

Response of macroalgae and macroinvertebrates to anthropogenic disturbance gradients in rocky shores



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ARTICLE INFO

Article history:

Received 8 August 2015

Received in revised form 12 October 2015

Accepted 14 October 2015

Available online 10 November 2015

Keywords:

Marine

Intertidal

Nutrient enrichment

Biological quality elements

Comparative response

Opportunist/sensitive taxa

ABSTRACT

The compliance of macroalgal and macroinvertebrate assemblages to anthropogenic disturbance gradients (e.g., nutrient enrichment) was investigated at intertidal rocky shores. Macroalgae and macroinvertebrates presented parallel behavior, both showing shifts in the communities' structural variation along the gradients, in which an higher number of opportunistic species (and higher abundances) were found in more stressful sites (close to the disturbance source), in contrast to less disturbed sites (far from the disturbance source), which showed higher presence of more sensitive species (and higher abundance of several of them).

The macroinvertebrate abundance and taxonomic composition, which are parameters required by the Water Framework Directive (WFD) to be included in tools for the ecological quality status assessment, responded to the disturbance gradient. Results suggest that the macroinvertebrate biological element might be considered an indicator of disturbance in intertidal rocky shores as good as the macroalgae, and therefore the development of a specific methodology based solely on benthic macroinvertebrates of rocky shores, presently a gap in the ecological quality status assessment for the WFD, seems feasible.

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1. Introduction

Rocky shores, similarly to many coastal systems, have been historically exposed to human pressures (e.g., wastewater discharges) (Mearns et al., 2014). Despite that, rocky shores are considered of great importance in marine ecosystems, providing valuable ecosystem services in terms of biological diversity, contribution to primary productivity, fisheries and tourism (Seitz et al., 2013).

In the last decade, the interest on coastal marine biological communities has increased, mainly concerning the classification of water bodies' quality status, which is an essential requirement in terms of the implementation of directives such as the Water Framework Directive (WFD, Directive 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) in Europe, or the Clean Water Act (CWA, 2002) in the United States, among others (Borja et al., 2008). The use of benthic communities in marine pollution assessments is based on the concept that they reflect not only conditions at the time of sampling but also conditions to which the community was previously exposed (Reish, 1987). Due

to their permanence over seasonal time scales, benthic communities may integrate the effects of long-term exposure to natural and anthropogenic disturbances (Borowitzka, 1972). Moreover, there is extensive literature about their taxonomy, ecology and distribution, and about responses to disturbance (e.g., Boaventura et al., 2002; Schiel, 2004; O'Hara et al., 2010; O'Connor, 2013; Cabral-Oliveira et al., 2014a,b).

In coastal waters (CW), where are included the rocky shores, the macroinvertebrates and macroalgae are some of the biological elements encompassed in such quality assessments. However, despite of the readiness of information on rocky shore intertidal communities, the use of both elements in assessment studies is many times hampered by the different methodologies usually applied to each element (e.g., sampling technique, data processing). Consequently, many studies have generally been based in visual census, or have been focused on relations between particular groups of macroinvertebrates and/or macroalgae (e.g., Bishop et al., 2002; Terlizzi et al., 2005; Pereira et al., 2006; De-la-Ossa-Carretero et al., 2010; Atalah and Crowe, 2012), and not using quantitative data and encompassing entire communities, as in the present work. Furthermore, despite the many historical papers analysing such communities (e.g., Littler and Murray, 1975; López Gappa et al., 1993; Archambault et al., 2001), studies on the structural variation

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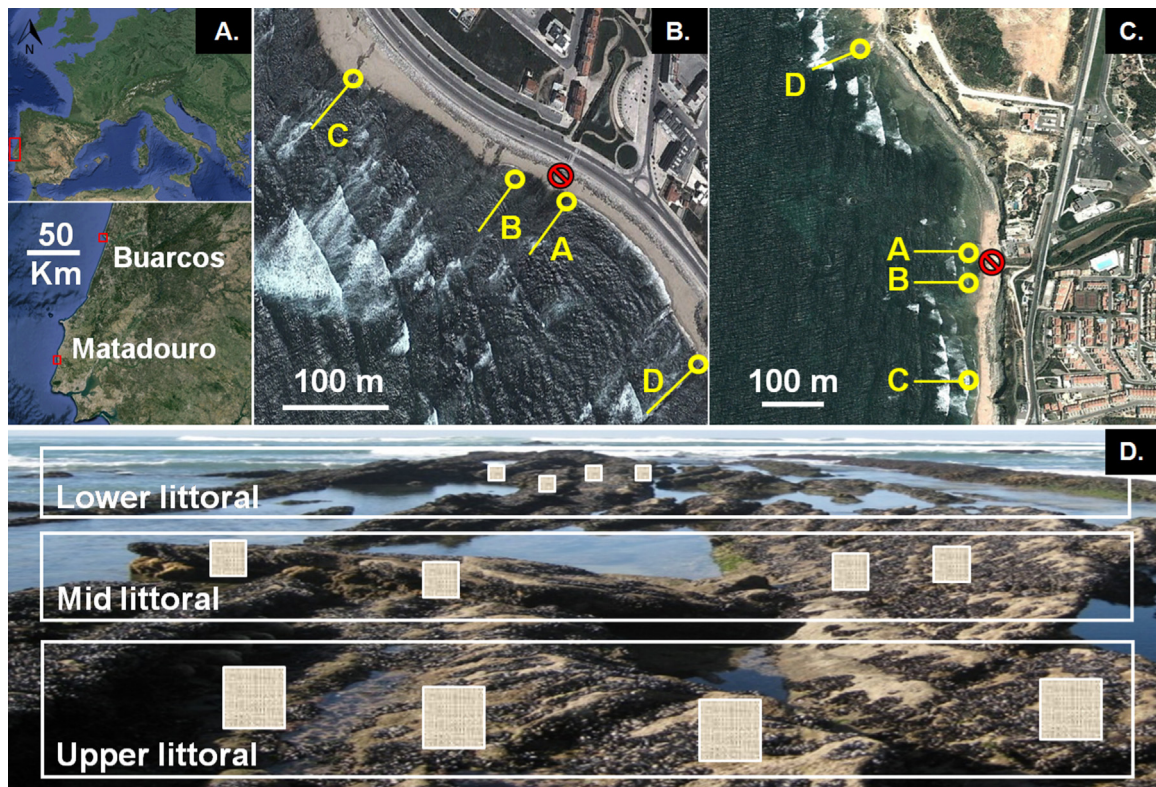


Fig. 1. Study sites location: A. Europe and Portugal; B. Buarcos; C. Matadouro; D. Sampling design. In both Buarcos and Matadouro sites (A–D) are placed gradually away from the source of pollution (SOP; ⊗ sign), with site D positioned as control.

of intertidal rocky shore macroalgae and macroinvertebrate communities, simultaneously, and inside the same disturbance gradient are not common.

The present work aimed to analyze the shifts shown by macroalgal and macroinvertebrate communities exposed to the same anthropogenic disturbance gradient (e.g., small nutrient enriched water discharges). More specifically: (a) to verify if the structural variation of macroinvertebrate and macroalgal communities follows similar patterns, and (b) to check if the variation in structure of each biological element is related to the disturbance level affecting the study areas.

2. Materials and methods

2.1. Study area

Two rocky shores located in the western Portuguese coast, Buarcos and Matadouro (Fig. 1A), were studied in this work. They are included in the Exposed (Buarcos) and Moderately Exposed (Matadouro) Atlantic Coast typologies (TICOR project, Bettencourt et al., 2004; available at <http://www.ecowin.org/ticor/>). Along this coast the prevailing current direction is from West-Northwest (Portuguese Coastal Current) with occurrences from Southwest (Portuguese Coastal Counter-Current) (Bettencourt et al., 2004). In Buarcos area, the Boa Viagem hill may lead to a current turnover from North-South to South-North orientation (Pais-Costa, 2011; personal observation). The most frequent wave period and wave height are in the range of 8–12 s and of 1–3 m, respectively. Tide is semidiurnal and the extreme spring tide ranges from 3.5 to 4 m (Boaventura et al., 2002; Bettencourt et al., 2004). Surface sea temperature ranges between 13 and 15 °C during winter and 20 and 22 °C during summer, and surface salinity varies between 35 and 36 (Boaventura et al., 2002).

Both rocky shores are situated in narrow sandy shores and limited landward by urban infrastructures, namely coastline protection adjacent to seaside avenues. Buarcos is approximately 150 km north to Matadouro and is more exposed to the Atlantic influence. There, the rocky surface is composed of slightly sloped shelving platforms. In Matadouro, wave energy is more attenuated, and the rocky surface is less irregular and more horizontal along the platforms.

The sampling areas at both shores were selected due to the presence of a point source of pollution (SOP) discharging almost directly on the upper intertidal zone (Fig. 1B and C). At each shore, although with a very low run-off, the discharge is continuous throughout the year, crossing urban centers (Buarcos – Figueira da Foz, and Matadouro – Mafra, with 62,125 and 76,685 residing inhabitants in 2011, respectively) and agricultural land before reaching the shore.

2.2. Sampling design

Four sites were selected at Buarcos and Matadouro to characterize the disturbance gradients, sites A, B and C distancing gradually from the SOP (about 30–40, 50–60 and 250–300 m, respectively), and site D situated opposite to the prevailing coastal current direction, used as control (300–500 m, respectively) (Fig. 1B and C). At each site three horizontal zones were selected, naturally defined by tides – upper littoral (submersed for ~25% of the tide period, ~6 h/day), mid littoral (submersed for ~50% of the tide period, ~12 h/day) and lower littoral (submersed for ~75% of the tide period, ~18 h/day). At each zone four random replicates (12 × 12 cm²) were sampled (Fig. 1D). Sampling was done twice in summer (August and September 2011), during low-water of spring tides, and pools and crevices were avoided. The samples (96 from each shore) were immediately preserved after sampling

in neutralized 4% formalin solution (prepared with sea water). In the laboratory, the samples were sieved through a 1 mm mesh and the organisms sorted out. Macroinvertebrates were preserved in 80% ethanol for posterior count and identification, while macroalgae were frozen. Taxonomy for macroinvertebrates and macroalgae (done to species level whenever possible) was standardized in accordance to the World Register of Marine Species (WoRMS, <http://www.marinespecies.org>) and the AlgaeBase (<http://www.algaebase.org>), respectively. Macroinvertebrates' biomass was determined as ash-free dry weight (AFDW) (loss of ignition after 8 h of incineration at 450 °C of specimens previously dried at 60 °C until weight stabilization). Macroalgae biomass was determined as dry weight (DW) (specimens dried at 60 °C until weight stabilization). Prior to data analysis, macroinvertebrate density and biomass data were standardized to ind m⁻² and g AFDW m⁻², respectively, and macroalgae biomass was standardized to g DW m⁻².

