

Bioindicators, Monitoring, and Management Using Mediterranean Seagrasses: What Have We Learned from the Implementation of the EU Water Framework Directive?

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Abstract Seagrasses are flowering plants that inhabit coastal and transitional waters. They colonize sedimentary seabeds (and to a lesser extent rocky substrates) and present unique adaptations to the marine environment. Seagrasses are especially sensitive to environmental deterioration and live in a world that is particularly threatened by human activity. The response of the plants and their associated communities to disturbances is relatively well known. This has facilitated the development of a large number of seagrass bioindicators based on biochemical, physiological, morphological, structural, demographic, and community measures, especially after the deployment of the EU Water Framework Directive (WFD) and to a lesser extent the implementation of the Marine Strategy Framework Directive.

Bioindicators are at the interface between science and policy. In order for their use by managers for different purposes (monitoring, water quality assessment, long-term changes, etc.) to be robust and consistent, a clear definition of management goals is needed. The development of bioindicators must also be based on careful

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evaluation together with rigorous and transparent selection processes to ensure their scientific credibility.

Here, we present bioindicator indices based on seagrasses that were developed with the context of the implementation of the WFD in Catalonia, NE Spain, to assess the ecological status of coastal and transitional water bodies. Ecological status includes aspects concerning both the quality of the biological community and the hydrological and chemical characteristics of the environment. For this reason, and to develop a WFD-compliant system for ecological status assessment based on Mediterranean seagrasses, we used multivariate techniques to combine different bioindicators, gathered from different levels within the biological organization, into single biotic indices (POMI and CYMOX, based on the species *Posidonia oceanica* and *Cymodocea nodosa*, respectively). We report how this was achieved and how the robustness and reliability of those indices were assessed through correlation with human pressures, uncertainty analysis, and intercalibration. Finally, besides their applicability, we discuss their shortcomings and what we, as seagrass biologists, have learned overall from responding to the challenges posed by the WFD and specifically by the part dealing with seagrasses.

Keywords Coastal waters, *Cymodocea nodosa*, Ecological status, Human pressures, *Posidonia oceanica*

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Acronyms

ACA	Catalan Water Agency
BQEs	Biological quality elements
EQR	Ecological quality ratio

ICCM	Intercalibration Common Metric
WB	Water body
WFD	Water Framework Directive (2000/60/EC)

1 The Concept of Bioindicator and Its Development at the Interface Between Science and Management

In a broad sense, “indicators” are signals that capture complex information in a simple but effective way. Although indicators are used in very different fields, including social sciences, marketing, economics, and business, they are particularly useful in environmental assessment and planning. Environmental indicators are at the interface between science and policy [1], and thus, to be helpful, both sides of that interface should adapt to, or at least understand, the constraints of the other. Specifically, managers should clearly define the problem under consideration [2], while scientists should make every effort to design indicators that are not only helpful in solving the problem but are also easy to understand and communicate [3].

Biological indicators or “bioindicators” are a subset of environmental indicators that rely on measurements of biological entities. Their use is highly recommended, because organisms provide an integrated response to environmental stress. The measurements on which bioindicators rely can be gathered from several levels of biological organization, from the subcellular to the community level. Measurements at lower levels (from molecular to individual, e.g., biochemical, genetic, or morphological traits) are usually more specific to stressors and respond faster than those at higher levels (from population to community, e.g., abundance, biomass, or taxonomic composition), which are more relevant to concepts such as ecological integrity [4]. Consequently, measurements at the molecular, cellular, or individual level present rapid time responses and great specificity, making them excellent early warning indicators, while measurements at the population or community level present longer response times, tend to be more integrative, and consequently are more relevant to assess conditions at the level of the ecosystem [5].

When bioindicators are selected for use in environmental assessment programs, managers should, first of all, clearly define the problem to be addressed and what kind of information is needed. For example, there could be an interest in assessing the actual condition of a system or in evaluating trends over time, or in evaluating the effects of a given coastal development (harbor construction, beach nourishment, etc.), or in establishing the causes behind an observed deterioration of the ecosystems [6]. In any of these scenarios, scientists should face the challenge of choosing from the literature or specifically designing robust and reliable bioindicators that are suitable for each specific goal. In all cases, the development of sets of bioindicators implies a step-by-step process in which the selection of adequate measurements and eventually their aggregation in the form of simple and readable indices are crucial milestones. The development of bioindicator sets

must be based in a rigorous and transparent selection process to ensure scientific credibility and adequacy with respect to the needs of managers [7].

Success in the use of biological indicators to solve environmental problems relies on the identification of an effective set of variables¹ to be measured. Some criteria these variables should meet are [6] easy to understand, simple to measure and cost effective, sensitive to stresses, predictable response to stress, low natural variability, and the provision of relevant information on environmental issues. Similarly, some additional requirements to be met by variables to be used as bioindicators are [8] relevance to ecological integrity, broad-scale applicability, early-detection capacity, feasibility of implementation, interpretability against reference conditions, and capacity to link ecosystem degradation with its causative stressors. The challenge is, therefore, to choose from among the hundreds of bioindicators proposed so far, or among the thousands of measurements that can be performed on biological entities, those that best fulfill the criteria mentioned above.

Recently, there has been a rapid increase in the development and application of ecological indicators. For instance, governments in the United States, Canada, Europe, and Australia are developing programs for routine reporting based on ecological indicators [3]. In Europe, the European Water Framework Directive establishes a framework for the protection of groundwater, inland surface waters, estuarine waters, and coastal waters. The WFD represents a challenge for water resource management in Europe, because, for the first time, water management is based on biological assessment of ecosystem status or health [9]. In coastal and transitional waters, the biological elements to be considered include phytoplankton, macrophytes (macroalgae and seagrasses), zoobenthos, and fish (only in transitional waters). The WFD and its implementation have generated a considerable amount of research on bioindicators and specifically on indicators based on seagrasses.

