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Changes in the composition and structure of Mediterranean rocky-shore communities following a gradient of nutrient enrichment: Descriptive study and test of proposed methods to assess water quality regarding macroalgae

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Abstract

Changes in the species composition and structure of Mediterranean macroalgal-dominated communities from the upper sublittoral zone are described along a gradient of nutrient enrichment coming from a urban sewage outfall. *Ulva*-dominated communities only appear close to the sewage outfall. *Corallina*-dominated communities replace ulvacean algae at intermediate levels of nutrient enrichment. *Cystoseira*-dominated communities thrive in the reference site but already appear at nutrient levels that are threefold higher than those reported from unpolluted sites. Assemblage variability of *Cystoseira*-dominated communities decreases along the gradient of nutrient enrichment. Methods based on the functional-form groups of macroalgae to assess the water quality provide equivocal results at intermediate levels of nutrient enrichment because species belonging to the same group can display a completely different response to pollution. Alternatively, methods based on indicator species showed correlated evidence among species abundances and pollution levels and seem to have better performances in water quality assessment.

Keywords: Macroalgae; Nutrient enrichment; Biological indicators; European Water Framework Directive; Mediterranean

1. Introduction

Eutrophication is an increasing problem in coastal waters all over the world. Waste water discharges close to the coastline cause deterioration in water quality and affect macroalgal communities thriving in rocky-shores (e.g. Bellan-Santini, 1968; Borowitzka, 1972; Munda, 1974; Littler and Murray, 1975). Organic and nutrient enrichment due to domestic wastes is today one of the main reasons explaining the deterioration of marine nearshore ecosystems (Flechter, 1996). This deterioration consists of changes both at the population, community or, even, at the ecosystem level (Soltan, 2001). According to Grime's theory of plant strategies (Grime, 1977) plants can be categorized into three primary strategies in accordance to its ecological abilities: (1) competitors, which occupy habitats of low stress and disturbance, (2) stress-tolerators which are able to grow in various stress conditions, and (3) ruderals (opportunists) which occupy highly disturbed areas. "Competitive species" are replaced by "stress-tolerant species" at intermediate levels of pollution, and "ruderal species" (always *sensu* Grime, 1977) take their place in highly polluted sites (e.g. Munda, 1974; Murray and Littler, 1978; Tewari and Joshi, 1988; Díez et al., 1999). These replacements imply a simplification of the architectural complexity of the communities due to (i) the reduction or disappearance of the engineering species (Fucales and Laminariales, but also other erect thick-bladed seaweeds) and (ii) a decrease in species richness (e.g. Borowitzka, 1972; Belsher, 1974, 1979; Gorostiaga and Díez, 1996; Middelboe and Sand-Jensen, 2000).

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In response to the increasing eutrophication problems and the greater social environmental concerns, policy-makers of developed countries are implementing initiatives aimed at reducing impacts of pollution in aquatic ecosystems for restoring water quality levels. In Europe, the Water Framework Directive (WFD, 2000/60/EC) promotes the use of biological indicators to assess water quality. Specifically, composition and abundance of aquatic flora is recommended as a key quality element to assess the ecological status of coastal waters. Different methodologies have been already proposed to evaluate the ecological status of a water body using macroalgae as indicators (Orfanidis et al., 2001; Giaccone and Catra, 2004; Pinedo et al., in press; Ballesteros et al., in press) but none of them has closely related the spatial boundaries between dominant macroalgae and pollution levels (e.g. nutrient concentration in seawater). In our opinion, understanding the relationship between specific and measurable sources of pollution and shifts in the structure of communities, is vital to develop useful tools for the diagnosis of eutrophication.

The assumptions underlying the different approaches that are currently being used as official methods in Greece (EEI method; Orfanidis et al., 2001) or several Mediterranean regions of Spain (CARLIT and BENTHOS methods; Ballesteros et al., in press; Pinedo et al., in press) are very different. The EEI method is based in the assignment of any species found in a sample to a ‘‘good’’ or a ‘‘bad’’ group according to its morphology, based on the functional-form group model of macroalgae described by Littler and Littler (1980). The EEI method is, thus, not based on expert judgement, nor on correlated evidence amongst distribution of species and water quality, but on rough but ecologically important attributes of algae. The BENTHOS method is based on the results obtained from an ordination analysis of samples and correlated evidence between community types and water quality. The CARLIT method uses the knowledge in the value of species/communities as bioindicators obtained from the BENTHOS procedure to obtain a quick assessment of water quality based on cartographical surveys. Both, the BENTHOS and CARLIT methods are thus only based on empirical evidence and expert judgement of the sensitivity of each species/community to pollution.

The purpose of this study is to assess the spatial variability of the upper sublittoral communities developed along a pollution gradient caused by the discharge of a domestic sewage outfall in order to identify relationships between dissolved nutrient concentration in seawater and benthic community structure. This study also serves to evaluate the usefulness of rocky-shore communities as bioindicators of environmental health and provides real data to test the accuracy of the proposed methodologies to assess ecological status of water bodies in the Mediterranean using macroalgae.

