ASSESSING A QUICK MONITORING METHOD USING ROCKY INTERTIDAL COMMUNITIES AS A BIOINDICATOR: A MULTIVARIATE APPROACH IN ALGECIRAS BAY

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Abstract. A multivariate approach was used to test the value of intertidal communities as a bioindicator of environmental conditions at Algeciras Bay, southern Spain. The study area is located in the Strait of Gibraltar and it is subjected to a variety of anthropic impacts. Eight localities (5 inside and 3 outside the bay) were selected, and four transects were undertaken in each locality to characterise the fauna and flora. The spatial distribution of the intertidal species reflected the physico-chemical conditions of Algeciras Bay. The stations located outside the bay, characterised by high hydrodynamism and dissolved oxygen and low sedimentation and turbidity, had a higher diversity and species richness than the inner stations. According to the BIO-ENV procedure and CCA, water turbidity was the factor which best correlated with the intertidal assemblages. SIMPER showed that the molluscs *Chthamalus stellatus*, *Mytilus* cf. *edulis*, *Littorina neritoides* and *Balanus perforatus*, and the algae *Gelidium pusillum*, *Corallina elongata*, *Asparagopsis armata*, *Colpomenia sinuosa* and *Fucus spiralis* were the species that most contributed to the dissimilarity between internal and external sites. The present study, based on the spatial distribution of intertidal taxa, yielded similar results to those previously obtained in the area with costly physico-chemical analysis based on complex matrices of subtidal epifaunal communities. Consequently, the intertidal sampling method proposed in this study is presented here as a quick, effective alternative strategy, and can be useful in environmental monitoring programs, since these communities are easily accessible and amenable to sample, and the sessile nature of the majority of the species makes future, long-term monitoring relatively simple.

Keywords: algae, Algeciras Bay, bioindicator, intertidal communities, macrofauna, spatial distribution

1. Introduction

Intertidal communities are located at the interface of the land and the sea, and play an important role in maintaining local ecological balance (Cai *et al.*, 2003). They are open ecosystems, with steep environmental gradients and their susceptibility to both terrestrial and marine disturbances makes them more vulnerable than sublittoral and offshore habitats (Thompson *et al.*, 2002).

In comparison with other marine environments, gaining access to intertidal ecosystems is generally easier, and these communities are more amenable to

management than open ocean habitats. Furthermore, the composition of sessile communities is particularly useful as a baseline for ecological monitoring because such organisms are unable to avoid disturbances in the marine environment and thus, the composition of the community reflects their common history (Fa *et al.*, 2002). However there are still considerable gaps in the available knowledge of these habitats (Thompson *et al.*, 2002).

Algeciras Bay is located in the eastern-most zone of the Strait of Gibraltar (Figure 1). It is an important industrial area, with chemical factories, refineries, thermal power plants, iron works, paper mills and ship yards, along with a major port (Conradi *et al.*, 1997). Recently, several studies have been conducted in Algeciras Bay using subtidal benthic communities as bioindicators (sponges: Carballo *et al.*, 1996; ascidians: Naranjo *et al.*, 1996; epifauna of algae and bryozoa: Conradi *et al.*, 1997, Sánchez-Moyano and García-Gómez, 1998; Sánchez-Moyano *et al.*, 2000a, b, 2002). These studies reflected clear differences in community composition between external and internal areas of the Algeciras Bay, showing a good correlation between subtidal fauna and environmental conditions. Although some preliminary results dealing with intertidal communities of Algeciras Bay have been already published (Fa *et al.*, 2003), the value of these communities as a bioindicator has not been tested yet.

The main objective of the present work was a spatial study of the intertidal communities in internal and external sites of Algeciras Bay, to test their value as bioindicator of environmental conditions. Taking into account that, nowadays, authorities and government institutions are demanding quick and effective environmental studies in coastal areas, it seems interesting to focus our efforts on the implementation of a monitoring tool, based on sessile intertidal fauna and flora. The sampling in

Figure 1. Map of Algeciras Bay showing the sampling localities. A: La Línea; B: Acerinox; C, D: Harbour; E, Saladillo; F, G: San García; H: Punta Carnero.

these intertidal ecosystems, unlike the previous studies conducted in Algeciras Bay, does not require scuba diving and is potentially useful in environmental monitoring programs since these communities are easily accessible and amenable to sampling. Also, the sessile nature of the majority of the species makes future, long-term monitoring relatively simple.

