al., 1995). The metric lower limit type is therefore evaluated as: (i) high, when the meadow grows towards deeper areas suggesting increased seagrass vitality and/or improvement of environmental conditions (progressive limit) or when the depth is limited by natural conditions (e.g. hydrodynamic activity or geomorphology) and not by the plant's photosynthetic compensation depth (totally erosive limits); (ii) good, when the meadow is stable and therefore its limit remains at photosynthetic compensation depth (sharp limits); (iii) moderate, when the meadow shows difficulties in developing at the limit depth, suggesting its slight deterioration (sparse limits); (iv) poor, when meadow conditions have clearly deteriorated (regressive limits) (Table 3). The EQR class values for the metric lower limit type are arbitrarily set by giving it the class centre value.

3.1.4. Evaluation of the metric shoot density

The results show that shoot density is significantly different, at intermediate depth (15 ± 1 m), in NSP and SP sites. The class boundaries for the shoot density are set according to the procedure described, and are reported in Table 3.

3.1.5. Evaluation of the metric shoot leaf surface

The results of the Factorial ANOVA show that there is a significant effect of "pressure*months". The categorised box plots reveal that shoot leaf surface in August and September has a lower variability (Fig. 4). This stability is probably due the reduced growth rates of the plant at that period (Bay, 1984; Alcoverro et al., 1997). August/September is therefore selected as reference period of the year for shoot leaf surface. Shoot leaf surface during this period is significantly different in NSP and SP sites (*t*-test,

Table 4
Frequency distribution (%) of lower limit types in SP and NSP sites.

	Erosive	Progressive	Sharp	Sparse	Regressive
NSP	80%	87%	69%	40%	10%
SP	20%	13%	30%	60%	90%

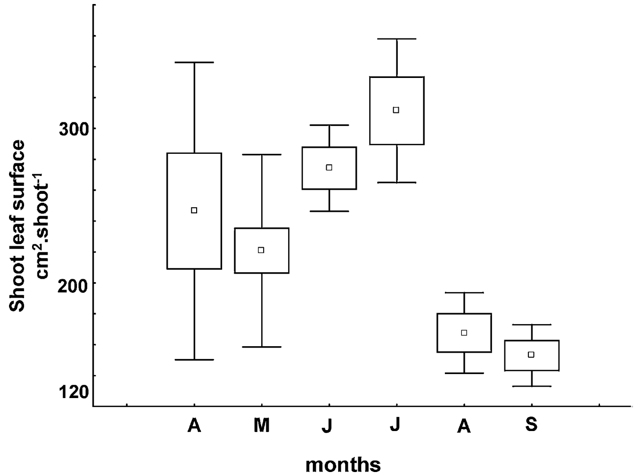

Fig. 4. Shoot leaf surface in relation to months at 15 ± 1 m depth (mean, SE, confidence interval).

$p < 0.005$). The Class boundaries are set according to the procedure described, on data relative to the months of August and September (Table 3).

Moreover, the selected metrics are all non-destructive measures except for shoot leaf surface. As destructive sampling is considered not to be in the spirit of environmental policies (Foden and Brazier, 2007; Pergent-Martini et al., 2006), and given the high correlation between shoot leaf surface and shoot length ($R^2 = 0.912$), shoot leaf surface can be replaced by shoot length, as non-destructive leaf biometry metric. Shoot length can be measured directly in the field or on collected shoots. Class boundary values for shoot length can be set using the same procedure as for the other metrics (Table 3).

3.1.6. The BiPo index: integration of metrics

The results of the harmonisation of individual metrics, through the calculation of EQR′ for each metric, are shown in Table 5. The EQR value of *P. oceanica* ecological status is then determined by integrating individual metrics, by averaging EQR′ scores:

$$\mathrm{EQR} = \frac{\mathrm{EQR}'_{\mathrm{LL\,depth}} + \mathrm{EQR}'_{\mathrm{LL\,type}} + \mathrm{EQR}'_{\mathrm{Density}} + \mathrm{EQR}'_{\mathrm{shoot\_leaf\,surface}}}{4} \quad (2)$$

Classification of *P. oceanica* ecological status is obtained using EQR results on the EQR scale defined in Fig. 3.

