



Mediterranean Marine Science

Vol 12, No 1 (2011)



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doi: 10.12681/mms.60

To cite this article:

ORFANIDIS, S., PANAYOTIDIS, P., & UGLAND, K. (2011). Ecological Evaluation Index continuous formula (EEI-c) application: a step forward for functional groups, the formula and reference condition values. *Mediterranean Marine Science*, *12*(1), 199–232. https://doi.org/10.12681/mms.60

Ecological Evaluation Index continuous formula (EEI-c) application: a step forward for functional groups, the formula and reference condition values

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Received: 30 November 2010; Accepted: 7 April 2011; Published on line: 20 April 2011

Abstract

The Ecological Evaluation Index continuous formula (EEI-c) was designed to estimate the habitat-based ecological status of rocky coastal and sedimentary transitional waters using shallow benthic macrophyte communities as bioindicators. This study aimed to remedy the weaknesses of the currently used EEI methodology in: (1) ecological status groups (ESG), (2) the formula, and (3) reference condition values.

A cluster analysis of twelve species traits was used to delineate ESGs. Two main clusters (ESG I, late-successional; ESG II, opportunistic) were identified that were hierarchically divided into three and two sub-clusters, respectively: ESG I comprised thick perennial (IA), thick plastic (IB) and shade-adapted plastic (IC) coastal water species, and angiosperm plastic (IA), thick plastic (IB) and shade-adapted plastic (IC) transitional water species. ESG II comprised fleshy opportunistic (IIB) and filamentous sheet-like opportunistic (IIA) species both in coastal and transitional waters.

To avoid discrete jumps at the boundaries between predefined ecological categories, a hyperbolic model that approximates the index values and expresses the ecosystem status in continuous numbers was developed. Seventy-four quantitative and destructive samples of the upper infralittoral *Cystoseira crinita* and coastal lagoon *Ruppia cirrhosa* communities from tentative pristine to less impacted sites in Greece verified 10 as an 'ideal' EEI-c reference condition value.

Keywords: Ecological status; Marine benthic macrophytes; Macroalgae; Angiosperms; Functional groups; Water Framework Directive.

Introduction

The European Water Framework Directive (WFD, 2000/60/EC) demands assessments of the ecological status of coastal and transitional waters by using biotic indices of different biology elements and type-specific reference conditions. One of the biological quality elements to be considered is benthic macrophytes (EC, 2000) including the two main groups of marine plants, macroalgae (seaweeds) and angiosperms (vascular plants). Although the WFD treats them separately, they have often been examined together because of morphological and functional similarities and the apparent overlap in habitats (ORFANIDIS *et al.*, 2001, 2008).

In order to implement the WFD in the Mediterranean Sea, different benthic macrophyte ecological quality indices are currently suggested for rocky coastal (GIACCONE & CATRA, 2004), (CARLIT; BALLESTEROS et al., 2007b), (BENTHOS; PINEDO et al., 2007), and sedimentary transitional waters (EXCLAME; DEROLEZ, 2007), (CYMOX; MASCARÓ et al., 2009), (MAQI; SFRISO et al., 2009), and one of them is applicable for both types (EEI; ORFANIDIS et al., 2001, 2003, 2008; PANAYOTIDIS et al., 2004). Other methodologies applied along Eastern Atlantic rocky coasts include the reduced species list index (RSL) (WELLS et al., 2007), the quality of rocky bottoms index (CFR) (JUANES et al., 2008) and opportunistic algal cover (KRAUSE-JENSEN et al., 2007).

The EEI, inspired by the 'alternative stable stages' theory (HOLLING, 1973; MAY, 1977), is based on the well-known pattern where anthropogenic stress, for example eutrophication and heavy metal pollution, shifts the ecosystem from being pristine, where late-successional species are dominant, to a degraded state, where opportunistic, nitrophilous species are dominant (Fig. 1; HOLLING, 1973; ODUM, 1985; TILMAN & LEHMAN, 2001). Such stepwise sudden qualitative and quantitative changes of marine plant communities correspond to phases I to III of increasing eutrophication but not to hypertrophic phase

IV where benthic macrophytes start to disappear (SCHRAMM, 1996). Human-induced shifts may be assessed by classifying benthic macrophytes into two functional groups that respond differently to environmental disturbance: the late-successional group with low growth rates and long life histories (Ecological Status Group I, mostly K-selection) and the opportunistic group with high growth rates and short life histories (ESG II, mostly r-selection) (MAC ARTHUR & WILSON, 1967). Certainly, no organism is completely r-selected or completely K-selected, but all must reach a compromise between the two extremes (r-, Kcontinuum) (PIANKA, 1970). Such a classification scheme is intended to combine ecophysiological traits like nutrient uptake, photosynthesis and growth rates with morphology and life-history strategies (LITTLER & LITTLER, 1980; LITTLER et al., 1983; see also PADILLA & ALLEN, 2000). Beside nutrients, light may also affect benthic macrophyte communities across eutrophication gradients (SCHRAMM & NIENHUIS, 1996; SCHRAMM, 1999), favouring species with shade-adapted characteristics (LOBBAN & HARRISON, 1994). The dominance of shade-adapted, slowgrowing species at moderately impacted sites (SOLTAN et al., 2001; PANAYOTIDIS et al., 2004; ORFANIDIS & PANAYO-TIDIS, 2005; AREVALO et al., 2007), which 'paradoxically' tolerate the strong irradiance of the upper infralittoral zone and behave as sun-adapted species (BELLAN, 1985), may be the combined result of these opposing selection pressures.

Several benthic macrophytes are known for their remarkable variations in morphology, physiology and life history, usually termed phenotypic plasticity, that enable them to adjust processes and structures to changing environmental factors



Fig. 1: Conceptual model of two alternative stable states of macroalgal communities across an ecological status gradient in coastal waters. A conventional (A) and dynamic (B) view of successional changes (Modified from ORFANIDIS *et al.*, 2005, 2008; VIAROLI *et al.*, 2008).

(ENSMINGER *et al.*, 2005). Such a plasticity may explain why certain slow growing (plastic) species such as *Corallina* (DÍ EZ *et al.*, 2009) or *Cymodocea* (CECERE *et al.*, 2009) also extend their distribution into the continuously changing and unpredictable waters.

On the one hand the EEI has been successfully implemented in coastal and transitional water ecosystems in Greece (ORFANIDIS *et al.*, 2001, 2008; PANAYO-TIDIS *et al.*, 2004; ORFANIDIS & PANAYOTIDIS, 2005) and in other Mediterranean Sea countries such as Slovenia (ORLANDO-BONACA *et al.*, 2008), Cyprus (CARLETTI & HEISKANEN, 2009), Italy (FALACE *et al.*, 2009) and Bulgaria (DENCHEVA, 2010). On the other hand, difficulties applying the EEI in specific coastal water sites in Spain (ARÉVALO *et* al., 2007), Croatia (IVEŠA et al., 2009) and Malta (AZZOPARDI & SCHEMBRI, 2009) were observed and discussed (ORFANIDIS, 2007; BALESTEROS et al., 2007a). Beside difficulties that may have been due to low data compatibility, the EEI was criticized for three reasons: (1) species of the same ESG may respond differently to similar stressors, such as species of the genera Cystoseira and Corallina, (2) the functional group approach used originally proposed to predict productivity and other ecological attributes (e.g. grazing resistance, competitive abilities, reproductive effort) and not water degradation (see also GUINDA et al., 2008; JUANES et al., 2008), and (3) the formula is non-continuous, i.e. one value for each ecological status class (ESC), allowing discrete jumps at the boundaries between these predefined ecological categories.

