BENTHIC MACROALGAE AS BIOINDICATORS OF THE ECOLOGICAL STATUS IN THE GULF OF TRIESTE

BENTOŠKE MAKROALGE KOT BIOINDIKATORJI EKOLOŠKEGA STANJA V TRŽAŠKEM ZALIVU

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Key words: macroalgae, EEI (Ecological Evaluation Index), ecological status, monitoring programme, Gulf of Trieste
Ključne besede: makroalge, EEI (Indeks ovrednotenja ekološkega stanja), ekološko stanje, program monitoringa, Tržaški zaliv

ABSTRACT

A preliminary assessment of macroalgae in Slovenian coastal waters led to a selection of seven sampling sites for the monitoring programme. The aim of the study was to verify whether the monitoring results confirm the first evaluation of the ecological status (ES) according to macrophytes. The ES was reconfirmed as *High/Good*.

IZVLEČEK

Na osnovi predhodne ocene makroalg v slovenskih obalnih vodah je bilo izbranih sedem vzorčnih mest za program monitoringa. Namen študije je bil preveriti ali rezultati monitoringa potrjujejo prvo oceno ekološkega stanja (ES) glede na makrofite. ES je bilo potrjeno kot *Zelo dobro/Dobro.*

1. INTRODUCTION

In the Mediterranean infralittoral rocky belt, two ecological quality indices are mostly used for assessing the ecological status (ES) of macroalgal communities: the Greek EEI (Ecological Evaluation Index) proposed by Orfanidis et al. (2001, 2003) and Panayotidis et al. (2004), and the Spanish CARLIT (cartography of littoral and upper-sublittoral rocky communities) proposed by Ballesteros *et al*. (2007). The EEI, which is a multimetric scale-based biotic index that reveals the response of macrophytes to anthropogenic stress, has been successfully used to assess the ES of Slovenian coastal waters (Orlando-Bonaca et al. 2008) and it is also planned to be used in the Italian part of the Gulf of Trieste (A. Falace, *pers. comm.*). Recently, the EEI was tentatively tested in Croatian Istrian waters as well (Iveša et al. 2008). Its basic concept, according to which "nutrients shift the ecosystem from a pristine state, where late-successional species are dominant, to a degraded state, where opportunistic species" prevail, is well documented (Giaccone 1993, Chryssovergis et Panayotidis 1995, Arévalo et al. 2007).

The results of the preliminary assessment of benthic macroalgae in Slovenian coastal waters (Orlando-Bonaca et al. 2008) led to a selection of seven sampling sites on km-scale, dominated

by late-successional species, for the surveillance monitoring programme, following the EEI successional model and according to the European Water Framework Directive.

The aim of the study was to verify whether the results of the first year monitoring programme confirm the preliminary assessment of benthic macrophytes or indicate different conditions/ situation of benthic macroalgae in Slovenian coastal waters.

2. MATERIAL AND METHODS

2.1 STUDY AREA AND SAMPLING PROCEDURE

The Gulf of Trieste is characterized by the largest tidal differences (semidiurnal amplitudes approach 30 cm) and the lowest winter temperatures (below 10°C) in the Mediterranean Sea (Boicourt et al. 1999). The Slovenian coastal sea covers the southern part of the Gulf of Trieste. Its coastline is approximately 46.7 km long. It is a shallow semi-enclosed gulf with a maximum depth of ca. 33 m in waters off Piran. The Slovenian coastal sea is affected by freshwater inflows and local sources of pollution (Turk 1999). In recent decades, it has suffered from many anthropogenic impacts such as intensive farming, mariculture, and sewage outfalls (Turk 1999). Many activities such as urbanisation and massive tourism have modified the natural shoreline.

During 2007, benthic macroalgae were sampled at seven monitoring sites selected in two water bodies: SI5VT4 and SI5VT5 (Figure 1). The first were characterized as “rocky shallow moderately exposed”, the second as “sedimentary shallow moderately exposed”. The sites have been located at regular distances of less than 5 km apart from each other, dominated by late-successional species. All the sites were sampled twice: in spring and in late summer. As a sampling site, an area of 10 x 10 m was considered. At each site, in a depth range of 2 to 4 m, three samples were randomly scraped from the bottom (20 x 20 cm). Such a surface (400 cm^2) is considered to be the minimal sampling area in the case of the Mediterranean infralittoral communities (Boudouresque et Belsher 1979). All samples were collected between 8 and 12 a.m. Each collected sample was placed in a plastic bag and all the material transported to the Marine Biology Station of the National Institute of Biology laboratory for analysis. The samples were then fixed in ethanol (70%).