2 Seagrasses: Flowering Plants on the Seabed

Seagrasses are flowering plants which, after evolving in the terrestrial environment, have secondarily (and “recently,” i.e., about 100 million years ago: the end of the Cretaceous) colonized coastal marine waters. As a result of this somewhat atypical history that parallels that of whales and other marine mammals, seagrasses present unique adaptations to marine conditions, including roots (and modified underground stems, called rhizomes), hydrophilic pollination, an internal gas circulation system (aerenchyma), and basal leaf meristems, among others. Despite their

¹As explained, indicators are variables measured for biological entities. These variables are sometimes referred to as *descriptors*, *attributes*, or *traits*. In the Water Framework Directive, they are often called *metrics*.

worldwide distribution (except Antarctica), seagrasses constitute a group poorly diversified and includes only ca. 70 species [10].

Unlike algae, seagrasses can colonize sedimentary bottoms, where they can attach (and from which they can take up nutrients), thanks to their roots. This capability to colonize sedimentary bottoms has allowed seagrasses to extend over thousands of square kilometers, constructing a habitat (called “meadow”) of high primary production which harbors hundreds of species that find food, substrate, or shelter there, with a global importance. Seagrass meadows are remarkable for the ecosystem services and goods they provide, including, among many others, beneficial effects on fisheries (playing a nursery role), shore protection, providing biodiversity hotspots, nutrient cycling, and constituting carbon sinks [11]. However, seagrasses are especially sensitive to environmental deterioration, and they are threatened by human activity. In recent decades, this has become a matter of concern, as seagrass meadows seem to be suffering worldwide regression [12]. This has generated a considerable amount on research on how seagrasses and their associated communities respond to man-made disturbances [13–16].

In the Mediterranean Sea, we find only five seagrass species (excluding the genus *Ruppia*). Of these five, one is an introduced species (*Halophila stipulacea*, a Lessepsian migrant), for the most part present in the Eastern basin, and two, belonging to the genus *Zostera* (*Z. noltii* and *Z. marina*), are relatively rare, with discontinuous distributions, mostly associated with brackish or extremely calm waters. The other two (*Posidonia oceanica* and *Cymodocea nodosa*) are much more abundant by far, and they extend over large coastal stretches.

P. oceanica and *C. nodosa* are both sensitive to environmental deterioration, just as other seagrasses are [17], although the former is more sensitive than the later, and their responses to specific disturbances (e.g., hypersalinity, trawling, eutrophication, coastal works, fish farming, etc.) have repeatedly been studied [18–23]. All these results, along with extensive knowledge of their biology and ecology [24], represent an excellent starting point for the identification of variables or descriptors whose association with given disturbances is well known and unequivocal, thereby providing a solid base for defining reliable bioindicators.

3 Digging Through Seagrasses in Search of Bioindicators

A number of variables (biological traits, attributes, etc.) associated with seagrasses and seagrass ecosystems have been reported in the literature to respond to environmental alterations. As outlined in Sect. 1, sensitivity to environmental stress is a necessary but not a sufficient condition for a variable to be used as a reliable bioindicator. Perhaps most critically, indicator responses must match the spatial and temporal scales that are appropriate for the specific management needs. In addition, a useful indicator would help identify the specific pressures (eutrophication, coastal development, fish farming, etc.) affecting the system, to allow managers to remedy the problem at its source. Consequently, before a bioindicator

is employed, it is essential to understand how it behaves and to determine if it fits the specific management goals. This may often require a specific validation process which, although time-consuming, will serve to ensure that the bioindicators chosen are reliable, sensitive, and managerially effective.

During the implementation of the WFD along the coast of Catalonia in NE Spain, the behavior of measurements based on both *P. oceanica* and *C. nodosa* was carefully assessed [25, 26]. For both species, the process followed included (1) screening for variables, (2) validation over an environmental gradient of stress, and (3) detection of potential redundancies among variables.

3.1 Screening for Candidate Variables

The most relevant variables (or metrics, following the WFD) were selected from a suite of measurements obtained from in-depth screening of the literature. The entire suite of candidate variables showed, in accordance with the published evidence, a clear response to stressors, although through a great variety of methods and spatial and temporal scales. A total of 59 and 54 candidate seagrass variables were selected for *P. oceanica* and *C. nodosa*, respectively, following the criteria given above. The complete lists of variables selected are in Table 1 of Martínez-Crego et al. [8] for *P. oceanica* and Oliva et al. [26] for *C. nodosa*.

3.2 Validation Over an Environmental Gradient

We assessed the behavior of the candidate variables over environmental quality gradients at spatial scales appropriate for the management objectives, i.e., the deployment of the WFD along the coast of Catalonia (approximately 700 km; see Fig. 1) for *P. oceanica* and within the transitional waters (approximately 100 km²; see Fig. 2) for *C. nodosa*.

For *P. oceanica*, we chose nine sites encompassing the maximum range of environmental quality in the area. A similar design was used for *C. nodosa* within the transitional waters. In both cases, every effort was made to capture the maximum spread of random spatial variability, performing nested sampling (scales of replication, 10 and 100 m). Seagrass samples were taken at a single depth (15 m for *P. oceanica*, 1 m for *C. nodosa*; see more on potential confounding effects of depth in Sect. 5) and within the shortest possible time (to avoid confounding effects of seasonality). In parallel, the environmental gradients were assessed using water column information (chlorophyll a, water transparency, salinity, and ammonium concentration in the water), sediment data (total phosphorus, ammonium concentration in pore water, and Hg and Pb content), and information obtained independently from other bioindicators (macroalgae [28]). Based on these environmental data, we attributed an environmental quality category (healthy, intermediate,

