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Can biotic indicators distinguish between natural and anthropogenic environmental stress in estuaries?

J.R. Tweedley[a*], R.M. Warwick[a,b], I.C. Potter[a]

[a] Centre for Fish and Fisheries Research, School of Veterinary and Life Sciences, Murdoch University, South Street, Perth, Western Australia 6150, Australia

[b] Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PLI 3DH, United Kingdom

*Corresponding author:

Tel.: +61 (0) 8 9239 8808

Fax: +61 (0) 8 9360 6303

E-mail address: j.tweedley@murdoch.edu.au

Abstract

Because estuaries are naturally stressed, due to variations in salinity, organic loadings, sediment stability and oxygen concentrations over both spatial and temporal scales, it is difficult both to set baseline reference conditions and to distinguish between natural and anthropogenic environmental stresses. This contrasts with the situation in marine coastal and offshore locations. A very large benthic macroinvertebrate dataset and matching concentrations for seven toxic heavy metals (i.e. Cr, Ni, Cu, Zn, Cd, Hg and Pb), compiled over three years as part of the UK's National Marine Monitoring Programme (NMMP) for 27 subtidal sites in 16 estuaries and 34 coastal marine sites in the United Kingdom, have been analysed. The results demonstrate that species composition and most benthic biotic indicators (number of taxa, overall density, Shannon-Wiener diversity, Simpson's index and AZTI's Marine Biotic Index [AMBI]) for sites in estuarine and coastal areas were significantly different, reflecting natural differences between these two environments. Shannon-Wiener diversity and AMBI were not significantly correlated either with overall heavy metal contaminant loadings or with individual heavy metal concentrations ('normalised' as heavy metal/aluminium ratios) in estuaries. In contrast, average taxonomic distinctness (Δ^+) and variation in taxonomic distinctness (Λ^+) did not differ significantly between estuarine and coastal environments, *i.e.* they were unaffected by natural differences between these two environments, but both were significantly correlated with overall heavy metal concentrations. Furthermore, Δ^+ was correlated significantly with the Cu, Zn, Cd, Hg and Pb concentrations and Λ^+ was correlated significantly with the Cr, Ni, Cu, Cd and Hg concentrations. Thus, one or both of these two taxonomic distinctness indices are significantly correlated with the concentrations for each of these seven heavy metals. These taxonomic distinctness indices are therefore considered appropriate indicators of anthropogenic disturbance in estuaries, as they allow a regional reference condition to be set from which significant departures can then be determined.

Keywords: Benthic macroinvertebrates; AMBI; taxonomic distinctness; metal contamination; estuarine waters; coastal waters.

Introduction

Analysis of the structure and composition of benthic macroinvertebrate faunas has become one of the mainstays of environmental quality assessments and led to the development of a wide variety of indicators for assessing the ecological status of estuaries and other transitional waters, either singly or in combination as multimetrics. Two major problems with this approach are the difficulties in setting baseline reference conditions (Borja et al., 2004; Borja and Tunberg, 2011; Warwick and Somerfield, in press) and distinguishing between the responses of these indictors to natural and anthropogenic environmental stresses (Dauvin, 2007; Elliott and Quintino, 2007; Dauvin and Ruellet, 2009). This is a particular problem in estuaries since their natural environmental conditions vary both spatially and temporally (Hutton et al., 2-15; Borja and Tunberg, 2011; Tweedley et al., 2012; Wetzel et al., 2012; Nebra et al., 2014; Brauko et al., 2015).