In parallel to biological sampling, water samples (~20 L) were collected at each site and at the SOP, for quantification of chlorophyll a (Chl.a) (µg L⁻¹) (Strickland and Parsons, 1972), total suspended solids (TSS) (g L⁻¹), particulate organic matter (POM) (g L⁻¹), and determination of dissolved nutrients concentration (mg L⁻¹) [N-NO₃, N-NO₂, N-NH₄, P-PO₄ (DIP) and silica]. For TSS, a pre-weighed filter (Whatman GF/C glass fiber filter – 47 mm diameter, 1.2 µm pore) was dried (60 °C until constant weight), re-weighed, and the suspended material content estimated as the weight difference (dry weight). POM was determined weighing the same filter after combustion (450 °C, 8 h) (ash weight). Nutrients were analyzed by colorimetric reaction using a Skalar San++® Continuous Flow Autoanalyzer (Skalar, 2010). Dissolved inorganic nitrogen (DIN) was determined as the sum of N-NO₃, N-NO₂ and N-NH₄. Simultaneously to sampling, water temperature (°C), conductivity (µS cm⁻¹), oxidation-reduction potential (ORP) (mV), salinity, dissolved oxygen (DO) (%) and pH parameters were measured in situ (using an YSI Professional Plus handheld multiparameter probe).

2.3. Data analysis

All statistical analyses were performed with PRIMER 6 + PERMANOVA© software (Clarke and Gorley, 2006; Anderson et al., 2008).

2.3.1. Environmental data

The environmental parameters (Env.) were used to visualize the sites' distribution inside the disturbance gradients by performing Principal Coordinate (PCO) analyses. It was firstly applied to Buarcos and Matadouro data conjunctly, to confirm that the gradients were similar at both shores. After this, PCO was applied to each shore data individually, to check the influence of the parameters inside the gradient within each shore. The Euclidean similarity measure was used in the calculation of similarity matrices, after square root transformation of data (except DIN, Chl.a and Silica, 1/X was used in these cases) to approach normality, followed by normalization.

Statistically significant differences between shores and between sampling sites within shore were tested with Permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). It was firstly applied to Buarcos and Matadouro data conjunctly, to test for differences between shores. It included two fixed factors, 'Shore' (two levels: Buarcos and Matadouro) and 'Site' (nested in Shore; five levels: SOP and sites A, B, C and D). After this, PERMANOVA was applied to each shore data individually, with only the factor "Site". The above mentioned similarity matrices were used. The statistical significance of variance components was tested using

9999 permutations and unrestricted permutation of raw data, with a significance level of $\alpha = 0.05$.

2.3.2. Biological data

Statistically significant differences were tested between shores, between sites within shore, between zones within site, and between zones across sites. Seven assemblage descriptors were used, five as multivariate data [macroalgae' biomass (B_{alg}) and presence/absence (P/A_{alg}) and macroinvertebrates' density (D_{inv}), biomass (B_{inv}), presence/absence (P/A_{inv})], and two as univariate data [macroinvertebrates' and macroalgae' richness (as number of species, S_{inv} and S_{alg} , respectively)]. For each descriptor, PERMANOVA was applied including three fixed factors, 'Shore' (two levels: Buarcos and Matadouro), 'Site' (nested in Shore; four levels: sites A, B, C and D) and 'Zone' (nested or non-nested in 'Site' to test within or across sites, respectively; three levels: upper, mid and lower littoral). For the multivariate descriptors Bray Curtis similarity measure was used in the calculation of similarity matrices, after fourth root transformation of data (to reduce natural dominance of species); for B_{alg} and P/A_{alg} it was included one *dummy variable* since there were samples without algae present, acceptable for their stressful nature (upper littoral). For the univariate descriptors, Euclidean distance was used, without transformation of data. The statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model for multivariate descriptors, and unrestricted permutation of raw data for univariate descriptors, with a significance level of $\alpha = 0.05$.

To infer about the agreement between assemblages, the relationships between the descriptors were analyzed with RELATE (comparative Mantel-type tests on similarity matrices), using the similarity matrices calculated earlier for PERMANOVA. Similarly, relationships were analyzed between each descriptor and Env. Accordingly, the data matrices were adjusted before calculating the similarity matrices: biotic data matrices were calculated as mean values per site of macroalgae biomass and macroinvertebrates density and biomass, and for Env. matrices the SOP was removed. Spearman correlation and 9999 permutations were used, with a significance level of $\alpha = 0.05$.

To identify the taxa (macroalgae and macroinvertebrates) which contribute mostly to the communities' structural variation between sites, Similarity Percentage Analysis (SIMPER) was applied to each B_{alg} and D_{inv} descriptors of Buarcos and Matadouro shores. Dissimilarities between groups were assessed using two-way crossed designs with factors 'Site' and 'Zone' (as for PERMANOVA) (with a 95% and 85% cut off for macroalgae and macroinvertebrates, respectively, and without transformation of data). After this, taxa showing higher contribution for dissimilarities between sites at each shore (first 10 macroalgae and first 20 macroinvertebrates) were selected from all comparisons (e.g., A–B, A–C), to show the structural variation among sites, by means of their abundances and sensitivity/tolerance to pollution. The sensitivity/tolerance to pollution was described for all taxa found, using the most recent literature. The macroalgae were assigned from a "Reduced Taxa List" (RTL) (Gaspar et al., 2012; Neto et al., 2012), in which macroalgae are grouped according to morphological and functional characteristics into one of two Ecological Status Groups (ESG), where ESG I includes late successional or perennial to annual taxa and ESG II includes opportunists or annual taxa. For the macroinvertebrates, the AZTI Marine Biotic Index – AMBI (Borja et al., 2000) list of November 2014 was used (available at <http://ambi.azti.es>). In such list, taxa are classified with consensus expert judgment into one of five Ecological Groups (EG I–V) regarding their responses to natural and man-induced changes in water quality, where increasing tolerance to pollution (organic enrichment) is expected from EG I to EG V.

2.3.3. Biological and environmental data

Distance-based linear modeling analyses (DistLM) (Legendre and Anderson, 1999) were performed to Buarcos and Matadouro data separately, to check the variation of D_{inv} and B_{alg} explained by Env. BEST was used as selection procedure and BIC (Bayesian Information Criterion) criterion, with 9999 permutations. Prior to DistLM, abiotic correlated variables (>0.9) were removed. Distance-based Redundancy Analysis (dbRDA) was performed to visualize the fitted models in the multi-dimensional space (McArdle and Anderson, 2001).

3. Results

3.1. Environmental data

At both shores, the environmental parameters salinity and conductivity showed similar behavior (Table 1); low salinity values were registered at the SOP (<1) and were 34–36 at all remaining sites (except at site A of Matadouro in August – 4.04). Water temperature (20 – 22°C) and pH (6.9 – 8.5) were usually higher at the SOP, while DO (72 – 77%) was lower there. Chl.a did not vary much between sites at Matadouro (0.3 – 2 mg L^{-1}), but at Buarcos the SOP (7.4 – 8.7 mg L^{-1}) registered much higher values than all the other sites (0.37 – 0.92 mg L^{-1}). POM and TSS behaved similarly, and showed different trends at Buarcos and Matadouro, with the SOP registering for both parameters higher values than the sites in Buarcos, contrary to Matadouro. Silica was much higher at the SOP (16 – 19 mg L^{-1}), followed by site A ($<0.63\text{ mg L}^{-1}$). DIN and DIP were generally higher at the SOP (maximum of 6.42 and 0.10 mg L^{-1} , respectively). The ORP (20 – 258 mV) was usually lower at the SOP and higher at site D.

The PCO ordination showed an overlap of the Buarcos and Matadouro sites (Fig. 2A). At each shore was observed a separation between the SOP and the remaining sites, followed by a gradual separation of site A, site B, and then sites C and D. In Buarcos the SOP was associated to higher DIP, POM, TSS, silica, DIN and Chl.a (as lower $1/\text{silica}$, $1/\text{DIN}$ and $1/\text{Chl.a}$, respectively, in PCO), and lower salinity, conductivity and DO (Fig. 2B). In Matadouro the pattern was similar, except the SOP which was associated to lower POM, TSS and Chl.a (as lower $1/\text{Chl.a}$ in PCO) (Fig. 2C).

Statistically significant differences were not found between Buarcos and Matadouro [PERMANOVA; pseudo- $F=1.8866$, $p(\text{perm})=0.3007$]. Differences between sites were found only in Buarcos, namely between the SOP and sites C [$t=2.6953$, $p(\text{MC})=0.0425$] and D [$t=3.0381$, $p(\text{MC})=0.0456$].

