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Research Article

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

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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-, K-continuum) (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, slow-growing species at moderately impacted sites (SOLTAN *et al.,* 2001; PANAYOTIDIS *et al.,* 2004; ORFANIDIS & PANAYOTIDIS, 2005; ARÉVALO *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

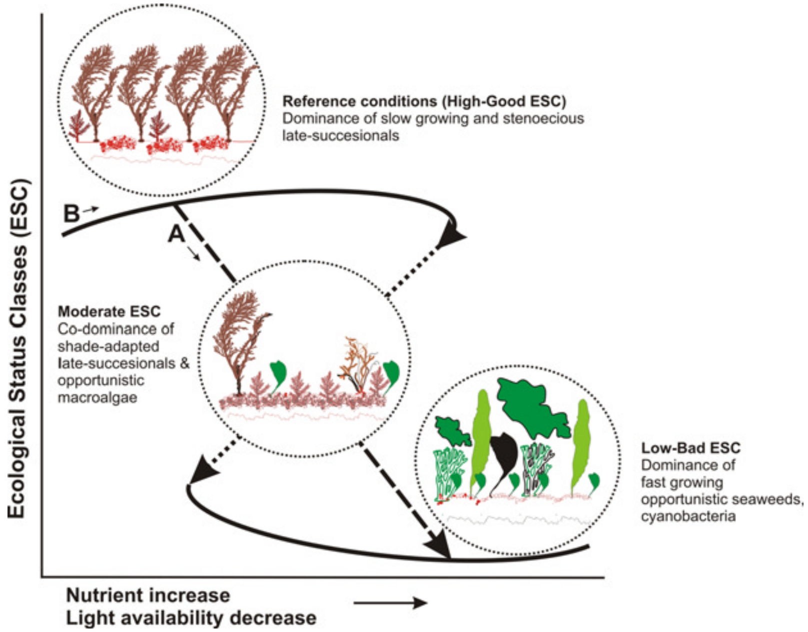

Fig. 1: Conceptual model of two alternative stable states of macroalgal communities across an ecological status gradient in coastal waters. A conventional (A) and dynamic (B) view of successional changes (Modified from ORFANIDIS *et al.,* 2005, 2008; VIAROLI *et al.,* 2008).

(ENSMINGER *et al.,* 2005). Such a plasticity may explain why certain slow growing (plastic) species such as *Corallina* (DÍEZ *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; PANAYOTIDIS *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 two-dimensional 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/km^2 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,

Table 1

Trait-scoring matrix of typical Mediterranean upper infralittoral coastal water taxa. a=LITTLER *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), g=FALACE AND BRESSAN (2006), h=FALACE *et al.* (2005), i=TYLER-WALTERS (2008), j=GARRABOU AND BALLESTEROS (2000), k=CHRYSSOVERGIS AND PANAYOTIDIS (1995), l=LAZARIDOU *et al.* (1997), m=DÍEZ *et al.* (1999), n=SOLTAN *et al.* (2001), o=CORMACI AND FURNARI (2003), p=PANAYOTIDIS *et al.* (2004), q=ORFANIDIS AND PANAYOTIDIS (2005), r=ARÉVALO *et al.* (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 and personal observations.

