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Assessing proposed modifications to the AZTI marine biotic index (AMBI), using biomass and production

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Abstract

Initially described in 2000, AZTI's Marine Biotic Index (AMBI) aims to assess alterations in communities of soft-bottom marine benthic macroinvertebrates caused by anthropogenic impacts. Although it was designed to be used in European estuaries and coasts this index, based on Pearson and Rosenberg's model of responses to organic enrichment, is being used successfully worldwide. Taking into account statistical difficulties associated with the use of raw abundance data, modifications to the index were recently proposed. These included transforming abundances prior to its calculation, or to use data other than abundances which might be more functionally relevant (such as biomass or production data). Using data from the Basque coast and estuaries (northern Spain), collected between 1995 and 2009, where the evolution of human pressures and restoration actions in the area may be taken into account, the performance of AMBI is compared to that of the proposed modifications in order to assess their usefulness. Despite large variations in the form and nature of the input data, all variations of AMBI index are shown to be highly correlated, even when presence/absence data are used. New boundaries between disturbance categories were calculated, reflecting inter-relationships between different forms of the index. The disturbance classification obtained from all variations using the recalculated boundaries agreed closely with that derived from AMBI. The finding that AMBI values calculated with presence/absence data are potentially useful opens up many possibilities, such as determining the status of assemblages retrospectively using historical data.

Key words: AMBI, AMBI modifications, benthic indicators, soft-bottom benthic macroinvertebrates, coastal waters, estuarine waters, Basque coast

1 Introduction

AMBI (AZTI's Marine Biotic Index: Borja *et al.*, 2000) is a univariate measure of community structure that uses information about proportional abundances of benthic macroinfaunal species in samples for its calculation. It is the average (within samples) of species scores, where each species has been assigned a score according to its sensitivity to anthropogenic stress (from I, sensitive, to V, first-order opportunistic), weighted by the abundances of the species and scaled to give continuous values between 0 (all species in ecological group I) to 6 (all species in ecological group V). This response is based upon the Pearson and Rosenberg's (1978) paradigm, which predicts an increase in the abundance of opportunistic species and a decrease of sensitive species, following an organic enrichment of the sediment.

Numerical abundances generally vary widely, even between replicate counts of the same species. This is, in part, a motivation for the routine pre-treatment of abundance data using some sort of transformation prior to conducting multivariate analyses (Clarke *et al.*, 2006). Changes in numbers within species may not be a good proxy for changes in ecosystem function. For example, a single individual of a small opportunistic species may not have the same functional importance as a single large bivalve or echinoderm. Motivated by such considerations, Warwick *et al.* (2010) proposed calculating AMBI using different types of input data, specifically numerical abundances (NAMBI), biomass (BAMBI) and production (PAMBI). They also suggested that pre-treating data prior to calculating the indices, using a spectrum of power transformations (square root, fourth root, logarithm, presence/absence) such as are routinely used in nonparametric multivariate analyses (Clarke, 1993), might usefully down-weight the influence of dominant species and give a better overview of the status of assemblages. To avoid confusion and facilitate comparisons with previous work, in what follows we will retain AMBI to refer to the index calculated using untransformed abundances.

Warwick *et al.* (2010) went on to demonstrate that, in a series of samples reflecting a strong organic enrichment gradient (Warwick and Clarke, 1993), a mild transformation (square root) improved the ability of AMBI-derived measures to discriminate samples along the gradient. They also showed that the measures calculated using different input data (raw and pre-treated abundance, biomass and production) were surprisingly strongly related to each other, although the relationships were non-linear.

Here, a large dataset of samples collected from the Basque coast of northern Spain, previously used in many studies describing and using AMBI (Borja *et al.*, 2000, 2003a, Muxika *et al.*, 2005, etc.), was used to assess the reproducibility of findings by Warwick *et al.* (2010) and to take forward some suggestions made in that work. Specifically: (1) the relationships between measures calculated using different input data (AMBI, NAMBI, BAMBI, PAMBI) were determined, together with the underlying patterns that underpin their calculation; (2) the effects of applying pre-treatments to the input data prior to calculating the indices on overall patterns in values of the indices were examined; (3) the relationships between different measures calculated following various pre-treatments and measured environmental variables were determined, and; (4) the variation in the differently-derived measures along selected temporal patterns was illustrated.

2 Materials and methods

2.1. Data description

The Basque Water Agency, by means of the Littoral Water Quality Monitoring and Control Network, has monitored Basque coastal and estuarine water quality since 1994 (Figure 1) (Borja *et al.*, 2009, 2010). This network comprises the analyses of both physico-chemical (in water, sediment and biota) and biological elements (phytoplankton, macroalgae, benthos and fishes). The data series includes 32 coastal and estuarine stations sampled from 1995 to 2009, together with 19 additional locations sampled since 2002.

Soft-bottom macrobenthic communities are sampled annually in winter, using a van Veen grab in sublittoral locations (0.07-0.1 m^2), combined with quadrats (0.5×0.5 m) sampled directly at intertidal locations (see Sampling Methods, in Borja *et al.*, 2003b, 2010). Three replicates are collected at each sampling station.

To explore the responses of benthic communities to abiotic factors and human pressures, data from the same sampling locations were used. These included: sediment characteristics (grain-size, organic matter content, redox potential, etc.), and concentrations of metals and organic compounds. For methods used in sampling and analyses, Rodríguez *et al.* (2006) and Tueros *et al.* (2008, 2009) can be consulted.

2.2.Data treatment

Production of each species within communities was approximated using values of abundance (A) and biomass (B) by the allometric equation:

$$P = \left(\frac{B}{A}\right)^{0.73} \times A,$$

where B/A is the mean body size and 0.73 is the average exponent of a regression of annual production on body size for macrobenthic invertebrates (Brey, 1990).

Abundance, biomass (dry biomass) and production data were transformed using a set of transformations of increasing severity: square root, fourth root and log (1+x) and presence/absence, and through the use of dispersion weighting (Clarke and Gorley, 2006), which down-weights clustered species.