3.2. Assemblage composition

A total of 59 macroalgae (Appendix A) and 179 macroinvertebrate (Appendix B) taxa were identified. In both Buarcos (42 macroalgae and 143 macroinvertebrate taxa) and Matadouro (49 macroalgae and 147 macroinvertebrate taxa) the macroalgae Rhodophyta showed higher species richness (35 and 34 taxa for Buarcos and Matadouro, respectively), followed by Chlorophyta (4 taxa) and Phaeophyceae (3 taxa) in Buarcos, and by Phaeophyceae (9 taxa) and Chlorophyta (6 taxa) in Matadouro. Higher biomass of Chlorophyta was registered at sites A and B, while higher biomass of Phaeophyceae and Rhodophyta was observed at sites C and D. The opportunist taxa (e.g., *Ulva* spp., filamentous Phaeophyceae, filamentous Rhodophyta) biomass was higher at sites A and B and lower at sites C and D, at both shores. On the other hand, the perennial species *Lithophyllum incrustans* and *Fucus spiralis* showed higher biomass at sites C and D (the latter being present only in Matadouro) and *Corallina* spp. showed higher biomass at site D. Regarding the macroinvertebrates, in both shores six groups

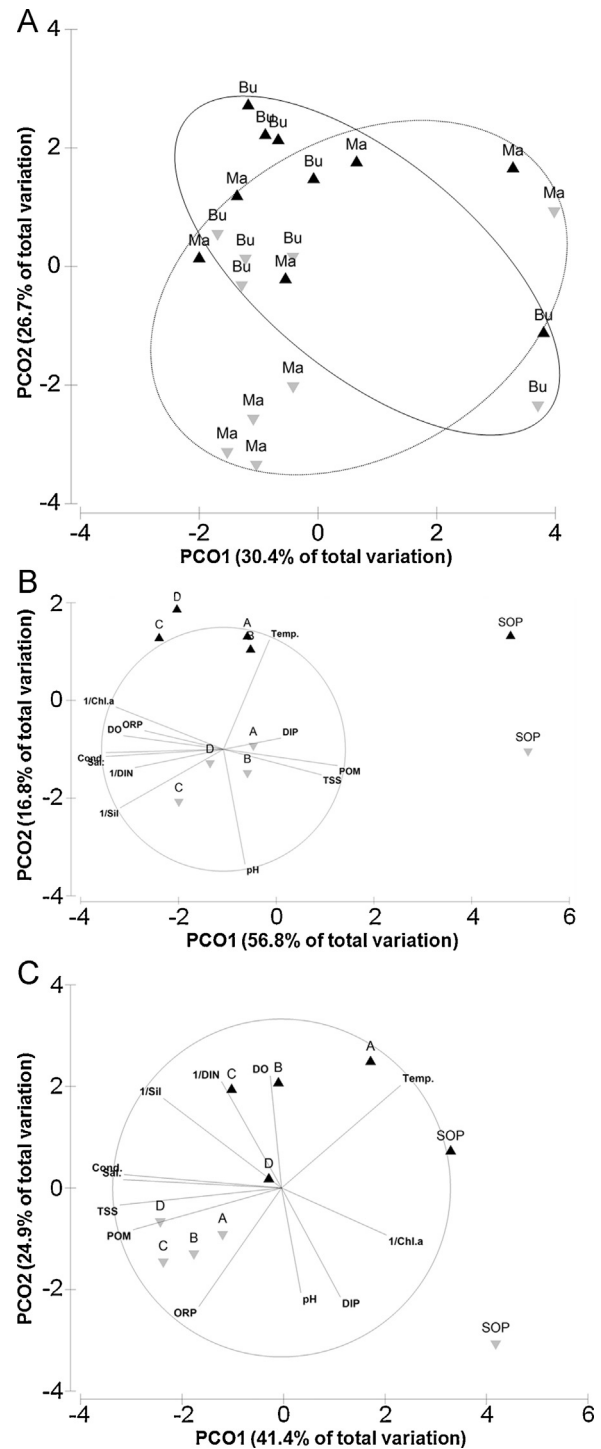


Fig. 2. Principal Coordinates Ordination (PCO) plots of the environmental parameters: A. Buarcos versus Matadouro shores; B. Buarcos; and C. Matadouro. Key: SOP= source of pollution; A, B, C and D= sites A, B, C and D. Black upward triangles = August, gray downward triangles = September.

constituted to about 90% of the total density and 95% of the total biomass. They were Cirripedia (essentially *Chthamalus montagui*), Bivalvia, Gastropoda, Polychaeta, Tanaidacea (essentially *Tanais dulongii*, contributed to density) and Polyplacophora (mainly *Acanthochitona* spp., contributed to biomass). It was found a higher species richness of Polychaeta (47 and 53 taxa for Buarcos and Matadouro, respectively), followed sequentially by Gastropoda, Amphipoda, Isopoda, Bivalvia, Decapoda, Insecta, Pycnogonida,

Table 1
Environmental parameters measured/estimated monthly (August and September) at the source of pollution (SOP) and sites A, B, C and D in Buarcos and Matadouro shores.

	August					September				
	SOP	A	B	C	D	SOP	A	B	C	D
Buarcos										
Temperature (°C)	22.0	21.5	20.5	20.1	21.2	19.9	19.2	19.1	17.8	18.7
pH	7.12	7.11	6.89	6.93	7.02	8.47	8.26	8.24	8.23	8.15
Salinity	0.94	35.23	35.29	35.37	35.34	0.74	36.12	35.88	36.34	36.26
DO (%)	74.6	149.0	94.6	125.5	125.3	77.7	114.2	109.1	124.1	107.4
ORP (mV)	125.0	114.9	123.8	149.5	211.5	94.3	151.8	148.8	134.6	168.8
Conductivity ($\mu\text{S cm}^{-1}$)	1659	49,705	48,834	48,472	49,557	1323	48,442	48,080	47,307	47,981
Chlorophyll a (mg m^{-3})	7.380	0.608	0.675	0.371	0.367	8.670	0.628	0.920	0.737	0.561
TSS (g L^{-1})	0.019	0.016	0.009	0.009	0.010	0.020	0.013	0.017	0.011	0.013
POM (g L^{-1})	0.0037	0.0019	0.0010	0.0010	0.0015	0.0045	0.0017	0.0018	0.0015	0.0016
Silica (mg L^{-1})	16.776	0.626	0.546	0.542	0.574	19.202	0.389	0.415	0.296	0.357
DIN (mg L^{-1})	0.600	0.446	0.303	0.265	0.382	0.773	0.632	0.386	0.219	0.303
DIP (mg L^{-1})	0.086	0.051	0.064	0.024	0.020	0.075	0.079	0.000	0.067	0.024
Matadouro										
Temperature (°C)	20.2	22.1	20.7	19.4	18.8	19.9	18.9	17.2	17.6	17.4
pH	7.51	8.14	7.65	7.55	7.50	8.43	8.05	8.15	8.03	7.94
Salinity	0.90	4.04	34.64	35.96	36.37	0.83	33.80	36.66	36.42	36.45
DO (%)	75.5	143.2	101.4	92.0	92.0	72.1	99.0	87.5	85.6	81.3
ORP (mV)	20.0	127.3	55.0	87.8	149.7	212.6	239.6	258.3	256.7	240.6
Conductivity ($\mu\text{S cm}^{-1}$)	1530	6899	48,250	48,615	47,940	1470	45,535	46,911	47,227	47,022
Chlorophyll a (mg m^{-3})	0.896	1.999	0.543	0.646	0.688	0.298	1.538	1.399	1.600	1.750
TSS (g L^{-1})	0.004	0.010	0.013	0.013	0.014	0.002	0.024	0.020	0.032	0.033
POM (g L^{-1})	0.0011	0.0021	0.0019	0.0020	0.0019	0.0012	0.0031	0.0030	0.0041	0.0032
Silica (mg L^{-1})	15.966	0.370	0.260	0.195	0.369	15.804	0.463	0.336	0.354	0.371
DIN (mg L^{-1})	0.282	0.433	0.164	0.240	0.586	6.422	0.586	0.370	0.281	0.319
DIP (mg L^{-1})	0.031	0.007	0.038	0.000	0.033	0.104	0.046	0.032	0.069	0.001

Polyplacophora and Cirripedia. The density of opportunist taxa (assigned to EG IV–V in AMBI), namely of Oligochaeta, the polychaetes *Boccardia polybranchia*, *Cirriformia tentaculata*, *Malacoceros fuliginosa* and *Tharyx* sp., and the insects Tipulidae, decreased away from the site (A) closest to the SOP. On the other hand, sensitive taxa (belonging to EG I, excluding *C. montagui*), namely the polychaetes *Ampharete* sp., *Euclymene palermitana*, *Serpula vermicularis* and *Travisia* sp., the amphipods *Ampelisca rubella* and *Maera grossimana*, the isopod *Paranthura nigropunctata*, the bivalves *Cardita calyculata*, *Hiatella arctica*, *Musculus costulatus*, *Psammobiidae* and *Striarca lactea*, and the gastropods *Crisilla semistriata*, *Patella* spp. (especially *P. ulyssiponensis*), *Rissoa parva*, *Skeneopsis planorbis* and *Tricolia pullus*, showed higher density in site D (sometimes in C). Nevertheless, some sensitive taxa showed higher abundance at sites (A and B) closest to the SOP, compared to the sites (C and D) furthest from the SOP, namely the amphipods *Apherusa cirrus* and

Photis longicaudata, and the gastropods *Gibbula* spp. and *Runcina coronata*.

Statistically significant differences (PERMANOVA; [Supplementary Material, Sheet 1](#)) were found between Buarcos and Matadouro for all tested descriptors, except S_{inv} [Pseudo- $F=0.0375$, $p(\text{perm})=0.8470$] and S_{alg} [Pseudo- $F=0.0769$, $p(\text{perm})=0.7833$]. Within both shores the pattern of descriptors B_{alg} , P/A_{alg} , D_{inv} , B_{inv} and P/A_{inv} was quite similar ([Fig. 3A, B, D, E and F](#)). Differences across sites, between zones within site and between zones across sites were found using these five descriptors. Generally, sites were different from each other, and so were zones within and across sites. Nevertheless, fewer differences were identified between sites A and B, which were closest to the SOP, between inner sites (B and C) inside the disturbance gradient, and between sites C and D, which were furthest from the SOP. Also, fewer differences exist between zones within sites (A and B) closest to

Table 2
RELATE results for environmental (Env.) and biotic descriptors of Buarcos and Matadouro rocky shores: B_{alg} , P/A_{alg} and S_{alg} are the macroalgae' biomass, presence/absence and number of taxa, and D_{inv} , B_{inv} , P/A_{inv} and S_{inv} are the macroinvertebrates' density, biomass, presence/absence and number of taxa. Higher relations (>0.9) are presented in bold and underlined. An asterisk (*) means the analyses were not statistically significant (significance level of $\alpha=0.05$).

