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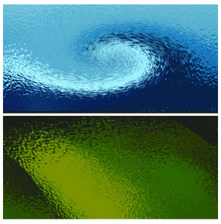

RESEARCH ARTICLE

A Comparison of Two Biotic Indices, AMBI and BOPA/BO2A, for assessing the Ecological Quality Status (EcoQS) of Benthic Macro-invertebrates.

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Abstract

1 - The assessment of quality status of Transitional Aquatic Ecosystems remains a challenge for the ecologists.

2 - Here, we compared the results of two common indices (AMBI - Ecological Groups and BOPA/BO2A -Taxonomic Sufficiency) from samples coming from the north-eastern Atlantic and Mediterranean Sea. Both biotic indices rely on distinct assessments of species sensitivity/tolerance.

3 - Six studies provided the raw data that permitted AMBI and BOPA to be compared. A total 922 data element was available, most of them from the Seine estuary (78%). The database was later divided into three sub-sets: French Atlantic Transitional Waters, Mediterranean Coastal Waters and Mediterranean Lagoons.

4 - Both indices' values demonstrated a strong correlation; however, the BOPA index had a tendency to overestimate the EcoQS compared to the values obtained from AMBI index, mainly due to discrepancies between ‘high’ and ‘good’ quality.

5 - New thresholds for BOPA/BO2A index are proposed in order to reduce this overestimation.

Introduction

In order to achieve the objectives of the European Water Framework Directive (WFD), common definitions for water quality status needed to be established. Benthic macro-invertebrates 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 (Borja *et al*., 2000; Dauvin *et al*., 2007). Thus, the composition and abundance of benthic macro-invertebrate fauna is one of the elements proposed in the WFD for assessing quality and for determining ecological status.

Ecological indicators are employed with the aim of supplying synoptic information about the state of ecosystems (Salas *et al*, 2006). In response to WFD implementation in coastal aquatic ecosystems, several indices were developed to infer environmental status from the assessment of benthic community condition: AMBI (Borja *et al*., 2000; Borja and Muxika, 2005), BENTIX (Simboura and Zenetos, 2002), BQI (Rosenberg *et al*., 2004), BOPA (Dauvin and Ruellet, 2007), M-AMBI (Muxika *et al*., 2007) and BO2A (Dauvin and

Ruellet, 2009). These indices summarise the ecological status or the ecological quality of a water body and allow the results to be easily interpreted. However, because all organisms are not equally sensitive to all types of anthropogenic disturbances and are thus likely to respond differently to different types of perturbations, one biotic index is unlikely to be universally applicable (Dauvin *et al*., 2007; Pranovi *et al*., 2007; Afli *et al*., 2008; Grémare *et al*., 2009).

Following an initial study that showed the effectiveness of an opportunistic polychaeta/amphipod ratio for identifying oil spill events (Gomez-Gesteira and Dauvin, 2000), the BOPA index was created, modifying this ratio to allow estuarine and coastal communities to be divided into the five classes suggested by the European directive (Dauvin and Ruellet, 2007): 'high' for unpolluted sites, 'good' for slightly polluted sites, 'moderate' for moderately polluted sites, 'poor' for heavily polluted sites and 'bad' for extremely polluted or azoic sites. This BOPA index was originally calibrated using the AMBI index. It respects two main principles: 1) the taxonomic sufficiency principle, and 2) the principle of antagonism between sensitive species and opportunistic species. Opportunistic polychaetes are known to be resistant to, indifferent to or favoured by organically enriched sedimentary matter, whereas amphipods form an abundant and ecologically-important zoological group that is more highly sensitive to contaminated sediments than other benthic macro-invertebrates (Dauvin and Ruellet, 2009). [NB: Some amphipod species of the genus *Jassa* Leach (Corophioidea: ischyroceridae) are not counted as sensitive species because they are part of the EG IV on the AZTI list (www.azti.es)].

BOPA uses frequency data and the proportion of organisms in each category (Pinto *et al*., 2009). The main advantages of the BOPA index are its independence from sampling protocols using several meshes sizes and several surface units for expressing abundances. An additional advantage is the reduced need for taxonomic knowledge. After the initial BOPA proposition, Dauvin and Ruellet (2009) proposed adding Clittellata (i.e., Hirudinea and Oligochaeta) to the opportunistic polychaeta in order to adapt BOPA index for application in the freshwater sectors of transitional waters, thus creating the Benthic Opportunistic Annelida Amphipods index (BO2A). In coastal sediment, as the Clitellata are generally absent or weakly represented, the BO2A and BOPA values are strictly or very similar.