For many of these indicators there is no universal reference condition. Such indicators include those that employ data on species diversity and abundance, either alone or in combination with other metrics, e.g. M-AMBI (Muxika et al., 2007), or those involving ratios between different taxa, e.g. the BOPA index (Dauvin and Ruellet, 2007) or functional groups, e.g. the Infaunal Trophic Index (Maurer et al., 1999). In such cases there are a number of possibilities for setting reference conditions (Borja et al., 2004; Borja and Tunberg, 2011). The best-scoring samples are typically taken as indicating the most pristine state. These are then used to establish local reference conditions that act as a baseline against which temporal changes or spatial differences can be assessed and which may vary among estuaries. It has been argued, however, that it is inappropriate to use a pristine state as a reference point against which potentially impacted sites can be evaluated (ICES, 2002). This point is particularly valid with estuaries, where all sites might be impacted to some degree and no appropriate reference sites may thus be available. In such circumstances, Borja et al. (2004) have proposed the use of 'virtual' reference locations, i.e. those "based upon experience gained of the area and conceived as 'potential' components – biological parameters, chemical concentrations, etc. - that should be present".

Rather than comparing data, real or virtual, among times or locations, an alternative approach is to apply measures that, in some sense, have expected values that reflect differences in environmental quality. Two indices, AZTI's Marine Biotic Index, AMBI (Borja et al., 2000;

2003) and taxonomic distinctness (Clarke and Warwick, 2001; Warwick and Clarke, 2001) adopt different approaches to setting reference conditions by using global or regional, rather than local data to establish baselines.

AMBI was designed to assess the environmental quality of European coastal waters by classifying their benthic macroinvertebrate species into five ecological groups on the basis of their known sensitivity to environmental stress. The designation of a species to an ecological group is drawn from the extensive literature on species in marine and transitional waters, supplemented by the consensus judgement of experts, with the index based on the relative abundances of species in each ecological group (Borja et al., 2000; Teixeira et al., 2010). The index has become an important element for assessing the ecological status of marine and transitional waters under the European Water Framework Directive, either alone or in combination with other metrics, such as species richness and Shannon-Wiener diversity (e.g. Borja et al., 2003; 2004; 2007; Blanchet et al., 2008). Based on survey data from a large number of sites in the north-eastern Atlantic, numerical limits for AMBI have been shown to reflect differences in ecological status, so that ecological status may be assigned using single samples, the ecological condition being based on a global comparison with other areas (Tweedley et al., 2014). Furthermore, in regions of the world where the sensitivity of species to pollution and disturbance is not well documented, calculating AMBI at the family level has proved effective (Tweedley et al., 2014). However, AMBI is essentially an indicator of organic enrichment and associated reduction in oxygenation of the sediments, properties that vary naturally and thus potentially confound any biotic response to anthropogenic contamination or disturbance (Wetzel et al., 2012; Tweedley et al., 2014; Brauko et al., 2015). Consequently, some authors have considered it an inappropriate tool for assessing disturbance levels in estuaries (Escavarage et al., 2004; Dauvin, 2007). The BENTIX index (Simboura and Zenetos, 2002) is essentially similar to AMBI, except that the species comprise three rather than five ecological groups. It is invariably correlated with AMBI and thus suffers from the same influences of natural variability.

Unlike AMBI and species richness measures, taxonomic distinctness measures of biodiversity are claimed to be relatively insensitive to natural changes in environmental conditions, but are sensitive to anthropogenic disturbance (Leonard et al., 2006). That paper included a preliminary analysis of benthic macroinvertebrate data collected as part of the United Kingdom's National Marine Monitoring Programme (NMMP), which is treated in much more detail here. Taxonomic distinctness indices, based on simple species lists (presence or absence of species, i.e. Δ^+ and Λ^+), provide a potential framework within which these measures can be tested for departure from expectation (see Warwick and Clarke, 2001). Variability in taxonomic distinctness, due to differences in natural environmental factors, generally falls within a predictable range, based on the null hypothesis that the species present are structured as if they are a random selection from the regional species pool. This expectation then becomes the baseline against which biodiversity change is determined, the concept of spatial or temporal baselines thus being replaced by the concept of a 'reference condition'. This potentially establishes a baseline in a region that is entirely impacted to some degree, and where no appropriate reference sites are available. To date, taxonomic distinctness has not been included in many papers comparing a plethora of other biotic indices employed to assess the environmental quality of estuaries (Hutton et al., 2-15; e.g. Cardoso et al., 2012; Wetzel et al., 2012; Nebra et al., 2014; Brauko et al., 2015).