	Morphology			Physiology				Life history		Distribution *		
Species	**External morphology[a]**	**Internal anatomy[a]**	**Texture[a]**	**Surface area/ volume (SA/V) ratio**	**Photosynthetic/ non-photosynthetic ratio**	**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]**
Ulva rigida	1 (thin tubular and sheet-like)	1 (uncorticated, one - several cells thick)	1 (soft)	3 (high)	1 (high)	1 (high)	1 (sun adapted)	1 (annual)	1 (opportunistic)	1 (rare, common during spring)	2 (common)	3 (abundant)
Porphyra elongata	1 (thin tubular and sheet-like)	1 (uncorticated, one - several cells thick)	1 (soft)	3 (high)	1 (high)	1 (high)	1 (sun adapted)	1 (annual)	1 (opportunistic)	1 (rare, common during spring)	2 (common)	3 (abundant)
Cladophora sp.	2 (delicately branched)	2 (uniseriate, multiseriate or lightly corticated)	1 (soft)	3 (high)	1 (high)	1 (high)	1 (sun adapted)	1 (annual)	1 (opportunistic)	1 (rare, common during spring)	2 (common)	3 (abundant)
Gracilaria gracilis	3 (coarsely branched upright)	3 (corticated)	2 (fleshy)	3 (high)	1 (high)	1 (high)	1 (sun adapted)	1 (annual)	1 (opportunistic)	1 (rare)	2 (common)	3 (abundant)
Cystoseira crinitophylla	4 (thick blades and branches)	4 (differentiated, heavily corticated thick walled)	3 (leather)	1 (low)	2 (low)	2 (low)	1 (sun adapted)	2 (perennial)	2 (late-successional)	3 (abundant)	1 (rare)	0 (absent)

(Continued)

Table 1 (Continued)

	Morphology			Physiology				Life history		Distribution *		
Species	**External morphology**[a]	**Internal anatomy**[a]	**Texture**[a]	**Surface area/ volume (SA/V) ratio**	**Photosynthetic/ non-photosynthetic ratio**	**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]
Cystoseira compressa	4 (thick blades and branches)	4 (differentiated, heavily corticated thick walled)	3 (leather)	1 (low)	2 (low)	2 (low)	1 (sun adapted)	3 (perennial through rosette-shaped form basis[g])	2 (late-successional)	2 (common)	2 (common)	1 (rare)
Cystoseira barbata	4 (thick blades and branches)	4 (differentiated, heavily corticated thick walled)	3 (leather)	1 (low)	2 (low)	2 (low)	1 (sun adapted)	3 (perennial stipe[h])	2 (late-successional)	2 (common, pristine closed Gulfs)	1 (rare)	1 (rare)
Corallina caespitosa	5 (articulated, calcareous, upright)	5 (calcified genicula, flexible intergenicula)	4 (stony)	2 (moderate[b])	2 (low)	2 (low)	2 (shade adapted, but sun adapted when nurients are high[d, e])	3 (perennial basis[i])	2 (late-successional)	2 (common)	3 (abundant)	1 (rare)
Lithophyllum sp.	6 (epilithic, prostrate, encrusting)	6 (calcified or non-calcified parallel cell rows)	4 (stony or tough)	1 (low)	2 (low)	2 (low)	2 (shade adapted[f])	2 (perennial[j])	2 (late-successional)	2 (common)	3 (abundant)	1 (rare)

(Continued)

Table 2

Trait-scoring matrix of selected traits of typical Mediterranean transitional water taxa. a=LITTLER *et al.* (1983), b=LITTLER AND ARNOLD (1982), c=(ORFANIDIS, unpublished data), d=CUNHA AND DUARTE (2005), e=MALEA *et al.* (2004), *=a very approximate estimation of abundance based on different sources. See Table 1 for more details.