The BOPA index has been applied in different situations, thus showing its effectiveness at distinguishing the presence of hydrocarbons (Gomez-Gesteira and Dauvin, 2000; Dauvin and Ruellet, 2007) and sewage discharges (de-la-Ossa-Carretero *et al*., 2009) in certain zones, such as oyster culture areas (Bouchet and Sauriau, 2008) or harbours (Ingole *et al*., 2009). However, this effectiveness is limited by an apparent overestimation of the ecological quality status (EcoQS) compared to the EcoQS from other indices.

In order for the various indices to be successfully implemented, they must be intercalibrated to make them comparables, and this intercalibration must identify the level of agreement between methodologies (Borja *et al*., 2007). For this reason, the EcoQS thresholds of the indices must be modified in order to produce the same, or at least similar, results for the assessment of the same area (Ruellet and Dauvin, 2007). The main objective of this paper is to correctly intercalibrate the BOPA/BO2A EcoQS classifications with the AMBI classifications. In this paper, we first review the results in the literature for ecological quality status (EcoQS) obtained with the BOPA index. Then, we propose new thresholds in order to adapt the EcoQS results produced by BOPA/BO2A for use in benthic coastal and transitional waters.

Materials and methods

In November 2009, we reviewed a total of 10 papers in which the BOPA index was employed in sites in the Atlantic Ocean and the Mediterranean Sea (Table 1 provides a list of the papers reviewed).

In these papers, both the BOPA index and the AMBI index were usually used to identify the ecological quality status (EcoQS) of macrobenthic communities. It was not always possible to have access to the raw data. Nevertheless, we managed to obtain the data for eight papers, which gave us a total of 1,093 values for both the BOPA and AMBI indices.

But 171 values corresponded to null BOPA values, i.e. BOPA = 0 when there were no opportunistic annelida or polychaeta or no sensitive amphipod species. After having excluded the null BOPA values, the remaining values which are taken into account in our comparison are 922 (Table 2). The data extracted from the MABES database (Seine Estuary MAcroBenthos, available via the data administrator of the GIP Seine Aval: nbacq@seine-aval.fr) made up 78% of the data (Table 2). Indices values were divided into three subsets according to where the samples on which the values were calculated were taken: French Atlantic transitional waters, Mediterranean lagoons and Mediterranean coastal waters.

Table 1 - List of articles in which the BOPA index was employed

Paper	Site	Applied Indices
Afli et al., 2008	Bizerte Lagoon, Bay of Tunis, Dkhila Coast (Tunisian Coast)	BOPA, AMBI, BENTIX, ITI
Bakalem et al., 2009	Bays of Fetzara, Jijel, Bejaia, Alger, Bou Ismail, Arzew & Oran (Algerian Coast)	BOPA, AMBI, M-AMBI, BENTIX, H', ITI
Blanchet et al., 2008	Marennes-Olèron Bay, Arcachon Bay, Seine Estuary (Western French Coast)	BOPA, AMBI, BENTIX, BQI, H'
Bouchet and Sauriau, 2008	Pertuis Charentais (Western French Coast)	BOPA, AMBI, BENTIX, H'
Dauvin et al., 2007	Seine Estuary (Western French Coast)	BOPA, AMBI, BQI
de-la-Ossa-Carretero et al., 2009	Castellon Coast (Eastern Spanish Coast)	BOPA
Munari and Mistri, 2007	Orbetello, Padrogiano, Tortolì, San Teodoro, (Tyrrhenian lagoons)	BOPA, AMBI, FINE
Munari and Mistri, 2008	Venice, Scardovari, Goro, Gorino, Comacchio, Lesina, Oran (Adriatic coastal lagoons)	BOPA, AMBI, H', FINE
Pravoni et al., 2007	Venice Lagoon	BOPA, AMBI, M-AMBI, BENTIX, H'
Lavesque et al., 2009	Arcachon Bay (Western French Coast)	BOPA, AMBI

Table 2 - Number of available BOPA data elements in the different data sets, the number of null values (BOPA= 0, when there were no opportunistic polychaetes in the samples that had at least 20 individuals), and the identification of marine sub-section concerned (N= numbers).

