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Review

Ecosystem health assessment using the Mediterranean seagrass *Posidonia oceanica*: A review

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ABSTRACT

The endemic Mediterranean seagrass *Posidonia oceanica* is a key species in coastal waters and it is widely employed as an ideal biological indicator for assessing the quality of water bodies, in accordance to the Water Framework Directive (WFD), as well as for assessing the health status of coastal ecosystems. In this contribution the current situation of the *P. oceanica* monitoring programs in the Mediterranean Sea is reviewed focusing on those descriptors adopted commonly by researchers and local administrators. The application of recently introduced approaches based on a set of synthetic ecological indices, namely the Conservation Index (CI), the Substitution Index (SI) and the Phase Shift Index (PSI), is also reviewed discussing their effectiveness in the context of ecosystem health assessment and of the requirements of the WFD. The CI, the SI and the PSI go beyond the quality of the water and, thus, the WFD, as they provide additional indications on past events of disturbance that affected a meadow, on potentiality of a meadow to recover, on quality of sediments, on biological pollutants. An integrated approach based on the descriptors of the water quality together with the three ecological indices of the ecosystem health (CI, SI, and PSI) is thus recommended in order to discriminate the main components affecting the status of coastal ecosystems. Application of the CI, the SI and the PSI should be experienced in different areas of the Mediterranean Sea for selecting appropriate reference sites and for formulating more generalized classifications that shift the requirements of the WFD toward the perspective of the ecosystem health evaluation.

Contents

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1. Introduction

Seagrass meadows are widely recognized as key ecosystems in shallow coastal waters (Short and Wyllie-Echeverria, 1996). Due to their wide distribution, their sedentary habit and their susceptibility to changing environmental conditions, seagrasses are habitually used as biological indicators of water quality and health (Pergent-Martini and Pergent, 2000; Bhattacharya et al., 2003), in accordance with the Annex V of the Water Framework Directive (WFD, 2000/60/EC) where seagrasses are listed as biological quality elements to be used in assessing the ecological status of coastal water bodies (Foden and Brazier, 2007). As many disturbances affecting coastal ecosystems do not necessarily compromise directly the quality of water (e.g. destructive fishing activities, pleasure boats anchoring, dragging, etc.), seagrasses have been shown to work properly as bioindicators also for assessing the health status of marine coastal environment (Pergent et al., 1995).

The endemic *Posidonia oceanica* (L.) Delile is the most important and abundant seagrass in the Mediterranean Sea, where it forms extensive meadows from the surface down to 40 m water depth and plays a major ecological role (Boudouresque et al., 2006), being able to build a ''matte'', a monumental construction resulting from horizontal and vertical growth of rhizomes with entangled roots and entrapped sediment (Francour et al., 2006). The indicator value of *P. oceanica* works at three levels: the ''individual'' level, where the phenology of the plant (especially leaf biometry) provides information about its status and growth condition (Buia et al., 2004; Leoni et al., 2006; Marbà et al., 2006); the ''population'' level, where the structure (e.g. density and/or cover) and morphology of the meadow (such as the presence of regressive structures: dead matte, intermatte channels, etc.) represent characteristic imprints of environmental conditions (Pergent et al., 1995; Montefalcone et al., 2008); the ''community'' level, where the associate flora and fauna (especially the leaves epiphytes) are similarly susceptible to environmental alterations (Ruiz et al., 2001; Cancemi et al., 2003; Balata et al., 2007).

Meadows of *P. oceanica* occur in coastal areas that are often subjected to intense human activities that inevitably affect *P. oceanica*, either directly by physical damages to the meadow (Meinesz et al., 1991) or indirectly through the impact on the quality of waters and sediments (Duarte, 2002). An alarming decline of the *P. oceanica* meadows has been reported in the Mediterranean Sea and mainly in the north-western side of the basin (Ardizzone et al., 2006; Boudouresque et al., 2006; Montefalcone et al., 2007a), where many meadows have already been lost during last decades (Marbà et al., 1996; Montefalcone et al., 2007b). Different efforts have been carried out in several countries in order to protect legally *P. oceanica*. The species is included in the Red List of marine threatened species of the Mediterranean (Boudouresque et al., 1990) and meadows are defined as priority natural habitats on Annex I of the EC Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora (EEC, 1992), which lists those natural habitat types whose conservation requires the designation of special areas of conservation, identified as Sites of Community Interest (SCIs).

According to the recognized ecological importance of *P. oceanica* meadows and to the necessity of managing properly the existing and the planned SCIs, it is crucial to dispose of adequate standardized monitoring plans to be adopted for evaluating the health status of the meadows. Similarly, the availability of tools for classifying the conservation status of the meadows is essential within the context of ecosystem health assessment. In this contribution the current situation of the *P. oceanica* monitoring programs in the Mediterranean Sea is reviewed with a brief but complete excursus on the methods and the descriptors adopted commonly by researchers and local administrators. Ecological indices are considered as one possible measure of the ecosystem status and they are often used to evaluate and assess ecological integrity of the system (Pinto et al., 2009). The application of recently introduced approaches based on a set of synthetic ecological indices, namely the Conservation Index (CI), the Substitution Index (SI) and the Phase Shift Index (PSI), is also reviewed focusing on their effectiveness in relation to the ecosystem health assessment and to the requirements of the WFD.

