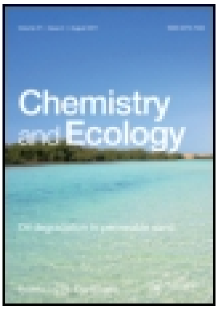

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gche20

Biotic indexes reveal the impact of harbour enlargement on benthic fauna

R. Riera [a] , Ó. Monterroso [a] , M. Rodríguez [a] & E. Ramos [a]
[a] Atlantic Environmental Research Center (CIMA SL), C/Arzobispo Elías Yanes, La Laguna, Tenerife, Canary Islands

Available online: 03 May 2011

Chemistry and Ecology
Vol. 27, No. 4, August 2011, 311–326

Biotic indexes reveal the impact of harbour enlargement on benthic fauna

R. Riera*, Ó. Monterroso, M. Rodríguez and E. Ramos

Atlantic Environmental Research Center (CIMA SL), C/Arzobispo Elías Yanes, La Laguna, Tenerife, Canary Islands

(*Received 3 May 2010; final version received 28 February 2011*)

Environmental effects of enlargement works in Puerto Calero (Lanzarote, Canary Islands) were analysed in the sediments using abiotic variables (total hydrocarbons, polyaromatic hydrocarbons, organic matter and granulometry) and three biotic indexes (AMBI, M-AMBI and BENTIX). A before–after/control–impact (BACI) design was developed with four sampling campaigns, before (November 2004), during (March and July 2005) and after (July 2006) works. Inner stations were characterised by high concentrations of pollutants (total hydrocarbons and polyaromatic hydrocarbons). A temporal trend (2004–2006) was observed in macrofaunal assemblage structure, and thus in AMBI, M-AMBI and BENTIX indexes. Macrobenthic assemblages also mirrored the pollution gradient, with bioindicator species in inner stations (the polychaete *Pseudopolydora paucibranchiata* and the mollusc *Abra alba*), sensitive species (the sipunculid *Aspidosiphon muelleri*, the crustacean *Pariambus typicus* and the polychaete *Aponuphis bilineata*) in outer stations and ubiquitous species (the polychaetes *Aricidea assimilis* and *Armandia polyophthalma*) in both stations (inner and outer).

Keywords: macrofauna; harbour; pollution; monitoring; AMBI; M-AMBI; BENTIX; biotic index; Canary Islands

1. Introduction

Assessment of the ecological integrity of benthic invertebrate communities in coastal areas has progressed in recent years due in large part to legislation such as the 'Clean Water Act' in the USA or the Water Framework Directive (WFD) [1] and 'Marine Strategy Directive' [2] in Europe. Such policies, albeit broad in definition, explicitly recognise the link between fauna, flora and habitat, and require appropiate strategies for assessing the relative importance, status or ecological integrity of water bodies [3]. A plethora of studies have demonstrated that benthic assemblages are useful indicators of environmental status, because they respond predictably to different natural and anthropogenic disturbances [4]. Moreover, macrobenthic populations can be used to understand the dominance of certain ecological factors, responsible for the structure and productivity of benthic communities [5].

*Corresponding author. Email: rodrigo@cimacanarias.com

ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757540.2011.570753
http://www.informaworld.com

Analysis of changes in benthic communities, using univariate and multivariate methods, has become an important tool in the assessment and monitoring of the biological effects of marine pollution. Univariate measures (e.g. Shannon's diversity, Pielou's evenness, Margaleff's richness) have the disadvantage of reducing a great amount of information to a single summary index. Furthermore, disturbed and a non-disturbed sites might have the same diversity, making it difficult to discern the changes produced by 'natural' variations from those produced by anthropogenic activities [6]. Multivariate methods (MDS, Cluster, Canonical analysis, etc.) are more sensitive than univariate methods at detecting community changes [7]. However, the results obtained are generally difficult for non-specialists to interpret, i.e. environmental impact studies and marine quality monitoring, which are addressed to policy-makers, stakeholders, etc.

Several researchers have developed biotic indexes to estimate macrobenthic community disturbance and to establish the ecological status of soft-bottom benthos [8,9]. All such studies have emphasised the importance of biological indicators for measuring the ecological quality of a studied marine environment [10,11]. Recent approaches have developed a biocriteria-based predefined reference condition and several deviations (disturbance classes) from this have been established [12,13]. Borja et al. [14] have proposed the utilisation of AZTI's Marine Biotic Index (AMBI), using macrobenthic assemblages as bioindicators of anthropogenic and natural disturbances. These authors have studied the response of soft-bottom communities to natural and man-induced changes in water quality. Such an approach has integrated the long-term environmental conditions in several European estuaries and coastal environments.

The AMBI and M-AMBI have been used in an increased number of studies for monitoring purposes and in analysis of impacts on soft-bottom benthic communities [15]. These indexes are based upon the proportion of species assigned to one of five levels of sensitivity to increasing degrees of disturbance, from very sensitive to opportunistic species. They have been been tested under different stress sources [16,17] and have been applied not only in Europe, but also in Asia [18], North Africa [19] and South America [20].

The BENTIX index [21] is a marine biotic index that was developed, like AMBI and M-AMBI, for the ecological and status classification of the marine environment using macroinvertebrates. BENTIX is designed to assess the environmental impact caused by general stressors and does not discriminate between natural and anthropogenic disturbances. BENTIX has been succesfully tested on the eastern coast of the Mediterranean Sea, and less intensively in other areas (western Mediterranean) [22].

The former biotic indexes (AMBI, M-AMBI and BENTIX) are classified into a category based on ecological groups [23], in which macrobenthic species are divided into previously defined ecological groups (EG) before determining the respective proportion of the different groups in the overall macrofaunal assemblage. AMBI, M-AMBI and BENTIX have not been used anteriorly in the Canarian archipelago and could be developed as an environmental tool to fulfil environmental requirements of the Water Framework Directive (WFD/2000/60/CEE).

The aim of this study is to test the use of biotic indexes (AMBI, M-AMBI and BENTIX) on data collected in a marina of the Canary Islands (Puerto Calero, Lanzarote) and its sensitivity to a spatial and temporal pollution gradient.