2. Materials and Methods

2.1. SAMPLING PROCEDURE

A total of eight sampling sites were selected along the Algeciras Bay (A–H) (Figure 1). Sites A, B, C, D and E are internal areas, while F, G, and H, are external, selected outside the Bay for comparative purposes.

To quantitatively assess changes in abundance and distribution of organisms, a systematic method based on fixed belt transects was selected as the sampling method (see Jones *et al.*, 1980; Fa, 1990). Sampling was conducted during August 2003. At each site, a generalised shore search was previously undertaken in order to both select the most appropriate location for the transects and also to ensure that it was as representative as possible of the area being studied (Fa *et al.*, 2002). Four transects were selected in each site. The stations per transect were marked at 25 cm intervals. A ruler, set square and rope were used to establish the different heights. The first height was the zero tidal level and the process was continued until the vertical height of 2 meters had been achieved, providing eight stations for each transect (Figure 2). Each station was sampled using quadrats of 25×25 cm, further subdivided into 25 square units of 5×5 cm with thick fishing line. The presence/absence of both species of algae and macrofauna was recorded for each unit. This method for estimation of abundance using data of presence/absence was employed for all species, to get homogeneous matrices of data instead of mixed matrices of abundances based on density for some species and matrices of cover percentages for others. The census based on presence/absence data was assessed visually on site but if the species could not be properly identified visually, it was removed for later identification in the laboratory. Only the visually distinctive species of algae and macrofauna were considered during sampling. Fa *et al.* (2002) reported the difficulties of on-site separation of very similar co-existing species such as *Mytilus edulis* Linnaeus, 1758 and *Mytilus galloprovincialis* Lamarck, 1819, whose morphs grade into each other. In the present study we have considered *Mytilus cf. edulis*. In addition to the biotic study, several environmental parameters were measured “in situ” in the water column at each sampling site (water temperature, pH, salinity, dissolved oxygen and turbidity). Three measurements were made for each parameter during August and mean values were used for statistical analysis. Water temperature and salinity were measured using a conductivimeter WTW LF-323, and pH using a pHmeter Hanna HI-8324. The

Figure 2. Sampling design conducted in each transect. Four transects were sampled for each locality.

dissolved oxygen was measured using an oxymeter WTW oxi-196, and turbidity was measured in nephelometric turbidity units (ntu) using a turbidimeter Hanna HI 93703.

2.2. STATISTICS

Taking into account that the objective of the present study is the comparison among sampling localities and not a detailed descriptive study of the different belts (heights) in each transect, the eight quadrats of each transect (Figure 2) were added, and considered as a whole area of 200 units per transect (25 units per square, and a total of 8 square per transect). Consequently, the data of abundance (expressed in frequency percentages) of species were calculated based on the presence/absence in 200 units distributed along the 2 meters vertical transect (Figure 2). This method, although involving a loss of information regarding to the different heights (belts) inside each transect, it is useful to characterise each sampling locality intertidal zone as a whole and get an integrated matrix with single data for each species in each transect (4 values for each sampling site) instead of getting 8 data (one for each height) for each species in each transect.

2.2.1. *Univariate Analysis*

The total number of species (S), the Shannon–Wiener diversity index (H') (Shannon and Weaver, 1963) and Pielou's evenness index (J) (Pielou, 1966) were calculated for each transect. Mean values of the four transects sampled in each locality and standard deviation values were calculated. Possible differences in these indices between internal and external sites were tested using the Kruskal–Wallis test, after verifying that hypothesis of normality (Kolmogorov–Smirnov test) was not passed; Levene's test showed homogeneity of variances, indicating that the Kruskal–Wallis test was adequate. For multiple post-hoc comparisons we used the Tamhane's test. For univariate analyses, the BMDP was used (Dixon, 1983).