	N data	N null values	N of retained values in the analysis	Marine sub-section concerned
MABES Dauvin et al., 2007; Dauvin & Ruellet, 2007, 2009	864	142	722	French Atlantic transitional waters
Blanchet et al., 2008	24	6	18	French Atlantic transitional waters
Bouchet and Sauriau, 2008	15	0	15	French Atlantic transitional waters
Lavesque et al., 2009	12	0	12	French Atlantic transitional waters
Bakalem et al., 2009	101	5	96	Mediterranean coastal waters
Afli et al., 2008	31	12	19	Mediterranean coastal waters Mediterranean lagoons
Munari and Mistri, 2007	30	4	26	Mediterranean lagoons
Pravoni et al., 2007	16	2	14	Mediterranean lagoons
Total	1093	171	922	

First, we determined the degree of correlation between values for both indices using Pearson coefficient for total area and each area sub-section. The significance of the correlation was set at 0.01. Then, we looked at the EcoQS classifications recorded for each site for both indices. These classifications were ranked from 1 to 5, from ‘high’ status to ‘bad’ status, giving us a numerical EcoQS value. We compared these values using the ratio, EcoQS (BOPA)/EcoQS (AMBI), to validate the EcoQS classifications of both indices. When this ratio is <1, BOPA had overestimated the EcoQS compared to AMBI; when this ratio is >1, BOPA had underestimated the EcoQS compared to AMBI. The frequency distribution of the BOPA and AMBI EcoQS values obtained from the compiled papers were plotted in order to obtain an overview of the probability of belonging to each EcoQS category for the BOPA and AMBI indices.

Linear regression was initially considered to calculate new BOPA thresholds values. However, while AMBI follows the normal law, BOPA follows an exponential law (Ruellet and Dauvin, 2007), and for that reason, it is not possible to use linear regression. Thus, the new BOPA thresholds were calculated from the AMBI thresholds using non-parametric regression through a pair wise comparison of both indices. Non-parametric regression can be used when the hypotheses of the more traditional regression methods cannot be verified or when the main interest is the predictive quality of the model and not its structure (Härdle, 1992).

Finally, new threshold values were applied to database, and the percentages of each EcoQS were calculated. Weighted Kappa analysis (Cohen, 1960; Landis and Kosch, 1977) was used to analyse the agreement between the indices for both the previous BOPA thresholds and the new thresholds proposed in this paper. The methodology proposed by Borja *et al*. (2007) was employed. The equivalence table from Monserud and 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 and Cohen, 1973) to the analysis to decrease importance of misclassification between close categories and increase importance between distant categories. The percentage of correspondence was also calculated for each threshold system.

Results

The Pearson coefficient showed a significant strong positive correlation between both indices in each area sub-section (Table 3). The highest value of Pearson coefficient was obtained for Mediterranean coastal waters, while the lowest value was obtained for Mediterranean lagoons.

Despite this correlation, the BOPA index had a general tendency to overestimate EcoQS compared to the classification obtained from AMBI index (Figure 1). The BOPA provided better EcoQS for 81 % of the sites. It underlined great differences in the Tyrrhenian and the Adriatic lagoons, and several sites on the Pertuis Charentais, Tunisian Coast and Algerian coast (Bejaia) had similar or worse EcoQS than the values provided by AMBI.

Our analysis of the frequency distribution of the BOPA values (Figure 2) highlighted a tendency to classify sites as "high": 54 % of the samples were classified as "high", whereas only 13.3 % were classified as "moderate", "poor" or "bad". On the other hand, the AMBI index (Figure 3) tended to classify sites as "good" (70.6 %), with only 10.3 % being classified as "moderate", "poor" or "bad". Given these results, it would seem that overestimation of the BOPA index was caused by the thresholds between high and good status.

Using the values predicted for the new thresholds with non-parametric regression (Figure 4) demonstrated that the greatest change occurred in the limit between "high" and "good", whereas the other limits scarcely changed. Consequently, though the limits between "good and moderate" and "poor and bad" decreased slightly, the limit between "moderate and poor" got slightly bigger (Table 4).

