Descriptors from *Posidonia oceanica* (L.) Delile meadows in coastal waters of Valencia, Spain, in the context of the EU Water Framework Directive

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~~We~~ Evaluations are provided of~~evaluate~~ 21 descriptors of *Posidonia oceanica* meadows along the coast of Valencia, ~~(~~Spain~~)~~, with a view to~~f~~ using these in implementing the European Water Framework Directive (WFD). The descriptors selected were known to respond to a variety of anthropogenic disturbances. Data ~~have been~~were collected at 17 locations during three consecutive years. A principal components analysis was used to classify the ecological status of each locality according to five classes as prescribed by the WFD. To identify the descriptors that contributed most to similarity among localities within each class and to dissimilarity between adjacent classes, a similarity percentage analysis was performed. We also correlated the descriptors with an independent set of indicators for various types of anthropogenic pressures on the water bodies associated with the different localities. The descriptors providing the most consistent information ~~in terms of~~on status as well as ~~showi~~demonstrating a significant relationship with estimated pressures were: shoot density, shoot foliar surface, dead-matte cover, meadow cover, herbivore pressure, rhizome baring/burial, foliar necrosis, percent of plagiotropic rhizomes and leaf-epiphyte biomass.

Key-words: Biological quality element, descriptors, *Posidonia oceanica*, seagras~~se~~s, Water Framework Directive.

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Introduction

The Water Framework Directive (WFD) establishes a comprehensive policy oriented towards the monitoring and protection of the ecological status of surface waters and groundwater within the European Union (EC, 2000), including marine coastal waters. Its main objective is to reach at least a "good ecological status" for all surface water bodies by 2015. The WFD also prescribes that the status of each water body is assessed based on biological, hydromorphological and physico-chemical quality elements. Biological quality elements (BQEs) are defined as organisms or groups of organisms that are sensitive to disturbances, including phytoplankton, macrophytes, benthic invertebrate fauna and transitional fish fauna.

The application of the WFD in coastal waters has resulted in the development of new methodologies focused mainly on invertebrates of soft-bottom benthos (Simboura *et al*., 2005; Dauvin and Ruellet, 2007), macroalgal communities (Ballesteros *et al*., 2007; Wilkinson *et al*., 2007), and seagrasses (Krause-Jensen *et al*., 2005; Romero *et al*., 2007).

Seagrass meadows represent an important ecosystem that is sensitive to changes in environmental quality (Short and Wyllie-Echeverria, 1996; Hemminga and Duarte, 2000). In the Mediterranean Sea, the dominant seagrass is *Posidonia oceanica* (L.) Delile, an endemic species that may form extensive meadows down to 40 m. These meadows constitute one of the most productive and valuable ecosystems in the Mediterranean (Jeudy De Grissac, 1979; Boudouresque and Meinesz, 1982). Despite being listed as a protected species in the Habitats Directive (EC, 1992), *P. oceanica* habitats are experiencing a widespread decline (Marbà *et al*., 1996). These losses are commonly attributed to human activities such as bottom-trawl fishing, coastal constructions, beach replenishment, fish farming and desalination plants (Sánchez-Lizaso *et al*., 1990; Delgado *et al*., 1999; Ruiz and Romero, 2003; Fernández-Torquemada *et al*., 2005; González-Correa *et al*., 2008). Given its broad distribution throughout the Mediterranean Sea and its sensitivity, *P. oceanica* might be used as an appropriate bioindicator (Pergent-Martini and Pergent, 2000) and has been proposed as one of the BQEs for coastal waters in the area (Casazza *et al*., 2006).

Descriptors that have been employed most often in research and monitoring programmes include shoot density, meadow and dead-matte cover, meadow limits, epiphytic coverage, leaf biometry, shoot balance and total non-structural carbohydrates content in rhizomes (Alcoverro *et al*., 2001; Krause-Jensen *et al*., 2004; Pergent-Martini *et al*., 2005; González-Correa *et al*., 2008). Our main objective is to evaluate some of those potential descriptors with a view of selecting appropriate indicators from the *Posidonia* ecosystem for application in implementing the WFD.