Biotic indices were calculated using raw (AMBI) and transformed abundance (NAMBI), biomass (BAMBI) and production (PAMBI) values (Warwick *et al.*, 2010) using AMBI 4.1 software (freely available at http://ambi.azti.es) and the February 2010 species list. This software provides values calculated for replicate samples, and average and standard deviations within stations and years of sampling. Guidelines derived from Borja and Muxika (2005) were used in the calculation of the measures.

As noted by Warwick *et al.* (2010), the mean AMBI score is often reduced to a simple integer scale or discretised into an even smaller number of status categories (e.g. AMBI≤1.2, unpolluted; 1.2<AMBI≤3.3, slightly polluted; 3.3<AMBI≤5, moderately polluted; 5.0<AMBI≤6.0, heavily polluted; AMBI>6.0, extremely polluted (Borja *et al.*, 2000) but the cut-off points and boundaries for such classifications need to be set at appropriate points on the scale, dependent on which combination of input data (abundance, biomass, production) and transformation is being used. The relationships between AMBI (calculated using raw abundance data) and indices calculated using different input data (biomass or production) and transformations across all samples (replicates) were fitted using quadratic trend lines. The formulae for the trend lines were then used to calculate values of the various indices corresponding to AMBI values separating status categories defined by Borja *et al.* (2000).

To analyse the agreement in the pollution classification between AMBI, NAMBI, BAMBI and PAMBI calculated from raw data, and those calculated from pre-treated data, a Kappa analysis was undertaken (Cohen, 1960). The level of agreement between the methods was established, based upon the equivalence table from Monserud and Leemans (1992), as used in the European intercalibration exercises, for the Water Framework Directive implementation (see Borja *et al.,* 2007). As the importance of misclassification is not the same between close categories (e.g., between undisturbed and slightly disturbed, or extremely disturbed and heavily disturbed) as between further categories (e.g., between undisturbed and moderately disturbed, or undisturbed and extremely disturbed), Cicchetti–Allison weights were applied to the analysis (Cicchetti and Allison, 1971).

Relationships between measured environmental variables and variation in the different biotic indices were explored using BVSTEP (Clarke and Warwick, 1998), a stepwise algorithm with forward selection and backward elimination steps. It aims to find a subset of variables which maximises a rank correlation (in this case the Spearman rank correlation) between a resemblance matrix derived from that subset of variables, and a predefined fixed resemblance matrix. Euclidean distance was used as resemblance measure, both for the fixed matrices derived from the biotic indices and for the subset matrices derived from the environmental variables. Environmental variables were log-transformed and normalised prior to the analyses. The significance of the correlation between the best subset and the fixed matrix, in each case, was assessed using a permutation procedure taking into account selection bias (Clarke *et al.*, 2008). Analyses were performed using PRIMER v6 (Clarke and Gorley, 2006).

In order to test whether changes in pressures at some selected sites were related to changes in the values of the indices, the significance of differences between values before and after the change in the pressure was tested by non-parametric Mann-Whitney tests. These stations were selected according to the known history in human pressures, described by Borja *et al.* (2009).

3 Results

The main characteristics of each sampled station can be seen at Table 1. On average, the analysed sediments can be classified as muddy sands (average sand content: 70%, SD=31; average silt/clay content: 25%; SD=30), with 4.9% of organic matter content (SD=4). However, estuarine sampling stations show a lower sand fraction on average and higher heterogeneity (average sand content: 57%, SD=30; average silt/clay content: 37%, SD=30) than coastal sampling stations (average sand content: 91%, SD=15; average silt/clay content: 6%, SD=15). As expected, estuarine sampling stations showed higher organic matter content (6% on average) than coastal sampling stations (3% on average). As a result, redox potential values were lower and more heterogeneous in estuarine stations (43 mV on average, SD=187) than in coastal ones (297 mV on average, SD=138).

These results are reflected also in the concentrations of metals and organic pollutants. For all of them, estuarine sampling stations present a wider range of values and higher average concentrations than coastal ones (Table 1). Iron and manganese are the only exceptions, having higher average concentrations and variability in coastal stations than in estuarine ones.

In terms of AMBI values, they range between 0 and 7 in estuarine stations and between 0.2 and 5.9 in coastal stations. Depending on the input data (abundance, biomass or production) and their pre-treatment, the minimum value in coastal stations can be even lower (0.03). On average (Table 2), the values for the different indices calculated range between 3.1 and 3.9 (SD=1.3-1.5) at estuarine sampling stations and between 1.3 and 1.6 (SD=0.9-1.0) at coastal ones.

Quadratic trend lines were used to illustrate how the relative abundance, biomass, production and number of species within each of the five ecological groups varied with increasing values of the different indices (Figure 2). The r^2 values (Table 3) indicate at least moderate correlation ($|r|>0.25$) *sensu* Colton (1974), and even excellent correlation ($|r|>0.75$), implying that the trend lines provide adequate to excellent illustrations of the variation in proportions. The results show that the shapes of the relationships between the relative proportions within ecological groups and the different indices are remarkably consistent, implying that the abundance-based conceptual model on which AMBI is based may be usefully extended to include biomass, production or proportions of species.

Comparisons between the different AMBI-based indices and AMBI (Figure 3) result in excellent correlations ($|r|>0.75$) (Table 4). Both estuarine and coastal sampling stations present the same kind of relationship, although NAMBI, BAMBI and PAMBI values (calculated from both raw data and pre-treated data) are usually higher in estuarine stations than in coastal ones. The equations obtained from these correlations were used to estimate the boundaries between disturbance levels for NAMBI, BAMBI and PAMBI calculated using raw and pre-treated data corresponding to the predefined (Borja *et al.*, 2000) boundaries for AMBI (Table 4).

Using those new boundaries equivalent disturbance classes can be used for all the variations of AMBI. Once all sampling stations were classified using these levels, kappa analyses were undertaken to explore the degree of agreement between the indices. The results show an excellent agreement between the classification obtained by AMBI calculated from raw data and NAMBI calculated from square root transformed data (Table 4). Good agreement ($0.55<$kappa coefficients≤ 0.70) was reached between AMBI and: BAMBI; BAMBI calculated from square root transformed data and log transformed data; PAMBI; and PAMBI calculated using log transformed data. For the remainder of indices the agreement with AMBI classification was very good ($0.70<$kappa coefficients≤ 0.85).