2.2.2. *Multivariate Analysis*

The affinities among stations based on the environmental parameters were established through cluster analysis using UPGMA (unweighted pair group method using arithmetic averages) (Sneath and Sokal, 1973) and euclidean distances. Abiotic data were $\log(x + 1)$ transformed (Guerra-García *et al.*, 2003). The affinities among transects based on biotic data were also established through cluster analysis using UPMA method, based on the Bray–Curtis similarity index (Bray and Curtis, 1957). The relationships between environmental measures and biotic assemblages were studied by Canonical Correspondence Analysis (CCA). This analysis is a direct gradient technique in which the resulting ordination of stations is directly related to the values of the environmental factors (Ter Braak, 1986). Relationships between multivariate biological structure and environmental variables were also examined using the BIO-ENV procedure (Clarke and Ainsworth, 1993). Percentage of similarity analysis (SIMPER) (Clarke, 1993) was used to determine the species involved in grouping of the different sampling localities. Multivariate analyses were carried out using the PRIMER package (Clarke and Gorley, 2001) and the PC-ORD programme (McCune and Mefford, 1997).

3. Results and Discussion

3.1. ENVIRONMENTAL VARIABLES

The general trend of the abiotic variables throughout the bay is shown in Figure 3. Temperature, pH and salinity present similar values in all localities sampled. Turbidity was higher inside the bay than in external localities, while dissolved oxygen concentrations were higher in the external sites. This is the expected trend in Algeciras Bay, since the configuration of the bay involves less water renovation inside than outside the bay; these results are in agreement with our previous studies conducted in Algeciras Bay which showed that the prevailing

Figure 3. Mean values and standard deviations ($n = 3$) of abiotic parameters measured in the present study.

conditions in the external zone are high hydrodynamics and low sedimentation rate, whereas the internal zone presents high sedimentation rate and higher values for organic matter (Carballo *et al.*, 1996; Naranjo *et al.*, 1996; Sánchez-Moyano *et al.*, 2002a, b).

3.2. DISTRIBUTION OF FAUNA AND ALGAE

The eight stations provided a total of 57 identified taxa, of which 22 were macrofauna, and 35 algae. Table I shows the overall dominance in the four transects (1–4) of the eight stations (A–H).

Most of the common species of macrofauna were widely distributed throughout the bay, such as the cirripeds *Chtamalus stellatus* and *Balanus perforatus*, and the molluscs *Littorina littorea*, *Littorina neritoides*, *Mytilus cf edulis*, *Patella caerulea*, *Patella rustica*, *Patella nigra* and *Siphonaria pectinata*. Especially remarkable is the presence of the endangered mollusc *Patella ferruginea* in the stations B and G. The limpet *Patella ferruginea* Gmelin, 1791 is the most endangered marine invertebrate in the western Mediterranean rocky shores (Laborel-Deguen *et al.*, 1991a; Ramos, 1998). Although its relative abundance in Palaeolithic and Neolithic deposits indicates an extensive former distribution in the western Mediterranean Basin (east coast of Italy, Mediterranean France, Iberian Peninsula, Morocco, Tunisia and the western Mediterranean islands), today its Mediterranean range has progressively contracted to a few restricted areas (Fischer-Piette, 1959; Cretella *et al.*, 1994; Templado, 1996) probably due to human pressure (Aversano, 1986). Although recently, several important populations of *P. ferruginea* have been found and studied in Ceuta, North Africa (Guerra-García *et al.*, 2004a, b), the presence of this species in the Iberian Peninsula is extremely reduced; the most recent cites of *P. ferruginea* in the Iberian Peninsula were reported by García-Gómez (1983) who recorded the species in Algeciras Bay, and Moreno (1992), who found two specimens in Cabo de Gata. Several specimens have been recently observed in the Gibraltar harbour by Templado and Fa in 2002 (pers. comm.). Consequently, it would be advisable to undertake a further study of these populations of *P. ferruginea* in the internal and external zone of Algeciras Bay in the near future. Little is known about the biology of *P. ferruginea* so far; this species has a very low growth and reproductive rate, reaching sexual maturity at the age of 2–3 years. It is a protandric species with relatively large sized ovocytes suggesting a short planktonic phase (Frenkiel, 1975; Templado, 1996), it is considered a k-strategist (Laborel-Deguen *et al.*, 1991b) and feeds mainly on cyanobacteria and the algae *Ralfsia* spp. and *Rissoella* spp.