2. Materials and methods

2.1. *Study area*

This study was conducted at Puerto Calero, on the east coast of Lanzarote (Canary Islands, NE Atlantic Ocean, 28°55′00″N/13°42′07″W) (Figure 1). Marina enlargement works consisted of the construction of a new dyke (30 m long) in the mouth of the harbour. This new section could

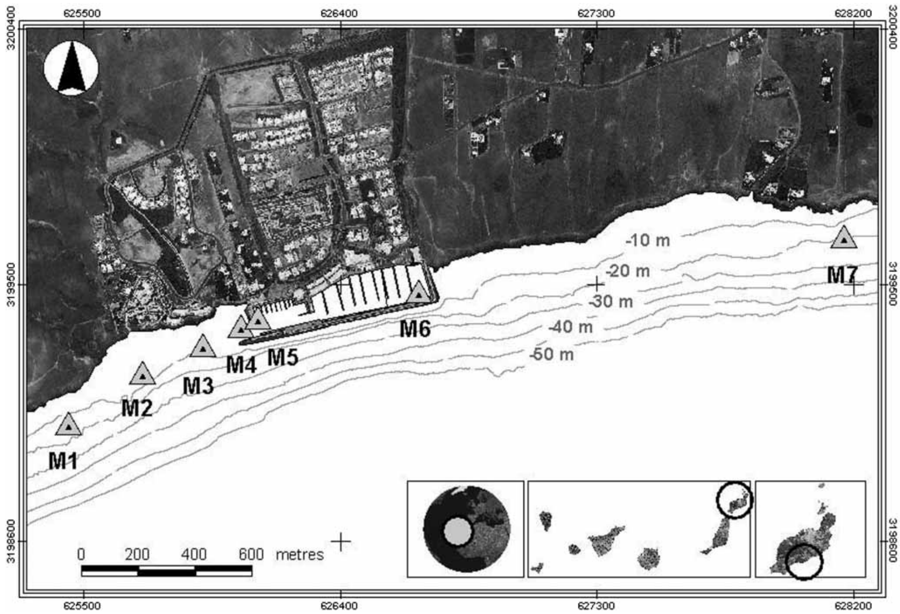

Figure 1. Location of the sampling stations in Puerto Calero.

shelter 20 berths for boats between 25 and 50 m long, and increases the capacity of the marina to 440 berths.

Samples were collected in four sampling campaigns, according to the harbour works: pre-conditions (first campaign: November 2004), harbour works (second and third campaigns: March and July 2005, respectively) and post-conditions (fourth campaign: July 2006).

2.2. *Sampling procedures*

We selected seven sampling stations in the study area. These stations can be divided into three groups: Control (stations M1 and M7), Influence (M2 and M3) and Impact (M4, M5 and M6) (Figure 1). The locations of the sampling points were arranged after carrying out ecocartographic and hydrodynamic studies in the sampling area. Sediment samples were collected manually by SCUBA divers at a depth of 5–25 m. Sediment cores (20 cm inner diameter) were pushed into the sediment to a depth of 20 cm. Three replicates were collected per station for faunistic analysis and one for sediment variables (granulometry, organic matter content and hydrocarbons).

Sediment samples for faunistic purposes were preserved in 10% seawater formaldehyde solution and decanted through a 0.5-mm mesh sieve. The fraction remaining on the mesh sieve was separated into different taxonomical groups under a binocular microscope and preserved in 70% ethanol. In the laboratory, macrofaunal specimens were determined to species level, whenever possible, by means of a binocular microscope or even in a LEICA DMLB microscope equipped with Nomarski interference contrast.

2.3. *Environmental variables*

Granulometry was obtained from subsamples of 100 g sediment. Samples were dried at air temperature, sieved on a stack of graded sieves from 0.063 to 2-mm mesh, and the residue on each was weighed [24]. The percentage of organic matter was determined according to Walkley [25].

Total hydrocarbons were extracted with CCl_4 and measured by infrared spectrometry. Concentrations of total hydrocarbons are given in ppm (mg·L^{-1}). Polyaromatic hydrocarbons (PAHs) were extracted with SPE cartridges. Organic compounds were retained in the solid phase and diluted later with hexane. The concentrated extract was injected into a gas chromatograph unit and determined and quantified using a mass spectrometer detector. Results were quantified with a regression curve and are given in ppb (μg·L^{-1}).

2.4. *Statistical analyses*

Biological descriptors of the community (abundance, species richness, Shannon's diversity and Pielou's evenness) were calculated. Differences in macrofaunal abundance were tested using one-way ANOVA after verifying normality using the Kolmogorov–Smirnov test and Levene's test for homogeneity of variances. When there was no normality and/or homogeneity of variances, the Kruskal–Wallis ANOVA (KW ANOVA) test was used.

Affinities among macrofaunal assemblages based on species composition were established using non-metric multidimensional scaling (n-MDS), being abundance data square-root transformed and the Bray–Curtis similarity index used. A two-way nested ANOSIM routine [26] was used to analyse differences among sampling stations groups (Control, Impact and Influence) within each sampling year (2004, 2005 and 2006). Multivariate analyses were carried out using the PRIMER 5.2. package [27].

2.5. *Biotic indices*

Classification of the identified species into the five ecological categories (I–V) was based upon the updated list of the AMBI programme (Table 1). AMBI and M-AMBI indexes were calculated using AMBI 4.1 software, freely available at www.azti.es. Guidelines in deriving these indexes [28] were also applied. Species not considered in the list were classified according to the literature [29], the authors acknowledge the ecological distribution and expertise of AZTI's scientists, with special reference to Dr A. Borja.

BENTIX is based, as AMBI and M-AMBI indexes, on the relative percentage of sensitive to tolerant species weighted with an ecological rationale to derive a simple formula which renders a five-scale classification scheme ranging from 2 to 6 and corrsponding with high, good, moderate, poor and bad ecological status. The developed formula is $[6 \times \%GI + 2 \times (\%GII + \%GIII)]/100$ and assigns the numerical factor '6' to the sensitive taxa (GI, group I) and the factor '2' to tolerant

Table 1. Summary of AMBI values and their equivalences (Borja et al. [14] and Muxica et al. [17]).

AMBI	Dominanting ecological group	Benthic community health	Site disturbance classification	Ecological status
$0.0 < AMBI \leq 0.2$	I–II	Normal	Undisturbed	High
$0.2 < AMBI \leq 1.2$		Impoverished		
$1.2 < AMBI \leq 3.3$	III	Unbalanced	Slightly disturbed	Good
$3.3 < AMBI \leq 4.3$	IV–V	Transitional to polluted	Moderately disturbed	Moderate
$4.3 < AMBI \leq 5.0$		Polluted		Poor
$5.0 < AMBI \leq 5.5$	V	Transitional to heavy pollution	Heavily disturbed	
$5.5 < AMBI \leq 6.0$		Heavy polluted		Bad
$6.0 < AMBI \leq 7.0$	Azoico	Azoic	Extremely disturbed	

Note: The ecological groups correspond to: I, sensitive to pollution; II, indifferent to pollution; III, tolerant to organic matter; IV, opportunistic of second order; V, opportunistic of first order (for details, see Borja et al. [14,16]).

taxa (GII and GIII). The BENTIX methodology and an extended list of species scores can be freely downloaded from http://www.hcmr.gr/english_site/services/env_aspects/bentix.html.

3. Results

3.1. *Abiotic factors*

The granulometric composition of the sediments from sampling stations is presented in Figure 2. Medium sands dominated in stations M2, M3 and M7. Fine sands were the most important granulometry fraction in station M5 and coarse sands dominated in the remaining stations studied (M1, M4 and M6). During the study, the sedimentary trend in inner stations (M4, M5 and M6) was a slight increase in coarse sedimentary types (medium sands, coarse sands and gravels). No discernible changes in granulometric composition were found in the Influence (M2 and M3) and Control (M1 and M7) stations.

In terms of sampling campaign, sedimentary changes were more important during the last campaign (after the enlargement works) reaching the granulometric composition present in the first campaign (before harbour works) (Figure 2).

