

Variation of structural and functional metrics in macrophyte communities within two habitats of eastern Mediterranean coastal lagoons: natural versus human effects

S. ORFANIDIS^{a,*}, M. PINNA^b, L. SABETTA^b, N. STAMATIS^a and K. NAKOU^a

^a National Agricultural Research Foundation, Fisheries Research Institute, 640 07 Nea Peramos, Kavala, Greece

^b Di.S.Te.B.A., Centro Ecotekne, Prov. Lecce-Monteroni 7 University of Salento, 31 00 Lecce, Italy

ABSTRACT

1. The variation of structural (multi-dimensional scaling plot of Bray–Curtis similarity, species number, Shannon–Weaver index, percentage coverage) and functional (Ecological State Group (ESG) I percentage coverage, ESG II percentage coverage, Ecological Evaluation Index (EEI)) metrics in benthic macrophyte communities was studied in two different habitats: (a) mud with submerged angiosperms (MA), and (b) mud with macroalgae (MM), in three eastern Mediterranean coastal lagoons. One lagoon was in northern Greece (Agiasma, Nestos Delta, Eastern Macedonia region) and two in south-eastern Italy (Cesine and Margherita of Savoia, Apulian region).

2. The aim was to differentiate metric variation caused by human or natural processes and thereby to (1) select reliable metrics and (2) develop user-friendly protocols for cost-effective monitoring programmes for coastal lagoon water quality.

3. Eight different sites dominated by macrophyte communities characterized by two angiosperms (*Ruppia cirrhosa* and *R. maritima*), two opportunistic macroalgae (*Ulva* sp. and *Chaetomorpha linum*), and Cyanobacteria colonies were quantitatively and destructively sampled twice.

4. Structural metrics showed highest heterogeneity at a local site-specific scale, while functional metrics showed highest heterogeneity at the scale of habitat. As a result the structural metrics appeared inappropriate as indicators of lagoon water quality changes. By contrast shifts of habitat dominated by angiosperms to opportunistic macroalgae owing to nutrient excess, especially nitrogen, can be identified by functional metrics, especially with EEI.

Copyright © 2008 John Wiley & Sons, Ltd.

Received 30 August 2007; Accepted 4 January 2008

KEY WORDS: benthic macrophytes; nested analysis; diversity indices; Ecological Evaluation Index–EEI; monitoring; WFD

*Correspondence to: Sotiris Orfanidis, National Agricultural Research Foundation, Fisheries Research Institute, 640 07 Nea Peramos, Kavala, Greece. E-mail: sorfanid@otenet.gr

INTRODUCTION

Marine benthic macrophytes (macroalgae, angiosperms) are key structural and functional components of many coastal lagoons, forming extensive (Verhoeven, 1979; Agostini *et al.*, 2003a,b), highly productive (Terrados and Ros, 1992; Rismondo *et al.*, 1997; Sfriso and Ghetti, 1998; Calado and Duarte, 2000; Menéndez, 2002; Agostini *et al.*, 2003c; Malea *et al.*, 2004) and spatio-temporally patchy habitats (Verhoeven, 1979, 1980). They are sensitive to anthropogenic stress (Dennison *et al.*, 1993; Duraco, 1995; Domin *et al.*, 2004; Krause-Jensen *et al.*, 2004), and have recently been incorporated as quality elements in water quality monitoring programmes (for US EPA see Gibson *et al.*, 2000; for WFD see EC, 2000). Habitat includes all aspects of an organism's life history, including how a particular location meets these needs relative to substrate, water quality etc. (Diaz *et al.*, 2004). The TWReferenceNET project adopted a two-level factorial classification of habitats, which includes substratum type (rock-sand-mud) and dominant vegetation (macroalgae, or Cyanobacteria-angiosperms alone or in coexistence with macroalgae, or Cyanobacteria-no vegetation).

Apart from difficulties related to the dynamic nature of ecosystems (see Orfanidis *et al.*, 2008), one of the main difficulties of using benthic macrophytes as bioindicators (*sensu* Doust *et al.*, 1994; Anderson, 1999), quality elements (*sensu* WFD) or state variables in monitoring programmes is the high temporal and spatial variability of communities between and within habitats. A theoretical framework that explains benthic vegetation dynamics with metrics that causally links the variability to natural or human processes is absent. In addition, a quantitative assessment of the composition of benthic macrophyte communities has been less scientifically explored in comparison with zoobenthic communities. This is, however, a prerequisite to analyse marine communities using statistical tools developed by Clarke and Warwick (1994). Other difficulties include long-term periodicity and slow recovery of angiosperms from extreme meteorological (storms) and hydrological (river floods) events, as well as angiosperm diseases, e.g. in *Zostera*, indicating the need for additional parameters, such as water and sediment nutrient concentrations, and light attenuation, to interpret macrophyte data (Gibson *et al.*, 2000; Ponti *et al.*, 2006).

The use of macrophyte community changes to evaluate and diagnose water quality status necessitates an understanding of the underlying causal ecological processes (Duraco, 1995; Rindi and Guiry, 2004). This requires characterization of both the spatial and temporal components of macrophyte community patterns and understanding how patterns and processes may interact (Wiens *et al.*, 1993; Underwood, 1997; Benedetti-Cecchi, 2001). Although natural and human

processes operate at more than one spatial and temporal scale, their effect can be most obvious on one of these scales (Benedetti-Cecchi, 2001; Benedetti-Cecchi *et al.*, 2001; Rindi and Guiry, 2004). Since coastal lagoons are often subdivided into different rather homogenous basins (Tagliapietra and Volpi Ghirardini, 2006) different ecological conditions can be met even in neighbouring basins. Therefore, changes in nutrient or light regimes could affect broad spatial scales or a whole basin of a lagoon, for instance by shifting communities and habitats. A conceptual model of this successional process, showing deterministic or dynamic changes in coastal lagoons is presented in this issue (Viaroli *et al.*, 2008). On the other hand, species diversity, for example, could be affected by local salinity gradients existing within a lagoon basin or habitat (Coutino and Seeliger, 1984; Middelboe *et al.*, 1998).

Biotic metrics, including biotic indices or parameters, represent an effort to describe different and complex aspects of communities or other different biological organizational levels by integrating them in a formula producing a single numerical output (see Orfanidis *et al.*, 2001; Ponti and Abbiati, 2004; Salas *et al.*, 2004; Reizopoulou and Nicolaidou, 2007; for a review see Diaz *et al.*, 2004). Diversity and abundance indices, for example, are abstractions of the highly complex structure of communities that may be useful for comparisons (Thiebaut *et al.*, 2002). This relatively low-cost approach effectively distinguishes responses to human impact from natural variability only when supported by quantitative data for precision and accuracy, recognition of spatial and temporal heterogeneity of communities, stress-response relationships, the contribution of multiple stressors (Niemi and McDonald, 2004; Niemi *et al.*, 2004), predictive modelling (Pykh *et al.*, 2000) and sound ecological theory (Orfanidis, 2007). Development of broadly applicable tools (Niemi *et al.*, 2004) linked to major theoretical frameworks is the only way to enhance interdisciplinarity (Austoni *et al.*, 2007) and integration (Jørgensen, 2006) in environmental management.

Macrophyte biotic indices used to evaluate water quality status in coastal lagoons (Sfriso *et al.*, 2002; Mouillot *et al.*, 2005) are often based on community composition analysis at the species level. However, a more predictive approach might be achieved by using appropriate functional classifications (Orfanidis *et al.*, 2001, 2003; see also Mouillot *et al.*, 2006). Such an approach could reduce the apparent community complexity (Steneck and Walting, 1982; Steneck and Dethier, 1994) allowing comparisons between communities with little species overlap at local, ecoregion or global scales. User-friendly protocols and cost-effective monitoring systems can also be developed. For seaweeds, Littler and Littler (1980) and Steneck and Walting (1982) proposed functional-form groups that are independent of phylogeny. Similarities in surface area/volume ratios (SA/V) can generally predict functions like nutrient uptake (Rosenberg and Ramus, 1984; Duke *et al.*,

1989; Hein *et al.*, 1995; Pedersen and Borum, 1997) or photosynthesis and growth rates (Nielsen and Sand-Jensen, 1990). Since the growth of a species under certain conditions seems to be related to species' competence to exploit the most abundant or limited resources, either through growth or colonization ability (Schramm, 1999; Worm and Karez, 2002), i.e. nutrients, light or space in the case of benthic macrophytes (Carpenter, 1990), such functions are critical to understand and predict macrophyte community changes along a pollution gradient (Pedersen and Borum, 1997). Based on growth (Nielsen and Sand-Jensen, 1990; Duarte, 1995), longevity and canopy traits, Orfanidis *et al.* (2001, 2003) have included angiosperms within this functional-form classification scheme. It is in accordance with r- and K-selection theory (MacArthur and Wilson, 1967), and it was used to classify the benthic macrophytes in two groups that respond differently to environmental disturbance: the late-successional group with low growth rates and long life cycles (Ecological State Group I, K-selection) and the opportunistic group with high growth rates and short life cycles (ESG II, r-selection). All seagrasses and seaweed species with a thick or calcareous thallus are included in the first group, whereas species with a filamentous, sheet-like or coarsely branched thallus and Cyanobacteria are included in the second group. The concepts of r- and K-selection are not absolute and are meaningful only by comparison. Certainly, no organism is completely r-selected or completely K-selected, but all must reach a compromise between the two extremes (r-, K- continuum) (Pianka, 1970). Such a classification scheme, although in some aspects provisional (Orfanidis *et al.*, 2003), seems to overcome failed predictions of the functional-form model of Littler and Littler (1980) in ecophysiological traits of species belonging to closely related functional groups, e.g. filamentous and foliose algae (Lotze and Schramm, 2000; Padilla and Allen, 2000), and combines ecophysiological traits such as nutrient uptake, photosynthesis, growth rates, and grazing resistance with life cycle strategy (r, K selection). The Ecological Evaluation Index (EEI; Orfanidis *et al.*, 2001, 2003) based on this scheme is designed to evaluate shifts in coastal and transitional ecosystems in which ESG I and II species dominate under oligo- or eutrophic conditions, respectively (Kautsky *et al.*, 1986; Schramm and Nienhuis, 1996 and references therein; Schramm, 1999). Taking into account the plant plasticity of ecophysiological traits (Schlichting, 1986), as well as recent progress in competition theory (Sommer and Worm, 2002), such an approach should be regarded as a model that needs to be also site- and species-specifically experimentally verified (Orfanidis *et al.*, 2001, 2003; Orfanidis, 2007).