	Morphology			Physiology				Life history		Distribution *		
Species	**External morphology[a]**	**Internal anatomy[a]**	**Texture[a]**	**Surface area/ volume (SA/V) ratio**	**Photosynthetic/ non-photosynthetic ratio**	**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]**
Ulva sp.	1 (thin tubular and sheet-like)	1 (uncorticated, one-several cells thick)	1 (soft)	2 (high)	1 (high)	1 (high)	1 (sun adapted)	1 (annual/ opportu-nistic)	1 (opportunistic)	1 (rare, common during spring)	2 (common)	3 (abundant)
Cladophora sp.	2 (delicately branched)	2 (uniseriate, multiseriate or lightly corticated)	1 (soft)	2 (high)	1 (high)	1 (high)	1 (sun adapted)	1 (annual/ opportu-nistic)	1 (opportunistic)	1 (rare, common during spring)	2 (common)	3 (abundant)
Gracilaria bursa- pastoris	3 (coarsely branched upright)	3 (corticated)	2 (fleshy)	2 (high)	1 (high)	1 (high)	1 (sun adapted)	1 (annual/ opportu-nistic)	1 (opportunistic)	1 (rare)	2 (common)	3 (abundant)
Cystoseira barbata	4 (thick blades and branches)	4 (differentiated, thick walled) heavily corticated	3 (leather)	1 (low)	2 (low)	2 (low)	1 (sun adapted)	2 (perennial to annual)	2 (late successional)	2 (common, pristine closed Gulfs)	1 (rare)	1 (rare)
Halopithys incurva	4 (thick blades and branches)	4 (differentiated, heavily corticated thick walled)	3 (leather)	1 (low)	2 (low)	2 (low)	1 (sun adapted)	2 (perennial to annual)	2 (late-successional)	2 (common, pristine closed Gulfs)	1 (rare)	1 (rare)
Hydrolithon sp.	5 (epiphytic, prostrate, encrusting)	5 (calcified)	4 (tough)	1 (low)	2 (low)	2 (low)	2 (shade adapted)	1 (annual)	2 (late successional)	2 (common)	3 (abundant)	1 (rare)
Cymodocea nodosa	6 (highly differentiated from foliose to cylindrical (leafs, rhizomes, roots, flowers, fruits)	6 (highly) differentiated (epidermis, mesophyll, vascular system)	3 (leather)	1 (low)	2 (low, in relative terms to macroalgae)	2 (low in comparison to macroalgae and fast in comparison to seagrasses)	1 (sun adapted)	2 (perennial, with annual cohortsd)	2 (late-successional)	3 (abundant)	2 (common)	1 (rare)

Table 2 (Continued)

Species	Morphology			Physiology				Life history		Distribution *		
	External morphology[a]	Internal anatomy[a]	Texture[a]	Surface area/volume (SA/V) ratio	Photosynthetic/non-photosynthetic ratio	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]
Ruppia sp.	6 (highly differentiated from foliose to cylindrical (leafs, rhizomes, roots, flowers, fruits)	6 (highly differentiated (epidermis, mesophyll, vascular system)	3 (leather)	1 (low)	2 (low, in relative terms to macroalgae)	2 (low in comparison to macroalgae and fast in comparison to seagrasses)	1 (sun adapted)	2 (perennial to annuale)	2 (late-successional)	3 (abundant)	2 (common)	1 (rare)

(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 hand-held 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-

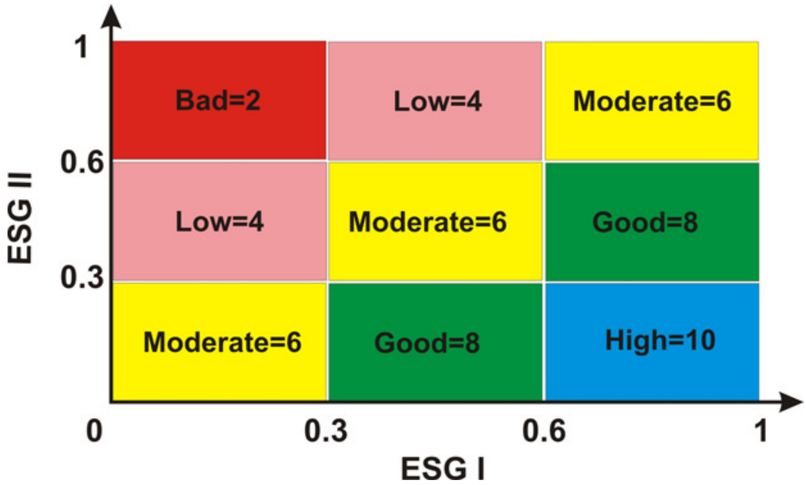

Fig. 2: Estimation of EEI and the equivalent ESCs from a matrix based on the mean abundance (%) of ESGs.

