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Response of benthic opportunistic polychaetes and amphipods index to different perturbations in coastal oligotrophic areas (Canary archipelago, North East Atlantic Ocean)

Rodrigo Riera[1,]* & Jose Antonio de-la-Ossa-Carretero[2]

1 Atlantic Environmental Marine Centre (CIMA SL), San Cristóbal de La Laguna, Spain
2 Department of Marine Sciences and Applied Biology, University of Alicante, Alicante, Spain

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Correspondence
Rodrigo Riera, Atlantic Environmental Marine Centre (CIMA SL), San Cristóbal de La Laguna, E-38206, Santa Cruz de Tenerife, Canary Islands, Spain.
E-mail: rodrigo@cimacanarias.com

*Present address: Department of Biodiversity, Qatar Environment and Energy Research Institute (QEERI), Doha, 5825, Qatar

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Abstract

Oligotrophic areas harbour low macrofaunal abundance and patchy distribution. In these areas it is necessary to test the reliability of biological indicators, especially those based on taxonomic sufficiency where the level of identification is balanced against the need for ecological information and could affect the efficiency of bioindicators. The BOPA (benthic opportunistic polychaetes and amphipods) index was applied in five coastal areas subjected to different perturbations (aquaculture, harbour, brine, sewage, and thermal pollution) in the Canary archipelago, an oligotrophic area of the Atlantic Ocean. Significant differences in the BOPA index between impact and control sites were only found in the area affected by a harbour. Perturbations such as aquaculture, brine or sewage discharge produce only a weak response of the BOPA index, whereas no effects were observed at thermal pollution-impacted locations. The BOPA index should be used with caution to establish the ecological status of coastal water bodies in the Canary Islands, since it was only reliable in strongly impacted regions (enlargement harbour works), but did not respond clearly to other man-induced perturbations.

Introduction

Anthropogenic activities are recognized as the main potential source of pollution for the coastal marine environment (GESAMP 2001). Currently, there are different methodologies used to determine the quality of coastal and transitional waters, assessing the ecological status (ES; Borja *et al.* 2004; Simboura *et al.* 2005; Dauvin *et al.* 2007). These methods have been developed in the European Water Framework Directive (2000/60/EC) (WFD) and lately came into effect in the Marine Strategy Framework Directive (MSFD) (2008/56/EC) (Borja *et al.* 2003, 2011). One of the biological quality elements proposed in the WFD is benthic invertebrate fauna.

Benthic organisms make good ecological indicators because they are relatively sedentary and thus are unable to avoid deteriorating water/sediment quality. They have relatively long life-spans, show marked responses to stress depending on their species-specific sensitivity/tolerance levels, and play a vital role in cycling nutrients and materials between the underlying sediment and the overlying water column (Gray *et al.* 1988; Dauer 1993; Borja *et al.* 2000; Dauvin *et al.* 2007).

Benthic assemblages often reflect pollution effects and are widely used to study the effects of marine perturbations (Gray *et al.* 1990). The relationships between macrofauna and the effect of pollutants on them have been extensively described in the last decades (*e.g.* Calabretta & Oviatt 2008; Callier *et al.* 2009). To summarize information provided by the status of these benthic communities, several biotic indices have been developed (see summary in Díaz *et al.* 2004). These indices are useful tools for communication with managers because they reduce complex scientific data, integrate different types of information, and produce results that can be easily interpreted in the perspective of environment quality management (Wilson & Jeffrey 1994; Chainho *et al.* 2007).

Some of these indices are based on the classification of species (or group of species) in several ecological groups representing the specific sensitivity to disturbance levels. Two of the most widely used indices are AMBI (Borja *et al.* 2000) and BENTIX (Simboura & Zenetos 2002). Unfortunately, both require taxonomic expertise to determine macrofauna to species level and this task is time-consuming, especially for certain families of polychaetes and amphipods (De Biasi *et al.* 2003). A number of alternative and cost-effective methods for benthic pollution monitoring have been proposed including the use of sediment profile imaging (SPI) (Karakassis *et al.* 2002), video recordings (Crawford *et al.* 2001), different sieve mesh sizes (*e.g.* 0.5 and 1 mm) (Thompson *et al.* 2003), the size fractionation of the macrobenthic biomass (Lamparidou *et al.* 2008) and different taxonomic resolution (phylum, family or genus level) (Dauvin *et al.* 2003). The main aim of the latter methods is to develop easy and cheap monitoring protocols, reducing significantly time and cost while maintaining the reliability to detect the level of environmental impact (Lamparidou *et al.* 2005).

There are indices, such as the benthic opportunistic polychaetes and amphipods (BOPA) index, in which the taxonomic effort is highly reduced (Gómez-Gesteira & Dauvin 2000; Dauvin & Ruellet 2007), since this index only requires identification of species of opportunistic polychaetes (Table 1), and counting and differentiating amphipods (except the genus *Jassa*) of the remaining organisms. Opportunistic polychaetes are resistant, indifferent or favoured by organically enriched sedimentary matter and belong to a group of opportunistic and tolerant species in AMBI and BENTIX. In contrast, amphipods are sensitive to significant increases in organic matter and most of them belong to a group of sensitive species in AMBI and BENTIX. The BOPA index could therefore be a reliable alternative to obtain similar results to more time-consuming former indices.

Despite the fact that the BOPA index has been applied previously in European coasts (Atlantic Ocean and Mediterranean basin) (Dauvin & Ruellet 2007; Munari & Mistri 2007, 2008; Pravoni *et al.* 2007; Blanchet *et al.* 2008; Bouchet & Sauriau 2008; Lavesque *et al.* 2009; de-la-Ossa-Carretero *et al.* 2009), BOPA has not been employed in the Macaronesian region (or Canary Islands). This area is characterized by low primary production rates (Arístegui *et al.* 2001), producing macrofaunal assemblages characterized by low abundances and intermediate-high species richness (Herrando-Pérez *et al.* 2001; Riera *et al.* 2011a,b). This high diversity increases the possibility of macrobenthic invertebrate congeners with different pollution tolerances (Resh & Unzicker 1975), producing the need to test indices such as BOPA that establish the same tolerance level for a group of congeners. Thus, the BOPA index could be under- or overestimated in these geographic regions, producing mistakes in the assessment of anthropogenic impacts.

The main aim of the present study was to analyse the applicability of the BOPA index in oligotrophic areas by checking its reliability and accuracy for different anthropogenic disturbances (fish cages, harbour enlargement, brine and sewage outfalls, and thermal pollution). The

Table 1. List of opportunistic polychaeta. Area where each species were present is indicated: (a) Barranco Hondo fish cages; (b) Calero Harbour; (c). Las Burras desalination plant; (d) Tarajalillo plant (brine + sewage); (e). UNELCO (Central Electric Power).