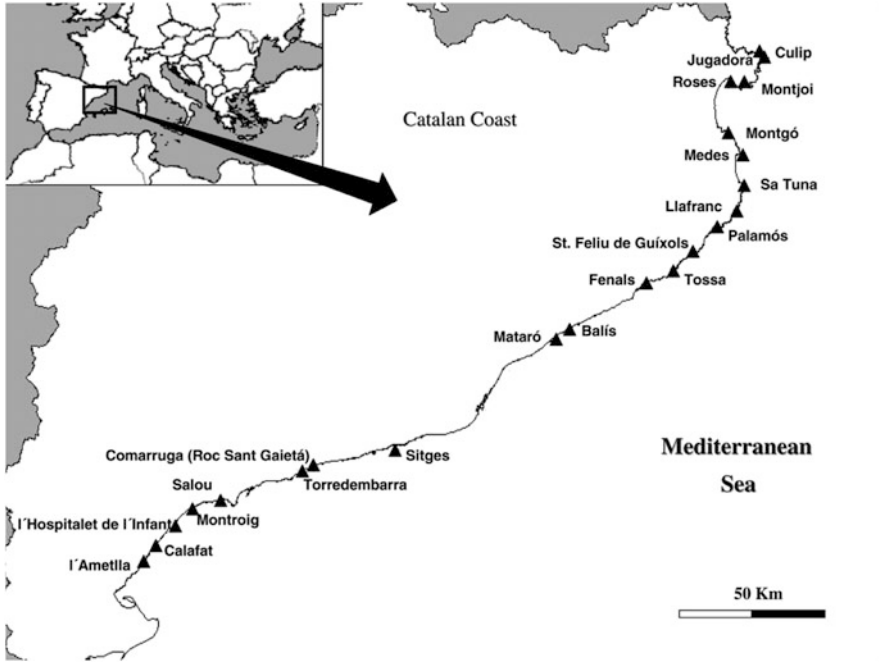


Fig. 1 Map of the Catalan coast including sampling sites for the POMI index and the seagrass (*Posidonia oceanica*) monitoring network (from Romero et al. [27])

unhealthy) to each of the sites, and we performed nested ANOVA (fixed factor, environmental quality; random factor, sites) for each of the candidate variables. We retained only those variables that displayed significant differences between the different environmental qualities. An initial remarkable fact was that only 22–24 (depending on the depth) of the potential 59 variables selected showed significant variability in accordance with the environmental status (in the case of *P. oceanica*) and 37 (out of 54) for *C. nodosa*. This indicates that a field evaluation at spatial scales that match those that are relevant for management is an unavoidable step in the selection of metrics.

3.3 Detecting Redundancies and Selection of Variables

Once the field validation was completed, we dropped the nonresponsive variables and performed a principal components analysis (PCA [29]) with the rest to identify common trends of variability within them, potential redundancies, and their correlation with environmental status. Based on this, and taking other aspects into account (cost, expertise needed, etc.), we selected a set of variables as biological indicators for WFD implementation (metrics). The variables selected for

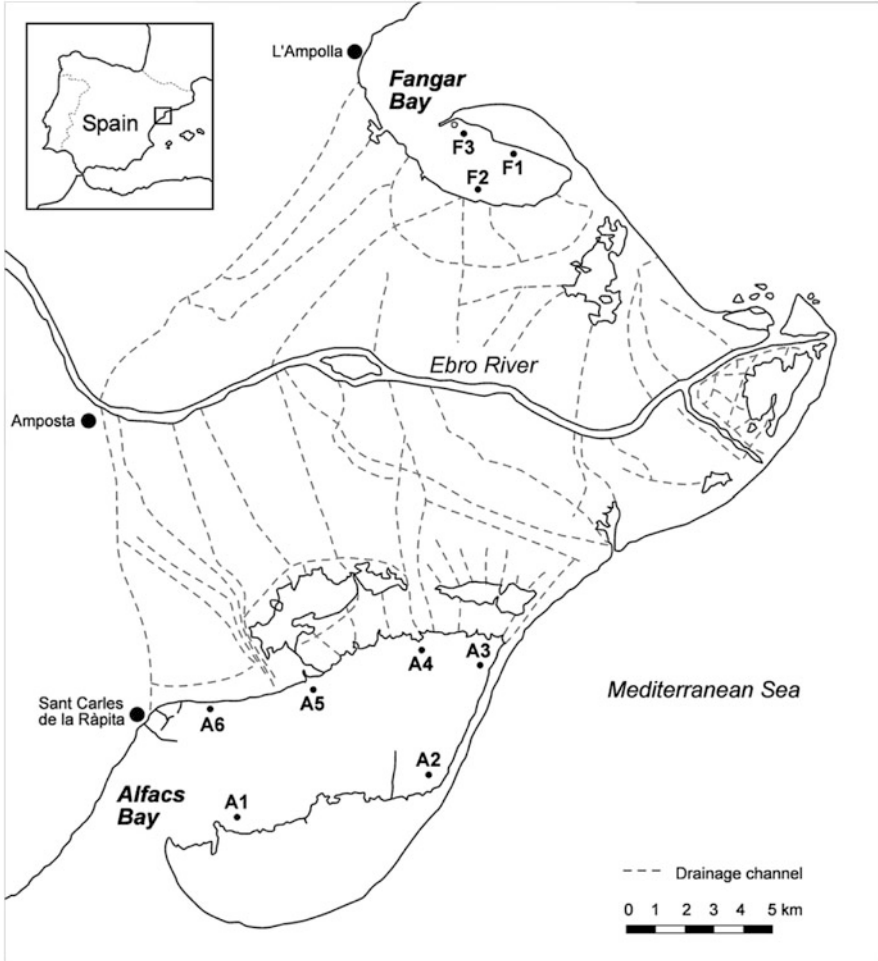


Fig. 2 Map of the study area and sampling sites where the CYMOX index based on the seagrass *Cymodocea nodosa* was applied (from Oliva et al. [26])

P. oceanica were phosphorus, nitrogen, and sucrose content in rhizomes, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ isotopic ratios in rhizomes, percentage of leaves with necrosis, shoot size, meadow cover, shoot density, percentage of plagiotropic rhizomes, nitrogen content of epiphytes, and copper, lead, and zinc content of rhizomes. For *C. nodosa*, they were root weight ratio (ratio between root and roots plus leaves weight), shoot size, epiphyte load, and N, $\delta^{15}\text{N}$, P, $\delta^{34}\text{S}$, Cd, Cu, and Zn content of rhizomes (Table 1).