Sampling stations presented low values of organic matter, with the exception of those registered in the second campaign (March 2005), with maximum values for a control station (2.06%) that apparently can be considered temporal (Table 2). No spatial (control–impact) or temporal (2004–2006) differences were found in the organic matter content of the studied sediments (Figure 3). Percentages of organic matter ranged from 0.01 to 1.5% and are considered typical of subtidal soft-bottoms from the Canary Islands (Riera, 2004) Thus, no organic enrichment was evident in the study site during the sampling period.

Total hydrocarbons and PAHs showed an important increase during the enlargement works at the marina. In the first campaign (November 2004) rather low values were encountered at all sampling stations, with the exception of 102.6 ppb total hydrocarbons in Station 6, which was probably due to an accidental oil spill inside the marina. Station M3 is clearly influenced by oil spills from diggers and bulldozers used in the work, with a high total hydrocarbon content (516 ppm in March 2005 and 141.1 ppm in July 2005) and PAH content (114 ppb in March 2005 and 148 ppm in July 2005) during the enlargement works (Tables 3 and 4).

Impact stations (M4, M5 and M6) were characterised by high values of both contaminants, with mean concentrations of 214 ppm PAHs and 87.3 ppb total hydrocarbons in March 2005, and 154 ppm PAHs and 371.6 ppb total hydrocarbons in July 2005. Certain high values at impacted sampling points (298 ppb PAHs in M6 and 872.3 ppb total hydrocarbons in M5) stand out from the remaining sampling stations, showing an increase in these pollutants over time during 2005. Total hydrocarbons and PAHs decreased during the last campaign (July 2006), with the exception of M5 (779.2 ppm total hydrocarbons and 201 ppb PAHs). In comparison with pristine conditions (November 2004), the concentration of total hydrocarbons remained low with the exceptions of M2 and M5. However, the PAH content showed an increase compared with 2004 values, with a mean concentration of 5 ppb in November 2004 and 66.3 ppb in July 2006 (Figures 4 and 5).

3.2. *Macrofauna*

A total of 4176 individuals, belonging to 211 species, were collected in the sampling stations (M1–M7) throughout the study period. The most abundant taxa were the sipunculid *Aspidosiphon muelleri* with 483 individuals, followed by the mollusc *Bittium latreillii* (339 individuals) and the caprellid *Pariambus typicus* (293 individuals). By contrast, 59 species were represented by one single individual.

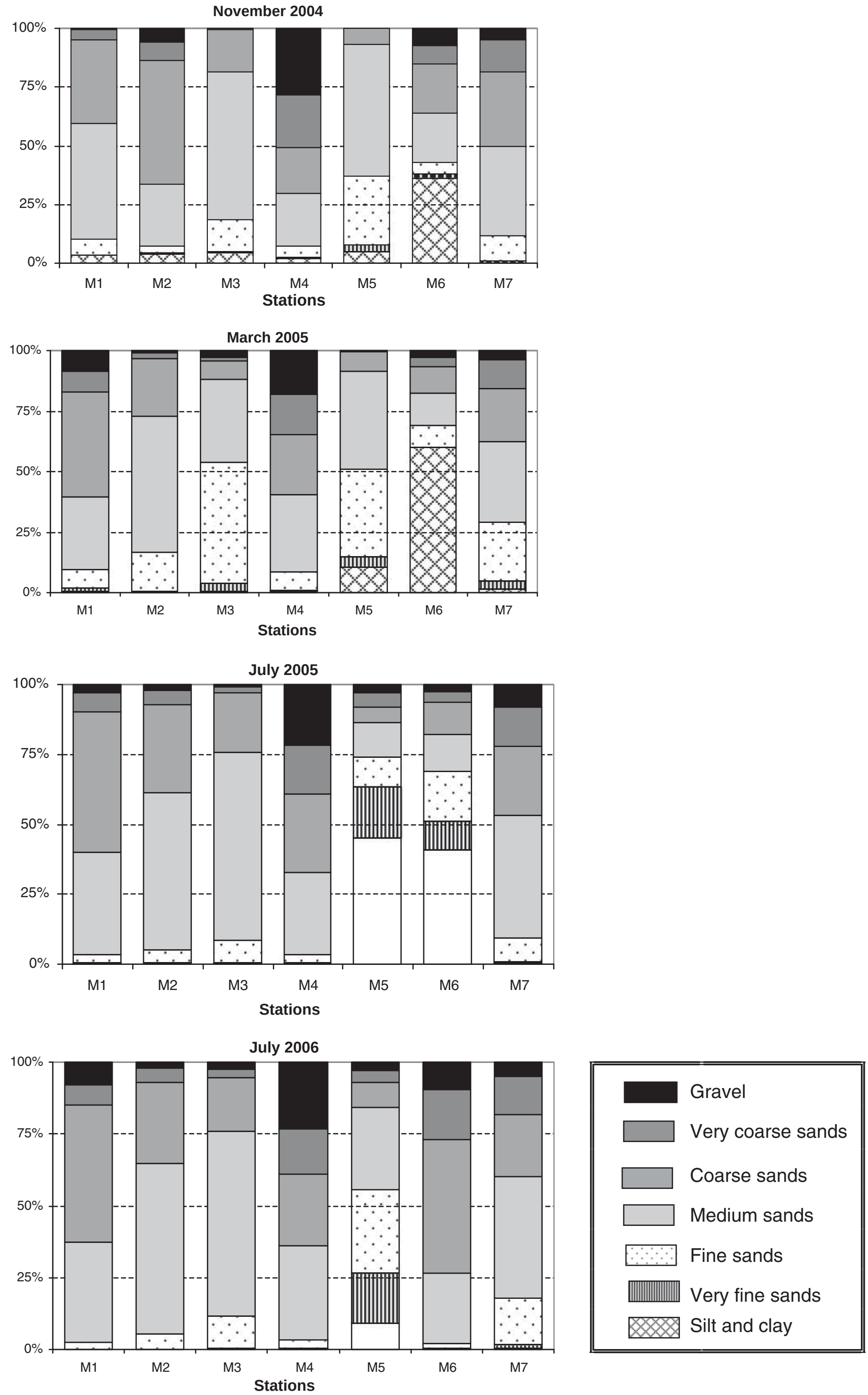

Figure 2. Granulometry of the sampling stations along the four sampling campaigns.

Table 2. Percentages of organic matter in sampling stations.

	Organic matter (%)			
Station	Nov 2004	Mar 2005	Jul 2005	Jul 2006
M1	0.36	2.06	0.24	0.05
M2	0.23	0.09	<0.01	0.33
M3	0.23	1.24	0.29	0.66
M4	0.18	1.05	0.29	0.76
M5	0.50	1.05	0.83	1.33
M6	0.27	1.38	0.2	0.28
M7	0.14	1.01	0.34	1.09

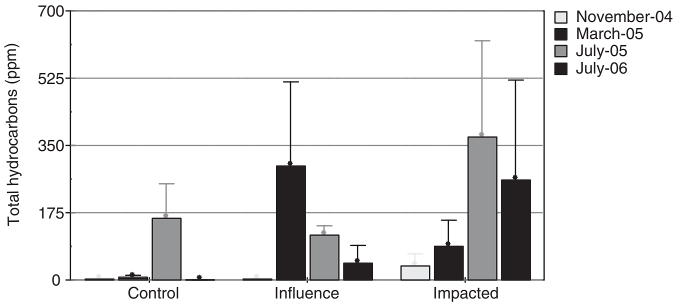

Figure 3. Concentration of total hydrocarbons (±SE) (ppm) in control, influence and impacted stations.