In order to see if the various indices responded differently to physico-chemical variables BEST analyses were carried out for estuarine sampling stations, for coastal sampling stations, and for all sampling stations together. Significant correlations ($p<0.05$) with combinations of physico-chemical variables were found for all the indices (Table 5), except BAMBI in coastal stations ($p=0.06$). Correlation coefficients were higher when all the stations were taken into account (rho≥ 0.32), than when estuarine and coastal sampling stations where treated separately (rho≥ 0.26 and rho≥ 0.16, respectively).

In estuaries, all the combinations of variables providing the best match to variation in the indices included the carbon to nitrogen ratio, and particulate organic nitrogen was also included in most of the combinations. At coastal sites, all of the best-matching combinations of variables included redox potential, and zinc concentration, gravel content and organic matter content were often included. When all stations were included, all of the best-matching combinations included sand content, particulate organic nitrogen, redox potential and copper concentration. Despite subtle variations, there is little evidence, therefore, that differently-derived variations of AMBI vary markedly in their responses to physico-chemical variables.

Some sampling stations were selected to compare the responses of variations of AMBI to known changes in pressures (Figures 4 and 5). In the inner part of the Nervión estuary (St. 3) all the indices showed a significant (Mann-Whitney U=0.0; p=0.000) improvement after 2002, once the biological treatment of the wastewaters discharged to the estuary commenced in 2001 (Borja *et al.*, 2006b, 2009). Before that milestone the sediment was azoic, but once it was colonized by some opportunistic species, macrofauna species were found in every campaign. The outer part of the Nervión estuary (St. 7) is very influenced by marine waters and the effects of the wastewater treatment on bottom water layers are not important. However, an increase was detected in all indices in 2002-2003 (Figures 4 and 5), after dredging was undertaken (2001) in the area where the sampling station is located (Borja *et al.,* 2009). AMBI also showed an increase in 1997, which could be related to the works carried out between 1995 and 1997 to construct new docks in the port that occupies all the outer part of the estuary (Borja *et al.,* 2009). However, if data are pre-treated this relative maximum decreases and the NAMBI value becomes more similar to BAMBI or PAMBI (Figure 5).

Finally, the increase in AMBI, BAMBI, PAMBI and p/a AMBI values detected between 1995 and 1996 at St. 41 (Figure 4) coincides with an increase of urban and industrial discharges through an outfall located near the sampling station (Borja *et al.*, 2009). Such discharges were discontinued in 2001, which allowed an important improvement in macrobenthic communities. All the indices calculated, except NAMBI from dispersion-weighting transformed abundances, BAMBI (both from raw biomass and log-transformed biomass) and PAMBI (both from raw production and log-transformed production), where able to find significant differences (Mann-Whitney U=1.0-9.0; p<0.05) between the period with discharges and the period after their removal.

4 Discussion

AMBI index was developed by Borja *et al.* (2000) inspired by the use by Grall and Glémarec (1997) of a biotic index which was defined on the abundance distribution of five ecological groups. These authors based their work on previous investigations by Hily (1984), Hily *et al.* (1986) and Majeed (1987). At the same time, all these findings were based on Pearson and Rosenberg (1978), who (1) identified some species which became dominant at different levels of organic enrichment or which were favoured by some level of it; and (2) predicted an increase in benthic abundance in organically enriched areas caused by a bloom of opportunistic species.

All those findings were based on raw abundance data. However, as Warwick *et al.* (2010) stated, ecological indices based on relative abundances of species are often over-sensitive to the super-abundance of one or a few dominants. Moreover, it is usual to find high variation in numerical abundance, even between replicate counts of the same species, which can affect to the robustness of the index. As a result, many authors support the transformation of the data, especially prior to multivariate analyses, in order to down-weight the dominant species (Clarke and Warwick, 2001). However, the results obtained in this paper show that, at least at the ecological group level, there are no large differences in their dominance distributions along a disturbance gradient represented by AMBI (Figure 2). Moreover, these distributions are even similar to that presented by Borja *et al.* (2000). These similarities are probably due to the fact that the variation between counts are not so high at ecological group level as at species level, which could make AMBI more insensitive to abundance changes of single species than other indices which work at species level. Nonetheless, it should be noted that the maximum dominance values, especially for ecological groups IV and V, are lower after presence/absence transformation. This lower dominance of opportunistic species would probably result in lower values, on average, when AMBI is calculated using presence/absence data than when it is calculated using raw data.

Moreover, the distributions of ecological groups' dominances were also very similar when biomass or production were used instead of abundance. As a result, assuming that biomass and production might have more ecological relevance than abundance (Warwick *et al.*, 2010), using them to derive BAMBI or PAMBI could make sense, at least when there is more confidence in biomass or production measures than in abundance.

As expected, the high similarities between the distributions of ecological groups' dominances led to excellent correlations *sensu* Colton (1974) between AMBI, BAMBI and PAMBI calculated using both raw and transformed data (Table 3). The quadratic correlations followed the same shape in estuarine and coastal sampling stations, although the latter presented lower average values. This means that all the indices calculated work in the same way in both environments, although it has been discussed the difficulty of distinguish between natural and human disturbance in estuarine systems (Dauvin, 2007; Elliott and Quintino, 2007). Hence, it is not possible to support the use of any of them above the remainder neither in estuaries nor in coastal areas. The researchers could test some of the AMBI variants and select the one which gives the best diagnostic for the dataset used.

The differences between coastal and estuarine sampling stations in the average values of the indices could be related to the higher pollutant concentrations (including organic matter content or more negative redox potential values) in the latter, as shown in Table 1. However, independently of the pollution level, some authors argue that biotic indices will always provide more negative results in estuaries than coasts because of the natural disturbance found in such variable (changes in salinity, emersion periods, etc.) and organically enriched environments (Dauvin, 2007). These conditions would benefit tolerant and opportunistic species, and are known as the Estuarine Quality Paradox (Elliott and Quintino, 2007).