In contrast to macrofauna, the algae showed greater discrimination between internal and external sites (Table I). Although some species were very common throughout the bay (e.g. *Corallina elongata*, *Ceramium rubrum*, *Gelidium pusillum*, *Hildenbrandia* sp. and *Ulva rigida*), the external localities San García (F, G) and Punta Carnero (H) were clearly richer in number of species than the internal sites. Species such as *Bryopsis plumosa*, *Caulacanthus ustulatus*, *Codium adherens*, *Cladophora* spp., *Fucus spiralis*, *Lithophyllum lichenoides* and *Rivularia bullata*, were exclusively distributed in these external sites, and other species such *Asparagopsis armata*, *Colpomenia sinuosa*, *Gelidium latifolium* and *Mesophyllum lichenoides*, although present in some internal sites, were much more abundant in the external localities.

TABLE I
List of species found in the present study and values of abundance (%) along each transect (four transects per locality)

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4	E1	E2	E3	E4	F1	F2	F3	F4	G1	G2	G3	G4	H1	H2	H3	H4	
FAUNA																																	
<i>Achantochitona fascicularis</i>	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
<i>Actinia equina</i>	-	-	-	-	0.5	0.5	0.5	0.5	2.5	3	1.5	-	-	-	-	0.5	0.5	-	-	-	-	-	-	1.5	3	1	0.5	1.5	0.5	0.5	-	-	-
<i>Anemonia sulcata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	0.5	0.5	-	0.5	0.5	-	-	-
<i>Balanus perforatus</i>	3	-	0.5	1	1	1	-	0.5	12	13	12.5	14.5	12.5	13.5	9	6.5	1.5	0.5	-	-	8	6.5	7.5	7	-	0.5	-	0.5	2	2	3	2	
<i>Bugula neritina</i>	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Chamaelea stellatus</i>	18	13	14	18.5	12.5	17.5	17.5	31.5	26	26.5	28.5	28	26	24	22	22.5	16	17.5	12.5	8.5	26	34.5	22	36	17.5	23	26	21	37.5	35.5	35	27.5	
<i>Coralliactis viridis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	
<i>Dendropoma paetrum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	
<i>Gibbula</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Ligia oceanica</i>	-	-	-	-	-	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Littorina littorea</i>	-	-	-	-	-	-	-	-	5	2.5	1	3	6.5	3	4	4.5	0.5	0.5	-	-	-	-	-	-	1	1	1	-	0.5	-	-	-	-
<i>Littorina neritoides</i>	5.5	0.5	0.5	0.5	8	7.5	9	3	-	-	-	-	-	-	-	-	4	3	5	2.5	11	8.5	11.5	18	13	22.5	10	10	4	6	5.5	4.5	
<i>Mytilus cf edulis</i>	-	-	-	-	11	6	7	8	4	2.5	4.5	2	11	14.5	9.5	14	1	-	-	0.5	1	1.5	3.5	1	0.5	-	1	1	5.5	8.5	2.5	4.5	
<i>Ocenebrina</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	
<i>Pachygrapsus marmoratus</i>	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Paracentrotus lividus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	0.5	-	-	-	-	-	-	0.5	-	
<i>Patella caerulea</i>	7	3	0.5	1	2	8	0.5	4.5	3	3.5	4.5	2.5	3.5	1.5	1.5	2.5	4.5	4.5	4.5	4	8.5	4	6	5.5	3.5	3	3	9.5	6	3.5	4	4.5	
<i>Patella ferruginea</i>	-	-	-	-	-	0.5	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	0.5	-	-	-	-	-	
<i>Patella nigra</i>	0.5	0.5	1	3	4	3.5	4.5	3.5	-	-	-	-	-	0.5	0.5	-	12	18	7.5	11	1	-	1	-	2.5	1	3.5	3	-	-	-	1	
<i>Patella rustica</i>	-	-	-	-	6	13	7	3.5	1	1.5	2	1	1.5	1	1	3.5	3	5	2.5	7.5	-	1.5	0.5	-	1.5	0.5	1	1	7	7.5	7.5	3.5	
<i>Pollicipes pollicipes</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	0.5	1	-	1
<i>Siphonaria pectinata</i>	-	1	-	1.5	-	-	8	3	-	-	-	-	-	-	1	1	8.5	8	4.5	2	-	2	-	-	2.5	0.5	2.5	2.5	2	0.5	0.5	0.5	
FLORA																																	
<i>Amphiroa rigida</i>	1	0.5	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Asparagopsis armata</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	3.5	6	7	3	3	4.5	3.5	6.5	4	3	4	
<i>Bryopsis plumosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	1	0.5	1	1.5	-	1	0.5	2	1.5	-	-	
<i>Caulacanthus ustulatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3.5	1.5	1	3.5	2	2	2	6	2.5	6	6.5	
<i>Ceramium rubrum</i>	8.5	8.5	4.5	4	-	1.5	-	-	-	-	-	5	6	5	4	-	-	-	-	-	2	2.5	2	3	4	6	5	6	6	4	5	1.5	
<i>Ceramium</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.5	-	0.5	1	1	1	1	
<i>Cladophora</i> sp1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	
<i>Cladophora</i> sp2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	1	0.5	1	-	0.5	-	-	-	-	-