This paper aimed to develop the EEI continuous formula (EEI-c) by remedying the weaknesses of the currently used EEI methodology in terms of the following aspects: (1) the identification of ESGs using trait combinations in relative terms of species morphology, physiology, life strategy and distribution, (2) the development of a formula that expresses the ecosystem status in continuous numbers, and (3) verification of EEI-c reference condition values in putatively pristine coastal and transitional water sites of Greece. A theoretical example will be provided in order to review the application of the EEI-c as a tool for estimating the ecological status of coastal and transitional waters of Mediterranean Sea under the prescriptions of the WFD and as a paradigm for a broader use.

Materials and Methods

a) Ecological status groups

Different morphological (external morphology, internal anatomy, texture), physiological (surface area/volume ratio, photosynthetic/non-photosynthetic ratio, photosynthetic performance, growth, light adaptation) and life history (longevity, succession) traits along with distributional data across eutrophication gradients were selected. They were relevant to nutrient and light responses in accordance with the functional-form model (LITTLER & LITTLER, 1980; LITTLER et al., 1983) and other relevant literature (see LOBBAN & HARRISON, 1994; SCHRAMM & NIENHUIS, 1996). In total, twelve traits of nine coastal and eight transitional water taxa typical of the Mediterranean Sea were used to identify their ESGs. The trait scoring range was derived from the literature (Table 1; Table 2) and personal observations.

b) Continuous formula

According to ORFANIDIS *et al.* (2001, 2003) the assemblage of benthic macrophytes in each sample is assessed according to the coverage (%) of species (less than 30% coverage, between 30 and 60% coverage, above 60% coverage) belonging to the predefined categories of ESG I and ESG II. All samples are given two scores and may therefore be represented in an ordinary twodimensional plane where the x-coordinate is the score in ESG I and the y-axis is the score in ESG II.

In order to establish an ecological index representing the degree of stress in each sample, the various combinations of scores in ESG I and II are classified as bad, low, moderate, good and high with the respective scores of 2, 4, 6, 8 and 10. A two-dimensional representation of this classification by using colours for the scores is shown in Figure 2. Since this preliminary index has discrete jumps at the boundaries between the five ecological categories, a hyperbolic model that approximates the index values was developed.

c) Reference conditions

The assessment of water quality within WFD is based on the extent of deviation from reference sites (benchmarks). To define existing reference sites with absent of or very low pressures following criteria developed by the Mediterranean Geographical Intercalibration Group (MED-GIG) for coastal macroalgae were used: (1) no settlement with more than 1000 inhabitants/km2 in the next 15 km and/or more than 100 inhabitants/km² in the next 3 km within that area (number of inhabitants is restricted to winter population), (2) no more than 10% of artificial coastline, (3) no harbour (more than 100 boats) within 3 km, (4) no beach regeneration within 1 km,

2007), s=MANGIALAJO et al. (2007); t=IVEŠA et al. (2009).*=a very approximate estimation of abundance and Ecological Status Classes (ESC) due to low data comparability (rare= <30%, common=30-60%, abundant=>60%). Information not cited is based on diverse sources g=FALACE AND BRESSAN (2006), h=FALACE et al. (2005), i=TYLER-WALTERS (2008), j=GARRABOU AND BALLESTEROS (2000), o=CORMACI AND FURNARI (2003), p=PANAYOTIDIS et al. (2004), q=ORFANIDIS AND PANAYOTIDIS (2005), r=ARÉVALO et al. Trait-scoring matrix of typical Mediterranean upper infralittoral coastal water taxa. a=LJTTLER *et al.* (1983), b=TAYLOR *et al.* (1999), c=LITTLER AND ARNOLD (1982), d=(ORFANIDIS, unpublished data), e=VERGARA AND NIELL (1993), f=MARTÍ *et al.* (2005), k=CHRYSSOVERGIS AND PANAYOTIDIS (1995), 1=LAZARIDOU et al. (1997), m=DÍEZ et al. (1999), n=SOLTAN et al. (2001), and personal observations. Table 1

		Morphology			Phys	siology		Life	history	D	Distribution *	
cies	External morphology ^a	Internal anatomy ^a	Texture ^a	Surface area/ volume	Photosynthetic/ non- photosynthetic	Photosynthetic performance ^c and growth ^d	Light adaptation	Longevity	Succession	Presence in high-good ESC ^{k-t}	Presence in moderate ESC ^{k-t}	Presence in poor-bad ESC ^{k-t}
				(SA/V) ratio	ratio)						
va rigida	1 (thin tubular	1 (uncorticated,	1 (soft)	3 (high)	1 (high)	1 (high)	1 (sun	1 (annual)	1	1 (rare,	2 (common)	3 (abundant)
	and sheet-like)	one - several cells thick)		1			adapted)		(opportunistic)	common during spring)		
nrphyra	1 (thin tubular	1 (uncorticated,	1 (soft)	3 (high)	1 (high)	1 (high)	1 (sun	1 (annual)	1	1 (rare,	2 (common)	3 (abundant)
mgata	and sheet-like)	one - several cells thick)	~				adapted)	·	(opportunistic)	common during spring)		
adophora sp.	2 (delicately	2 (uniseriate,	1 (soft)	3 (high)	1 (high)	1 (high)	1 (sun	1 (annual)	1	1 (rare,	2 (common)	3 (abundant)
	branched)	multiseriate or					adapted)		(opportunistic)	common		
		lightly corticated)								during spring)		
racilaria	3 (coarsely	3 (corticated)	2 (fleshy)	3 (high)	1 (high)	1 (high)	1 (sun	1 (annual)	1	1 (rare)	2 (common)	3 (abundant)
acilis	branched upright)						adapted)		(opportunistic)			
stoseira	4 (thick blades	4 (differentiated,	ء س ر	1 (low)	2 (low)	2 (low)	1 (sun	2	2 (late-	3 (abundant)	1 (rare)	0 (absent)
ntophylla	and branches)	neavity corticated thick walled)	(leather)				adapted)	(perennial)	successional)			
												Ontinued)

	Presence in poor-bad ESC ^{k-t}	1 (rare)	1 (rare)	1 (rare)	1 (rare)
Distribution *	Presence in moderate ESC ^{kt}	2 (common)	1 (rare)	3 (abundant)	3 (abundant)
	Presence in high-good ESC ^{k-t}	2 (common)	2 (common, pristine closed Gulfs)	2 (common)	2 (common)
history	Succession	2 (late- successional)	2 (late- successional)	2 (late- successional)	2 (late- successional)
Life	Longevity	3 (perennial through rosette- shaped form basis ^g)	3 (perennial stipe ^h)	3 (perennial basis ['])	2 (perennial ⁱ)
	Light adaptation	1 (sun adapted)	1 (sun adapted)	2 (shade adapted, but sun adapted when nurients are high ^d , ^e)	2 (shade adapted ^f)
siology	Photosynthetic performance ^c and growth ^d	2 (low)	2 (low)	2 (low)	2 (low)
Phys	Photosynthetic/ non- photosynthetic ratio	2 (low)	2 (low)	2 (low)	2 (low)
	Surface area/ volume (SA/V) ratio	1 (low)	1 (low)	2 (mode- rate ^b)	1 (low)
	Texture ^a	3 (leather)	3 (leather)	4 (stony)	4 (stony or tough)
Morphology	Internal anatomy ^a	4 (differentiated, heavily corticated thick walled)	4 (differentiated, heavily corticated thick walled)	5 (calcified genicula, flexible intergenicula)	6 (calcified or non-calcified parallel cell rows)
	External morphology ^a	4 (thick blades and branches)	4 (thick blades and branches)	5 (articulated, calcareous, upright)	6 (epilithic, prostrate, encrusting)
	Species	Cystoseira compressa	Cystoseira barbata	Corallina caespitosa	Lithophyllum sp.