As abovementioned, because of the down-weighting of the relative abundance of dominant species, which usually are opportunistic species, the transformation of the data led to lower index values, especially above an AMBI of *ca.* 3. This is reflected in the threshold values calculated for the different indices (Table 4), which are systematically lower than the inter-class boundaries for AMBI. When these boundaries were used to define the level of alteration provided by each of the indices, a good level of agreement was found between them. Again, this suggests that all the variations of AMBI, BAMBI and PAMBI give the same information, and that the same results can be obtained independently of the index used or the level of transformation of the input data (from none to presence/absence).

This agreement among the variations paves the way to the use of DNA identification techniques, which are nowadays developing and give qualitative presence/absence results, in environmental quality assessment studies. They are already being used for zooplankton community analyses from bulk samples (Machida *et al.*, 2009) and even for meiofaunal community analyses (Creer *et al.*, 2010). Hence, the use of these novel techniques could make the assessment of the marine quality faster, by applying to this genetic identification the presence/absence AMBI.

As expected from the environmental data, the parameters which drive the values of the indices vary from estuarine to coastal sampling stations (Table 5). Hence, in estuaries those related to eutrophication or organic enrichment, carbon, to nitrogen ratio and particulate organic nitrogen content, were the main parameters which explained the variances of the indices. Conversely, at coastal sampling stations, redox potential, zinc concentration, gravel content and organic matter content were the main parameters, although the correlation coefficients were always lower than for estuarine stations. Finally, the highest correlations were found when all sampling stations were studied together, being sand and particulate organic nitrogen content, redox potential and copper content, systematically correlated with all the indices. These variables are related to the historical human pressures within the Basque coast and estuaries, which mainly includes urban and industrial waste discharges, dredging and sediment disposal, and hydromorphological alterations (Borja *et al.,* 2006a). Hence, all AMBI, NAMBI, BAMBI and PAMBI calculated from raw and transformed data respond basically to the same physico-chemical factors. However, it should be noted that BAMBI calculated from raw biomass data, always presented the lowest correlation coefficient values, to the point that there is no significant correlation between BAMBI and physico-chemical variables in estuarine sampling stations. These findings do not exactly match the conclusions of Warwick *et al.* (2010), who found that a marginally better relationship with the impact axis of the meta-analysis was achieved by a moderate transformation of the data, with a decline which followed the increasing severity of transformations.

As it has been discussed, all indices provide very similar results and it is not yet feasible to determine if it is better to calculate AMBI from abundance data, from biomass data or from production data, or if the subsequent index will be more sensitive to pressures or impacts if the input data are pre-treated or not. In fact, all the indices react to changes in pressures in a similar way and follow similar improvement or degradation paths after those changes.

In general, given the relationships between the indices (Figure 3), AMBI values would be expected to be higher than BAMBI, PAMBI or p/a NAMBI values, but given the distribution of proportions within different sensitivity categories (Figure 2) responses would be expected to be similar. The results (Figure 4) show great consistency between the responses of different AMBI-derived measures to anthropogenic pressures.

5 Conclusions

The underlying contributions from different species with differing tolerance to pollution, when aggregated to proportions in samples, show great similarities whether abundances, biomass or production (raw or transformed) are used to determine their contributions. Therefore variations of AMBI index are highly correlated.

Once the boundaries between disturbance classes are calculated from the correlations between the variants, the disturbance classifications obtained from all variations agree with that derived from AMBI very closely.

For historical data, inventories, etc., p/a AMBI could provide a valid proxy to AMBI, which could be useful to define reference AMBI values. This p/a AMBI could be also very useful when DNA techniques are used for species identification.

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Table 1. Average and standard deviation of general sediment composition parameters, metal concentration and organic compounds concentration, for estuarine (n=302) and coastal (n=191) sampling stations separately and for all together. Key: OM= organic matter; POC= particulate organic carbon; PON= particulate organic nitrogen; C/N= carbon over nitrogen ratio; Hg_i= inorganic mercury; ∑lPAH= sum of light PAHs (2 or 3 rings); ∑hPAH= sum of heavy PAHs (4-6 rings), ∑PAH= sum of all PAHs; ∑PCB= sum of PCB; ∑DDT= sum of DDT, DDD and DDE.

		Estuarine stations		**Coastal stations**		**All stations**	
		Average	**Standard Deviation**	**Average**	**Standard Deviation**	**Average**	**Standard Deviation**
General	**Gravel (%)**	6.59	13.24	2.30	6.06	4.92	11.15
	Sand (%)	56.72	30.44	91.20	15.41	70.15	30.86
	Silt/Clay (%)	36.70	30.31	6.50	14.97	24.94	29.59
	OM (%)	6.17	4.47	2.84	2.14	4.87	4.03
	POC (mol·kg^{-1})	2.88	1.24	2.25	1.08	2.63	1.21
	PON (mol·kg^{-1})	0.128	0.086	0.032	0.028	0.090	0.084
	C/N	49.39	68.48	98.41	62.97	68.48	72.36
	Redox (mV)	42.9	187.5	296.9	137.8	141.8	213.4
Metals (mg·kg^{-1})	**Cd**	0.91	1.85	0.23	0.32	0.65	1.46
	Cr	51.3	40.6	29.0	22.3	42.6	36.2
	Cu	85.1	123.9	27.2	25.9	62.6	100.0
	Fe	40,302	25,930	53,890	56,229	45,594	41,743
	Hg_i	0.664	1.363	0.573	1.163	0.628	1.277
	Mn	508	545	683	760	576	654
	Ni	37.4	18.6	27.4	13.3	33.5	17.6
	Pb	114.4	144.0	60.7	31.8	93.5	113.9
	Zn	345.1	302.7	174.9	146.5	278.8	260.3
Organics (µg·kg^{-1})	**∑lPAH**	339.6	1,212.1	41.8	176.6	223.6	927.4
	∑hPAH	3,430.2	11,899.7	348.6	1,268.9	2,230.1	9,099.5
	∑PAH	3,769.9	12,925.3	390.5	1,438.4	2,453.7	9,889.2
	∑PCB	73.1	192.2	19.5	9.2	52.2	146.6
	∑DDT	6.5	38.2	3.4	4.7	5.3	28.7

Table 2. Average and standard deviation of AMBI and each of its variants. Key for pre-treatments: sr= square root; fr= fourth root; log= logarithm; dw= dispersion weighting; p/a= presence/absence.