Table 3. Concentration of total hydrocarbons in sampling stations.

	Total hydrocarbons (ppm)			
Station	Nov 2004	Mar 2005	Jul 2005	Jul 2006
M1	1	11.2	249.9	0.1
M2	1	73.8	93.3	88.9
M3	1	516	141.1	0.1
M4	1	0.1	138.2	0.1
M5	1	220	872.3	779.2
M6	102.6	41.8	104.7	0.1
M7	1	0.1	68.9	0.1

A total of 15 taxonomic groups (Amphipoda, Cumacea, Decapoda, Echinodermata, Isopoda, Mollusca, Nematoda, Nemertea, Oligochaeta, Ostracoda, Phoronidea, Polychaeta, Sipuncula, Tanaidacea and Turbellaria) were collected throughout the study period. The most abundant group were the polychaetes (1776 individuals), followed by amphipods (871 individuals) and molluscs (563 individuals). Isopods and nematodes were scarce throughout the study period, with three and seven individuals, respectively (see Table S1 – available online only).

Species richness varied throughout the sampling period, ranging from 25 (Impact 2005) to 95 taxa (Control 2005), with minimum values in impacted stations (Table 5). Macrofaunal abundances varied among sampling years (2004–2006), however, impacted stations were always characterised by the lowest macrofaunal abundances. These variations in abundance ranged from 13.08 ± 10.2 (Impact 2005) to 85.75 ± 32.5 individuals per 0.1 m^2 (Control 2005). Differences in macrofaunal abundances were more acute during enlargement works (2005), compared with before and after conditions (Figure 6A). Pielou's evenness (J') and Shannon's diversity (H') showed variations among sampling years and depending on the location of sampling locations (Control, Impact or

Table 4. Concentration of polycyclic aromatic hydrocarbons in sampling stations.

	PAH (ppb)			
Station	Nov 2004	Mar 2005	Jul 2005	Jul 2006
M1	3	69	144	31
M2	5	33	418	34
M3	5	114	148	57
M4	4	182	165	30
M5	1	161	125	201
M6	9	298	171	35
M7	8	129	126	76

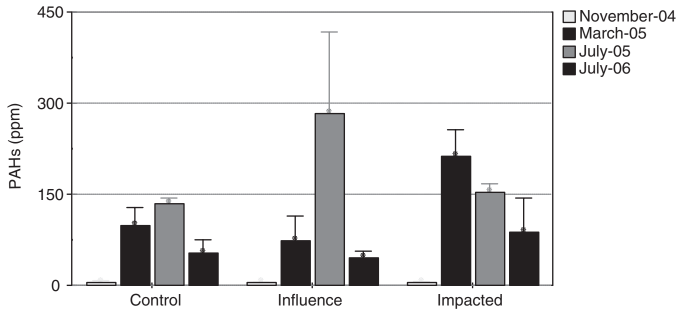

Figure 4. Concentration of polycyclic aromatic hydrocarbons (PAHs) (±SE) (ppb) in control, influence and impacted stations.

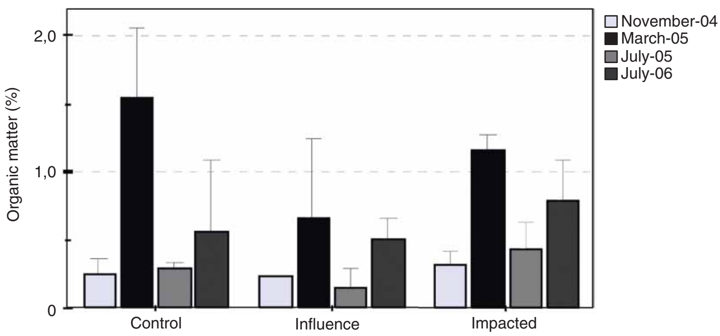

Figure 5. Organic matter content (±SE) (%) in control, influence and impacted stations.

Table 5. Univariate parameters of the studied macrofaunal assemblage structure throughout the sampling period.

	Parameter			
	No. species	Individuals (*N*)	Evenness (*J*')	Diversity (*H*′)
Control 2004	66	66.67 ± 25.4	0.74	3.08
Influence 2004	76	78.56 ± 38.9	0.68	2.93
Impact 2004	34	31.33 ± 25.6	0.81	2.87
Control 2005	95	85.75 ± 32.5	0.72	3.28
Influence 2005	90	42.17 ± 25.6	0.78	3.50
Impact 2005	25	13.08 ± 10.2	0.55	1.79
Control 2006	49	56 ± 18.9	0.59	2.30
Influence 2006	61	43.44 ± 25.4	0.83	3.42
Impact 2006	30	34.83 ± 12.6	0.63	2.13