Material and methods

Study area and sampling design

The study was conducted along the coast of Valencia (Spain) during September-October in 2005, 2006 and 2007. Sampling was done by SCUBA divers at 17 locations (Figure 1) in the depth range of 14-17 m, because meadows at these depths are usually not affected by natural alterations, such as caused by waves or storms (Krause-Jensen *et al*., 2004). The locations were selected based on existing knowledge of the status of their respective *Posidonia* meadows. At each locality, three sampling sites separated by hundreds of meters were randomly selected to prevent spatial pseudo-replication. At each site, three 40x40 cm quadrats were randomly selected to measure shoot density, percentage of plagiotropic rhizomes and rhizome baring. Alive and dead *Posidonia* cover was estimated as the proportion of alive and dead patches on three replicate 20 m transects. In addition, ten shoots were harvested at random and transported to the laboratory for further analysis (Table 1).

Analysis of data and metric selection

As a first selection of potentially useful descriptors based on the existing literature (Pergent-Martini *et al*., 2005; Romero *et al*., 2007), we have chosen 21 metrics known to respond to a variety of anthropogenic disturbances and that appear to contribute most information on meadow quality (Table 1). We performed a univariate analysis of variance (ANOVA; Underwood, 1997) to estimate the variability for each descriptor and to test whether differences in the mean values of the various descriptors varied significantly among locations. The linear model for this analysis was defined as:

$X_{ijk} = \mu + L_i + s_{j(i)} + \varepsilon_{ijk}$ $i=1,\ldots,17,\ j=1,\ldots,3,\ k=1,\ldots,3$

where μ is the overall mean, L_i is a fixed effect for location *i*, $s_{j(i)}$ is a random effect for site *j* within location *i* and $\varepsilon_{ijk)}$ is the residual error for the *k*th observation of site *j* within location *i*.

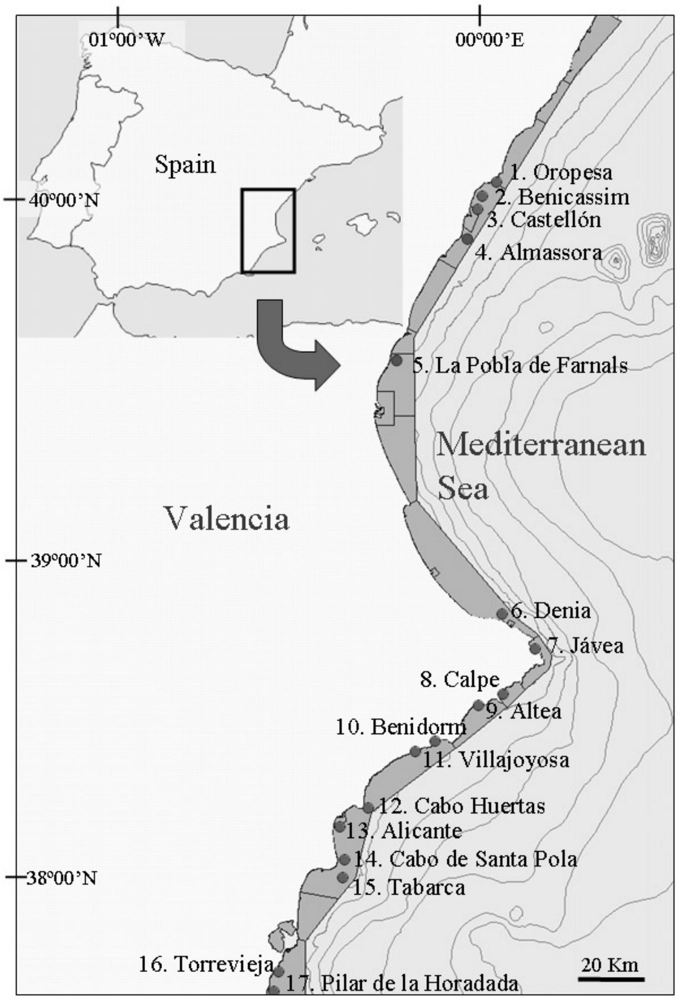

Figure 1. Map of the coast of Valencia (East Spain) with the 17 sampling locations indicated as well as the water bodies distinguished.