2. Situation of the *Posidonia oceanica* monitoring programs

The proper management of the *P. oceanica* meadows requires standardized methodologies of study, to be applied by both researchers and administrators, enabling comparable results on the scale of the whole Mediterranean basin (Pergent-Martini et al., 2005). Specific national *P. oceanica* monitoring plans adopted for managing the SCIs and for the procedures of environmental impact evaluation schedule a number of analyses to be performed in order to assess the health status of the meadows (Cicero and Di Girolamo, 2001; Buia et al., 2004; Boudouresque et al., 2006; Romero et al., 2007). These methods have been recently reviewed in order to identify the most adopted methodologies by the Mediterranean researchers and to select the most suitable descriptors of the status of *P. oceanica* (Leoni et al., 2003; Pergent-Martini et al., 2005). Among the standardized methods usually adopted for this purpose, ''destructive'' and ''not destructive'' techniques can be recognized (Buia et al., 2004), linked with the necessity or not, respectively, to collect *P. oceanica* shoots samples.

The analyses at the individual level (the plant) and most of the analyses at the community level (the associate organisms of leaves and rhizomes) require collection of shoots, thus being defined as destructive techniques. The mean number of sampled and measured *P. oceanica* shoots ranges from about 10 to 20 shoots (Pergent-Martini et al., 2005). On the contrary, analyses at the population level (the meadow) and some of the analyses at the community level, i.e. the mobile fauna associated to the meadow, require simply underwater surveys for collecting data, thus being defined as not destructive techniques. In Table 1 the most employed analyses by the Mediterranean research laboratories have been listed (Leoni et al., 2003; Buia et al., 2004; Pergent-Martini et al., 2005; Boudouresque et al., 2006).

The shoot density can be viewed as the most adopted standardized descriptor in the *P. oceanica* monitoring plans (Pergent-Martini et al., 2005) because provides important information about vitality and dynamic of the meadow and proves effective in revealing the human influence on the

Table 1 – The analyses on *Posidonia oceanica* most routinely employed by the Mediterranean research laboratories, separated in destructive and not destructive techniques.

Destructive techniques	Not desctructive techniques
Epiphytic assemblages of leaves and rhizomes: • Quantitative analysis of biomass (Buia et al., 2004) and coverage (Morri, 1991) • Qualitative analysis of specie composition (Balata et al., 2007)	Shoot density (number of shoots per square metre) and its classification following the absolute scales by Giraud (1977) and by Pergent et al. (1995)
Leaf biometry and related descriptors (Giraud, 1977): • Type of leaves (adult, intermediate or juvenile) • Number of leaves per shoot • Length and width of leaves • Leaf surface area per shoot and per square metre (the latter defined as the ''Leaf Area Index'') • Presence of dead brown tissue • Percentage of broken leaves (Coefficient A) and the cause of their damage (water movement or grazing), referring to the protocol of Boudouresque and Meinesz (1982)	Upper and lower limits of the meadow: • Bathymetric position of limits • Typology of the lower limit, according to Meinesz and Laurent (1978) and to Pergent et al. (1995) • Monitoring the position of limits over time, throughout fixed marks (''balise''), in situ photographs, aerial diachronic photographs (Pergent-Martini et al., 2005)
Indirect estimation of the past primary production of leaves and rhizomes, throughout: • Lepidochronology (Pergent, 1990) • Internodal length (Peirano, 2002) • Plastochrone interval (Cebrian et al., 1994)	Structure of the matte: • Presence of intermatte channels and of dead matte • Measuring the baring of the rhizomes as defined by Boudouresque et al. (2006) • Evaluating the homogeneity and the compactness of the matte and measuring the percentage of plagiotropic rhizomes and the thickness of the matte (Pergent-Martini et al., 2005) Percentage of bottom covered by living *P. oceanica* (see also Table 2) Relative shoot density (number of shoots per square metre multiplied for the cover of living *P. oceanica*) (Romero, 1986) Mobile fauna associated to the meadow and the presence of other macrophytes (Pergent-Martini et al., 2005)

environment (Pergent et al., 1995). The absolute scales available for the shoot density (Giraud, 1977; Pergent et al., 1995) provide classifications of the status of the meadow that can be used in the ecosystem health assessment. The absolute scale proposed by Pergent et al. (1995) has been recently revised by Buia et al. (2004) and by Boudouresque et al. (2006), whom avoided the use of the adjectives ''normal, abnormal and sub-normal'' adopted originally for classifying the shoot density, being difficultly interpreted. Alternatively, they recognized three distinct classes of bed according to its density: beds in equilibrium, disturbed beds, and very disturbed beds. Another useful classification of the meadow health is based on the relative shoot density (Romero, 1986), which recognizes meadows in a not satisfactory conservation status, meadows in a satisfactory conservation status, and meadows in an exceptional conservation status (Regione Liguria, 2003). This latter classification is an adequate tool for verifying the requirement of the EC Directive 92/43/EEC, which states that ''a SCI must be preserved in a satisfactory conservation status'' (EEC, 1992).