	Estuarine stations		**Coastal stations**		**All stations**	
	Average	**Standard Deviation**	**Average**	**Standard Deviation**	**Average**	**Standard Deviation**
AMBI	3,896	1,425	1,614	1,037	3,024	1,701
sr AMBI	3,728	1,324	1,604	0,937	2,917	1,576
fr AMBI	3,599	1,270	1,594	0,889	2,833	1,499
log AMBI	3,663	1,290	1,594	0,917	2,873	1,536
dw AMBI	3,299	1,443	1,450	0,939	2,593	1,559
BAMBI	3,091	1,508	1,303	0,959	2,408	1,584
sr BAMBI	3,200	1,407	1,397	0,899	2,511	1,516
fr BAMBI	3,324	1,317	1,492	0,862	2,624	1,465
log BAMBI	3,098	1,500	1,308	0,957	2,414	1,580
PAMBI	3,301	1,495	1,365	0,974	2,561	1,621
sr PAMBI	3,367	1,375	1,459	0,901	2,638	1,529
fr PAMBI	3,419	1,297	1,525	0,867	2,695	1,474
log PAMBI	3,300	1,464	1,376	0,961	2,565	1,597
p/a AMBI	3,467	1,239	1,582	0,854	2,747	1,437

Table 3. r^2 obtained for the quadratic regressions undertaken between the relative abundance of each of the ecological groups (%n= numerical abundance; %b= biomass; %p= production; %s= species number) and AMBI, BAMBI, PAMBI and p/a AMBI (n=1,845).

	AMBI				**BAMBI**	**PAMBI**	**p/a AMBI**
	%n	**%b**	**%p**	**%s**	**%b**	**%p**	**%s**
EG I	0.888	0.439	0.548	0.774	0.905	0.891	0.875
EG II	0.477	0.233	0.294	0.434	0.617	0.543	0.501
EG III	0.648	0.477	0.525	0.487	0.770	0.724	0.584
EG IV	0.095	0.064	0.075	0.122	0.179	0.129	0.124
EG V	0.921	0.677	0.769	0.563	0.901	0.910	0.806

Table 4. Results obtained after quadratic regression between AMBI and each of the variations (n=602). For quadratic regressions, r^2 and coefficients obtained for each of the terms in the equation (a: intercept; AMBI, $AMBI^2$ $AMBI^3$ $AMBI^4$: slope for each of the terms) are shown. For boundaries, interpolated inter-class benchmarks are shown for undisturbed/slightly disturbed (1.2), slightly/moderately disturbed (3.3), moderately/heavily disturbed (5.0) and heavily/extremely disturbed (6.0). Kappa coefficients obtained to assess the agreement between AMBI and each of its variations is also shown, as well as the interpretation of the coefficients after Monserud and Leemans (1993). Key for pre-treatments: sr= square root; fr= fourth root; log= logarithm; dw= dispersion weighting; p/a= presence/absence.

	Quadratic regression						**Boundaries**				**Kappa analysis**	
	r^2	**a**	**AMBI**	**$AMBI^2$**	**$AMBI^3$**	**$AMBI^4$**	**1.2**	**3.3**	**5.0**	**6.0**	**Coefficient**	**Interpretation**
sr AMBI	0.98	0.230	0.650	0.223	-0.061	0.005	1.23	3.21	4.63	5.63	0.88	excellent
fr AMBI	0.94	0.396	0.322	0.450	-0.122	0.010	1.24	3.16	4.29	5.21	0.80	very good
log AMBI	0.96	0.267	0.498	0.360	-0.101	0.008	1.23	3.21	4.41	5.34	0.85	very good
dw AMBI	0.94	0.029	0.892	-0.006	-0.013	0.002	1.07	2.73	4.21	5.45	0.78	very good
BAMBI	0.83	0.241	0.491	0.148	-0.042	0.005	0.98	2.49	3.92	5.22	0.65	good
sr BAMBI	0.88	0.328	0.372	0.282	-0.078	0.007	1.06	2.67	3.92	5.06	0.68	good
fr BAMBI	0.90	0.436	0.211	0.451	-0.123	0.011	1.15	2.88	4.01	5.07	0.72	very good
log BAMBI	0.84	0.246	0.482	0.155	-0.044	0.005	0.98	2.49	3.93	5.21	0.65	good
PAMBI	0.89	0.153	0.663	0.069	-0.020	0.002	1.02	2.67	4.22	5.46	0.70	good
sr PAMBI	0.92	0.306	0.442	0.271	-0.074	0.007	1.11	2.83	4.15	5.25	0.72	very good
fr PAMBI	0.92	0.435	0.221	0.468	-0.126	0.011	1.18	2.98	4.07	5.05	0.73	very good
log PAMBI	0.90	0.220	0.543	0.147	-0.038	0.004	1.03	2.70	4.22	5.46	0.70	good
p/a AMBI	0.89	0.562	-0.023	0.691	-0.186	0.015	1.24	3.14	4.02	4.94	0.72	very good

Table 5. Results obtained from BEST analysis, for estuarine and coastal sampling stations separately, and for all sampling station together. Key: rho= correlation coefficient; p= significance; vars= variables which best explain the variance of the index; n= numerical abundance; b= biomass; p= production; p/a= presence/absence; 1= %gravel; 2= %sand; 4= log(%organic matter); 5= log(COP); 6= log(NOP); 7= C/N ratio; 8= redox potential; 9= log(Cd); 11= log(Cu); 14= log(Mn); 17= log(Zn); 20= log(nPAH) ; 22= log(DDT).