Table 1 (Continued)

(Continued)

ARNOLD (1982), $c=(ORFANIDIS, unpublished data), d=CUNHA AND DUARTE (2005), <math>e=MALEA \ at \ al. (2004), *=a very approximate$ Trait-scoring matrix of selected traits of typical Mediterranean transitional water taxa. a=LITTLER et al. (1983), b=LITTLER AND Table 2

		Presence in poor-bad	ESCkt	3 (abundant)	3 (abundant)		3 (abundant)	1 (rare)	1 (rare)	1 (rare)	1 (rare)
	Distribution *	Presence in moderate	ESC ^{k-t}	2 (common)	2 (common)		2 (common)	1 (rare)	1 (rare)	3 (abundant)	2 (common)
•6111	, .	Presence in high-good	ESC ^{k-t}	1 (rare, common during spring)	1 (rare,	during spring)	1 (rare)	2 (common, pristine closed Gulfs)	2 (common, pristine closed Gulfs)	2 (common)	3 (abundant)
	history	Succession		1 (opportunistic)	1 (concerninicatio)	(opportunition)	1 (opportunistic)	2 (late successional)	2 (late- successional)	2 (late successional)	2 (late- successional)
	Life	Longevity		1 (annual/ opportu- nistic)	1 (annual/	opportu- nistic)	1 (annual/ opportu- nistic)	2 (perennial to annual)	2 (perennial to annual)	1 (annual)	2 (perennial, with annual cohortsd)
		Light adaptation		1 (sun adapted)	1 (sun	auapicu)	1 (sun adapted)	1 (sun adapted)	1 (sun adapted)	2 (shade adapted)	1 (sun adapted)
	iology	Photosynthetic performance ^c	and growth ^d	1 (high)	1 (high)		1 (high)	2 (low)	2 (low)	2 (low)	2 (low in comparison to macroalgae and fast in comparison to seagrasses)
ice Daseu un c	Phys	Photosynthetic/ non-	photosynthetic ratio	1 (high)	1 (high)		1 (high)	2 (low)	2 (low)	2 (low)	2 (low, in relative terms to macroalgae)
Innual		Surface area/	volume (SA/V) ratio	2 (high)	2 (high)		2 (high)	1 (low)	1 (low)	1 (low)	1 (low)
		Texture ^a		1 (soft)	1 (soft)		2 (fleshy)	3 (leather)	3 (leather)	4 (tough)	3 (leather)
Countrat	Morphology	Internal anatomy ^a		1 (uncorticated, one-several cells thick)	2 (uniseriate,	lightly corticated)	3 (corticated)	4 (differentiated, thick walled) heavily corticated	4 (differentiated, heavily corticated thick walled)	5 (calcified)	6 (highly)6 (pidernis,(epidernis,mesophyll,vascularsystem)
	I	External morphology ^a		1 (thin tubular and sheet-like)	2 (delicately		3 (coarsely branched upright)	4 (thick blades and branches)	4 (thick blades and branches)	5 (epiphytic, prostrate, encrusting)	6 (highly differentiated from foliose to cylindrical (leafs, rhizomes, roots, flowers, fruits)
		Species		Ulva sp.	Cladophora sp.		Gracilaria bursa- pastoris	Cystoseira barbata	Halopithys incurva	Hydrolithon sp.	Cymodocea nodosa

	Presence in poor-bad ESC ^{k-t}	1 (rare)
Distribution *	Presence in moderate ESC ^{k-t}	2 (common)
	Presence in high-good ESC ^{k-t}	3 (abundant)
history	Succession	2 (late- successional)
Life	Longevity	2 (perennial to annuale)
	Light adaptation	1 (sun adapted)
siology	Photosynthetic performance ^c and growth ^d	2 (low in comparison to macroalgae and fast in comparison to seagrasses)
Phy	Photosynthetic/ non- photosynthetic ratio	2 (low, in relative terms to macroalgae)
	Surface area/ volume (SA/V) ratio	1 (low)
	Texture ^a	3 (leather)
Morphology	Internal anatomy ^a	 6 (highly differentiated (epidermis, mesophyll, vascular system)
	External morphology ^a	6 (highly differentiated from foliose to cylindrical (leafs, rhizomes, roots, flowers, fruits)
	Species	Ruppia sp.

(5) no industries within 3 km, (6) no fish farms within 1 km, (7) no desalination plants within 1 km, and (8) no evidence of perennial species (*Cystoseira* for coastal and angiosperms for transitional waters) regression due to other unconsidered impacts. For this survey the available data from year 2001 were used.

From 26 'Natura 2000' Aegean sites (Fig. 3) passed the above criteria, 62 quantitative and destructive samples were collected using a metallic frame (20 x 20 cm or 25 x 25 cm) during the period 1999-2000 (Table 3). They were representative of the photophilic *Cystoseira crinita* Duby community (MOLINIER, 1960) of 'Infralittoral rock moderately exposed or sheltered from wave actions and/or currents and tidal streams' (EUNIS code A3.2, A3.3) (EU Habitats Directive Annex I code 1170). These data are part of the Hellenic 'NATURA 2000' database built up by a scientific consortium (PANAYOTIDIS *et al.*, 2001).

Two sites in the Fanari lagoon (Fig. 4) were visited in November 2000 and July 2001 (see ORFANIDIS *et al.*, 2001). Similar sites were sampled again in July 2009. Five destructive random quadrates were sampled in each site using a metallic handheld box corer (17 cm x 17 cm x 15 cm; length x width x height; see ORFANIDIS *et al.*, 2008). They were representative of the 'Brackish coastal lagoons' (EUNIS) (EU Habitats Directive Annex I code 1150).

In the laboratory formalin-fixed samples were very carefully sorted and the species were identified at species and functional group levels. No detailed taxonomic analysis of cyanobacterial colonies was undertaken. In order to estimate percentage coverage, a transparent square PVC container, filled with tap water and with a square matrix divided into 100 squares on the bottom, was used. The surface covered in ver-

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Fig. 2: Estimation of EEI and the equivalent ESCs from a matrix based on the mean abundance (%) of ESGs.



Fig. 3: Map of putatively pristine sites in Greece.

tical projection floating in the seawater by each sorted taxon was quantified as follows: coastal sample coverage was analysed semi-quantitatively using a BRAUN-BLANQUET (1932) class midpoints scale with seven levels [r (rare)=0.01%, + (<1%)=0.5%, 1 (1-5%)=3%, 2 (5-25%)=15%, 3 (25-50%)=37.5%, 4 (50-75%)=62.5%, 5 (75-100%)=87.5%], whereas lagoon sample coverage was analysed quantitatively as the percentage of the sampling surface.

NATURA	Sampling	Mean	Geographical area	No.
code	time	depth (m)		samples
GR2420001	February 2000	0.45	Kafireas	3
GR3000003	May 1999	0.5	Sxinias-Marathonas	2
GR3000004	May 1999	0.5	Braurona	1
GR3000005	May 1999	0.5	Sounio, Partoclos	1
GR4110004	November 1999	0.5	Kaloni Gulf, Lesvos Island	1
GR4210004	June 1999	0.41	Kastelorizo, Ro, Strogili Ils	3
GR4210005	June 1999	0.5	Rhodes, Akramytis, Armenistis	2
GR4220010	October 1999	0.5	NW. Kythnos, Kefalos Cape	1
GR4220012	June 2000	0.5	N. Amorgos, Kinavos, Levitha, Mauria Ils	3
GR4220013	June 2000	0.5	Small Cyklades	2
GR1150007	Jully 1999	0.38	Limenaria, Thasos Island	4
GR1150009	September 1999	0.35	Eleutheron Gulf, Kavala	2
GR4110001	August 1999	0.34	Limnos Island	7
GR4110002	August 1999	0.43	Agios Eustratios Island	3
GR4120003	June 2000	0.3	Samos Island	2
GR4120004	November 1999	0.1	Fournoi Island	1
GR4130001	October 1999	0.4	N. Chios, Oinousses Ils	2
GR4210007	November 1999	0.23	Nisiros Island	4
GR4210008	November 1999	0.3	Kos Island	3
GR4220008	October 2000	0.25	Sifnos Island	4
GR4220017	October 2000	0.25	Despotiko Island	4
GR4210001	October 1999	0.37	Kasos Island	3
GR4210011	October 1999	0.3	Syrna Island	1
GR4220004	September 1999	0.2	Sikinos Island	1
GR4220006	September 1999	0.5	Polyaigos Island	1
GR4220007	September 1999	0.5	Antimilos Island	1
GR1130009	November 2000	0.7	Fanari Lagoon, Thrace	2
GR1130009	July 2001	0.6	Fanari Lagoon, Thrace	2
GR1130009	July 2009	0.65	Fanari Lagoon, Thrace	10

 Table 3

 Background data of reference site sampling protocol.