Patterns of variation in structural (multi-dimensional scaling plot of Bray–Curtis similarity, species number, Shannon–Weaver index, percentage of total coverage) and functional (ESG I percentage coverage, ESG II percentage coverage, and

EEI) metrics on a hierarchy of different scales at two habitats (mud with macroalgae [MM], mud with angiosperms [MA]) were studied in randomly selected eastern Mediterranean coastal lagoons (Agiasma from Greece, and Cesine, Margherita of Savoia from Italy). Regarding ecosystems as hierarchically organized systems (O'Neil, 1988) such a sampling design will contribute to efforts to differentiate metric variation caused by human or natural processes and thereby to (1) select reliably interpretable metrics and (2) develop user-friendly protocols for cost-effective monitoring programmes for coastal lagoon water quality.

MATERIAL AND METHODS

Study area

This study was conducted between November 2004 and August 2005 in three eastern Mediterranean coastal lagoons (Figure 1), one on the north Greek coast (Agiasma, Nestos Delta, Eastern Macedonia region) and two on the south-east Italian coast (Cesine, and Margherita of Savoia, Apulian region).

Agiasma (Figure 1) belongs to the Nestos Delta lagoons (40.55°N, 24.40°E), which is an internationally protected Ramsar and Natura 2000 site (code GR1150010). Intensive agriculture and the construction of new hydroelectric dams in the upper reaches of the Nestos river are the current main threats to the ecosystem. Agiasma is a shallow (mean depth *ca* 1 m) lagoon covering an area of 367 ha, having two narrow outlets to the sea. Extensive fish aquaculture, in which fish immigrants are prevented from returning to the sea by a system of mesh frames (metal grids) and a stationary entrapment system (fish barrier), is the main commercial use of the lagoon. The outlet B remains open only during stocking, from mid-February to May, whereas outlet A is open throughout the year. A longitudinal salinity gradient exists from the outlets to the main freshwater sources in the north-western part of the lagoon, with salinity in the central area ranging between 22 and 29 PSU. Two different areas separated from each other by a metal grid were studied, one dominated by *Ruppia cirrhosa* (Petagna) Grande (habitat MA) and the other dominated by *Ulva* sp. and Cyanobacteria (habitat MM). Mean nutrient concentrations for MA and MM in the water column based on sampling during this study were 5.4 µmol L⁻¹ TDIN (total dissolved inorganic N) and 2.67 µmol L⁻¹ SRP (soluble reactive P) and 5.85 µmol L⁻¹ TDIN, 1.17 µmol L⁻¹ SRP. Agiasma is regarded as one of the less affected of the Nestos Delta lagoons.

Cesine is a shallow (mean depth 80 cm) lagoon in south-east Italy (40.21°N, 18.23°E) covering an area of 620 ha (Figure 1). It is designated as a special protection area and a nature

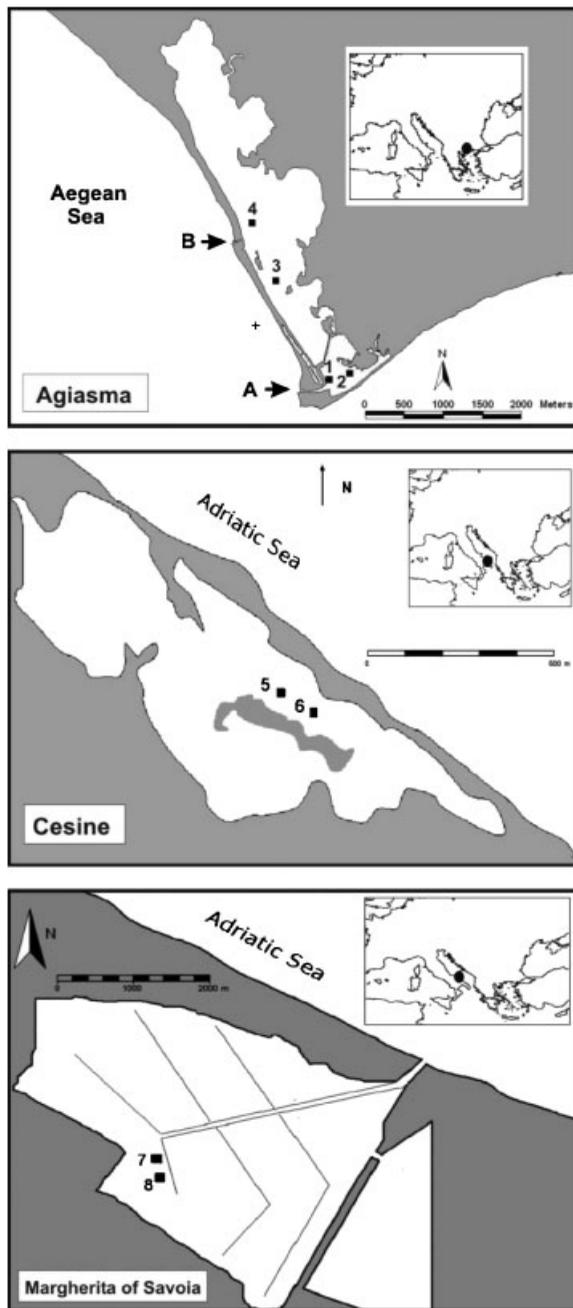


Figure 1. Map of the studied areas. Arrows indicate the outlets of Agiasma lagoon. Sites containing mud with macroalgae and mud with angiosperms habitats in Agiasma lagoon 1, 2 and 3, 4, respectively. Sites containing mud with angiosperms habitats in Cesine lagoon 5, 6. Sites containing mud and macroalgae in Margherita of Savoia lagoon 7, 8.

reserve. The creation of coastal dunes has prevented water from returning to the sea, thereby producing a geohydrographic site among the dunes. Water salinity is determined by the tide and varies between 5 and 15 PSU, except during summer and autumn when salinity can increase to 28–30 PSU because of occasional dune breaks and the input of sea water. Rainfall is the only fresh water source. The lagoon is covered by extensive meadows of *Ruppia maritima* L. (habitat MM). Mean nutrient concentrations in the water column based on sampling during this study were $3.90 \mu\text{mol L}^{-1}$ TDIN and $0.10 \mu\text{mol L}^{-1}$ SRP. Cesine is regarded as one of the most pristine lagoons in the Apulian region.

Margherita of Savoia is the biggest salt marsh area in Italy (Figure 1) located along the Italian southern coast line of the Adriatic Sea (41.24°N ; 16.04°E). The total area covered by water is 4000 ha, and the average water depth is about 2.5 m. Parts of this ecosystem are designated as wetland of international importance, protected by the Ramsar Convention and proposed as a Site of European Interest. A canal 2350 m long and with a depth of 4 m connects the salt marsh area with the Adriatic Sea. Mean nutrient concentrations in the water column based on sampling during this study were $19.86 \mu\text{mol L}^{-1}$ TDIN and $0.07 \mu\text{mol L}^{-1}$ SRP. Margherita of Savoia is regarded as one of the most degraded lagoons of the Apulian region.

Sampling design and collection of data

Variability was examined at three levels: habitat, time, and site (Figure 2). Two habitats were sampled: 'Mud with macroalgae' MM in the lagoons of Agiasma and Margherita of Savoia, and 'Mud with angiosperms' MA in the lagoons of Agiasma and Cesine. In each habitat two (four in total) sites, i.e. areas 10×10 m were randomly selected 200–800 m apart (Figure 1). At each site five quadrats were sampled by throwing randomly the sampler from the boat (Figure 2). Sampling was carried out during two randomly selected time periods: sampling in Agiasma took place on 3 November 2004 and 12 July 2005; in Margherita of Savoia on 9 December 2004 and 4 May 2005; in Cesine on 6 December 2004 and 4 August 2005.

The sampling was destructive, using a metal hand-held box corer ($17 \text{ cm} \times 17 \text{ cm} \times 15 \text{ cm}$; length \times width \times height), which was vertically pushed through the benthic vegetation and sediment. From each sample the existing vegetation (seaweeds, seagrasses leaves and roots, Cyanobacteria colonies) was carefully removed and placed individually in airtight plastic bags, where it was fixed in 4–5% formalin in sea water for a few seconds. The excess formalin solution was later removed from the plastic bag, which was then sealed, labelled, and stored in a plastic box.

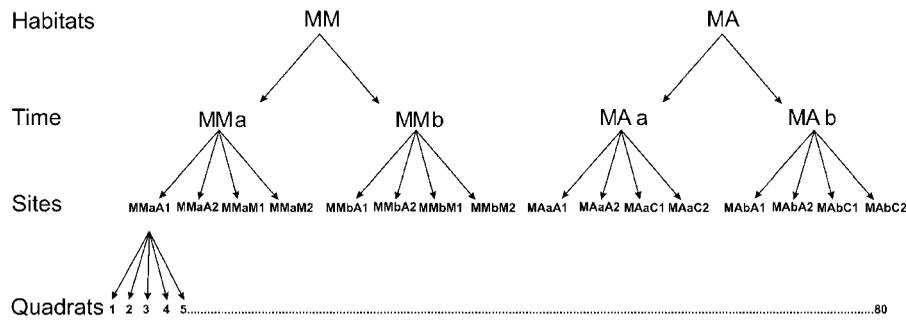


Figure 2. Hierarchical sampling design used in this study. Five quadrats were nested within each of four sites, nested within two sampling times, nested within two habitats. MM = mud with macroalgae, MA = mud with angiosperms, a = sampling period a, b = sampling period b, A = Agiasma lagoon, M = Margherita of Savoia lagoon, C = Cesine lagoon, 1 = site 1, 2 = site 2.