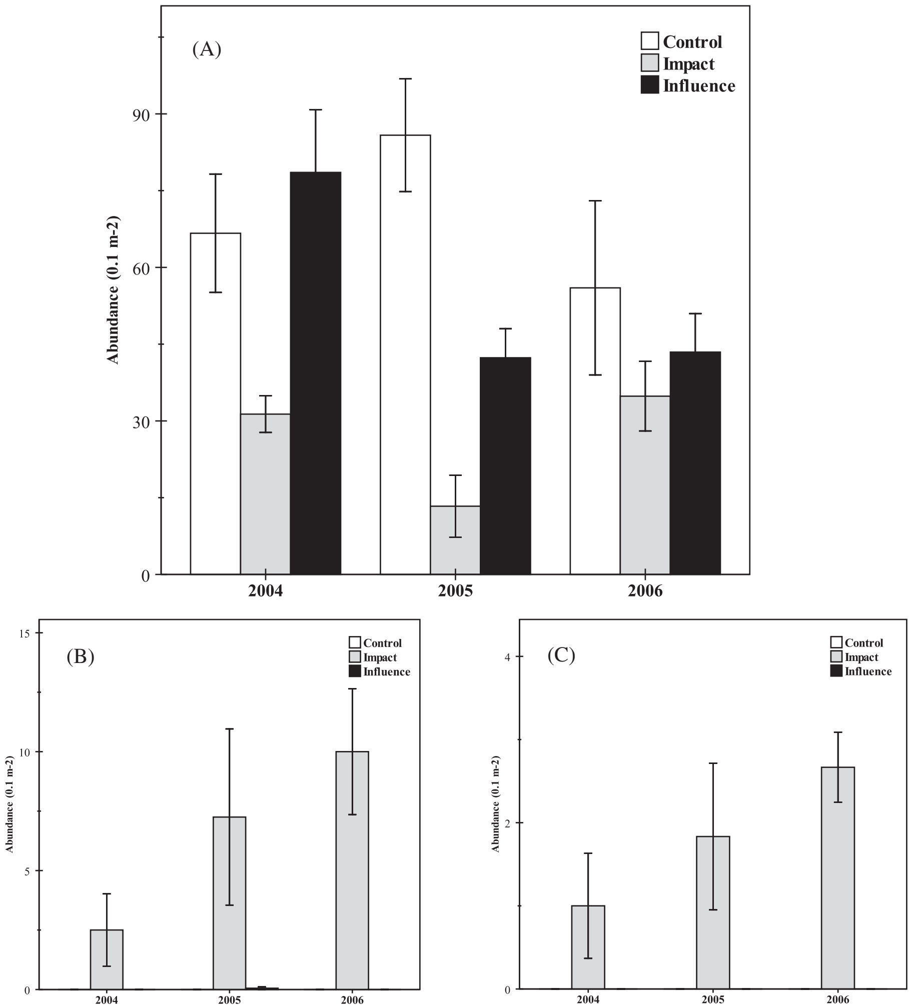

Figure 6. (A) Overall macrofauna, (B) *Pseudopolydora* cf. *paucibranchiata*, (C) *Abra alba* abundances (±SE) throughout the study period.

Influence). Minimum values of J' (0.72) and H' (1.79) were found in impacted stations during 2005. Maximum values of J' were found in Influence stations during 2006 (0.83) and maximum values of H' were observed in Influence stations during 2005 (3.50).

No seasonal variations in macrofaunal abundances were observed throughout the sampling period (one-way ANOVA, $F = 1.779$; $p = 0.178$), however, highly significant differences were found among Control, Influence and Impact stations (one-way ANOVA, $F = 10.735$; $p = 0.0001$) (Table 6).

Macrofauna assemblage structure varied greatly throughout the study period (two-way nested ANOSIM, $R = 0.663$; $p = 0.001$), with an impoverished assemblage in Impact stations, compared with Influence and Control stations, considering each sampling year separately (2004, 2005 and 2006). These differences were consistent and can be observed in 2D ordination space (Figure 7), Impact stations being separated from the other sampling groups (Influence and Control), with the except of M5 from the first campaign (November 2004) which was not environmentally altered before enlargement works.

Table 6. Results of univariate ANOVA testing for differences in overall macrofaunal abundance, *Pseudopolydora* cf. *tridentata* and *Abra alba* abundances among sampling stations groups (Control, Impact and Influence) throughout the study period (2004–2006).

		Overall macrofaunal abundance			*P*. cf. *tridentata* abundance			*A. alba* abundance		
Source of variation	df	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*
Impact (I)	2	8674.88	10.735	**0.0001**	277.68	19.286	<**0.0001**			0.456*
Year (Y)	2	1843.19	1.779	0.178	24.78	1.085	0.344			≪**0.0001***

Notes: Significant differences ($p < 0.01$) are highlighted in bold. *Kruskal–Wallis ANOVA test was carried out.

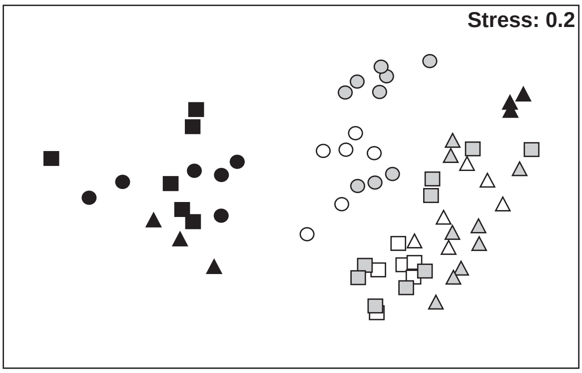

Figure 7. Ordination plot (nMDS, stress = 0.2) showing similarities in macrofaunal assemblage structure among sampling station groups. Centroids for each distance are plotted. Triangles: 2004; squares: 2005; circles: 2006. White: control stations, grey: influence stations; black: impact stations.

The polychaete *Pseudopolydora* cf. *paucibranchiata* (Figure 6B) and the mollusc *Abra alba* (Figure 6C) can be considered as bioindicator species of pollution, since they were almost exclusively present in Impact stations, especially during the last two sampling years, when pristine conditions disappeared completely. *P*. cf. *paucibranchiata* and *A. alba* showed highly significant variations among sampling stations (Control, Impact and Influence) (*P*. cf. *paucibranchiata*, one-way ANOVA, $F = 19.286$, $p < 0.0001$; *A. alba*; K–W ANOVA, $H = 26.458$, $p << 0.0001$). These species were scarcely observed in non-affected sampling stations (Influence and Control). In terms of temporal variations, no significant differences were found in either taxa (*P*. cf. *paucibranchiata*, one-way ANOVA, $F = 1.085$, $p = 0.344$; *A. alba*, K–W ANOVA, $H = 0.795$, $p = 0.456$) (Table 6).

3.3. *Biotic indexes (AMBI, M-AMBI and BENTIX)*

3.3.1. *First campaign (November 2004)*

The AMBI index varied between 0.44 (M2) and 2.57 (M6). All stations presented AMBI values of non-disturbed sites, although station M6 is considered a slightly disturbed site. This station is dominated by species belonging to ecological group III, typical of unbalanced macrofaunal communities. M-AMBI index values ranged from 0.53 (M6) to 0.97 (M1), all stations are characterised by high (M1, M2 and M4) and good (M3, M5 and M7) status, with the exception of M6 which is of moderate status. BENTIX values ranged from 2.70 (M6) to 4.91 (M2) and all stations are characterised as unpolluted areas, with the exception of M4 (BENTIX = 4), which is classified as slightly polluted and M6 classified as heavily polluted (Table 7).

Table 7. Percentages of ecological groups, AMBI, M-AMBI and BENTIX indexes in sampling stations.

	Nov 2004							Mar 2005							Jul 2005							Jul 2006						
	M1	M2	M3	M4	M5	M6	M7	M1	M2	M3	M4	M5	M6	M7	M1	M2	M3	M4	M5	M6	M7	M1	M2	M3	M4	M5	M6	M7
Group I (%)	67	78.2	81.9	50	70	17.6	71.1	30.2	44.1	76.3	54.1	9.1	0	42.6	30.2	73.1	57.4	59	0	5.7	41.9	77.9	43.8	65.6	66.4	34.1	2.9	92.1
Group II (%)	12.5	19	2.8	27.6	2.9	0	19.1	16.9	29.4	5.3	39.2	0	6.7	26.3	13.5	18.3	18.3	32.7	0	4.5	18.3	13.2	41.3	25	11.8	2.4	3.9	6.4
Group III (%)	18.2	2.8	8.8	22.4	21.4	73.5	5.4	51.9	23.5	7.9	6.8	0	0	31.1	54.2	7.7	18.3	4.4	0	26.1	39.2	8.8	12.5	3.1	19.7	11.8	76.5	1
Group IV (%)	2.3	0	6.5	0	5.7	8.8	4.4	0	0	10.5	0	90.9	93.3	0	0.5	1	6.1	2	100	63.6	0	0	1.3	6.3	0	50.6	16.7	0.5
Group V (%)	0	0	0	0	0	0	0	1.1	2.9	0	0	0	0	0	1.6	0	0	2	0	0	0.5	0	1.3	0	2	1.2	0	0
AMBI	0.91	0.45	0.63	1.13	0.97	2.57	0.66	1.64	1.16	0.79	0.8	4.09	4.3	1.33	1.85	0.58	1.08	0.83	4.5	3.6	1.47	0.46	1.03	0.62	0.9	2.8	3.06	0.12
M-AMBI	0.97	0.86	0.79	0.89	0.69	0.54	0.82	0.92	0.83	0.71	0.85	0.29	0.26	0.76	0.9	0.91	0.92	0.77	0.23	0.45	0.87	0.85	0.83	0.74	0.96	0.56	0.44	0.78
BENTIX	4.68	4.91	5.27	4	4.8	2.70	4.84	3.21	3.76	5.05	4.16	2.91	2	3.70	3.21	4.93	4.30	4.36	2	2.27	3.67	5.11	3.76	4.62	4.65	3.37	2.12	5.68