3.2. Case study: evaluation P. oceanica ecological status around Corsica

Of the 15 sites monitored along the Corsican coastline 4 are classified as being in high ecological status, 9 in good ecological status, and 2 in moderate ecological status (Table 6). This result is in accordance with what is expected, considering the few sources of anthropogenic pressures on the island. The population density is on average very low (279 000 total inhabitants, approximately 32 inhabitants/km^2; source: INSEE) and the major sources of pressure are the commercial ports of Ajaccio and Bastia, which are also the largest towns of the island. This is also supported by the relationship between EQR results and pressure assessment results (Fig. 5), for which the linear regression presents an R^2 of 0.659.

Consistently two of the sites with the highest EQRs (Macinaggio and Porto) belong to protected areas (respectively the Natural Reserve of the Finocchiarola isles and the Marine Protected Area of Scandola), whereas sources of pressure in sites with the lowest

Table 5
The BiPo index: evaluation and harmonisation of metrics.

Class		RC	High	Good	Moderate	Poor	Bad(3)
Lower limit depth	Values (m)	38	>31	31–25	25–19	<19	n/a
	EQR′	1	((X − 31)/7) × 0.225) + 0.775	((X − 25)/6) × 0.225) + 0.55	((X − 19)/6) × 0.225) + 0.325	((X/19) × 0.225) + 0.1	0.05
Lower limit type	Values	n/a	Progressive and erosive limits	Sharp limits	Sparse limits	Regressive limits	n/a
	Supporting parameters(1)		>70% cover or>70% plagio	<70% cover and <70% plagio	<15% cover, %plagio n/a	recent dead matte	n/a
	EQR′	1	0.89	0.66	0.44	0.21	0.05
Shoot density	Values (shoots m^2)	599	>339	339–239	239–172	<172	n/a
	EQR′	1	((X − 339)/260) × 0.225) + 0.775	((X − 239)/100) × 0.225) + 0.55	((X − 172)/67) × 0.225) + 0.325	((X/172) × 0.225) + 0.1	0.05
Shoot leaf surface(2)	Values (cm shoot^{-1})	310	>200	200–152	152–119	<119	n/a
	EQR′	1	((X − 200/133) × 0.225) + 0.775	((X − 152)/48) × 0.225) + 0.55	((X − 119)/33) × 0.225) + 0.325	((X/119) × 0.225) + 0.1	0.05
Or shoot length	Values (mm)	955	>812	812–651	651–481	<481	n/a

RC: reference conditions.
(1) % cover and % plagiotropic rhizomes (plagio) are used to support characterisation of lower limit types.
(2) In cases of sampling constraints or historical data, boundary values determined for the late summer can be adapted to early summer or spring, by normalising them against shoot leaf surface reference conditions. Reference conditions for shoot leaf surface are 290 cm shoot^{-1} for spring (April–May), 381 cm shoot^{-1} for early summer (June), and 443 cm shoot^{-1} for full summer (July).
(3) Bad Status corresponds to a recorded recent die-off of the meadow (<5 years).

Table 6
Evaluation of the sites monitored in Corsica according to the BiPo index.