Table 3 - Pearson coefficient values and significance level for the BOPA and AMBI indices for the total area and each area sub-section.

BOPA / AMBI	Pearson coeficient	Sig. level	N
Total	0.707	0.000	922
Atlantic transitional waters	0.701	0.000	767
Mediterranean coastal waters	0.878	0.000	110
Mediterranean lagoons	0.605	0.000	45

Table 4. BOPA thresholds from Dauvin and Ruellet (2007) and the new BOPA thresholds proposed.

	Thresholds from Dauvin and Ruellet (2007)	**New thresholds proposed**
High-Good	0.04576	0.02452
Good-moderate	0.13966	0.13002
Moderate-bad	0.19382	0.19884
Poor-bad	0.26761	0.25512

By applying these new thresholds, we obtained changes in the percentages of each EcoQS category (Figure 5).

Using these new thresholds, we obtained more similar EcoQS category frequencies for AMBI and BOPA. The percentage of samples classified as "high" was reduced to 35.36 %, while the percentage of "good" samples increased to 48.7 %. Still, differences between the classifications resulting from both indices remain, especially for Atlantic transitional waters and for Mediterranean lagoons, where AMBI continues to show higher percentages of good EcoQS. On the other hand, similar percentages of EcoQS categories for both indices were found for Mediterranean coastal waters. The results of Kappa agreement analysis for both indices (Table 5) indicate an increase in the Kappa values, except for Mediterranean lagoons, and increase in the percentage of matching using the new thresholds proposed. The increase in matching was around 10 % for the coincidence classifications for the total values.