(Continued on next page)

TABLE I
(Continued)

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4	E1	E2	E3	E4	F1	F2	F3	F4	G1	G2	G3	G4	H1	H2	H3	H4		
<i>Cladophora</i> sp3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3.5	3	3.5	3	3.5	3.5	5.5	0.5	4	2	1	2	-
<i>Codium adhaerens</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	0.5	1	1.5	-	1	1	-	0.5	4.5	8.5	-	
<i>Codium fragile</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	
<i>Colpomenia sinuosa</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5	-	-	6	4	2	5.5	9	7.5	8	7.5	-	
<i>Corallina elongata</i>	15	14	6	10	14.5	17.5	15.5	13.5	7	3.5	3.5	8.5	0.5	-	-	-	11	13	14.5	13	12.5	12.5	11	8	12.5	14	12	13	8.5	10.5	16.5	7	-	
<i>Cytosseira</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Dicryota dichotoma</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	0.5	0.5	-	-	0.5	0.5	-	-	-	-	-	-	-	-
<i>Enteromorpha cf linza</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Enteromorpha cf compressa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.5	1.5	-	-	-	-	-	-	-	0.5	-	-	-	-
<i>Fucus spiralis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Gelidium latifolium</i>	-	-	-	-	-	-	-	-	-	4	2	2	2	-	-	-	-	-	-	-	-	0.5	-	-	7	7.5	2.5	2	2.5	11	4	9.5	-	-
<i>Gelidium pusillum</i>	3.5	2.5	2.5	5	2.5	-	1	3	5.5	3.5	6	2.5	-	-	-	-	1	1	3	3	13.5	10	6.5	10.5	12.5	14	8.5	11	7	11	11.5	9.5	-	-
<i>Gigartina</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	0.5	-	-	-	-	-	0.5	-	-	-	-
<i>Halopteris scoparia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	1	0.5	-	1.5	-	-	
<i>Hildenbrandia</i> sp	-	7.5	8.5	4.5	15.5	7	13.5	8.5	-	-	-	-	-	-	-	-	7.5	4	4	4.5	2.5	3.5	5.5	1.5	4	3.5	4	3.5	1.5	1	0.5	2.5	-	
<i>Laurencia</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	0.5	-	
<i>Liagora</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Lichina pignaea</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	1	0.5	-	-	-	-	-	-	-	-
<i>Lithophyllum lichenoides</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	16.5	9	11.5	9.5	6.5	7	6	-	-
<i>Lithophyllum</i> sp	-	-	-	-	4	1.5	2	5	2.5	-	-	1.5	-	-	-	-	8.5	3	3	4	9	3.5	3	2	2	3	1.5	2	-	-	-	-	0.5	-
<i>Lithotamniacea</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	1.5	-	-	1	1.5	3	-	-	-	-	-	0.5	-
<i>Mesophyllum lichenoides</i>	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	1	1	1.5	4.5	0.5	3.5	2	1	4	7	4.5	3	2.5	0.5	1	0	-	-
<i>Placodium cartilagineum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	0.5	1.5	-
<i>Rivularia bullata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	2.5	9	4	1	9	2.5	0.5	-	5.5	0.5	-	-
<i>Rodomeleacea</i> sp	0.5	0.5	0.5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	0.5	-	-	1	-	-	-	1.5	-	-	-	-	-
<i>Ulva rigida</i>	-	-	-	-	-	-	-	-	3.5	6.5	6	7.5	1	3.5	3.5	2.5	-	-	-	-	3.5	1	1.5	1	1.5	0.5	0.5	2.5	1.5	2.5	1.5	1.5	-	-
<i>Valonia utricularis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.5	-	-	-	-	-	0.5	1	-