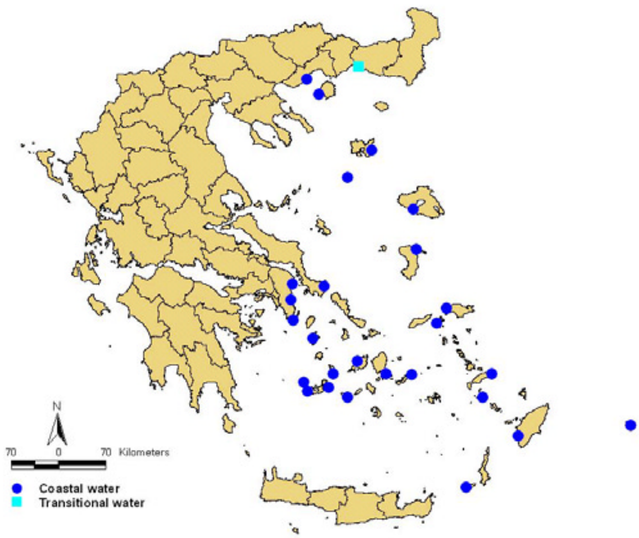

Fig. 3: Map of putatively pristine sites in Greece.

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.

Table 3
Background data of reference site sampling protocol.

NATURA code	Sampling time	Mean depth (m)	Geographical area	No. 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

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 crinita* 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

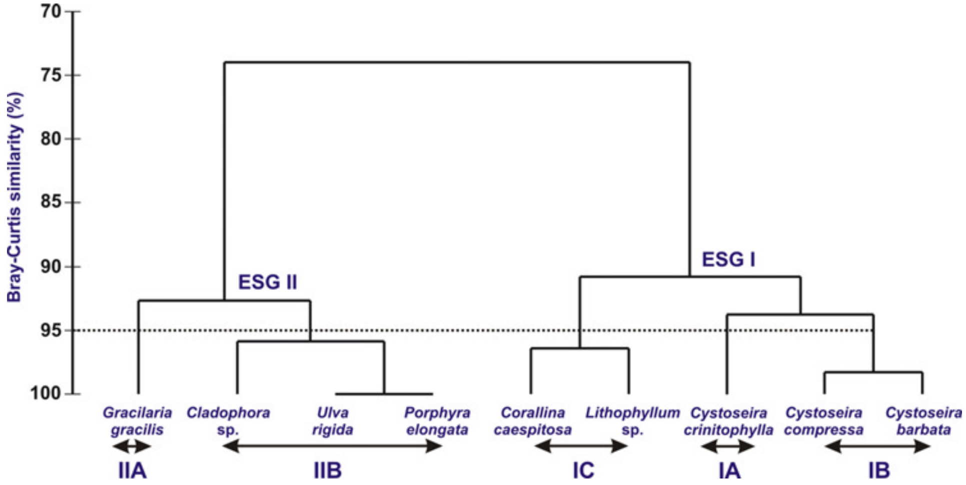

Fig. 4: Similarity cluster derived from character sets (Table 1) showing the classification of selected coastal water macroalgae in five functional groups. ESG = Ecological Status Group; ESG I=late-successional; ESG II=opportunistic; ESG IA=thick perennial; ESG IB=thick plastic; ESG IC=shade-adapted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