Data analysis

Taxa similarities were investigated using cluster analysis (group average) based on the Bray-Curtis similarity index of taxa trait scores after being Log(x+1) transformed. Untransformed data were analysed by SIMPER to identify species abundance and contribution (%) in the *Cystoseira crini*- *ta* community. All calculations were performed using the PRIMER v. 5.0 software package (CLARKE & GORLEY, 2001). STATISTICA 7 software was used to illustrate the hyperbolic function of the EEI-c, whereas EXCEL SOLVER software was used to estimate the parameters of the continuous formula. The EEI-c was calculated



Fig. 4: Similarity cluster derived from character sets (Table 1) showing the classification of selected coastal water macroalgae in five functional groups. ESG = Ecological Status Group; ESG I=late-successional; ESG II=opportunistic; ESG IA=thick perennial; ESG IB=thick plastic; ESG IC=shade-adapted plastic; ESG IIA=fleshy opportunistic; ESG IIB=filamentous sheet-like, opportunistic.

at the sample level according to the ESGs (IA, IB, IC, IIA, IIB) and using the newly developed continuous hyperbolic formula.

Results

a) Ecological status groups

Bray-Curtis similarity cluster analysis of the taxa presented in Tables 1 and 2 is shown in Figures 4 and 5. At 95% similarity two main clusters representing ESG I (slowgrowing, late-successional species) and ESG II (fast-growing, opportunistic species) were present in coastal and transitional water taxa. They were divided into three and two subclusters, respectively, as follows:

Coastal waters

1) *Cystoseira crinitophylla* Ercegovic formed one group that represents slow-growing, sun-adapted species with a thick, differentiated thallus and long life histories. They form late-successional communities, mainly in pristine environments due to their high demands for light and their high internal nutrient reserves (ESG IA). Other representatives of this group that only exist in coastal waters are certain *Cystoseira* species, for example *Cystoseira crinita* Duby and *C. mediterranea* Sauvageau. The angiosperm *Posidonia oceanica* (L.) Delile which only rarely exists in hard substratum habitats can tentatively be classified within this group.

2) Cystoseira compressa (Esper) Gerloff & Nizamuddin and C. barbata (Stackhouse) C. Agardh formed one group that represents slow-growing, sun-adapted species with a thick, differentiated thallus and high adaptive plasticity, which can survive adverse conditions by having a perennial stipe or basis. They form late-successional communities in pristine and moderately degraded environments (ESG IB). Other representatives of this group are the species Cystoseira compressa, Cystoseira barbata and



Fig. 5: Similarity cluster derived from character sets (Table 2) showing the classification of selected transitional water benthic macrophytes in five functional groups. ESG IA=angiosperm, plastic; ESG IB=thick, plastic; ESG IC=shade-adapted, plastic; ESG IIA=fleshy opportunistic; ESG IIB=filamentous sheet-like opportunistic. See Figure 2 for more details.

Sargassum vulgare. The angiosperm genera *Cymodocea* and *Zostera* which only rarely exist in hard substratum habitats can tentatively be classified within this group.

3) Corallina caespitosa R. H. Walker, J. Brodie & L. M. Irvine and Lithophyllum sp. formed one group that represents slow growing, shade-adapted calcareous jointed and crustose species that are resistant to herbivores and to adverse hydrodynamic conditions (ESG IC). They form late-successional communities in pristine and moderately degraded coasts. Other representatives of this group are Corallina granifera J.Ellis & Solander and Haliptilon virgatum (Zanardini) Garbary & H. W. Johansen. The non-calcareous crusts such as Ralfsia can tentatively be classified within this group.

4) *Gracilaria gracilis* (Stackhouse) M. Steentoft, L. M. Irvine & W. F. Farnham formed one group that represents fast-growing, sun-adapted, coarsely-branched species that can grow in all environments, but only to high abundances in degraded environments (ESG IIA). Other representatives of this group are species of the genera *Laurencia* and *Caulerpa*.

5) Ulva rigida C. Agardh, Porphyra elongata (Areschoug) Kylin and Cladophora sp. formed one group that represents fast-growing, sun-adapted filamentous and sheet-like species with high reproductive capacity and short life histories. They can grow in all environments but at high abundances they often form blooms, for example green tides, in highly degraded environments (ESG IIB). Other representatives of this group are species of the genera Ulva and Chaetomorpha. Cvanobacteria, although unique in several aspects of their biology, are provisionally included in this group because they can also be abundant in degraded environments under nitrogen limiting conditions.

Transitional waters

1) Cymodocea nodosa (Ucria) Ascher-

son and *Ruppia* sp. formed one group that represents relatively slow-growing (Table 2), sun-adapted perennial to annual marine angiosperms with high adaptive plasticity. They form late-successional communities in pristine and moderately degraded conditions (ESG IA). Other representatives of this group are species of the genus *Zostera*.

2) Cystoseira barbata and Halopithys incurva (Hudson) Batters formed one group that represents slow-growing, sun-adapted perennial to annual macroalgae. They form late-successional communities in pristine and moderately degraded environments (ESG IB). Other representatives of this group are species of the genus Sargassum.

3) *Hydrolithon* sp. formed a group that represents shade-adapted, slow-growing red algal calcareous crusts living mainly as epiphytes on seaweed thalli or angiosperm leaves (ESG IC).

4) *Gracilaria bursa-pastoris* (S. G. Gmelin) P. C. Silva and *Ulva* sp., *Cladophora* sp. formed groups similar to those of coastal water ESG IIA and ESG IIB groups, respectively.

The key functional traits and names of benthic macrophyte ESGs are summarized in Table 4. A provisional classification of the Mediterranean benthic macrophytes of coastal and transitional waters into ESGs is given in Tables 5 and 6, respectively.

Ecological status group value

Each ESG was valued differently within the main groups, ESG I and II. For ESG I, the criteria were phenotypic plasticity and light adaptation (e.g., *Cystoseira crinitophylla* <*C. compressa* <*Corallina* spp.), whereas for ESG II the criterion was growth rate (e.g. *Gracilaria* spp. <*Ulva* spp.). The mean group coverage (%) for an assemblage was estimated as follows: ESG I (% coverage) = [(IA*1) + (IB*0.8) + (IC*0.6)]ESG II (% coverage) = [(IIA*0.8) + (IIB*1)]

b) The continuous formula

To avoid discrete jumps at the boundaries between predefined ecological categories, the EEI-c was defined as a continuous function. A hyperbolic model was developed approximating the index values (Fig. 6). It is composed of two nonlinear forms (i.e., parabolic functions) of ESG I (the xaxis) and ESG II (the y-axis), respectively, and one interaction term (hyperbolic function). Since the axes represent coverage in percentages (i.e. 35% rather than 0.35) the index may be defined via the second order polynomial:

$$p(x,y) = a + b^{*}(x/100) + c^{*}(x/100)^{2} + d^{*}(y/100) + e^{*}(y/100)^{2} + f^{*}(x/100) * (y/100)$$

where x is the score in ESG I, y is the score in ESG II and a, ..., f are the coefficients of the hyperbola. However, an unwanted property of the polynomials is that they have no boundaries. In order to keep the values below 1 we simply cut off the polynomial values above 1; that is, we redefined the polynomial by:

$$f(x,y) = min\{1, p(x,y)\}$$

Finally, since we wanted index values between 2 and 10, the ecological index may be defined as

$$ESI(x,y) = 2 + 8*min\{1, p(x,y)\}$$

The six parameters $\{a,b,c,d,e,f\}$ are estimated in such a way that the difference between the hyperbola, ESI (x,y),

 Table 4

 Key functional traits and names of benthic macrophyte Ecological Status Groups.