In the laboratory, the formalin preserved samples were first washed in tap water for a few seconds, passed through a double sieve of 1 mm and 500 μm and then transferred to sea water. Benthic macrophytes were then very carefully sorted and species were identified to functional group level and as far as possible to species level using a stereoscope and a binocular microscope. Taxonomically difficult taxa were consistently summarized to genus level as 'spp'. No detailed taxonomic analysis of Cyanobacteria colonies was undertaken.

In order to estimate percentage coverage, a transparent double bottom square PVC container, filled with sea water and having at its bottom a square 17 \times 17 cm matrix divided in 100 squares was used. The surface covered by each sorted taxon in vertical projection floating in sea water was quantified as percentage of coverage (2.89 cm^2 = 1% sampling surface). The percentage coverage of epiphytes on seagrass leaves was roughly assessed without removal of the epiphytes from the host plants. The total coverage often exceeded 100% due to the presence of different layers in the vegetation, i.e. mainly canopy and understorey layers. For species present with insignificant abundance a coverage value of 0.01% was allocated. From each sample, voucher specimens of taxonomically difficult taxa were fixed in 3–5% formalin sea water, which were deposited in the Fisheries Research Institute for future study.

Analysis of data

Multivariate analyses were based on mean coverage data, per site $n = 5$, after a 4th-root transformation. The similarity of the sites was investigated using non-parametric multidimensional scaling analysis based on the Bray–Curtis similarity index. The ANOSIM test was used to verify the statistical significance of the ordination analysis. Species contributing most to the dissimilarity among the ordination clusters of sites were investigated using SIMPER analysis (Carr, 1997). Two-way

nested ANOSIM (Clarke, 1993) was performed to test for differences between habitats and sites for each sampling period separately. All calculations were performed using the PRIMER v. 5.0 software package.

Six metrics related to community structure (multi-dimensional scaling plot of Bray–Curtis similarity, species number, Shannon–Weaver index (\log_2), percentage of total coverage) and function (ESG I percentage coverage, ESG II percentage coverage, and EEI) were estimated. The calculation of diversity indices was based on coverage measurements, as suggested by Boudouresque (1971), and was performed using the PRIMER software. The occurrence of a single taxon in several samples limits the calculation of other known biodiversity indices, e.g. Pielou evenness. The abundance of the two Ecological State Groups (ESG I, ESG II) and the Ecological Evaluation Index (EEI) for each site were calculated according to Orfanidis *et al.* (2001, 2003). Each sampling site was classified into one of five Ecological Status Classes (ESC) after a cross-comparison of the average coverage values of ESG I and II in accordance with Orfanidis *et al.* (2001, 2003). In order to use EEI as a trend index ($\text{EEI}_{\text{trend}}$), each sample was classified in one of the five ESC values after a cross-comparison of the coverage value of the ESG I and II on a matrix. The optimal sampling frequency for determining EEI at the site-scale was assessed by calculating EEI using 1 to 5 randomly selected samples.

All metrics were analysed using parametric nested analysis of variance (ANOVA) with habitat (two levels), time (two levels) within each habitat, site (two levels) within each time, habitats and site. While habitat was regarded as fixed, time and site were regarded as random factors. Since the homogeneity of variances could not be achieved by data transformation (\log , Sqr , Sin), parametric ANOVA on non transformed data at different significance levels was used ($\alpha = 0.5, 0.01, 0.001$) under the prerequisite that >40 df in the residual. The key analyses were repeated on non-transformed data using

Kruskal–Wallis non-parametric ANOVA. A pattern was accepted only when non-parametric and parametric nested ANOVA gave similar results ($P < 0.05$). To determine the random scales with greatest variability, an analysis of variance on untransformed data was used to estimate variance components at site and time scales. All ANOVA and variance tests were carried out using the STATGRAFICS v. 7.1 software package.

RESULTS

Structural metrics

Bray–Curtis multidimensional scaling ordination of the sites (Figure 3) showed a very different pattern for the first and the

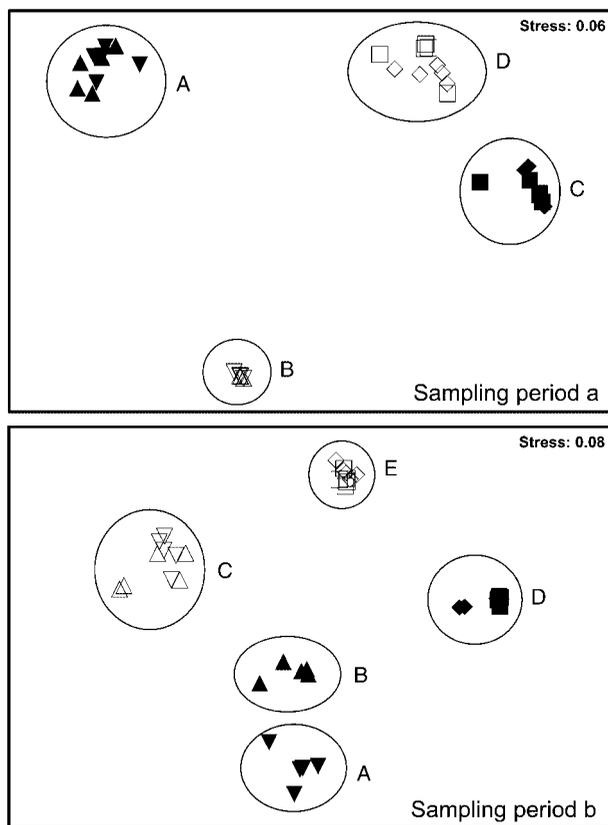


Figure 3. nMDS ordination plots of two sampling periods (a, b) samples. Black and white (empty) colours indicate the habitats 'mud with submerged angiosperms' and 'mud with macroalgae', respectively. Up and down triangles indicate the sites 1 and 2 of the Agiasma lagoon, respectively. Squares and rhombs indicate the sites 1 and 2 of Cesine–Margherita of Savoia lagoons, respectively. Capital letters indicate different assemblages.

second sampling period, where four (A–D) and five (A–E) different assemblages were identified, respectively. The ANOSIM test showed that these assemblages during both sampling periods were significantly different at a level 0.1% (global $R = 1$). This was reflected by high dissimilarities among samples taken from different sites of the same habitats in different lagoons, but also in the case of the second sampling period in the Agiasma lagoon, by high dissimilarities between sites of the same habitat in the same lagoon. An analysis of the contribution of each taxon to the dissimilarity between assemblages, as shown by the SIMPER analysis (Tables 1 and 2), showed that the differences during the first sampling period were mainly due to Cyanobacteria colonies and the species *R. maritima*, *R. cirrhosa*, and *Chaetomorpha linum* (O. F. Müller) Kützing (Table 2) and the differences during the second sampling period due to the species *Ulva* sp., *R. maritima*, *R. cirrhosa*, and *C. linum* (Table 3).

A nested analysis of similarity (ANOSIM) revealed significant differences for both sampling periods among sites ($R = 0.69–0.76$, $P = 0.001$) but not among habitats (Table 3). This indicates that there were significant differences among sets of samples from similar habitats. In the MM habitat *Ulva* sp. and Cyanobacteria colonies dominated the Agiasma lagoon and *C. linum* the Margherita of Savoia lagoon. In the MA

Table 1. SIMPER analysis of the sampling period a results. Groups A–D corresponds to Figure 3, sampling period a groups. AD = average dissimilarity

Species	Average abundance in 1st group	Average abundance in 2nd group	Contribution (%)
Groups A vs. B (AD = 99.96)			
Cyanobacteria	0.01	55.00	75.81
Groups A vs. C (AD = 99.98)			
<i>Ruppia maritima</i>	0.00	44.30	67.35
<i>Ruppia cirrhosa</i>	10.25	0.00	17.18
Groups B vs. C (AD = 99.99)			
Cyanobacteria	55.00	0.01	55.48
<i>Ruppia maritima</i>	0.00	44.30	42.29
Groups A vs. D (AD = 99.63)			
<i>Chaetomorpha linum</i>	0.00	77.50	66.80
<i>Ruppia cirrhosa</i>	10.25	0.00	9.54
Groups B vs. D (AD = 99.99)			
<i>Chaetomorpha linum</i>	0.00	77.50	49.76
Cyanobacteria	55.00	0.01	37.39
Groups C vs. D (AD = 92.14)			
<i>Chaetomorpha linum</i>	0.00	77.50	57.94
<i>Ruppia maritima</i>	44.30	5.00	29.57

Table 2. SIMPER analysis of the sampling period b results. Groups A–E corresponds to Figure 3, sampling period b groups. AD = average dissimilarity

Species	Average abundance in 1st group	Average abundance in 2nd group	Contribution (%)
Groups A vs. B (AD = 37.75)			
<i>Ruppia cirrhosa</i>	72.00	66.00	64.75
<i>Cladophora</i> sp.	9.60	0.00	16.63
Groups A vs. C (AD = 97.12)			
<i>Ulva</i> sp.	0.60	120.00	52.48
<i>Ruppia cirrhosa</i>	72.00	0.05	38.21
Groups B vs. C (AD = 99.92)			
<i>Ulva</i> sp.	0.00	120.00	59.06
<i>Ruppia cirrhosa</i>	66.00	0.05	36.09
Groups A vs. D (AD = 98.62)			
<i>Ruppia cirrhosa</i>	72.00	0.00	48.81
<i>Ruppia maritima</i>	0.00	34.70	23.92
Groups B vs. D (AD = 99.97)			
<i>Ruppia cirrhosa</i>	66.00	0.00	47.44
<i>Ruppia maritima</i>	0.00	34.70	29.50
Groups C vs. D (AD = 99.99)			
<i>Ulva</i> sp.	120.00	0.00	60.33
<i>Ruppia maritima</i>	0.00	34.70	22.23
Groups A vs. E (AD = 92.22)			
<i>Ruppia cirrhosa</i>	72.00	0.00	53.50
<i>Chaetomorpha linum</i>	0.00	45.00	32.72
Groups B vs. E (AD = 99.98)			
<i>Ruppia cirrhosa</i>	66.00	0.00	48.95
<i>Chaetomorpha linum</i>	0.00	45.00	37.87
Groups C vs. E (AD = 98.93)			
<i>Ulva</i> sp.	120.00	0.00	62.25
<i>Chaetomorpha linum</i>	0.66	45.00	28.39
Groups D vs. E (AD = 99.01)			
<i>Chaetomorpha linum</i>	0.00	45.00	38.69
<i>Ruppia maritima</i>	34.70	0.55	30.80
<i>Lophosiphonia subadunca</i>	22.00	0.00	19.80

habitat *R. maritima* dominated the Cesine lagoon and *R. cirrhosa* the Agiasma lagoon (Tables 2 and 3).