Describing the features of both the upper and the lower limit of the meadow and monitoring over time their positions are commonly adopted procedures for evaluating the evolution of the meadow in term of stability, improvement or regression that is linked to water transparency, hydrodynamic regimes, sedimentary balance and human actions along the coastline (Pergent et al., 1995). As many *P. oceanica* meadows underwent regression during last decades (Marbà et al., 1996; Ardizzone et al., 2006; Boudouresque et al., 2006), especially in their deep portions (Montefalcone et al., 2007a), the existing classifications of the lower limit (Meinesz and Laurent, 1978; Pergent et al., 1995; Buia et al., 2004) have been recently revised (Montefalcone et al., 2006a; Montefalcone, 2007). The three classic typologies of natural ''healthy'' limit, with their respective ''regressive'' typologies characterized by the occurrence of dead matte in correspondence of the limit itself, have been renamed as follow: natural and regressive shaded limit (Fig. 1a and b respectively), natural and regressive sharp limit (Fig. 1c and d respectively), natural and regressive erosive limit (Fig. 1e and f respectively) with a pronounced step of matte. Each of the three typologies of regressive limit may also show further peculiarities and three novel regressive limits were thus described (Montefalcone, 2007): regressive limit with patches (Fig. 1g), characterized by the presence of residual patches of living *P. oceanica* (either with a pronounced step of matte or without) among the dead matte occurring just beyond the limit; regressive limit with living belts (Fig. 1h), characterized by belts (Buia et al., 2004) of living *P. oceanica* (either with a pronounced step of matte or without) developing orthogonal to the coastline just beyond the limit and among the dead matte; regressive limit with dead belts (Fig. 1i), where belts of dead matte (either with a pronounced step of matte or