The aim of this study was to compare the inferences drawn from AMBI and taxonomic distinctness for a very large and extensive benthic macroinvertebrate dataset, for which corresponding sediment contaminant data (i.e. toxic heavy metal concentrations) were also available. If any of the indices are to be a useful indicator of environmental condition, they should clearly be correlated with pollution status. Although Shannon-Wiener diversity has no reference condition, the relative performance of this very widely used index is also included for comparative purposes.

Material and Methods

Source of data

The data for benthic macroinvertebrates and metal concentrations employed in this study were obtained as part of the UK's NMMP. The NMMP benthic survey has involved a massive sampling and analytical effort by several teams of workers in the different regions. While the methodology has been standardized as far as possible, this standardization is not perfect. Five replicate samples of benthic macroinvertebrates were collected using 0.1 m^2 Day grabs or box corers at each of 67 locations in estuarine and coastal marine areas around the UK coast on one, two or three occasions between February and June in 1999, 2000 and 2001 (CEFAS, 2004). The concentrations of chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), mercury

(Hg) and lead (Pb), together with that of aluminium (Al), were measured in five replicate samples from 61 of the 67 sites. Thus, composite faunal and metal data were available for 61 sites. These sites comprised 27 subtidal sites in 16 estuaries (Forth, Tweed, Tyne, Wear, Tees, Humber, Thames, Medway, Tamar, Pool Harbour, Severn, Dovey, Mawddach, Mersey, Bann and Lough Foyle) and 34 sites in coastal areas (Fig. 1). The faunal densities and metal concentrations of the five replicate samples from each site in each of the three years were averaged and used in the following analyses.

Sieve mesh sizes of either 0.5 or 1.0 mm were used for extracting the macrofauna from the sediment. As taxonomic distinctness metrics are based on presence/absence data, they would not be expected to be affected by this difference in mesh size. Furthermore, Warwick et al. (2006) found that the species diversity in samples collected from the same location and sieved at 0.5 and 1.0 mm did not differ.

Full details of field sampling and calculation of the metal concentrations are given in Davies et al. (2001). Benthic macroinvertebrates were identified to the lowest possible taxonomic level, which was usually species (i.e. 92%).

Statistical analyses

Statistical analyses were undertaken to determine the following. 1) Do the values for 12 univariate biotic indicators and the faunal composition differ significantly between environments (estuarine and coastal areas) and years? 2) Are Shannon-Wiener diversity, AMBI, average taxonomic distinctness (Δ^+) and variation in taxonomic distinctness (Λ^+) correlated with heavy metal concentrations? 3) Do species belonging to the same AMBI ecological group exhibit similar patterns of density across estuaries? All statistical analyses were performed using a Beta test version of the PRIMER v7 multivariate statistics software package (Clarke et al., 2014a) with the PERMANOVA+ add on (Anderson et al., 2008), except for Pearson correlation coefficients which were calculated in MINITAB v16.

Biotic indicators in estuarine and coastal areas

PERMANOVA was employed to determine whether the values for 12 univariate biotic indicators in estuarine and coastal areas were significantly different. The 12 indicators were number of taxa, overall density of benthic macroinvertebrates, Shannon-Wiener diversity, Simpson's index, Δ^+, Λ^+, AMBI and the proportion of individuals assigned to each of the five AMBI ecological groups (I, II, III, IV and V).