Site	Lower limit depth (m)	Lower limit type			Shoot density (shoots m^{-2})	Shoot length (mm shoot^{-1})	EQR	Class
		Type	Cover (%)	Plagio (%)				
Macinaggio	38.0	Erosive	22.0	20.6	243.8	978.1	0.856	High
Cap Sagro	33.0	Sharp	13.0	0.6	270.8	989.7	0.778	High
Toga	24.2	Regressive	19.0	9.1	230.7	853.8	0.509	Moderate
Arinella	26.8	Sharp	55.0	30.9	246.4	913.9	0.680	Good
Diana	36.1	Regressive	39.0	0.5	315.1	830.8	0.659	Good
Secteur Est	36.9	Sparse	15.0	n/a	325.0	903.0	0.752	Good
La Chiappa	35.3	Sharp	19.0	11.0	304.0	839.3	0.759	Good
Lavezzi	30.3	Progressive	62.0	18.0	169.3	914.7	0.709	Good
Porto Pollo	32.2	Sharp	33.0	90.0	256.3	730.0	0.729	Good
La Parata	35.3	Sparse	10.0	n/a	176.1	732.1	0.555	Good
Sagone	33.2	Progressive	23.5	76.5	472.4	576.7	0.762	Good
Porto	36.5	Progressive	6.8	95.7	338.4	785.8	0.802	High
Calvi	37.8	Sharp	25.0	2.9	310.4	887.1	0.803	High
Ile Rousse	35.8	Regressive	15.0	0.0	208.3	810.7	0.552	Good
Canari	27.0	Sparse	5.0	0.6	280.2	690.8	0.547	Moderate

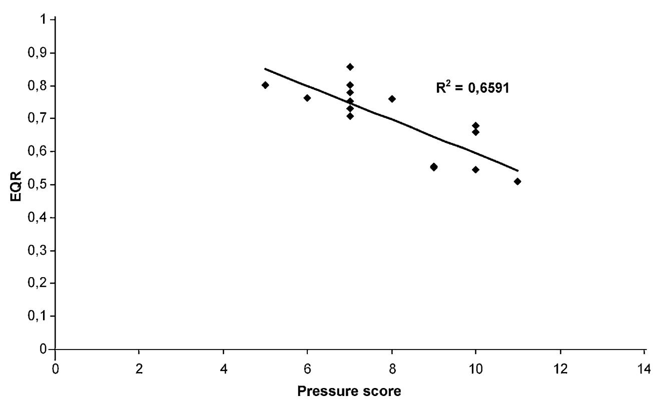

Fig. 5. Relationship between the EQR determined by the BiPo index in Corsica and the scores resulting from the pressure assessment according to Lopez y Royo et al. (2009).

EQRs (Canari and Toga), have been reported in literature. The discharges of the previous asbestos mine of Canari (11 million tons of rubble in the sea between 1948 and 1965) still have an effect on *P. oceanica* (Lafabrie et al., 2008). The recent coastal development in Toga (i.e. enlargement of the recreational port) increased water turbidity (Pergent et al., 2008), affecting the *P. oceanica* ecosystem, already under threat for its proximity to the largest commercial port and town of Corsica (i.e. Bastia). It is also interesting to note that two sites classified as good have an EQR in proximity of the Good/Moderate boundary (La Parata and Ile Rousse). La Parata is situated at 500 m from a fish farm (visible in the satellite images), whereas Ile Rousse is located in proximity of an urban wastewater collector (Pergent et al., 2008) which, at the moment, is not associated with an operational wastewater treatment plant (source: EAURMC). This suggests that the index is able to indicate sites that are the most liable to undergo deterioration in the future.

4. Discussion

The biotic index based on *P. oceanica* (BiPo), developed to evaluate ecological status, responds to the requirements that gave rise to its development.

The classification index developed is in accordance with the EU Water Framework Directive requirements (EC, 2000), as: (i) it has been developed on the basis of a pressure analysis in order to determine the characteristics of the quality element under disturbance, (ii) reference conditions have been defined using a combination of spatial data and modelling, (iii) ecological status is assessed in one of the five required classes, (iv) the EQR scale ranges from 1 (best conditions) to 0 (worst conditions) and actual observed conditions refer to the defined reference conditions. Furthermore, the classification index is in agreement also with the decisions taken within the Mediterranean Intercalibration Group (MedGIG), working group established by the European Commission for intercalibration of coastal waters classification in the Mediterranean ecoregion (Med-GIG, 2007). The classification index corresponds to the agreed EQR scale as well as to the conceptual definition of classes (Med-GIG, 2007).