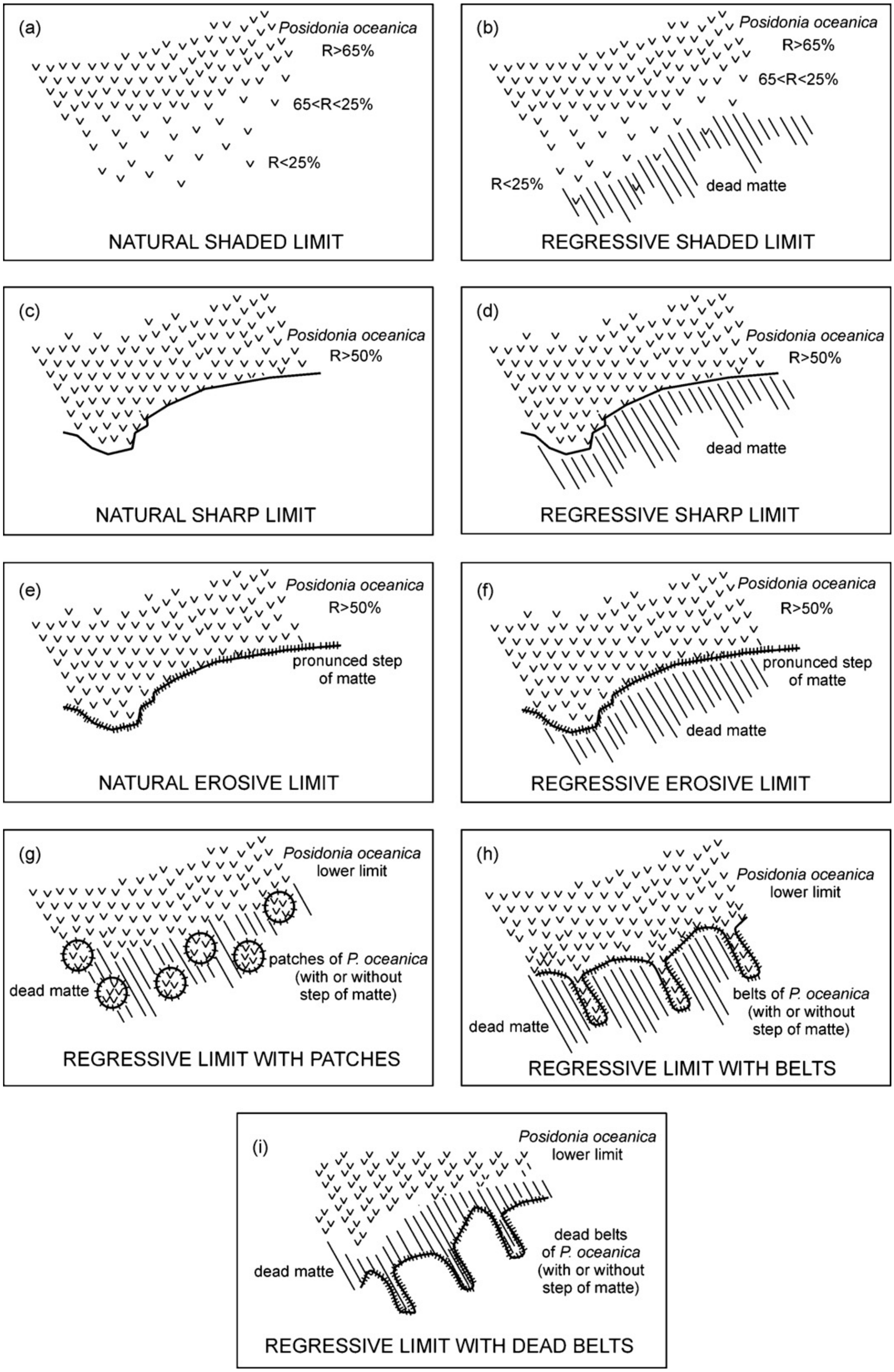

Fig. 1 – Distinct typologies of natural (a, c, e) and regressive (b, d, f, g, h, i) *Posidonia oceanica* lower limits. *R* = percentage of bottom covered by living *P. oceanica*. From Montefalcone (2007), modified.

without), developing orthogonal to the coastline, occur just beyond the limit.

Different methods have been proposed for measuring the cover of living *P. oceanica*: (1) it can be estimated visually by two divers independently diving some metres above the bottom on a defined seabed surface area (Buia et al., 2004); (2) using a grid designed on a transparent support where the diver swims at about 3 m above the bottom and observes the presence/

Table 2 – Comparison between five common methods for the underwater assessment of the *Posidonia oceanica* bottom cover.

Methods	Advantages	Disadvantages
Visual estimation (Buia et al., 2004)	Rapidity, immediate results	Subjectivity. Influenced by the seasonal dynamics of canopy and by condition of visibility. Lack of standardized values of seabed surface area
Grid on a transparent support (Leriche et al., 2006)	Rapidity	Comparability with a true cover. Influenced by seasonal dynamics of canopy and by condition of visibility. Lack of standardized values of seabed surface area
Vertical photography (Romero, 1985)	Rapidity, objectivity, reference collections	Influenced by seasonal dynamics of canopy and by condition of visibility. Difficulty in maintaining the zenith by diver. Lack of standardized values of seabed surface area
Grid on a frame laid on the seafloor (Pergent-Martini et al., 2005)	Rapidity, objectivity	Comparability with a true cover
Line Intercept Transect (LIT) (Montefalcone et al., 2007b)	Objectivity, accuracy	Slowness

absence of *P. oceanica* (Leriche et al., 2006); (3) using a vertical photography (Romero, 1985); (4) estimating the shoots repartition within a grid on a frame laid on the seafloor (Pergent-Martini et al., 2005); (5) using the Line Intercept Transect (LIT) methodology (Montefalcone et al., 2007b). In Table 2 the supposed advantages and disadvantages for each method have been discussed. Only the visual method proposed by Buia et al. (2004), which is likely to be the most rapid one, and the photographic analysis (Romero, 1985) allow for the true plan view cover estimation; the latter, however, shows little usage in the current literature. The two methods adopting the grid (Pergent-Martini et al., 2005; Leriche et al., 2006) allow for the *P. oceanica* frequency estimation. Data recorded using the LIT (Montefalcone et al., 2007b) allow for the measure (and not the estimation) of the linear cover of *P. oceanica*; it has been recently demonstrated that the plan view cover data collected with the visual estimation and the linear cover data collected with the LIT are very similar (Wilson et al., 2007). For many of the above-mentioned methodologies, i.e. the visual estimation, the photography and the grid on a transparent support, there is not, at present, consensus about standardized values of the seabed surface area to be adopted, so that the results are not always comparable.

3. Evidences from not destructive methodologies

Ecosystem status evaluation based on ecological synthetic indices is today widespread (Pinto et al., 2009). Ecological indices succeed in ‘‘capturing the complexities of the ecosystem yet remaining simple enough to be easily and routinely monitored’’ (Dale and Beyeler, 2001) and may therefore be considered ‘‘user-friendly’’ (Winter et al., 2005). Many ecological indices are currently employed in the seagrass monitoring plans, e.g. the biomass of leaves and rhizomes, the Leaf Area Index (Buia et al., 2004), the Epiphytic Index (Morri, 1991), the bottom cover, etc. Following the requirements of the WFD (Foden and Brazier, 2007), Romero et al. (2007) proposed a multivariate index (POMI), based on a number of structural and functional descriptors of *Posidonia oceanica*, in order to assess the ecological status of coastal waters. However, some of the descriptors used for computing the POMI index show little usage in the *P. oceanica* monitoring plans, e.g. the chemical and biochemical composition and the contamination of *P. oceanica* (Pergent-Martini et al., 2005).