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Furthermore, we composed two virtual sites, one with the best values observed for all individual metrics (highest values for "positive" metrics and lowest for the "negative" ones) to serve as a reference condition and one with the worst values. These were used together with the data for the 17 localities to carry out a principal component analysis (PCA). The first component of this PCA was translated into an ecological quality ratio (EQR) on a scale 0 to 1 by dividing it by the component value of the reference condition. ~~as defined by the WFD~~. Subsequently, we applied the class boundaries established in the EU intercalibration meetings (MedGIG, 2007) to these EQR values to classify the current ecological status of the *Posidonia* meadows at each site according to five classes (high, good, moderate, poor and bad). To elucidate the contribution of each metric to the similarity among localities within a class and to the dissimilarity between adjacent classes, the SIMPER (similarity percentage analysis) routine was used (PRIMER-E software, Plymouth, UK).

Table 1. Summary of the *P. oceanica* descriptors evaluated and methods employed for measurements in the field (F) or in the laboratory (L).

Metric (and abbreviation)	Method and units
Herbivore pressure (Herb)	L: percentage of leaves with herbivore marks per shoot
Leaf epiphyte biomass (Epi)	L: dry weight after removal from leaves (mg cm^{-2})
Caulerpa racemosa cover (Cr)	F: % cover of this invasive macroalga on three 20 m transects
Meadow cover (Cover)	F: % alive patches on three 20 m transects
Dead matte cover (Dead M)	F: % dead patches on three 20 m transects
Shoot density (Shoot dens)	F: alive shoots in three random 40x40 cm quadrats (n m^{-2})
Plagiotropic rhizomes (Pl rhi)	F: % in three random 40x40 cm quadrats
Rhizome baring/burial (Rhi b)	F: length from sediment to leaf ligula (cm)
Shoot biomass (Shoot B)	L: dry weight of leaves without epiphytes (g $shoot^{-1}$)
Shoot foliar surface (Shoot FS)	L: surface area of leaves (cm^2 $shoot^{-1}$)
Number of leaves (N^{er} leav)	L: count (n $shoot^{-1}$)
Maximum leaf length (Leaf L)	L: per shoot (cm)
Leaf width (Leaf W)	L: average per shoot (mm)
Foliar necrosis (Necros)	L: % leaves with necrosis marks per shoot
Vertical rhizome elongation (Rhi el)	L: growth of vertical rhizomes* (mm yr^{-1} $shoot^{-1}$)
Rhizome production (Rhi P)	L: dry weight of vertical rhizomes* (g yr^{-1} $shoot^{-1}$)
Leaf production (Leaf P)	L: number produced* (n yr^{-1} $shoot^{-1}$)
Sheath length (She L)	L: average per year* (mm)
Starch content (Starch)	L: from rhizome dry tissue** (mg g^{-1} DW)
Sucrose content (Sucrose)	L: from rhizome dry tissue** (mg g^{-1} DW)
Total non-structural carbohydrate (TNC)	L: from ground rhizome dry tissue** (mg g^{-1} DW)

* estimated by lepidochronology (Pergent, 1990)
** extracted in 80ºC ethanol and analysed following Alcoverro *et al.* (1999).