Species	Area	Species	Area
Aonides oxycephala (Sars, 1862)	a,b,c,d,e	*Pygospio elegans* Claparède, 1863	a
Capitellidae sp.	d	*Prionospio* Malmgren, 1867	a,d
Capitella minima Langerhans, 1881	a,b,c,e	*Prionospio steenstrupi* Malmgren, 1867	a,b,c,d,e
Caulleriella alata (Southern, 1914)	a,b	*Scolelepis tridentata* (Southern, 1914)	b,e
Caulleriella bioculata (Keferstein, 1862)	a	*Pseudopolydora* Czerniavsky, 1881	b
Cirratulus cirratus (O. F. Müller, 1776)	e	*Pseudopolydora paucibranchiata* (Okuda, 1937)	b
Cirriformia tentaculata (Montagu, 1808)	a	*Pygospio elegans* Claparède, 1863	a
Cirrophorus armatus (Glémarec, 1966)	e	*Rhynchospio glutaea* (Ehlers, 1897)	a
Cirrophorus lyra (Southern, 1914)	e	*Scolelepis* Blainville, 1828	a
Dispio uncinata Hartman, 1951	a,b	*Scolelepis squamata* (O.F. Muller, 1806)	b,c,d
Laonice cirrata (M. Sars, 1851)	b	*Scolelepis tridentata* (Southern, 1914)	b
Leiochrides africanus Augener, 1918	e	*Spio filicornis* (Müller, 1776)	a,c,d,e
Malacoceros fuliginosus (Claparède, 1870)	d	*Spionidae* sp.	e
Mediomastus fragilis Rasmussen, 1973	e	*Spiophanes bombyx* (Claparède, 1870)	b
Microspio mecznikowianus (Claparède,1869)	e		

response of BOPA was compared with other indices, such as Shannon diversity index, BENTIX and AMBI to determine the impact of reducing the taxonomic effort in monitoring assessment studies.

Material and Methods

Study area

Five areas subjected to different anthropogenic disturbances were used in the present study: fish cages (Barranco Hondo), harbour enlargement works (Calero harbour), brine (Las Burras), brine and sewage (Tarajalillo) and thermal pollution (UNELCO). The study sites were located in three islands of the Canary archipelago: Tenerife (Barranco Hondo and UNELCO), Lanzarote (Calero harbour) and Gran Canaria (Las Burras and Tarajalillo) (Fig. 1).

Barranco Hondo (fish cages) (coordinates: 28°22′50″ N, 16°21′03″ W; Tenerife, Canary Islands). This cage cultured gilt-head seabream (*Sparus aurata*) (110 t·year^{-1}) and seabass (*Dicentratus labrax*) (90 t·year^{-1}) throughout the study. Six stations were sampled, two impacted stations (St. 1 and 2) beneath fish cages, and four controls (Control Sand: St. 3 and 4, Control *Cymodocea nodosa*: St. 5 and 6) (>0.5 km from fish cages). Control stations were not affected by organic load from aquaculture farms as shown in sediment and water column samples (R. Riera, O. Monterroso, M. Rodriguez, unpublished data) and comparable biotic and abiotic variables (*e.g.* grain size composition and sedimentary organic content) with affected stations. Samples were collected during six campaigns (April 2007, November 2007, March 2008, June 2008, September 2008 and December 2008).

Calero Harbour (harbour enlargement) (28°55′00″ N, 13°42′07″ W; Lanzarote, Canary Islands). Marina enlargement works consisted of the construction of a new dyke (30 m long) in the mouth of the harbour. This new section could shelter 20 berths for boats between 25 and 50 m long, increasing the capacity of the marina to 440 berths.

The impact mainly consisted of changes in turbidity and the dominance of finer grain size (silt/clay and very fine sands) and, to a lesser extent, an increase of total hydrocarbons (>100 ppm) and polycyclic aromatic hydorcarbons (PAHs; >100 ppb) in inner parts of the harbour during works. Six sampling stations were sampled, two impacted (St. 1 and 2), in the inner part of the harbour; two influenced (St. 3 and 4) in the mouth of the harbour; and two controls (St. 5 and 6) (>0.5 km from the harbour). All stations were dominated by fine-grained sediments and a low concentration of organic matter content (<1%). Samples were collected along four sampling campaigns, according to the harbour works: pre-conditions (1st campaign: November 2004), harbour works (2nd and 3rd campaigns: March and June 2005, respectively) and post-conditions (4th campaign: June 2006). More details are provided in Riera *et al.* (2011a).

Las Burras (brine outfall) (27°76′48″ N, 15°55′86″ W; Gran Canaria, Canary Islands). The impact consisted of high concentrations of salinity on the surroundings of the brine outfall (47–50 psu) due to the continuous brine discharge of 17,000 m^3·day^{-1}. Nine stations were sampled, three at 0 m from the outfall (St. 1, 2 and 3, Impacted, 45.6 ± 1.2 psu), three at 15 m (St. 4, 5 and 6, Influenced, 38.7 ± 0.7 psu) and three at 30 m far from the outfall (St. 7, 8 and 9, Control, 36.6 ± 0.2 psu) through three radial transects. Samples

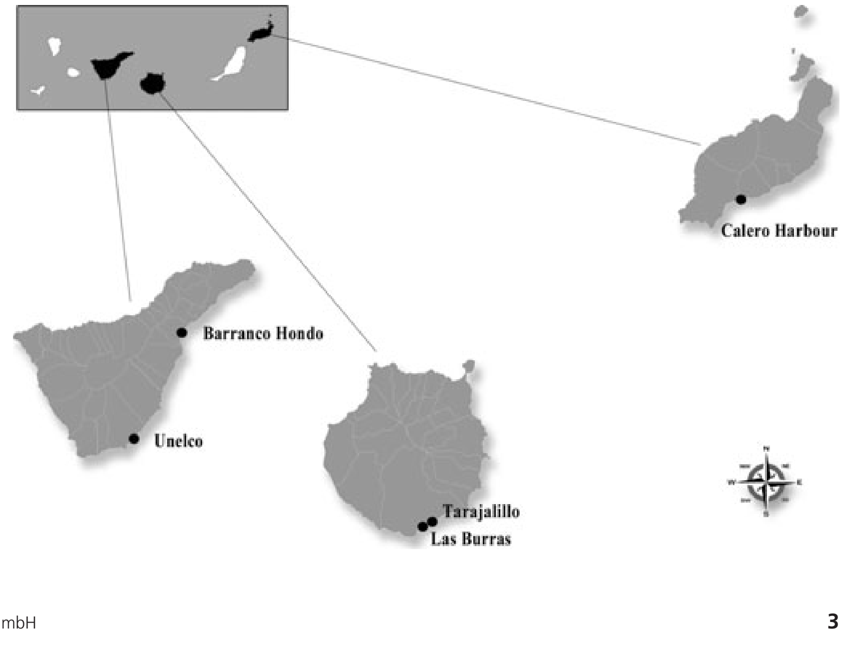

Fig. 1. Map of the study area, showing study areas in Tenerife (Barranco Hondo and UNELCO), Gran Canaria (Tarajalillo and Las Burras) and Lanzarote (Calero Harbour).

were collected in May 2008 and January 2009. Sedimentary composition varied at impacted stations, being dominated by coarse-grained sediments, whereas non-affected stations (15 and 30 m) were characterized by fine sands. Variations in sedimentary composition were observed between both surveys, because of swell periods during winter season (Riera *et al.* 2012).