The number of taxa and overall density of benthic macroinvertebrates (no. m^{-2}) and the Shannon-Wiener diversity, Simpson's index, Δ^+ and Λ^+ were calculated from the mean values for data for the five samples collected at each site in each year using the DIVERSE routine. Average taxonomic distinctness provides a measure of the average spread of species across higher taxa, whereas Λ^+ is a measure of the evenness of the spread of species across higher taxa (Warwick and Clarke, 1995; Clarke and Warwick, 2001). A low Δ^+ and/or high Λ^+ indicates that the fauna has responded to environmental perturbation (Warwick and Clarke, 1998). The same data matrix was also analysed using the AMBI software v5.0 to calculate, for each site, the AMBI score and the proportion of individuals assigned to each of the five AMBI ecological groups (I, II, III, IV and V). Note that the data for any sample, in which the percentage contribution of individuals from taxa not in the extensive AMBI database was ≥20%, was not included in the above analyses (Borja and Muxika, 2005).

The data for each of the above 12 univariate biotic indicators was subjected to two-way Permutational Analysis of Variance (PERMANOVA; Anderson et al., 2008) to determine whether the values for each of those measures differed significantly between the sites in estuarine and coastal areas and among the three years, and whether there was an interaction between environment and year. The null hypothesis that there was no significant difference was rejected if the significance level (P) was <0.05. Prior to undertaking these analyses, 'draftsman plots' of the values for each pair of univariate measures at each site were examined visually to assess whether the values for each variable were heavily skewed and, if so, which type of transformation would satisfy the assumption of homogeneity of variances. These plots demonstrated that overall density required a $\log_e$ (x+1) transformation and that the proportion of individuals assigned to each of the five AMBI ecological groups required a square-root transformation.

Faunal composition in estuarine and coastal areas

PERMANOVA, a non-metric multidimensional scaling (nMDS) ordination plot and a shade plot were employed to elucidate whether the composition of the benthic macroinvertebrate faunas in estuaries and coastal areas were different and, if so, the species that were responsible for those differences.

The average density of each invertebrate taxon at each of the 27 estuarine and 34 coastal sites in 1999, 2000 and 2001 was fourth-root transformed to down-weight the contributions of taxa with consistently relatively high values. The resultant data were then used to construct a Bray-Curtis resemblance matrix, which was subjected to a two-way PERMANOVA to determine whether species composition differed between environments (estuarine and coastal areas) and between years and whether there was an interaction between environment and year. As this test demonstrated that environment, but not year, was significant and that there was no interaction (see results), the average fourth-root transformed density of each invertebrate taxon at each estuarine and coastal site was used to construct a Bray-Curtis resemblance matrix, which was then subjected to non-metric multidimensional scaling (Clarke, 1993) to produce an ordination plot.

A shade plot, derived from the fourth-root transformed faunal data for each site (as above), was used to visualise the trends exhibited by the densities of the various taxa across the sites in estuarine and coastal areas. This shade plot is a simple visualisation of the frequency matrix, where a white space for a taxon demonstrates that the taxon was never collected, while the depth of shading from grey to black is linearly proportional to the density of that taxon (Clarke et al., 2014b; Valesini et al., 2014). As the dataset contained as many as 862 taxa, the taxa employed for this shade plot were restricted to those which contributed ≥10% to the overall density at any site, i.e. 29 taxa. There has been some reordering of the taxon and sample axes, as advocated by Clarke et al. (2014a), purely to aid visualisation, and with the samples from estuarine and coastal areas kept separate on the sample axis. Note, however, that such re-orderings are irrelevant to multivariate analyses of those samples.

Relationships between heavy metals and biotic indicators

Pearson correlation coefficients were used to determine whether Shannon-Wiener diversity, AMBI, Δ^+, Λ^+ and were correlated with levels of heavy metal contamination in estuaries, as defined by Principal Component Analysis (PCA). The relationship between the densities of taxa belonging to different AMBI ecological groups and the level of heavy metal contamination was explored using a shade plot and coherent species analysis.