2. Materials and methods

2.1. Study site

Sampling was conducted from August 2002 to September 2003 along a stretch of coast measuring 1.5 km at the vicinity of Tossa de Mar (Spain, Northwestern Mediterranean), in the influence area of a sewage effluent from a treatment plant. The discharge outfall is located at the

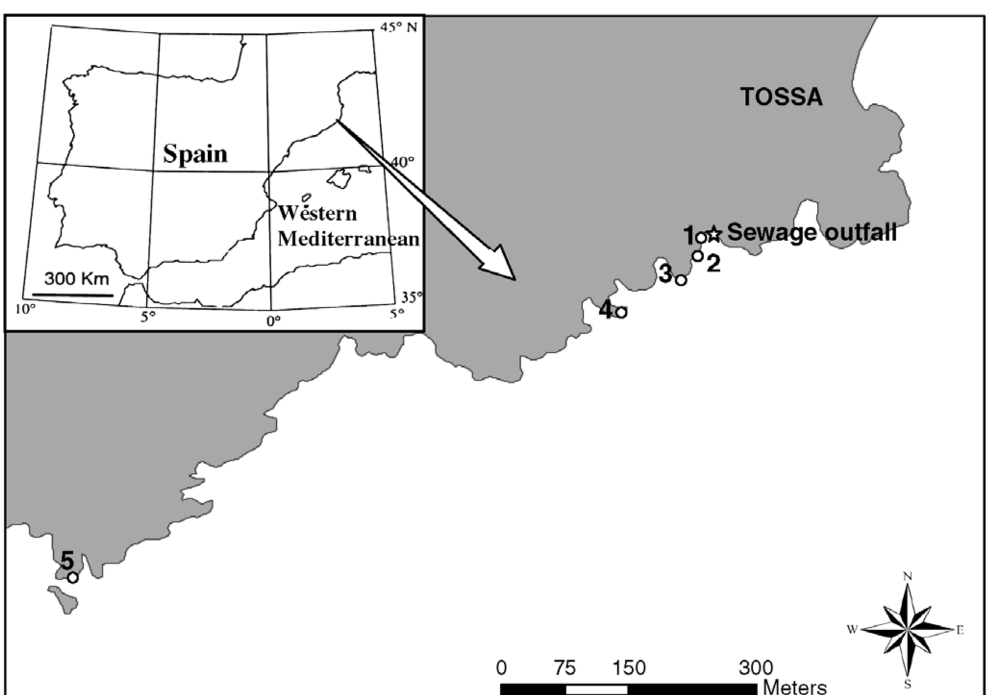

Fig. 1. Location of study area and sampling sites. Sewage outfall is indicated.

coastline (Fig. 1) in an environmentally homogenous, granitic, highly exposed, sea-cliff, where anthropogenic pressures were limited.

Tossa de Mar is a first rank tourist destination (Sardá et al., 2005), whose sewage waters are composed only of domestic waste. There is no industrial development in the area and agriculture is extremely reduced. Most of the land (>90%) is covered by extensive Mediterranean forests of cork oak and thus pollution coming from run-off waters is expected to be low. Before discharge to the sea, domestic waters receive two types of treatments: (i) biologic treatment in winter when total population is low; and (ii) primary and biologic treatment during summer in order to decrease the higher impact due to increased tourist population. The sewer outfall discharges an average of 2054 m^3/day (720 m^3/day in winter). Waste water from the outfall usually flows to the south along the coast following the direction of dominant currents.

2.2. Sampling design and analytical procedures

Five sampling sites were situated at increasing distances from the outfall (2, 8, 84, 163 and 1350 m), the most distant being used as reference site (Fig. 1). Sites were selected to be as similar as possible with respect to orientation, coastal slope and wave exposure in order to decrease community variability due to environmental factors other than water quality.

Sampling was conducted in the community developing at the upper-most level of the sublittoral zone (0.1–0.3 m depth below the mean sea level). Three replicates were randomly collected at each sampling site every 40 days for a year. Every replicate was taken by scraping off all organisms from a 15×15 cm^2 surface using a hammer and a chisel. This surface is large enough to be representative of the complexity (number of species, diversity, biomass, coverage) of the sampled communities (Ballesteros, 1988a,b, 1992). Samples were preserved in formaline:seawater at 4% and subsequently sorted in the laboratory. Algae and invertebrates were identified to species level and quantified in terms of coverage (horizontal surface measured after spreading the algal thalli into a laboratory tray; Ballesteros, 1992). Coverage of some encrusting species that could be destroyed when sampling was estimated *in situ*. Species with a negligible abundance were assigned coverage of 0.1 cm^2.

Three water samples were collected at each sampling site every 20 days during one year with clean plastic bottles and frozen until chemical analyses were done. Water samples were analyzed for dissolved nutrients (silicates, phosphates, nitrates, nitrites and ammonia) on a Bran-Luebbe® TRAACS 2000 Autoanalyzer.

2.3. Community data analysis

Differences among sites for community data and nutrients analysis were determined by non-parametric tests (Kruskall–Wallis) as normality was never met after several data transformation using SPSS statistical package (version 13.0).

K-Dominance curves of coverage data were obtained using PRIMER statistical package (version 5.0) in order to compare diversity patterns between different sites (Lambshead et al., 1983).