Tarajalillo (brine and sewage outfall) (27°77′45″ N, 15°51′90″ W; Gran Canaria, Canary Islands). The impact consisted of high salinity concentrations at the brine outfall (49–53 psu), due to the continuous brine discharge of 14,000 $m^3 \cdot day^{-1}$ and occasional dumping (*ca.* 10,000–50,000 $m^3 \cdot month^{-1}$) of domestic sewage with brine. Sampling effort and disposition were the same as in Las Burras (see above) (0 m, 48.7 ± 1.4 psu; 15 m, 38.9 ± 0.9 psu, 30 m 36.7 ± 0.2 psu), as were the sampling campaign periods. Variations of sedimentary organic matter composition were recorded, being larger in impacted stations. Variations in sedimentary composition were observed between both surveys (May 2008 and January 2009) because of rough seas triggering sediment resuspension during swell periods in winter.

UNELCO (thermal pollution) (28°04′59″ N, 16°28′57′′ W; Tenerife, Canary Islands). The impact consisted of an increase of 4–6 °C in the proximity of the turbines (bottom temperature). Five stations were sampled, one situated at the mouth of the turbines (St. 1), two as influenced (St. 2 and 3) (<200 m southwards from the turbines) and two (St. 4 and 5) as control (>1 km north of the turbines). Two sampling campaigns were conducted (July 2003 and January 2004). More details are provided in Riera *et al.* (2011b). Sedimentary composition at impacted and control locations remained dominated by fine-grained sediments.

In all study areas, macrofaunal samples were collected by SCUBA divers using sediment cores (20 cm inner diameter) that were pushed into the sediment to a depth of 20 cm. Three replicates were collected in each sampling site, except in UNELCO (thermal pollution), where a mean of 10 replicates were collected in each site. Samples were preserved in a 10% seawater formaldehyde solution and subsequently decanted through a 0.5-mm-mesh sieve. The fraction remaining was separated into different taxonomical groups under a binocular microscope, and preserved in 70% ethanol. Macrofaunal specimens were determined to species level and counted, whenever possible, by means of a binocular microscope, or in a LEICA DMLB microscope equipped with Nomarski interference contrast.

The BOPA index, Shannon diversity index, AMBI and BENTIX were calculated. The BOPA index was calculated according to the guidelines of Dauvin & Ruellet (2007): $BOPA = \log[(fp_{op}/(f_a + 1) + 1]$, where fp_{op} is the opportunistic polychaete proportion of all fauna (0–1) and f_a is the amphipod (excluding *Jassa*) proportion of all fauna (0–1). The BOPA index ranges from 0, when there are no opportunistic polychaetes, to 0.30103, when there are only opportunistic polychaetes reflecting the most disturbed situation. The Shannon diversity index (H') was calculated using this algorithm: $H' = -\Sigma pi \log_2 p_i$, where p_i is the proportion of abundance of the number of species i in a community where species proportions are p_i, p_2, p_3 … p_n (Magurran 1988). The AMBI (AZTI Marine Biotic Index) developed by Borja *et al.* (2000) considers the proportions of five ecological groups (GI, GII, GIII, GIV and GV, established according to a gradient of tolerance to organic matter enrichment by the formula: AMBI = [(0) (%GI) + (1.5) (%GII) + (3) (%GIII) + (4.5) (%GIV) + (6) (%GV)]/100. AMBI ranges from 0 when sediment is unpolluted, 6 when is heavy polluted, and 7 when the sediment is azoic. The BENTIX index (Simboura & Zenetos 2002) was designed to fit the Mediterranean benthic ecosystem. It is based on the concept of indicator groups and uses the relative contribution of tolerant (GT) and sensitive species (GS) in the fauna weighted analogously to derive a single formula: BENTIX = [(6) (%GS) + (2) (%GT)]/100, where the numerical factor '6' is assigned to the sensitive species group GS and the factor '2' to the tolerant species groups GT. Thus BENTIX can produce a series of continuous values from 2 (heavily polluted) to 6 (pristine), being 0 when the sediment is azoic. AMBI and BENTIX were calculated using free available software at www.azti.es and www.hcmr.gr, respectively.

Non-parametric multivariate techniques were used to compare the composition of species of each study area. All multivariate analyses were performed using the PRIMER v. 6 statistical package (Clarke & Warwick 1994). Triangular similarity matrices were calculated through the Bray–Curtis similarity coefficient using square root-transformed mean abundances. A graphical representation of multivariate patterns of community composition was obtained by non-metric multidimensional scaling (nMDS) and a bubble plot correlated BOPA values of each station with community composition. Two-way crossed ANOSIM for each study case was used to test the differences between location (impact, control and influence) throughout sampling campaigns. The RELATE procedure was used to test the Spearman correlation between similarity matrices of stations and BOPA values.

BOPA values were examined using 3-factor analyses of variance (ANOVA) with location (impact, influence and control), stations and sampling campaigns as factors, except in the cases of Barranco Hondo fish cages and UNELCO thermal central, where the required ANOVA was an asymmetric model. For Barranco Hondo, asymmetrical design was used to test differences between impact and both control areas. Two separate ANOVAs testing differences between impact and influence area, and impact and control areas were performed in UNELCO, since a single model encompassing comparisons of the three positions (impact, influence and control) would have had a too complex asymmetry.

Prior to ANOVA, the homogeneity of variance was tested using Cochran's test. Data were $\sqrt{x}$ + 1-transformed since variances were significantly different; however, variance remained heterogeneous. It was therefore decided to analyse untransformed data, as ANOVA is robust to heterogeneity of variances, particularly for large balanced experiments (Underwood 1997). We set a more conservative significance level of 0.01 to reduce the probability of a Type I error. The SNK test (Student–Newman–Keuls) was used to determine which samples were implicated in the differences.