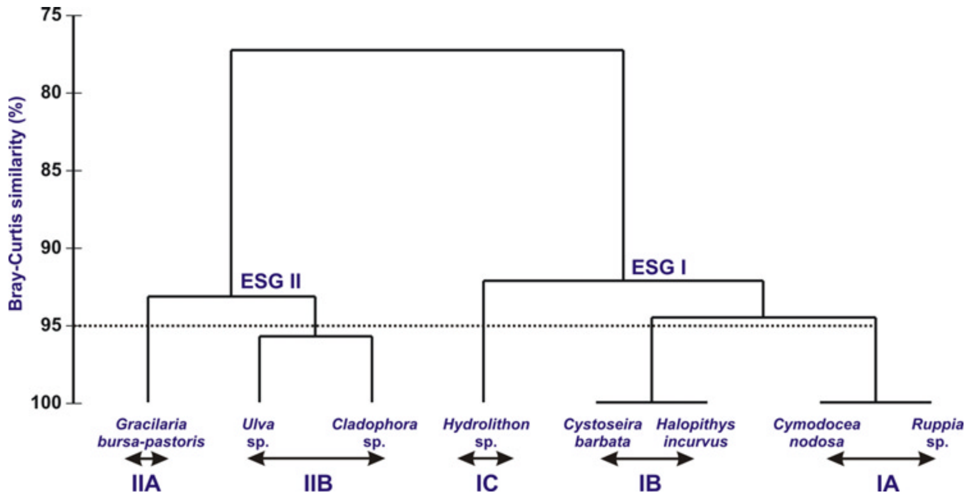

Fig. 5: Similarity cluster derived from character sets (Table 2) showing the classification of selected transitional water benthic macrophytes in five functional groups. ESG IA=angiosperm, plastic; ESG IB=thick, plastic; ESG IC=shade-adapted, plastic; ESG IIA=fleshy opportunistic; ESG IIB=filamentous 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.* Cyanobacteria, 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 x-axis) 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 $a, \ldots, f$ 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 traits	ESG IA	ESG IB	ESG IC	ESG IIA	ESG IIB
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-successional	late-successional	late-successional	opportunistic	opportunistic
B. Transitional 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:

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

Ecological quality ratio

To ensure comparability in accordance with the WFD (REFCOND, 2003), the EEI-c 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:

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

Table 5
An indicative classification of coastal water Mediterranean upper infralittoral benthic macrophyte taxa into Ecological Status Groups.

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

(Continued)

Table 5 (Continued)

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			

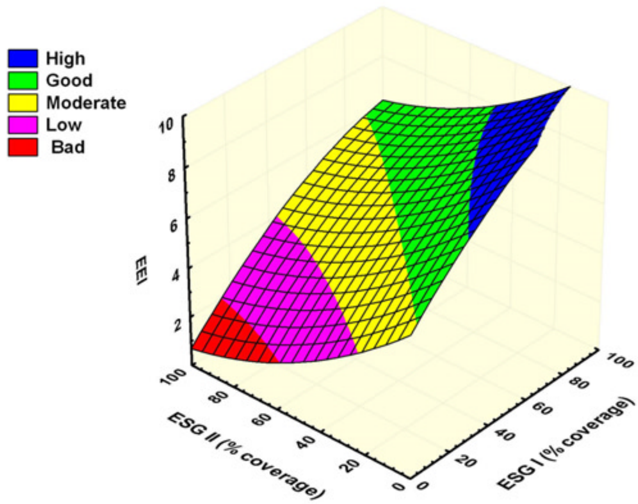

Fig. 6: An illustration of EEI-c hyperbolic function.

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 contribution 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-