3.3.2. *Second campaign (March 2005)*

AMBI index values varied between a minimum of 0.79 (M3) and a maximum of 4.3 (M6). Sampling stations were characterised as non-disturbed (M3 and M4) or slightly disturbed (M1, M2 and M7) and moderately disturbed (M5 and M6) sites. Stations M5 and M6 were dominated overwhelmingly by the ecological group IV (90.9% in M5 and 93.3% in M6). M-AMBI values ranged from 0.25 (M6) and 0.92 (M1). Sediments from M5 and M6 were classified as poor status, and the remainder were characterised as good, with the exception of M1 (high status). BENTIX values ranged from 2 (M6) to 5.05 (M3). Inner stations of the marina are characterised as heavily polluted (M5 and M6) and the remainder as slightly polluted (M1, M2 and M7) and unpolluted (M3 and M4) (Table 7).

3.3.3. *Third campaign (July 2005)*

AMBI index values varied between 0.58 (M2) and 4.5 (M6). Sampling stations were characterised as non-disturbed (M2, M3, M4 and M7), slightly disturbed (M1) and moderately disturbed (M5 and M6). M5 and M6 were dominated by species belonging to ecological group IV (transitional to polluted communities), 63.6% in M6 and 100% in M5. M-AMBI values ranged from 0.23 (M5) and 0.92 (M3). Soft-bottom macrofaunal communities were classified as high status (M1, M2, M3 and M7), good status (M4), moderate status (M6) and poor status (M5). BENTIX values ranged from 2 (M5) to 4.93 (M2). Inner stations were characterised as heavily polluted (M5 and M6) and the remainder were classified as slightly polluted (M1) or unpolluted (M2, M3, M4 and M7) (Table 7).

3.3.4. *Fourth campaign (July 2006)*

AMBI index values varied between 0.12 in M7 and 3.1 in M6. Sampling stations were characterised as non-disturbed (M1, M2, M3, M4 and M7) and slightly disturbed (M5 and M6). Stations M5 and M6 were dominated by species belonging to ecological group III (M6) and IV (M5). M-AMBI index values ranged from 0.44 (M6) to 0.96 (M4). Sampling stations were classified as high status (M1 and M4), good status (M2, M3 and M5) and moderate status (M6). BENTIX values ranged from 2.12 (M6) to 5.68 (M7). Inner stations were characterised as heavily (M6) and moderately (M5) polluted areas. The remaining sampling stations were classified as slightly polluted (M2) and unpolluted (M1, M3, M4 and M7) (Table 7).

3.3.5. *Station M5 (Impact station)*

This station was characterised by important variations of AMBI index along the four sampling campaigns. In November 2004, the AMBI index was 0.97, typical of non-disturbed ecosystems, whereas whilst in 2005 (March and July) the AMBI index reached values typical of moderately disturbed ecosystems (4.09 in March and 4.5 in July). A decrease in the AMBI index was observed during the last campaign (July 2006) to a value of 2.80, characteristic of slightly disturbed ecosystems. The high abundances in the second (March 2005) and third (July 2005) campaigns were due to the high numbers of the spionid polychaete *Pseupolydora* cf. *paucibranchiata*. M-AMBI reached minimum values during enlargment works (March 2005, 0.290; July 2005, 0.227) and a recovery was observed in 2006 (0.558), although it did not reach 2004 values (0.695). BENTIX varied throughout the study period, reaching the highest value during the first campaign (November 2004) with 4.8, typical of unpolluted areas. However, during the enlargement works, BENTIX decreased to 2.91 (March 2005) and 2 (July 2005), typical of moderately and heavily polluted areas. In 2006, BENTIX showed a partial recovery, with 3.37, typical of slightly polluted areas (Table 7).

3.3.6. *Station M6 (Impact station)*

This station was characterised by important variations in the AMBI index along the four samplign campaigns. In November 2004, AMBI was 2.57, typical of slightly disturbed ecosystems, whereas in 2005 (March and July) AMBI reached values typical of moderately disturbed ecosystems (4.3 in March 2005 and 3.6 in July 2005). A decrease in AMBI was observed during the last campaign (July 2006) to 3.06, characteristic of slightly disturbed ecosystems. The high values of AMBI during 2005 were due to the increase of abundances of the spionid polychaete *Pseudopolydora* cf. *paucibranchiata* and, to a lesser extent, the mollusc *Abra alba* and the amphipod *Corophium acutum*. M-AMBI reached minimum values in March 2005 (0.257) with a tendency to recover in July 2005 (0.449) and 2006 (0.438), however, M-AMBI in pre-condition period reached a maximum value in this station (0.54). BENTIX showed slight differences throughout the study period, with Station 6 characterised as a slightly polluted area in November 2004 (2.7). In the following campaigns (March 2005, July 2005 and July 2006), BENTIX classified Station 6 as a heavily polluted area, with values ranging from 2 to 2.27 (Table 7).

Spearman's correlation between the environmental variables (organic matter, silt/clay, total hydrocarbons, PAH and depth) and biotic indexes (AMBI, M-AMBI and BENTIX) confirmed the existence of significant correlations among them. AMBI was significantly positive correlated with silt/clay content, total hydrocarbons and PAH. The opposite pattern was observed with M-AMBI and BENTIX, being significantly negative correlated to the former parameters. There were negative correlations among AMBI and M-AMBI and BENTIX, the last two being positively correlated with each other. Organic matter was not significantly correlated with any environmental variable of a biotic index. Silt and clay content showed significantly positive correlations with total hydrocarbons and PAHs (Table 8).

In short, the macrofaunal community structure of Puerto Calero was characterised by opportunistic species (the polychaete *P*. cf. *paucibranchiata* and the mollusc *Abra alba*), present only in inner stations, sensitive species (the sipunculid *Aspidosiphon muelleri*, the crustacean *Pariambus typicus* and the polychaete *Aponuphis bilineata*), present only in outer stations, and ubiquitous species (the polychaetes *Aricidea assimilis* and *Armandia polyophthalma*) present in both stations. The concentration of total hydrocarbons and PAHs in the studied sediments were key to the structure of macrofaunal assemblages in Puerto Calero, with a drastic change in inner stations of the marina compared with non-disturbed conditions (outer stations). These shifts were recorded in the three biotic indexes analysed in the study (AMBI, M-AMBI and BENTIX), which showed differences throughout the phases of the enlargement work in the marina (before, during and after).