To define the ecological status of each sample it is necessary to calculate the ecological quality ratio (EQR). The EQR is the ratio between the observed value and a reference value of this index in an unperturbed environment, ranging from 0 for a bad quality environment to 1 for a high quality environment. Several methods were developed to establish this reference situation (Borja *et al.* 2012). In this case, the reference situation was considered for each location in stations with the highest value for the Shannon diversity index, absence of opportunistic polychaetes for BOPA and dominance of sensitive species for AMBI and BENTIX.

The agreement and correlation between BOPA and each index was analysed. Thresholds presented by De-la-Ossa-Carretero & Dauvin (2010) for BOPA, by Vincent *et al.* (2002) for the Shannon diversity index, by Borja *et al.* (2000) for AMBI and by Simboura & Zenetos (2002) for BENTIX were used to establish the ecological status classification. The Pearson correlation coefficient was calculated between the EQRs of each index. Weighted Kappa analysis (Cohen 1960; Landis & Kosch 1977) was used to evaluate the agreement, employing the methodology proposed by Borja *et al.* (2007). The equivalence

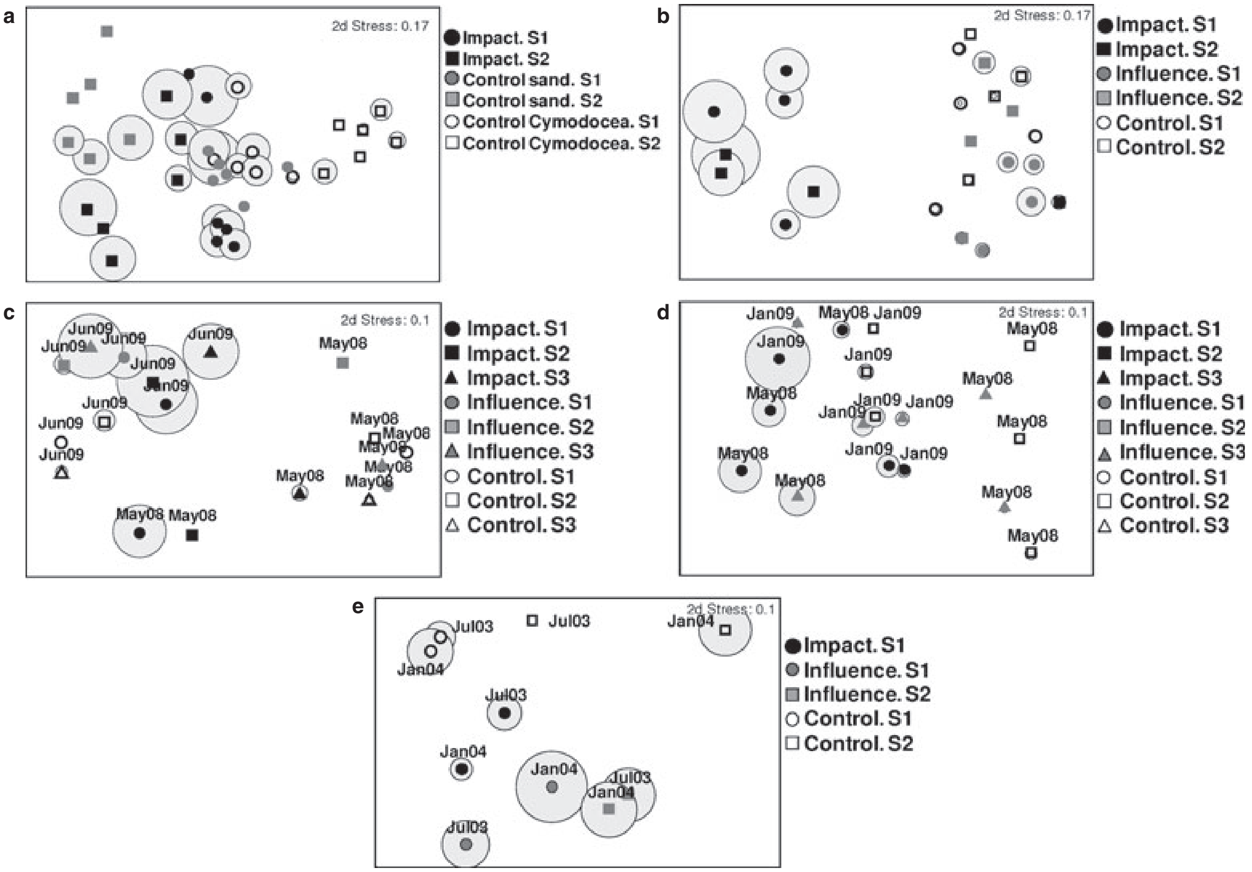

Fig. 2. nMDS ordination plot of benthic community abundance and associated stress values for each station in each study area. BOPA values of each station were represented by bubble plot representing: (a) Barranco Hondo fish cages; (b) Calero Harbou; (c) Las Burras desalination plant; (d) Tarajalillo plant (brine + sewage); (e) UNELCO (Central Electric Power).

table from Monserud & Leemans (1992) was used to establish the level of agreement of the two indices. In addition, since the importance of misclassification is not the same between close categories (*e.g.* between high and good, or poor and bad) as between distant categories (*e.g.* between high and moderate, or high and bad), we chose to apply Fleiss–Cohen weights (Fleiss & Cohen 1973).

Results

Analysis of the community composition of Barranco Hondo area showed that fish cages did not produce a clear effect in benthic fauna (Fig. 2a). ANOSIM showed significant differences among location groups (Global R) but the pairwise test (Table 2) showed that differences were mainly between the 3 control of *Cymodocea* and impact, and not between impact and control sand. The BOPA index showed a significant correlation with these changes (Rho: 0.189, $P < 0.05$).

MDS plots of community composition affected by Calero Harbour showed a clear segregation of the stations in the inner part of the harbour (Fig. 2b). ANOSIM detected significant differences among impact, influence and control stations (Table 2). The BOPA index showed a significant correlation with detected changes, increasing in impacted stations with respect to control and influence (Rho: 0.325, $P < 0.005$).

Table 2. Results of two-way crossed ANOSIM testing for differences among locations (impact, influence, control) across all campaigns, except Bco. Hondo, where the levels for locations were impact, control sand and control *Cymodocea*.