		B_{inv}	P/A_{inv}	S_{inv}	B_{alg}	P/A_{alg}	S_{alg}	Env.
Buarcos	D_{inv}	<u>0.972</u>	<u>0.976</u>	0.701	0.690	0.639	0.498	0.503
	B_{inv}		<u>0.953</u>	0.687	0.673	0.623	0.472	0.523
	P/A_{inv}			0.701	0.657	0.613	0.471	*
	S_{inv}				0.624	0.577	0.500	*
	B_{alg}					<u>0.951</u>	0.782	0.408
	P/A_{alg}						0.807	0.408
	S_{alg}							*
	D_{inv}	<u>0.984</u>	<u>0.977</u>	0.680	0.778	0.711	0.477	*
	B_{inv}		<u>0.957</u>	0.688	0.770	0.703	0.486	*
Matadouro	P/A_{inv}			0.674	0.774	0.694	0.462	*
	S_{inv}				0.646	0.653	0.564	*
	B_{alg}					<u>0.927</u>	0.650	*
	P/A_{alg}						0.715	*
	S_{alg}							*

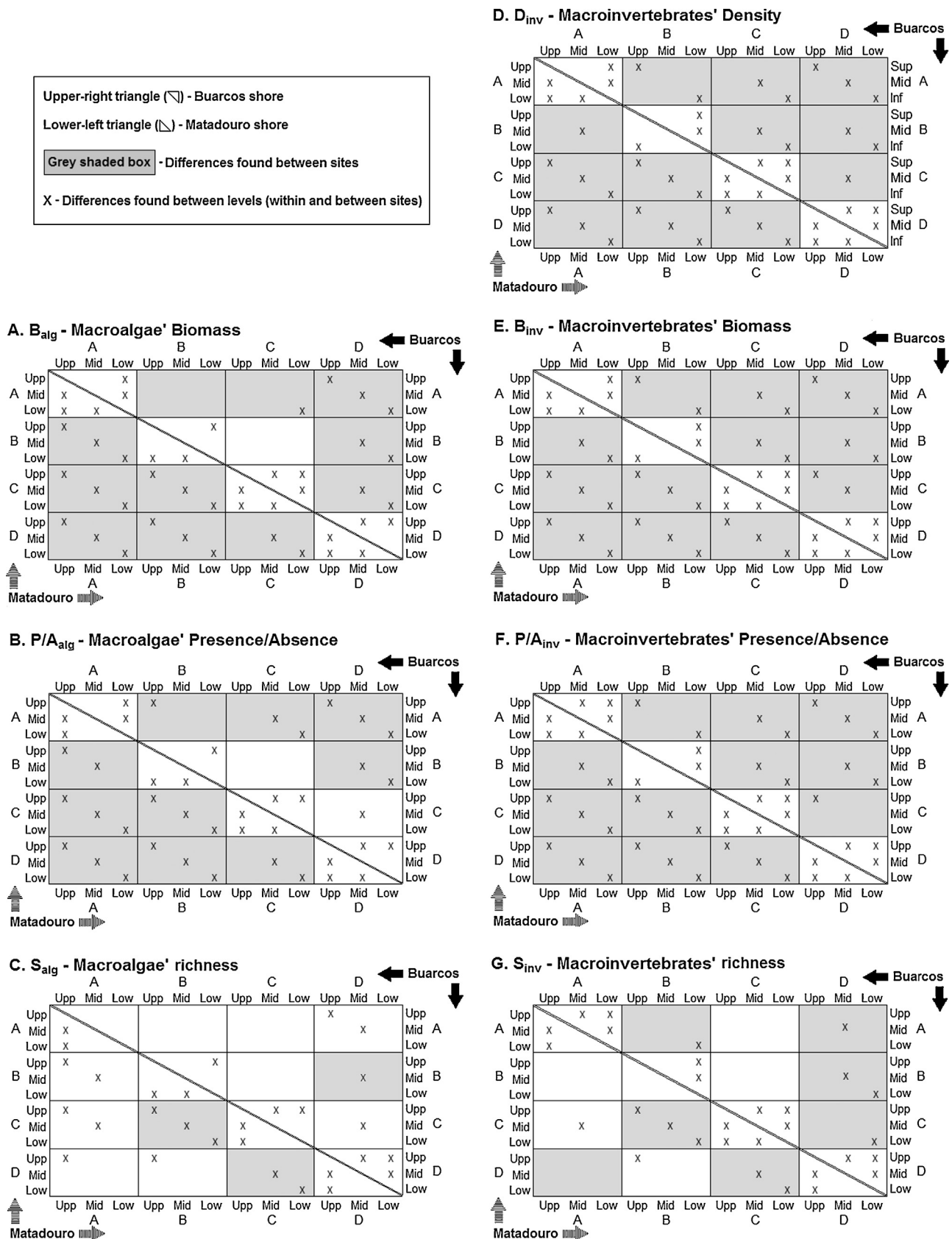


Fig. 3. Summarized PERMANOVA results for the assemblage descriptors: A. B_{alg} ; B. P/A_{alg} ; C. S_{alg} ; D. D_{inv} ; E. B_{inv} ; F. P/A_{inv} ; and G. S_{inv} in Buarcos (upper-right triangles) and Matadouro (lower-left triangles) shores. Key: A, B, C and D = sites A, B, C and D. Upp, Mid and Low = Upper, Mid and Lower littoral zones.

the SOP, and between zones across those sites. Fewer differences were also found between zones across sites (C and D) furthest from the SOP. The S_{alg} and S_{inv} were the descriptors with most distinct pattern showing, among descriptors, the fewest differences for all tested terms (Fig. 3C and G, respectively). Generally, the biggest differences were found between the site (D) furthest from the SOP and the other sites, and in zones between that site and the other sites. Simultaneously, fewer differences exist between zones within sites (A and B) closest to the SOP.

There were significant relationships with high correlation (RELATE) both at Buarcos and Matadouro, not only within the macroalgae and macroinvertebrates descriptors (>0.9 between $B_{alg}-P/A_{alg}$, $D_{inv}-B_{inv}$, $D_{inv}-P/A_{inv}$, and $B_{inv}-P/A_{inv}$), but also between descriptors of both biological elements, namely $B_{alg}-D_{inv}$ (0.69 and 0.78 in Buarcos and Matadouro, respectively) (Table 2). Also, there are significant relationships between biotic descriptors and Env. in Buarcos, namely between Env.- B_{alg} , Env.- B_{inv} , Env.- D_{inv} and Env.- P/A_{alg} .

The patterns in the communities' structural variation (SIMPER dissimilarities between sites and zones) support previous results for B_{alg} and D_{inv} in Buarcos (Supplementary Material, Sheet 2) and Matadouro (Supplementary Material, Sheet 3). Regarding B_{alg} (Fig. 4A–C and Appendix A), in Buarcos dissimilarity was higher between sites B and D (85%) and lower between sites B and C (76%). Dissimilarity was also higher (94%) between the upper and mid zones, and between the upper and lower zones. In Matadouro, dissimilarity was higher between sites B and C (78%) and lower between sites C and D (65%), sites B and D (66%) and sites A and B (67%). Dissimilarity was also higher (89%) between the upper and mid zones, and between the upper and lower zones.

Five taxa contributed mainly to the dissimilarities between sites – *Ulva* spp. (leaf-like forms), *Corallina* spp. and *Porphyra* spp. in Buarcos, and the former two plus *L. incrustans* and *F. spiralis* in Matadouro. In both shores *Corallina* spp. (EG I) was much variable in biomass across sites, although registering higher values in the site (D) furthest from the SOP. Sensitive taxa increased in biomass from the site (A) closest to, to the site (D) furthest from, the SOP (or showed higher biomass further away – sites C and D – than closer – sites A and B – to the SOP), namely *Lithophyllum incrustans* (EG I), *F. spiralis* (EG I), *Mastocarpus stellatus* (EG I), *Pterosiphonia complanata* (EG I), *Bifurcaria bifurcata* (EG I), *Dictyota dichotoma* (EG II) and *Leathesia marina* (EG II). In contrast, tolerant taxa (EG II opportunists) decreased in biomass in that direction (or presented higher biomass in sites A and B comparing to sites C and D), namely *Ulva* spp. (leaf-like forms), *Ulva* spp. (tubular-like forms), *Porphyra* spp., *Rhodochorton* spp./*Rhodothamniella* spp. and *Ceramium* spp. Four taxa contributed mainly to the dissimilarities between zones – *Ulva* spp. (leaf-like forms), *Corallina* spp. and *Osmundea pinnatifida* in Buarcos, and the former two plus *L. incrustans* in Matadouro.

Regarding D_{inv} (Fig. 4D–F and Appendix B), in Buarcos, dissimilarity was higher between sites A and B (78%) and lower between sites C and D (53%). Dissimilarity was also higher between the upper and lower zones (85%). In Matadouro, dissimilarity was higher between sites A and C (81%) and lower between sites C and D (63%). Dissimilarity was also higher between the upper and lower zones (90%).

Three species were top contributors to dissimilarities between sites in both shores – *C. montagui*, *Mytilus galloprovincialis* and *Rissoa parva*. *Chthamalus montagui* (EG I) and *M. galloprovincialis* (EG III) showed high variability in density across sites, despite *M. galloprovincialis* registering higher biomass at sites (A and B) more proximate to the SOP (especially in Buarcos). *Rissoa parva* (EG I) increased in density from the site (A) closest to, to the site (D) furthest from, the SOP (or showed higher density further away – sites C and D – than closer – A and B – to the SOP). The same was observed for other (less) contributing taxa such as the sensitive *Dynamene*

bidentata (EG II), *Melarhapha neritoides* (EG II) (which was not found in site A in Matadouro), *Psammobiidae* (EG I), *Patella depressa* (EG I) and *Acanthochitona* spp. (EG I) (which also showed higher biomass in sites C and D). In contrast, the opportunists *Oligochaeta* (EG V), *Omalogyra atomus* (EG III) (which only occurred in Matadouro), *Chironomidae* (EG III) and *Nemertea* (EG III) decreased in density in that direction (or showed higher density in sites A and B comparing to sites C and D). Six taxa contributed mainly to the dissimilarities between zones – *C. montagui*, *M. galloprovincialis*, *R. parva* and *S. alveolata* in Buarcos, and these four plus *M. neritoides* and *Barleeia unifasciata* in Matadouro.

3.3. Environmental and biological relationships

Prior to the DistLM, correlated variables (>0.9) from each shore were removed: in Buarcos, pH (correlated with salinity and silica), silica (correlated with conductivity, pH, salinity and temperature), temperature (correlated with conductivity, salinity and silica) and TSS (correlated with POM), and in Matadouro, conductivity (correlated with DO and salinity), DO (correlated with conductivity and salinity), pH (correlated with Chl.a) and TSS (correlated with POM).

