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## 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.

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### Introduction

The European Water Framework Directive (WFD, 2000/60/EC) demands assess-

ments 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 bio-

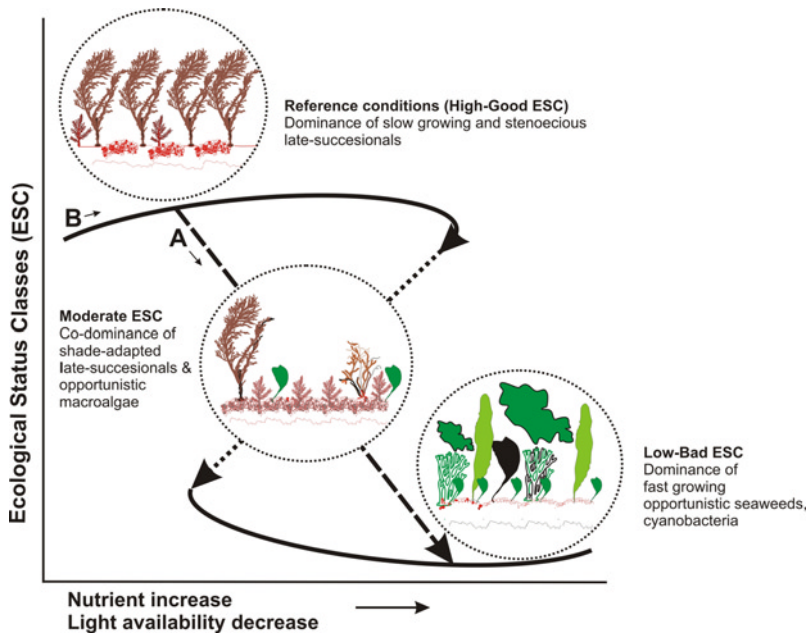
logical quality elements to be considered is benthic macrophytes (EC, 2000) including the two main groups of marine plants, macroalgae (seaweeds) and angiosperms (vascular plants). Although the WFD treats them separately, they have often been examined together because of morphological and functional 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) (MACARTHUR & 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 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/km<sup>2</sup> in the next 15 km and/or more than 100 inhabitants/km<sup>2</sup> 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=CHRYSOSOVERGIS 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=AREVALO *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.

| Species                         | Morphology                       |   |                      | Physiology                       |   |   | Life history     |               |                       | Distribution *                          |  |  |
|---------------------------------|----------------------------------|---|----------------------|----------------------------------|---|---|------------------|---------------|-----------------------|---|--|--|
|                                 | External morphology <sup>a</sup> | Internal anatomy <sup>a</sup>                       | Texture <sup>a</sup> | Surface area/volume (SA/V) ratio | Photosynthetic/non-photosynthetic ratio | Photosynthetic performance <sup>c</sup> and growth <sup>d</sup> | Light adaptation | Longevity     | Succession            | Presence in high-good ESC <sup>kt</sup> | Presence in moderate ESC <sup>kt</sup> | Presence in poor-bad ESC <sup>kt</sup> |
| <i>Uva rigida</i>               | 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)                           |
| <i>Porphyra elongata</i>        | 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)                           |
| <i>Cladophora</i> 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)                           |
| <i>Gracilaria gracilis</i>      | 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)                           |
| <i>Cystoseira crinitophylla</i> | 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)

| Species                     | Morphology                           |   | Physiology           |                                  |   |   | Life history   |   | Distribution *        |   |  |  |
|-----------------------------|--------------------------------------|---|----------------------|----------------------------------|---|---|--|---|-----------------------|---|--|--|
|                             | External morphology <sup>a</sup>     | Internal anatomy <sup>a</sup>                       | Texture <sup>a</sup> | Surface area/volume (SA/V) ratio | Photosynthetic/non-photosynthetic ratio | Photosynthetic performance <sup>e</sup> and growth <sup>d</sup> | Light adaptation   | Longevity   | Succession            | Presence in high-good ESC <sup>kt</sup> | Presence in moderate ESC <sup>kt</sup> | Presence in poor-bad ESC <sup>kt</sup> |
| <i>Cystoseira compressa</i> | 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 <sup>b</sup> ) | 2 (late-successional) | 2 (common)                              | 2 (common)                             | 1 (rare)                               |
| <i>Cystoseira barbata</i>   | 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 <sup>b</sup> )                             | 2 (late-successional) | 2 (common, pristine closed Gulfs)       | 1 (rare)                               | 1 (rare)                               |
| <i>Corallina caespitosa</i> | 5 (articulated, calcareous, upright) | 5 (calcified genicula, flexible intergenicula)      | 4 (stony)            | 2 (moderate <sup>b</sup> )       | 2 (low)                                 | 2 (low)   | 2 (shade adapted, but sun adapted when nurients are high <sup>d, e</sup> ) | 3 (perennial basis)   | 2 (late-successional) | 2 (common)                              | 3 (abundant)                           | 1 (rare)                               |
| <i>Lithophyllum</i> 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)  | 2 (perennial)   | 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=LITTLE *et al.* (1983), b=LITTLE 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.