Table 1 List of selected metrics to construct the POMI and CYMOX indices (from Oliva et al. [26] and Romero et al. [27])

Biological organization level	POMI metrics	CYMOX metrics
Sub-individual	P, N, and sucrose content in rhizomes	N and P content in rhizomes
	$\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ isotopic ratios in rhizomes	$\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ isotopic ratios in rhizomes
	Pb, Cu, and Zn content in rhizomes	Cd, Cu, and Zn content in rhizomes
Individual	Percentage of leaves with necrosis	Shoot size
	Shoot size	
Population	Meadow cover	Root weight ratio (ratio between root and roots plus leaves biomass)
	Shoot density	
	Percent of plagiotropic rhizomes	
Community	N content in epiphytes	Epiphyte load

4 Integrating Time Scales: Multivariate Biotic Indices

In the preceding sections, we have stressed the requirements that a “perfect” bioindicator should meet. Unfortunately, there is no single variable (plant trait, ecosystem attribute) that adequately meets all those requirements. A reasonable alternative, always keeping in mind the management objectives, is the combined use of different variables; this approach has a number of advantages. On the one hand, the results for these variables can be presented independently and their values interpreted individually. If the set has been chosen properly, all the needs regarding sensitivity to and specificity for different environmental stressors will be covered. On the other hand, the set of variables can be numerically aggregated into a single index. Such aggregated indices are usually called multimetric indices or multivariate indices if multivariate statistical techniques are used for the aggregation. For the WFD we developed multivariate indices for both seagrass species by (1) integrating the variables, (2) setting reference conditions, and (3) assessing the relationship between pressures and impacts.

4.1 Integrating Selected Variables

The integration of the different variables was based on PCA. As all the metrics selected were correlated to environmental quality (see preceding sections), there was substantial common variability, which was clearly reflected in the clustering of the variables along the first axis [26, 27] (see Figs. 3 and 4, for *P. oceanica* and *C. nodosa*, respectively). The scores of the sites for the first component were then

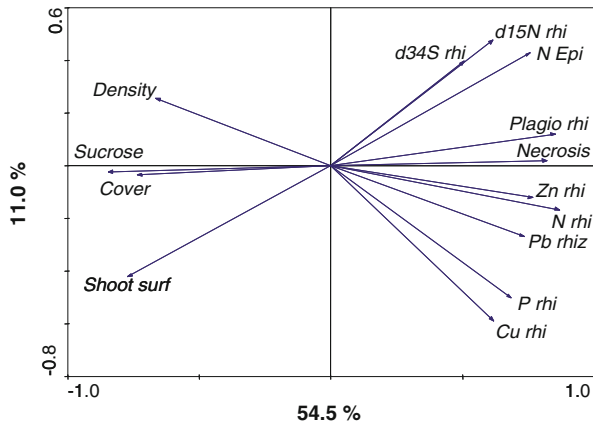


Fig. 3 Factor loading of the different metrics used to construct the *Posidonia oceanica*-based POMI index including the variability explained (%). The factors include shoot surface, percent of necrosis in leaves, nitrogen content in rhizomes, phosphorus content in rhizomes, sucrose in rhizomes, $\delta^{15}\text{N}$ isotopic ratio in rhizomes, $\delta^{34}\text{S}$ isotopic ratio in rhizomes, trace metals in rhizomes (zinc, lead, and copper), meadow cover, shoot density, percent of plagiotropic rhizomes, and epiphyte nitrogen content (from Romero et al. [27])

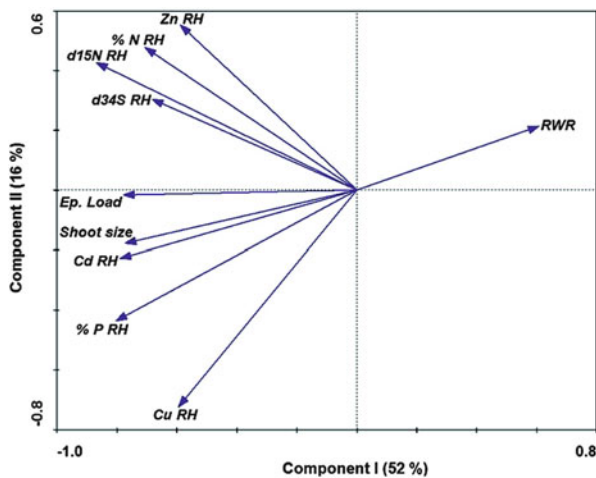


Fig. 4 Factor loading of the different metrics used to construct the *Cymodocea nodosa*-based CYMOX index including the variability explained (%). The factors include root weight ratio, shoot size, nitrogen content in rhizomes, phosphorus content in rhizomes, $\delta^{15}\text{N}$ isotopic ratio in rhizomes, $\delta^{34}\text{S}$ isotopic ratio in rhizomes, trace metals in rhizomes (zinc, cadmium, and copper), and epiphyte load (from Oliva et al. [26])

taken as an expression of their ecological status and in order to fulfill the WFD requirements, scaled to the interval 0–1 (ecological quality ratio, EQR) using reference conditions (see below).

4.2 *Setting Reference Conditions*

Reference conditions are usually understood as those corresponding to undisturbed, near-pristine sites; therefore, they present the optimal value for a given bio-indicator. Reference conditions are commonly used (and specifically in the WFD) as a kind of benchmark against which actual conditions are compared, and the difference (or distance) between reference and actual conditions is used to express the status of the system under analysis. Therefore, defining adequate reference conditions is a critical step and often represents a challenge, because in most coastal regions (at least in Europe), areas that are unambiguously devoid of all anthropogenic impact are extremely scarce.