The DistLM (BEST) models were constituted by six abiotic variables for B_{alg} and D_{inv} of both shores (BIC = 38.49, $R^2 = 0.933$ and BIC = 44.56, $R^2 = 0.943$, respectively, for Buarcos; BIC = 45.54, $R^2 = 0.931$ and BIC = 44.88, $R^2 = 0.917$, respectively, for Matadouro). For Buarcos, using B_{alg} , the first two axes captured 56.2% of the fitted variation, which is 53.0% of the total variation. Sites C and D were associated to higher ORP, and lower conductivity, DIN (as higher 1/DIN in dbrDA) and Chl.a (as higher 1/Chl.a in dbrDA), contrary to sites A and B (Fig. 5A). Using D_{inv} the first two axes captured 52.5% of the fitted variation, representing 48.9% of the total variation. Sites C and D were associated to higher ORP, and lower DIN (as higher 1/DIN in dbrDA) and Chl.a (as higher 1/Chl.a), contrary to sites A and B (Fig. 5B).

For Matadouro, using B_{alg} , the first two axes captured 68.0% of the fitted variation, corresponding to 63.3% of the total variation. Sites C and D were essentially associated to lower ORP and temperature, contrary to sites A and B (Fig. 5C). Using D_{inv} , the first two axes captured 60.4% of the fitted variation, which is 55.4% of the total variation. Sites C and D were mainly associated to lower ORP, and associated in minor degree to higher POM and DIN (as lower 1/DIN in dbrDA), and lower silica (as higher 1/silica in dbrDA) (Fig. 5D).

4. Discussion and conclusions

4.1. Macroalgal and macroinvertebrate communities' response to disturbance

In rocky shores macroalgae constitute habitats for macroinvertebrates seeking for food, shelter from predators, or avoiding desiccation and thermal stress (Bustamante et al., 2014). Therefore, the response of both macroalgae and macroinvertebrates communities' structure (abundance or richness) under the same environmental influences should, at least in a certain extent, be equivalent. Although in the present work we could only consider prompt measures of environmental factors (Env.), discarding variations through time, the statistical analysis performed showed that, at least in the periods considered, those parameters were generally distinct between the sampling sites, namely regarding the source of pollution (SOP). Levels of disturbance appeared to be similar in both shores, with stronger influence of the SOP at proximate sites (A and B).

The macroalgal assemblages are known to respond to human induced pressures (e.g., organic enrichment), and species may

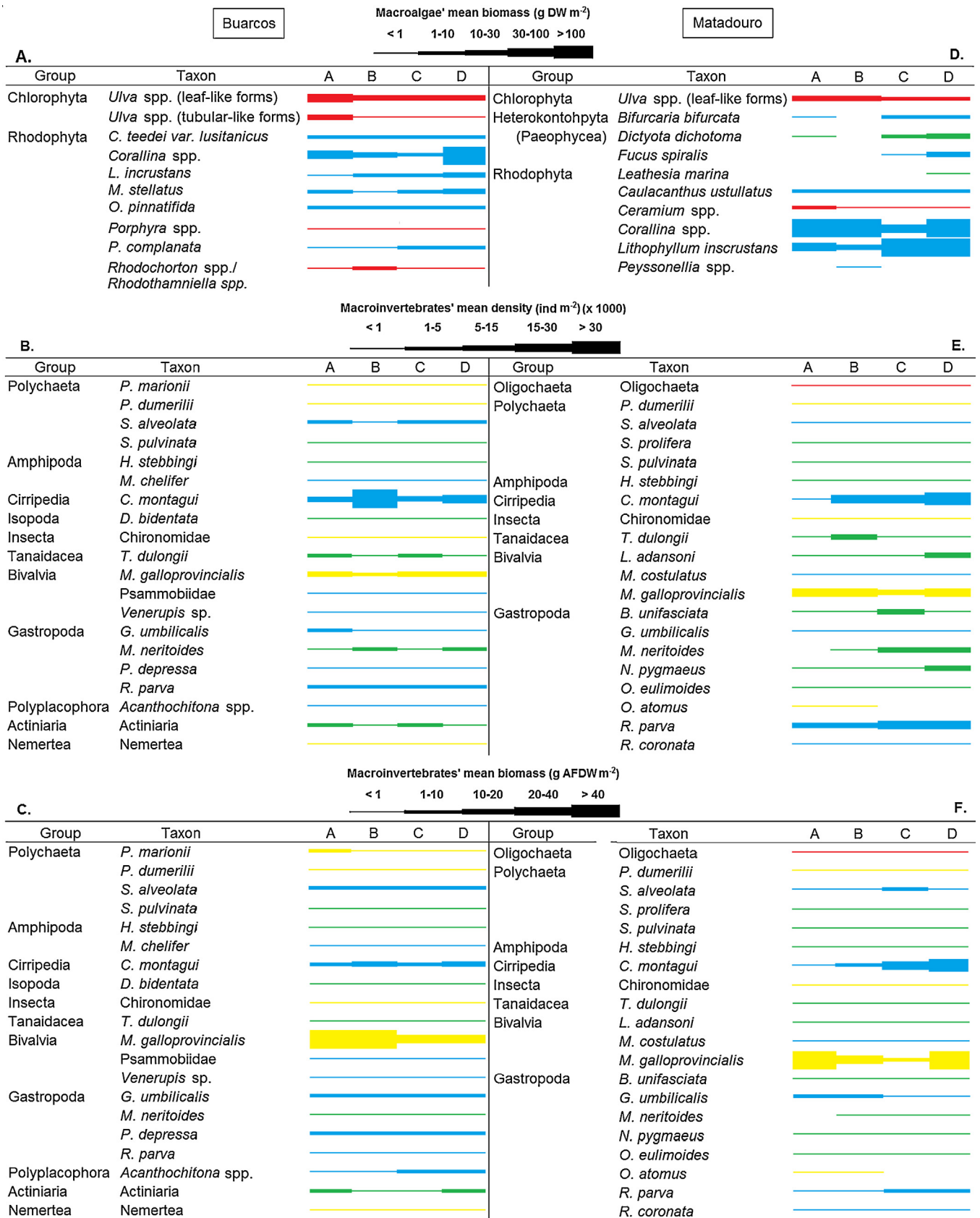


Fig. 4. Taxa contributing to the communities' structural variation between sites A, B, C and D, and their abundances (macroalgae' biomass, and macroinvertebrates' density and biomass), in Buarcos (A, B, C and D, respectively) and Matadouro (D., E. and F., respectively). [Taxa are alphabetically ordered within higher groups. Key: Thickness of bars represents different abundance classes. Color of bars represent opportunism of taxa: Macroalgae – black: opportunist taxa from EG II; dark gray: non-opportunistic taxa from EG II; and light gray: taxa from EG I (Gaspar et al., 2012; Neto et al., 2012); Macroinvertebrates – black: taxa from EG I; dark gray: taxa from EG III; light gray: taxa from EG II; and white: taxa from EG I (AMBI species list of November 2014).]

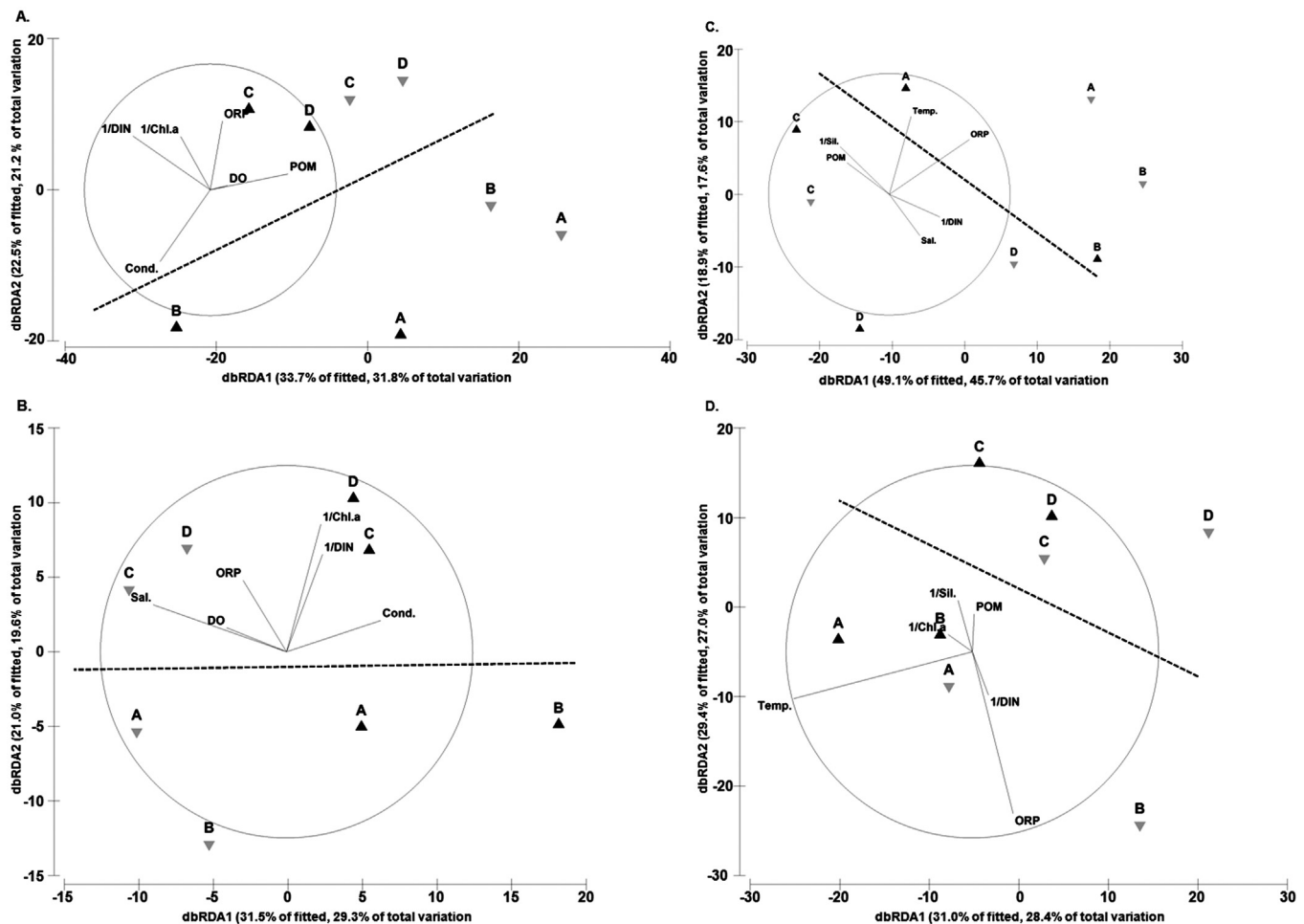


Fig. 5. Two-dimensional distance-based redundancy analysis (dbrDA) ordination based on the best set of environmental variables (using BEST as selection procedure and BIC as selection criterion) and macroalgal and macroinvertebrate data from Buarcos (A. and B., respectively) and Matadouro (C. and D., respectively). Key: Black upward triangles = August, gray downward triangles = September. A dashed line is aiding to visualize the separation of sites A and B from sites C and D.