As natural concentrations of metals can vary by several orders of magnitude, depending on the granulometry and mineral composition of the sediment (Turekian and Wedepohl, 1961; Wong and Moy, 1984), it is important to determine what proportion of each of Cr, Ni, Cu, Zn, Cd, Hg and Pb in the sediments originate from natural sources and what proportion is derived from anthropogenic sources. Aluminium is a major component of the aluminosilicate mineral fraction of sediments and is commonly used as a "normalizer" when assessing the degree of anthropogenic enrichment (Schropp et al., 1990; Ho et al., 2012; Mil-Homens et al., 2013). This is because its concentration is related to the geology of the sediment and is thus not affected by anthropogenic sources (Schropp and Windom, 1988). As the relative proportion of other metals to aluminum is relatively constant, the concentrations of those other metals are expressed as heavy metal:Al ratios (Schropp and Windom, 1988). By taking into account natural differences in mineralogy and granulometry, these ratios could then be used to compare the degree to which the sediments were contaminated by the seven toxic heavy metals as a result of anthropogenic activities.

The ratio of each of the seven toxic heavy metals to aluminum were normalized by subtracting the mean for that ratio across all sites from each of the individual values of that ratio at each site and then dividing by its standard deviation (Clarke et al., 2014a). This allows each of the heavy metals to contribute equally, despite differences in the magnitude of the ratio between individual heavy metals and aluminum. These pretreated data were then subjected to PCA to determine objectively the level of heavy metal contamination at each site using the PC1 scores (Leonard et al., 2006). Pearson correlation coefficients for each of Shannon-Weiner diversity, AMBI, Δ^+ and Λ^+ *vs* both PC1 and each of the seven heavy metal:Al ratios were calculated, together with their significance levels.

A second shade plot was produced to illustrate the trends exhibited by the densities (fourth-root transformed) of the taxa at each estuarine site vs the score on the PC1 axis for heavy metal contamination. This plot included data for all 67 species (and the few other taxa) that contributed ≥5% to the overall density at any one of the 27 estuarine sites and which together contributed as much as 92% to the total number of individuals sampled in estuaries. The 67 species were ranked according to their AMBI ecological groups from V at the top to I at the bottom and, within each score, according to their overall density across all estuarine sites. Estuarine sites were ranked from most to least contaminated, as determined by the PC1 scores and their AMBI disturbance classification calculated using the data for the full suite of 296 taxa recorded in estuaries.

Coherent species analysis (Somerfield and Clarke, 2013; Veale et al., 2014) was employed to determine whether the pattern of change in the relative densities of the 67 key species (i.e. those contributing ≥5% of the overall density at any site) was consistent across estuarine sites. This test identifies each group of species whose patterns of relative densities over samples (estuarine sites) are indistinguishable within a group and statistically significant from other such groups. As the pattern of relative densities of species belonging to different AMBI ecological groups should differ across estuarine sites (according to their levels of heavy metal contamination), species in the same AMBI ecological group should occur in the same coherent species group(s).

For coherent species analysis, the data matrix of the raw densities of each key species was standardised to account for differences in the overall densities among estuarine sites and employed to construct a resemblance matrix using a species-standardized form of Bray–Curtis similarity, *i.e.* Whittaker's index of association (Whittaker, 1952). This resemblance matrix was then subjected to hierarchical agglomerative clustering with group-average linking (CLUSTER) and an associated Similarity Profiles (SIMPROF) test employing the type 3 SIMPROF permutation procedure (Somerfield and Clarke, 2013).

Results

Biotic indicators in estuarine and coastal areas

The dataset comprised 862 taxa, predominantly identified to species (92%), belonging to 14 phyla, with 296 taxa from sites in estuaries, 784 from sites in coastal areas and 218 shared between these two environments (Table 1). Three major phyla, Annelida, Crustacea and Mollusca, comprised at least ~90% of the taxa in all cases. Only four of the total of 33 echinoderm species were found in estuaries, due to their well-documented intolerance of reduced salinity (Brusca and Brusca, 2003).

