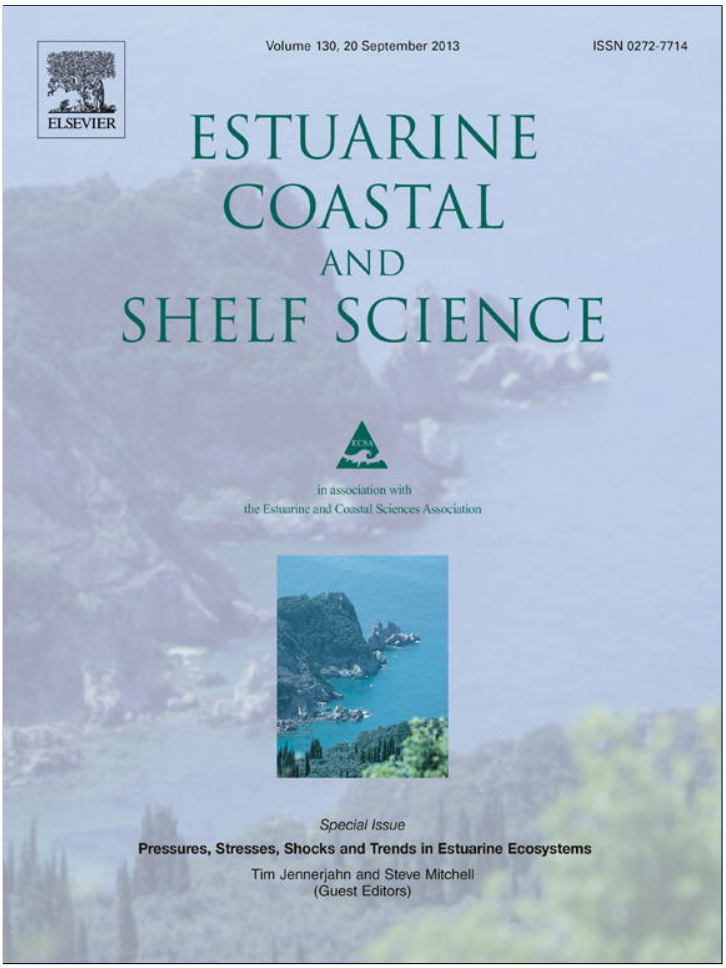

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Effectiveness and consistency of a suite of descriptors for assessing the ecological status of seagrass meadows (*Posidonia oceanica* L. Delile)

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ABSTRACT

The increasing rate of human-induced environmental changes on coastal marine ecosystems has created a demand for effective descriptors, in particular for those suitable for monitoring the status of seagrass meadows. Growing evidence has supported the useful application of biochemical and genetic descriptors such as secondary metabolite synthesis, photosynthetic activity and genetic diversity. In the present study, we have investigated the effectiveness of different descriptors (traditional, biochemical and genetic) in monitoring seagrass meadow conservation status. The *Posidonia oceanica* meadow of Monterosso al Mare (Ligurian sea, NW Mediterranean) was subjected to the measurement of bed density, leaf biometry, total phenols, soluble protein and photosynthetic pigment content as well as to RAPD marker analysis. This suite of descriptors provided evidence of their effectiveness and convenient application as markers of the conservation status of *P. oceanica* and/or other seagrasses. Biochemical/genetic descriptors and those obtained by traditional methods depicted a well conserved meadow with seasonal variability and, particularly in summer, indicated a healthier condition in a portion of the bed (station C), which was in agreement with the physical and sedimentological features of the station. Our results support the usefulness of introducing biochemical and genetic approaches to seagrass monitoring programs since they are effective indicators of plant physiological stress and environmental disturbance.

1. Introduction

Seagrass meadows are considered an engineering ecosystem that plays a major ecological, geological and economic role in the shallow coastal waters around the world (Spalding et al., 2003). *Posidonia oceanica* (L.) Delile is the dominant endemic seagrass in the Mediterranean sea (Procaccini et al., 2003), covering 37,000 km^2 which corresponds to about 1–2% of the sea bottom (Pasqualini et al., 1998; Boudouresque et al., 2006). *Posidonia* meadows are a benchmark for monitoring the environmental health of aquatic systems, and several governments and institutions worldwide have recognized the meadows as bioindicators (Council of Australian Governments Water Reform Framework of 1994, in Australia and New Zealand; Water Framework Directive 2000/60/EC, in the European Union).