We also correlated estimates of the six anthropogenic pressures that were considered most relevant for *Posidonia* meadows (i.e. coastal construction, beach regeneration, urban sewage, industrial sewage, pollution from rivers and channels, and pollution from agricultural soil use; Table 2) with the mean value of each descriptor for the ten water bodies distinguished (Figure 1). Based on all results, the nine most promising descriptors to evaluate anthropogenic impacts were selected for re-running the PCA and the classification in WFD categories.

Table 2. Data on the main anthropogenic pressures per kilometre coastline by water body (WB; cf. Figure 1) as derived from Agència Catalana de l'Aigua (2006).

	Location	Coastal constructions (km artificial coastline)	Beach regeneration (m^3 sand added)	Urban sewage (kg COD d^{-1})	Industrial sewage (kg COD d^{-1})	Rivers and channels (kg BOD5 d^{-1})	Agricultural soil use (ha x mm precipitation yr^{-1})
WB 1	1	0.04	0.3	11.0	0.0	11.1	52.4
WB 2	2-4	0.33	34.5	93.0	144.2	125.7	49.8
WB 3	6	0.03	0.3	16.0	11.0	20.8	30.7
WB 4	7	0.01	0.1	3.5	0.0	3.7	12.6
WB 5	8	0.04	1.1	47.5	0.0	2.7	10.9
WB 6	9	0.10	5.6	41.6	0.0	37.8	9.5
WB 7	10-12	0.17	20.4	35.6	0.0	0.0	5.2
WB 8	13	0.49	90.5	399.9	47.0	0.9	0.8
WB 9	14-15	0.13	14.0	2.0	11.6	2.6	7.4
WB 10	16-17	0.14	8.0	0.5	0.0	3.6	12.8

Results

The results of the ANOVA showed that all descriptors evaluated differed significantly among locations, suggesting that all were potentially useful and none could be rejected *a priori*. Therefore, all descriptors were used in a PCA (Figure 2a), the first two axes of which (PC1 and PC2) explained 69% and 12% of the variance, respectively. Results from this PCA were also applied to obtain the classification of the 17 locations in terms of five status classes (high, good, moderate, poor and bad) based on the EQR derived from the PC1, where the intermediate class boundaries are somewhat arbitrary (Figure 2b).