	R	Significance level, %
Fish farm (Bco. Hondo)		
Global	0.204	4.6
Impact *versus* Control sand	−0.083	66.3
Impact *versus* Control Cymodocea	0.542	0.1
Control sand *versus* Control Cymodocea	0.125	18.9
Harbour enlargement (Calero Harbour)		
Global	0.681	0.1
Impact *versus* Influence	0.813	2.5
Impact *versus* Control	0.688	3.7
Influence *versus* Control	0.75	3.7
Brine discharge (Las Burras)		
Global	0.646	0.2
Impact *versus* Influence	0.667	1
Impact *versus* Control	0.926	1
Influence *versus* Control	0.389	3
Brine discharge (Tarajalillo)		
Global	0.28	4.6
Impact *versus* Influence	0.222	16
Impact *versus* Control	0.648	2
Influence *versus* Control	−0.037	56
Thermal central disposal (UNELCO)		
Global	−0.063	65.3
Impact *versus* Influence	−0.5	88.9
Impact *versus* Control	−0.5	88.9
Influence *versus* Control	0.25	44.4

The effect of brine discharge was reflected in Las Burras community composition (Fig. 2c) and ANOSIM detected significant differences among impact, influence and control stations, especially between impact and control stations (Table 2). BOPA showed a significant correlation with changes in community composition (Rho: 0.262, $P < 0.05$), but differences in BOPA seemed related more to changes between sampling campaigns (Fig. 2c).

Similarly, ANOSIM reflected that brine discharge from Tarajalillo desalination plant affected community composition, detecting significant differences between control and impact stations (Table 2, Fig. 2d). BOPA values were also correlated with these changes in community (Rho: 0.319, $P < 0.01$).

ANOSIM did not detect changes in community composition related to thermal central disposal presence (Table 1, Fig. 2e), and RELATE did not detect significant correlations between BOPA values and variability among stations (Rho: −0.104, $P > 0.05$).

ANOVA of BOPA values only detected significant differences related to proximity to perturbance in Calero harbour, where differences for factor location (Table 3) were significant due to the higher BOPA values in St. 1 and 2, especially accentuated during the last three sampling campaigns (Fig. 3b). For Barranco Hondo (fish cages), significant differences were only detected among stations of control locations (Table 3). Differences between impact and control areas were not significant, although higher BOPA values were usually detected in stations beneath fish cages (Fig. 3a).

With respect to Las Burras, significant differences for factor campaign were detected (Table 3) due to the high temporal variability among sampling campaigns (Fig. 3c). Effects of both brine discharges (Las Burras and Tarajalillo) were weakly reflected in the BOPA index, whose higher values was usually obtained in outfall stations. ANOVA detected significant differences in the interaction between campaign and site in Tarajalillo (Table 3), since higher values of BOPA were obtained in St. 1 during the first campaign (Fig. 3d).

Finally, regarding UNELCO (thermal pollution), BOPA values did not correctly respond to the heat water outfall (Fig. 3e). ANOVA detected significant differences in BOPA between impact and influence stations (Table 3) but these differences were due to low index values at impact stations.

Comparing the EQR of BOPA and the other biotic indices, the highest correlation was obtained with AMBI

Table 3. Results of ANOVA for BOPA for the factors: locations (impact, control and influence), station (not present in thermal central disposal) and campaign.

	Source	df	MS	F	F *versus*	P
Fish farm (Bco. Hondo)	Locations	2	0.00016	3.77	Station (Loc)	0.15$^{\text{n.s.}}$
	Loc: Imp. *versus* Con.	1	0.00031	50.76	Control	0.09$^{\text{n.s.}}$
	Loc: Con.	1	0.00001	0.10	St. (Con.)	0.78$^{\text{n.s.}}$
	Station (Loc.)	3	0.00004	2.51	RES (Locations)	0.07$^{\text{n.s.}}$
	Station (Loc: Imp.)	1	0.00001	0.32	RES (Impact)	0.58$^{\text{n.s.}}$
	Station (Loc: Con.)	2	0.00006	4.75	RES (Controls)	<0.01*
	Campaign	5	0.00012	3.26	Camp. × St. (Loc.)	0.03$^{\text{n.s.}}$
	Camp. × Loc.	10	0.00002	0.44	Camp. × St. (Loc.)	0.90$^{\text{n.s.}}$
	Camp. × Loc.: Imp.*versus* Con.	5	0.00002	3.20	Camp. × Con.	0.11$^{\text{n.s.}}$
	Camp. × Loc.: Con.	5	0.00001	0.26	Camp. × St. (Con.)	0.93$^{\text{n.s.}}$
	Camp. × Station (Locations)	15	0.00004	2.12	RES (Location)	0.02$^{\text{n.s.}}$
	Camp. × Station (Loc.: Imp.)	5	0.00005	1.92	RES (Impact)	0.13$^{\text{n.s.}}$
	Camp. × Station (Loc.: Cont.)	10	0.00003	2.33	RES (Control)	0.02$^{\text{n.s.}}$
	RES (Locations)	72	0.00002			
	RES: Impact	24	0.00003			
	RES: Controls	48	0.00001			
Harbour enlargement (Calero Harbour)	Location	2	0.1087	69.9	Stat (Loc.)	<0.01*
	Station (Loc.)	3	0.0016	1.1	RES	0.36$^{\text{n.s.}}$
	Campaign	3	0.0179	6.67	Camp. × Stat. (Loc.)	0.01$^{\text{n.s.}}$
	Loc. × Camp.	6	0.0142	5.29	Camp. × Stat. (Loc.)	0.01$^{\text{n.s.}}$
	Camp. × Stat. (Loc.)	9	0.0027	1.9	RES	0.07$^{\text{n.s.}}$
	RES	48	0.0014			
Brine discharge (Las Burras)	Location	2	0.006	8.25	Stat (Loc.)	0.02$^{\text{n.s.}}$
	Station (Loc.)	6	0.0007	0.52	RES	0.79$^{\text{n.s.}}$
	Campaign	1	0.0099	11.68	Camp. × Stat. (Loc.)	<0.01*
	Loc. × Camp.	2	0.0022	2.61	Camp. × Stat. (Loc.)	0.15$^{\text{n.s.}}$
	Camp. × Stat. (Loc.)	6	0.0008	0.61	RES	0.72$^{\text{n.s.}}$
	RES	36	0.0014			
Brine discharge (Tarajalillo)	Location	2	0.0082	2.55	Stat (Loc.)	0.16$^{\text{n.s.}}$
	Station (Loc.)	6	0.0032	7.64	RES	<0.0005***
	Campaign	1	0.0004	0.11	Camp. × Stat. (Loc.)	0.75$^{\text{n.s.}}$
	Loc. × Camp.	2	0.0008	0.22	Camp. × Stat. (Loc.)	0.81$^{\text{n.s.}}$
	Camp. × Stat. (Loc.)	6	0.0036	8.62	RES	<0.0005***
	RES	36	0.0004			
Thermal central disposal (UNELCO)	Impact *versus* Influence					
	Locations: Imp.& Inf.	2	0.0054	3.67	RES	0.03$^{\text{n.s.}}$
	Loc: Imp. *versus* Inf.	1	0.0105	7.06	RES	<0.01*
	Loc: Influence	1	0.0004	0.27	RES	0.61$^{\text{n.s.}}$
	Campaign	1	0.0010	0.68	RES	0.41$^{\text{n.s.}}$
	Camp × Loc.: Imp.& Inf.	2	0.0018	1.24	RES	0.30$^{\text{n.s.}}$
	Camp × Loc.: Imp. *versus* Inf.	1	0.0020	1.37	RES	0.25$^{\text{n.s.}}$
	Camp: Inf.	1	0.0016	1.10	RES	0.30$^{\text{n.s.}}$
	RES	64	0.0015			
	Impact *versus* Control					
	Locations: Imp.& Con.	2	0.0003	0.83	RES	0.44
	Loc: Imp. *versus* Con.	1	0.0006	1.61	RES	0.21
	Loc: Control	1	0.0000	0.03	RES	0.85
	Campaign	1	0.0014	3.59	RES	0.06
	Camp × Loc.: Cont.& Inf.	2	0.0012	3.26	RES	0.05
	Camp × Loc.: Cont. *versus* Inf.	1	0.0021	5.42	RES	0.02
	Camp × Loc.: Cont.	1	0.0004	0.83	RES	0.37
	RES	54	0.0004			