PERMANOVA demonstrated that number of taxa, overall density, Shannon-Wiener diversity, Simpson's index, AMBI and the contributions by individuals belonging to AMBI ecological groups I, II, III and V all differed significant between estuarine and coastal areas, but not between years, and that the interaction between environment and year was also not significant (Table 2). Number of taxa, Shannon-Wiener diversity and Simpson's index were all greater at coastal than estuarine sites, while overall density and AMBI were greater in estuaries (Fig. 2). The greater AMBI scores in estuarine than coastal areas was due to individuals belonging to AMBI ecological group V making a greater contribution in estuaries, whereas the reverse was true with the contributions by individuals in ecological groups I, II and III (Fig. 2). PERMANOVA demonstrated, however, that Δ^+, Λ^+ and the contribution of individuals in AMBI ecological group IV did not differ significantly between estuarine and coastal areas and, in none of these cases, was year or the interaction between environment and year significant (Table 2).

Faunal composition in estuarine and coastal areas

Two-way PERMANOVA demonstrated that the compositions of the benthic macroinvertebrate faunas differed significantly between the sites in estuarine and coastal areas (P = 0.001), but not between years (P = 0.775), and that the interaction between environment and year was not significant (P = 0.829). These differences are clearly identifiable visually on the nMDS ordination plot, derived from the fourth-root transformed densities of all taxa, with the points for the estuarine sites lying on the left side and showing limited overlap with those for the coastal areas on the right (Fig. 3). The trends exhibited on the shade plot, derived from the fourth-root transformed densities the 29 taxa that contributed ≥10% of the overall density at any site averaged across the three years, illustrate which taxa are particularly important in distinguishing between the faunas in estuaries and coastal areas (Fig. 4). Thus, from the shade plot, it is evident that the difference between environments is due, in particular, to the presence of a greater proportion of opportunistic annelid species with high AMBI scores in estuaries than coastal areas, with four oligochaete species of the genus *Tubificoides* and the polychaete *Capitella capitata* complex particularly abundant in the former environment. A few species, such as the errant polychaete *Nephtys hombergii,* occurred in approximately similar densities in the two environments, while others were more frequently present or only recorded in coastal areas, e.g. *Nephtys cirrosa* and the amphipod *Haustorius arenarius,* respectively (Fig. 4). This latter species is characteristic of clean mobile sands and has a low AMBI ecological group score. Despite these differences in species composition and their characteristic AMBI scores, and as already noted, Δ^+ and Λ^+ do not differ significantly between sites in estuarine and coastal areas (Fig. 2).

Relationships between heavy metals and biotic indicators

The mean and minimum concentrations of each of the seven heavy metals examined, expressed as mg kg^{-1} of dry sediment, were greater for estuaries than coastal areas and the same was true for the maximum of all metals except Cr (Table 3). Furthermore, the high maximum concentration of 281 for Cr was due to the extreme value for one of the 34 sites, with the values for all other sites less than 218.

Shannon-Wiener diversity was highly correlated with number of taxa and Simpson's index, with Pearson's correlation coefficient values of 0.70 and 0.89, respectively, and is thus subsequently used as a proxy for those two indices. The PCA plot for heavy metals:Al ratios shows a group of estuarine and coastal sites with overlapping PC1 scores, and a number of estuarine sites with exceptionally low scores (i.e. high heavy metal:Al ratios), reflecting the range of values being far larger for estuaries than coastal areas (Fig. 5). The PC1 axis explained 51% of the total variation in high heavy metal:Al ratios and PC2 26%, with each of the other axes contributing less than 9% of the variation.

For estuaries, the Pearson correlation between Shannon-Wiener diversity and the PC1 score was low and non-significant and the same was true for AMBI (Table 4). In contrast, there was a high and significant correlation with both Δ^+ and Λ^+, with the Pearson correlation coefficients demonstrating that Δ^+ increased with decreasing heavy metal concentration, i.e. positive scores with PC1, while the reverse was true for Λ^+, i.e. negative scores with PC1 (Table 4).