Species identification of macroalgae was carried out in the laboratory by using a binocular microscope and a microscope in accordance with Ribera et al. (1992), Gallardo et al. (1993), Gomez Garreta et al. (2001) and Bressan et Babbini (2003). Each sample was sorted carefully and the surface covered by each species (the vertical projection) was quantified in cm^2 (4 cm^2 = 1% of the sampling surface). Only species covering at least 1% of the sampling area were assessed. In cases where the coverage of morphologically similar species could not be measured precisely, these species were grouped together (as spp.).

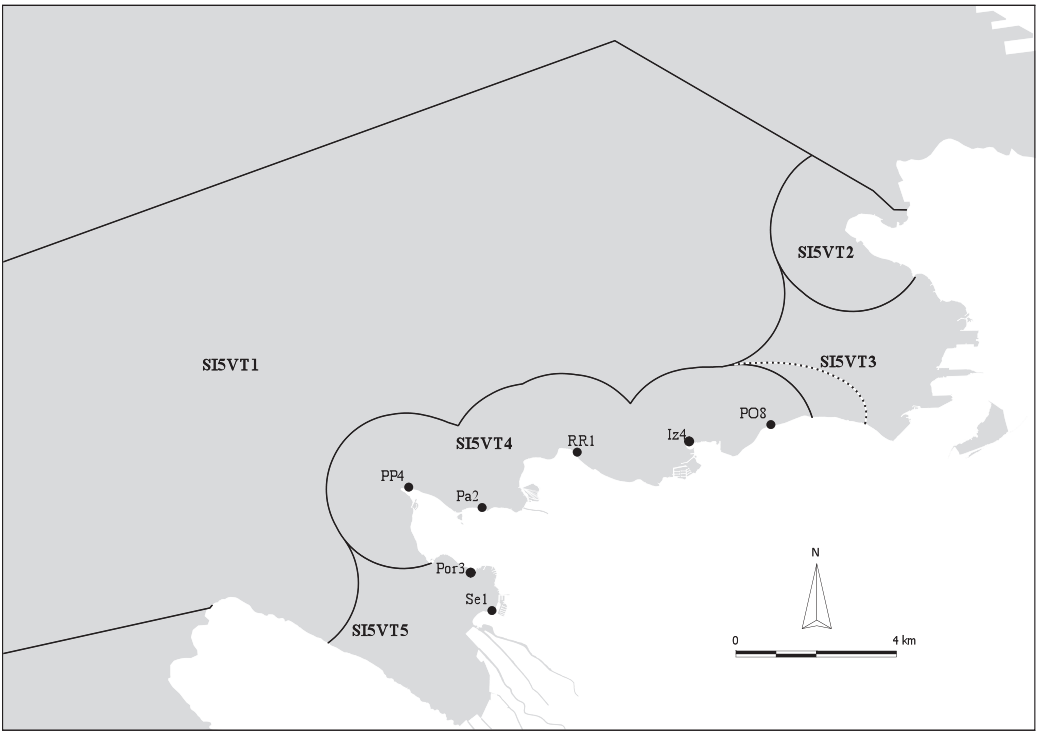

Figure 1: The study area (Slovenian coastal waters) with seven sampling sites for the 2007 monitoring programme.
Figure 1: Obravnavano območje (slovenske obalne vode) s sedmimi vzorčnimi mesti za program monitoringa v letu 2007.

2.2 DATA ANALYSIS

The macrophyte species were divided into two Ecological State Groups (ESG). In ESG I, the thick leathery, articulate upright calcareous and crustose calcareous species, most of them *k*-selected species, were grouped. In ESG II were grouped the foliose, the filamentous and the coarsely branched upright species, most of them *r*-selected species.