A correspondence analysis (CA; Benzecri, 1973) was performed on species coverage data using CANOCO package (version 4.5) in order to establish the affinities between samples. The mean of each three replicates by period was used to characterize the community at each sampling date. Species occurring in less than 2% of samples were excluded from the analysis. Pearson correlations between nutrient concentration and principal axes (I and II) obtained from the CA were performed to test relationships between pollution and principal axes. No transformation of data was done.

3. Results

There is a strong gradient of dissolved nutrient concentrations in seawater along the five sampling sites, decreasing from sites 1 to 5 (Fig. 2). Significant differences in concentrations of all dissolved nutrients are found between sites (Kruskall–Wallis H test for all dissolved inorganic nutrients measured, all with $p < 0.0001$). Sites 1 and 2 are completely different from all other sites regarding all the nutrients. Site 3 mainly differs from site 4 and 5 by the concentration of phosphates and nitrites and site 4 and 5 only have significant differences in phosphate concentrations.

The total number of species found at each site increases at increasing distances from the outfall but becomes stabilized at sites 4 and 5. The highest differences are found between site 1 and 2 (52% increase) and between site 2 and 3 (39% increase) (Table 1). Green algae are the qualitatively dominant group at site 1 while red algae dominate

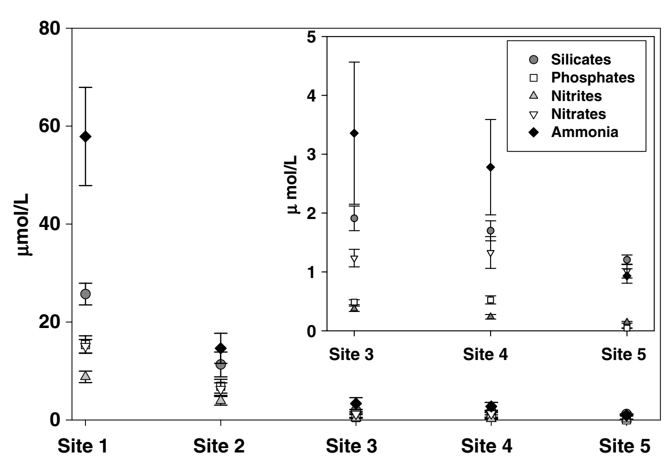

Fig. 2. Mean (±SE) annual dissolved inorganic nutrient concentration in seawater (silicates, phosphates, nitrates, nitrites and ammonia) at each sampling sites.

Table 1
Total number of species collected for each algal group and for main macroinvertebrates at each site

	Site 1	Site 2	Site 3	Site 4	Site 5
Rhodophyta	8	22	34	44	43
Phaeophyta	2	8	14	14	16
Chlorophyta	9	10	20	16	13
Macroinvertebrates	3	6	7	5	6
Total	22	46	75	79	78

at all other sites. The number of brown algae is particularly low near the outfall (Table 1).

Annual average of species coverage for the five sampling sites is presented in Table 2. Site 1 was completely dominated by the green alga *Ulva rigida*, together with other species of the genus *Ulva* and *Cladophora*, and the presence of the red algae *Gelidium pusillum* and *Corallina elongata*. Coverage of macroinvertebrates was almost negligible (Fig. 3). *C. elongata* quantitatively dominated the community at site 2 (Fig. 3), being responsible for the dominance of red algae in this site. The abundance of *U. rigida* was greatly reduced and only another coralline alga (*Lithophyllum incrustans*) showed a relatively high cover. Two macroinvertebrates displayed high abundances at this site: the small sea cucumber *Ocnus brunneus* and the limpet *Patella* sp.. *Cystoseira mediterranea* was the dominant species in sites 3, 4 and 5 (Fig. 3) and no significant differences were observed in coverage among these sites for this species ($p > 0.05$). However, differences among sites 3, 4 and 5 were obtained for other abundant species (*C. elongata*, *Jania rubens* and *U. rigida*). Sites 3 and 4 presented a significant higher coverage of *C. elongata* and *U. rigida* than site 5 ($p < 0.05$ and $p < 0.005$, respectively). In contrast, coverage of *J. rubens* in sites 3 and 4 was significantly lower ($p < 0.001$) than in site 5. Thus, there was an increase in the coverage of *J. rubens* and a decrease in the coverage of *C. elongata* and *U. rigida* with increasing distances to the outfall. Other important species for the community of *C. mediterranea* were the red algae *Ceramium rubrum*, *Callithamnion tetragonum*, *Boergeseniella fruticulosa* and *Polysiphonia mottei*, all of them growing as epiphytes over *C. mediterranea*. Barnacles (*Balanus perforatus*) and mussels (*Mytilus galloprovincialis*) were the most abundant macroinvertebrates in sites 3, 4 and 5.

K-Dominance curves display different lengths and shapes according to the community considered (Fig. 4). Curves change from a quadrangular to a diagonal shape and from shorter to longer at increasing distances from the outfall. Thus, the community dominated by *U. rigida* shows the lowest diversity and the community of *C. mediterranea* the highest, being the *C. elongata* community of intermediate diversity.