| Species                          | Morphology  |   |                      | Physiology                       |  |  |                   | Life history                       |                       |   | Distribution *                         |  |  |
|----------------------------------|---|---|----------------------|----------------------------------|--|--|-------------------|------------------------------------|-----------------------|---|--|--|--|
|                                  | External morphology <sup>a</sup>  | Internal anatomy <sup>a</sup>                                     | Texture <sup>a</sup> | Surface area/volume (SA/V) ratio | Photosynthetic non-photosynthetic ratio  | Photosynthetic performance <sup>c</sup> and growth <sup>d</sup>          | Light adaptation  | Longevity                          | Succession            | Presence in high-good ESC <sup>kt</sup> | Presence in moderate ESC <sup>kt</sup> | Presence in poor-bad ESC <sup>kt</sup> |  |
| <i>Ulva</i> 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/opportunistic)           | 1 (opportunistic)     | 1 (rare, common during spring)          | 2 (common)                             | 3 (abundant)                           |  |
| <i>Cladophora</i> sp.            | 2 (delicately branched)   | 2 (uniserial, multiseriate or lightly corticated)                 | 1 (soft)             | 2 (high)                         | 1 (high)                                 | 1 (high)   | 1 (sun adapted)   | 1 (annual/opportunistic)           | 1 (opportunistic)     | 1 (rare, common during spring)          | 2 (common)                             | 3 (abundant)                           |  |
| <i>Gracilaria bursa-pastoris</i> | 3 (coarsely branched upright)   | 3 (corticated)  | 2 (fleshy)           | 2 (high)                         | 1 (high)                                 | 1 (high)   | 1 (sun adapted)   | 1 (annual/opportunistic)           | 1 (opportunistic)     | 1 (rare)                                | 2 (common)                             | 3 (abundant)                           |  |
| <i>Cyrtosira barbata</i>         | 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)                               |  |
| <i>Halophilys incurva</i>        | 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)                               |  |
| <i>Hydrolythion</i> 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)                               |  |
| <i>Cymodocea nodosa</i>          | 6 (highly differentiated from foliose to cylindrical (leaves, 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 cohorts) | 2 (late successional) | 3 (abundant)                            | 2 (common)                             | 1 (rare)                               |  |



Table 2 (Continued)

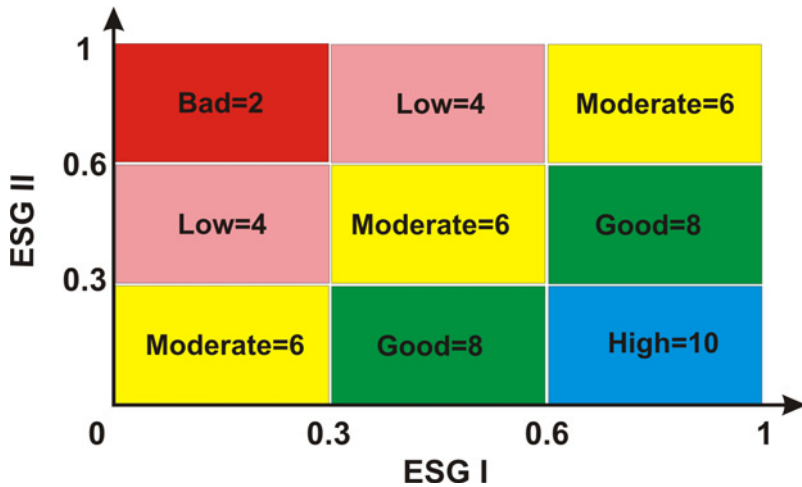
| Species           | Morphology   |   | Physiology           |                                  |  |  | Life history     |                          | Distribution *        |   |  |  |
|-------------------|--|---|----------------------|----------------------------------|--|--|------------------|--------------------------|-----------------------|---|--|--|
|                   | External morphology <sup>a</sup>   | Internal anatomy <sup>a</sup>                                     | Texture <sup>a</sup> | Surface area/volume (SA/V) ratio | Photosynthetic/non-photosynthetic ratio  | Photosynthetic performance <sup>c</sup> and growth <sup>d</sup>          | Light adaptation | Longevity                | Succession            | Presence in high-good ESC <sup>kt</sup> | Presence in moderate ESC <sup>kt</sup> | Presence in poor-bad ESC <sup>kt</sup> |
| <i>Ruppia</i> sp. | 6 (highly differentiated from foliose to cylindrical (leaves, 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 an-giosperms for transitional waters) regression due to other unconsidered impacts. For this survey the available data from year 2001 were used.