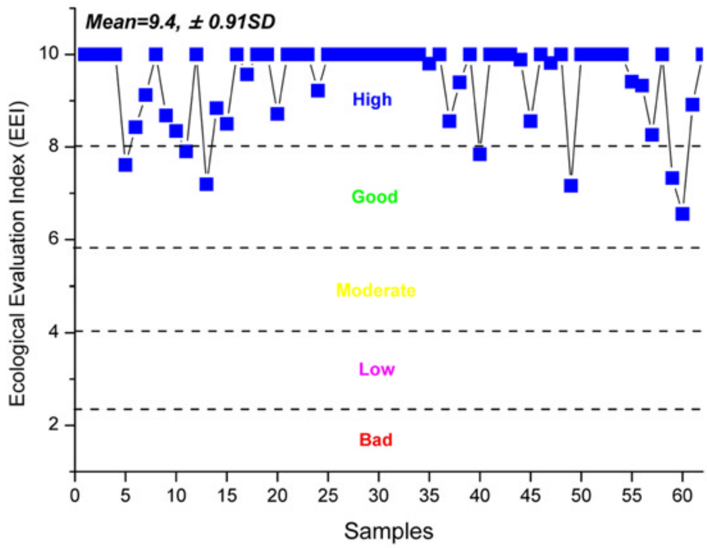

Fig. 7: Variation of EEI-c in Aegean, Greece reference site samples. See Tables 3 and 7 for more details.

Table 6
An indicative classification of transitional water Mediterranean benthic macrophyte taxa into Ecological Status Groups.

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

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 Class boundaries of transitional and coastal waters based on the Ecological Evaluation Index continuous formula (EEI-c) applied in 2000 theoretical values.

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

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-

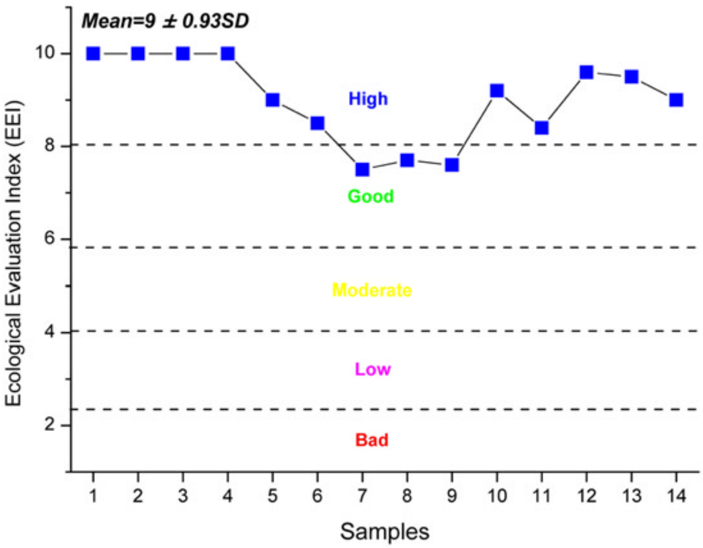

Fig. 8: Variation of EEI-c in Fanari lagoon site samples. See Tables 3 and 7 for more details.

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):

$$\text{ESG I}=(80\text{x}1)+(40\text{x}0.8)+(30\text{x}0.6)=130,$$
$$\text{ESG II}=(15\text{x}0.8)+(5\text{x}1)=17$$

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 (9.8x0.7)+(7.8x0.3)=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 EEI-c this corresponded to good ESCs (EEI-c=8.07):

$$\text{ESG I}=(60\text{x}1)+(10\text{x}0.8)+(10\text{x}0.6)=74$$
$$\text{ESG II}=(35\text{x}0.8)+(10\text{x}1)=38$$