Species number, Shannon–Weaver index, and percentage coverage metrics showed significant differences in their mean values for the site scale in the non-parametric and nested parametric ANOVA (Table 4). Nested ANOVA indicated that the F-values for site were maximal. The number of species (24 taxa were identified in total) per sample ranged from one at the sites of the MM habitat in the Agiasma lagoon during the first

Table 3. Results of two-way ANOSIM tests examining differences between habitats, and between sites within habitats. Two separate tests for the different sampling times were performed

Time factor	No of permutations	Clarke's R	Significance (%)
Autumn			
Habitat	35	−0.5	100
Site	Too many	0.69	0.1
Summer			
Habitat	35	−0.073	62.9
Site	Too many	0.759	0.1

sampling period (MMaA1, MMaA2) to nine at Site 2 of the MM habitat in the Margherita of Savoia lagoon (MMbM2; mean value = 7) and Site 1 of the MA habitat in the Agiasma lagoon (MAbA1; mean value = 8), both during the second sampling period (Figure 4). The Shannon–Weaver index ranged from 0 at the sites of the MM habitat in the Agiasma lagoon (MMaA1, MMaA2) to 1.8 at Site 2 of the MM habitat in the Margherita of Savoia lagoon (MMaM2; mean value = 0.96), both during the first sampling period (Figure 4). The maximum mean value (1.09) was found at Site 1 of the MA habitat in the Cesine lagoon during the second sampling period (MAbC1). Coverage values (%) ranged from 1.5% (mean value = 14.8%) at Site 1 of the habitat MA in the Agiasma lagoon during the first sampling period (MAaA1) to 303% (mean value = 171%) at Site 1 of the MM habitat in the Agiasma lagoon during the second sampling period (MMbA1; Figure 4). Components of variation calculated on each of the random scales tested for the diversity and coverage metrics (Figure 5) indicated that the site scale alone (species number) or along with residuals (Shannon–Weaver index, Coverage %) were most important in explaining variance.

Functional metrics

The functional metrics showed significant differences in their mean values for the habitat and site scales in the non-parametric and nested parametric ANOVA (Table 4). Nested ANOVA indicated that the F-values for habitat were larger than those for site. ESG I showed significant differences in its mean values for the site scale with both parametric and non-parametric ANOVA but only significant differences with the non-parametric ANOVA for the habitat scale (Table 4). Coverage (%) of ESG I species ranged from 0 at several sites of the habitat MM to 120% (mean value = 66%) at Site 2 of the MA habitat in the Agiasma lagoon during the second sampling period (MAbA2; Figure 6). The maximum mean value (72%) was found at Site 1 of the habitat MA in the Agiasma lagoon during the second sampling period (MAbA1). ESG II and EEI_{trend} showed significant differences in their

Table 4. Comparisons between habitat, time and site for structural (species number, Shannon–Weaver index, % coverage) and functional (Ecological State Group I % coverage, ESG II % coverage, and Ecological Evaluation Index-EEI) metrics using nested parametric and non-parametric ANOVA

Source of variation	Structural					Functional				
	Parametric			Non-parametric		Parametric			Non-parametric	
	df	MS	<i>F</i>	df	χ^2	df	MS	<i>F</i>	df	χ^2
Species number						ESG I				
Habitat	1	17.11	0.51	1	13.09***	1	29121.80	9.57	1	57.80***
Time (Habitat)	2	33.46	1.46	3	20.66***	2	3044.02	3.04	3	59.60***
Site (Habitat*Time)	12	22.90	46.39***	15	61.99***	12	1000.97	4.81***	15	64.00***
Residual	64	0.49				64	207.94			
	Shannon–Weaver index (<i>H'</i>)					ESG II				
Habitat	1	0.59	0.55	1	1.80	1	100323.61	50.56*	1	64.80***
Time (Habitat)	2	1.06	1.50	3	12.40**	2	1984.24	0.41	3	65.20***
Site (Habitat*Time)	12	0.71	8.61***	15	48.00***	12	4866.23	5.92***	15	67.20***
Residual	64	0.08				64	822.32			
	Coverage (%)					EEI _{trend}				
Habitat	1	21341.68	2.76	1	7.20**	1	396.05	93.19*	1	23.23***
Time(Habitat)	2	7738.76	1.41	3	14.00**	2	4.25	0.99	3	28.39***
Site(Habitat*Time)	12	5488.05	5.34***	15	28.80*	12	4.28	3.43***	15	50.18***
Residual	64	1027.36				64	1.25			

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

mean values for both habitat (ESG II $P = 0.019$, EEI $P = 0.0106$) and site (both $P \leq 0.000$) scales in nested parametric ANOVA (Table 4). In the non-parametric ANOVA for both metrics $P < 0.000$. Coverage (%) of ESG II species ranged from close to 0 at several sites of the habitat MA during the first sampling period to 303 (mean value = 171) at Site 1 of the habitat MM in the Agiasma lagoon during the second sampling period (MMbA1; Figure 6). Values of EEI_{trend} ranged from 2 at several sites of the habitat MM to 10 at Sites 1 (MAbA1, mean value = 9.2) and 2 (MAbA2, mean value = 8.4) of the habitat MA in the Agiasma lagoon during the second sampling period and at Site 2 (MAbC2, mean value = 8) of the Cesine lagoon during the first sampling period (Figure 6). Components of variation calculated on each of the random scales tested for the functional metrics indicated that the site scale along with residuals (ESG I, ESG II) or residuals (EEI_{trend}) was most important in explaining variance (Figure 5).

Using data from both sampling periods, the sites of the MA habitat of the Agiasma and Cesine lagoons were classified as 'good' ESC, whereas the sites of the MM habitat of the Agiasma and Margherita of Savoia lagoons were classified as 'bad' ESC. To estimate optimal sampling frequency per sampling period per sampling site, EEI was estimated using 1, 2, 3, 4 and 5 samples (Figure 7), and 4 or 5 samples of 0.0289 m² per sampling period were found to be sufficient to discriminate between sustainable (good to high;

EEI ≥ 6) and non-sustainable (bad to moderate; EEI < 6) coastal lagoons.

DISCUSSION

The results of this study indicate for the first time considerable differences between structural and functional metrics of benthic macrophyte communities at a variety of scales, within and among two habitats of selected Eastern Mediterranean coastal lagoons (Figures 3–6, Table 4). These results should take into account two biostatistic constraints: (1) absence of a hierarchical spatial scale design from cm/m to kms within habitats in order to indicate all scale-based spatial heterogeneity of macrophyte communities owing to size heterogeneity of the tested habitats within the lagoons, typical for most Mediterranean coastal lagoons (Basset *et al.*, 2006), (2) existence of both habitats in only one lagoon (Agiasma) could constrain separation of the effects of habitat from lagoon, such lagoon effects, however, are not studied in this work. The influence of lagoon, catchments, landscape or even greater spatial scales on habitat variance is a challenging topic because of the complex and heterogeneous nature of lagoons (Kjerfve, 1994) that needs further research.

In this study benthic vegetation was the determining factor of the two tested habitats, MM and MA, comprising

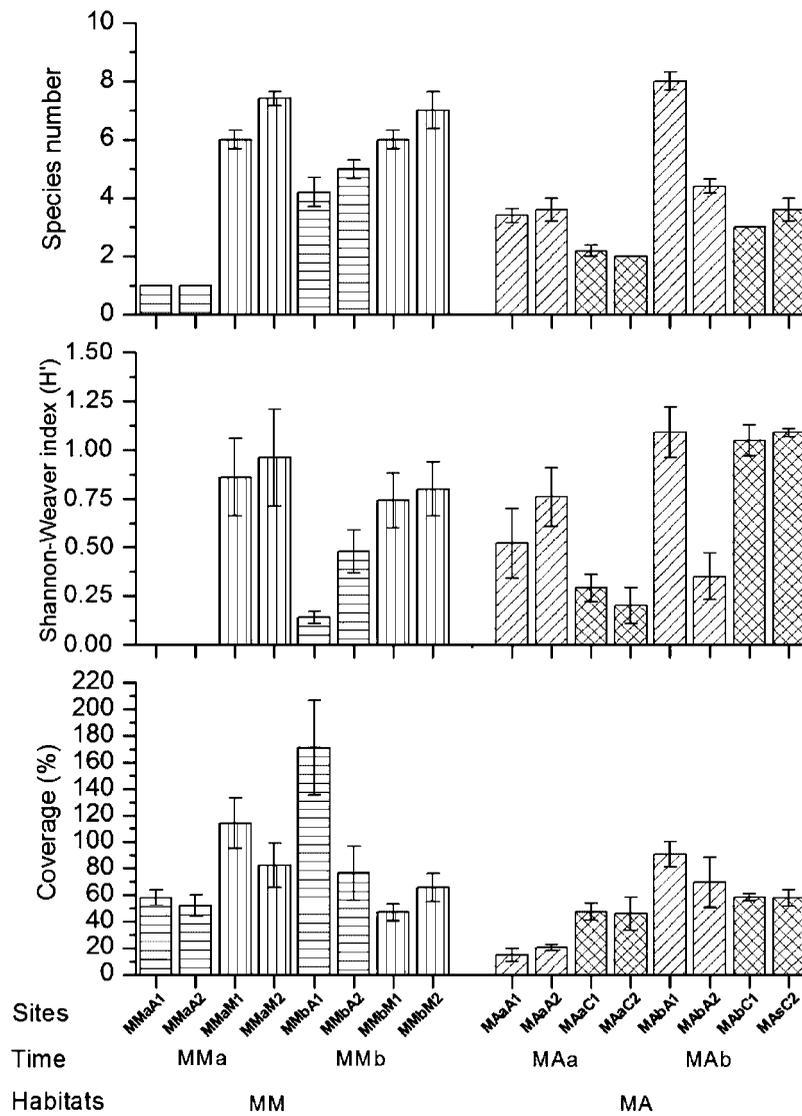


Figure 4. Mean values (\pm SE) of species number, Shannon–Weaver index and percentage coverage metrics. There are five quadrats in each of four sites, in each two sampling times, in each two habitats. See Figure 2 for more information.