Index	**Pre-treatment**	**Estuarine stations**			**Coastal stations**			**All stations**		
		rho	**p**	**vars**	**rho**	**p**	**vars**	**rho**	**p**	**vars**
AMBI	**none**	0.320	0.01	4,6,7	0.238	0.01	8	0.426	0.01	2,4,6,8,11
	√n	0.340	0.01	6,7	0.225	0.01	8	0.431	0.01	2,6,8,11
	√√n	0.348	0.01	6,7	0.249	0.01	1,2,4,7,8,17	0.423	0.01	2,6,8,11
	log(n+1)	0.353	0.01	6,7	0.243	0.01	1,2,4,8,17	0.428	0.01	2,6,8,11
	dw	0.321	0.01	4,6,7,8	0.223	0.01	1,2,8,17,20	0.398	0.01	2,4,6,7,8,11
BAMBI	**none**	0.257	0.01	4,7,8	0.156	0.06	1,4,7,8,9,17	0.318	0.01	2,4,6,8,11
	√b	0.289	0.01	4,6,7,8,14,22	0.201	0.01	1,4,7,8,17	0.356	0.01	2,4,6,8,11
	√√b	0.311	0.01	6,7	0.245	0.01	1,2,4,8,17	0.391	0.01	2,6,8,11
	log(b+1)	0.258	0.01	4,7,8	0.158	0.04	1,4,7,8,9,17	0.321	0.01	2,4,6,8,11
PAMBI	**none**	0.259	0.01	4,7,14	0.180	0.01	4,5,7,8,17	0.349	0.01	2,4,6,8,11
	√p	0.298	0.01	4,6,7,14	0.227	0.01	2,4,8,17	0.385	0.01	2,6,8,11
	√√p	0.320	0.01	6,7	0.254	0.01	1,2,4,8,17	0.405	0.01	2,6,8,11
	log(p+1)	0.270	0.01	4,7,14	0.180	0.02	2,7,8,17	0.357	0.01	2,4,6,8,11
p/a AMBI		0.338	0.01	6,7	0.255	0.01	1,2,4,7,8,17	0.408	0.01	2,6,7,8,11

Figure captions

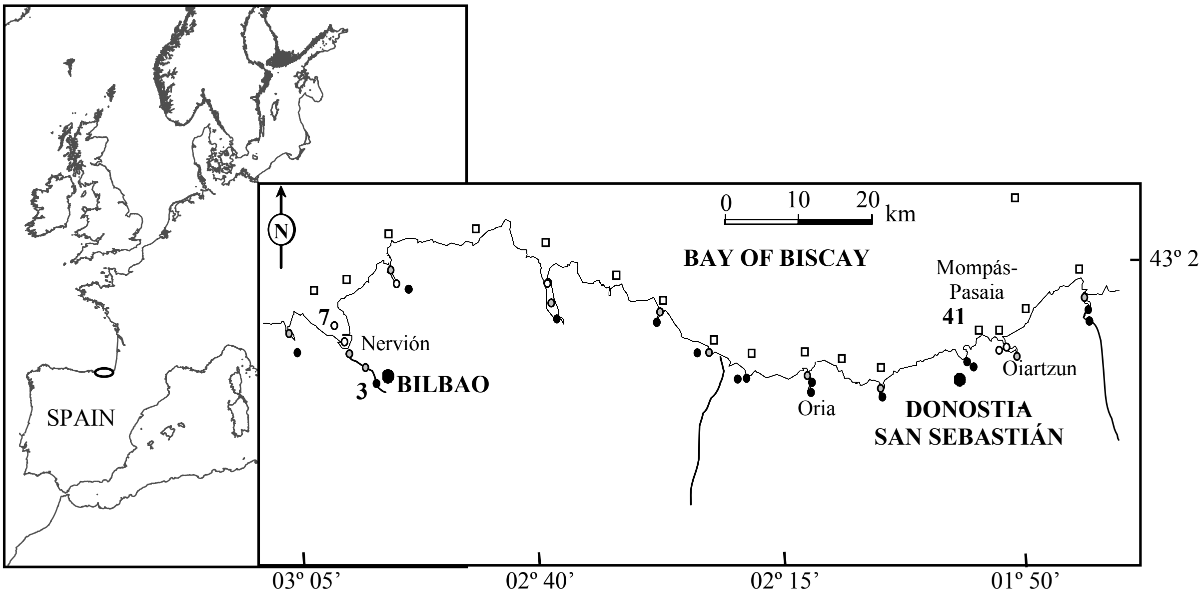
Figure 1. Sampling stations within the Littoral Water Quality Monitoring and Control Network of the Basque Country. Those that have been used for the analysis of temporal trends are numbered (3=inner Nervión; 7=outer Nervión; 41=Mompás-Pasaia). Key: squares= coastal stations; white circles= euhaline estuarine stations; grey circles= polyhaline estuarine stations; black circles= meso- and oligohaline estuarine stations.

Figure 2. Distribution of the relative abundances (n= numerical abundance; b= biomass; p=production; s= species number) of the ecological groups (EG), along AMBI, BAMBI, PAMBI and p/a AMBI scales, fitted by quadratic regression.

Figure 3. Correlation between AMBI and some of the variants –AMBI calculated after dispersion weighting (dw), square root (sr), forth root (fr) and logarithm (log) transformations of the numerical abundances, BAMBI, PAMBI, p/a AMBI.

Figure 4. AMBI, BAMBI, PAMBI and p/a AMBI results for some selected sampling stations. See location in Figure 1.