Not destructive approaches have been recently proposed for monitoring *P. oceanica* meadows within the issue of ecosystem health assessment, based on the application of three synthetic ecological indices, namely the Conservation Index (CI), the Substitution Index (SI) and the Phase Shift Index (PSI). The CI, the SI and the PSI represent an useful tools for assessing the quality and the health of coastal environments in their whole, not only for assessing the quality of the water bodies. All the three indices fit several of the criteria listed by Dale and Beyeler (2001): they are easily measured, anticipatory, integrative, and sensitive to stress. Another important advantage is that the three indices do not require the collection of plants, whereas several standardized descriptors employed currently for assessing the status of the *P. oceanica* meadows are destructive (Leoni et al., 2003; Buia et al., 2004; Pergent-Martini et al., 2005; Boudouresque et al., 2006). The CI, proposed by Moreno et al. (2001) for assessing the health status of the *P. oceanica* meadows in Spanish waters, measures the proportional abundance of dead matte relative to living *P. oceanica* (see also Table 3 for its formula). Boudouresque et al. (2006) highlighted the CI as a proper tool for measuring the anthropogenic disturbances affecting a meadow, notwithstanding that its punctual value might not be always significant as dead matte areas also originate from natural causes (i.e., hydrodynamic regime). Application of the CI proved effective in evaluating the health status of the Ligurian *P. oceanica* meadows (Montefalcone et al., 2006a,b, 2007a,b), where most of the observed dead matte areas are likely to be the result of human induced disturbances that affected coastal waters from 1960 (Bianchi and Peirano, 1995). The CI may also be a useful tool for assessing the evolution over time of the meadow, which reflects situation of stability, improvement or regression.

Table 3 – Assumed regional scales for the Conservation Index, the Substitution Index and the Phase Shift Index.

Conservation Index: CI = $P/(P + D)$
where P is the percent cover of living *Posidonia oceanica* and D is the percent cover of dead matte
1 - CI < 0.3: bad conservation status
2 - CI between 0.3 and 0.5 excluded: poor conservation status
3 - CI between 0.5 and 0.7 excluded: moderate conservation status
4 - CI between 0.7 and 0.9 excluded: good conservation status
5 - CI ≥ 0.9: high conservation status
Substitution Index: SI = $S/(S + P)$
where S is the percent cover of substitutes and P is the percent cover of living *P. oceanica*
1 - SI < 0.1: no (SI = 0) or little substitution; high conservation status
2 - SI between 0.1 and 0.25 excluded: low substitution; good conservation status
3 - SI between 0.25 and 0.4 excluded: moderate substitution; moderate conservation status
4 - SI between 0.4 and 0.7 excluded: significant substitution; poor conservation status
5 - SI ≥ 0.7: strong substitution; bad conservation status
Phase Shift Index:
PSI = $\{[D/(P + D) \times 1] + [Cn/(P + Cn) \times 2] + [Cp/(P + Cp) \times 3] + [Ct/(P + Ct) \times 4] + [Cr/(P + Cr) \times 5]\}/6$
where D is the percent cover of dead matte, P that of living *P. oceanica*, Cn of *Cymodocea nodosa*,
Cp of *Caulerpa prolifera*, Ct of *C. taxifolia*, and Cr of *C. racemosa*
1 - PSI < 0.08: no (PSI = 0) or early stage of phase shift; high conservation status
2 - PSI between 0.08 and 0.16 excluded: low phase shift; good conservation status
3 - PSI between 0.16 and 0.25 excluded: moderate phase shift; moderate conservation status
4 - PSI between 0.25 and 0.5 excluded: significant phase shift; poor conservation status
5 - PSI ≥ 0.5: strong phase shift, irreversible; bad conservation status
Regional scale from Montefalcone et al. (2007a), modified

The CI does not contemplate the possibility of dead meadows to be recolonized by other species, named as substitutes (Montefalcone et al., 2006a). The SI (see Table 3 for its formula) has therefore been proposed for measuring the amount of replacement of *P. oceanica* by the other common native Mediterranean seagrass *Cymodocea nodosa* (Ucria) Ascherson and by the three species of green algae genus *Caulerpa*: the native *Caulerpa prolifera* (Forsskål) Lamouroux and the two alien invaders *C. taxifolia* (Vahl) C. Agardh and *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman and Boudouresque (Montefalcone et al., 2007a). The SI, applied repeatedly in the same meadow, can objectively measure whether the substitution is permanent or progressive or, as hypothesized by Molinier and Picard (1952), will in the long term facilitate the reinstallation of *P. oceanica*. While the application of the CI is obviously limited to those seagrass species that form a matte, the SI can be applied to all cases of substitution between two different seagrass species and between an alga and a seagrass.