The EEI is a number ranging from 2 to 10. To determine the EEI of water bodies, the following procedure was used (Orfanidis et al. 2001, 2003):

The sampling area (where the bottom is rocky) was divided into non-overlapping permanent lines (PL) and several relevées of benthic vegetation were obtained from each. According to the Impress (2003), the length of each segment of rocky coast was defined with regard to known and possible pressures (maritime traffic, mariculture, municipal waste waters, harbours, industry, agriculture, etc.) as well as geomorphology of the coast and seabed.

In each relevé, the absolute abundance (%) of each ESG was estimated by its coverage.

The average coverage (%) of ESG I and II are cross compared in a matrix to determine the ES of the PLs in a range of five categories from high to bad. A numerical scoring system was used to express the ES categories to a numerical value (bad = 2, low = 4, moderate = 6, good = 8, high = 10).

The length of each PL was multiplied by its ES value and divided by the sum of all the lengths of the PLs. The length-weighted values were then summed to obtain EEI and the ES category of each water body (> 8 = high, 8->6 = good, 6->4 = moderate, 4->2 = low, 2 = bad).

3. RESULTS

3.1 STRUCTURAL AND FUNCTIONAL ANALYSIS

The list of macrophyte species and their average coverage (%) recorded in 2007 is presented in Table 1. Forty-one (41) taxa were identified in total, with 20 Rhodophyceae (dominating qualitatively), 12 Phaeophyceae (dominating quantitatively) and 9 Chlorophyceae.

Twenty-four (24) of the taxa belong to ESG I and 17 to ESG II. Using EEI, the seven sampling sites were classified into five Ecological Status Classes (Figures 2 and 3). Five sites were evaluated as *High*, while two were assessed as *Moderate*.

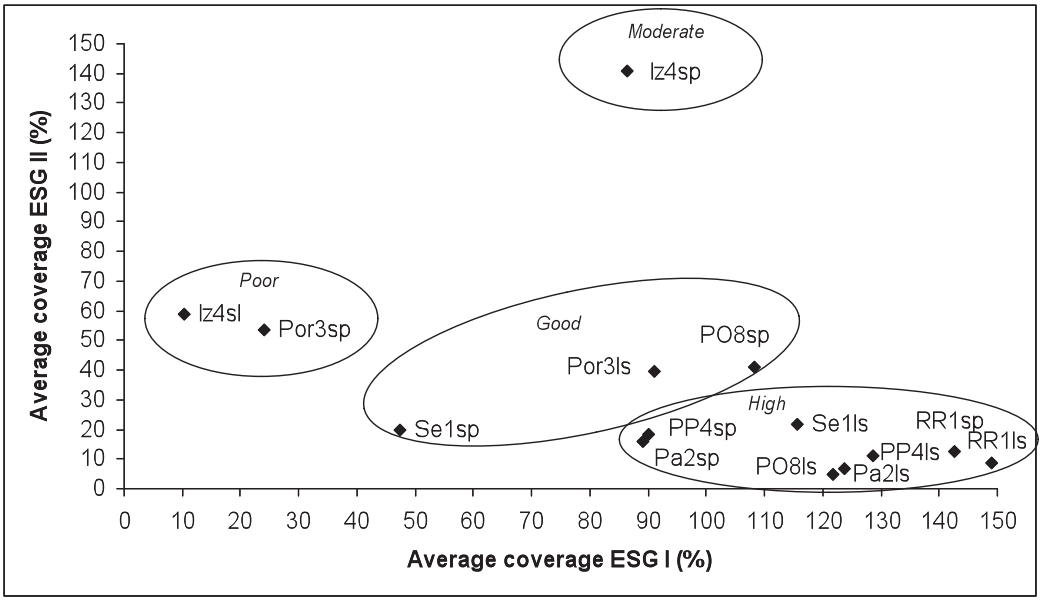

Figure 2: Macroalgal coverage (%) and classification into ecological categories by the use of EEI for samples from 2007 (sp = spring samples, ls = late summer samples).

Slika 2: Pokrovnost makroalg (%) in razvrstitev v ekološke kategorije z uporabo EEI za vzorce iz leta 2007 (sp = spomladanski vzorci, ls = poznopoletni vzorci).

At three sampling sites (RR1, Pa2 and PP4), the ES of macroalgae was evaluated as *High* during spring and late summer as well (Figure 2). In the spring period, *Cystoseira* species were dominant, while in the late summer *Halopithys incurva* and *Padina pavonica* were the most abundant species.