Figure 5. AMBI, BAMBI and PAMBI results calculated both from raw data and from transformed data for St. 7. See location at Figure 1.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Mompás-
Pasaia
41
7
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BILBAO
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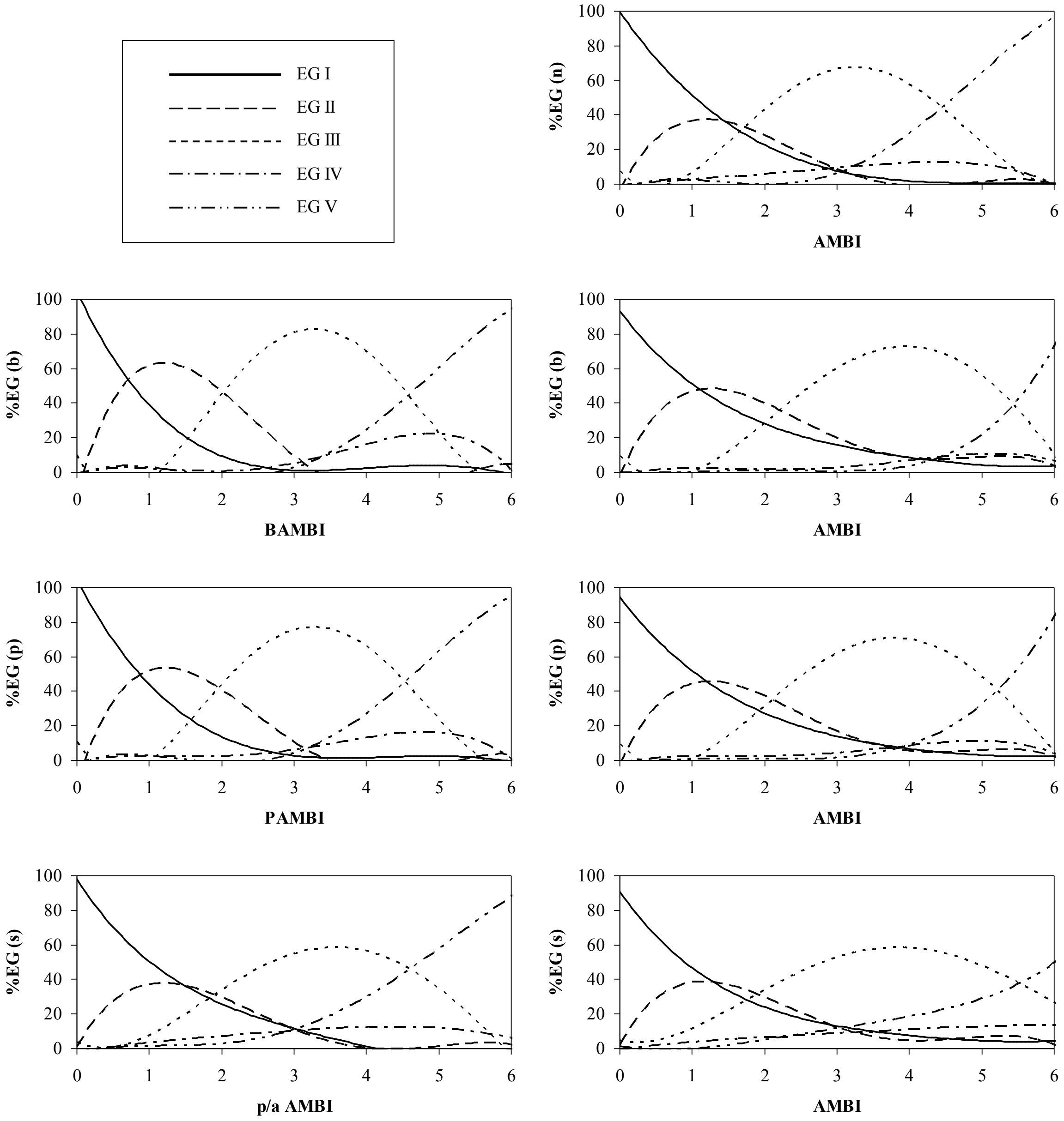
DONOSTIA
SAN SEBASTIÁN
Oiartzun
03º 05’
02º 40’
02º 15’
01º 50’

Figure 1


EG I
EG II
EG III
EG IV
EG V
%EG (n)
AMBI
%EG (b)
BAMBI
%EG (b)
AMBI
%EG (p)
PAMBI
%EG (p)
AMBI
%EG (s)
p/a AMBI
%EG (s)
AMBI
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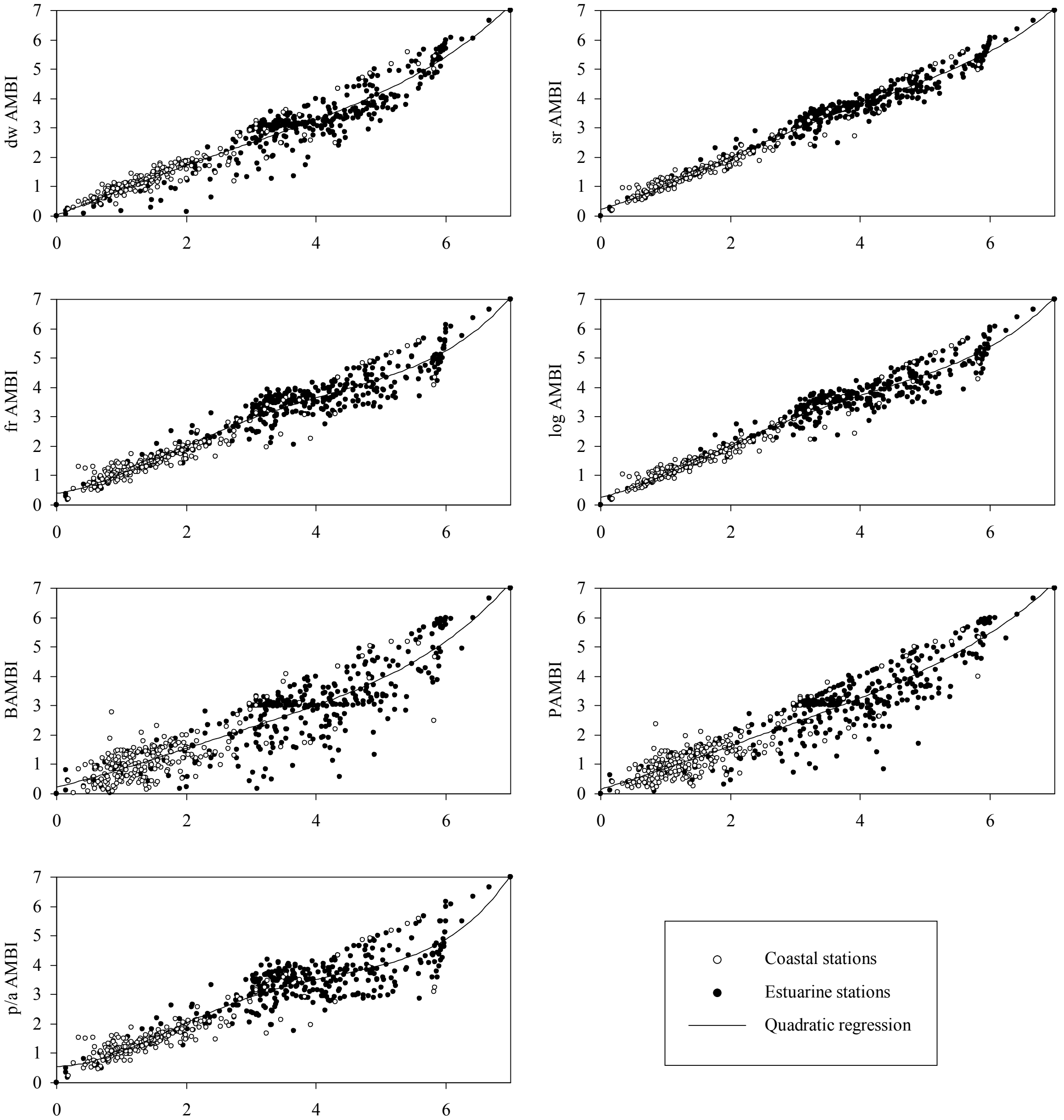
60
80
100
0
1
2
3
4
5
6