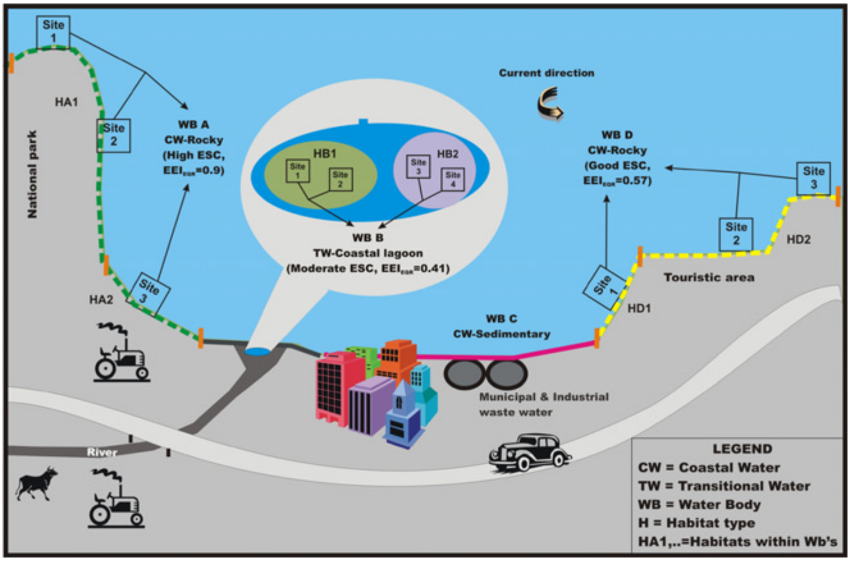

Fig. 9: A hypothetical coastal zone including coastal and transitional water bodies of different Ecological Status Classes.

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 extreme eutrophication conditions (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 coverage (%)	Contribution (%)	Cumulative (%)
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 & L. M. Irvine	2.64	0.48	96.4
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 & Y.M.Chamberlain	0.52	0.15	98.24
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; RODRÍGUEZ-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 compressa* and *C. barbata* (FALACE *et al.,* 2005; FALACE AND BRESSAN, 2006) can extend their distribution from pristine to moderately degraded coastal waters (LAZARIDOU *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, slow-growing, 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 μM, P=2 μM), 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 & PANAYOTIDIS, 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

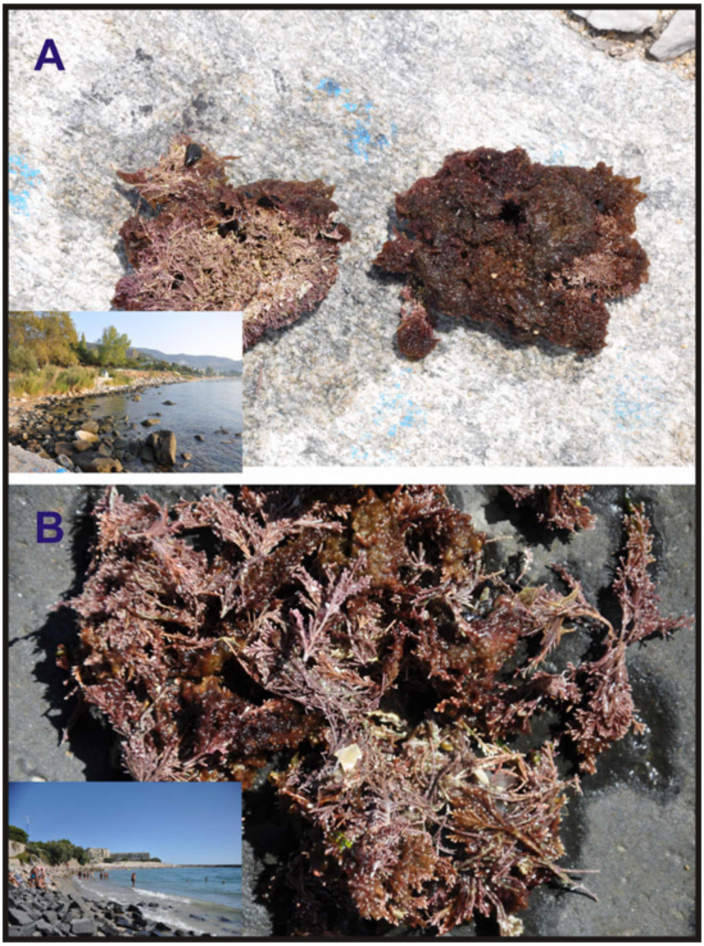

Fig. 10: *Corallina* spp. covered with filamentous epiphytes sampled in Kavala, Greece (A) and Sète, France (B) during summer 2010.

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 EEI-c 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; BALLESTEROS, 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.

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