Table 1: List of species and average coverage (42 samples) of macrophytes at monitoring sampling sites in 2007 expressed as % of the sampling surface. ESG = Ecological State Groups.

Tabela 1: Seznam vrst in povprečna pokrovnost (42 vzorcev) makrofitov na vzorčnih mestih v letu 2007, izražena kot % vzorčevalne površine. ESG = Ecological State Groups (ekološki razredi).

ESG	SPECIES	Coverage (%)
ESG I	*Acetabularia acetabulum* (Linnaeus) P.C. Silva	0.08
	Alsidium corallinum C. Agardh	4.54
	Anadyomene flabellata J.V. Lamouroux	0.02
	Corallina officinalis Linnaeus	0.27
	Cystoseira barbata (Stackhouse) C. Agardh	7.75
	Cystoseira compressa (Esper) Gerloff & Nizamuddin	10.92
	Cystoseira corniculata (Turner) Zanardini	0.46
	Cystoseira sauvageauana G. Hamel	0.83
	Cystoseira sp.	0.27
	Flabellia petiolata (Turra) Nizamuddin	0.75
	Halimeda tuna (J. Ellis & Solander) J.V. Lamouroux	4.79
	Haliptilon virgatum (Zanardini) Garbary & H.W. Johansen	3.46
	Halopithys incurva (Hudson) Batters	19.71
	Hydrolithon spp.	0.85
	Jania sp.	0.85
	Lithophyllum spp.	0.06
	Lithothamnion spp.	0.63
	Padina pavonica (Linnaeus) Thivy	30.08
	Peyssonnelia polymorpha (Zanardini) F. Schmitz	1.42
	Peyssonnelia squamaria (S.G. Gmelin) Decaisne	2.73
	Phyllophora sp.	0.48
	Pseudolithophyllum sp.	0.21
	Rhodymenia sp.	0.10
	Zanardinia prototypus (Nardo) Nardo	0.29
ESG II	*Ceramium* spp.	0.15
	Chondria spp.	0.04
	Cladophora spp.	6.21
	Codium bursa (Olivi) C. Agardh	0.06
	Codium vermilara (Olivi) Chiaje	1.79
	Dictyota dichotoma (Hudson) J.V. Lamouroux	5.85
	Dictyota linearis (C. Agardh) Greville	0.85
	Dictyopteris polypodioides (A.P. de Candolle) J.V. Lamouroux	2.52
	Gelidium spp. J.V. Lamouroux	0.06
	Gigartina sp.	5.42
	Halopteris spp. Kützing	0.38
	Laurencia spp.	0.15
	Polysiphonia spp.	0.08
	Pterocladiella capillacea (S.G. Gmelin) Santelices & Hommersand	0.02
	Sphacelaria spp.	5.06
	Ulva spp.	0.19
	Valonia utricularis (Roth) C. Agardh	0.71

At two sampling sites (PO8 and Se1), the spring *Good* ES turned into *High* Ecological Status during the late summer (Figure 2). At both sites, *Cystoseira* species were missing, while *H. incurva, Alsidium corallinum* and *P. pavonica* dominated quali-quantitatively. At Por3 site, the spring *Poor* ES improved into a Good ES in late summer, principally due to a very high coverage of *P. pavonica.*

At Iz4 site, the spring *Moderate* Ecological Status, with *Cystoseira compressa* as the dominant ESG I species, deteriorated into a *Poor* ES during the late summer, with a high coverage of species from ESG II and a drastic decrease in the coverage of *C. compressa*.

Using spatial scale weighted EEI, the two water bodies were classified in Ecological Status Classes (Table 2). While the ESC in water body SI5VT4 was evaluated as *High*, the ESC in water body SI5VT5 was assessed as *Good*.