No.	Functional	ESG IA	ESG IB	ESG IC	ESG IIA	ESG IIB
	traits					
A. (Coastal waters	•	•	•	•	
1.	Thallus morphology	thick	thick	calcareous upright and calcareous and non-calcareous crusts	fleshy	filamentous and leaf-like
2.	Growth	slow	slow	slow	fast	fast
3.	Light adaptation	sun-adapted	sun-adapted	shade-adapted	sun-adapted	sun-adapted
4.	Phenotypic plasticity	no	yes	yes	yes	yes
5.	Thallus longevity	perennial	perennial thallus basis or stipe	perennial thallus basis	annual	annual
6.	Succession	late-	late-	late-	opportunistic	opportunistic
		successional	successional	successional		
B. T	ransitional waters					
1.	Thallus morphology	angiosperm	thick	calcareous and non-calcareous crusts	fleshy	filamentous and leaf-like
2.	Growth	slow	slow	slow	fast	fast
3.	Light adaptation	sun-adapted	sun-adapted	shade-adapted	sun-adapted	sun-adapted
4.	Phenotypic plasticity	yes	yes	yes	yes	yes
5.	Thallus longevity	perennial to annual	perennial to annual	annual	annual	annual
6.	Succession	late- successional	late- successional	late- successional	opportunistic	opportunistic

and the preliminary step function (Fig. 5) is minimized. Using 2000 theoretical values, SOLVER in EXCEL gave the estimates:

$$a = 0.4680$$
 $b = 1.2088$ $c = -0.3583$
 $d = -1.1289$ $e = 0.5129$ $f = -0.1869$

Ecological quality ratio

To ensure comparability in accordance

with the WFD (REFCOND, 2003), the EEIc values ranging from 2 to 10 can be transformed into Ecological Quality Ratios from 0 to 1 (EQR, i.e. the ratio between the value of the observed biological parameter for a given surface water body and the expected value under the reference conditions), as follows:

EEI- c_{EQR} =1.25*(EEI-cvalue/RCvalue)-0.25, where RC=10

Table 5

No.	Taxon	ESG	No.	Taxon	ESG	No.	Taxon	ESG
1	Acetabularia	IC	64	Drachiella	IIA	127	Padina	IB
2	Acinetospora	IIB	65	Dudresnaya	IIB	128	Pedobesia	IIB
3	Acrochaetium	IIB	66	Ectocarpus	IIB	129	Penicillus	IIB
4	Acrodiscus	IIA	67	Entocladia	IIB	130	Petalonia	IIB
5	Acrosorium	IIA	68	Erythrocladia	IIB	131	Peyssonnelia	IC
6	Acrothamnion	IIB	69	Erythroglossum	IB	132	Phaeophila	IIB
7	Aglaothamnion	IIB	70	Erythropeltis	IIB	133	Phyllophora	IIA
8	Aglaozonia	IB	71	Erythrotrichia	IIB	134	Pleonosporium	IIB
9	Ahnfeltiopsis	IIA	72	Falkenbergia	IIB	135	Plocamium	IB
10	Alsidium	IIA	73	Feldmannia	IIB	136	Pneophyllum	IC
11	Amphirhoa	IC	74	Flabellia	IC	137	Polysiphonia	IIB
12	Anadyomene	IC	75	Fosliela	IC	138	Porphyra	IIB
13	Anotrichium	IIB	76	Ganonema	IC	139	Porphyrostromium	IIB
14	Antithamnion	IIB	77	Gastroclonium	IIA	140	Posidonia	IA
15	Antithamnionella	IIB	78	Gelidiella	IIA	141	Pringsheimiella	IIB
16	Asparagopsis	IIA	79	Gelidium	IIA	142	Pseudobryopsis	IIB
17	Asperococcus	IB	80	Giffordia	IIB	143	Pseudochlorodesmis	IIB
18	Auduniella	IIB	81	Gigartina	IIA	144	Pseudocrouania	IIB
19	Bangia	IIB	82	Goniotrichum	IIB	145	Pterocladia	IIA
20	Blastophysa	IIB	83	Gracilaria	IIA	146	Pterocladiella	IIA
21	Blidingia	IIB	84	Gracilariopsis	IIA	147	Pterosiphonia	IIB
22	Boergeseniella	IIA	85	Grateloupia	IIA	148	Pterothamnion	IIB
23	Botryocladia	IIA	86	Griffithsia	IIB	149	Radicilingua	IIA
24	Bryopsis	IIB	87	Gulsonia	IIB	150	Ralfsia	IC
25	Callithamnion	IIB	88	Halimeda	IC	151	Rhizoclonium	IIB
26	Caulacanthus	IIA	89	Haliptilon	IC	152	Rhodophyllis	IB
27	Caulerpa	IIA	90	Halodictyon	IIB	153	Rhodothamnionella	IIB
28	Centroceras	IIB	91	Halopitys	IB	154	Rhodymenia	IIA
29	Ceramium	IIB	92	Halopteris	IIA	155	Ruppia	IB
30	Chaetomorpha	IIB	93	Halurus	IIB	156	Rytiphlaea	IB
31	Champia	IIA	94	Halymenia	IIA	157	Sahlingia	IIB
32	Chondracanthus	IIA	95	Herposiphonia	IIB	158	Sarconema	IIA
33	Chondria	IIA	96	Hincksia	IIB	159	Sargassum	IB
34	Chondrophycus	IIA	97	Hydroclathrus	IIA	160	Schizymenia	IIA
35	Chondrus	IA	98	Hydrolithon	IC	161	Schottera	IIA
36	Choreonema	IC	99	Hypnea	IIA	162	Scinaia	IIA
37	Chroodactylon	IIB	100	Hypoglossum	IIA	163	Scytosiphon	IIB
38	Chrysymenia	IIA	101	Jania	IC	164	Spermothanmion	IIB
39	Chylocladia	IIA	102	Kallymenia	IIA	165	Sphacelaria	IIA
40	Cladophora	IIB	103	Kuckuckia	IIB	166	Sphaerotrichia	IIB

An indicative classification of coastal water Mediterranean upper infralittoral benthic macrophyte taxa into Ecological Status Groups.

