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Marine Pollution Bulletin 48 (2004) 405–408
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

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The biotic indices and the Water Framework Directive: the required consensus in the new benthic monitoring tools

In recent years several benthic biotic indices have been proposed for use in marine waters. One of them, named AMBI (AZTI Marine Biotic Index), was created by the authors of these comments and has been applied to different geographical areas, under various impact sources (Borja et al., 2000, 2003a).

Further, the European Water Framework Directive (WFD; Directive 2000/60/EC) develops the concept of Ecological Quality Status (EcoQ) for the assessment of the biological quality of water masses.

Simboura (2003), replying to Borja et al. (2003a), suggests that another index, named Bentix (Simboura and Zenetos, 2002), could be more appropriate to use than AMBI in assessing the EcoQ in Mediterranean waters.

1. The origin of the AMBI

The AMBI was designed to establish the ecological quality of European coasts, investigating the response of soft-bottom communities to changes in water quality. Hence, the AMBI offers a 'pollution classification' of a particular site, representing the benthic community 'health' (sensu Grall and Glémarec, 1997).

The theoretical basis of AMBI is that of the ecological strategies of the *r*, *k* and *T* (Pianka, 1970) and the progressive steps in stressed environments (Bellan, 1967; Pearson and Rosenberg, 1978). Most of the concepts developed within the AMBI are based upon previous proposals: (i) the species should be classified into five ecological groups (EG) (Glémarec and Hily, 1981; Grall and Glémarec, 1997); and (ii) with a scale values from 0 to 7 (Hily, 1984; Majeed, 1987).

However, the most novel contribution of the AMBI was the formula [1] permitting the derivation of a series of continuous values, with several thresholds in the scale, based upon the proportions amongst the five EG (see Fig. 2, in Borja et al., 2000). These thresholds are coincident with the benthic community health proposed by Grall and Glémarec (1997) (see Table 1, in Borja et al., 2000), whose sources can be found in Bellan (1967).

Our early studies have never established equivalence between the AMBI values and the 'ecological status' (ES) classification of the WFD, as in Simboura and Zenetos (2002). Only recently has such equivalence been proposed (Borja et al., 2003b); however, this is not coincident with that used by Simboura (2003). Our proposal has been based upon the interpretation of the five ES level definitions in the WFD, as outlined below.

(i) High status: "The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions. All the disturbance-sensitive taxa associated with undisturbed conditions are present". Hence, this definition could be associated to 'normal' and 'impoverished' benthic community health, dominated by the EG I (species very sensitive to pollution). Thus, we proposed the same thresholds as in the 'unpolluted' areas ($0 < \text{AMBI} \leqslant 1.2$) (sensu Borja et al., 2000).

(ii) Good status: "The level ... is slightly outside the range associated with the type-specific conditions. Most of the sensitive taxa of the type-specific communities are present". This definition could be associated with the 'unbalanced' benthic community health, dominated by EG III (species tolerant to an excess of organic matter). Thus, we proposed the same thresholds as in the 'slightly polluted' areas ($1.2 < \text{AMBI} \leqslant 3.3$) (sensu Borja et al., 2000).

(iii) Moderate status: "The level ... is moderately outside the range associated with the type-specific conditions. Taxa indicative of pollution are present. Many of the sensitive taxa of the type-specific communities are absent". This definition should not be interpreted as being associated with polluted areas (as Simboura proposes); rather it should be associated with transitional conditions between 'unbalanced' and 'polluted' benthic community health. Hence, we consider it more correct to assign this status to a situation intermediate between the dominance of EG III and EG IV and V (opportunistic species) ($3.3 < \text{AMBI} \leqslant 4.3$) (sensu Borja et al., 2000).

(iv) Poor status: "Water showing evidence of major alterations to the values of the biological quality

[1] $\text{AMBI} = \{(0 \times \%\text{GI}) + (1.5 \times \%\text{GII}) + (3 \times \%\text{GIII}) + (4.5 \times \%\text{GIV}) + (6 \times \%\text{GV})\}/100$.