Results from the correspondence analysis are presented in Fig. 5. The first and second main axes explain respectively 35% and 21% of the total variance. Three major groups of samples are highlighted by the analysis. The first group is composed by all the samples from site 1, situated very close to the outfall, dominated by *U. rigida*; they display positive values on the first axis. The second group is defined by samples corresponding to site 2, dominated by *C. elongata* and without *C. mediterranea*; they display high and positive values on the second axis. The third group is composed by samples corresponding to sites 3, 4 and 5, covered by *C. mediterranea*. Samples corresponding to site 3 are widely distributed along the second axis indicating a gradient of abundance in *C. elongata*; even in one case (samples showing slightly positive values on the first axis) there is a relatively high abundance of *U. rigida*. Samples of sites 4 and 5 also display a certain distribution along the second axis, indicating variability among the relative abundances of *C. elongata* or *J. rubens*. Discontinuities are sharp between samples from site 1, site 2 and samples from other sites. Sites 3, 4 and 5 only differ in the variability of their samples, being the highest in site 3 and the lowest in site 5. The second axis is a quadratic function of the first (Guttman effect), indicating that samples are arranged along a strong environmental gradient (Hill, 1973). In fact, the first axis is strongly correlated with all the measured nutrients ($p < 0.001$) and the second axis is also correlated with dissolved nutrients but only when samples from the site 1 are not considered (Table 3). That means that the first axis separates communities growing in waters diverging by an order of magnitude in dissolved nutrients concentrations whilst the second axis segregates communities situated along a gradient of lower dissolved nutrients concentrations that remain amongst the same order of magnitude.

4. Discussion

The considerable variation found in the dissolved nutrients concentrations among sites correspond with differences in the species composition and in the main structural parameters of the studied communities situated in the upper sublittoral zone. As it has been stated in other studies (Borowitzka, 1972; Belsher, 1974; Littler and Murray, 1975; Rodríguez-Prieto and Polo, 1996; Díez et al., 2003) both the number of species and the alpha-diversity of the communities decrease as sites approach the outfall. Ordination of these communities in a factorial space obtained by applying a CA to the coverage values of the species in the samples, and the strong correlation between the main factorial axes and the dissolved nutrients concentrations in seawater indicates that the main reason of community change is due to water quality. The community dominating at high nutrient levels is composed of green algae (Ulvaceae) as it has been repeatedly reported in the literature (Golubic, 1970; Littler and Murray, 1975; Fairweather, 1990; Díaz et al., 2002). In fact, ulvacean algae can be considered as ''ruderal species'' and in natural ecosystems they usually develop in places with high nutrient levels (e.g. river mouths, transitional waters) or places subjected to high levels of disturbance (e.g. sand abrasion)