df, degrees of freedom; MS, medium squares; F of each factor = MS factor/F. Levels of significance: n.s., no significant difference; *P < 0.01; **P < 0.001, ***P < 0.0005.

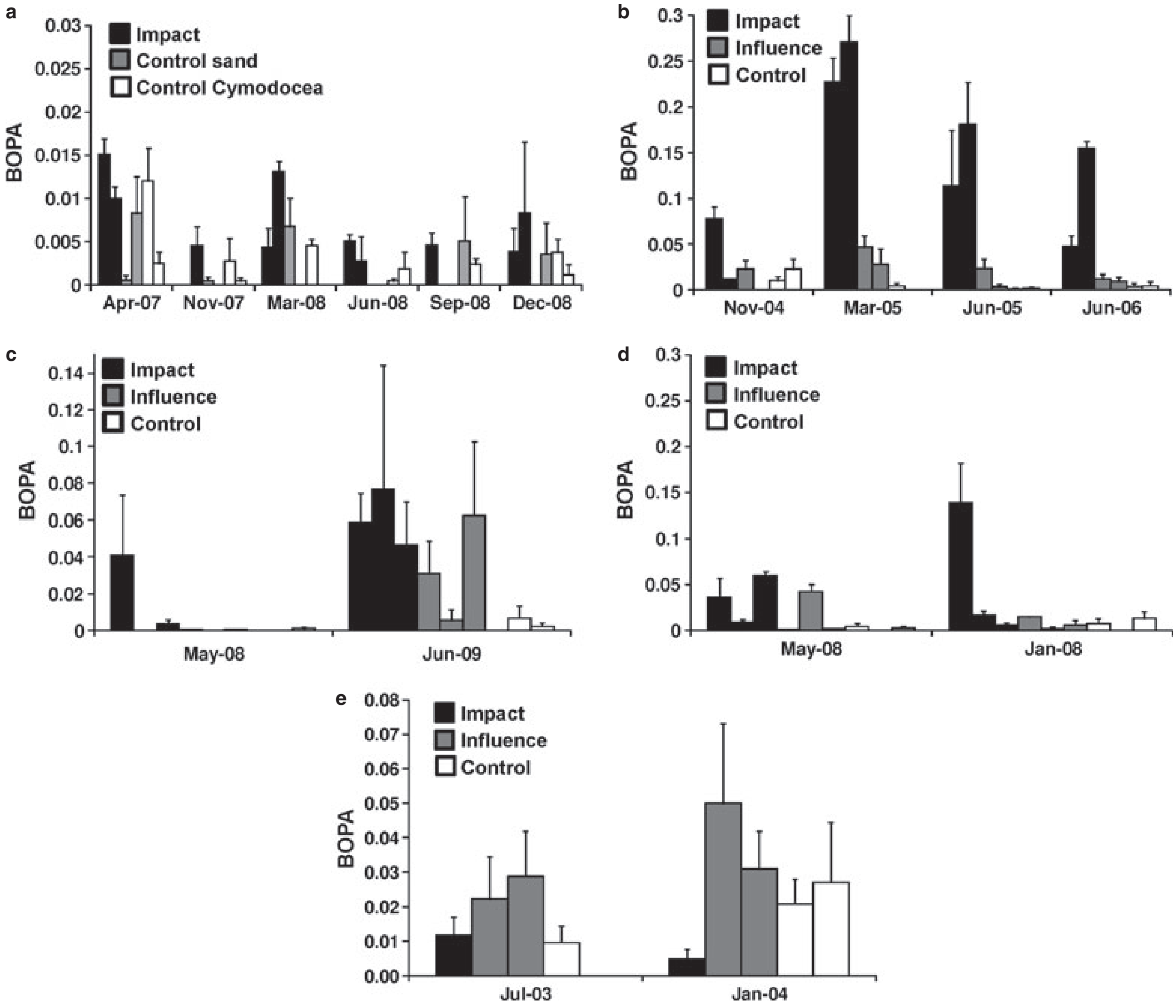

Fig. 3. Mean and standard errors of the BOPA index for each station in each study area. (a) Barranco Hondo fish cages. (b) Calero Harbour. (c) Las Burras desalination plant. (d) Tarajalillo plant (brine + sewage). (e) UNELCO (Central Electric Power). Bar color indicates location related to distance to impact (black: impact, gray: influence and white: control), except in the case of Barranco Hondo fish cages where gray: control sand and White: control *Cymodocea nodosa.*

(Table 4). Among areas, the highest correlation was obtained with AMBI in Calero Harbour area, where the BENTIX and Shannon indices also showed a significantly strong correlation with BOPA. Lower and non-significant correlations were obtained in fish cages in such a way that a significant correlation (negative) was only obtained between the BOPA and Shannon diversity index. AMBI obtained weak significant correlations in areas affected by brine discharge and thermal disposal, with the Shannon index showing lower or negative correlation values. Higher and significant correlation values were obtained with BENTIX in the Tarajalillo and UNELCO areas, but the correlation between BOPA and BENTIX was not significant in the Las Burras area.

Despite these correlations, most of the cases showed a very low or null concordance (Table 4). Good levels of concordance were only obtained in the case of harbour enlargement with the Shannon diversity index and AMBI.