0025-326X/$ - see front matter
doi:10.1016/j.marpolbul.2003.10.024

Table 1
AMBI and Bentix indices values and the derived EcoQ, calculated using the same data of Saronikos Gulf (see Borja et al., 2003a)

Stations	S1	S2	S10	S3	S4	S5
AMBI	4.55	3.96	3.68	2.45	2.32	1.99
Bentix	2.1	2.33	2.64	3.53	3.64	3.96
EcoQ-AMBI*	M	M	M	G	G	G
EcoQ-AMBI**	P	M	M	G	G	G
EcoQ-Bentix	P	P	M	G	G	G
EcoQ old methods	P	M	M	G	G	G

Key: G—good ES; M—moderate ES; P—poor ES. *Results proposed by Simboura (2003); **Results using the scale proposed by Borja et al. (2003b).

elements for the surface water body type, in which the relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions''. This definition could be interpreted as being associated with 'polluted' areas and those 'transitional towards heavy pollution', dominated by EG IV and V (opportunistic species) ($4.3 < \text{AMBI} \leqslant 5.5$) (sensu Borja et al., 2000).

(v) Bad status: ''Water showing evidence of severe alterations . . . in which large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent''. However, it is not possible to associate exclusively this definition to azoic conditions, as Simboura proposes. It should include those areas of 'heavily polluted' health, dominated by the EG V (first-order opportunistic species) ($5.5 < \text{AMBI} \leqslant 7$) (sensu Borja et al., 2000).

Hence, in using the AMBI, we advise the use of the 'site pollution classification', when evaluating the health of the community under the influence of a source of impact, and the 'ES' classification when applying the WFD (see Table 1, in Borja et al. (2003b)).

2. Advantages in using the AMBI

(i) The AMBI has been validated against a series of chemical contaminants (Borja et al., 2000), both in estuaries and coastal habitats.

(ii) The AMBI has been verified successfully in relation to a very large set of environmental impact sources, including drill cutting discharges, submarine outfalls, harbour and dyke construction, heavy metal inputs, eutrophication processes, diffuse pollutant inputs, recovery in polluted systems under the impact of sewerage schemes, dredging processes, mud disposal, sand extraction and oil spills (Borja et al., 2000, 2003a,b; Casselli et al., 2003; Forni and Occhipinti Ambrogi, 2003; Bonne et al., 2003; Gorostiaga et al., in press).

(iii) The AMBI is very easy to use, having freely-available software, including a continuously updated species list, incorporating more than 2700 taxa (www.azti.es/ingles).

(iv) The AMBI is particularly efficient in detecting the impact gradients, both temporal and spatial (Muxika et al., 2003).

(v) The AMBI has been verified in a very large number of geographical areas, including both Atlantic (Borja et al., 2000, 2003a; Bonne et al., 2003; Gorostiaga et al., in press; and the following personal communications: María José Gaudencio, Susan Smith, Alison Miles, Mike Bailey, Mark Davison, Hocen Bazairi, Antonio Rodríguez-Martín) and Mediterranean (Borja, 2003a; Casselli et al., 2003; Forni and Occhipinti Ambrogi, 2003; AZTI, various unpublished reports; and further personal communications: Vivianne Solís-Weiss and Susana Pinedo) coasts.

3. Some problems associated with the use of the AMBI

Even the advantages described above, in the use of the AMBI as a 'tool' for detecting and evaluating impacts, some problems have been identified by users:

(i) The robustness of this index is reduced when only a very low number of taxa (1–3) and/or individuals are found in a sample. In these cases, a more detailed analysis and discussion of the results are recommended.

(ii) In order to avoid ambiguous results, it is preferable to calculate the AMBI values for each of the replicates, then to derive the mean value. This approach is useful when some of the replicates do not contain any taxa (Borja et al., 2003a).

(iii) When the percentage of taxa which are not assigned is high (>20%), the results should be evaluated with care.

(iv) As commented upon by Simboura (2003), the assignation of a taxa to one of the five EG could lead to misclassification problems (this applies even if there only two EG, as in Simboura and Zenetos (2002)).

(v) The different thresholds in the site pollution classification, or even in the ES, depend upon the thresholds established in the AMBI scale values (these thresholds are based upon ecological aspects of the community health). Changing the thresholds would alter the final classification.