Table 2
Mean annual percentage coverage of species at each sampling site

Species	Site 1	Site 2	Site 3	Site 4	Site 5
Cystoseira mediterranea Sauvageau	–	–	342.690	362.690	265.622
Ulva rigida C. Agardh	509.678	6.505	20.219	1.831	0.048
Corallina elongata Ellis and Solander	12.513	230.645	71.949	74.019	38.264
Jania rubens (Linné) Lamouroux	0.977	0.285	9.130	28.877	93.747
Mytilus galloprovincialis Lamarck, 1819	0.111	6.506	41.119	57.801	18.862
Balanus perforatus Bruguiére, 1789	–	0.249	17.640	17.410	13.088
Lithophyllum incrustans Philippi	–	20.011	10.441	4.839	5.084
Ceramium rubrum (Hudson) C. Agardh	–	0.328	11.049	10.752	7.067
Callithammion tetragonum (Withering) S.F. Gray	–	2.457	16.928	5.041	0.792
Ocnus brunneus Forbes and Goodsir, in Forbes, 1841	–	23.502	0.040	–	–
Mytilaster minimus (Poli, 1795)	0.007	2.107	3.793	7.050	5.571
Gelidium pusillum (Stackhouse) Le Jolis	11.673	0.002	1.263	2.500	1.451
Boergeseniella fruticulosa (Wulfen) Kylin	–	–	2.031	8.805	4.124
Polysiphonia mottei Lauret	–	0.005	10.092	2.433	0.331
Valonia utricularis (Roth) C. Agardh	–	–	2.055	5.510	2.120
Hypnea musciformis (Wulfen) Lamouroux	–	–	–	0.782	3.605
Grateloupia filicina (Lamouroux) C. Agardh	1.885	–	0.077	0.516	1.546
Enteromorpha sp.	3.174	–	0.003	–	–
Patella sp.	0.015	2.985	0.027	–	0.023
Ectocarpus siliculosus (Dillwyn) Lyngbye	–	0.005	2.482	0.402	–
Enteromorpha compressa (Linné) Greville	1.894	0.013	0.734	0.009	–
Peyssonnelia harveyana J. Agardh	–	–	0.782	0.415	1.287
Halopteris scoparia (Linné) Sauvageau	2.243	0.002	0.046	0.063	0.006
Cystoseira compressa (Esper) Gerloff and Nizamuddin	–	–	–	0.157	1.989
Gelidium latifolium (Greville) Bornet and Thuret	–	–	0.174	1.195	0.536
Gigartina acicularis (Roth) Lamouroux	–	–	0.844	0.283	0.714
Cladophora sp.	1.644	0.002	0.002	0.002	0.005
Porphyra leucosticta Thuret in Le Jolis	0.745	0.705	0.106	0.069	–
Cladophora sericea (Hudson) Kützing	1.131	–	0.006	0.350	0.025
Modiolus sp.	–	0.211	0.195	0.517	0.579
Ceramium diaphanum (Lightfoot) Roth	–	0.536	0.338	0.187	0.229
Gastroclonium clavatum (Rothpletz) Ardissone	–	0.006	0.008	0.325	0.916
Bryopsis duplex De Notaris	–	0.722	0.139	0.327	0.028
Enteromorpha clathrata (Roth) Greville	1.191	0.002	0.002	0.002	–
Petalonia fascia (O. F. Müller) Kuntze	–	–	–	–	1.073
Rhodymenia ardissonei J. Feldmann	–	–	0.004	0.214	0.841
Aphanocladia stichidiosa (Funk) Ardré	0.903	0.022	0.007	0.005	–
Cladophora laetevirens (Dillwyn) Kützing	0.754	–	0.002	0.002	0.002
Gymnogongrus griffithsiae (Turner) Martens	–	–	0.023	0.113	0.582
Ceramium tenerrimum (Mertens) Okamura	–	0.609	0.072	0.002	–
Bryopsis plumosa (Hudson) C. Agardh	–	–	0.598	–	0.023
Rhodophyllis divaricata (Stackhouse) Papenfuss	–	–	0.414	0.109	0.097
Callithamnion granulatum (Ducluzeau) C. Agardh	–	–	0.461	0.034	0.086
Cladophora hutchinsiae (Dillwyn) Kützing	0.057	0.015	0.052	0.098	0.202
Feldmannophycus rayssiae (J. and G. Feldmann) Augier and Boudour	–	–	–	0.170	0.250
Osmundea truncata (Kützing) K.W. Nam and Maggs	–	–	–	0.057	0.314
Feldmannia caespitula (J. Agardh) Knoepffler-Péguy	–	–	–	0.279	0.072
Falkenbergia rufolanosa (Harvey) Schmitz (stadium)	0.263	0.002	0.010	0.016	0.024
Sphacelaria cirrosa (Roth) C. Agardh	–	–	0.028	0.167	0.120
Ceramium ciliatum (Ellis) Ducluzeau v. *robustum* (J. Agadh) Mazoyer	0.298	–	–	0.015	–
Ceramium echionotum J. Agardh	–	–	0.002	0.258	0.015
Cladophora albida (Hudson) Kützing	0.228	–	0.002	0.003	0.003
Rhizoclonium tortuosum (Dillwyn) Kützing	–	–	0.091	0.120	0.023
Lomentaria clavellosa (Turner) Gaillon	–	0.003	0.087	0.106	0.005
Chondria boryana (De Notaris) De Toni	–	–	–	–	0.172
Herposiphonia tenella v. *secunda* (C. Agardh) Hollenberg	–	0.023	0.042	0.074	0.031
Cladophora coelothrix Kützing	–	–	0.025	0.119	0.015
Laurencia obtusa (Hudson) Lamouroux	–	–	–	0.128	0.030
Aglaozonia parvula (Greville) Zanardini (stadium)	–	0.031	0.124	–	0.002
Lophosiphonia sp.	–	0.015	0.048	–	0.093
Crouania attenuata (C. Agardh) J. Agardh	–	–	–	0.127	0.024
Halopteris filicina (Grateloup) Kützing	0.074	0.002	0.003	0.048	0.009
Colpomenia sinuosa (Mertens ex Roth) Derbès and Solier	–	0.004	0.015	0.002	0.061

(*continued on next page*)

Table 2 (*continued*)