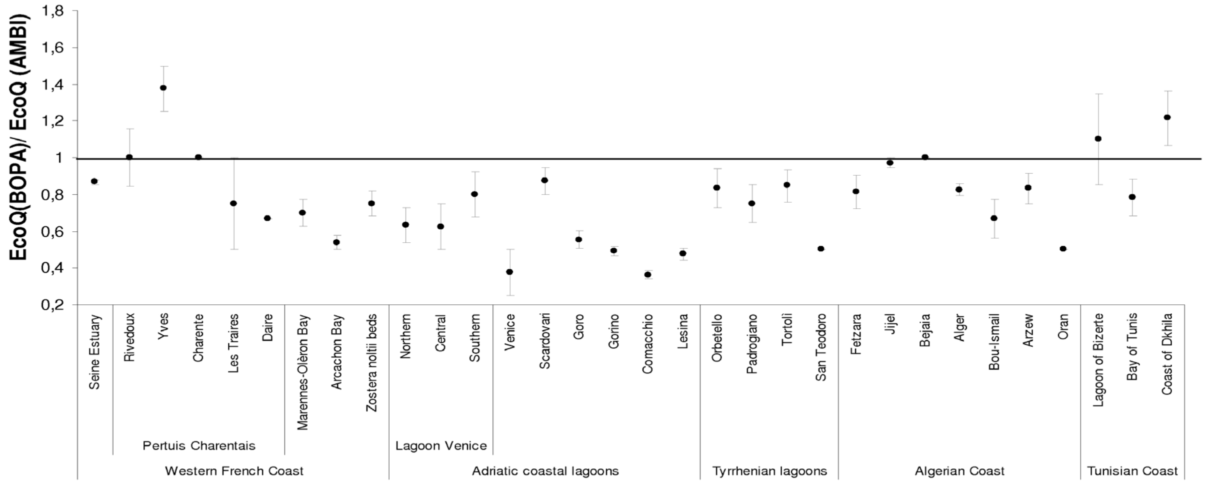
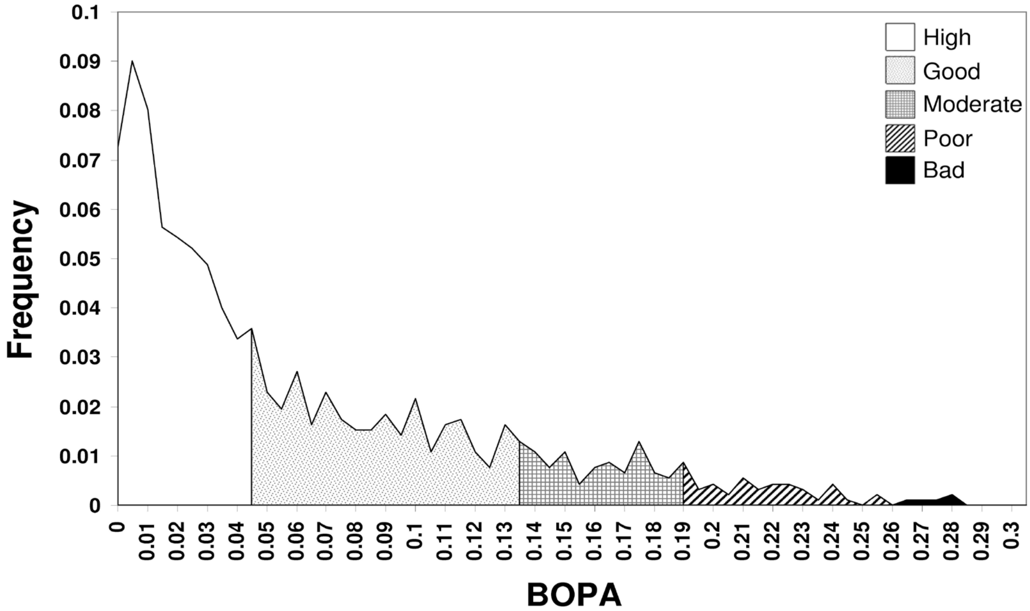
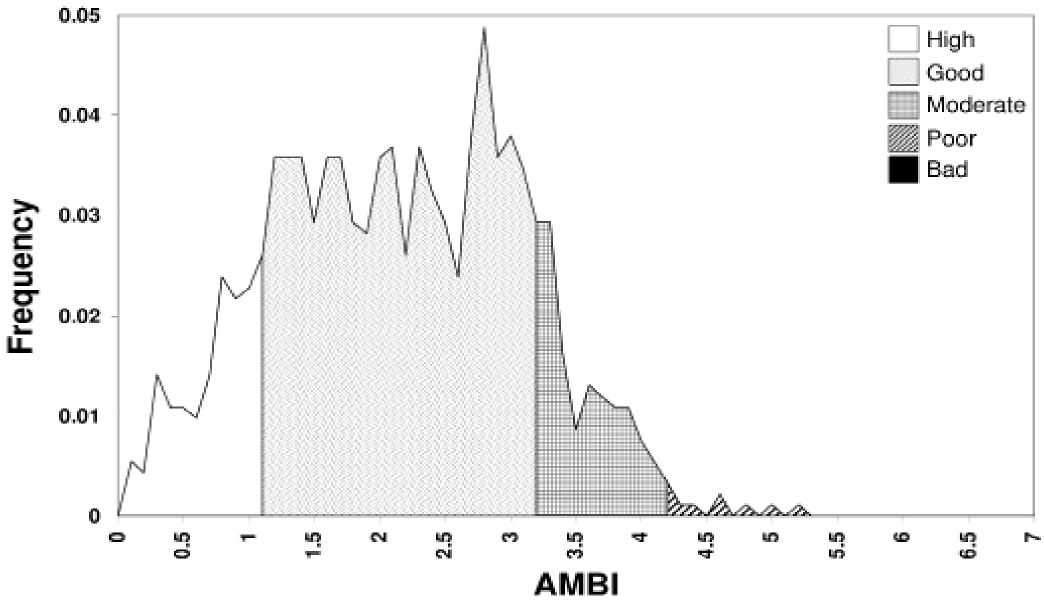


Figure 1. Mean and standard error of the ratio of both indices' EcoQS classifications [EcoQS (BOPA)/ EcoQS (AMBI)]. When this ratio is <1, BOPA has overestimated the EcoQS compared to AMBI; when this ratio is >1, BOPA has underestimated the EcoQS compared to AMBI.



Fifure 2. Frequency distribution of BOPA values. Thresholds taken from Dauvin and Ruellet (2007).



Fifure 3. Frequency distribution of AMBI values. Thresholds taken from Borja *et al*. (2000).