opportunistic algae (*Ulva* sp. and *Chaetomorpha linum*, Cyanobacteria) members of ESG II, and angiosperms (*Ruppia cirrhosa* and *R. maritima*) members of ESG I, respectively. These habitat related vegetation differences were better indicated by functional metrics than by structural ones, by reducing the spatial and temporal complexity of macrophyte communities to the habitat scale. Such a broad spatial scale variance pattern is very likely to be related to environmental factors or to chronic pollution (Wiens *et al.*,

1993; Underwood, 1997; Benedetti-Cecchi, 2001; Benedetti-Cecchi *et al.*, 2001) and therefore these metrics can be useful in water quality assessment.

From all functional metrics tested the biotic index EEI classified the sampling sites into ‘bad’ and ‘good’ ESC. The habitat MA of Agiasma and Cesine lagoons were indicated as less affected sites and classified as ‘good’ ESC. Indeed mean nitrogen concentrations, one of the main factors influencing macrophyte communities shifts in coastal lagoons (Howarth

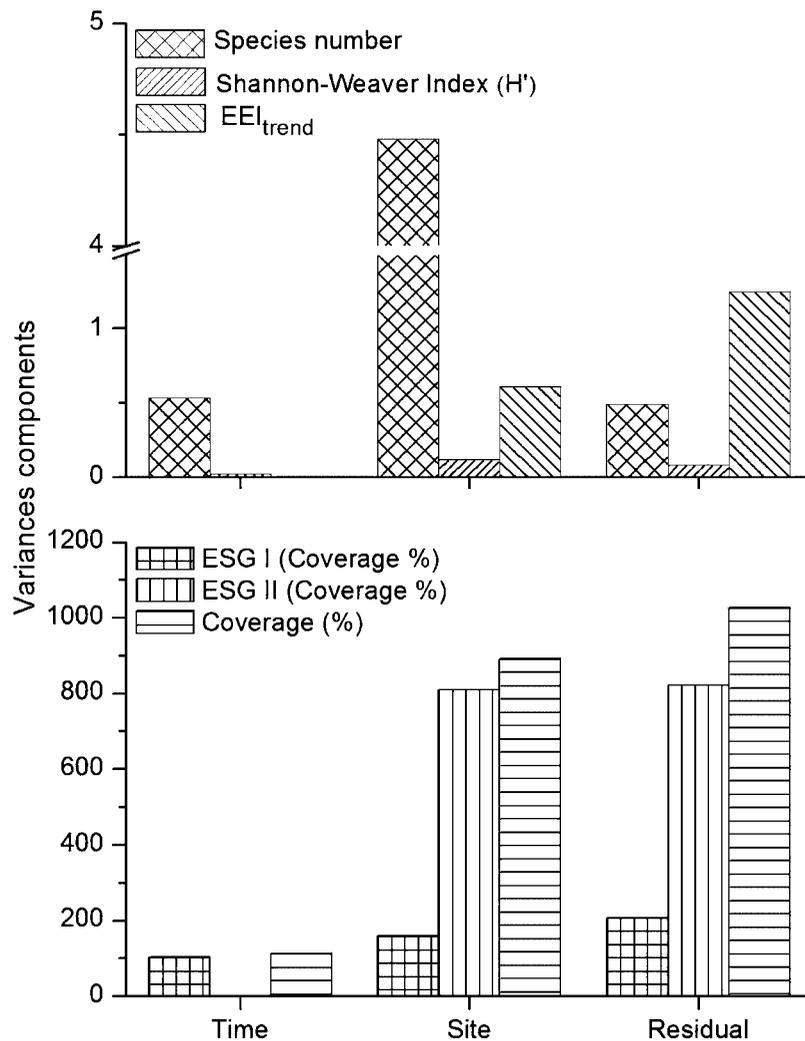


Figure 5. Components of variation in different metrics: species number, Shannon–Weaver index, coverage (%), ESG I, ESG II, EEI_{trend} indices.

and Marino, 2006; Viaroli *et al.*, 2008), in MA-Agiasma were $5.4 \mu\text{mol L}^{-1}$ TDIN, and in MA-Cesine $3.9 \mu\text{mol L}^{-1}$ TDIN and thereby were a little lower than in MM-Agiasma ($5.85 \mu\text{mol L}^{-1}$ TDIN) and much lower than MM-Margherita of Savoia ($19.86 \mu\text{mol L}^{-1}$ TDIN). Although high variability of coastal lagoon abiotic factors due to climatic and hydrological influence was noticed elsewhere (Petihakis *et al.*, 1999; Basset *et al.*, 2001; Orfanidis *et al.*, 2005) the nutrient values measured in this study should be regarded as representative. Moreover, since sampling in MA-Agiasma was undertaken during the period of restricted sea water exchange with the sea because of

fish aquaculture practices the measured values should rather over-estimate lagoon trophic conditions.

Seasonal growth of perennial angiosperms (Verhoeven, 1979, 1980; Calado and Duarte, 2000; Menéndez, 2002; Agostini *et al.*, 2003c; Malea *et al.*, 2004) exhibiting high and low coverage values in summer and autumn–winter periods, respectively, seems not to constrain use of EEI, which is effective at low percentage coverage values ranging from 0 to ca. 60%.

Input of nutrients and changes in light transparency are considered among the processes affecting the growth of

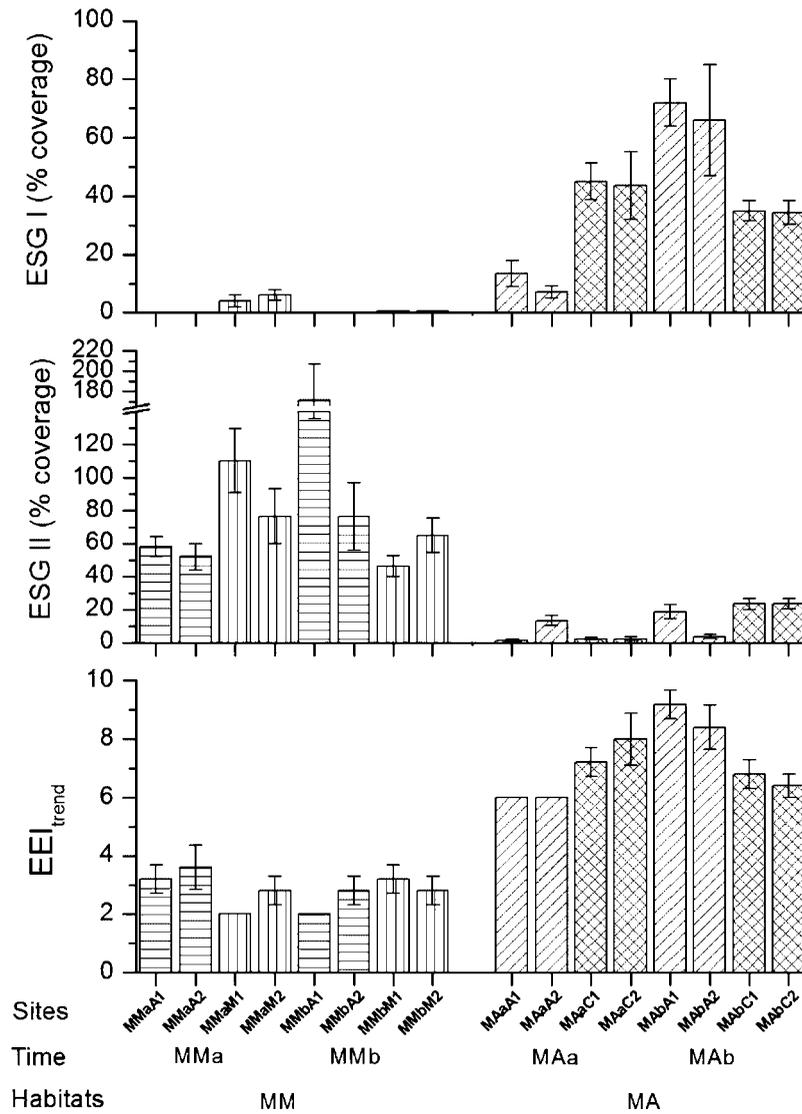


Figure 6. Mean values (\pm SE) of ESG I, ESG II, and EEI_{trend} metrics. There are five quadrats in each of four sites, in each two sampling times, in each two habitats. See Figure 2 for more information.

macroalgae and angiosperms, most conspicuously, in coastal lagoons (Cloern, 2001; De Jonge *et al.*, 2002). Under nutrient excess and turbid conditions, species composition shift from angiosperms to dominance of opportunistic and often bloom forming macroalgae (Harlin, 1995; Schramm and Nienhuis, 1996; Viaroli *et al.*, 2008). This may be due to the efficient nutrient assimilation of opportunistic macroalgae (Thompson and Valiela, 1999) and their non-linear and self-accelerating

response after crossing certain nutrient boundaries (Duarte, 1995). Furthermore, opportunistic macroalgae demand lower light levels for growth than rooted angiosperms (Congdon and McComb, 1979; Lüning, 1990; Kenworthy and Fonseca, 1996; Hemminga and Duarte, 2000). Under oligotrophic and highly transparent conditions angiosperms are at an advantage over seaweeds by using nutrients from the sediment (Borum, 1996; Hemminga and Duarte, 2000). Other factors that can trigger