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(Continued)

Table 5	(Continued)
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No.	Taxon	ESG	No.	Taxon	ESG	No.	Taxon	ESG
41	Cladostephus	IIA	104	Kuetzingiella	IIB	167	Sphondylothamnion	IIB
42	Codium	IIB	105	Laurencia	IIA	168	Spongites	IC
43	Colpomenia	IIA	106	Lejolisia	IIB	169	Spyridia	IIB
44	Corallina	IC	107	Liagora	IC	170	Stictyosiphon	IIB
45	Corallophila	IIB	108	Liebmannia	IIB	171	Stilophora	IIB
46	Corynophlaea	IIB	109	Lithophyllum	IC	172	Stylonema	IIB
47	Cottoniella	IIB	110	Lobophora	IIA	173	Stypocaulon	IIA
48	Crouania	IIB	111	Lomentaria	IIA	174	Taenioma	IIB
49	Culteria	IB	112	Lophosiphonia	IIB	175	Taonia	IB
50	Cyanobacteria	IIB	113	Melobesia	IC	176	Titanoderma	IC
51	Cymodocea	IB	114	Mesogloia	IIA	177	Tricleocarpa	IC
52	Cystoseira	IA	115	Mesophyllum	IC	178	Ulotrix	IIB
53	Cystoseira barbata	IB	116	Monosporus	IIB	179	Ulva	IIB
54	Cystoseira compressa	IB	117	Monostroma	IIB	180	Ulvella	IIB
55	Dasya	IIB	118	Myriactula	IIB	181	Valonia	IIB
56	Dasycladus	IIA	119	Myrionema	IIB	182	Vaucheria	IIB
57	Derbesia	IIB	120	Nanozostera	IB	183	Womersleyella	IIB
58	Dermatolithon	IC	121	Nemastoma	IIA	184	Wrangelia	IIB
59	Dictyopteris	IIA	122	Neosiphonia	IIB	185	Zanardinia	IIA
60	Dictyota	IIA	123	Neurocaulon	IIA	186	Zonaria	IIA
61	Digenea	IB	124	Nitophyllum	IIA	187	Zostera	IB
62	Dilophus	IIA	125	Osmundaria	IIA			
63	Dipterosiphonia	IIB	126	Osmundea	IIA			



Fig. 6: An illustration of EEI-c hyperbolic function.

where EEI- c_{EQR} values for coastal waters in Greece higher than 0.48 (±0.09SD) indicate sustainable ecosystems of good or high ESC, whereas EEI-c values lower than 0.48 indicate that the ecosystems should be restored to a higher ESC (Table 7).

c) Reference conditions

Aegean coastal sites

One hundred and thirteen (113) taxa were identified in total (73 Rhodophyceae, 25 Phaeophyceae, and 15 Chlorophyceae) in the *Cystoseira crinita* community of the Aegean reference condition sites (PANAYOTIDIS *et al.*, 2007). Twenty taxa of them contributed cumulatively by 99% in the community, whereas 3 taxa contributed cumulatively by 90% (Table 8). Besides *Cystoseira crinita*, which includes the morphologically similar species *C. crinitophylla*, other *Cystoseira* species with a high contribu-

tion in the community were C. compressa and C. barbata. Species with a lower contribution not included in Table 8 were C. brachycarpa var. balearica (Sauvageau) Giaccone, C. corniculata (Turner) Zanardini, C. mediterranea, and C. schiffneri G. Hamel. The understory layer of the community was dominated by the red coralligenous algae Corallina granifera, C. caespitosa and Jania rubens (Linnaeus) J. V. Lamouroux, and the brown alga Padina pavonica (Linnaeus) Thivy. Cystoseira crinita's epiphytes distinguished as: 1) filamentous green (Cladophora spp.), brown [Sphacelaria cirrosa (Roth) C.Agardh] and red [Herposiphonia secunda (C. Agardh) Ambronn] algae, and 2) encrusting red algae [Lithophyllum cystoseirae (Hauck) Heydrich and Hydrolithon spp.] These species were classified into five ESG. Five species were classified into ESG IA, nine species into ESG IB, twenty-three species into ESG IC, thirty-three species into ESG IIA, and forty-



Fig. 7: Variation of EEI-c in Aegean, Greece reference site samples. See Tables 3 and 7 for more details.

Table	6
Lanc	v

No.	Taxon	ESG	No.	Taxon	ESG
1	Acanthophora	IIA	32	Hincksia	IIB
2	Acetabularia	IIC	33	Hydrolithon	IIC
3	Acrothamnion	IIB	34	Hypnea	IIA
4	Agardhiella	IIA	35	Lamprothamnion	IB
5	Alsidium	IIA	36	Laurencia	IIA
6	Anotrichium	IIB	37	Lithophyllum	IC
7	Antithamnion	IIB	38	Lophosiphonia	IIB
8	Bangia	IIB	39	Monostroma	IIB
9	Blidingia	IIB	40	Nanozostera	IA
10	Boergeseniella	IIA	41	Nitophyllum	IIA
11	Callithamnion	IIB	42	Phaeophyla	IIB
12	Ceramium	IIB	43	Pneophyllum	IC
13	Chaetomorpha	IIB	44	Polysiphonia	IIB
14	Chondria	IIA	45	Porphyra	IIB
15	Chondrophycus	IIA	46	Pterothamnion	IIB
16	Cladophora	IIB	47	Rhizoclonium	IIB
17	Cyanobacteria	IIB	48	Ruppia	IA
18	Cymodocea	IA	49	Rytiphlea	IB
19	Cystoseira	IB	50	Rhodophylis	IIA
20	Dasya	IIB	51	Sargassum	IB
21	Dictyota	IIA	52	Solieria	IIA
22	Entocladia	IIB	53	Sphacelaria	IIA
23	Erythropeltis	IIB	54	Spyridia	IIA
24	Erythrotrichia	IIB	55	Stylonema	IIB
25	Fucus	IB	56	Ulotrix	IIB
26	Gastroclonium	IIA	57	Ulva	IIB
27	Gracilaria	IIA	58	Ulvella	IIB
28	Gracilariopsis	IIA	59	Undaria	IB
29	Griffithsia	IIB	60	Valonia	IIB
30	Halopitys	IIA	61	Vaucheria	IIB
31	Herposiphonia	IIB	62	Zostera	IA

An indicative classification of transitional water Mediterranean benthic macrophyte taxa into Ecological Status Groups.

three species into ESG IIB. While the mean coverage (%) of ESG II species was 22.89%, the mean coverage (%) of ESG I species was 111.34%. All of these EEI-c samples were either classified as high (89%) or good (11%) ESCs, with a mean value of 9.4 (Fig. 7). They verified 10 as an 'ideal' EEI-c

reference condition value for the Greek coastal waters.

Fanari coastal lagoon

Four macroalgae (*Chondria capillaris* (Hudson) M. J. Wynne, *Chaetomorpha mediterranea* (Kützing) Kützing, *Cladopho*-

Table 7

Ecological Status	EEI-c boundary	EEI-c _{EQR} boundary	No. of theoretical
Classes	values	values	values
High	9.72±0.46SD	0.97±0.06SD	334
Good-High	8.09±0.74 SD	$0.76 \pm 0.09 SD$	193
Good-Moderate	5.84±0.70 SD	$0.48 \pm 0.09 SD$	617
Moderate-Low	4.04±0.68 SD	0.25±0.08SD	383
Bad	2.34±0.78 SD	0.04±0.10SD	473

Ecological Status	Class boundaries	of transiti	onal and coastal	waters based on	the Ecological
Evaluation	Index continuous	formula (1	EEI-c) applied in	a 2000 theoretica	l values.

ra dalmatica Kützing and Cyanobacteria) and one angiosperm species (*Ruppia cirrhosa* (Petagna) Grande) were identified in total in the Fanari coastal lagoon. The species were classified into three ESGs. One species was classified into ESG IA, one species into ESG IIA, and two species into ESG IIB. While the mean coverage (%) of ESG II species was 29.77%, the mean coverage (%) of ESG I species was 104.64%. All of these EEI-c samples were either classified as high (79%) or good (21%) ESCs, with a mean

value of 9 (Fig. 8). They verified 10 as an 'ideal' EEI-c reference condition value for the Greek transitional waters.

d) A theoretical example

A hypothetical coastal zone was divided into four water bodies (WB): two coastal (A, D) with hard substrata, one coastal (C) and one transitional (B) with a sedimentary substratum (Fig. 9). The EEI-c can be applied in shallow (depth<1m) and vegetated (% coverage>10%) sites of the rocky coastal and sed-



Fig. 8: Variation of EEI-c in Fanari lagoon site samples. See Tables 3 and 7 for more details.

imentary transitional (salinity>10psu) WBs.