Figure 4. Mean values and standard deviations ($n = 4$ transects) of descriptive parameters in each sampling locality.

Confirming the above trend, the mean values of number of species and diversity per locality were significantly higher in the external sites (F–H) than in the internal localities (A–E) (Figure 4 and Table II). The number of species clearly differentiated the two groups of sites (external vs internal); however the evenness values were similar in all the localities and no statistical differences could be obtained (Table II). This similarity in evenness values determined that Shannon diversity values, although higher in external sites, were not as discriminative than the number of species.

These differences of species richness and diversity between internal and external sites, shown by the univariate analysis based on the study of the intertidal community, are in agreement with previous studies focused in subtidal communities of Algeciras Bay. For example, Conradi *et al.* (1997) showed that the amphipod population associated with the bryozoan *Bugula neritina* had a higher diversity and species richness in the stations located at the mouth of the bay than the inner

TABLE II
Results of the Kruskal–Wallis for the data of number of species, diversity (H') and evenness (J)

	K	Homogeneous group
<i>Number of species</i>	28.06***	<u>ABCDE</u> <u>FGH</u>
<i>Diversity (H')</i>	29.34***	<u>AD</u> <u>BCE</u> <u>FGH</u>
<i>Evenness (J)</i>	16.17n.s.	<u>ABCDEFGH</u>

Note. n.s., not significant, *** $p < 0.001$. The homogeneous group according to the Tamhane's test ($p < 0.05$) are indicated underlined with a continuous line.

stations with low water movement and higher sedimentation. Sánchez-Moyano and García-Gómez (1998) and Sánchez-Moyano *et al.* (2002a, b) studied the spatio-temporal distribution of the epifaunal community of macrofauna living on the alga *Halopteris scoparia*, collected by scuba in several stations of Algeciras Bay, and reported that in the external zone a greater number of species appeared. Carballo *et al.* (1996) and Naranjo *et al.* (1996) exploring the value of sponges and ascidians, respectively, as marine bioindicators in Algeciras Bay, also found significantly richer number of species and diversity values in the external sites of San García and Punta Carnero.