Despite the ecological and economic importance of seagrass beds, an increasing number of reports document the ongoing loss of seagrass biomass in several countries, with a global decline rate estimated at 2–5% per year (Duarte, 2002; Orth et al., 2006). The decline of *Posidonia oceanica* meadows is mainly due to human-induced disturbances through the modification of the hydrological regime and littoral transport (Ruiz and Romero, 2003), pollution and eutrophication (Balestri et al., 2004; Burkholder et al., 2007), aquaculture (Pergent-Martini et al., 2006; Apostolaki et al., 2009) and anchoring (Milazzo et al., 2004; Montefalcone et al., 2008). Growing evidence suggests that seagrass meadows are also vulnerable to climate change (e.g. Short and Neckles, 1998; Duarte et al., 2008) due to the impact of higher temperatures on shoot survival (Duarte, 2002; Marbà and Duarte, 2010). Consequently, the

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development and combination of sensitive and measurable descriptors, which may reveal environmental alterations, are becoming essential for monitoring ecosystem health.

Research, coastal management and environmental policies have been focussing on seagrasses because of their essential ecological role and significance as bioindicators. Biochemical and genetic descriptors such as secondary metabolite synthesis, e.g. phenol compounds (Migliore et al., 2007; Pergent et al., 2008; Arnold et al., 2012; Santoso et al., 2012), photosynthetic activity (photosynthetic pigments) and/or oxidative stress (Sureda et al., 2008; Marín-Guirao et al., 2011; Sandoval-Gil et al., 2012) and the genetic diversity of seagrasses (Alberto et al., 2001; Micheli et al., 2005; Rotini et al., 2011) have been successfully utilized as environmental biomarkers. However, these descriptors are still underutilized in *Posidonia* meadow monitoring, so far few comprehensive methodological studies on descriptors for *Posidonia oceanica* have been reported. The aim of this study was to test the effectiveness of different descriptors (traditional, biochemical and genetic) in assessing the conservation status of the Monterosso al Mare meadow located in the Ligurian sea (NW Mediterranean).

2. Material and methods

2.1. Sampling site and strategies

The *Posidonia oceanica* plants utilized to perform the analyses were collected from the Monterosso meadow (La Spezia, Italy). This meadow, spanning from Punta Mesco to Monterosso al Mare, covers a surface of 30 ha and is included in the "Cinque Terre" National Park (Ligurian Sea). The meadow lies on a soft bottom, and the seagrass cover ranges from 100% to 20%, with lower values in proximity to Monterosso al Mare. The lower limit, at ca. 20 m depth, is a regressive limit (according to the classification of Pergent et al., 1995), characterized by the presence of dead *matte* (Cavazza et al., 2000).

Samples were obtained by scuba diving in June 2010 (summer sampling) and February 2011 (winter sampling). Three stations at 12–14 m depth were chosen (St. W: West, 44°08′56″N, 9°38′94″; St. C: Central, 44°08′39″N, 9°38′72″; St. E: East, 44°08′48″N, 9°38′28″; see Fig. 1). Seven sites were randomly chosen at each station, along a 50 m transect. At each site, shoot density was estimated and 3 orthotropic shoots were collected for the subsequent laboratory analyses.

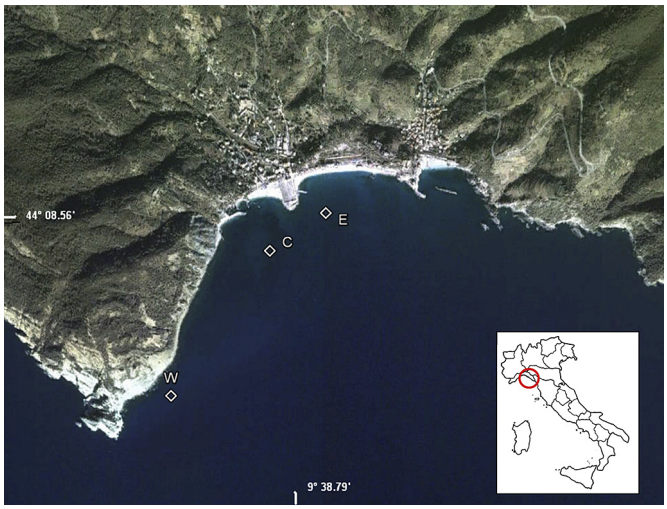

Fig. 1. Map showing the location of the three sampling stations in the Monterosso *Posidonia oceanica* meadow (La Spezia, Italy). W = Western, C = Central, E = Eastern.

2.2. Bed density and leaf biometry

Shoot density was estimated following Peirano et al. (2011) by counting the number of shoots in 6 sub-quadrats (20 × 20 cm), randomly selected on a gridded quadrat of 1 m^2. The counts were replicated randomly, three times along the transect. The value obtained was expressed as number of shoots/m^2. In the laboratory, *Posidonia oceanica* shoots were separated into rhizomes and leaves and then biometric measurements were done. Leaf length, leaf width and leaf number per shoot were measured. These measurements allowed the calculation of the mean leaf area per shoot (cm^2/shoot) according to Buia et al. (2004). Rhizomes and leaves were then washed in distilled water and cleaned with a blade to remove epiphytes, sheets and cortical tissues. Clean rhizomes and leaves were frozen in liquid nitrogen and then stored separately at −80 °C until analysed.