In the case of the Western Mediterranean, potential reference sites can be found in zones far from human pressures, such as some island areas (e.g., Corsica, Sardinia, and the Balearic archipelago). However, the problem of the lack of comparability between ultraoligotrophic insular waters and the oligo-mesotrophic waters of continental coasts is a serious drawback of this approach. Other possible reference areas are marine reserves [30]; however, communities within marine reserves, although protected from major human impacts, can be subjected to drivers of change beyond the protective regulations (eutrophication, climate change, invasive species, changes in land uses, etc. [31]). To overcome these constraints, and for the seagrass-based indices developed for the coast of Catalonia, a reference frame was constructed using a modeling type of approach. For each variable, data from all the sites were pooled and ranked from best to worse, which were increasing or decreasing values depending of the variable under consideration. The average of the values above the 90th percentile was considered the reference (optimal) value for each specific variable, while the average of the values below the 10th percentile was considered the worst possible value. This strategy agrees with the modeling approach proposed in the WFD [32]. Arguments supporting it are given in Romero et al. [27].

4.3 *Defining the Indices*

We included the optimal (reference) and worst sites in the PCA described in Sect. 4.1 as passive objects (software used: CANOCO v 4.5 [33]), and their scores on the first axis (Figs. 5 and 6, for *P. oceanica* and *C. nodosa*, respectively) represented the two extreme conditions of the system. We calculated the EQR (following the WFD, comprised between 1 and 0, as mentioned above) as

$$\text{EQR}_x = (\text{CI}_x - \text{CI}_{\text{worst}}) / (\text{CI}_{\text{optimal}} - \text{CI}_{\text{worst}}) \quad (1)$$

where EQR_x is the EQR of the site x and CI_x , $\text{CI}_{\text{optimal}}$, and CI_{worst} are the scores on the first axis of the PCA of sites x , optimal and worst, respectively.

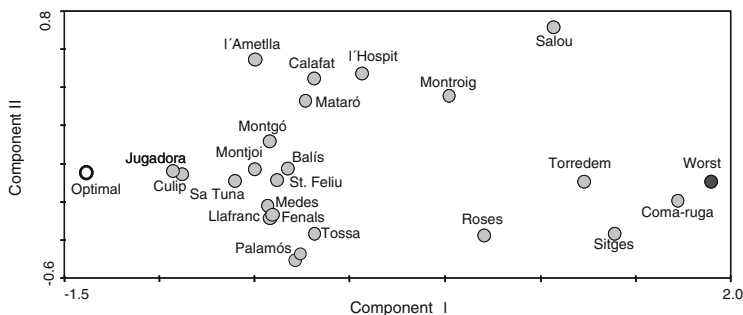


Fig. 5 PCA ordination of the studied sites (factor scores) along the Catalan coast for POMI, showing the optimal and worst sites. The sites are those depicted in Fig. 1 (from Romero et al. [27])

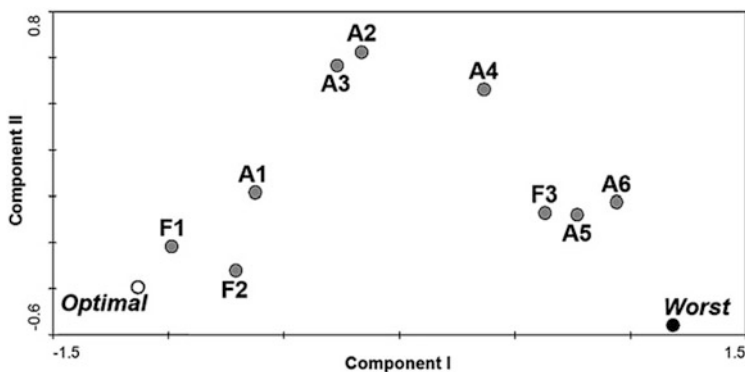


Fig. 6 PCA ordination of the studied sites in transitional waters for CYMOX (factor scores) showing the optimal and worst sites as supplementary objects. The sites are those depicted in Fig. 2 (from Oliva et al. [26])

As the WFD requires classifying the ecological status into one of five classes, from high to bad, the boundaries between classes have to be set within the 1 to 0 EQR scale. Since the relationship between pressures and the EQR was found to be linear, we simply divided the scale 0 to 1 into five equal classes (Table 2A, for *C. nodosa*, the CYMOX index). However, considering that *P. oceanica* is highly sensitive to anthropogenic disturbances, we constructed a slightly different index called POMI. This is based on the assumption that meadow disappearance has been reported in environmental conditions under which other biological assemblages persist [34, 35]. According to that we defined the bad class for the POMI index as the ecological status in which *P. oceanica* cannot survive. In other words, wherever and whenever a *P. oceanica* bed is able to survive, even heavily degraded, the ecological status is above bad. We arbitrarily assigned the range from 0 to 0.099 to this bad ecological status. The other EQR boundaries were obtained by dividing the remaining scale (from 0.1 to 1) into four categories of equal amplitude (0.225 each;

Table 2 Boundaries between the different ecological status classes (from Oliva et al. [26] and Romero et al. [27])

EQR (CYMOX)	Ecological status	Colour code
A: CYMOX		
1.00–0.80	High	Blue
0.79–0.60	Good	Green
0.59–0.40	Moderate	Yellow
0.39–0.20	Poor	Orange
0.19–0.00	Bad	Red
B: POMI		
1.000–0.775	High	Blue
0.774–0.550	Good	Green
0.549–0.325	Moderate	Yellow
0.324–0.100	Poor	Orange
0.099–0.000	Bad	Red

see Table 2B). Therefore, when *P. oceanica* exists, the EQR is computed as follows:

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

being EQR'_x the EQR for site x (where living *P. oceanica* exists) and EQR_x obtained from formula (1).