3.3. MULTIVARIATE ANALYSIS

The cluster analysis using the environmental matrix of abiotic parameters showed that the two groups of localities (external vs internal) could be differentiated (Figure 5A), mainly based on the low values of turbidity and high values of dissolved

Figure 5. Dendrograms of similarity between the stations. (A) based on the environmental data; (B) based on the species composition.

oxygen in the external sites F–H (Figure 3). The cluster analysis based on the abundance values of the intertidal community species of the four transects in each locality (Figure 5B) was very similar to that obtained with the abiotic parameters and showed two groups of stations. The first group included the external sites (F–H), characterised by high values of species richness and diversity, and the second group included the remaining sites. This group, was divided into two subgroups; one of them including the transects of the harbour sites (C, D) and the other one including the remaining transects (A, B, E). The sampling sites C and D were located within the harbour walls and the waves produced by the ferries and boats moving across the harbour could influence the species composition of the intertidal communities of these two sites.

When the SIMPER analysis was applied to detect the species contributing most to the dissimilarity between the two groups of stations (A–E: internal vs. F–H: external), the outstanding organisms were the molluscs *Chtamalus stellatus* and *Littorina neritoides*, and the alga *Gelidium pusillum*, all of them more abundant in the external sites. The whole list of taxa listed in order of decreasing contribution to the average dissimilarity between the two groups of sites up to 90% of accumulated total dissimilarity is included in Table III.

The Canonical Correspondence Analysis (CCA) confirmed the ordination of the localities previously obtained by the Cluster analysis. The external sites (F–H) appeared together in the positive part of axis 1 and negative part of axis 2, mainly determined by higher oxygen concentrations (Figure 6). Sites C and D, located within the harbour walls, were also grouped apart, and the remaining stations were characterised by higher values of turbidity. Axis 1 seems to separate the stations according to the oxygen and salinity gradient, and axis 2 is mainly correlated with the water turbidity. This parameter, which showed the highest correlation in the CCA analysis (Table IV), seems to be one of the most important factor affecting algae and macrofauna distribution. In fact, according to the BIO-ENV analysis, the best combination to explain the biological data was a single variable, turbidity ($r = 0.40$). Consequently, turbidity seems to be an interesting parameter to take into account in future environmental control programmes conducted in Algeciras Bay. This measure is easily and quickly undertaken with a turbidimeter, it is strongly related to the amount of solids in suspension (pers. obs.) and it is much quicker to obtain than the estimation of suspension solids by filtering water (see Guerra-García and García-Gómez, 2001).

3.4. CONCLUSIONS AND ENVIRONMENTAL IMPLICATIONS FOR FUTURE PROGRAMMES OF MONITORING

Authorities and Governmental Institutions are increasingly demanding quick and effective environmental studies in coastal areas, as useful tools for monitoring programmes. This fact precludes developing series of data based on complex matrices of various taxonomic groups whose identification requires great effort and time.

TABLE III

Average abundances of the most relevant species of the sampling sites located in the internal area of Algeciras Bay (A–E) (INT) and external area (F–H) (EXT)

Taxa	Abund. INT.	Abund. EXT.	Av. dis.	Ratio	Dis.%	Cum. dis%
<i>Chtamalus stellatus</i>	20.03	28.46	4.83	1.56	9.28	9.28
<i>Littorina neritoides</i>	2.45	10.38	4.24	1.82	8.13	17.41
<i>Gelidium pusillum</i>	2.28	10.46	4.17	4.68	8.00	25.41
<i>Balanus perforatus</i>	5.13	3.25	2.59	1.27	4.98	36.92
<i>Corallina elongata</i>	9.03	11.50	2.36	1.11	4.53	41.45
<i>Mytilus cf edulis</i>	4.78	2.54	2.23	1.21	4.29	45.74
<i>Asparagopsis armata</i>	0.05	4.33	2.20	4.68	4.22	49.96
<i>Colpomenia sinuosa</i>	0.10	4.25	2.06	1.30	3.95	53.91
<i>Fucus spiralis</i>	0.00	3.83	1.89	1.30	3.63	57.54
<i>Hildenbrandia</i> sp	4.25	2.79	1.83	1.53	3.52	61.06
<i>Patella nigra</i>	3.50	1.08	1.68	0.80	3.22	64.28
<i>Caulacanthus ustulatus</i>	0.00	3.29	1.66	2.41	3.19	67.47
<i>Rivularia bullata</i>	0.00	3.13	1.60	2.69	3.08	70.55
<i>Cladophora</i> sp3	0.00	3.13	1.60	2.07	3.06	73.61
<i>Ceramium rubrum</i>	2.35	3.92	1.52	1.98	2.92	76.53
<i>Patella rustica</i>	3.00	2.63	1.49	1.24	2.85	79.38
<i>Mesophyllum lichenoides</i>	0.43	2.46	1.11	1.42	2.13	81.51
<i>Lithophyllum</i> sp	1.75	2.21	1.08	1.25	2.07	83.58
<i>Ulva rigida</i>	1.70	1.58	1.02	1.70	1.96	85.55
<i>Patella caerulea</i>	3.33	5.08	0.93	1.58	1.78	87.33
<i>Siphonaria pectinata</i>	1.93	1.13	0.86	1.00	1.65	88.97
<i>Codium adherens</i>	0.00	1.63	0.81	1.29	1.56	90.53