2.3. Total phenols

Total phenols were quantified in rhizomes from the three orthotropic shoots sampled at each site. Phenolic compounds were extracted from 100 mg (fresh weight) of apical, intermediate and basal sections of each rhizome and ground in liquid nitrogen using a mortar and pestle. Total phenol content from the extracts was determined following the protocol of Migliore et al. (2007). Two different extractions were done for each sample, and all the extracts were read in duplicate. Final results were expressed as milligrams of phenolic compounds per gram of rhizome fresh weight and are the arithmetic means of four measurements.

2.4. Soluble protein and photosynthetic pigment content in leaves

Soluble protein and photosynthetic pigment content were quantified in leaves from one shoot for each site. Soluble protein extraction procedure was adapted to *Posidonia oceanica* from Polle et al. (1993). Soluble protein content in the extracts was determined spectrophotometrically using a dye-binding assay (Coomassie Brilliant Blue G-250 dye; Bradford, 1976) and bovine serum albumine as a standard (BioRad). *Posidonia oceanica* foliar photosynthetic pigments were extracted and quantified according to Wellburn (1994). Briefly, pigments (i.e. chlorophyll *a* and *b* and total carotenoids) were extracted in dimethyl-formamide (100 mg fresh weight: 5 ml solvent) inside a glass tube sealed with a cotton plug and kept in the dark overnight at 4 °C. Extractions were made in two replicates for each sample and each extract was read in duplicate. The absorbances of the extract were read at 480, 646.8, 663.8 and 750 nm in a Schimadzu UV-260 spectrophotometer, using a glass cuvette. The formulas to calculate the concentration of pigments in the extract are reported in the Supplemental data (Table A). Final results were expressed as milligrams of pigment per gram of foliar dry weight and are means of four measurements.

2.5. RAPD genetic analysis

Genetic analyses were performed on three shoots collected at each of the seven sites of the three stations in summer 2010. According to Micheli et al. (2005), after morphological measurements, the young leaves of the plants were washed in distilled water, frozen in liquid nitrogen and stored at −80 °C while awaiting RAPD genetic analyses. DNA was extracted and amplified by TAQ Polymerase (Applied Biosystems), and PCR was carried out using 10 primers (see Supplemental Data, Table B). The amplification products were then separated by agarose gel electrophoresis (1.4%), stained with ethidium bromide, visualized on UV light and photographed. The DNA fingerprints obtained were checked for

qualitative differences, i.e. the presence (1) or absence (0) of the bands. Data were reported in a matrix and processed to determine the percentage of polymorphisms at each of the three stations. The percentage of polymorphisms was calculated as the number of polymorphic bands out of the total number of bands (mono- and polymorphic).

2.6. Statistical analysis

For density, biometry and biochemical markers, the differences between seasons and stations were analysed through ANOVA. Levene's test was used for testing the homogeneity of group variances and post-hoc comparisons of means were performed through Tukey's test. Kruskall–Wallis ANOVA multiple comparison test and Mann–Whitney *U* test were used when data did not satisfy the homoscedasticity assumptions (Levene's test). For genetic analyses, Principal Coordinates (PCoA, NT-SYS software; Rohlf, 1993) was performed to elucidate the distribution of the samples by deriving genetic distances.

3. Results

3.1. Bed density and leaf biometry

The mean (±S.D.) shoot density of the Monterosso meadow measured in summer and winter was 259.7 ± 75.5 and 260.2 ± 34.0 shoots/m^2, respectively (Fig. 2A), with no significant differences among stations and seasons (Table 1c). Despite the lack of statistical significance, it must be noted that the box-plot of the summer measurements showed a doubled value of the interquartile range (IQR, i.e. the difference between the upper and lower quartiles), indicating a higher variability in shoot density. The number of leaves per shoot and leaf width did not show differences between seasons or among stations (Fig. 2B and D and Table 1b). The mean leaf length per shoot was significantly higher in summer than in winter (Fig. 2C and Table 1c), whereas the mean leaf area per shoot (Fig. 2E) showed: (1) significant differences between seasons in each station (Table 1b); and (2i) significant differences between station C and stations E and W in summer (see Table C in supplemental data). The complete dataset is reported in the Supplemental data (Table D).

3.2. Total phenols

The mean (±S.D.) total phenol content in the entire rhizome was significantly higher in summer than in winter, with 27.62 ± 3.80 mg/g vs 22.31 ± 5.83 mg/g fresh weight (FW), respectively (Fig. 3 and Table 1a). The values of station C showed significant differences both in summer and in winter sampling when compared to those of stations E and W (Table C supplemental data); furthermore, the box-plot for station C phenol content showed the lowest values of IQR both in summer and winter, accounting for the minimal variability at this station. Lastly, we also found a decreasing gradient of the total phenol content within the rhizome, starting from the apical to the basal section of each rhizome (Fig. 3B and C). The complete dataset is reported in the Supplemental data (Table E).