Species	Site 1	Site 2	Site 3	Site 4	Site 5
Padina pavonica (Linné) Thivy	–	–	0.078	–	0.002
Ceramium strictum Harvey	–	–	0.018	0.049	0.002
Pseudochlorodesmis furcellata (Zanardini) Børgesen	–	0.057	0.002	0.002	0.005
Sphacelaria tribuloides Meneghini	–	–	0.005	0.002	0.057
Sphacelaria rigidula Kützing	–	–	0.015	0.006	0.034
Cladophora pellucida (Hudson) Kützing	–	0.037	0.011	0.003	–
Enteromorpha prolifera (Müller) J. Agardh	–	0.048	0.002	–	–
Enteromorpha ramulosa (Smith) Hooker	–	–	0.002	0.048	–
Antithamnionella elegans (Berthold) Price and John	–	0.017	0.016	0.002	0.006
Dasya sp.	–	–	0.003	0.005	0.032
Ceramium codii (Richards) Mazoyer	–	0.002	0.023	0.003	0.002
Ceramium flaccidum (Kützing) Ardissone	–	–	0.005	0.003	0.020
Dictyota dichotoma (Hudson) Lamouroux	–	–	0.003	0.006	0.015
Antithamnion cruciatum (C. Agardh) Nägeli	–	–	–	0.023	–
Monosporus pedicellatus (Smith) Solier in Castagne	–	–	–	–	0.023
Ceramium tenuissimum (Roth) J. Agardh	–	0.015	–	0.002	0.002
Herposiphonia tenella (C. Agardh) Ambronn	–	–	0.002	0.015	0.002
Aglaophenia kirchenpaueri (Heller, 1868)	–	–	0.002	0.002	0.009
Callithamnion corymbosum (Smith) Lyngbye	–	–	0.003	0.002	0.006
Aglaothamnion tenuissimum (Bonnemaison) Feldmann-Mazoyer	–	0.003	–	–	0.007
Sphacelaria sp.	–	–	–	0.002	0.007
Chondria capillaris (Hudson) M.J. Wynne	–	–	–	–	0.008
Codium vermilara (Olivi) Delle Chiaje	–	–	–	0.008	–
Peyssonnelia dubyi Crouan and Crouan	–	–	–	–	0.008
Audouinella sp.	–	0.002	–	0.002	0.003
Hincksia mitchelliae (Harvey) P.C. Silva	–	0.002	0.002	0.002	–
Cutleria adspersa (Mertens ex Roth) De Notaris	–	0.003	–	0.002	–
Champia parvula (C. Agardh) Harvey	–	–	–	0.002	0.002
Ectocarpus fasciculatus Harvey	–	–	0.002	–	0.002
Hincksia secunda (Kützing) P.C. Silva	–	0.004	–	–	–
Audouinella daviesii (Dillwyn) Woelkerling	–	–	–	0.003	–
Aglaothamnion caudatum (J. Agardh) Feldmann-Mazoyer	–	–	–	0.002	–
Blidingia minima (Nägeli ex Kützing) Kylin	–	–	0.002	–	–
Cladophora lehmanniana (Lindenberg) Kützing	–	–	–	–	0.002
Cladophora prolifera (Roth) Kützing	–	–	0.002	–	–
Cladophora rupestris (Linné) Kützing	–	0.002	–	–	–
Dictyopteris polypodioides (de Candolle) J.V. Lamouroux	–	–	0.002	–	–
Gymnothamnion elegans (Schousboe ex C. Agardh) J. Agardh	–	–	0.002	–	–
Taonia atomaria (Woodward) J. Agardh	–	–	–	–	0.002
Trailliella intricata Batters (stadium)	–	0.002	–	–	–
Bryopsis muscosa Lamouroux	–	–	–	–	0.001
Chaetomorpha aerea (Dillwyn) Kützing	0.001	–	–	–	–

(authors' personal observations). The concentrations at which ulvacean algae develop in this study are very high for mediterranean water standards (e.g. 50–70 μmol NH_4^+ l^{-1}, 12–18 μmol NO_3^- l^{-1}; 12–18 μmol PO_4^{3-} l^{-1}). The complete dominance of *Ulva* in site 1 and the absence of other species can be related to the high levels of ammonium which seem to trigger the development of thionitrophilic algae and prevent the growth of most algae (Waite and Mitchell, 1972). *C. elongata*, a species well-known as a substitute of fucoid algae in a wide array of situations involving different kinds of disturbances (Belsher, 1979; Ballesteros, 1988a; Soltan et al., 2001; Benedetti-Cecchi et al., 2001) but without a marked preference for thionitrophilic environments (Boudouresque, 1985), is the dominant species in a site still displaying high nutrient levels (around 15 μmol NH_4^+ l^{-1}, 6 μmol NO_3^- l^{-1}, and 6 μmol PO_4^{3-} l^{-1}), far above the normal levels obtained in unpolluted coastal mediterranean environments. *C. elongata* can be considered as a ''stress-tolerant species'' as calcification makes it resistant to abrasion and predation and heterotrichy allows its survival during unfavourable periods and a rapid recovery after disturbances (Littler and Kauker, 1984). In the field, *C. elongata* completely replaces *C. mediterranea* in environments where *Cystoseira* is not able to develop (e.g. places subjected to low irradiance levels, overgrazed areas, polluted areas) (Ballesteros, 1988a). *C. mediterranea* is a ''competitive'' species which, like other *Cystoseira* species, almost exclusively thrives in anthropogenically undisturbed sites (Bellan-Santini, 1968; Belsher, 1979; Hoffmann et al., 1988; Ballesteros et al., 1998; Thibaut et al., 2005). In this study *C. mediterranea* is only dominant at lower values of dissolved nutrients concentrations in seawater than those found in the communities of *U. rigida* and *C. elongata*. However, *C. mediterranea* seems to resist a certain degree of pollution since nutrient levels in sites 3 and 4 are two to threefold higher than those found in

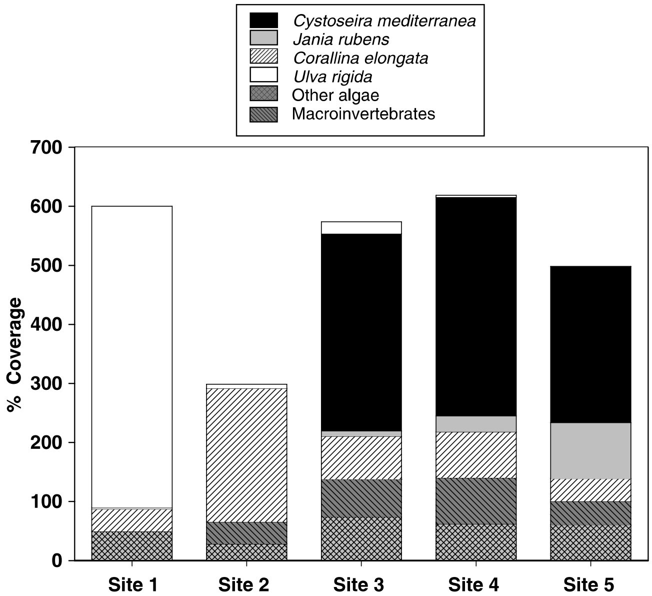

Fig. 3. Mean annual percentage coverage of dominating species and groups at each sampling sites.