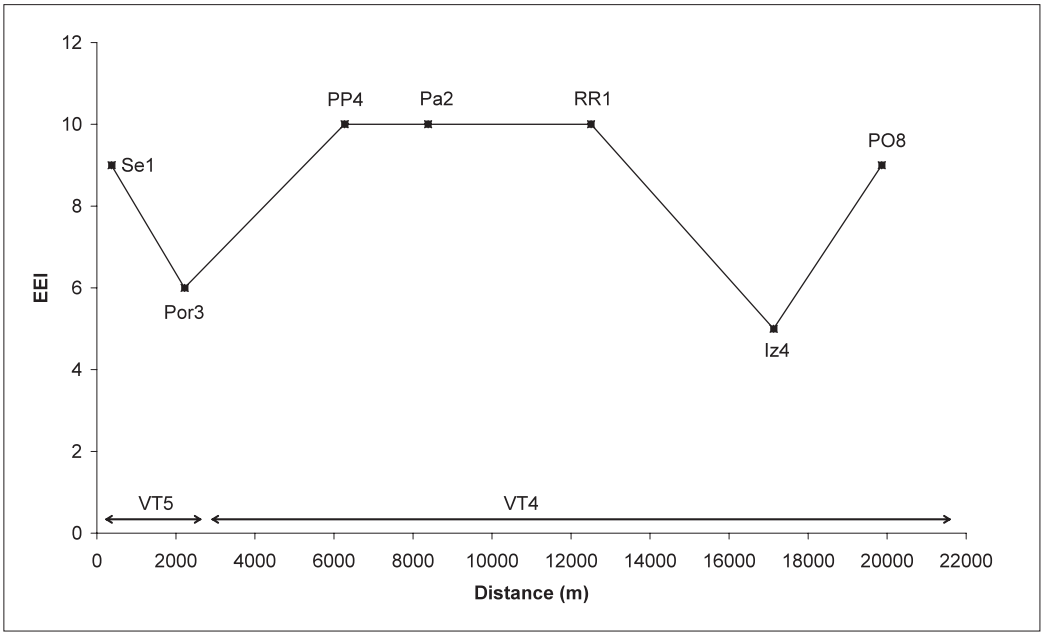

Figure 3: Classification of monitoring sampling sites for the year 2007 into ecological categories by the use of EEI.
Slika 3: Razvrstitev vzorčnih mest za monitoring v letu 2007 v ekološke razrede po EEI.

Table 2: Ecological status achieved by two Slovenian coastal water bodies in 2007.
Tabela 2: Ekološko stanje, ki sta ga leta 2007 dosegli dve obalni vodni telesi.

WB	Site	Length (m)	EEI	Length x EEI	Weighted EEI	ESC
SI5VT4	PO8	2934.10	9	26406.89	**9.24**	**HIGH**
	Iz4	1470.25	5	7351.23		
	RR1	3203.80	10	32037.96		
	Pa2	2485.25	10	24852.52		
	PP4	3391.24	10	33912.41		
SI5VT5	Por3	889.96	6	5539.78	**7.26**	**GOOD**
	Se1	645.80	9	5812.19		

4. DISCUSSION

The EEI results for five of the seven sampling sites confirm those from the preliminary study (Orlando-Bonaca et al. 2008). The 2007 sampling allowed the observation of seasonal differences in species composition and coverage between spring and late summer samples. At almost all sampling sites, the ES was higher in late summer, with the exception of Iz4. At this site, the late summer situation was worse than that in spring, while in the late summer of 2006 the ES was evaluated as *High,* with *A. corallinum* as the dominant species. In 2007 samples, this red alga covered only 6% of the sampling area (average value). We suppose that in 2007 the Iz4 site was subject to high nutrients inputs. This assumption would explain why in spring samples, only *C. compressa* was present and very abundant among *Cystoseira* species (with thallus almost 70 cm high). The morphological plasticity of the thallus of *C. compressa*, with a luxuriant form with erect fronds in spring and a rosette-shaped form in late summer, autumn and winter, was previously described for the Gulf of Trieste by Falace et al. (2005). Giaccone et al. (1994) reported that under particular ecological conditions other species than *C. crinita* become very abundant in the association *Cystoseiretum crinita*e Molinier, 1958, forming recognisable subassociations. *Cystoseiretum crinitae* subass. *Cystoseiretosum compressae* was defined as dominant at unperturbed sites with mild pollution (Giaccone et al. 1994, Cormaci et al. 2003). The coexistence of the late-successional and opportunistic species (*e.g.* intermediate disturbance hypothesis by Connell 1978) forms communities that are indicative of intermediate conditions, which reflects the situation at Iz4 site.