3.3. Soluble protein and photosynthetic pigment content in leaves

The foliar soluble protein content (Table 2) was always significantly lower in winter than in summer at all the sampling stations (Table 1a), but the differences between sampling stations were not statistically significant. In leaves, both the total chlorophyll (Chl_{tot}) and the total carotenoid (Car_{tot}) content slightly increased from summer to winter at all the stations (Fig. 4A and B), but this increase was not statistically significant (Table 1c). Conversely, *Chl-a*/*Chl-b*, Chl_{tot}/Prot and Chl_{tot}/Car_{tot} ratios were significantly higher in winter than in summer (Fig. 4C–E, Table 1b and c). The complete dataset is reported in the Supplemental data (Tables F and G).

3.4. RAPD genetic analysis

Ten RAPD primers generated a total of 132 bands, with fragments ranging in size from 0.2 to 3.8 kb (Table 3). The mean percentage of polymorphisms was 40.82%, 61.66% and 35.64% at stations W, C and E, respectively. The percentage of polymorphisms detected by each primer set was calculated (Table 3); the primer

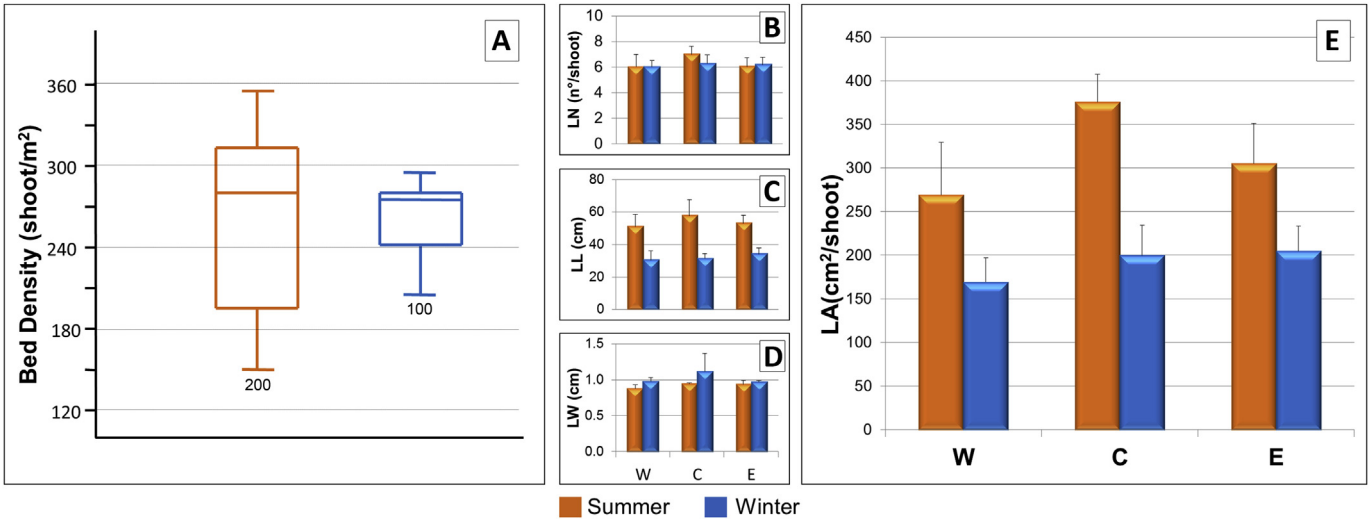

Fig. 2. Bed density and shoot biometry of *Posidonia oceanica* in summer 2010 and winter 2011 in W, C, and E sampling stations (W = Western, C = Central, E = Eastern). Shoot density values (A) represented as box-plots: the box contains 50% data (the extremes of that box are the Q1 and Q3, 1st and 3rd quartiles), the internal horizontal segments represent the median of the distributions (Q2 value, 2nd quartile), "whiskers" range from the lowest to the highest value; under each box-plot the interquartile range value is reported (IQR = Q3 − Q1). Mean values of leaf number per shoot (B), leaf length (C), leaf width (D) and leaf area per shoot (E); error bars represent standard deviation (LN = leaf number; LL = leaf length; LW = leaf width; LA = leaf area).

Table 1
Summary of statistical analysis results (n.s. = not significant; * = 0.05 > p > 0.01, ** = 0.01 > p> = 0.001, *** = p > 0.001).