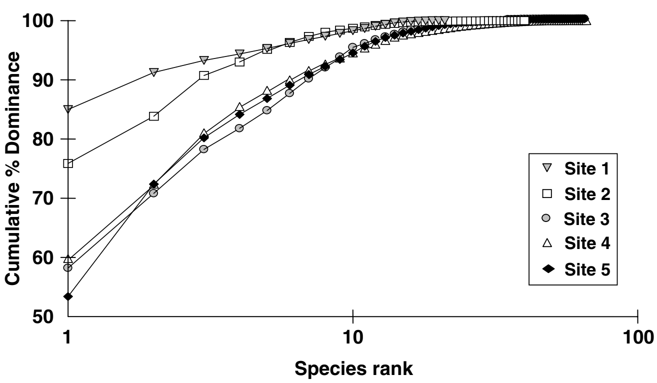

Fig. 4. *K*-Dominance curves for mean coverage at each sampling sites.

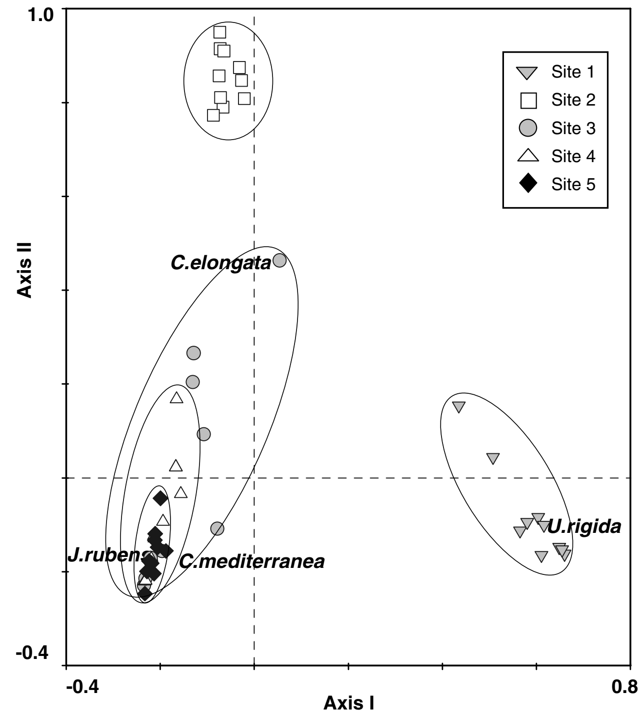

Fig. 5. Correspondence analysis (CA) ordination plot showing the distribution of samples using mean coverage data of the species (three replicates) at each sampling date. Only dominant species (*Cystoseira mediterranea*, *Corallina elongata*, *Jania rubens* and *Ulva rigida*) are represented in the ordination.

Table 3
Results from multiple correlations between CA scores for axis I and II and dissolved nutrients concentrations: (a) all samples considered and (b) all samples except those collected in site 1

	Axis I		Axis II	
(*a*)				
Silicates	0.748	***	0.068	n.s.
Phosphates	0.703	***	0.084	n.s.
Nitrites	0.613	***	0.076	n.s.
Nitrates	0.749	***	0.033	n.s.
Ammonia	0.687	***	−0.064	n.s.
(*b*)				
Silicates	0.359	*	0.505	***
Phosphates	0.379	*	0.534	***
Nitrites	0.363	*	0.478	**
Nitrates	0.317	*	0.480	**
Ammonia	0.372	*	0.449	**

* $p < 0.05$; n.s.: non-significant.
** $p < 0.005$.
*** $p < 0.001$.

the reference situation (site 5) and in nearby unpolluted areas (Ballesteros, 1989). Sites 3 and 4, situated relatively close to the outfall (in the range of hundreds of meters), and displaying a relatively high nutrient concentration and also a high variability in these concentrations (see Fig. 2), also show a high variability in the species composition and in the relative abundances of the species thriving in the community, stated by the high dispersion of the samples in the correspondence analysis (see Fig. 5). Although samples collected in these sites usually have a high coverage of *C. mediterranea*, they still display a relatively high amount of *C. elongata* and (sometimes) *U. rigida*, being the coverage of the red epiphytic articulated coralline *J. rubens* rather low. Site 5, situated far away from the outfall, in a place not affected by sewage waters, shows dissolved nutrients concentrations in seawater similar to that detected in other non-impacted coastal Mediterranean environments (e.g. Ballesteros, 1992), with ammonium and nitrate levels situated usually below 1 μmol l^{-1} and dissolved phosphate levels usually situated close to its detection levels (<0.04 μmol l^{-1}). This site shows also the lowest sample variability, which is probably related to seasonal changes (Ballesteros, 1991) that have been already described for this community (Ballesteros, 1988b).