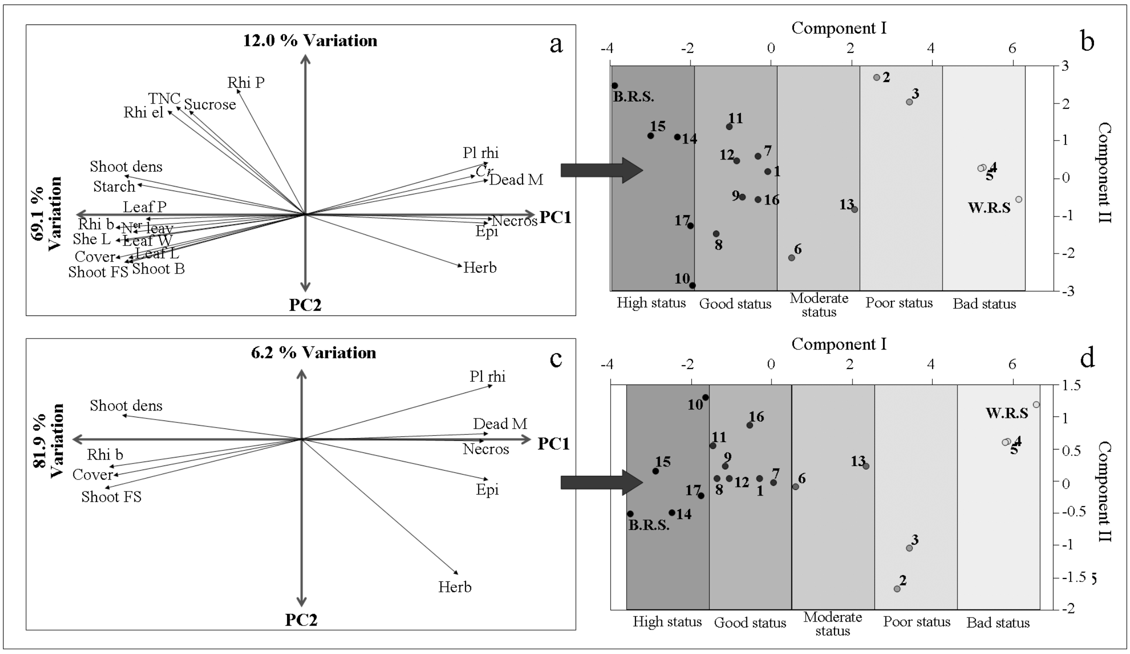

Figure 2. Results of the Principal Components Analysis applied (a) to all *Posidonia* descriptors evaluated (for abbreviations see Table 1) and (c) to a selected group of nine descriptors, and (b and d, respectively) the associated PCA ordination diagrams of the localities studied (for locality numbers see Figure 1; B.R.S.: Best Reference Site, W.R.S.: Worst Reference Site).

The similarity percentage analysis showed that the descriptors that contributed more than 5% on average to similarity among localities within each status class were shoot density, shoot foliar surface, herbivore pressure, meadow cover, and maximum leaf length (Table 3a). The metrics that contributed more than 5% on average to dissimilarity were shoot density, dead-matte cover, meadow cover, shoot foliar surface, and *C. racemosa* cover (Table 3b).

Table 4 shows all correlations found between descriptors and estimates of the six selected anthropogenic pressures. High positive correlations were found between dead-matte cover and coastal constructions and between proportion of plagiotropic rhizomes and industrial sewage; a high negative correlation occurred between meadow cover and industrial sewage.

Table 3. Analysis of (a) similarity within WFD classes and (b) dissimilarity between neighbouring WFD classes for individual *P. oceanica* descriptors (cf. Table 1) by year.

a. Similarity

Within WFD class	High			Good			Moderate			Poor		Weighted
Year	2005	2006	2007	2005	2006	2007	2005	2006	2007	2005	2006	average
Shoot dens	25.4	20.1	26.3	26.2	18.7	24.1	18.2	13.7	17.1	18.5	12.1	24.2
Shoot FS	21.1	13.2	19.7	20.9	12.4	18.9	19.3	9.3	15.1	18.0	8.1	19.4
Herb		7.2	10.9	4.3	7.9	12.3	4.3	8.9	13.0	4.3	8.0	8.9
Cover	15.7	10.2	14.0	13.4	9.8	13.7	12.7	2.9	7.2			8.6
Leaf L	13.0	7.5	11.8	13.4	7.2		10.3	6.0	9.8	11.1		8.1
Dead M							11.7	6.9	11.0	15.7	11.1	2.8
Necros				5.0	3.7			7.3	8.2		9.0	1.7
TNC		10.1			9.5			9.0			8.0	1.5
Rhi b	4.8	3.5	4.3	3.6	2.6	3.4						1.3
Starch		8.3			7.6				7.7		6.3	1.2
N^{er} leav	3.3		3.4			3.5	5.7	2.7	3.6	3.8		0.8
Sucrose		6.0			5.5				4.8		4.2	0.8
Cr								5.3		10.4	11.1	0.8
Pla rhi								3.8	5.7		5.5	0.5