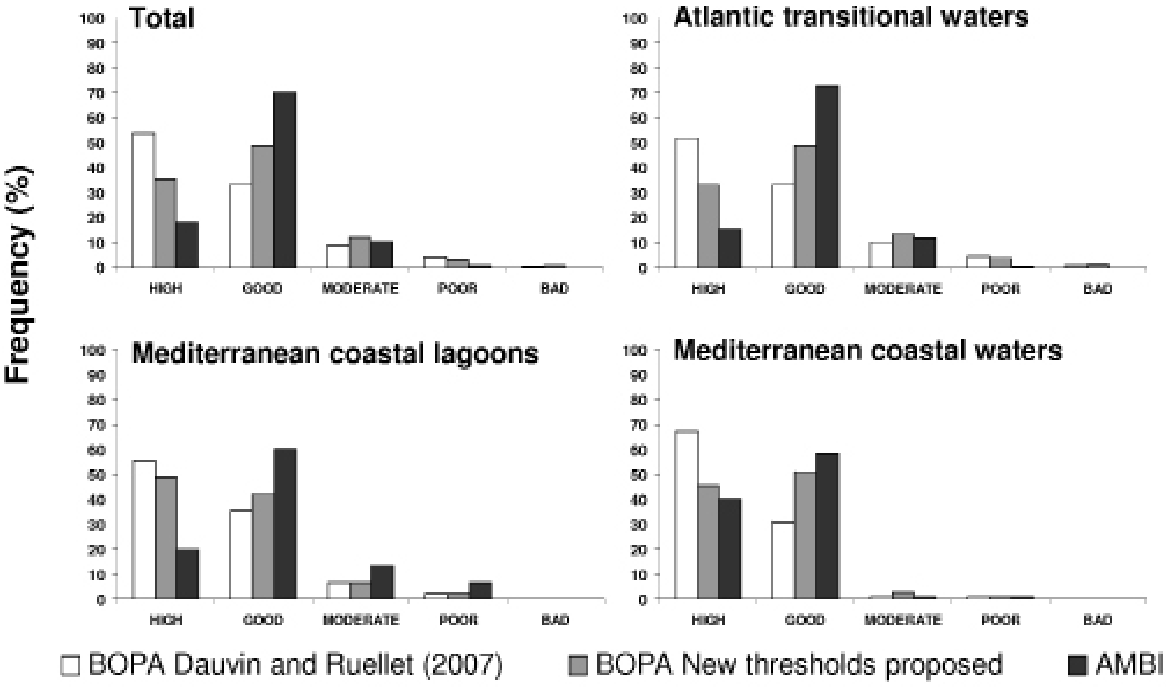


Figure 4. Percentage of each Ecological Quality Status (EcoQS) derived from BOPA [thresholds from Dauvin and Ruellet (2007) and this article] and AMBI for the total area and each area sub-section.