For the last two observations, it is evident that a consensus is needed between the scientific community. Such consensus can be achieved readily with the accumulated knowledge available on this subject, as used in the AMBI (for details, see Borja et al. (2000, 2003a)).

4. Comparing the AMBI and the bentix index

The Bentix index (Simboura and Zenetos, 2002) is based upon the AMBI. These authors propose that the modification "lies in the reduction of the (five) EG involved in the formula, in order to avoid errors in the grouping of the species, and reduce effort in calculating the index, without at the same time loosing its discriminative power or sensitivity". Hence, the original five EG are reduced to three: sensitive to disturbance; second-order and first-order opportunistic.

Another change is related to the formula, [2] which reduces the effective power of three EG to only two (sensitive and opportunistic), and changes the range of the scale (from 2 to 6 instead from 0 to 6). Finally, the scale is inverted and changes the thresholds of the pollution classification and the ES. These are, in fact, similar for both of the concepts.

In our opinion all of these changes are not justified, from an ecological perspective; likewise, the method is not validated in an effective way. The authors do not compare both approaches within the context of a series of samples, sources of pollution, geographical areas, etc. Further, the results are not analysed statistically, in order to establish a series of justified conclusions.

Moreover, the consequences of misclassification of the species, to the EG, could be more serious within the context of a lower number of EG. Thus, the Bentix index is sensitive to this particular problem, as the species are assigned finally only to two EG, not having accommodation of tolerant species. Hence, in the case of communities dominated by tolerant species (e.g. *Corophium* sp. or *Abra* sp.), classified within EG III (AMBI) or 2 (Bentix), the final assessment would be 'good' and 'poor' status, respectively. This example suggests that the Bentix tends towards extreme values in the ES, because only azoic sediments could be assessed as being worse than this particular classification.

On the other hand, a similar erroneous assessment occurs with the Bentix when a problem of misclassification occurs; e.g. if the same above mentioned species were assigned erroneously to EG II (AMBI) or 1 (Bentix); this would result in 'good ES', according to the AMBI (the same assessment as previously) and 'high ES', according to Bentix (the opposite than that proposed previously). This result demonstrates that misclassification in the Bentix leads to considerable erroneous assessments.

Hence, it is considered that some of the problems in the use of the AMBI, as outlined by Simboura (2003), are only personal opinions; likewise, that they are based upon incorrect concepts, as outlined below.

Considering benthic invertebrate data obtained from the Saronikos Gulf, the latter author compares the application of the AMBI and Bentix indices, with the previous assessments undertaken by univariate and multivariate analytical methods. Both indices detect the pollution gradient over the area (Table 1), being the results like mirror images, because of the inverted scale used in the case of Bentix.

The most important difference among the three methods (AMBI, Bentix and multivariate methods), in this example, is the classification scale. Although Simboura (2003) does not explain what is the method in establishing the EcoQ, based upon univariate and multivariate methods, this provides a comparative basis for the remaining two methods (AMBI and Bentix). Even though it is not mentioned by Simboura (2003), each of the methods fails in the assessment of one of the sampling stations: S1 (AMBI) and S2 (Bentix) (Table 1). However, this is based upon the EcoQ proposed by Simboura (2003), but use of the AMBI (Borja et al., 2003b) does not fail at any of the sampling stations (Table 1).

Finally, when studying the potential of AMBI and Bentix indices to discriminate differences amongst sampling locations and along a known gradient, a critical analysis should be undertaken. The analysis should incorporate all the error components relevant to the programme objectives; further, it should separate extraneous variability, to reveal the true environmental signal in the indicator data (Jackson et al., 2000). Following this analysis, the discriminatory ability should be evaluated against programme data quality objectives, demonstrating how sample size, species assignation, thresholds, etc., affect the precision and confidence levels of the reported results. Similarly, how these variables may be optimised to attain the specific objectives. This concept can be undertaken on the basis of power analysis in the hypothesis testing, to evaluate the probability of failing to reject the 'null hypothesis' when the alternative hypothesis is true (Jackson et al., 2000).

[2] $\text{Bentix} = \{(6 \times \%\text{GI}) + 2 \times (\%\text{GII} + \%\text{GIII})\}/100$.

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