Within WB A, two coastal lines inhabited by different habitat types (HA1, HA2) were identified covering an area of 70 and 30%, respectively. In each habitat one or more permanent sites (10 m x 10 m; length)x width), apart by a distance of kilometres, with a well-developed (climax) macrophyte community were selected. At each site three random samples (25 cm x 25 cm; length x width) were taken two or more times a year in different seasons (preferably not in winter). The mean absolute coverage (%) of ESGs IA, IB, IC, IIA and IIB of a site sample of HA1 was 80, 40, 30, 15 and 5, respectively. By using the EEI-c this corresponded to high ESCs (EEI-c=10):

ESG I=(80x1)+(40x0.8)+(30x0.6)=130, ESG II=(15x0.8)+(5x1)=17

The average EEI-c value for all site samples was 9.8. Similarly, the average EEI-c value of all site samples of HA2 was 7.8.

The overall ESC of WB A was $(9.8\times0.7)+(7.8\times0.3)=9.2$, which corresponded to a high ESC and to EEI-c_{EQR}=0.9, reference conditions EEI=10. The ESC of WB D was estimated in a similar way.

Within WB B, a coastal lagoon, two habitat types (HB1, HB2) were identified covering an area of 40 and 60% of the coastal lagoon, respectively. In each habitat at least two permanent sites (15 m x 15 m; length x width), apart by a distance of kilometres, with a well-developed macrophyte community were selected. At each site four to five random samples (box corer; 17 cm x 17 cm x 15 cm; length x width x height) were taken once a year during summer. The mean absolute coverage (%) of ESGs IA, IB, IC, IIA and IIB in a site sample of HB1 was 60, 10, 10, 35 and 10, respectively. By using the EEIc this corresponded to good ESCs (EEI-c=8.07):

ESG I=
$$(60x1)$$
+ $(10x0.8)$ + $(10x0.6)$ =74
ESG II= $(35x0.8)$ + $(10x1)$ =38



Fig. 9: A hypothetical coastal zone including coastal and transitional water bodies of different Ecological Status Classes.

The average EEI-c value for all samples was 7.6. Similarly, the average EEI-c value of all site samples of HB2 was 3.8. The overall ESC of the coastal lagoon was (7.6x0.4)+(3.8x0.6)=5.3, which corresponds to moderate ESC and to EEI-c_{EQR}=0.41, reference conditions EEI-c=10.

Discussion

The EEI-c classification scheme can be applied (see theoretical example) in vegetated coastal and transitional water habitats where benthic macrophyte growth is not limited by hard substratum absence and by low salinity, respectively. To avoid any natural environmental gradient bias such as light attenuation down to water column sampling should be realized in shallow waters.

EEI-c takes into consideration species' functional attributes in an attempt to understand response mechanisms and to predict how communities are affected by human-induced stress. This is an effective way of reducing and capturing complexity in a restricted number of ESGs in that they are be regarded as recognizable units along a continuum, each containing considerable variation.

Trait selection for ESG identification and value is justified by the EEI-c hypothesis that mainly nutrient and light resource allocation strategies, including plastic and non-plastic responses, seem to be mainly involved in species adaptation across a water pollution gradient. Indeed, growth under certain conditions seems to be related to a species competence in exploiting the most abundant or limited resources, either through growth or colonization ability (CARPENTER, 1990; SCHRAMM, 1999; WORM & KAREZ, 2002). Any direct physiological responses such as heavy metal or ammonium toxicity, although they have been hypothesized (see GRÉMARE et al., 1998), might be ecologically relevant only under eutrophication conditions extreme (BURKHOLDER et al., 2007; LEONI et al., 2008), especially when multi-stressor interactions are considered (CLOERN, 2001). In agreement with this, laboratory heavy metal toxicity tests have indicated that benthic macrophytes are relative tolerant to heavy metals, with heavy metal toxicity depending on the cellular sites availability, which became increased with the fast growth of species, leading to enhanced toxicity tolerance (ORFANIDIS et al., 2009; PAPATHANASIOU et al., 2009). Other toxic substances, however, such as herbicides, affect sea grasses (NIELSEN & DAHLLÖF, 2007) and may therefore contribute to a shift in primary producers in transitional waters.

A biological index, BIOSTRESS, based on the same hypothesis, was suggested by UGLAND et al. (2008) in order to assess the community stress induced by human activity. This index is based on the relative abundances of various predefined opportunistic species with different tolerances to pollution. In our terminology, the BIOSTRESS index is based on the ecological species group consisting of pollution indicators (ESG II). It should be realized that the EEI-c allows for a finer resolution since it is based on the functional traits of two different ecological status groups. The idea of using functional indices to indicate resource availability changes that can alter species composition and abundance is supported by ecological theory (SCHEFFER et al., 2001), monitoring datasets (ORFANIDIS et al., 2008), and modelling (SPATHARIS et al., 2011).

The Mediterranean Sea, including the present study sites, shallow rocky coasts and coastal lagoons (Table 8, results section), is

Table 8
Cystoseira crinita community of the reference coastal sites of Aegean Sea, Greece,
identified by simper analysis.

Species	Mean	Contribution	Cumulative
	coverage (%)	(%)	(%)
Cystoseira crinita Duby	55.57	74.08	74.08
Corallina granifera J. Ellis & Solander	11.86	8.38	82.46
Cystoseira compressa (Esper) Gerloff & Nizamuddin	15.05	7.17	89.64
Jania rubens (Linnaeus) J. V. Lamouroux	7.84	3.77	93.41
Anotrichium barbatum (C. Agardh) Nägeli	2.61	1.2	94.61
Padina pavonica (Linnaeus) Thivy	3.57	0.79	95.4
Herposiphonia secunda (C. Agardh) Ambronn	1.39	0.52	95.92
Corallina caespitosa R. H. Walker, J. Brodie &	2.64	0.48	96.4
L. M. Irvine			
Cladophora spp.	2.41	0.42	96.81
Sphacelaria cirrosa (Roth) C. Agardh	0.9	0.4	97.21
Lithophyllum cystoseirae (Hauck) Heydrich	1.52	0.36	97.57
Dasya rigidula (Kützing) Ardissone	0.38	0.27	97.84
Laurencia obtusa (Hudson) J.V. Lamouroux	1.02	0.25	98.09
Hydrolithon farinosum (J. V. Lamouroux) D.Penrose	0.52	0.15	98.24
& Y.M.Chamberlain			
Cystoseira barbata (Stackhouse) C. Agardh	2.91	0.15	98.39
Asparagopsis armata Harvey	0.93	0.14	98.53
Herposiphonia secunda var. tenella (C. Agardh) Ambronn	1.1	0.14	98.67
Halopteris scoparia (Linnaeus) Sauvageau	2.02	0.14	98.81
Melobesia membranacea (Esper) J.V.Lamouroux	0.28	0.14	98.94
Dictyota dichotoma (Hudson) J. V. Lamouroux	1.05	0.1	99.05

inhabited by well-stratified climax benthic macrophyte communities suitable for indicating anthropogenic stress: they are easily accessible and directly exposed to human activities (PANAYOTIDIS *et al.*, 2004), and the competition among benthic macrophytes for resources may be high, whereas disturbance and physical stresses may be low (GRIME, 1979; CARPENTER, 1990). In oligotrophic and highly transparent pristine rocky coasts, slow growing, non-plastic *Cystoseira* species (ESG IA) may dominate (HOFFMAN *et al.*, 1988; RODRÍ GUEZ-PRIETO & POLO, 1996; BALLESTEROS *et al.*, 1998; BENEDETTI-CECCHI *et al.*, 2001; MANGALAJIO *et al.*, 2008; present study) by efficiently using their internally stored nutrient reserves to support growth during periods of nutrient shortage. Species of this group do not inhabit transitional waters, which are ecotones between land, sea and freshwater with continuous fluctuations (KJERFVE, 1994).