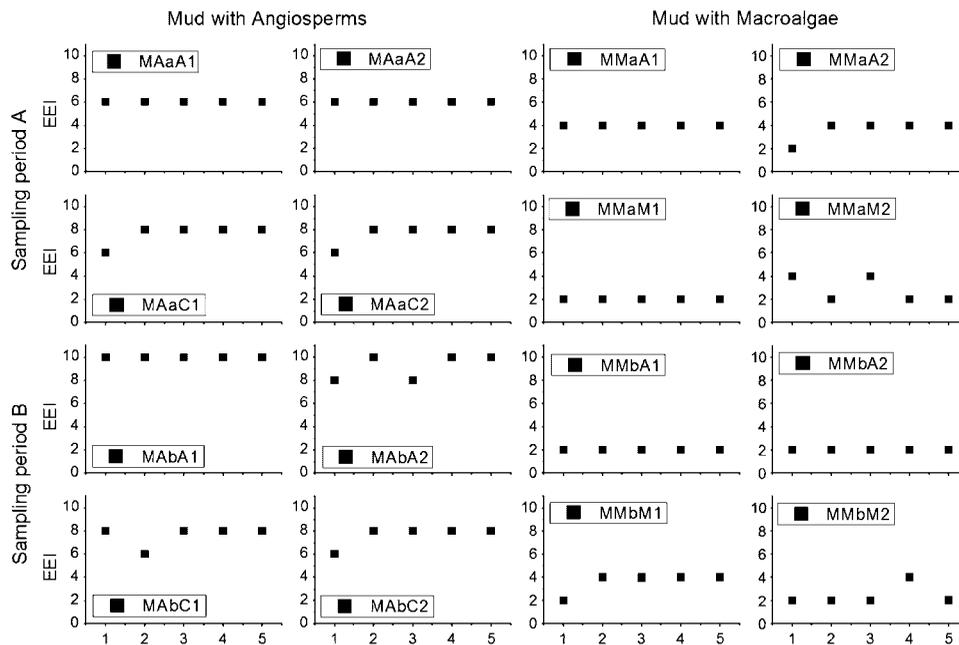


Figure 7. Changes of EEI in relation to sampling frequency per sampling period per sampling site in two habitats of Agiasma, Cesine and Margherita of Savoia lagoons. See Figure 2 for more information.

this switch, e.g. hydrographic change, grazing, etc., cannot be excluded (Scheffer, 1997), especially when interactions with other stressors are considered (Cloern, 2001).

The sites studied belong to the same biogeographical sub-region of the eastern Mediterranean and the species composition of the less affected MA habitat in the different lagoons was dominated by two different species of the same genus: *Ruppia cirrhosa* in the Agiasma lagoon and *R. maritima* in the Cesine lagoon. As a result, high dissimilarities became apparent among samples from this habitat in the Agiasma and Cesine lagoons. Furthermore, in the Agiasma lagoon high dissimilarities between sites of the same habitat were obvious during the second sampling period. The latter observation is probably due to differences in confinement (*sensu* Guelorget and Perthuisot, 1992) between the sites. Site 1 has better water circulation as it is located between the permanent (A) and the seasonally activated (B) Agiasma lagoon outlets. Therefore, although multidimensional plots have successfully been used locally to examine differences in species composition between reference and affected sites (Clarke, 1993; Warwick and Clarke, 1993), they can less easily be used as metrics to assess the water quality status at the broad spatial scale of a biogeographical region as required by WFD. Another obstacle in using such an approach even at a local scale is the establishment of reference

conditions. Pristine coastal lagoons no longer exist because of urban, industrial and agricultural effluents, aquaculture exploitation, habitat modification and introduction of exotic species (Sfriso *et al.*, 1992; De Casabianca *et al.*, 1997; Verlaque, 2001; Viaroli *et al.*, 2001, 2006). Type-specific reference conditions needed by the WFD to evaluate the ecological status (EC, 2000) further decreases the list of available reference sites (Basset *et al.*, 2006) in this biogeographical region.

Ruppia is a cosmopolitan genus, tolerant of salinity fluctuations, and characteristic of many coastal brackish waters and inland salt-water habitats (Verhoeven, 1979; Calado and Duarte, 2000; Menéndez, 2002; Mannino and Sarà, 2006). *Ruppia cirrhosa* and *R. maritima* in Europe grow in salinities between 3 and 100 PSU and 0.6 and 27 PSU, respectively (Verhoeven, 1979). This remarkable salinity tolerance indicates *Ruppia* species, especially *R. cirrhosa*, as 'ideal' bioindicators for coastal lagoons because possible community changes and/or discontinuities do not have to be attributed to salinity fluctuations. It also might offer an explanation why *R. cirrhosa* grows in the brackish to saline Agiasma lagoon and *R. maritima* in the fresh to brackish Cesine lagoon.

Macrophyte diversity indices, as in other lagoons (Middelboe *et al.*, 1998; Kunii and Minamoto, 2000; Curiel

et al., 2004; Mannino and Sarà, 2006), were in general low in this study and site-specific (Figure 4, Table 4). A decrease in macrophyte diversity from the entrance to the inner parts of estuaries (Munda, 1978; Kautsky, 1995) and coastal lagoons (Coutino and Seeliger, 1984; Orfanidis *et al.*, 2000), in MA-Agiasma during sampling period b, suggests either the existence of physiological stress due to strong salinity gradients (Coutino and Seeliger, 1984) or spore, fragment or propagule dispersal restriction (confinement) or interactions between them. An extensive study of macroalgal species diversity in the Danish estuaries indicated a rather complex pattern where species number of macroalgae increased with salinity and declined with nutrient concentrations (Middelboe *et al.*, 1998). Surfaces provided by hard substrata and by seagrass leaves increases the colonization ability of macroalgae thereby increasing their diversity (Coutino and Seeliger, 1984; Middelboe *et al.*, 1998; Mannino and Sarà, 2006) and affecting community composition (Nedwell *et al.*, 2002). These results are in agreement with Thiebaut *et al.* (2002) who indicated that species diversity indices are inadequate to evaluate water quality in freshwater ecosystems.

Maximum *Ulva* coverage, i.e. biomass, was observed at Site 1 of the habitat MM, which is one of the most polluted areas of the Agiasma lagoon (Stamatis *et al.*, 2006). Such macroalgal blooms of fast-growing species like *Ulva* that occur frequently in eutrophic estuarine and lagoonal ecosystems are generally explained by high nutrient availability (Schramm and Nienhuis, 1996; Rafaelli *et al.*, 1998). *Ulva* growth in different Mediterranean coastal lagoons similarly seems to be controlled by summer temperature, which exceeds the uppermost limit for optimal growth (ca. 23°C; De Casabianca and Posada, 1998; De Casabianca *et al.*, 2002). *Ulva* coverage at Site 1, located close to the outlet was double that of Site 2, located in the inner parts of the lagoon. Tidal exchange or wind forced seawater flushing moderates the thermal regime close to the lagoon outlet (pers. observation), benefiting the growth of *Ulva*. In addition, repeated flushing may favour *Ulva* growth because *Ulva* spp. are capable of rapid uptake and accumulation of nutrients (Pedersen and Borum, 1997) during the outflow periods that can be utilized later when nutrient-poor, clearer sea water flows into the lagoon from the sea (Schramm, 1999). Changes in the phosphorus to nitrogen ratio after *Ulva* decay combined with wind-driven resuspension phenomena or seasonal changes in rainfall (Orfanidis *et al.*, 2005) may favour the growth of Cyanobacteria during autumn and winter months in MM habitat of Agiasma (Viaroli *et al.*, 2008). The intensity of such changes will be related to the dimensions of the lagoon (Holling, 1992). Small (sub) basins like that of the MM habitat of the Agiasma lagoon are probably unable to buffer seasonal variations.

CONCLUSIONS

Nutrients, especially nitrogen excess, shifts the coastal lagoon habitat from late-successional angiosperms to dominance by opportunistic macroalgae. Such a community switch is better indicated by functional metrics, especially the biotic index EEI, which showed higher community heterogeneity at the habitat scale. Therefore, cost effective macrophyte monitoring programmes for coastal lagoon water quality need habitat replication and use of EEI. Future hierarchically designed studies at biogeographical or global scales, in space and time, will further increase our ability to diagnose macrophyte communities' heterogeneity.

ACKNOWLEDGEMENTS

This research was supported by the TWReferenceNET EU INTERREG IIIB project. SO and NS are very grateful to members of the Agricultural Fisheries Cooperative of Keramoti Lagoons for their support with field sampling.

REFERENCES

- Agostini S, Capiomont A, Marchand B, Pergent G. 2003a. Distribution and estimation of basal area coverage of subtidal seagrass meadows in a Mediterranean coastal lagoon. *Estuarine Coastal and Shelf Science* **56**: 1021–1028.
- Agostini S, Marchand B, Pergent G. 2003b. Temporal and spatial changes of seagrass meadows in a Mediterranean coastal lagoon. *Oceanologica Acta* **25**: 297–302.
- Agostini S, Pergent G, Marchand B. 2003c. Growth and primary production of *Cymodocea nodosa* in a coastal lagoon. *Aquatic Botany* **76**: 185–193.
- Anderson A. 1999. My bioindicator or yours? Making the selection. *Journal of Insect Conservation* **3**: 61–64.
- Austoni AG, Giordani P, Viaroli J, Zaldivar M. 2007. Application of specific exergy to macrophytes as an integrated index of environmental quality for coastal lagoons. *Ecological Indicators* **7**: 229–238.
- Basset A, Carlucci D, Fiocca A, Vignes F. 2001. Water transparency and health of coastal salt marshes: simple enclosure experiments of nutrient dynamic. *Aquatic Conservation: Marine and Freshwater Ecosystems* **11**: 273–279.
- Basset A, Sabetta L, Fonnesu A, Mouillot D, Do Chi D, Viaroli P, Giordani G, Reizopoulou S, Abbiati M, Carrada GC. 2006. Typology in Mediterranean transitional waters: new challenges and perspectives. *Aquatic Conservation: Marine and Freshwater Ecosystems* **16**: 441–455.
- Benedetti-Cecchi L. 2001. Variability in abundance of algae and invertebrates at different spatial scales on rocky sea shores. *Marine Ecology Progress Series* **215**: 79–92.