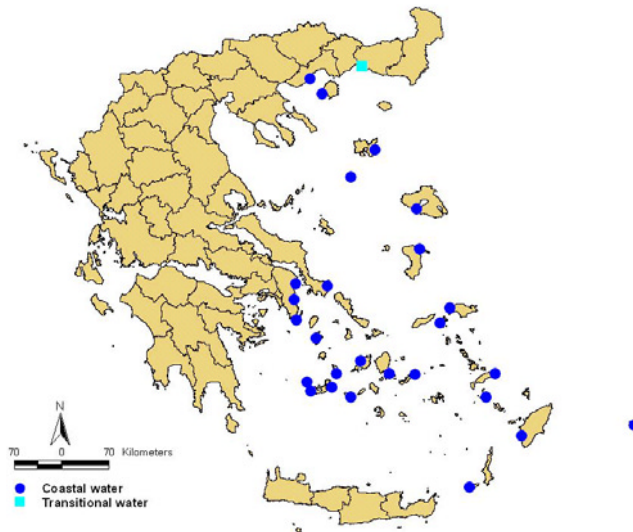
From 26 ‘Natura 2000’ Aegean sites (Fig. 3) passed the above criteria, 62 quantitative and destructive samples were collected using a metallic frame (20 x 20 cm or 25 x 25 cm) during the period 1999-2000 (Table 3). They were representative of the photophilic *Cystoseira crinita* Duby community (MOLINIER, 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.

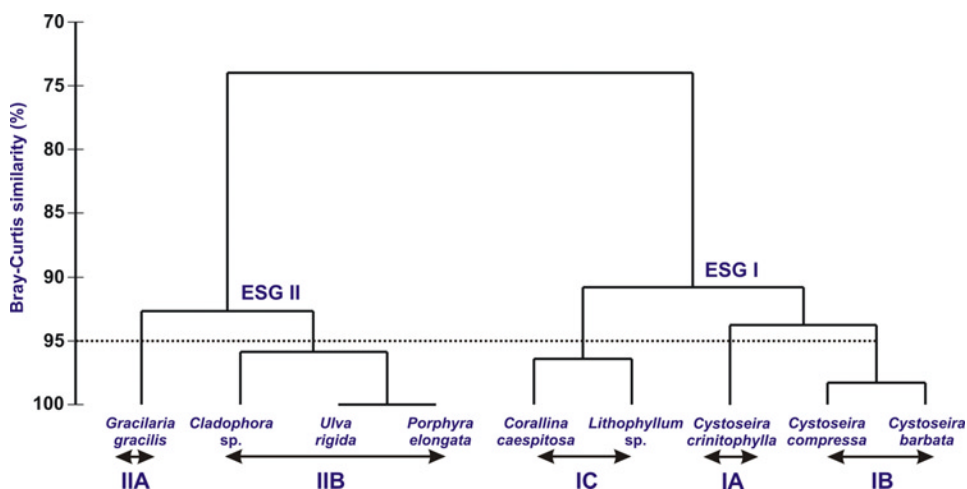
**Table 3**  
**Background data of reference site sampling protocol.**

| 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 IIs            | 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 IIs | 3           |
| GR4220013   | June 2000      | 0.5            | Small Cyclades                           | 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 IIs                  | 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  $\text{Log}(x+1)$  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 (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