For Por3 site, the 2007 results indicate that the ES evaluated in 2006 was too high. *P. pavonica* was confirmed as the dominant species from ESG I. The species is representative of the association *Cystoseiretum crinita*e Molinier, 1958, but it forms large enclaves where the environmental factors prevent the growth of *Cystoseira* species. The turbid conditions at Por3 site (*own observations*) reduce the light penetration and thus prevent development of the highest photophilic layer, composed mostly of brown algae with thick blades and branches. Since long-lived genera like those from the order Fucales follow long-term periodicity, their absence from a site should be regarded as indicative of environmental degradation, when correlated with key abiotic parameters, like nutrient inputs and light attenuation (see Gibson *et al*. 2000).

Despite the above mentioned differences for two sampling sites, the EEI assessment of the ES of two water bodies in 2007 is in agreement with the preliminary study (Orlando-Bonaca et al. 2008) and existing human pressures. On the basis of the obtained data for the year 2007, the ES of Slovenian coastal waters was reconfirmed as *High/Good* in terms of the European Water Framework Directive criteria. This result is generally in agreement with the preliminary evaluation of the ES of the Istrian coast near Rovinj (Iveša et al. 2008), where a *Good* ES was achieved with sampling macroalgae within the very same depth range. Iveša et al. (2008), like Arévalo et al. (2007), expressed scepticism about the correct evaluation of the role of algae *C. compressa* and *Corallina elongata* that belong to ESG I, but in the CARLIT method they are associated with intermediate ES levels. In Slovenian coastal waters, these algae do not provide equivocal results. Our data for Iz4 site

demonstrate that where a relevant nutrient input is present, *C. compressa* is surrounded by a thick layer of algae from ESG II. According to EEI, this site was evaluated as *Moderate,* which is the ES that would probably be reached by CARLIT as well. *C. elongata* was not found in Slovenian coastal waters, but the congeneric *C. officinalis* is usually present (although never abundant) only in samples evaluated as *Good/High* ES (Orlando-Bonaca et al. 2008).

Since monitoring programmes are the main drivers to determine long-term changes in coastal environment due to natural cycles or anthropogenic activities, and since no long-term data series for benthic macrophytes are available for the Slovenian coastal waters, these long-term field investigations are recommended to be carried on.

5. SUMMARY

The Ecological Evaluation Index (EEI) has been used to assess the Ecological Status (ES) of Slovenian coastal waters. The results of this preliminary assessment of benthic macroalgae led to a selection of seven sampling sites, dominated by late-successional species, for the surveillance monitoring programme. The aim of the study was to verify if the results of the monitoring programme confirm the first assessment or indicate different conditions of benthic macroalgae in Slovenian coastal waters. The sampling was performed seasonally, in spring and late summer. Despite variations for two sampling sites, the EEI assessment of the ES in 2007 is in good agreement with the preliminary study and was reconfirmed as *High/Good*. Since monitoring programmes are the main drivers to determine long-term changes in coastal environment due to natural cycles or anthropogenic activities, it is recommended that these long-term field investigations are carried on.

POVZETEK

Za oceno ekološkega stanja (ES) slovenskih obalnih voda je bil uporabljen Indeks ovrednotenja ekološkega stanja (EEI). Na podlagi rezultatov predhodne ocene bentoških makroalg je bilo za nadzorni monitoring izbranih sedem vzorčnih mest, s prevladujočimi vrstami z dolgo vegetacijsko dobo. Namen študije je bil preveriti ali rezultati monitoringa potrjujejo prvo oceno ali pa kažejo na drugačno stanje bentoških makroalg v slovenskih obalnih vodah. Vzorčenje je bilo opravljeno sezonsko, spomladi in pozno poleti. Kljub razlikam na dveh vzorčnih mestih je ocena ES v letu 2007 potrdila rezultate predhodne študije in je ES bilo potrjeno kot *Zelo dobro/Dobro*. Upoštevaje dejstvo, da so programi monitoringa glavno gonilo pri ugotavljanju dolgoročnih sprememb v obalnem okolju zaradi naravnih ciklov ali antropogenih vplivov, je nadvse priporočljivo, da se dolgoročne terenske raziskave nadaljujejo.

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