- Benedetti-Cecchi L, Pannacchiulli F, Bulleri F, Moschella PS, Airoldi L, Relini G, Cinelli F. 2001. Predicting the consequences of anthropogenic disturbance: large-scale effects of loss of canopy algae on rocky shores. *Marine Ecology Progress Series* **214**: 137–150.
- Borum J. 1996. Shallow waters and land/sea boundaries. *Coastal and Estuarine Studies* **52**: 179–203.
- Boudouresque CF. 1971. Méthodes d'étude qualitative et quantitative du benthos (en particulier du phytobenthos). *Tethys* **3**: 79–104.
- Calado G, Duarte P. 2000. Modelling growth of *Ruppia cirrhosa*. *Aquatic Botany* **68**: 29–44.
- Carpenter RC. 1990. Competition among marine macroalgae: a physiological perspective. *Journal of Phycology* **26**: 6–12.
- Carr MR. 1997. *Primer User Manual*. Plymouth Marine Laboratory: UK.
- Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**: 117–143.
- Clarke KR, Warwick RM. 1994. *Change in Marine Communities: an Approach to Statistical Analysis and Interpretation*. Plymouth Marine Laboratory: Plymouth.
- Cloern JE. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series* **210**: 223–253.
- Congdon RA, McComb AJ. 1979. Productivity of *Ruppia*: seasonal changes and dependence on light in an Australian Estuary. *Aquatic Botany* **6**: 121–132.
- Coutino R, Seeliger U. 1984. The horizontal distribution of the benthic algal flora in the Patos Lagoon estuary, Brazil, in relation to salinity, substratum and wave exposure. *Journal of Experimental Marine Biology and Ecology* **80**: 247–257.
- Curiel D, Rismondo A, Bellemo G, Marzocchi M. 2004. Macroalgal biomass and species variations in the lagoon of Venice (Northern Adriatic Sea, Italy): 1981–1998. *Scientia Marina* **68**: 57–67.
- De Casabianca ML, Posada F. 1998. Effect of environmental parameters on the growth of *Ulva rigida*, Thau lagoon, France. *Botanica Marina* **41**: 157–165.
- De Casabianca ML, Laugier T, Marinho-Soriano E. 1997. Seasonal changes of nutrients in water and sediment in a Mediterranean lagoon with shellfish farming activity (Thau Lagoon, France). *ICES Journal of Marine Science* **54**: 905–916.
- De Casabianca ML, Barthelemy N, Serrano O, Sfriso A. 2002. Growth rate of *Ulva rigida* in different Mediterranean eutrophicated sites. *Bioresource Technology* **82**: 27–31.
- De Jonge VN, Elliott M, Orive E. 2002. Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. *Hydrobiologia* **475/476**: 1–19.
- Dennison WC, Orth RJ, Moore KA, Stevenson JC, Carter V, Kollar S, Berstrom PW, Batiuk RA. 1993. Assessing water quality with submerged aquatic vegetation. *BioScience* **43**: 86–94.
- Diaz RJ, Solan M, Valente RM. 2004. A review of approaches for classifying benthic habitats and evaluation habitat quality. *Journal of Environmental Management* **73**: 165–181.
- Domin A, Schubert H, Krause JC, Schiewer U. 2004. Modelling of pristine depth limits for macrophyte growth in the southern Baltic Sea. *Hydrobiologia* **514**: 29–39.
- Doust JL, Schmidt M, Doust LL. 1994. Biological assessment of aquatic pollution: a review, with emphasis on plants as biomonitors. *Biological Reviews* **69**: 147–186.
- Duarte CM. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* **41**: 87–112.
- Duke CS, Litaker W, Ramus J. 1989. Effects of temperature, nitrogen supply, and tissue nitrogen on ammonium uptake rates of chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum*. *Journal of Phycology* **25**: 113–120.
- Duraco MJ. 1995. Indicators of seagrass ecological conditions: an assessment based on spatial and temporal changes associated with the mass mortality of the tropical seagrass, *Thalassia testudinum*. In *Changes in Fluxes in Estuaries: Implications for Science to Management*, Dyer KR, Orth RJ (eds). Olsen and Olsen: Fredensborg, Denmark.
- EC. 2000. *Directive 2000/60/EC, Establishing a framework for community action in the field of water policy*. European Commission PE-CONS 3639/1/100 Rev 1: Luxemburg.
- Gibson GR, Bowman ML, Gerritsen J, Snyder BD. 2000. *Estuarine and coastal marine waters: bioassessment and biocriteria technical guidance*. EPA 822-B-00-024. US Environmental Protection Agency, Office of Water: Washington, DC. Available online at <http://www.epa.gov/ost/biocriteria/States/estuaries/Estuaries-final.pdf>: Washington, DC.
- Guelorget O, Perthuisot JP. 1992. Paralic ecosystem. Biological organisation and functioning. *Vie et Milieu* **42**: 215–251.
- Harlin MM. 1995. Changes in major plant groups following nutrient enrichment. In *Eutrophic Shallow Estuaries and Lagoons*, McComb AJ (ed.). Institute for Environmental Science, Murdoch University, CRC Press: Murdoch, Australia; 173–187.
- Hein M, Pedersen MF, Sand-Jensen K. 1995. Size dependent nitrogen uptake in micro- and macroalgae. *Marine Ecology Progress Series* **118**: 247–253.
- Hemminga MA, Duarte CM. 2000. *Seagrass Ecology*. Cambridge University Press.
- Holling CS. 1992. Cross-scale morphology, geometry, and dynamics of ecosystems. *Ecological Monographs* **62**: 447–502.
- Howarth RW, Marino R. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. *Limnology and Oceanography* **51**: 364–376.
- Jørgensen SE. 2006. Application of holistic thermodynamic indicators. *Ecological Indicators* **6**: 24–29.
- Kautsky H. 1995. Quantitative distribution of sublittoral plant and animal communities along the Baltic Sea gradient. In *Biology and Ecology of Shallow Coastal Waters. Proceedings of the 28th European Marine Biology Symposium*, Eleftheriou

- A, Ansell AD, Smint CJ (eds). Olsen and Olsen: Fredensborg, Denmark; 23–30.
- Kautsky N, Kautsky H, Kautsky U, Waern M. 1986. Decreased depth penetration of *Fucus vesiculosus* (L.) since the 1940's indicates eutrophication of the Baltic Sea. *Marine Ecology Progress Series* **28**: 1–8.
- Kenworthy WJ, Fonseca MS. 1996. Light requirements of seagrasses *Halodule wrightii* and *Syringodium filiforme* derived from the relationship between diffuse light attenuation and maximum depth distribution. *Estuaries* **19**: 740–750.
- Kjerfve B. 1994. *Coastal Lagoon Processes*. Elsevier Science Publishers: Amsterdam.
- Krause-Jensen D, Greve TM, Nielsen K. 2004. Eelgrass as bioindicator under the European Water Framework Directive. *Water Resources Management* **19**: 63–75.
- Kunii H, Minamoto K. 2000. Temporal and spatial variation in the macrophyte distribution in coastal lagoon Lake Nakaumi and its neighbouring waters. *Journal of Marine Systems* **26**: 223–231.
- Littler MM, Littler DS. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *American Naturalist* **116**: 25–44.
- Lotze HK, Schramm W. 2000. Ecophysiological traits explain species dominance patterns in macroalgal blooms. *Journal of Phycology* **36**: 287–295.
- Lüning K. 1990. *Seaweeds: Their Environment, Biogeography and Ecophysiology*. John Wiley & Sons.
- MacArthur RH, Wilson EO. 1967. *The Theory of Island Biogeography*. Princeton University Press: Princeton.
- Malea P, Kevrekidis Th, Mogias A. 2004. Annual versus perennial growth cycle in *Ruppia maritima* L.: temporal variation in population characteristics in Mediterranean lagoons (Monolimni and Drana Lagoons, Northern Aegean Sea). *Botanica Marina* **47**: 357–366.
- Mannino AM, Sarà G. 2006. The effect of *Ruppia cirrhosa* features on macroalgae and suspended matter in a Mediterranean shallow system. *Marine Ecology* **27**: 350–360.
- Menéndez M. 2002. Net production of *Ruppia cirrhosa* in Ebro Delta. *Aquatic Botany* **73**: 107–113.
- Middelboe AL, Sand-Jensen K, Krause-Jensen D. 1998. Patterns of macroalgal species diversity in Danish estuaries. *Journal of Phycology* **34**: 457–466.
- Mouillot D, Gaillard S, Aliaume C, Verlaque M, Belsher T, Troussellier M, Chi TD. 2005. Ability of taxonomic diversity indices to discriminate coastal lagoon environments based on macrophyte communities. *Ecological Indicators* **5**: 1–17.
- Mouillot D, Spatharis S, Reizopoulou S, Laugier T, Sabetta L, Basset A, Do Chi T. 2006. Alternatives to taxonomic-based approaches to assess changes in transitional water communities. *Aquatic Conservation: Marine and Freshwater Ecosystems* **16**: 469–482.
- Munda IM. 1978. Salinity dependent distribution of benthic algae in estuarine areas of Icelandic fjords. *Botanica Marina* **21**: 451–468.
- Nedwell DB, Sage AS, Underwood GJC. 2002. Rapid assessment of macro algal cover on intertidal sediments in a nutrified estuary. *The Science of Total Environment* **285**: 97–105.
- Nielsen SL, Sand-Jensen K. 1990. Allometric scaling of maximal photosynthesis growth rate to surface/volume ratio. *Limnology and Oceanography* **35**: 177–181.
- Niemi G, McDonald ME. 2004. Application of ecological indicators. *Annual Review Ecology, Evolution, and Systematics* **35**: 89–111.
- Niemi G, Wardrop D, Brooks R, Anderson S, Brady V, Paerl H, Rakocinski C, Brouwer M, Levinson B, McDonald M. 2004. Rationale for a new generation of indicators for coastal waters. *Environmental Health Perspectives* **112**: 979–986.
- O'Neill RV. 1988. Hierarchy theory and global change. In *Scales and Global Change*, Rosswall T, Woodmansee RG, Risser PG (eds). Wiley: New York; 29–45.
- Orfanidis S. 2007. Comments on the development of new macroalgal indices to assess water quality within the Mediterranean Sea. *Marine Pollution Bulletin* **54**: 626–627.
- Orfanidis S, Stamatis N, Tsiagga A, Ragias V, Schramm W. 2000. Eutrophication and marine benthic vegetation in the Lagoon of Vassova, Prefecture of Kavala. *Proceedings of 6th National Symposium on Oceanography & Fisheries* **1**: 429–433.
- Orfanidis S, Panayotidis P, Stamatis N. 2001. Ecological evaluation of transitional and coastal waters: a marine benthic macrophyte-based model. *Mediterranean Marine Science* **2**: 45–65.
- Orfanidis S, Panayotidis P, Stamatis N. 2003. An insight to the ecological evaluation index (EEI). *Ecological Indicators* **3**: 27–33.
- Orfanidis S, Stamatis N, Ragias V, Schramm W. 2005. Eutrophication patterns in an eastern Mediterranean coastal lagoon: Vassova, Delta Nestos, Macedonia, Greece. *Mediterranean Marine Science* **6**: 17–29.
- Orfanidis S, Reizopoulou S, Basset A. 2008. Transitional states in transitional waters (editorial). *Aquatic Conservation: Marine and Freshwater Ecosystems* **18**: S1–S3.
- Padilla DK, Allen BJ. 2000. Paradigm lost: reconsidering functional form and group hypotheses in marine ecology. *Journal of Experimental Marine Biology and Ecology* **250**: 207–221.
- Pedersen MF, Borum J. 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. *Marine Ecology Progress Series* **161**: 155–163.
- Petihakis G, Triantafyllou G, Koutsoubas D, Allen I, Dounas C. 1999. Modeling the annual cycle of nutrients and phytoplankton in a Mediterranean lagoon (Gialova, Greece). *Journal of Marine Environmental Research* **48**: 37–58.
- Pianka ER. 1970. On r- and K-selection. *American Naturalist* **104**: 592–597.
- Ponti M, Abbiati M. 2004. Quality assessment of transitional waters using a benthic biotic index: the case study of the Pialassa Baiona (Northern Adriatic Sea). *Aquatic*