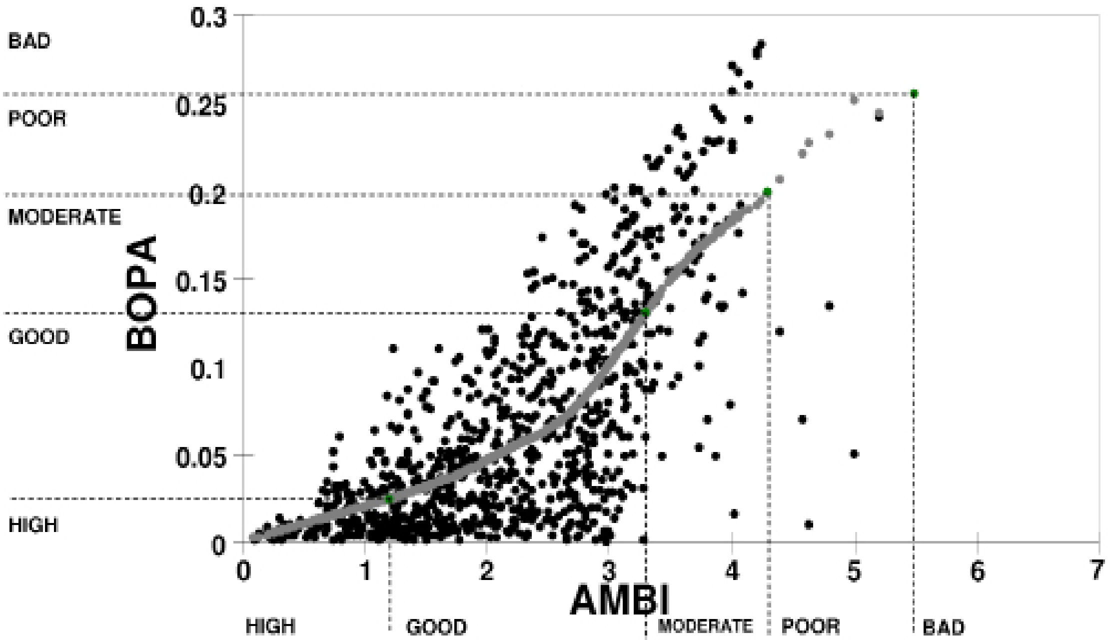


Figure 5.Non-parametric regression (grey points) between the BOPA and AMBI values (black points). New thresholds for the BOPA index were predicted based on the AMBI thresholds (Borja et al., 2000).

Table 5 - Kappa values, levels of agreement (based on Monserud and Leemans, 1992), and percentage of correspondence (% matching) between AMBI and BOPA were calculated for the thresholds proposed by Dauvin and Ruellet (2007) and new thresholds proposed in this paper for total area and each area sub-section.

BOPA / AMBI	Kappa analysis		% matching
	Kappa value	Level of agreement	
Total			
Thresholds proposed by Dauvin and Ruellet (2007)	0.56	Good	50.54%
New thresholds proposed in this paper	0.59	Good	61.28%
Atlantic transitional waters			
Thresholds proposed by Dauvin and Ruellet (2007)	0.56	Good	47. 98%
New thresholds proposed in this paper	0.59	Good	59.19%
Mediterranean lagoons			
Thresholds proposed by Dauvin and Ruellet (2007)	0.38	Low	48.89%
New thresholds proposed in this paper	0.37	Low	51.11%
Mediterranean coast			
Thresholds proposed by Dauvin and Ruellet (2007)	0.74	Very good	69.09%
New thresholds proposed in this paper	0.81	Very good	80%

The Kappa values indicate that the agreement between the two indices remained similar, except for Mediterranean coastal waters where the increase was greater. Despite obtaining higher matching percentages with the new thresholds proposed, Mediterranean lagoons had lower Kappa values due to an increase of misclassifications in the "high" EcoQS category.

Discussion

In spite of its initially demonstrated effectiveness for identifying polluted areas (Gomez-Gesteira and Dauvin, 2000; Dauvin and Ruellet, 2007; Bouchet and Sauriau, 2008, de-la-Ossa-Carretero *et al.*, 2009; Ingole *et al.*, 2009), the BOPA index appears to regularly overestimate the EcoQS values compared to AMBI index, ranking the sites in the same order but disagreeing on the precise EcoQS level for each site. AMBI and BOPA don't use the same model of sensitivity/tolerance to pollution. The BOPA index (Dauvin and Ruellet, 2007) uses an opportunistic polychaete/amphipod ratio (Gomez-Gesteira and Dauvin, 2000), then the BO2A uses an opportunistic annelida/ amphipod ratio, while AMBI (Borja *et al.*, 2000) uses a theoretical model in which the various species are divided into five ecological groups (EG) according to their sensitivity to organic pollution. However, the values for both BOPA and AMBI indices showed high correlations in the various sub-sections, especially in Mediterranean coastal waters. The fact that most of the amphipods, with the exception of one genus (*Jassa*), belong to EG1, and all the opportunistic polychaetes belong to EG4 and EG5 (see www.azti.es) could justify the variability of AMBI compared to BOPA. In other words, the high correlation between BOPA and

AMBI could be due to the fact that both indices classify the taxa in the same way: opportunistic polychaetes (BOPA group) belong to EG4 and EG5 (AMBI group), and most of amphipods (BOPA group) belong to EG1 (AMBI group).

Despite this high correlation and the fact that the BOPA index was calibrated using the AMBI thresholds (Dauvin and Ruellet, 2007), the BOPA index overestimated the EcoQS, mainly because it classified most of the samples as "high" EcoQS while AMBI classified them as "good". The two indices had different frequency distributions (Ruellet and Dauvin, 2007) due to the laws that the indices follow: BOPA follows an exponential law, which tends to produce low values and thus "high" EcoQS, and AMBI follows a normal law, which tends to produce higher values and thus "good" EcoQS. Comparing and inter-calibrating these indices for a large number of sites could probably overcome this problem (Dauvin *et al*., 2007; Ruellet and Dauvin, 2007; Bouchet and Sauriau, 2008). Adjusting the limits between the EcoQS categories would insure better agreement between the methods (Borja *et al*., 2007) and produce the same EcoQS assessment for a given zone (Ruellet and Dauvin, 2007). These adjustments should partially resolve the overestimation problem.