respond positively or negatively according to their tolerance to the disturbance, which may result in pronounced shifts from pristine reference conditions to degraded quality states (Odum, 1985; Orfanidis et al., 2011; Gaspar et al., 2012). In the present work, when comparing sampling sites in each shore (Buarcos and Matadouro shores), the predicted gradient of disturbance was visible. Site (A), nearest to the SOP, was more stressful than the one located further away from the SOP (D). Despite that there was not great variation in richness between sites inside the gradient; such stressful environment has resulted in differences in species composition and abundance. Higher biomass of opportunist taxa (considering all the opportunists: e.g., *Ulva* spp., filamentous Phaeophyceae, filamentous Rhodophyta) was found closer to the SOP (and decreasing to the furthest site from the SOP). On the other hand, higher biomass of perennial species like *Corallina* spp., *F. spiralis* and *L. incrustans*, which have been regarded as sensitive and indicative of good environmental health (Gaspar et al., 2012; Neto et al., 2012), was found further from the SOP. These results were expected since Chlorophyta opportunists are usually dominating species in degraded areas and may cause the diminishing of other less competitive species, while Phaeophyceae and Rhodophyta species usually show a decline in richness and abundance in those areas (Gaspar et al., 2012). These shifts in the communities were verified by performing several statistical routines (e.g., SIMPER, PERMANOVA), which

showed several macroalgal community descriptors, namely the macroalgal biomass (B_{alg}), were indicative of more similar structure either between the first two sites, between the inner sites, or between the last two sites in the gradient.

The overall findings for the macroalgal communities in the present work are indicative of their already acknowledged potential to respond to anthropogenic disturbance (e.g., Borowitzka, 1972; Juanes et al., 2008; Neto et al., 2012), being useful for detecting such anthropogenic disturbances by presenting changes in species composition and abundance, which are required by the WFD to be included in tools for the ecological quality status assessment (WFD, 2000).

Regarding the macroinvertebrates, similarly to the macroalgae, certain groups are known to generally respond positively to pollution, being tolerant to more stressful environments and therefore increasing in abundance, such as nemerteans (e.g., Fraschetti et al., 2006; Cabral-Oliveira et al., 2014b) and polychaets (e.g., Dauer and Conner, 1980; Cabral-Oliveira et al., 2014b), while other more sensitive groups usually decrease in abundance, such as gastropods (e.g., Airolidi, 2003; Terlizzi et al., 2005) and crustaceans (e.g., De-la-Ossa-Carretero et al., 2010). In the present work the macroinvertebrate communities were also responding to the disturbance gradient found, with some of those groups, and also many individual taxa, showing increased abundance in

sites closer to the SOP (e.g., Oligochaeta, Insecta and Nemertea), while other (e.g., Gastropoda) showed higher density in sites further to the disturbance. The species *M. galloprovincialis* and *C. montagui* were dominant in the communities and were highly variable in abundance (density and biomass) between sites, which has been already referred by other authors (e.g., Boaventura et al., 2002; Pereira et al., 2006). Nevertheless, *M. galloprovincialis* (EG III in AMBI) was usually more abundant in sites near the SOP (especially regarding biomass), while *C. montagui* (EG I in AMBI) was usually more abundant in sites further from the disturbance, namely in Matadouro. There, the decreased abundance of *C. montagui* at sites closer to the SOP may be explained by the increase of algae richness and biomass, which may have altered the substratum type and the surface topography (important in the settlement and recruitment of sessile marine invertebrates) (e.g., Knox, 2001). Such increase in macroalgal and macroinvertebrate richness has already been reported near impacted areas, owing to intermediate disturbance which may increase availability of resources, promoting less competitive exclusion and therefore the co-existence of species (Connell, 1978; Magurran and McGill, 2010; Cabral-Oliveira et al., 2014b; Díez et al., 2014). Within the macroinvertebrate communities there were also taxa that did not relate to the disturbance gradients. Some amphipods (and few other crustaceans), usually regarded as sensitive, occurred in high densities near the disturbance, but those could be related to the presence of *Corallina* spp., acting as refuge and increasing the heterogeneity of the substrate (Fish and Fish, 2001; Cabral-Oliveira et al., 2014b). Also, some herbivore gastropods (namely *Gibbula* spp., EG I in AMBI) may be benefiting from the increase of primary producers (e.g., macroalgae) richness and abundance (Knox, 2001).

Similarly to the macroalgae, the overall findings for the macroinvertebrate communities indicate changes in the communities relating to a disturbance gradient. Nevertheless, the community descriptors based in macroinvertebrates, namely the macroinvertebrate density (D_{inv}), seem to have better captured the changes in structure between sites in the gradient. Not only they pointed either for higher differences between the exterior sites in the gradient, or for higher relation between the first two, and the last two sites in the gradient, but also showed higher variability (regarding density data) within sites closer to the disturbance, decreasing to sites further from the disturbance, as described by O'Connor (2013) for other rocky shore benthic assemblages when impacted. These findings indicate that benthic invertebrates are a good indicator of stress and pollution in rocky shores, and corroborate what has been described for other coastal ecosystems (e.g., Borja et al., 2000; Marques et al., 2009).

4.2. Comparison of the macroalgal and macroinvertebrate biological elements

The present work, to the authors' knowledge, is one of the few that studied quantitatively (number of individuals, biomass, richness and taxonomic composition) the structural variation of the communities of two biological elements – macroalgae and macroinvertebrates – at intertidal rocky shores, with data gathered from the same set of samples. A strong parallelism was found between both macroalgal and macroinvertebrate biological elements in response to disturbance. Several descriptors based on each biological element showed identical behavior, namely the macroalgae' biomass (B_{alg}) and the macroinvertebrates' density (D_{inv}), which were the ones that better captured the disturbance gradients analyzed in the study (especially D_{inv}). This suggests that either one or the other biological element could be selected when studying the quality condition of intertidal rocky shores.

The descriptor D_{inv} was able to capture the disturbance gradients and, therefore, indicators based on this descriptor should cover the requirement of the WFD for CW when it demands the use of biological parameters such as abundance and taxonomic composition on the assessment of benthic macrofauna.

In the scope of European Directives, namely of the WFD, several are the ecological indicators based only on the biological quality element macroalgae to assess quality of rocky shores (e.g., Ballesteros et al., 2007; Juanes et al., 2008; Neto et al., 2012; Gall and Duff, 2014). Although the combined use of macroinvertebrates and macroalgae in rocky shore assessments may not be in disagreement with WFD requirements (Díez et al., 2012), according to the present findings the exclusive use of macroinvertebrates seems very much possible. Furthermore, using each biological element separately could allow conducting more dedicated surveys, with appropriate sampling methods, in the most feasible season (e.g., June to late September for rocky shore macroalgae; Juanes et al., 2008; Gaspar et al., 2012; Neto et al., 2012; and winter for soft-bottom macroinvertebrates in open coastal waters; Muxika et al., 2007; Teixeira et al., 2009), which in turn could produce more appropriate data from each element.

If an ecologically sound and consistent pattern of variation can be observed for indicators associated individually to macroalgae and macroinvertebrate communities, then, the use of measures relying on features from these communities should be possible to use and to combine in order to assess separately the quality status of each one of these biological elements. For the studied macroinvertebrate communities, after the preliminary application of sensitivity classes from AMBI to species identified, is highly promising that such kind of indicator has the potential to be developed (after convenient adaptation to rocky shore species) and to integrate a future assessment tool. It was also observed a variation of the species present on sites under different disturbance levels (sensitive species and pollution tolerant species presented opposite tendencies along the disturbance gradient) and between shores (SIMPER results; Fig. 4), which suggests the use of an indicator based on the parameter taxonomic composition is also promising and possible to be developed aiming to integrate future assessment methods. Similarly to other methods developed for the assessment of soft bottoms, such as the Benthic Assessment Tool – BAT (Teixeira et al., 2009) and the multivariate method AMBI – M-AMBI (Muxika et al., 2007), it would be possible to develop a specific methodology to rocky shores but based exclusively on macrobenthic fauna of this intertidal habitat.

The present work suggests the use of indicators (e.g., diversity and functional) based on macroinvertebrate communities to integrate specific assessment tools for intertidal rocky shores. A further challenge will be eventually to develop and test a suitable multi-metric assessment tool (presently a gap) compliant with the European WFD requirements.

Acknowledgements

The authors wish to thank the MARE colleagues who helped in the field and laboratory work. Research was carried out in scope of the MARE – Marine and Environmental Sciences Centre (UID/MAR/04292/2013) strategic plan. The work was supported by the Fundação para a Ciência e a Tecnologia (FCT) through the Portuguese Operational Human Potential Program (POPH), the Portuguese National Strategic Reference Framework (QREN Portugal) and the Portuguese Ministry of Science, Technology, and Higher Education (MCTES) through PhD grants attributed to P.A.V. (SFRH/BD/74524/2010), A.J.P.C. (SFRH/BD/108224/2015) and R.G. (SFRH/BD/82014/2011), and through the contract of J.M.N. held in the “Ciência 2008 – Program”.

Appendix A. List of macroalgal taxa found in sites A, B, C and D in Buarcos and Matadouro shores. Presence of species in each site is shown with a cross (x). Biomass of higher groups, and total number of species (S) in each site are shown and highlighted in gray. Each species' Ecological Group (EG) and opportunism are shown.