4.4 Assessing Pressure-Impact Relationships

Human activities (or drivers of change) that can potentially and adversely affect the status of ecosystems are considered pressures. When such an adverse effect actually occurs and produces a change in the status of the ecosystem, it is considered that an impact has taken place. This causal chain, known as DPSIR (Driver-Pressure-Status-Impact-Response) approach [36], has been established as an analytical framework for the determination of pressures and impacts under the WFD. In the context of European coasts, population density, tourism, industries, shipping activities, agriculture, fishing, and aquaculture are highlighted as the main drivers causing pressures on coastal ecosystems [37]. As a part of the WFD deployment, biotic indices used to assess ecological status under the WFD should be able to reflect the pressures acting on the water bodies. Therefore, we tested the sensitivity of the two seagrass-based indices (POMI and CYMOX) to pressures, in order to ensure their suitability for monitoring programs. For the analysis of the POMI index, pressures on coastal waters were estimated based on the document [38]. The main pressures considered were urban sewage discharge (kg/day/km coast), urban soil surface (ha/km coast), tourism pressure (rooms/km of coast), and harbor pressure (number of moorings/km of coast). All the pressures were normalized and reduced and then summed to estimate an aggregate pressure for each water

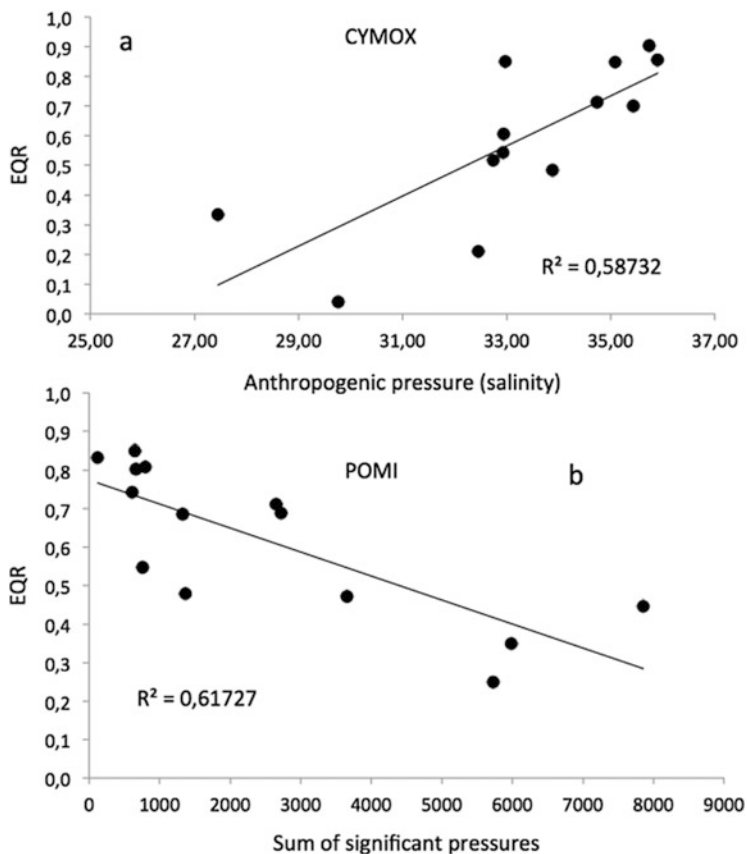


Fig. 7 Relationship between the CYMOX index (a) and the POMI index (b) and anthropogenic pressures. For the CYMOX index, anthropogenic pressure is represented by the salinity gradient, which decreases with increasing pressure. For the POMI index anthropogenic pressures are represented by a sum of significant pressures including sewage, urban use, tourism, and the presence of harbors

body where *P. oceanica* is currently present ($n = 17$, covering $>80\%$ of the coastline). POMI and the aggregate pressure were significant and negatively associated (Fig. 7b, $R^2 = 0.617$, $p < 0.05$). In the case of CYMOX, we considered that the main drivers of pressures affecting transitional waters were freshwater loadings (carrying nutrients, organic matter, and other pollutants). Accordingly, to test the CYMOX index, we assessed the correlation between the index values and salinity, which showed a positive and significant correlation (Fig. 7a, $R^2 = 0.588$ and $p < 0.05$).

To further assess the sensitivity of our indices to pressures, we explored the relationship between land uses and the POMI index [39]. Land uses were quantified from public databases in SIG format (*Mapa de Cobertes del Sòl de Catalunya*, <http://www.creaf.uab.es/mcsc/>) for coastal stretches corresponding to each water

body and extending 1.5 km inland. The three main land use categories considered were urban, natural, or nonirrigated agricultural and irrigated agricultural. Significant correlations between land use categories and ecological status, as estimated by POMI, were found. The category “natural or nonirrigated agricultural” was positively correlated with POMI values ($R^2 = 0.668$, $p < 0.01$). The surface occupied by the categories “urban” and “agricultural irrigated” correlated negatively with POMI ($R^2 = 0.679$ and $R^2 = 0.446$, respectively; p from 0.01 to 0.05). The direct relationship between land uses and coastal water status has profound implications for management and stresses the fact that to a large degree coastal water quality depends on human activity on land.