Figure 2


dw AMBI
sr AMBI
fr AMBI
log AMBI
BAMBI
PAMBI
p/a AMBI
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7
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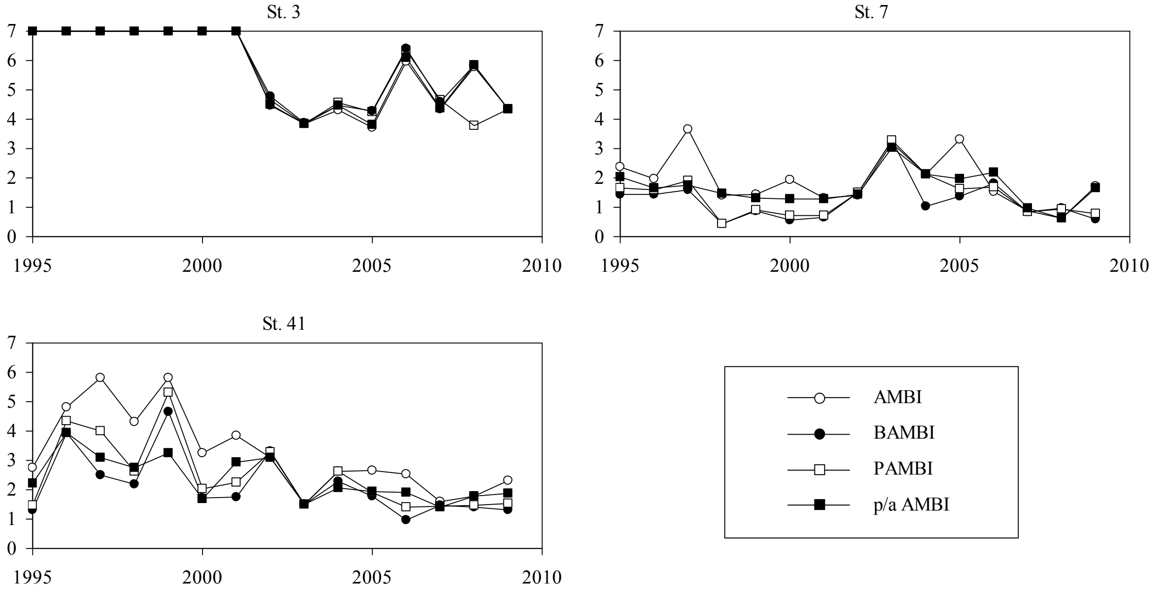
○ Coastal stations
● Estuarine stations
Quadratic regression

Figure 3


St. 3
St. 7
St. 41
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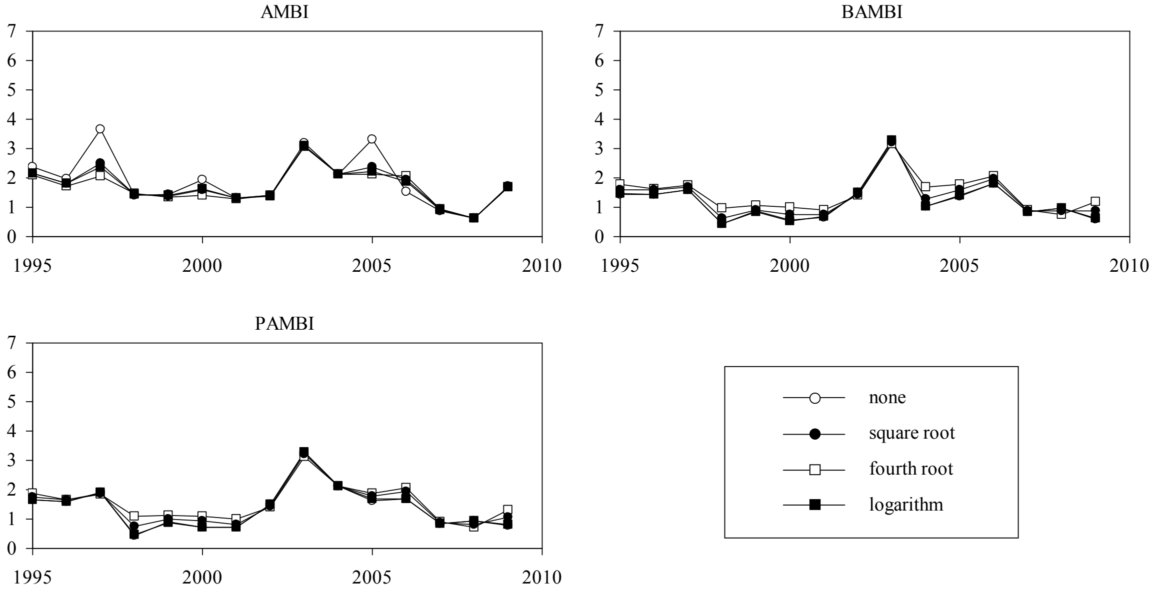
4
5
6
7
1995
2000
2005
2010
AMBI
BAMBI
PAMBI
p/a AMBI

Figure 4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PAMBI
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6
7
1995
2000
2005
2010
none
square root
fourth root
logarithm

Figure 5