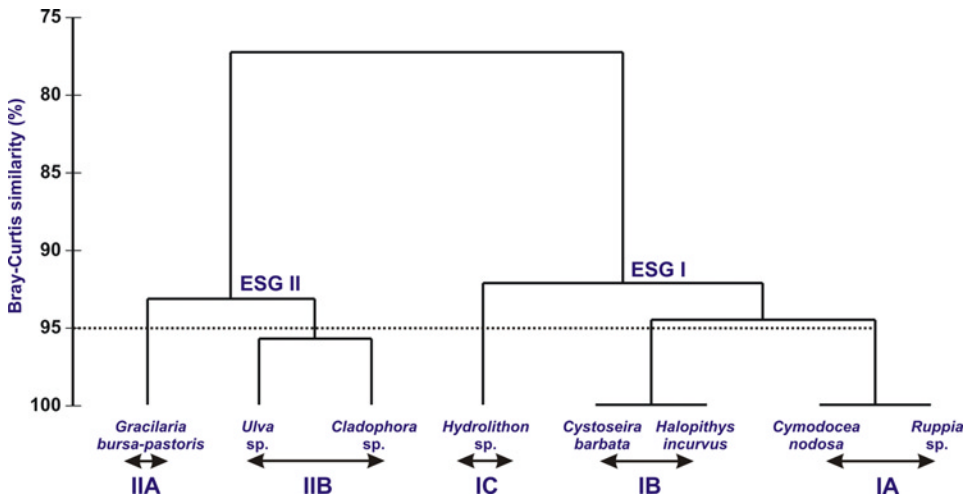


Fig. 5: Similarity cluster derived from character sets (Table 2) showing the classification of selected transitional water benthic macrophytes in five functional groups. ESG IA=angiosperm, plastic; ESG IB=thick, plastic; ESG IC=shade-adapted, plastic; ESG IIA=fleshy opportunistic; ESG IIB=filamentous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

ly 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 crinophylla* < *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:

$$\begin{aligned} \text{ESG I (\% coverage)} &= \\ &[(IA*1) + (IB*0.8) + (IC*0.6)] \\ \text{ESG II (\% coverage)} &= \\ &[(IIA*0.8) + (IIB*1)] \end{aligned}$$

#### **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:

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

where  $x$  is the score in ESG I,  $y$  is the score in ESG II and  $a, \dots, f$  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                   |
|-------------------------------|-----------------------|---------------------|----------------------------------|---|---------------|---------------------------|
| <b>A. Coastal waters</b>      |                       |                     |                                  |   |               |                           |
| 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>B. Transitional waters</b> |                       |                     |                                  |   |               |                           |
| 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:

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

where  $RC = 10$

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

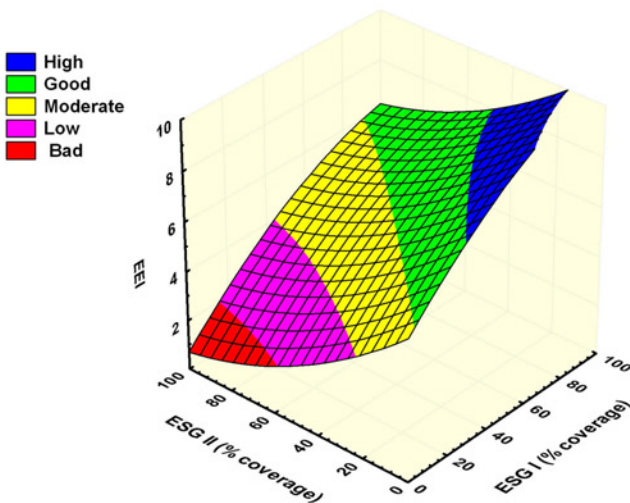
| No. | Taxon                   | ESG | No. | Taxon                 | ESG | No. | Taxon                     | ESG |
|-----|-------------------------|-----|-----|-----------------------|-----|-----|---------------------------|-----|
| 1   | <i>Acetabularia</i>     | IC  | 64  | <i>Drachiella</i>     |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

(Continued)