- Conservation: Marine and Freshwater Ecosystems* **14**: S31–S41.
- Ponti M, Pinna M, Vadrucci MR, Orfanidis S. 2006. Biotic indices of transitional ecosystem health. Technical report of TWReferenceNET — EU INTERREG IIIB PROJECT 3B073, Management and Sustainable Development of Protected Transitional Waters. Available online at [http://www.ecologia.unile.it/e-portal/Modules/Econtent/Portale/index.html\(WP2, Assessment of proposed descriptors performance\)](http://www.ecologia.unile.it/e-portal/Modules/Econtent/Portale/index.html(WP2, Assessment of proposed descriptors performance)).
- Pykh YA, Kennedy ET, Grant WE. 2000. An overview of systems analysis methods in delineating environmental quality indices. *Ecological Modelling* **130**: 25–38.
- Raffaelli DG, Raven JA, Poole LJ. 1998. Ecological impact of green macroalgal blooms. *Oceanography and Marine Biology: An Annual Review* **36**: 97–125.
- Reizopoulou S, Nicolaidou A. 2007. Index of size distribution (ISD): a method of quality assessing for coastal lagoons. *Hydrobiologia* **577**: 141–149.
- Rindi F, Guiry M. 2004. Composition and spatio temporal variability of the epiphytic macroalgal assemblage of *Fucus vesiculosus* (Linnaeus) at Clare Island, Mayo, western Ireland. *Journal of Experimental Marine Biology and Ecology* **311**: 233–252.
- Rismondo A, Curiel D, Marzocchi M, Scattolin M. 1997. Seasonal patterns of *Cymodocea nodosa* biomass and production in the lagoon of Venice. *Aquatic Botany* **58**: 55–64.
- Rosenberg G, Ramus J. 1984. Uptake of inorganic nitrogen and seaweed surface area:volume ratios. *Aquatic Botany* **19**: 73–96.
- Salas F, Neto JM, Borja A, Marques JC. 2004. Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems: the case of Mondego estuary (Portugal). *Ecological Indicators* **4**: 215–225.
- Scheffer M. 1997. *The Ecology of Shallow Lakes*. London: Chapman and Hall.
- Schlichting CD. 1986. The evolution of phenotypic plasticity in plants. *Annual Review Ecology and Systematics* **17**: 667–693.
- Schramm W. 1999. Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. *Journal of Applied Phycology* **11**: 69–78.
- Schramm W, Nienhuis PH. 1996. *Marine benthic vegetation. Recent changes and the effects of Eutrophication*. Springer: New York.
- Sfriso A, Ghetti PF. 1998. Seasonal variation in biomass, morphometric parameters and production of seagrasses in the lagoon of Venice. *Aquatic Botany* **61**: 1–17.
- Sfriso A, Pavoni B, Marcomini A, Orto A. 1992. Macroalgae, nutrient cycles, and pollutants in the lagoon of Venice. *Estuaries* **15**: 517–528.
- Sfriso A, La Rocca B, Godini E. 2002. Inventario dei taxa macroalali in tre aree della laguna di Venezia a differente livello di trofia. *Società Veneziana Scienze Naturali* **27**: 85–99.
- Sommer U, Worm B. 2002. *Competition and Coexistence*. Ecological studies 161. Springer-Verlag.
- Stamatis N, Christoforidis A, Sakellarides Th, Konstantinou I, Orfanidis S, Albanis T. 2006. Heavy metals and POPs across different habitat-type sediments of a reference condition lagoon: Agiasma, Nestos Delta, Greece. In *Transitional States in Transitional and Coastal Waters: Identifying Mechanisms & Developing Indicators of Habitat or Water Quality Shifts. Proceedings of a Workshop*, Orfanidis S, Basset A (eds). Kavala, Greece.
- Steneck R, Dethier MN. 1994. A functional group approach to the structure of algal dominated communities. *Oikos* **69**: 476–498.
- Steneck RS, Watling L. 1982. Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. *Marine Biology* **68**: 299–319.
- Tagliapietra D, Volpi Ghirardini A. 2006. Notes on coastal lagoon typology in the light of the EU Water Framework Directive: Italy as a case study. *Aquatic Conservation: Marine and Freshwater Ecosystems* **16**: 457–467.
- Terrados J, Ros JD. 1992. Growth and primary production of *Cymodocea nodosa* (Ucria) Ascherson in a Mediterranean coastal lagoon: the Mar Menor (SE Spain). *Aquatic Botany* **43**: 63–78.
- Thiebaut G, Guérol F, Muller S. 2002. Are trophic and diversity indices on macrophyte communities pertinent tools to monitor water quality? *Water Research* **36**: 3602–3610.
- Thompson SM, Valiela I. 1999. Effect of nitrogen loading on enzyme activity of macroalgae in estuaries in Waquoit Bay. *Botanica Marina* **42**: 519–529.
- Underwood AJ. 1997. *Experiments in Ecology. Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press: Cambridge.
- Verhoeven JTA. 1979. The ecology of *Ruppia*-dominated communities in western Europe. I. Distribution of *Ruppia* representatives in relation to their autecology. *Aquatic Botany* **6**: 197–268.
- Verhoeven JTA. 1980. The ecology of *Ruppia*-dominated communities in western Europe. III. Aspects of production, consumption and decomposition. *Aquatic Botany* **8**: 209–253.
- Verlaque M. 2001. Checklist of the macroalgae of the Thau Lagoon (Hérault, France): a hot spot of marine species introduction in Europe. *Oceanologica Acta* **24**: 29–49.
- Viaroli P, Azzoni R, Bartoli M, Giordani G, Tajè L. 2001. Evolution of the trophic conditions and dystrophic outbreaks in the Sacca di Goro lagoon (Northern Adriatic Sea). In *Structures and Processes in the Mediterranean Ecosystems*, Faranda FM, Guglielmo L, Spezie G (eds). Springer Verlag: Milano; 467–475.
- Viaroli P, Giordani G, Bartoli M, Naldi M, Azzoni R, Nizzoli D, Ferrari I, Zaldívar JM, Bencivelli S, Castaldelli G, Fano EA. 2006. The Sacca di Goro lagoon and an arm of the Po River. In *The Handbook of Environmental Chemistry. Volume H: Estuaries*, Wangersky PJ (ed.). Springer-Verlag: 197–232.
- Viaroli P, Bartoli M, Giordani G, Naldi M, Orfanidis S, Zaldívar JM. 2008. Community shifts, alternative stable

- states, biogeochemical controls and feedbacks in eutrophic coastal lagoons: a brief overview. *Aquatic Conservation: Marine and Freshwater Ecosystems* (this issue).
- Warwick RM, Clarke KR. 1993. Comparing the severity of disturbance: a meta-analysis of marine macrobenthic community data. *Marine Ecology Progress Series* **92**: 221–231.
- Wiens JA, Stenseth NC, Van Horne B, Ims RA. 1993. Ecological mechanisms and landscape ecology. *Oikos* **66**: 369–380.
- Worm B, Karez R. 2002. Competition, coexistence and diversity on rocky shores. In *Competition and Coexistence. Ecological studies 161*, Sommer U, Worm B (eds). Springer-Verlag; 133–163.