Group	Taxon	Buarcos				Matadouro				EG	Opportunism
		A	B	C	D	A	B	C	D		
Chlorophyta		63.24	26.02	26.00	23.62	35.26	39.01	9.50	10.03		
	<i>Bryopsis plumosa</i>							x		II	Yes
	<i>Cladophora</i> spp.	x	x	x	x		x		x	II	Yes
	<i>Codium</i> spp.	x			x	x		x		I	
	<i>Ulva clathrata</i>						x			II	Yes
	<i>Ulva</i> spp. (Leaf-like forms)	x	x	x	x	x	x	x	x	II	Yes
	<i>Ulva</i> spp. (Tubular-like forms)	x	x	x	x	x				II	Yes
Heterokontophyta (Phaeophyceae)		0.33	0.85	0.81	2.82	6.54	1.09	13.90	39.59		
	<i>Bifurcaria bifurcata</i>					x		x	x	I	
	<i>Cladostephus spongiosus</i>					x				I	
	<i>Colpomenia peregrina</i>					x	x	x		II	
	<i>Cystoseira tamariscifolia</i>					x		x	x	I	
	<i>Dictyota dichotoma</i>	x	x	x	x	x		x	x	II	
	<i>Ectocarpales/Sphacelaria</i> spp.	x	x		x	x	x	x	x	II	Yes
	<i>Fucus spiralis</i>							x	x	I	
	<i>Leathesia marina</i>								x	II	
	<i>Halopteris scoparia</i>	x		x	x	x		x	x	I	
Rhodophyta		79.92	43.95	39.82	152.86	175.45	273.08	283.52	430.72		
	<i>Acrosorium ciliolatum</i>			x		x				I	
	<i>Ahnfeltiopsis devoniensis</i>	x	x	x	x					I	
	<i>Asparagopsis armata</i>					x	x	x	x	II	
	<i>Boergeseniella</i> spp.	x		x	x	x	x	x	x	II	Yes
	<i>Callithamnion tetricum</i>	x		x	x			x		II	Yes
	<i>Caulacanthus ustullatus</i>	x				x	x	x	x	I	
	<i>Ceramium</i> spp.	x	x	x	x	x	x	x	x	II	Yes
	<i>Champia parvula</i>					x	x	x		I	
	<i>Chondracanthus acicularis</i>	x	x	x				x		I	
	<i>Chondracanthus teedei</i>					x	x	x	x	I	
	<i>Chondracanthus teedei</i> var. <i>lusitanicus</i>	x	x	x	x					I	
	<i>Chondria coerulescens</i>	x	x	x	x		x	x	x	I	
	<i>Chondrus crispus</i>	x	x							I	
	<i>Corallina</i> spp.	x	x	x	x	x	x	x	x	I	
	<i>Cryptopleura ramosa</i>	x	x	x	x			x	x	I	
	G1 ^a	x		x	x	x	x	x	x	II	Yes
	G2 ^b	x	x	x	x			x	x	II	Yes
	G3 ^c					x	x			II	Yes
	<i>Gastroclonium reflexum</i>	x	x		x	x	x	x	x	II	
	<i>Gellidium corneum</i>								x	I	
	<i>Gellidium pulchellum</i>	x	x		x					I	
	<i>Gellidium pusillum</i>						x	x		I	
	<i>Gigartina pistillata</i>			x	x			x		I	
	<i>Gracilaria gracilis</i>	x						x		II	
	<i>Grateloupia filicina</i>									I	
	<i>Gymnogongrus griffithsiae</i>	x				x				I	
	<i>Halurus equisetifolius</i>			x						I	
	<i>Hypnea musciformis</i>						x			II	
	<i>Hypoglossum hypoglossoides</i>		x	x	x	x		x	x	I	
	<i>Jania rubens</i>	x		x		x		x	x	I	
	<i>Lomentaria articulata</i>	x	x		x					II	
	<i>Lythophyllum incrustans</i>	x	x	x	x	x	x	x	x	I	
	<i>Mastocarpus stellatus</i>	x	x	x	x					I	
	<i>Nitophyllum punctatum</i>								x	I	
	<i>Ophidocladus simpliciusculus</i>	x	x	x	x	x		x	x	II	Yes
	<i>Osmundea hybrida</i>					x	x	x	x	I	
	<i>Osmundea pinnatifida</i>	x	x	x	x					I	
	<i>Peyssonnelia</i> spp.		x				x			I	
	<i>Plocamium cartilaginum</i>			x	x	x	x	x	x	II	
	<i>Polysiphonia</i> spp.	x	x	x	x	x	x	x	x	II	Yes
	<i>Porphyra</i> spp.	x	x	x	x					II	Yes
	<i>Pterosiphonia complanata</i>	x	x	x	x	x		x	x	I	
	<i>Pterosiphonia pennata</i>	x	x	x	x	x	x	x	x	II	Yes
	<i>Rhodochorton</i> spp./ <i>Rhodothamniella</i> spp.	x	x	x	x			x		II	Yes
Opportunist taxa		65.41	30.75	27.67	26.55	41.86	41.48	6.94	12.94		
Total S		35	28	31	32	31	24	37	31		

^a G1: Rhodomelaceae except *Chondria* spp., *Osmundea* spp., *Pterosiphonia* spp. and *Boergeseniella* spp. (e.g., *Aphanocladia* spp., *Ctenosiphonia* spp., *Herposiphonia* spp., *Heterosiphonia* spp., *Leptosiphonia* spp., *Lophosiphonia* spp.).

^b G2: Red Uniseriate filamentous forms except *Rhodochorton* spp. and *Rhodothamniella* spp. (e.g., *Acrochaetium* spp., *Audouinella* spp., *Colaconema* spp., *Bangia* spp., *Stylonema* spp.).

^c G3: Ceramiales except *Ceramium* spp., *Callithamnion tetricum* and *Halurus equisetifolius* (e.g., *Aglaothamnion*, *Anotrichium*, *Anthithamnion*, other *Callithamnion*, *Compsothamnion thuyoides*, *Halurus flosculosus*, *Pleonosporium borrii*, *Dasya* spp.).

Appendix B. List of macroinvertebrate taxa found in sites A, B, C and D in Buarcos and Matadouro shores. Presence of species in each site is shown with a cross (x). Density of higher groups, and total number of species (S) in each site are shown and highlighted in gray. Each species' Ecological Group (EG) is shown.

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
Oligochaeta	Oligochaeta	144.7	92.6	26.0	11.6	358.8	538.2	55.0	23.1	V
Polychaeta		6157.4	2106.5	4751.2	3414.4	1736.1	1252.9	4233.2	1446.8	
	<i>Ampharete</i> sp.			x	x			x	x	I
	<i>Aonides oxycephala</i>	x	x	x	x	x	x	x	x	III
	<i>Arabella iricolor</i>							x	x	I
	<i>Boccardia polybranchia</i>					x	x	x	x	IV
	<i>Brania pusilla</i>			x		x	x			II
	<i>Cirriformia tentaculata</i>	x	x		x	x	x	x	x	IV
	<i>Euclymene palermitana</i>							x		I
	<i>Eulalia viridis</i>	x	x	x	x	x	x	x	x	II
	<i>Fabricia stellaris</i>			x		x	x	x	x	II
	<i>Glycera</i> sp.	x	x	x				x		II
	<i>Harmothoe</i> sp.	x	x		x					II
	<i>Lepidonotus clava</i>	x	x	x	x	x	x	x	x	II
	<i>Lumbrineris latreilli</i>					x	x	x		II
	<i>Lysidice ninetta</i>							x		II
	<i>Malacoceros fuliginosa</i>	x	x	x		x	x		x	V
	<i>Maldane</i> sp.						x			I
	<i>Microspio atlantica</i>				x					III
	<i>Myrianida</i> sp.	x		x	x					II
	<i>Mysta picta</i>	x	x		x			x	x	III
	<i>Mysta</i> sp.2	x		x	x					III
	<i>Naineris laevigata</i>				x	x	x	x	x	I
	<i>Naineris quadricuspida</i>	x				x	x	x	x	I
	<i>Neanthes nubila</i>		x	x	x	x		x		III
	Nereidinae	x	x	x	x					III
	<i>Odontosyllis ctenostoma</i>	x	x	x	x		x	x	x	II
	<i>Oriopsis armandi</i>				x					II
	<i>Perinereis cultrifera</i>	x	x	x	x	x	x	x	x	III
	<i>Perinereis marionii</i>	x	x	x	x	x	x	x	x	III
	<i>Perinereis oliveirae</i>	x	x		x				x	III
	<i>Perinereis</i> sp.			x	x					III
	<i>Pholoe</i> cf. <i>synophthalmica</i>	x	x	x	x	x		x		II
	<i>Platynereis dumerilii</i>	x	x	x	x	x	x	x	x	III
	<i>Polycirrus</i> sp.	x	x	x	x	x	x	x	x	IV
	<i>Protoarcia oerstedi</i>					x				III
	<i>Pseudopotamilla reniformis</i>								x	II
	<i>Sabellaria alveolata</i>	x	x	x	x	x	x	x	x	I
	<i>Sabellaria spinulosa</i>	x	x	x	x	x	x	x	x	I
	<i>Scolecipis (Scolecipis) cantabra</i>								x	II
	<i>Scoletoma impatiens</i>	x	x	x	x	x	x	x	x	II
	<i>Serpula vermicularis</i>							x		I
	<i>Sphaerosyllis</i> sp.	x	x	x	x	x		x	x	II
	<i>Sphaerosyllis taylori</i>								x	II
	<i>Spio filicornis</i>							x		III
	<i>Spirobranchus lamarcki</i>	x	x	x	x	x	x	x	x	II
	<i>Spirobranchus triqueter</i>	x		x				x		II
	<i>Sthenelais boa</i>	x	x		x					II
	<i>Syllides edentatus</i>							x		II
	<i>Syllis amica</i>	x	x	x	x	x	x	x	x	II
	<i>Syllis armillaris</i>	x		x	x					II
	<i>Syllis corallicola</i>		x	x				x		III
	<i>Syllis garciai</i>	x	x	x	x	x	x	x	x	II
	<i>Syllis gerlachi</i>	x	x	x		x	x	x	x	II
	<i>Syllis gracilis</i>	x	x		x	x		x	x	III
	<i>Syllis hyalina</i>	x	x	x	x			x	x	II
	<i>Syllis prolifera</i>	x	x	x	x	x	x	x	x	II
	<i>Syllis pulvinata</i>	x	x	x	x	x	x	x	x	II
	<i>Syllis rosea</i>	x	x	x	x	x		x		II
	<i>Syllis</i> sp.							x		II
	<i>Syllis vivipara</i>	x	x	x	x		x		x	II
	<i>Tharyx</i> sp.	x	x	x	x	x	x	x	x	IV
	<i>Travisia</i> sp.	x	x	x	x	x		x	x	I
	<i>Vermiliopsis</i> sp.							x		II
Amphipoda		749.4	824.7	445.6	914.4	680.0	850.7	1053.2	448.5	
	<i>Abludomelita gladiosa</i>		x							III
	<i>Abludomelita obtusata</i>			x						III
	<i>Ampelisca rubella</i>				x	x	x	x		I
	<i>Ampelisca</i> sp.					x	x	x	x	I
	<i>Amphilocheus spencebatei</i>					x	x	x	x	II
	<i>Amphithoe gammaroides</i>								x	II