The new BOPA thresholds proposed in this paper which can be also adopted for the BO2A produced more limited high and good EcoQS classifications, which increased the conformity of the BOPA results with those produced by AMBI, thus resulting in a better agreement in Mediterranean coastal waters and in French Atlantic transitional waters. Nonetheless, the misclassification of these close categories (high and good) remained due to the inherently different mathematical nature of the indices. AMBI produces a greater range of the "good" EcoQS (Simboura and Reizopoulou, 2007), while BOPA tends to produces low values and thus "high" EcoQS, especially in environments where amphipod species are abundant and diverse. For example, Bakalem *et al*. (2009) did not find any moderate EcoQS in Algeria's shallow fine sand communities with the BOPA index due to this community's high diversity of amphipods, and de-la-Ossa-Carretero *et al*. (2009) obtained good EcoQS in sites affected by sewage discharges in the western Mediterranean's fine sand community.

Despite the fact that opportunistic polychaetes represent the majority (over 70%) of the opportunistic benthic macro-invertebrate species on the list used by AMBI (www.azti.es), there are situations in which opportunistic polychaetes are only present in small numbers, if they exist at all. In fact, 18% of BOPA values in the literature were 0, due to the complete disappearance of this taxa. If little to no opportunistic polychaete is in the samples, BOPA produces low or null values, thus classifying samples as high EcoQS. In order to remedy this problem, Dauvin and Ruellet (2009) proposed Benthic Opportunistic Annelida Amphipods index (BO2A), which adds clitellata to the opportunistic polychaete. This addition permits BO2A to be used all along the estuarine continuum. Moreover, although both indices do not produce identical EcoQS for all the samples, as do BENTIX and AMBI (Ruellet and Dauvin, 2007), the mean EcoQS calculated for a given body of water can be identical. Nevertheless, some particular situations, i.e. when non-polychaeta opportunistic species dominated such as the bivalve *Corbula gibba*, show divergence between high values of AMBI given a moderate to poor EcoQS and low values of BOPA or BO2A corresponding to good or high EcoQS. But, these cases remain limited in particular environment like in the harbours (see Bakalem *et al*., 2009).

Among the remaining BOPA misclassifications, those between close categories are not as important as those

between distant categories. Although the WFD requires a five-category quality classification system, for environmental managers and policymakers, the most important boundary is that between ‘‘moderate’’ and ‘‘good’’ (Munari and Mistri, 2007). Diagnostic discordances between acceptable and non-acceptable situations were lower than 10 %.
However, the situation is different for Mediterranean lagoons, where the agreement between the two indices continued to be slight. Given the high variability of environmental parameters in these lagoons (e.g., salinity, dissolved oxygen, temperature), the species living in such environments adapt to this variability (Cognetti, 1992) and become tolerant of changes. In addition, the common presence of macroalgae, which could be related to contamination problems (Pranovi *et al*., 2007), is associated with high amphipod abundance, and this is probably sufficient for BOPA to increase the ecological quality of the site (Munari and Mistri, 2008). On the other hand, AMBI index showed a poor discriminating power classifying some polluted Mediterranean lagoons (Pranovi *et al*., 2007, Simboura and Reizopoulou, 2008), and since it was used to recalibrated BOPA, it could led some misclassifications.
Finally, because the BOPA index is not based on the same ecological model of species sensitivity/tolerance to increasing organic matter input, like AMBI or BENTIX, BOPA can provide new information to a multi-index approach. The BOPA/BO2A index also limits the taxa misclassification caused by too many ecological groups (Ruellet and Dauvin, 2007): amphipods are unanimously recognized as being sensitive to organic enrichment and the list of opportunistic annelida is rarely contested, whereas species sensitivity/tolerance levels vary depending on the region (Grémare *et al*, 2009). In contrast with the other biotic indices, the BOPA/BO2A index proved to be relatively independent of the habitat characteristics in such areas as Atlantic transitional waters (Blanchet *et al*., 2008).

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