With respect to the percentage of each ecological status derived from BOPA (Fig. 4), non-acceptable status (moderate, poor or bad) was only found in stations affected by harbour enlargement (52%) and in one station affected by brine discharge from both the Las Burras and Tarajalillo areas. Most of the impacted stations from the other areas were classified as having a good or high status by BOPA.

Regarding the other biotic indices, in the area affected by fish cages (Fig. 4a), AMBI classified most of the sta-

Table 4. N (number of pair of data), Pearson correlation coefficient and significance level (values in bold indicate significant correlation; $P \leq 0.05$), Kappa values and levels of agreement between Ecological Quality Ratio of BOPA with Shannon Diversity Index, AMBI and BENTIX.

	N	Pearson coefficient^{p}	Kappa value	Level of agreement
Shannon diversity index				
Global	388	$\mathbf{0.13^{0.012}}$	0.08	Very low
Fish farm (Bco. Hondo)	108	$\mathbf{-0.41^{0.0001}}$	0.00	Null
Harbour enlargement (Calero Harbour)	72	$\mathbf{0.72^{0.0001}}$	0.64	Good
Brine discharge (Las Burras)	54	$\mathbf{-0.31^{0.022}}$	−0.10	Null
Brine discharge (Tarajalillo)	54	$0.14^{0.305}$	0.14	Very low
Thermal central disposal (UNELCO)	100	$0.10^{0.305}$	0.09	Very low
AMBI				
Global	388	$\mathbf{0.41^{<0.0001}}$	0.22	Low
Fish farm (Bco. Hondo)	108	$0.14^{0.152}$	0.01	Null
Harbour enlargement (Calero Harbour)	72	$\mathbf{0.82^{<0.0001}}$	0.68	Good
Brine discharge (Las Burras)	54	$\mathbf{0.32^{0.024}}$	0.09	Very low
Brine discharge (Tarajalillo)	54	$\mathbf{0.36^{0.007}}$	0.12	Very low
Thermal central disposal (UNELCO)	100	$\mathbf{0.35^{0.000}}$	0.05	Very low
BENTIX				
Global	388	$\mathbf{0.21^{<0.0001}}$	0.05	Null
Fish farm (Bco. Hondo)	108	$-0.14^{0.138}$	0.00	Null
Harbour enlargement (Calero Harbour)	72	$\mathbf{0.59^{<0.0001}}$	0.54	Moderate
Brine discharge (Las Burras)	54	$0.09^{0.526}$	0.01	Null
Brine discharge (Tarajalillo)	54	$\mathbf{0.52^{<0.0001}}$	0.02	Null
Thermal central disposal (UNELCO)	100	$\mathbf{0.44^{<0.0001}}$	0.20	Low

tions as having a good or high status, whereas the Shannon diversity index and BENTIX classified more than 50% of stations as having a non-acceptable status and both indices assigned a higher percentage of moderate, poor and bad status to *Cymodocea nodosa* control stations. With respect to Calero Harbour (harbour enlargement), all indices classified impacted stations as having a lower ecological status. The Shannon diversity index and BENTIX were stricter, since they produced lower percentages of acceptable conditions of impacted stations; however, both indices also classified some control stations as having a non-acceptable status.

In the area affected by brine discharge (Las Burras) (Fig. 4c), the Shannon index classified a high percentage of control stations as having a non-acceptable status, whereas impacted stations were classified as having a high and good status. AMBI gave similar percentage of non-acceptable status to control and impacted stations, whereas BENTIX assigned high percentages of non-acceptable status to all locations independently of the presence of brine discharge. For the other area affected by brine discharge, Tarajalillo, both the Shannon index and BENTIX assigned a worse ecological status to impacted stations; however, as occurred in Las Burras, BENTIX also assigned a high percentage of non-acceptable status to control stations. AMBI assigned most of stations of this area, a good or high status. Finally, the effect of thermal central disposal was not reflected in the ecological status classification; the Shannon diversity index assigned a higher percentage of acceptable status to impacted stations, AMBI classified most of the stations as having a good or high status independently of the presence of disposal, and BENTIX assigned a higher percentage of non-acceptable status to influence stations but not to impact stations.

Discussion

The BOPA index only registered clearly the impact produced by harbour enlargement works, increasing significantly in the inner part of the harbour. BOPA assigned a non-acceptable ecological status to impacted stations, since the start of enlargement works in Calero Harbour. The effectiveness of BOPA has been reported previously in different situations, distinguishing the presence of hydrocarbons (Gómez-Gesteira & Dauvin 2000; Dauvin & Ruellet 2007; Joydas *et al.* 2011), oyster culture areas (Bouchet & Sauriau 2008), harbours (Ingole *et al.* 2009) and monitoring sewage outfall impacts (de-la-Ossa-Carretero *et al.* 2009). In the enlargement works of Calero harbour, chronic pollution (PAHs, hydrocarbons and heavy metals) is significant (Riera *et al.* 2011a). However, other environmental stressors (*e.g.* turbidity, oxygen depletion and sediment load) could explain differences in the BOPA index in Calero harbour, since concentrations of hydrocarbons were lower than levels considered to produce adverse biological effects in marine assemblages (*e.g.* Long *et al.* 1995).

Other impacts such as brine discharge or fish cages were only weakly registered by an increase of BOPA values in impact stations with respect to control stations, the index response not being significant. In the case of Barranco Hondo, changes of benthic community composition are highly related to habitat, since most changes were detected in vegetated areas inhabited by *Cymodocea nodosa*. BOPA values from previously published papers seemed to range according to type of seabed or benthic community. Actually, the BOPA index responded