**Table 5 (Continued)**

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



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

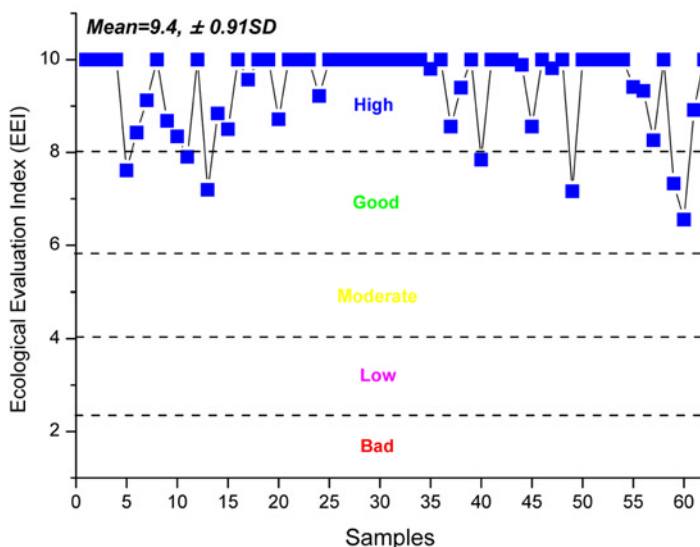
where  $EEI-c_{EOR}$  values for coastal waters in Greece higher than 0.48 ( $\pm 0.09SD$ ) indicate sustainable ecosystems of good or high ESC, whereas  $EEI-c$  values lower than 0.48 indicate that the ecosystems should be restored to a higher ESC (Table 7).

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

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

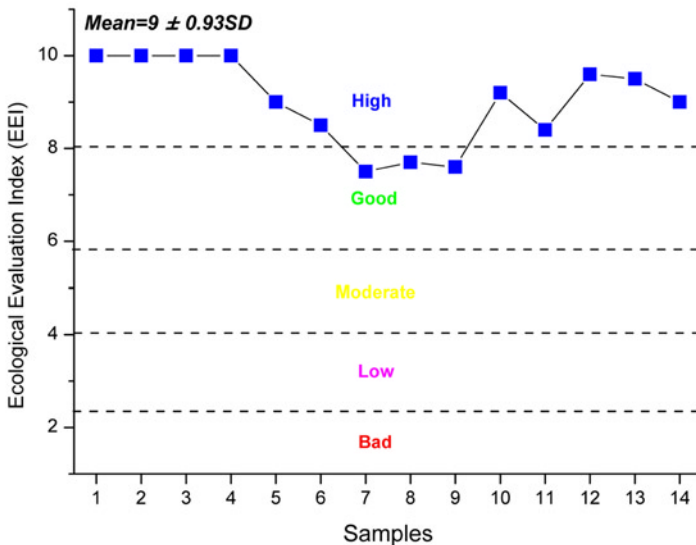
| Ecological Status Classes | EEI-c boundary values | EEI-c <sub>EQR</sub> boundary values | No. of theoretical values |
|---------------------------|-----------------------|--------------------------------------|---------------------------|
| High                      | 9.72±0.46SD           | 0.97±0.06SD                          | 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):

$$\text{ESG I} = (80 \times 1) + (40 \times 0.8) + (30 \times 0.6) = 130,$$

$$\text{ESG II} = (15 \times 0.8) + (5 \times 1) = 17$$

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  $(9.8 \times 0.7) + (7.8 \times 0.3) = 9.2$ , which corresponded to a high ESC and to  $\text{EEI-c}_{\text{EQR}} = 0.9$ , reference conditions  $\text{EEI} = 10$ . 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):