A. One-way ANOVA

Source of variation	Phenol (mg/g FW)				Protein (mg/g DW)			
	df	MS	F	p	df	MS	F	p
Season	1	867.9176	36.95	***	1	1389.43	97.72	***
Residual	124	23.4896			40	14.22		
Station (summer)	2	67.13885	5.29	***	2	28.07	1.36	n.s.
Residual	60	25.44544			18	20.61		
Station (winter)	2	245.0315	9.63	***	2	14.02	2.19	n.s.
Residual	60	12.69405			18	6.32		

B. Two-way ANOVA

Source of variation	Leaf number (per shoot)				Leaf length (cm)				LA (cm^2/shoot)				*Chl-a/b*				Chl_{tot}/Prot			
	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p
Station	2	1.556	3.26	*	2	56.22	1.46	n.s.	2	16460.7	10.31	***	2	0.011	0.33	n.s.	2	0.048	5.19	**
Season	1	0.381	0.80	n.s.	1	5092.71	132.46	***	1	165703.7	103.75	***	1	0.270	8.26	***	1	0.382	41.07	***
Station * season	2	0.738	1.54	n.s.	2	52.79	1.37	n.s.	2	6684.6	4.19	*	2	0.011	0.34	n.s.	2	0.013	1.37	n.s.
Residual	36	0.478			36	38.45			36	1597.1			36	0.033			36	0.009		

C. Kruskal–Wallis ANOVA

Source of variation	Density (shoot/m^2)				Leaf width (cm)				Chl_{tot} (mg/g DW)				Car_{tot} (mg/g DW)				Chl_{tot}/Car_{tot}			
	n	df	H	p	n	df	H	p	n	df	H	p	n	df	H	p	n	df	H	p
Season	108	1	0.093	n.s.	21	2	6.063	*	42	1	1.291	n.s.	42	1	0.404	n.s.	42	1	5.666	**
Station	108	2	5.608	n.s	21	2	1.989	n.s.	42	2	5.139	n.s.	42	2	5.142	n.s.	42	2	1.381	n.s.

UB24 gave the highest number of fragments ranging from 0.4 to 3.5 kb, and primer BY12 amplified the highest molecular weight product of 3.8 kb. Primers UB28 and BY11 detected the highest percentage of polymorphisms in station C, 80.0% and 81.8%, respectively. Principal coordinate analysis (PCoA) was applied to genetic distances among individual shoots (Fig. 5, the first two axes accounted for 42.6% of variation).

4. Discussion

The *Posidonia oceanica* meadow of Monterosso al Mare was studied by a combination of descriptors including those traditionally adopted in seagrass monitoring programs (i.e. shoot density and leaf area) and a new generation of descriptors that are still underemployed (i.e. biochemical and genetic analysis). Most of