5 Evaluating Uncertainty Associated with Ecological Status Classification

A common concern of those involved in the use and design of bioindicators is the natural variability (i.e., not related to human impacts) of the variables measured. For example, most seagrass attributes exhibit marked seasonality and/or strong bathymetric dependence, which can potentially confound the interpretation of values. In these cases, the problem can be easily fixed, sampling at a specific time of year and at a fixed depth. However, seagrass attributes (such as those included in the POMI and CYMOX indices) also show variability at different spatial scales (e.g., clonal integration, microhabitat, sediment patchiness, etc.), which is not always obvious to the researcher. This variability can result in unintentionally misclassifying water bodies and wrong management decisions, both in terms of action and inaction, which may in turn have high associated social and economic costs. Obviously, as in any sampling, this (random) variability should be taken into account through adequate replication. However, it is not always straightforward how to deal with this variability. Indices aggregating different attributes are common in most WFD approaches, including the examples reported here (i.e., CYMOX and POMI). In these cases, while the variability of each individual indicator (metric) can be easily assessed through replication, and expressed using the usual statistics means (standard deviation, confidence interval, etc.), the variability of the composite index cannot be directly evaluated. To address this problem, a number of techniques can be applied (bootstrapping, uncertainty assessment, error propagation techniques, etc.). Here we briefly describe one particular exercise that we conducted with POMI in order to evaluate different sources that contribute to the variability of the final index, as reported in Bennett et al. [40]. We identified relevant factors that could introduce uncertainty into the ecological status classification: small-scale (tens of meters) spatial variability, medium-scale (hundreds of meters) spatial variability, large-scale (tens of kilometers, but always within the same water body, i.e., a zone considered homogeneous from the point of view of the ecological status) spatial variability, depth (5 and 15 m), interannual variability

(variability between consecutive years within the 4-year period over which samples can be measured according to the WFD), and surveyor error (variability between surveyors, as some of the attributes such as density and cover are estimated directly in situ by divers). The main source of variability was, as expected, depth, which accounted for more than 60% of variability, leading to the conclusion that depth should remain fixed or be controlled in monitoring programs based on *P. oceanica*. In contrast, the variability in POMI scores between different surveyors was extremely low, explaining less than 1% of total variability, while interannual variability accounted for approximately 5% of the total. Additionally, variability at medium and large spatial scales did not differ from variability at the small spatial scale, which suggest that replication within scales of tens of meters is enough to capture most spatial variability.

Based on the results of this variability assessment, we evaluated the probability of misclassification of a given water body, as a function of the value of the POMI index obtained (EQR). Without entering into excessive detail (see [40, 41]), it has to be noted that the risk of misclassification, provided the design is controlled (e.g., fixed depth, fixed season, etc.), is low (as low as $<0.1\%$) for values far from the thresholds between classes, but increases significantly (up to 50%) for values within an interval of ± 0.1 EQR from the threshold value or with uncontrolled designs (Fig. 8). The issue of how to deal with this risk of misclassification remains an open question. It would seem reasonable, when this risk is high and before assigning a

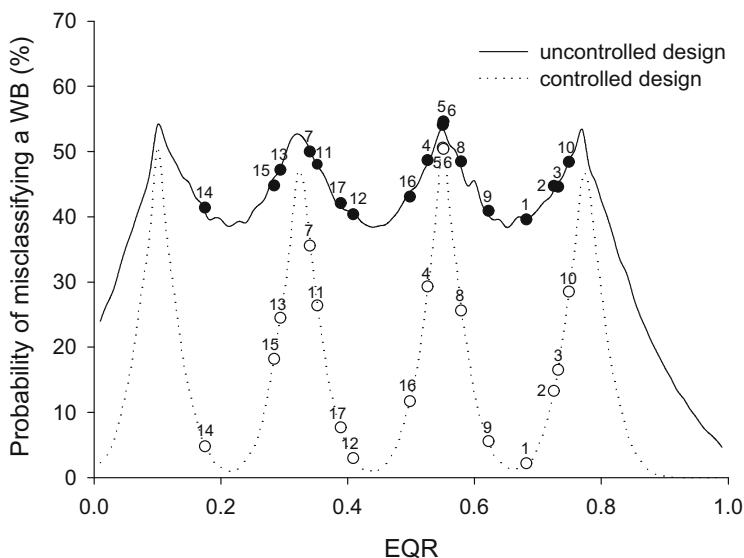


Fig. 8 Uncertainty analyses applied to establish the probability of misclassifying the ecological status class among EQR values calculated within a water body (WB) with a controlled design (fixed depth, fixed sites, fixed years, fixed zones, fixed surveyors) and an uncontrolled design where these variables are not fixed. *Full and open circles* represent the actual probability of misclassification for 17 Catalanian WB (in numbers) (from Bennett et al. [40])

water body status (and especially if the uncertainty is between the moderate and good classes), to obtain additional data on environmental conditions from independent sources (additional seagrass variables, physicochemical variables, etc.). The same kind of analysis was performed with CYMOX, with quite similar results [42].

6 Harmonizing Indices: Making Sure the Indices of Different Member States Compare Across the EU

A critical component of the WFD is the aim of ensuring the methods employed by different member states are intrinsically comparable, so that ecological status categories have the same meaning across the EU. This has required a process of careful intercalibration between assessment methods, which has proven to be extremely hard, as the first choice for intercalibration (applying different methods to the same set of sites) was ruled out due to budgetary constraints (however, see below). The intercalibration exercise was led by the EU and its main aim was to guarantee that the five classes required by the WFD represented equal levels of environmental health/deterioration across all EU member states, independently of the assessment method used. When complete, this will eventually result in the harmonization of all the different methods used by member states so that the definition of ecological status will not vary across water bodies and will be independent of the method used for its assessment. In the case of Mediterranean seagrasses, several assessment methods based on different indices (all related to the seagrass *P. oceanica*) were compared against each other. Specifically three methods were presented and intercalibration was successfully completed on them: POMI (Spain—Catalonia, Balearic Islands, Murcia, Andalusia, and Croatia [27]), Valencian CS (Spain, Valencia [43]), and PREI (France, Italy, Cyprus [44]). The methods were all multimetric, but differed in two aspects: (1) the individual indicators (i.e., metrics) used and (2) how the individual indicators were aggregated or combined to produce values on a unique EQR 0 to 1 scale. The more the two aspects differ, the more difficult it is to compare the indices, especially in the absence of a common experimental approach. However, as most of the methods included, as a metric or at least as complementary observations, values on leaf length, shoot density, and lower limit typology [45], an ICCM (intercalibration common metric) was constructed based on these three variables. Then, the relationship of the ICCM to (1) human pressures and (2) each one of the different methods assessed was evaluated using linear regression. The fitted linear models were used to detect whether the boundaries good/moderate and high/good corresponded, for all the methods, to the same level of human pressure. Although some methods needed some fine tuning, finally all of them were successfully intercalibrated, despite their differences.