One of the main advantages of the BiPo index is that it has been developed on the basis of a western Mediterranean dataset and on the basis of a homogeneous evaluation of anthropogenic pressures; it can therefore reflect the meadow quality status in most of the area. Thus the index may be applied to an extensive geographical area. On the other hand, the use of such a geographically extended database may also limit the consideration of potential regional or biogeographical variability in *P. oceanica* meadows. A few studies have suggested that natural sources of variability may affect *P. oceanica* meadow dynamics on a large scale (Duarte, 1991; Alcoverro et al., 1995; Marbà et al., 1996; Greve and Binzer, 2004). However, (i) none of these studies indicate that natural sources of variability are the main causes of differences in health of meadows located in different geographical areas, (ii) nor do they define the characteristics which would enable to identify different biogeographical areas; (iii) a number of studies also suggest that overall values allow to determine meadow deterioration across geographical areas or latitudinal gradients (Pergent et al., 1995; Marbà et al., 1996; Marbà et al., 2005; Pergent-Martini et al., 2005). Moreover, at this stage, within the Angiosperm Expert Group in the MedGIG (WFD WG), typologies (*sensu* WFD; EC, 2000) have not been considered relevant for *P. oceanica* ecological status evaluation (Med-GIG, 2007), and experts have not suggested any other subdivision of the Western Mediterranean for this purpose. The only way to consider potential biogeographical variability of *P. oceanica* meadows would be by applying political or administrative boundaries, which is exactly what this study proposes to overcome. Thus, according to these considerations, the approach at the level of Western Mediterranean is the most appropriate; and it is also in line with the "ecosystem approach" recently adopted by a number of policies in Europe and the Mediterranean, such as the WFD (EC, 2000), the Marine Strategy Framework Directive (EC, 2008), and the MEDPOL programme, phase IV, of the Barcelona Convention (UNEP, 2005). However, if in future typologies or biogeographical areas are clearly defined, the BiPo index could be adapted to these by applying the same methodology to the relative subsets of data or by simply modifying the reference conditions accordingly. Furthermore, the application at the scale of the Corsican coastal waters shows that the BiPo index adequately reflects ecological status (see Section 3.2), and supports the fact that, data pooling at the level of Western Mediterranean does not affect the reliability of the BiPo index at regional scale. However to ensure the basin-wide applicability of the index, it would be necessary to apply it to other areas of the Mediterranean or to perform an intercalibration with other WFD-compliant classification systems that have been developed (Casazza et al., 2006; Romero et al., 2007).

Another important element to consider in the effectiveness of an ecological index is the adequacy of its response time, in relation to its objectives. If it has been extensively demonstrated that *P. oceanica* meadows respond relatively quickly to disturbance (Ruiz and Romero, 2001; Leoni et al., 2006), literature on response time to environmental quality improvement are scarce. The four metrics adopted respond to disturbance on annual basis (Pergent et al., 1995; Pergent-Martini et al., 2005) or less (Ruiz and Romero, 2001; Pergent-Martini et al., 2005; Leoni et al., 2006). In terms of recovery however, their response time varies. In particular, concerning lower limit depth significant changes may not be detectable for a longer period of time, given the low growth rates of *P. oceanica* (Marbà and Duarte, 1998; Gonzalez-Correa et al., 2005), for which horizontal growth is on average 1–6 cm $year^{-1}$ (Marbà et al., 1996; Marbà and Duarte, 1998). However the other three metrics can recover on annual or 3 year basis. Concerning the lower limit type, the proportion of plagiotropic rhizome increases relatively quickly (<3 years; Francour et al., 1999) and moreover variations up to a few centimetres of the position of the limit may be detected using the balisage technique (Pergent et al., 1995; Boudouresque et al., 2006), facilitating the detection of a progression (or regression) of the limit. Concerning shoot density, consistent interannual improvement of *P. oceanica* shoot density has been identified (Pergent-Martini et al., 2000). In addition, according to Marbà et al. (2005), *P. oceanica* recruitment rates range between 5.26 and 62.8 shoots m^{-2} $year^{-1}$, significant improvements in shoot density should therefore be detectable over relatively short periods of time. Finally, leaf biometry can improve on a monthly to annual basis (Pergent-Martini et al., 2005; Leoni et al., 2006). Therefore, despite the low growth recovery rates of *P. oceanica*, the combination of the metrics selected for the BiPo index allow the detection of both deterioration and improvement in ecological status on a 1–3 year basis. In this context, it is important to note that the EU-WFD requires the evaluation of ecological status to be performed once every river basin management plan when "surveillance" monitoring is sufficient (i.e. every 6 years), and twice in cases in which "operational" monitoring is necessary (i.e. every 3 years) (EC, 2000). Thus response time of the BiPo metrics is more than adequate with respect to this time frame.