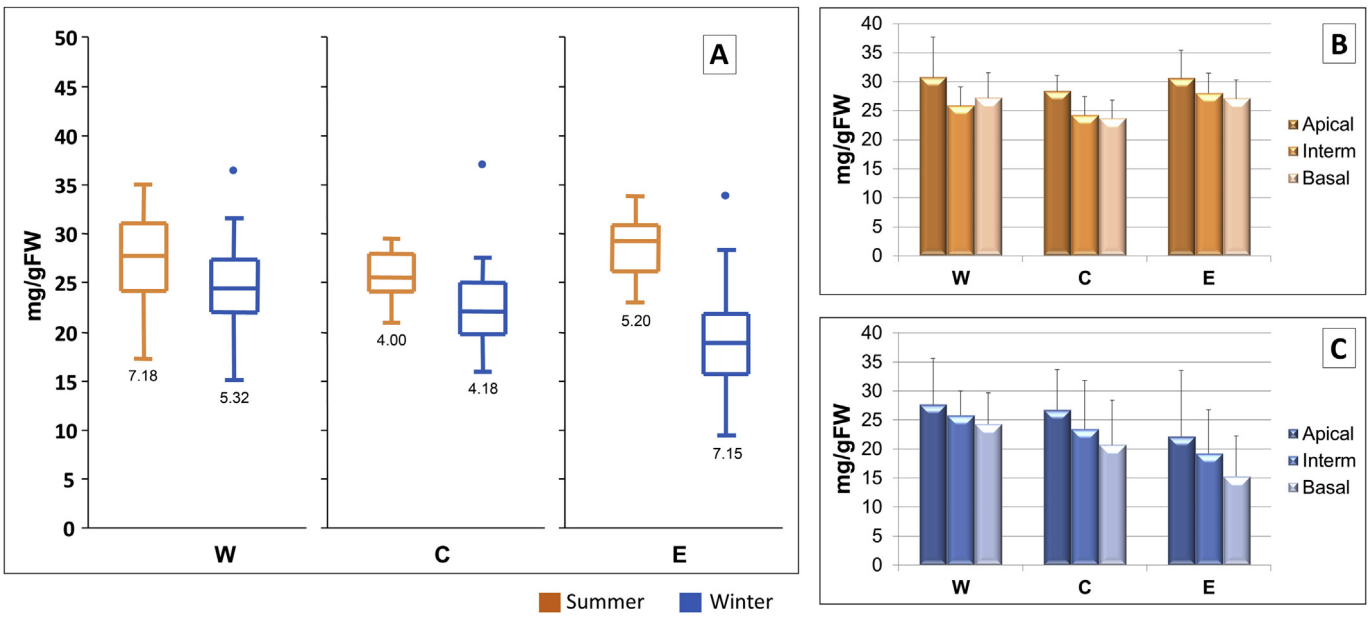

Fig. 3. Total phenol content in *Posidonia oceanica* rhizome collected in summer 2010 and winter 2011 in W, C and E sampling stations (W = Western, C = Central, E = Eastern). Total phenol concentrations in the entire rhizome (A) represented as box-plots: the box contains 50% data (the extremes of that box are the Q1 and Q3, 1st and 3rd quartiles), the internal horizontal segments represent median of the distributions (Q2 value, 2nd 513 quartile), "whiskers" range from the lowest to the highest value; under each box-plot the interquartile range value is reported (IQR = Q3 − Q1). Mean total phenol concentrations (B, C) in the apical, intermediate and basal rhizome sections; error bars represent standard deviation.

Table 2
Soluble protein content in *Posidonia oceanica* leaves (mean ± S.D.) collected in summer 2010 and winter 2011, in W, C and E sampling stations (W = Western, C = Central, E = Eastern).

	Soluble protein content (mg/g DW)		
	W	C	E
Summer	19.32 ± 5.42	23.27 ± 4.24	20.71 ± 3.81
Winter	10.36 ± 2.89	10.461 ± 2.81	7.96 ± 1.65

Table 3
Amplification range generated, and polymorphism detected, by RAPD markers in *Posidonia oceanica* plants collected in summer 2010, in W, C and E sampling stations (W = Western, C = Central, E = Eastern).

Primer	Amplification range (kb)	Polymorphism (%)		
		E	C	W
BY11	0.50–1.9	33.33	81.82	72.73
BY12	0.45–3.8	58.33	40.00	70.00
BY13	0.50–3.5	54.55	45.45	0.00
BY15	0.55–3.2	33.33	78.57	53.85
UB24	0.50–2.2	9.09	62.50	73.33
UB26	0.45–2.1	33.33	46.15	9.09
UB28	0.50–2.5	27.27	80.00	50.00
DN4	0.40–3.5	36.36	45.45	36.36
DN5	0.30–2.7	33.33	66.67	0.00
DN6	0.20–1.7	37.50	70.00	42.86

them consistently detected the seasonal variability and the ecological status of the meadow.

4.1. Traditional descriptors

According to the classification proposed by Pergent et al. (1995) and modified by Buia et al. (2004), the meadow should be considered a "disturbed bed" as the mean shoot density is low, although high variability, particularly in the summer, was observed. Meadow leaf biometrics varied neither among stations nor between seasons, with the exception of leaf length which changed with the seasons. As a consequence, the synthetic descriptor "leaf area per shoot" captured the longer leaf lengths in the spring/summer. There was also a significant increase in leaf area per shoot in the summer at station C, confirming the suitability of this descriptor for short-term analyses (Peirano et al., 2011).

4.2. Biochemical descriptor: total phenol content

The total phenol content within the rhizome showed a gradient of decreasing concentration from the apical to the basal section, as already recorded in plants from other meadows (Fresi et al., 2004; Migliore et al., 2007; Rotini et al., 2011). When it was evaluated in the rhizome as a whole, the mean total phenol content showed significant seasonal variations, with higher concentrations in summer than in winter. The seasonal difference in phenol content is novel information; it could be ascribed to increased plant growth in summer, which implies the synthesis of structural phenols contributing to the building of new cells (Vanholme et al., 2010). Nevertheless, rhizomes have quite a long lifespan and, compared to leaves, undergo minor fluctuations in common physiological processes, including the synthesis and accumulation of phenolic compounds (Migliore et al., 2007). Hence, the significant phenol content increase can be potentially ascribed to disturbance events affecting the meadow in summer. The relationship between phenol content and disturbance has been previously observed in several meadows exposed to different environmental pressures, e.g., turbidity and pollution (Migliore et al., 2007; Rotini et al., 2011); ocean acidification (Arnold et al., 2012); competition with invasive seaweed (Pergent et al., 2008); metal contamination (Ferrat et al., 2003); infection by *Labyrinthula* (Vergeer and Develi, 1997). Furthermore, the punctual response of this descriptor to stress conditions supports its feasibility as an early warning indicator. In addition, phenols are known to be toxic to nematodes (Badra et al., 1979; Tominaga et al., 2003) and due to their rapid response to environmental variations nematodes are considered a good bioindicator (Giovannetti et al., 2010; Losi et al., 2012). Therefore, phenols might be a good alternative to examining the nematode