This comparability between methods was confirmed by the experimental exercise performed by Spanish, French, and Italian teams and reported in Lopez y Royo

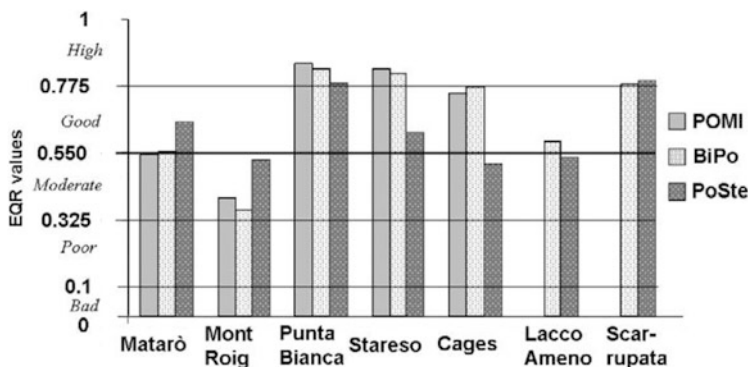


Fig. 9 EQR values and classification of sites according to the POMI index, BiPo index, and PoSte index, all three based on the seagrass *Posidonia oceanica* and applied in Catalonia (Spain), Corsica (France), and Campania (Italy) (from Lopez y Royo et al. [46])

et al. [46]. Joint sampling was performed in different areas of the Western Mediterranean at sites that encompass a gradient of human pressure. From the simultaneous data collection, three indices (POMI, BiPo, and PoSte; see references in [46]) were computed for each site, and then each one was compared to the others. Two of the indices, POMI and BiPo, showed a very good relationship with human pressures and were in almost complete agreement ($R^2 = 0.987$). In contrast, one of the methods, the PoSte index, clearly diverged from the other two as it was based on a different rationale for defining reference conditions and used a different response scale (Fig. 9). Taken altogether, this intercalibration approach highlights two main points: (1) indices with very different metrics can still provide completely reliable and comparable results; (2) indices that are based on different conceptual approaches (e.g., in their definition of what is near-pristine status) may diverge considerably.

7 Conclusions: What Have We Learned and Questions That Remain Open

The implementation of the WFD for the coastal waters of Catalonia stimulated a considerable amount of work and specifically a huge effort in building, validating, and analyzing seagrass-based bioindicators. From all this effort, summarized in the present document, a few basic conclusions emerge: (1) it is necessary to validate the response of individual metrics to human pressures at spatial and temporal scales suitable for the monitoring programs; (2) it can be extremely useful to use different metrics simultaneously, to cover different requirements of monitoring programs (early detection, specificity to stressors, relevance to ecosystem integrity, etc.); (3) it is a crucial careful assessment of the variability of such metrics and, based on

this, a careful sampling design; (4) in-depth knowledge of the behavior of each individual indicator (e.g., response time, potential hysteretic properties, etc.) is necessary; and (5) PCA has a great potential as an optimal method not only to aggregate different metrics into a single index objectively but also to reveal redundancy among metrics.

The indices based on seagrasses reported here (POMI and CYMOX) have a number of advantages as detailed below:

1. The individual metrics selected to construct the indices are gathered from several levels of biological organization and encompass different time responses to stress and different specificity to stressors. Thus, they represent a good integrated measure of ecosystem status. Specifically, biochemical and physiological measurements are typically not influenced by hysteretic properties, making them much better candidate bioindicators to detect recovery in environmental conditions over time scales relevant for management.
2. Under the WFD, individual metrics are aggregated to construct a single indicator expressing the distance between the present status and an ideal status of the system. In our indices, we use multivariate statistical techniques based on PCA to extract a common variability (associated with ecosystem status). This not only takes into account redundancy among individual metrics, but it probably introduces less bias than expert judgment scoring, a common practice in implementing multimetric indices.
3. Both indices (POMI and CYMOX) show good correlation to human pressures and, therefore, seem to be good indicators as required by the WFD for management purposes.

However, some open questions remain. We would like to stress three of them.

First, the application of multivariate indices that incorporate a large number of metrics, such as CYMOX and POMI, is time and resource consuming and could require some degree of expertise. Of course, this is an unavoidable counterpart to the strengths of the indices reported above. However, and following the needs of managers and the objectives of specific monitoring programs, the multivariate nature of POMI and CYMOX allows for simplification; for example, if there is no need for great precision or robustness, in this respect, we tested the reliability of POMI-9 (using only nine metrics, instead of 14) with good results [43]. Further simplifications, tailored to manager needs and budgets, could be explored in the future.

Second, a crucial aspect of the classification of water bodies based on their ecological status is the reference frame used. Bad or incorrect references can lead to misclassifications with undesired consequences for managers. The issue of how to define reference conditions is not a closed discussion, especially in the case of indices based on *P. oceanica*, for which some of the metrics used show very slow recovery. More research is needed, with inputs from different fields (paleoecology, modeling, etc.) to refine this crucial aspect.

Third, we have shown how large the uncertainty is in close to the boundary values between classes. Once this uncertainty has been recognized and properly

quantified, how it should be incorporated into the decision-making process associated with environmental monitoring remains an open question.

Overall, bioindicators have proved to be stimulating and fertile ground for research, at the interface between science and management, where research into ecology finds considerable social utility.

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