The observed changes (regression and substitution) in the *P. oceanica* ecosystems motivated the proposal of the PSI, another synthetic ecological index that identifies and measures the intensity of the phase shift (Montefalcone et al., 2007a,b) occurring within the ecosystem (see Table 3 for its formula). The PSI provides a synthetic evaluation of the irreversibility of changes undergone by a regressed meadow. The biological characteristics and the reproductive processes of *P. oceanica* are not conducive to a rapid re-colonisation of dead matte (Meinesz et al., 1991). If a potentiality of recovery still exists in a meadow showing few and small dead matte areas, a large-scale regression of *P. oceanica* meadow must therefore be considered almost irreversible on human-life time scales. On the contrary, the comparatively fast growth of all the potential substitutes (Montefalcone et al., 2007a) can make them to persist forever. Long-term monitoring is the only way to track the future evolution of such a phase shift and to appreciate any potentiality of recovery, and the set of the three ecological indices may be a useful tool to this purpose. Developing the capacity to predict the future distribution of substitutes is essential for their early detection and control; the CI, the SI and the PSI may also be applied for predicting the susceptibility of *P. oceanica* meadows to invasions and for quantifying the degree of replacement in degraded environments.

For the CI, the SI and the PSI, regional scales for classifying their values have been proposed (Montefalcone et al., 2007a). The merely local scales that were originally proposed for the CI (Moreno et al., 2001) and for the SI (Montefalcone et al., 2006a) are well suited for monitoring the evolution over time of a single meadow, thus highlighting its potentiality of restoration. In contrast, regional scales are better suited to compare synoptically different meadows in region-wide studies and are more easily handled for management purposes. The regional scale of the PSI had five levels of ecosystem quality whereas the scales of the CI and the SI had originally four levels, which were calculated with the method proposed by Moreno et al. (2001) averaging data over six Ligurian meadows taken together and not over a single meadow (Montefalcone et al., 2007a). In this contribution, the regional scales of the CI and the SI have been modified and a new level for each of them has been introduced after a synoptic analysis performed on a total of 17 Ligurian meadows investigated in the recent years (Montefalcone, 2007), which represent nearly half of the total number of meadows in the Region (Diviacco and Coppo, 2007). Following the procedure proposed by Bianchi (2007) for classifying the quality of the marine territory, a frequency distribution analysis of all the values of the CI (Fig. 2a) and the SI (Fig. 2b) per transect has been carried out and five levels of ecosystem quality were selected so that each level had a similar number of transects. Five levels of ecosystem status

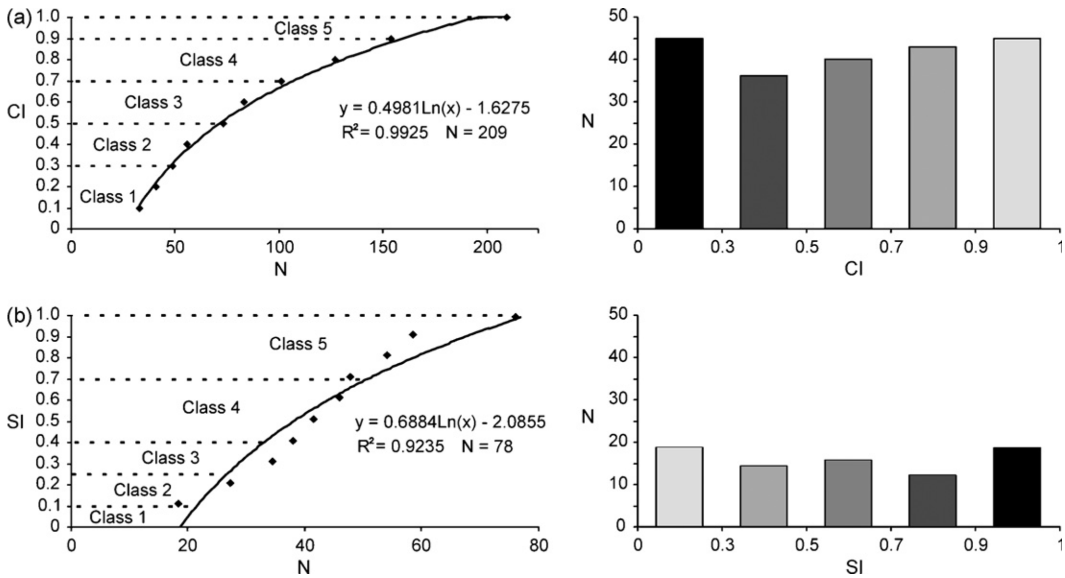

Fig. 2 – Procedure to assess the five levels (Class 1–5) of ecosystem quality through cumulative frequency distribution analysis of the values of the Conservation Index (CI) (a) and the Substitution Index (SI) (b) per transect. N = number of transects.