Appendix B (Continued)

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
	<i>Aora typica</i>							x		I
	<i>Apherusa cirrus</i>					x	x		x	I
	<i>Apherusa jurinei</i>		x	x	x	x	x	x	x	I
	<i>Apohyale prevostii</i>		x	x	x					II
	<i>Caprella acanthifera</i>	x	x	x	x	x		x		II
	<i>Caprella equilibra</i>	x	x	x	x	x		x	x	II
	<i>Dexamine spinosa</i>		x	x		x		x	x	III
	<i>Elasmopus rapax</i>	x	x	x	x	x	x	x	x	III
	<i>Gammaropsis maculata</i>		x		x			x		I
	<i>Hyale perieri</i>	x	x	x	x	x		x	x	II
	<i>Hyale pontica</i>	x								II
	<i>Hyale</i> sp.			x						II
	<i>Hyale stebbingi</i>	x	x	x	x	x	x	x	x	II
	<i>Jassa ocia</i>		x	x	x					V
	<i>Jassa pusilla</i>							x		V
	Lysianassidae			x						I
	<i>Maera grossimana</i>	x		x	x					I
	<i>Melita palmata</i>	x	x	x	x					I
	<i>Microdeutopus chelifer</i>	x	x	x	x	x	x	x		I
	<i>Microdeutopus damnoniensis</i> (nomen nudum)	x	x	x	x					I
	<i>Parajassa pelagica</i>	x			x					II
	<i>Photis longicaudata</i>	x	x							I
	<i>Podocerus variegatus</i>		x						x	III
	<i>Protomeadia fasciata</i>	x								II
	<i>Stenothoe monoculoides</i>			x		x	x	x	x	II
Cirripedia		14,236.1	51,342.6	11,571.2	24,508.1	2.9	8822.3	14,351.9	18,906.3	
	<i>Chthamalus montagui</i>	x	x	x	x	x	x	x	x	I
	<i>Balanus</i> sp.	x			x				x	I
Decapoda		83.9	43.4	31.8	43.4	150.5	52.1	292.2	34.7	
	<i>Cancer</i> sp.				x					II
	<i>Carcinus maenas</i>	x				x				III
	<i>Liocarcinus navigator</i>					x	x			I
	<i>Pachygrapsus marmoratus</i>	x			x				x	II
	<i>Pilumnus hirtellus</i>	x	x	x	x	x	x	x		I
	<i>Pirimela denticulata</i>	x	x	x	x	x	x	x	x	I
	<i>Xantho pilipes</i>	x			x	x		x		I
Isopoda		491.9	439.8	448.5	587.4	199.7	338.5	260.4	289.4	
	<i>Campecopea hirsuta</i>	x	x	x	x		x	x	x	II
	<i>Cymodoce truncata</i>		x	x	x		x	x	x	I
	<i>Dynamene bidentata</i>	x	x	x	x				x	II
	<i>Dynamene edwardsi</i>	x	x	x	x		x		x	II
	<i>Dynamene magnitorata</i>		x	x	x	x	x	x	x	II
	<i>Eurydice pulchra</i>					x	x		x	I
	<i>Gnathia maxillaris</i>	x	x	x	x	x		x		I
	<i>Idotea granulosa</i>	x			x					II
	<i>Idotea pelagica</i>	x	x	x	x	x	x	x	x	II
	<i>Ischyromene lacazei</i>	x	x	x	x		x	x	x	II
	<i>Lekanesphaera levii</i>					x	x	x	x	III
	<i>Paranthura nigropunctata</i>			x	x	x		x		I
	<i>Cleantis prismatica</i>					x				II
Tanaidacea		2387.2	575.8	1522.0	607.6	546.9	2083.3	865.2	104.2	
	<i>Apseudes talpa</i>	x	x	x	x					II
	<i>Tanais dulongii</i>	x	x	x	x	x	x	x	x	II
Insecta		225.7	367.5	110.0	188.1	1061.9	636.6	98.4	95.5	
	Chironomidae	x	x	x	x	x	x	x	x	III
	Tipulidae	x	x	x	x	x	x	x	x	IV
Bivalvia		11,999.4	3339.1	7624.4	11,519.1	7106.5	12,916.7	5934.6	11,033.0	
	<i>Cardita calyculata</i>					x	x	x	x	I
	<i>Hiatella arctica</i>	x	x	x	x	x	x	x	x	I
	<i>Irus irus</i>	x				x	x	x	x	I
	<i>Lasaea adansonii</i>					x	x	x	x	II
	<i>Musculus costulatus</i>	x	x	x	x	x	x	x	x	I
	<i>Mytilus galloprovincialis</i>	x	x	x	x	x	x	x	x	III
	<i>Parvicardium pinnulatum</i>					x	x	x	x	I
	Psammobiidae	x	x	x	x	x	x	x	x	I
	<i>Striarca lactea</i>			x						I
	<i>Tellina</i> sp.	x	x							I
	<i>Venerupis</i> sp.	x	x	x	x	x	x	x	x	I
Gastropoda		4239.0	8046.9	5795.7	9207.2	8579.3	5049.2	16,886.6	16,316.6	
	<i>Crisilla semistriata</i>			x	x	x		x		I

Appendix B (Continued)

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
	<i>Aplysia punctata</i>	x		x	x	x		x	x	I
	<i>Barleeia unifasciata</i>					x	x		x	II
	<i>Bittium reticulatum</i>							x		I
	<i>Buccinum humphreysianum</i>				x	x			x	II
	<i>Cerithiopsis tubercularis</i>	x	x		x		x	x	x	I
	<i>Epitonium clathratulum</i>	x	x		x				x	I
	<i>Gibbula cineraria</i>					x	x	x	x	I
	<i>Gibbula pennanti</i>	x	x	x	x	x	x	x	x	I
	<i>Gibbula umbilicalis</i>	x	x	x	x	x	x	x	x	I
	<i>Melarhaphe neritoides</i>	x	x	x	x		x	x	x	II
	<i>Nassarius incrassatus</i>		x		x	x				II
	<i>Nassarius pygmaeus</i>	x	x	x	x	x	x	x	x	II
	<i>Nassarius reticulatus</i>					x			x	II
	<i>Nucella lapillus</i>		x	x	x	x	x		x	II
	<i>Nudibranchia</i>		x	x	x		x	x		II
	<i>Ocenebra erinaceus</i>	x	x	x	x					II
	<i>Ocenebra aciculata</i>				x	x	x	x	x	II
	<i>Odostomia eulimoides</i>	x	x	x	x	x	x	x	x	II
	<i>Omalogyra atomus</i>	x				x	x			III
	<i>Onchidella celtica</i>								x	I
	<i>Opalia crenata</i>	x	x	x						I
	<i>Phorcus lineatus</i>	x	x	x	x				x	I
	<i>Patella depressa</i>	x	x	x	x	x	x	x	x	I
	<i>Patella</i> sp.	x			x					I
	<i>Patella ulyssiponensis</i>	x	x	x	x	x	x	x	x	I
	<i>Patella vulgata</i>	x	x	x	x			x		I
	<i>Rissoa parva</i>	x	x	x	x	x	x	x	x	I
	<i>Runcina coronata</i>	x	x	x	x	x	x	x	x	I
	<i>Siphonaria pectinata</i>						x	x		I
	<i>Skeneopsis planorbis</i>				x				x	I
	<i>Testudinalia testudinalis</i>	x			x					I
	<i>Tricolia pullus</i>	x		x	x	x	x	x	x	I
	<i>Urosalpinx cinerea</i>	x	x		x	x	x	x	x	II
Polyplacophora		202.5	133.1	347.2	546.9	269.1	460.1	321.2	303.8	
	<i>Lepidochitona (Lepidochitona) cinerea</i>	x	x	x	x	x	x	x	x	II
	<i>Acanthochitona</i> spp. (<i>A. crinita</i> and <i>A. fascicularis</i>)	x	x	x	x	x	x	x	x	I
Ophiuroidea	Ophiuroidea	173.6	156.3	144.7	188.1	8.7	23.1	14.5		II
Echinoidea	Echinoidea		23.1		5.8	2.9	2.9	23.1	11.6	I
Anthozoa	Actiniaria	1443.9	735.0	1452.5	613.4	124.4	115.7	312.5	243.1	II
Ascidacea	Ascidacea			2.9				2.9		III
Echiura	Echiura				2.9					II
Nemertea	Nemertea	818.9	596.1	269.1	254.6	147.6	315.4	115.7	182.3	III
Picnogonida		211.2	81.0	37.6	81.0	26.0	17.4	11.6	11.6	
	<i>Achelia echinata</i>	x	x	x	x			x	x	I
	<i>Anoplodactylus pygmaeus</i>	x	x			x			x	II
	<i>Anoplodactylus virescens</i>	x	x	x	x	x	x	x		II
	<i>Callipallene brevirostris</i>								x	II
Sipuncula	<i>Golfingia</i> sp.	x	x	x	x	x	x	x		I
Turbellaria	Leptoplanidae (<i>Notoplana</i> sp. and <i>Leptoplana</i> sp.)	26.0	60.8	14.5	17.4	11.6		2.9	2.9	II
Opportunist taxa		358.8	298.0	147.6	130.2	775.5	758.1	133.1	81.0	IV and V
Sensitive taxa (except <i>C. montagui</i>)		8857.1	8023.7	10,477.4	10,069.4	8521.4	6004.1	17,008.1	12,323.5	I
Total S		105	101	103	115	99	88	114	103	

Appendix C. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2015.10.038>.

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