$$\text{ESG I} = (60 \times 1) + (10 \times 0.8) + (10 \times 0.6) = 74$$

$$\text{ESG II} = (35 \times 0.8) + (10 \times 1) = 38$$

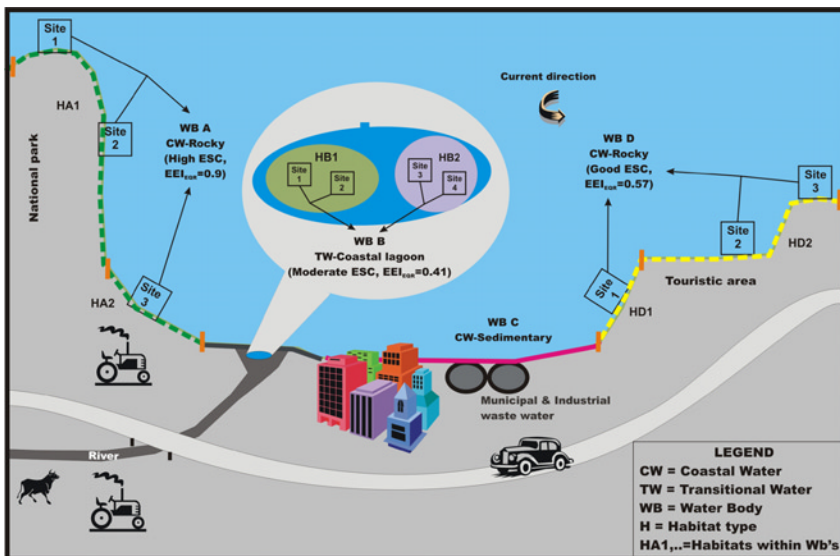


Fig. 9: A hypothetical coastal zone including coastal and transitional water bodies of different Ecological Status Classes.

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  $(7.6 \times 0.4) + (3.8 \times 0.6) = 5.3$ , 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 hy-

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

tial 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  $\mu$ M, P=2  $\mu$ M), was able to compensate for an effective quantum yield ( $\Delta F/Fm'$ ) decrease due to relatively high laboratory cultivation irradiance (55-60  $\mu$ mol 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; CHRYSOVERGIS & PANAYOTIDIS, 1995; LAZARIDOU *et al.*, 1999; SCHRAMM, 1999; DÍAZ *et al.*, 2002; PANAYOTIDIS *et al.*, 2004; ARÉVALO *et al.*, 2007; WELLS *et al.*, 2007).

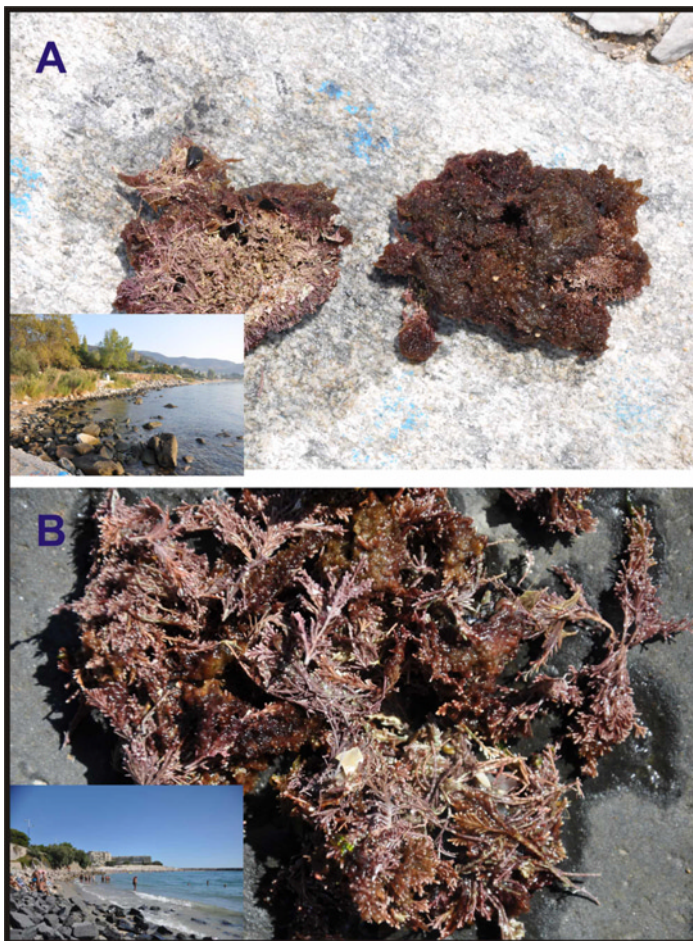
The EEI classification scheme regards *Corallina* spp. as a late successional species (ESG I) that has a closer relationship to canopy-forming species than to opportunistic (ESG II) species, in accordance with its embedded information (AUSTONI *et al.*, 2007). Such a view initiated discussions with the 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 as-



sessed *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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