are now available for all the three ecological indices (CI, SI, and PSI) (Table 3). In such a way, the classifications of the three indices are comparable to the classification of the ecological quality of water bodies established by the WFD (2000/60/EC). However, the Annex V of the WFD states that the classification should be based on the deviation of the status of the biological quality element from its potential status under pristine (i.e. undisturbed or nearly undisturbed) conditions, named reference conditions. The ratio between the actual status of a given biological quality element in a given body of surface water and its status in the reference conditions is called EQR (Ecological Quality Ratio). The regional scales of the CI, the SI and the PSI were elaborated without referring to reference conditions but as the results of preliminary attempts on the Ligurian *P. oceanica* meadows, which can be also viewed as the meadows developing at the highest latitudes in the Mediterranean Sea and within a situation of extreme anthropization of the coastline. The WFD high ecological status, which equates with no changes from the reference condition, can be equated in the ecosystem health context with no changes from the reference condition structure of a system showing an optimum vigour (Tett et al., 2007). In the scheme of the three ecological indices, the reference conditions may be represented by a meadow showing no dead matte areas (CI = 1), by a meadow showing no substitutes (SI = 0) and by a meadow showing only living *P. oceanica* (PSI = 0). The WFD moderate ecological status corresponds to the class 3 of each ecological index (see Table 3), which can be referred to the status of a meadow that appears only little changed but is approaching the limits of its resistance to disturbance, and so could easily shift into a degraded status which equates with WFD poor or bad status (class 2 and 1, respectively, for the CI and class 4 and 5, respectively, for the SI and the PSI).

The regional scales of the CI, the SI and the PSI provide useful tools for classifying the status of the meadow and those "threshold limits" requested by plans for seagrass conservation (Bell et al., 2001), similarly to the absolute scales proposed for the shoot density classification (Giraud, 1977; Pergent et al., 1995). For instance, the value of CI = 0.7 (see Table 3) may represent the threshold limit for discriminating meadows in a "satisfactory conservation status" from meadows in a "not satisfactory conservation status", as requested by the EC Directive 92/43/EEC for the SCIs management (EEC, 1992). Widespread loss of seagrasses has prompted efforts to restore meadows in many coastal areas: successful restoration of *P. oceanica* is more than likely to depend on the degree of regression and phase shift undergone by the meadow, and the ecological indices may be proposed also for evaluating feasibility of restoration plans. For instance, where the phase shift is strong (class 5, PSI $\geq$ 0.5), the meadow has no real potentiality for recovery: attempts to re-establish *P. oceanica* here might be a waste of time and money. On the contrary, meadows showing from early to moderate levels of phase shift (from class 1 to class 3, PSI $<$ 0.25) could still fully recover thanks to the removal of the major causes of disturbance and to specific restoration programs.

4. Protocol for collecting data to be computed in the ecological synthetic indices

Scuba diving is required for collecting data for the CI, the SI and the PSI. Most of the standardized methodologies used for monitoring *Posidonia oceanica* request the direct underwater work of divers, especially when collection of biological samples is necessary. Surveys can be carried out either along underwater transects or in random punctual stations. Collecting data in random punctual stations is the most rapid method, although a significant number of stations must be positioned within a meadow in order to cover its whole extent.

Transects can be laid on the bottom either perpendicular to the coastline (depth transect) (Montefalcone et al., 2006a) or parallel to the coastline (Line Intercept Transect, LIT) (Montefalcone et al., 2007b). The direction of the transect is maintained using an underwater compass. The depth transect, which is the most time-consuming methodology for collecting ecological indices data, is fixed by a nylon line marked every 5 m and is about 100 m long. The depth transect is effective for revealing differences along a bathymetrical gradient and it well suits when large-scale cartographies of the seagrass assemblages are requested (Bianchi et al., 2004; Montefalcone et al., 2006a). The LIT is fixed by a centimetre-marked line and is about 25 m long; the LIT, working at constant depth, is likely to be a good compromise between accuracy and dive effort and proves effective when a specific depth range must be investigated (Montefalcone et al., 2007b).

Every 10 m, in the case of depth transect, or in at least 5 replicated points (separated by 15–20 m to each other) in each punctual station, divers record on a PVC slate the species encountered (in this case *Posidonia oceanica*, *Cymodocea nodosa*, *Caulerpa prolifera*, *C. taxifolia* and *C. racemosa*), the occurrence of dead matte (also reporting when it is re-colonised by any of the substitutes), and their percentage of bottom cover. The bottom cover can be estimated using one of the standardized procedures proposed by Romero (1985), Buia et al. (2004), Pergent-Martini et al. (2005) and Boudouresque et al. (2006) (see also Table 2). When the species form mixed assemblages their contribution is portioned proportionally to bottom cover so that cover evaluation always totals 100%. Dead matte may be found uncolonised by any of the four substitutes or may be re-colonised by them: in this latter case the percent cover, which cannot exceed 100% by definition, is computed collectively for the association “dead matte + the substitute” and the resulting value is successively assigned to dead matte only when computing the CI, and to the substitutes only when computing the SI (see Table 3).