We have shown in this study that the nutrient enrichment made by the sewage outfall was hardly detectable at

100 m and its effects were completely absent at 1000 m. This could seem to be a very reduced effect but Tossa de Mar is a small village situated in a relatively isolated area and domestic sewage discharges come from a population of 4500 inhabitants in winter and 20,000 in summer (Sardá et al., 2005). Therefore, the overall impact of the discharge of dissolved nutrients from heavily populated areas such as the metropolitan area of Barcelona (3,500,000 inhabitants) should be great, as demonstrated by other studies (Ballesteros et al., in press; Pinedo et al., in press). Also and unfortunately, nutrients are not the only chemical compounds that are likely to produce shifts in macroalgal communities. There is a wide array of substances, from metals to several persistant organic compounds (POC) whose concentration has increased in several coastal areas all over the world. According to Moschella et al. (2005) more than 100,000 anthropogenic compounds are released into the aquatic environment and nobody knows the environmental effects of most of them, nor the multiple effects resulting from their interactions. In this study we have only tackled the effects of the sewage from a tourist village whose residual waters are nutrient enriched but they are presumably devoid of several harmful compounds poured out by industries or used in agriculture. Thus, sewage waters coming from industrial effluents or run-off waters from agricultural areas may have different (and probably enhanced) impacts on macroalgal communities to those reported here.

Results obtained in this study can also serve to test the validity of the methodologies currently in use to assess water quality of Mediterranean coastal environments using macroalgae. In the community approach used by Pinedo et al. (in press) and in the cartographical approach proposed by Ballesteros et al. (in press) continuous and structurally complex forests of *Cystoseira* spp. (with the exception of *Cystoseira compressa*) are indicators of high ecological status. The decrease of *Cystoseira* abundance implies a lowering of water quality, and assemblages dominated by *C. elongata* indicate intermediate levels of water quality (moderate to poor ecological status). Blooms of ulvacean algae in the upper sublittoral zone and total absence of *Cystoseira* spp. always indicate a poor to bad ecological status. This classification closely agrees with the findings obtained in the present study.

The approach used by Orfanidis et al. (2001) and Panayotidis et al. (2004) is based on the assumption that functional-form groups of macroalgae as described by Littler and Littler (1980) and Littler et al. (1983) can be used as biological indicators regarding water quality. In fact, Orfanidis et al. (2001) do not apply the Littler's categories but distinguish two main groups of algae based on a oversimplification of the functional-form groups of Littler and Littler (1980). According to Orfanidis et al. (2001) benthic communities of pristine sites should be dominated by perennial species (i.e. algae with a thick or calcareous thallus) whilst degraded sites should shelter a high number and coverage of opportunistic species (i.e. coarsely-branched, sheet-like and filamentous algae). They developed the "Ecological Evaluation Index" (EEI) based on the relative abundances of perennial and opportunistic species in a community. Application of the EEI index to the samples collected in this study scores site 1 as a bad ecological status site and the rest of sites as high ecological status (Table 4). Therefore, the classification provided by the EEI index does not agree with the water quality found at intermediate levels of pollution. This disparity could be due to differences in the communities from the Eastern Mediterranean, where the EEI index was developed, and communities found in the Northwestern Mediterranean. However, and in our opinion, the functional-form group approach has some flaws in classifying species according to their sensitivity to pollution because, amongst other reasons, it is not able to differentiate between perennial species which are stress tolerant and those which are not. For example, both *C. mediterranea* and *C. elongata* are perennial species but its response in front of pollution or any other kind of disturbance is completely different. Moreover, even two species belonging to exactly the same functional-form group in the sense of Littler et al. (1983) or situated in the same taxonomical group (Genus, Family) can display a completely different response to pollution as demonstrated in the present study. This is the case of *C. elongata* and *J. rubens*, both in the Jointed Calcareous group and members of the Family Corallinaceae, but displaying a completely different pattern of distribution along the studied pollution gradient. Another example is provided by several algae belonging to the Filamentous or Sheet-like groups such as most of the members of the Order Ceramiales which only thrive in good to high ecological status sites (e.g. this study, but see also Belsher, 1979). Moreover, it has to be remembered that the functional form group hypothesis was originally proposed to predict productivity and other ecological attributes (e.g. grazing resistance, competitive abilities, reproductive effort), but not resistance to pollution, from morphological features of the species. According to our results, an opportunistic life strategy seems not to be the only way to resist pollution and a sheet-like or a filamentous morphology cannot guarantee pollution resistance. Expert knowledge on the distributional patterns and natural history of the species and field studies focused to provide correlational evidences among pollution levels and species abundances is, in our opinion, the best

Table 4
Mean annual percentages of coverage of species belonging to the thick leathery and calcareous functional form groups (ESGI, corresponding to the Ecological State Group I in Orfanidis et al. (2001)) and to the coarsely branched, filamentous and sheet-like functional form groups (ESGII, corresponding to the Ecological State Group II in Orfanidis et al., 2001) and Ecological Status of the water bodies calculated according to EEI index

	Site 1	Site 2	Site 3	Site 4	Site 5
% ESGI	2.5	95.4	86.1	91.5	94.0
% ESGII	97.5	4.6	13.9	8.5	6.0
Ecological status	Bad	High	High	High	High

approach to describe the vegetation changes along pollution gradients and to create suitable and accurate indexes based on macroalgae as pollution indicators.

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