b. Dissimilarity

Between WFD classes	High ↔ Good			Good ↔ Moderate			Moderate ↔ Poor			Weighted
Year	2005	2006	2007	2005	2006	2007	2005	2006	2007	average
Shoot dens	18.8	15.3	22.2	29.4	14.4	13.3	7.9	6.5	11.9	12.6
Dead M	13.4	8.3	11.3	24.6	14.9	18.5	10.7	12.5	10.0	11.2
Cover	17.7	3.5	5.8	7.6	14.1	13.9	16.5	12.0	18.0	9.8
Shoot FS	10.8	8.0	17.1	9.8	8.9	10.5	9.3	5.9		6.4
Cr					12.9	15.6	32.3	20.2	20.3	5.1
Leaf L	3.3	4.0	8.1	8.5	4.2		6.9			2.4
Rhi b	7.1	4.5	6.6		3.2			2.8	5.0	1.8
Pla rhi		4.6	7.0		5.3	7.5			4.7	1.5
Herb	19.5			9.0			6.7	6.2	8.2	1.5
Necros			13.3			10.8			14.4	1.2
TNC		10.1			4.5			5.4		0.7
Sucrose		14.4			4.6			5.8		0.6
Starch		4.1								0.04
Epi				3.5						0.03

Only those contributing up to 90 % in any class or year are indicated; descriptors are ranked according to their weighted average contribution.

Based on these analyses, we selected nine descriptors that were significantly correlated with different human pressures and that also contributed most to the (dis-)similarity in the classification of localities. Number of leaves was left out because many other descriptors were also significantly correlated with the same pressures while it did not contribute much to the classification. Although not contributing much either, epiphyte biomass was retained, because it was one of the few to be correlated with river discharges. The final selection included: shoot density, shoot foliar surface, dead-matte cover, meadow cover, herbivore pressure, rhizome baring/burial, necrosis, plagiotropic rhizomes and epiphyte biomass. We then repeated the principal component analysis with these nine metrics (Figure 2c) to investigate whether the meadow classification remained stable (Figure 2d). This appeared to be largely the case. Although some differences can be observed in the distribution of the localities on the second axis (Figure 2d), the PC2 only explained 6% of variance and did not seem to be related with the status of *Posidonia* meadows. Furthermore, the PC1 explained a larger part of the variance (82% *versus* 69%), suggesting that this selection of descriptors provided a better discrimination tool for WFD quality classes than the original list.

Table 4. Correlation coefficients (*: p<0.05; **: p<0.01; ***: p<0.001) between average values of *P. oceanica* descriptors (cf. Table 1) per water body (only those showing at least one significant correlation are included) and the six components of anthropogenic pressure selected (n=10).

	Coastal constructions	Beach regeneration	Urban sewage	Industrial sewage	Rivers and channels	Agricultural soil use
Dead M	0.92 ***	0.86 **	0.80 **	0.83 **	0.54	0.20
Cover	- 0.69 *	- 0.62 *	- 0.57	- 0.89 ***	- 0.69 *	- 0.44
Necros	0.76 *	0.80 **	0.84 **	0.61 *	0.32	0.17
Shoot FS	- 0.65 *	- 0.62 *	- 0.59	- 0.70 *	- 0.45	- 0.39 *
Shoot dens	- 0.64 *	- 0.67 *	- 0.75 *	- 0.53	- 0.39	- 0.20
Pla rhi	0.56	0.43	0.36	0.96 ***	0.87 **	0.56
Epi	0.24	0.17	0.22	0.61 *	0.67 *	0.39
Herb	0.60	0.66 *	0.68 *	0.22	0.05	- 0.02
Rhi b	- 0.48	- 0.48	- 0.55	- 0.62 *	- 0.49	- 0.46
N^er leav	- 0.74 *	- 0.66 *	- 0.57	- 0.69 *	- 0.38	- 0.33

Discussion

In evaluating the 21 *Posidonia* descriptors, ANOVA, PCA, similarity analysis and correlations with anthropogenic pressures have been employed to select the most appropriate ones for implementation of the WFD. The final suite includes metrics relating to the community (herbivore pressure and epiphyte biomass), the population (shoot density, proportion of plagiotropic rhizomes, dead matte cover and meadow cover) and to individual plants (rhizome baring/burial, shoot foliar surface, and foliar necrosis). Meadow cover, shoot foliar surface and shoot density were negatively correlated with most of these pressures.