In the case of LIT, divers record the intercept to the nearest centimetre corresponding to the point where the key attributes (i.e. the species and the dead matte) change under the line. In each LIT, the length of each key attribute (L_x) is the distance occurring between two recorded intercepts, and it is calculated by subtraction. The percent cover ($R\%$) of each key attribute along a LIT of 25 m length is calculated by the following formula: $R\% = \sum(L_x/25 \times 100)$.

5. Discussion

There is a difference in the conceptual framework of the ecosystem health assessment and that of the WFD in its Annex V. The latter focuses on the quality of the water bodies and sees all changes from reference conditions as a degradation of ecological quality, whereas the concept of ecosystem health implies that the status of a marine ecosystem is modulated by the quality of the water body, as well as by all disturbances that may alter its structure and health without affecting, apparently, the water quality (as, for instance, disturbances like anchoring, trawling, dragging and biological pollution). In addition, the status of a meadow is linked to its historical memory of the environmental situations in which it developed and, consequently, to all disturbances it suffered in the past. A healthy ecosystem functions well and is able to resist or recover from disturbance: it has quantifiable components of vigour, organization, resistance to disturbance, and resilience (Tett et al., 2007). Due to the low resilience of *Posidonia oceanica* (Procaccini et al., 1996), the species has a strong and very long biological memory of past events of regression: the recovery of a regressed *P. oceanica* meadow may take several years (Boudouresque et al., 2006), independently from the quality of the water. The deep portions of a *P. oceanica* meadow are usually affected by disturbances linked with the quality of water, as the increased water turbidity, whereas the shallow portions of a meadow are frequently affected by physical damages caused by coastal constructions, anchoring, etc. (Ardizzone et al., 2006). The ecosystem health assessment, thus, should be done combining the descriptors of the water quality (e.g. the leaf biometry, the epiphytic coverage, the POMI index, etc.), as requested by the WFD (Foden and Brazier, 2007), with the ecological indices of the ecosystem integrity and health, like the CI the SI and the PSI: the use of several indices is always advised in order to get a better evaluation of the benthic community health and, preferentially, in association with other parameters (Pinto et al., 2009). The CI, the SI and the PSI, therefore, go beyond the quality of the water and, thus, the WFD requirements, as they provide additional indications on the status of the environment, as the potentiality of the meadow to recover from past events of disturbance, the quality of sediments, the biological pollutants.

Notwithstanding that a clear-cut distinction is not likely, the integrated approach here proposed (i.e. combining the descriptors of water quality with the ecological indices of ecosystem health) will allow to understand whether the status of coastal environments is more linked to the quality of water or to other kinds of disturbance. The application of the CI, the SI, and the PSI may consequently become a complementary tool to more traditional analytical descriptors (Buia et al., 2004; Boudouresque et al., 2006) for assessing the health status of the *P. oceanica* meadows and, indirectly, of the coastal ecosystems. Analyses such as phenology, epiphytic coverage or lepidochronology are irreplaceable for in-depth studies (Pergent et al., 1995); they are, however, comparatively costly, time-consuming, especially in terms of laboratory work and sample management, and destructive. In comparison, the three ecological indices may represent a convenient not destructive protocol that provides immediate information about the ecosystem health and that may be adopted in the specific monitoring plans for managing properly the SCIs. Moreover, the CI and the not destructive descriptors working at the population (meadow) level showed more effective than destructive descriptors working at the individual (plant) level when assessing the impact of boat anchoring (Montefalcone et al., 2006b, 2008), one of the main impact affecting *P. oceanica* meadows (Francour et al., 2006).

Most of the *P. oceanica* meadows have been classified as SCI (Relini, 2000); the European Community requires plans for SCI management (EEC, 1992) and asks that the priority habitats contained in SCI are in a satisfactory condition, meaning that the overall health status of the habitat must be preserved (and not only the quality of the water). Thus, the availability of a synthetic and objective way to evaluate the condition of the *P. oceanica* meadows is crucial for both the selection of a potential SCI and the management of an established SCI. Although a great attention on the problem of the water quality has been posed in the recent years and the criteria for the selection of both the descriptors and the reference conditions have been defined (WFD, 2000/60/EC), the ecosystem health assessment still requires standardized procedures. Application of the CI, the SI and the PSI should be experienced in different areas of the Mediterranean Sea in order to select opportune reference sites and to formulate more generalized (i.e., absolute) classifications of these ecological indices that shift the requirements of the WFD toward the perspective of the ecosystem health evaluation.

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