Table 8. Correlation matrix (Spearman) for environmental variables and biotic indexes (AMBI, M-AMBI and BENTIX).

	OM	Silt/clay	TH	PAH	AMBI	M-AMBI	BENTIX	Depth
OM	1							
Silt/clay	0.471	1						
TH	0.234	0.747*	1					
PAH	0.120	0.687*	0.756*	1				
AMBI	0.342	0.702*	0.995**	0.698*	1			
M-AMBI	0.213	−0.687**	−0.976**	−0.675*	−0.876**	1		
BENTIX	0.222	−0.699*	−0.388*	−0.387*	−0.948**	0.444**	1	
Depth	0.102	0.107	0.210	0.08	0.211	0.134	0.189	1

Notes: OM, organic matter; TH, total hydrocarbons; PAH, polycyclic aromatic hydrocarbons. $^{*}p < 0.05$; $^{**}p < 0.01$.

4. Discussion

In marinas, the accumulation of organic matter and petroleum hydrocarbons, within muddy sediments, might promote the establishment of anoxic conditions in bottom sediments; consequently, opportunistic and/or resistant species could become dominant in the macrofaunal community structure [30,31].

In this study, macrobenthic assemblages showed a drastic change in stations directly affected by dyke construction, with a clear dominance of the spionid polychaete *P*. cf. *paucibranchiata*. The most affected stations (M5 and M6) were characterised by a different macrobenthic assemblage structure throughout the study period (2004–2006), however, this was more acute during dyke construction (2005) with a clear dominance of ecological groups III and IV (89–100%) in M5 and M6. AMBI values increased in the inner stations during the enlargement work (2005), however, a decrease in AMBI was observed after the work (2006), although this did not reach pre-conditions (2004) values. The same pattern was found with M-AMBI, with minimum values during work in March 2005, and recovery in July 2005 and 2006. M-AMBI values during the pre-conditions period (2004) were higher than in 2006. Thus, AMBI and M-AMBI varied according to pre-conditions (November 2004), work in progress (March and July 2005) and post-conditions (July 2006), in agreement with the significant increase in hydrocarbons during the work (2005).

Outer stations were characterised by a more diverse macrofaunal assemblage, typical of Canarian subtidal sandy seabeds [32,33]. AMBI values ranged from 1.12 to 1.85, depending on the importance of ecological group III (species tolerant to an excess of organic enrichment, typical of slight unbalanced situations) in the macrofaunal assemblage structure. M-AMBI values showed small variations throughout the study period, typical of undisturbed ecosystems.

These results support the usefulness of AMBI in similar conditions (high hydrocarbon content), as shown for other geographical areas [17,20], with a positive correlation between AMBI and the total hydrocarbon concentration.

Former studies support the results obtained here, with a pollution gradient in marina sediments. Carrier et al. [34] showed the influence of sediment toxicity (heavy metals: Cu, Cd, Pb and Zn) and the percentage of fine grain sediments on AMBI, and therefore, the studied macrofaunal assemblages. They observed an increasing gradient of contamination from outside to inside the two studied marinas, with the innermost stations characterised by high AMBI values (moderately and heavily disturbed). In addition to the former effects of heavy metals and sediment grain size, the high level of organic matter (8–12%) may have affected the macrofaunal species composition, and therefore AMBI. Borja et al. [35] observed a reduction in M-AMBI values in sediments affected by marina construction, mainly due to habitat loss and disturbances to food webs which provoked a drastic change in benthic assemblages structure. Thus, M-AMBI detects benthic impacts produced by different hydro-morphological pressures (dredging, dumping, dyke construction), as well as the recovery of benthic assemblages after the cessation of work [35].

In this study, we observed the same trend in the BENTIX index throughout the study period (before, during and after work) as occurred with AMBI and M-AMBI. Minimum values of BENTIX were observed in inner stations M5 and M6 (BENTIX = 2) in March 2005 and July 2005, respectively. The highest values of BENTIX were obtained in M3 in November 2004 (5.27) and station 7 in July 2006 (5.68), in control stations before and after enlargement works.

Simboura [36] recommends using AMBI rather than BENTIX in places where specific richness is low and total abundance is high, considering that AMBI more accurately defines the ecological groups of the sample species (five ecological groups for AMBI compared with only two for BENTIX). However, in this study, the BENTIX index showed the same reliability as AMBI and M-AMBI.

The reliability of the three indexes was confirmed, and although not formerly applied in the Canarian archipelago they can be considered a useful tool for ecological assessment studies in marinas of the Canary Islands. Thus, the use of AMBI, M-AMBI and BENTIX in this study constitutes the first step in the development of different methodologies to assessing benthic quality in the sandy seabeds of the Canary Islands, however, comparison studies with other biotic indexes (e.g. IBI, BOPA) should be undertaken to improve the assessment of ecological integrity in the Macaronesian geographical region (Azores, Madeira, Selvagens Islands, Canary Islands and Cape Verde islands).

Further field work in marinas, aquaculture farms, pipelines and other pollutant sources (brine discharges, thermal pollution, etc.) is necessary to establish AMBI, M-AMBI and BENTIX as a suitable tool for coastal monitoring assessment studies in the Canary Islands.

Acknowledgements

The authors wish to thank the staff of Puerto Calero for the logistic help during sampling campaigns. To Puerto Calero S.A. for financial support of this study. We are grateful to Dr J. Núñez (University of La Laguna) for determining the polychaete species of the first campaign (November 2004). To Dr Óscar Pérez (Centro de Investigaciones Medioambientales del Atlántico, CIMA SL) for preparing the map of the study area and his constructive comments on an earlier draft of the manuscript. We are also grateful to Fernando Tuya (Edith Cowan University, Australia) for English correction and careful reading of the final manuscript.