For a selection of metrics to be applied in future monitoring programmes, cost-effectiveness is an important issue and therefore they should be easily measured and applied. Although some descriptors require that samples are analyzed in the laboratory (e.g. shoot foliar surface, foliar necrosis and epiphyte biomass), these metrics can be rapidly collected at relatively low costs. More complex and time-consuming analyses that could in some cases be subject to analytical errors might be rejected on the basis of not providing much additional information (Krause-Jensen *et al.*, 2004).

Sampling design may be a critical issue for the validity of the results obtained. Because some descriptors (such as meadow cover or shoot density) may show a high variability at small or medium spatial scales (Panayotidis *et al.*, 1981; Balestri *et al.*, 2003), we recommend utilizing a nested sampling design with an adequate spatial replication. Furthermore, seasonal variation at the community, population and individual plant level must be taken into account. Descriptors such as shoot foliar surface and epiphyte biomass should be sampled during a fixed period of the year to avoid any confounding effect of seasonality.

We emphasize that *P. oceanica* is a species with slow growth rates compared to other seagrass species (Bay, 1984; Ruiz and Romero, 2003). Thus, the selection of indicators provided here may be unsuitable for other species. The selection may also have to be adapted to regional conditions. However, it seems important to use a reasonable range of metrics to determine the ecological status of a water body based on seagrass. While using less descriptors could lead to erroneous classifications, using too many would be costly in terms of time and money. Because the WFD allows for a revision of the BQE classification methodologies every reporting cycle of 6 years, the data collection for *Posidonia* meadows will be continued.

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Table 1. Summary of the *P. oceanica* descriptors evaluated and methods employed for measurements in the field (F) or in the laboratory (L).

Metric (and abbreviation)	Method and units
Herbivore pressure (Herb)	L: percentage of leaves with herbivore marks per shoot
Leaf epiphyte biomass (Epi)	L: dry weight after removal from leaves (mg cm^{-2})
Caulerpa racemosa cover (Cr)	F: % cover of this invasive macroalga on three 20 m transects
Meadow cover (Cover)	F: % alive patches on three 20 m transects
Dead matte cover (Dead M)	F: % dead patches on three 20 m transects
Shoot density (Shoot dens)	F: alive shoots in three random 40x40 cm quadrats (n m^{-2})
Plagiotropic rhizomes (Pl rhi)	F: % in three random 40x40 cm quadrats
Rhizome baring/burial (Rhi b)	F: length from sediment to leaf ligula (cm)
Shoot biomass (Shoot B)	L: dry weight of leaves without epiphytes (g $shoot^{-1}$)
Shoot foliar surface (Shoot FS)	L: surface area of leaves (cm^2 $shoot^{-1}$)
Number of leaves (N^{er} leav)	L: count (n $shoot^{-1}$)
Maximum leaf length (Leaf L)	L: per shoot (cm)
Leaf width (Leaf W)	L: average per shoot (mm)
Foliar necrosis (Necros)	L: % leaves with necrosis marks per shoot
Vertical rhizome elongation (Rhi el)	L: growth of vertical rhizomes* (mm yr^{-1} $shoot^{-1}$)
Rhizome production (Rhi P)	L: dry weight of vertical rhizomes* (g yr^{-1} $shoot^{-1}$)
Leaf production (Leaf P)	L: number produced* (n yr^{-1} $shoot^{-1}$)

Sheath length (She L)	L: average per year* (mm)
Starch content (Starch)	L: from rhizome dry tissue** (mg g^{-1} DW)
Sucrose content (Sucrose)	L: from rhizome dry tissue** (mg g^{-1} DW)
Total non-structural carbohydrate (TNC)	L: from ground rhizome dry tissue** (mg g^{-1} DW)

* estimated by lepidochronology (Pergent, 1990)
** extracted in 80°C ethanol and analysed following Alcoverro *et al*. (1999).