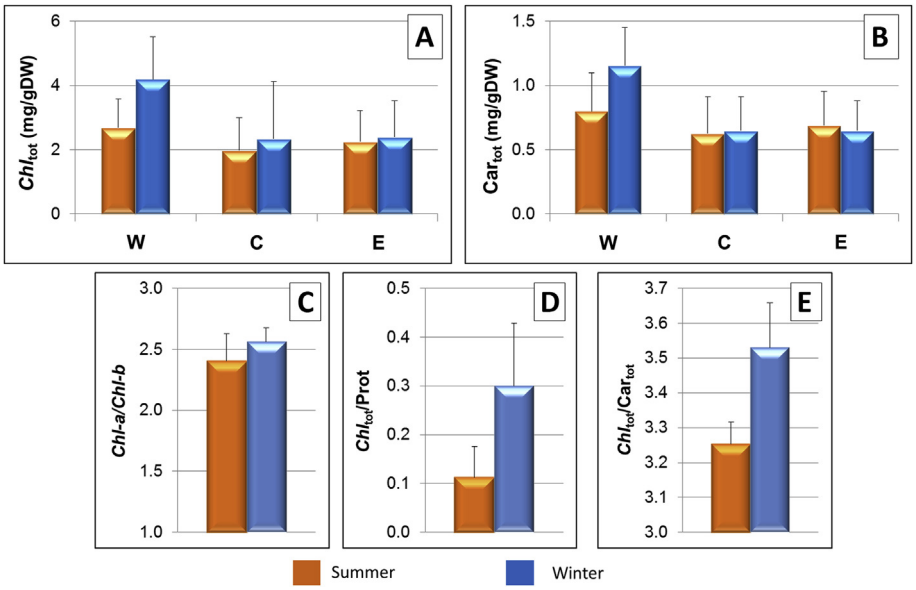

Fig. 4. Photosynthetic pigment concentrations in *Posidonia oceanica* leaves collected in summer 2010 and winter 2011 in W, C and E sampling stations (W = Western, C = Central, E = Eastern). Mean total chlorophyll (C_{tot}, A) and total carotenoid (Car_{tot}, B) concentrations; mean values of ratios *Chl-a*/*Chl-b* (C), C_{tot}/Pr (D) and Chl_{tot}/Car_{tot} (E); error bars represent standard deviation (*Chl-a* = chlorophyll *a*; *Chl-b* = chlorophyll *b*; Chl_{tot} = total chlorophyll; Car_{tot} = total carotenoid; Pr = soluble proteins).

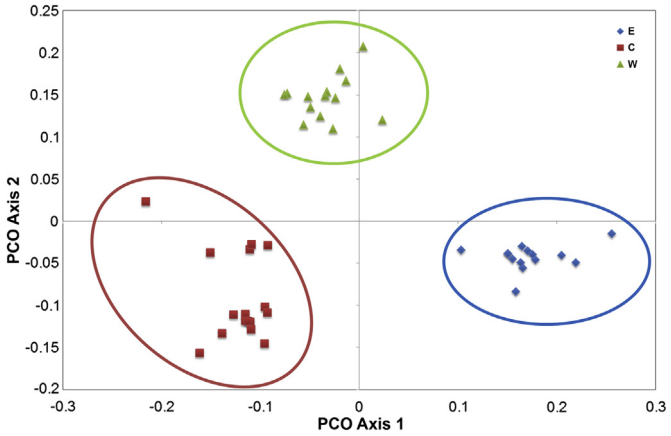

Fig. 5. Principal Coordinates analysis of genetic distances among *Posidonia oceanica* shoots collected in summer in W, C and E sampling stations (W = Western, C = Central, E = Eastern) in the Monterosso meadow. Principal Coordinates (PCO) Axis 1 and 2 account for 24.8% and 17.8% of the variation, respectively.

community especially in areas of limited taxonomic expertise. However, more work needs to be done correlating the increases in phenols that we observed with changes in nematode communities to ensure interchangeability.

4.3. Biochemical descriptors: chlorophylls, carotenoids and proteins

Plant chlorophyll (*Chl-a* and *Chl-b*) and carotenoid contents are lower in winter due to the reduction of light intensity, with an increased *Chl-a*/*Chl-b* ratio from summer to winter. An increase in *Chl-a* relative to *Chl-b* is commonly related to an increased excitation energy transfer capacity compared to the capacity to capture light (Lichtenthaler and Babani, 2004). The total content of foliar soluble proteins is also lower in winter. The low values can be related to the lower levels of Rubisco, which accounts for 50% of total leaf soluble proteins (Parry et al., 2003), and other Calvin Cycle enzymes (Bjorkman, 1981); these enzymes are involved in the down-regulation of the Calvin cycle in response to the lower availability of light during winter. A higher chlorophyll *a*/*b* ratio a lower total chlorophyll to carotenoid ratio (Lichtenthaler and Babani, 2004) and a lower total chlorophyll to soluble protein ratio (Bjorkman, 1981) are common responses to higher light intensities. We found a lower Chl_{tot}/Car_{tot} ratio during summer that was synergistically due to both lower total chlorophyll and lower total carotenoid content during summer. The lower chlorophyll content might be a consequence of its degradation which can be related to temperature and light stress, common during summer.