The plastic species *Cystoseira compres*sa and *C. barbata* (FALACE *et al.*, 2005; FALACE AND BRESSAN, 2006) can extend their distribution from pristine to moderately degraded coastal waters (LAZA-RIDOU *et al.*, 1997; PANAYOTIDIS *et al.*, 2004; ARÉVALO *et al.*, 2007; IVEŠA *et al.*, 2009) and fall within ESG IB. While Cystoseira compressa is a typical inhabitant of artificial substrates in harbours (THIBAUT et al., 2005b; pers. obs.), C. barbata is one of a few Cystoseira species inhabiting coastal lagoons (FALACE et al., 2009; SFRISO et al., 2009). Cymodocea nodosa and Ruppia sp., angiosperms with high phenotypic plasticity (MALEA et al., 2004; ORFANIDIS et al., 2010), behave in similar way. They may take advantage over macroalgae in oligotrophic conditions by using nutrients from the sediment and they may sustain growth in degraded conditions until light, due to epiphyte load, or high water turbidity become limiting (HEMMINGA & DUARTE, 2000).

On moderately impacted coasts, slowgrowing, shade-adapted calcareous species (ESG IC) often dominate (PANAYOTIDIS et al., 2004; ORFANIDIS & PANAYOTIDIS, 2005; ARÉVALO et al., 2007; IVEŠA et al., 2009). Of these species, the *Corallina* spp. deserve more attention due to their notorious presence and wide distribution across degradation gradients. They can inhabit less impacted conditions when canopy forming species are absent due to disturbance (such as articulated corallines; BENEDETTI-CECCHI et al., 2001) or artificial substratum (GACIA et al., 2007) to moderately degraded conditions (PANAYOTIDIS et al., 2004; PINEDO et al., 2007; BALESTEROS et al., 2007b). This high degree of fitness maintenance over broad ranges of environmental conditions seems to be facilitated through compensatory plastic responses of morphology (ALGARRA & NIELL, 1987) and physiology (ALGARRA et al., 1991; VERGARA & NIELL, 1993) to irradiance. Despite the repressing role of high irradiance levels on phycobiliprotein synthesis resulting in pigment degradation under N limitation, under N-sufficient conditions a partial r-phycoerythrin synthesis was observed (VERGARA & NIELL, 1993) that may support survival in sunny upper infralittoral zones. This pattern was also recently confirmed where Corallina thalli, only when cultivated in high nutrient concentrations (N=60 μ M, P=2 μ M), was able to compensate for an effective quantum yield $(\Delta F/Fm')$ decrease due to relatively high laboratory cultivation irradiance (55-60 µmol photons m⁻² s⁻¹) (Orfanidis, unpublished data). Additionally, coralline species prevent overgrowth by fleshy algae by using different mechanisms including the synthesis of antifouling compounds, the creation of refuges for herbivores that controls epiphytes, and thallus shedding and microtopography (see DALEO et al., 2006). Under high N supply coralline algae were outcompeted by faster growing green algae and cyanobacteria (GOLUBIC, 1970; LITTLER & MURRAY, 1975; CHRYSSOVERGIS & PANAYO-TIDIS, 1995; LAZARIDOU et al., 1999; SCHRAMM, 1999; DÍAZ et al., 2002; PANAYOTIDIS et al., 2004; ARÉVALO et al., 2007; WELLS et al., 2007).

The EEI classification scheme regards Corallina spp. as a late successional species (ESG I) that has a closer relationship to canopy-forming species than to opportunistic (ESG II) species, in accordance with its embedded information (AUSTONI et al., 2007). Such a view initiated discussions with the CARLIT classification group which values Corallina spp. rather differently, i.e., as being in the middle and closer to opportunistic species (8 out of 20 on the sensitivity level) (ARÉVALO et al., 2007; PINEDO et al., 2007; BALESTEROS et al., 2007b). Such an assessment, although it can describe Corallina as an indicator of moderately degraded conditions, underestimates its overall ecological role, which is emphasized by EEI. The EEI classification previously assessed *Corallina* as an indicator of moderate conditions only when its epiphytes, mainly filamentous species and cyanobacteria, were taken under consideration (Fig. 10). In a recent EEI-c classification scheme, however, this role was further clarified and received a different value from canopy forming species.

Nutrient excess and turbid conditions favour the growth of opportunistic macroalgae (HARLIN, 1995; SCHRAMM & NIENHUIS, 1996; VIAROLI *et al.*, 2008) due to their efficient nutrient assimilation (THOMPSON & VALIELA, 1999). As mainly mono-layered, opportunists, macroalgae may also demand lower light quantities for growth than perennial, multi-layered canopy forming macroalgae or rooted angiosperms (LOBBAN & HARRISON, 1994; HEMMINGA & DUARTE, 2000). The higher growth rates and reproduction capacity of leaf-like and filamentous mostly



Fig. 10: Corallina spp. covered with filamentous epiphytes sampled in Kavala, Greece (A) and Sète, France (B) during summer 2010.

green algae than coarsely branched fleshy species used to classify them in the ESG IIA and ESG IIB subgroups, respectively (LOBBAN & HARRISON, 1994). Although green macroalgae are the best competitors under highly degraded conditions (see above), several studies indicated that articulate corallines are generally more abundant than coarsely branched algae in anthropogenically degraded coasts (MURRAY & LITTLER, 1984; AIROLDI et al., 1995; BENEDETTI-CECCHI et al., 2001). Such patterns, which may be the combined result of factors other than light and nutrients, such as grazing or wave action, do not hinder EEIc applications. For example, a site in California, USA, that was investigated by MURRAY & LITTLER (1984), can be classified as poor to moderate ESC (EEI-c=4.5). Attention should be given when the EEI-c is applied to data referred as 'crusts' or 'turfs' because they could include different ESGs (see MURRAY & LITTLER, 1984; EDWARDS, 1998).

The EEI-c is based on marine benthic macrophytes inhabiting the water column (macroalgae and leaves of angiosperms) as well as the sediment (rhizome and roots of angiosperms) of coastal and transitional waters. Therefore, it provides a unifying habitat-based framework for an integrated ecological status evaluation. Its new continuous hyperbolic formula avoids discrete jumps at the boundaries between ESCs and it thereby improves regression analyses with continuous environmental variables.

The EEI-c is based on the absolute abundance of the ESGs and it is closely related to ecosystem functions or processes, e.g., nutrient cycling (ASMUS & ASMUS, 2000) and fish production (FONSECA *et al.*, 1996a, b). High values of the EEI-c, which are very close to the ideal value of 10 (Figs 7 and 8), indicate the existence of high ecologically and economically valued communities (see COSTANZA *et al.*, 1997) of high resilience (SCHEFFER *et al.*, 2001; PERKOL-FINKEL & AIROLDI, 2010). These communities exist in the Mediterranean Sea only in putatively pristine sites described as reference condition ecosystems (MOLINIER, 1960; GROS, 1978; VERLAQUE, 1987; BALLESTE-ROS, 1988; PANAYOTIDIS *et al.*, 2001; THIBAUT *et al.*, 2005a; MANGIALAJO *et al.*, 2007; present study) in the WFD ecological status assessment.

The EEI-c was designed to (1) cover the prerequisites of the European WFD, which is the operational tool for setting the objectives for water protection in Europe (EC 2000), and (2) to offer water managers worldwide a tool for comparing, ranking and setting management priorities at different spatial levels. Conceptually, it is more appropriate for assessing the impact of chronic pressures such as eutrophication, sedimentation, aquatic habitat destruction, pollution by organic matter, and general degradation.

Acknowledgements

We are very grateful to Inka Bartsch for constructive discussions on earlier drafts of this manuscript. We thank Miss K. Nakou for her technical assistance.

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