Note. Species are listed in order of decreasing contribution to the average dissimilarity (Av. dis.) between the two groups up to 90% of accumulated total dissimilarity (Cum. dis%). The ratio indicates Dis/Standard deviation. The total average dissimilarity between groups is 52.1%.

In Algeciras Bay, several studies had been conducted previously to test the value of subtidal communities as bioindicator of environmental conditions. These studies had been focused on subtidal epifauna living on algae or bryozoans, and subtidal sponges or ascidians. In both cases, scuba diving was necessary for sampling and great efforts were required for identification due to the difficulties in taxonomy of epifaunal groups of molluscs, polychaetes and crustaceans, and also in sponges or ascidians. All these previous studies showed strong correlation between spatial distribution of biological taxa and the environmental parameters measured, and differentiated the external *versus* the internal sites in Algeciras bay based on the faunal composition. In the present study we have tested the values of intertidal communities as bioindicators in the same environment as the previous studies,

Figure 6. Graph representation of the localities and species with respect to the first two axes of the Canonical Correspondence Analysis (CCA). The species included are those which most contributed to the dissimilarity between internal and external sites according to the Simper (Table III). See also Table IV.

Algeciras Bay, and we have obtained similar results: a good correlation between environmental measures and biotic data, and a clear separation between internal and external sites based on the biotic data. However, we should also consider that there may be circumstances where the use of one habitat or the other could be preferable (e.g. an oil spill near shore would suggest intertidal monitoring, but discharge of non-buoyant effluent from a submarine outfall might be better monitored subtidally).

The method used in the present study (Figure 2) does not require scuba diving, and is based only on the most distinct and conspicuous species of alga and sessile macrofauna (epifaunal community is not considered). Consequently, the spatial study of the intertidal communities following the sampling method proposed in

TABLE IV
Summary results of the canonical correspondence analysis

	Axis 1	Axis 2	Axis 3
Species-environment correlation	0.99	0.99	0.99
Percentage of species variance	33.7	28.4	11.4
Correlation with environmental variables			
Turbidity	–	0.77	–
Oxygen	0.44	–0.56	–
Salinity	–0.52	–	–
pH	–	0.49	–

Note. Only environmental variables significantly correlated with CCA axes at $p < 0.05$ are included.

this study is presented here as an alternative strategy and it can be a useful tool to characterise the interest of a site from a biotic point of view. Since this kind of sampling is less demanding in terms of time and resources than others currently in use, this method can easily be repeated periodically to detect natural or anthropogenic alterations in the intertidal community. Therefore, this tool could be useful for monitoring purposes. For example, Algeciras harbour is currently beginning to be affected by considerable remodeling which is surely affecting the communities of the whole bay. We plan to use the method proposed herein as a control method for monitoring the environmental conditions of Algeciras Bay during the remodeling of the harbour, or to detect changes due to accidental oil spills since there is intense marine traffic in the bay. This study reflects that a spatial approach dealing with the most distinctive species of intertidal communities can yield similar results to those obtained with costly physico-chemical analysis and based on complex matrices of epifaunal communities.

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