4.4. Genetic descriptors: RAPD markers

RAPD genetic polymorphisms accounted for differences among the specimens from each station. PCoA, applied to genetic distances among individual shoots, produced a clear clustering in three groups, each including samples from the same station. The distribution of the first two principal coordinates highlighted the highest variability found in station C, where the percentage of polymorphisms was also the highest. This genetic difference among the stations was previously reported by Micheli et al. (2012) and ascribed to external genetic input due to *Posidonia oceanica* fruits being carried ashore by the Corsica currents (Aliani et al., 2006; Micheli et al., 2010).

Biochemical/genetic descriptors and those obtained by traditional methods depicted healthy plants, i.e. a well conserved meadow, showing seasonal variation, with a higher stress level during summer. Higher anthropogenic pressure during summer can be conceived because of Monterosso al Mare is a famous tourist destination. Furthermore, all the descriptors coherently highlighted a difference between station C and stations W and E, particularly in summer. The healthier status of plants from station C is in agreement with the physical and sedimentological features of the station, where the action of the currents determines a coarse-grained sandy substratum and lower turbidity, the most favourable conditions for *Posidonia oceanica* growth (Góngora Gonzáles et al., 1996; Cavazza et al., 2000).

The suite of descriptors utilized in this study provided evidence of their effectiveness and convenient application as markers of the status of *Posidonia oceanica* and/or other seagrasses. Each of the descriptors utilized in this study is able to depict the physiological state of the plant at a given time and under a specific environmental condition. Biochemical descriptors (phenols, chlorophylls, carotenoids and proteins) are dynamic entities varying in the organism, as they are modulated by external and internal environmental changes. Some of them (as phenols) have reached a higher level of maturity while others have to be tested further, on different meadows, to determine their capability to describe the physiological status of the plants. However, the capability of all these descriptors to disentangle seasonal and/or spatial differences has been highlighted by our results. Hence, if the analysed parameters change with the stressful conditions posed by nature, then they might be good indicators of other stressful conditions, including anthropogenic ones. A different approach is the use of genetic descriptor (RAPD markers) to depict the conservation status of the meadow. Seagrass ecosystems are able to adapt and reshape themselves through their reservoir of genetic diversity (Procaccini et al., 2007). The genetic descriptor highlights the result of different environmental pressure experienced by the plants in the past: high genetic variability is maintained by outbreeding but also by good environmental conditions, and by the absence of directional selection. The extent of genetic variability is involved in the ability of populations to face stress.

Biochemical and genetic approaches might be usefully introduced in seagrass monitoring programs since they represent effective indicators of plant physiological stress and environmental disturbance. Although they require a somewhat higher level of laboratory competence than the traditional biometric analyses of shoots, the technical needs can be easily fulfilled by the laboratories in charge of the environmental monitoring activities. Furthermore, due to the low amount of biological material necessary for each analysis, it is possible to measure all the descriptors in a single shoot, achieving a double result: (1) to get a picture of the health status of each individual plant, thus improving our understanding of stress-response processes in seagrasses; and (2) to reduce the impact of “destructive” indicators. The use of these descriptors can also reduce the costly and timely consuming activities of diving surveys. According to the variability of the disturbance (e.g. nutrient input, decrease of water transparency, modified hydrodynamics, type of pollution), a proper combination of descriptors can be used. As already stated (Rotini et al., 2011), the combination of descriptors can be utilized under the framework of the epidemiological approach, i.e. the independent use of various lines of evidence, validated by the weight of the evidence (Chapman et al., 2002; Adams, 2003). This epidemiological approach has been successfully applied in estuarine ecosystems to evaluate whether the changes produced in a community structure were due to environmental pressure or natural variability (Sanz-Lázaro and Marin, 2009; Benedetti et al., 2012).

5. Conclusions

The increasing rate of human-induced environmental change in coastal ecosystems has created a demand for effective descriptors,

in particular for those suitable for monitoring the status of seagrass meadows. Biomonitoring is an expensive and time-consuming activity, and the appropriate choice of indicators is crucial. This work represents a contribution to the complex and relevant issue of *Posidonia oceanica* monitoring and opens the door to examining the useful application of the same descriptors to other seagrass species.

Authors' contributions

AR, LM and CM conceived and designed the study. AP designed the sampling strategy, performed sampling and biometrical analyses. AR performed biochemical analyses, both in Tor Vergata and in the Algae Group (Faro, University of Algarve – Gambelas Campus). AB and CM did the genetic analyses. JS, IB and RS set up the methods for pigment and protein analyses, respectively; LM and AR analysed data and produced graphics and figures. LM, AR, CM and IB wrote the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2013.06.015.

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