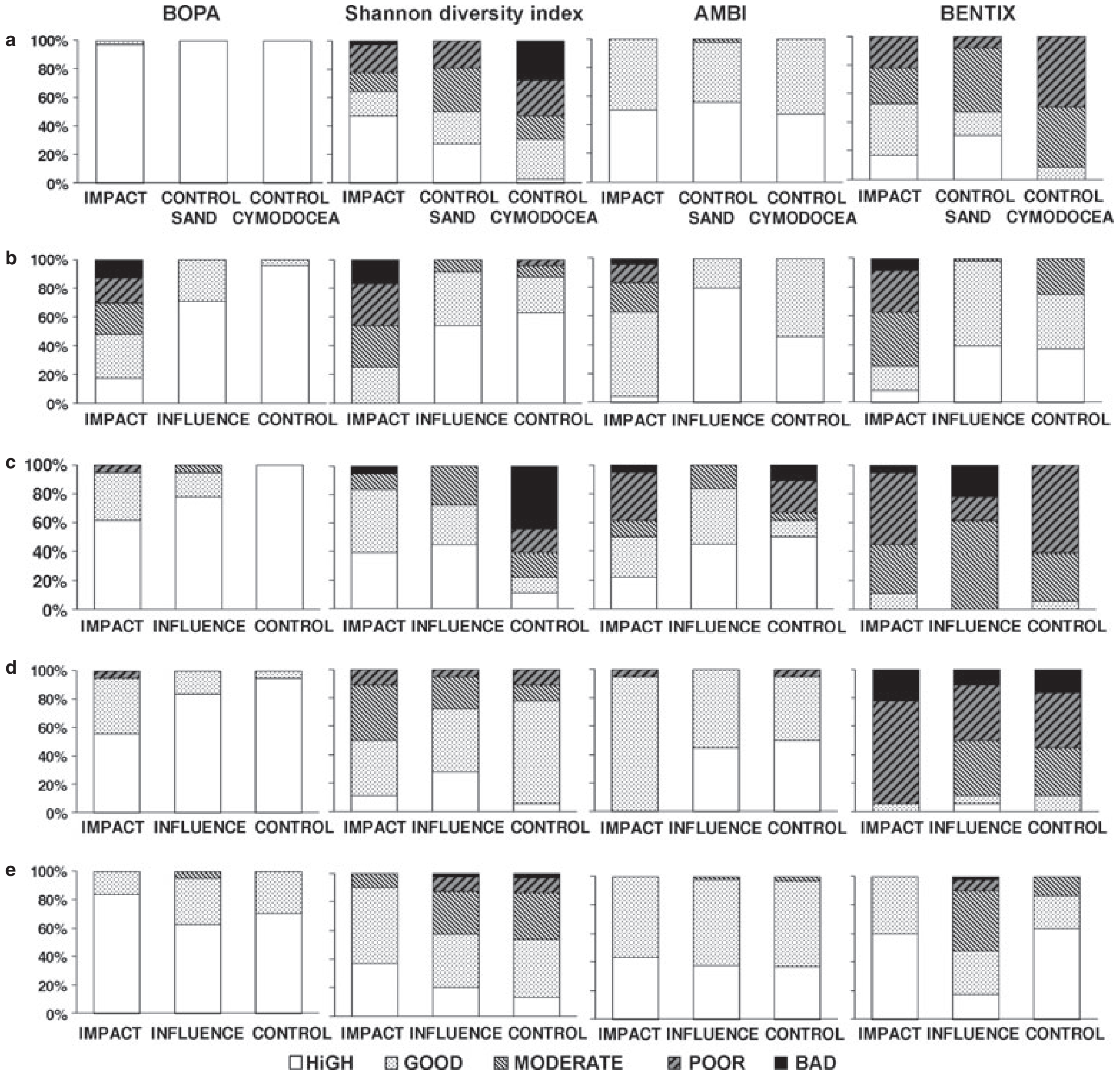

Fig. 4. Percentage of each ecological status derived from BOPA, Shannon Diversity Index, AMBI and BENTIX. (a) Barranco Hondo fish cages. (b) Calero Harbour. (c) Las Burras desalination plant. (d) Tarajalillo plant (brine + sewage). (e) UNELCO (Central Electric Power).

correctly when a similar community was studied (*e.g.* Bakalem *et al.* 2009; de-la-Ossa-Carretero *et al.* 2009). However, Munari & Mistri (2008) detected discrepancies in BOPA response due to macroalgae presence in Adriatic transitional waters. Changes in BOPA values were detected in vegetated areas inhabited by *Cymodocea nodosa* meadows with respect to sandy unvegetated seabeds. Macrofauna assemblages from *C. nodosa* meadows harbour higher species richness and abundances compared with adjacent non-vegetated substrates (Brito *et al.* 2005), thus the differences found in the BOPA index could be due to macrofaunal assemblage structure.

With respect to areas affected by brine discharges, both Las Burras and Tarajalillo showed changes in benthic community composition due to disposal presence, although differences among campaigns were reflected strongly in the community of both areas. It could be that the BOPA index did not respond significantly to these impacts because BOPA values were strongly affected by temporal variation. Seasonal variability on grain size composition, due to resuspension during swell periods in winter, masked brine effects on benthic fauna (Riera *et al.* 2012), suggesting that the BOPA index could not discern disposal impact in the sampling campaigns.

Finally, community composition was not affected by electric central disposal (UNELCO) and therefore BOPA did not detect any effects of thermal pollution. Changes in grain size composition seemed to explain BOPA values, rather than any other impact (temperature increases) (Riera *et al.* 2011a,b). These sedimentary shifts are triggered by the continuous sediment resuspension from hot water flow of the turbines (UNELCO). Moreover, thermal effluents are spatially limited and instantaneous water interchange occurs at the outfall (Riera *et al.* 2011b).

The opportunistic polychaeta list established for BOPA is based on the tolerance level to organic matter enrichment (Dauvin & Ruellet 2007); brine discharges and thermal pollution would have different effects and species sensitive to organic pollution could be tolerant to these kinds of pollution or *vice versa* (Del-Pilar-Ruso *et al.* 2008). In this way, it may be necessary to modify the opportunistic species considered, depending on pollution source.

Compared with the other biotic indices, BOPA only showed a good agreement with the Shannon diversity index and AMBI in the case of harbour enlargement, where all the indices seemed to respond to the impact. For the remaining areas, BOPA did not agree with the other indices. However, those indices did not seem to respond correctly to the other impacts, being affected by habitat (Shannon diversity index and BENTIX in the fish cage area), establishing higher percentages of non-acceptable status to control stations (*e.g.* Shannon diversity index in Las Burras desalination plant), giving similar percentages of non-acceptable status to control and impacted stations (*e.g.* AMBI in Las Burras) or assigning high percentages of non-acceptable status to all locations independently of the impact (BENTIX in Las Burras and Tarajalillo desalination plants).

The accuracy of a biological index on differing spatial spaces and under differing benthic conditions is a difficult task to obtain, and the BOPA index could respond to characteristics of the environment or habitat rather than to anthropogenic pressures. Moreover, the Canary Islands are exposed to the continuous Canary Current, with a mean velocity of 10–30 $\text{cm}\cdot\text{s}^{-1}$ throughout the year (Batten *et al.* 2000). Thus, point-source environmental perturbations (*e.g.* sewage, cages, etc.) could be dispersed by the current; no highly polluted coastal areas are found in the Canary archipelago. At first instance, the accuracy level of the BOPA index is similar to levels obtained by other indices. These approaches could be preferable in monitoring environmental studies that need a first approach to the ecological status of macrofauna assemblages. As stated above, the BOPA index is less time-consuming and can be used by consultants without a background on macrofaunal identification.

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