References

[1] A. Borja, *The European Water Framework Directive: a challenge for nearshore, coastal and continental shelf research*, Cont. Shelf Res. 25(14) (2005), pp. 1768–1783.
[2] A. Borja, *The new European Marine Strategy Directive: difficulties, opportunities, and challenges*, Mar. Pollut. Bull. 52 (2006), pp. 239–242.
[3] A. Borja, D. Dauer, R. Díaz, R.J. Llansó, I. Muxika, G. Rodríguez, and L. Schaffner, *Assessing estuarine benthic quality conditions in Chesapeake Bay: a comparison of three indexes*, Ecol. Indic. 8(4) (2007), pp. 395–403.
[4] C. Ritter and P.A. Montagna, *Seasonal hypoxia and model of benthic response in a Texas bay*, Estuaries 22 (1999), pp. 7–20.
[5] J.I. Sáiz-Salinas, *Evaluation of adverse biological effects induced by pollution in the Bilbao estuary (Spain)*, Environ. Pollut. 96 (1997), pp. 351–359.
[6] R.M. Warwick and K.R. Clarke, *Comparing the severity of disturbance: a meta-analysis of marine macrobenthic community data*, Mar. Ecol. Prog. Ser. 92 (1993), pp. 221–231.
[7] R.M. Warwick and K.R. Clarke, *A comparison of some methods for analyzing changes in benthic community structure*, J. Mar. Biol. Assoc. UK 71 (1991), pp. 225–244.
[8] C. Hilly, H. Le Bris, and M. Glémarec, *Impacts biologiques des emissaries urbains sur les écosystèmes benthiques*, Oceanis 12 (1986), pp. 419–426.
[9] J. Grall and M. Glémarec, *Using biotic indexes to estimate macrobenthic community perturbations in the Bay of Brest*, Estuar. Coast Shelf Sci. 44 (1997), pp. 43–53.
[10] V.D. Engle, J.K. Summers, and G.R. Gaston, *Benthic indexes of environmental condition of Gulf of Mexico estuaries*, Estuaries 17 (1994), pp. 372–389.
[11] S.B. Weisberg, J.A. Ranasinghe, D.M. Dauer, L.C. Schaffner, R.J. Díaz, and J.B. Frithsen, *An estuarine benthic index of biotic integrity (B-IBI) for Chesapeake Bay*, Estuaries 20 (1997), pp. 149–158.
[12] D.M. Dauer and R.V. Alden, *Long-term in the macrobenthos and water quality of the Lower Chesapeake Bay*, Mar. Pollut. Bull. 30 (1995), pp. 840–850.
[13] R.F. Van Dolah, J.L. Hyland, A.F. Holland, J.S. Rosen, and T.R. Snoots, *Benthic index of biological integrity for assessing habitat quality in estuaries of the southeastern USA*, Mar. Environ. Res. 48 (1999), pp. 269–283.
[14] A. Borja, J. Franco, and V. Pérez, *A Marine Biotic Index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments*, Mar. Pollut. Bull. 40 (2000), pp. 1100–1114.
[15] I. Muxica, L. Ibaibarriaga, J.I. Sáiz, and A. Borja, *Minimal sampling requirements for a precise assessment of soft-bottom macrobenthic communities, using AMBI*, J. Exp. Mar. Biol. Ecol. 349 (2007), pp. 323–333.
[16] A. Borja, I. Muxica, and F. Franco, *The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts*, Mar. Pollut. Bull. 46 (2003), pp. 835–845.
[17] I. Muxica, A. Borja, and W. Bonne, *The suitability of the Marine Biotic Index (AMBI) to new impact sources along European coasts*, Ecol. Indic. 5 (2005), pp. 19–31.
[18] L. Cai, N.F.Y. Tam, T.W.Y. Wong, L. Ma, Y. Gao, and Y.S. Wong, *Using benthic macrofauna to assess environmental quality of four intertidal mudflats in Hong Kong and Shenzhen coast*, Acta Oceanol. Sin. 22 (2003), pp. 309–319.

[19] H. Bazairi, A. Bayed, and C. Hily, *Structure et bioévaluation de l'état écologique des communaute's benthiques d'un écosystème lagunaire de la Côte Atlantique marocaine*, Comp. Rend. Biol. 328 (2005), pp. 977–990.

[20] P. Muniz, N. Venturini, A.M.S. Pires-Vanin, L.R. Tommasi, and A. Borja, *Testing the applicability of a Marine Biotic Index (AMBI) to assessing the ecological quality of soft-bottom benthic communities, in the South America Atlantic region*, Mar. Pollut. Bull. 50 (2005), pp. 627–637.

[21] N. Simboura and A. Zenetos, *Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index*, Med. Mar. Sci. 3(2) (2002), pp. 77–111.

[22] N. Simboura, E. Papathanassiou, and D. Sakellariou, *The use of a biotic index (Bentix) in assessing long-term effects of dumping coarse metalliferous waste on soft bottom benthic communities*, Ecol. Indic. 7 (2007), pp. 164–180.

[23] J.C. Dauvin, G. Bellan, and D. Bellan-Santini, *Benthic indicators: from subjectivity to objectivity – Where is the line?* Mar. Pollut. Bull. 60 (2010), pp. 947–953.

[24] J.B. Buchanan and J.M. Kain, *Measurement of the physical and chemical environment*, in *Methods for the Study of Marine Benthos*, N.A. Holme and A.D. McIntyre, eds., IBP Handbook No. 16, Blackwell, Oxford, 1971, pp. 30–58.

[25] A. Walkley, *A critical examination of a rapid method for determination of organic carbon in soils – effect of variations in digestion conditions and of inorganic soil constituents*, Soil Sci. 63 (1947), pp. 251–257.

[26] K.R. Clarke, *Non-parametric multivariate analysis of changes in community structure*, Aust. J. Ecol. 18 (1993), pp. 17–143.

[27] K.R. Clarke and R. Warwick, *Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation*, Plymouth Marine Laboratory, Plymouth, UK, 1994.

[28] A. Borja and I. Muxica, *Guidelines for the use of AMBI (AZTI's marine biotic index) in the assessment of the benthic ecological quality*, Mar. Pollut. Bull. 50 (2005), pp. 787–789.

[29] R. Riera, *Biodiversidad meiofaunal de las playas de Los Abrigos del Porís y de Los Cristianos en la isla de Tenerife: estructura y dinámica de sus comunidades*, PhD thesis, University of La Laguna, 2004.

[30] T. Pearson and R. Rosenberg, *Macrobenthic succession in relation to organic enrichment and pollution of the marine enviromment*, Ocean. Mar. Biol. Ann. Rev. 16 (1978), pp. 229–311.

[31] M.J. Cardell and R. Sardá, *Spatial changes in sublittoral soft-bottom polychaete assemblages due to river inputs and sewage discharges*, Acta Oecol. 20 (1999), pp. 343–351.

[32] S. Herrando-Pérez, G. San Martín, and J. Núñez, *Polychaete patterns from an oceanic island in the eastern Central Atlantic: La Gomera (Canary Archipelago)*, Cah. Biol. Mar. 42 (2001), pp. 275–287.

[33] Ó. Monterroso, R. Riera, and J. Núñez, *Subtidal soft-bottoms macroinvertebrate communities from the Canary Islands: an ecological approach*, Braz. J. Ocean, in press.

[34] M.D. Carrier, R.L. Fletcher, C.H. Thorp, and D. Fichet, *Macrofaunal community responses to marina-related pollution on the south coast of England and west coast of France*, J. Mar. Biol. Assoc. UK 89(1) (2009), pp. 19–29.

[35] A. Borja, I. Muxica, and J.G. Rodríguez, *Paradigmatic responses of marine benthic communities to different anthropogenic pressures, using M-AMBI, within the European Framework Directive*, Mar. Ecol. 30 (2009), pp. 214–227.

[36] N. Simboura, *Bentix index vs Biotic index in monitoring: an answer to Borja et al., 2003*, Mar. Pollut. Bull. 48 (2004), pp. 403–404.