Finally, another important advantage of the BiPo is its cost; it is in fact a cost and time effective method. The descriptors taken into account only require 6 types of measures (lower limit depth, lower limit type, % plagiotropic rhizomes, % cover, shoot density and leaf biometry) which are simple, straightforward and widely used (Pergent-Martini et al., 2005; Lopez y Royo et al., 2007). Work in the field by scuba is necessary in any *P. oceanica* sampling programme. The worktime in scuba for the BiPo is limited, and will require one dive, or at a maximum two repetitive dives. Thereafter, the descriptors involve very little laboratory and interpretation effort, as the only laboratory measures are leaf biometry measurements and estimation of type of limit from photographs. In terms of cost, the low work-time and the little equipment required keep the cost of sampling each site low. In addition to its cost-efficiency, the BiPo can entail a non-destructive sampling approach, as it does not necessarily require any *P. oceanica* shoots to be collected. This is noticeable in the context of existing regional and local *P. oceanica* monitoring programmes so far, as they all require shoot collection as part of their sampling design. Considering that on average between 20 and 100 shoots are collected on each site for monitoring purposes (MATT, 2001a; SINPOS, 2001; Pergent et al., 2005; Romero et al., 2007), a non-destructive approach would be in line with the spirit of the WFD (Foden and Brazier, 2007), would correspond to the legal status of *P. oceanica* (Pergent-Martini et al., 2006) and would support the notion of threatened or protected species. Indeed, *P. oceanica* (i) is listed as strictly protected species in the Convention on the Conservation of European Wildlife and Natural Habitats, (ii) is listed as priority habitat in the EU Habitats Directive, and (iii) is a priority species in the UNEP-MAP Action Plan for the Conservation of marine vegetation in the Mediterranean.

The proposed biotic index based on *P. oceanica* (BiPo) allows a reliable, standard and cost-effective evaluation of the meadow ecological status, in accordance with the EU-WFD requirements. Moreover the BiPo answers the main criteria defined for the selection of an ecological indicator (Blandin, 1986; Dale and Beyeler, 2001; Salas et al., 2006): (i) its is sensitive to disturbance and responds to improvements or deterioration of environmental conditions within the required time frame, (ii) it synthesises complex information in a simple and reliable way, (iii) its application is simple and cost-effective, (iv) it may be applied in an extensive geographical area, (v) it is relevant to policy and management requirements, in particular to WFD requirements. Furthermore, the use of the BiPo suggests a two-fold application, one as baseline evaluation of the seagrass status in the western Mediterranean, and the other as a tool or one of the components for an overall assessment of water quality. The index would greatly benefit from a wider application within the Mediterranean basin, and from an intercalibration with existing methods for ecological status evaluation, in order to further test and validate the method proposed. Nevertheless, its overall characteristics make it a simple, efficient and useful tool for management and conservation issues.

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