The correlation between Shannon-Wiener diversity and each of the seven individual heavy metal:Al ratios at the estuarine sites was low and non-significant, as it also was for AMBI (Table 4). In contrast, the correlation between Δ^+ and each of Cu, Zn, Cd, Hg and Pb was significant and the same was true for the correlation between Λ^+ and each of Cr, Ni, Cu, Cd and Hg. Thus, one or both of these two taxonomic distinctness indices were significantly correlated with the values for each of the seven heavy metals:Al ratio.

The relationship with the PC1 scores for sites in coastal areas was not significant with either Shannon-Wiener diversity (r = 0.118; p = 0.505) or AMBI (r = -0.124; p = 0.485). Although Δ^+ or Λ^+ were also not significantly correlated with PC1, there was evidence of a weak relationship in the case of Δ^+ (r = -0.315; p = 0.069). It should be recognized, however, that the sediment heavy metal concentrations at these sites, which covered a narrow range, were low (Fig. 5) and thus unlikely to have a conspicuous impact on the benthic macroinvertebrate fauna.

A shade plot showing the 67 key species ordered by decreasing AMBI ecological groups (species with the same score ordered by decreasing overall density) against estuarine sites ordered by decreasing heavy metal contamination (i.e. increasing PC1 scores) showed little evidence that species belonging to high AMBI ecological groups were more abundant in the more contaminated sites or vice versa (Fig. 6). In fact, several species were present in the majority of sites, despite their allocation to different AMBI ecological groups, e.g. *Tubificoides pseudogaster* and *Tubificoides benedii* (V), *Ophryotrocha hartmanni* and *Tharyx* sp. (IV), *Streblospio shrubsolii* and *Hydrobia ulvae* (III) and *N. hombergii* (II) (Fig. 6).

Coherent species analysis assigned the subset of 67 species into four main groups, *i.e.* those containing more than two taxa (Fig. 7), based on their patterns of relative densities across the estuarine sites. When the species on the dendrogram were coded according to their AMBI ecological group, there was no evidence of species belonging to the same group occurring predominantly in the same coherent species group. Instead, species classified in AMBI ecological groups of II-V were spread across most of the coherent species groups (Table 5). There were only five species belonging to AMBI ecological group I in the subset of 67 key species.

Discussion

Taxonomic distinctness metrics are measures of biodiversity that have a wide range of applications and, unlike many environmental indicators such as AMBI, BENTIX, BQI and BOPA, were not developed specifically for environmental impact assessment, although they have been widely used in this context (Warwick and Clarke, 1998). Average taxonomic distinctness and variation in taxonomic distinctness, in particular, have been used to study relatively large-scale patterns of biodiversity in various groups of organisms, assess the effects of various anthropogenic impacts on biodiversity, evaluate the representativeness of surrogates for biodiversity estimation and (by comparison of recent and fossil death assemblages) predict the effects of climate change (see reviews by Warwick and Clarke, 2001; Leonard et al., 2006; Warwick, 2008). In summary, the advantages of these measures are that they are independent of sample size or sampling effort and are minimally affected by small variations in habitat type.

They can be used for data consisting simply of species lists rather than quantitative measures of abundance, which makes sample analysis less labour intensive and hence less expensive. A regional reference condition can be set, from which significant departures can be determined.

This study has clearly demonstrated that, for a wide range of estuaries around the UK, taxonomic distinctness indices, based on species presence/absence data, overcome the problems that have beset other indicators commonly used to evaluate the environmental quality of estuaries. These indices are unaffected by the naturally stressful conditions found in these systems, but are sensitive to contaminant loadings, at least with respect to toxic heavy metals. Whether this holds true for other contaminants (e.g. polyaromatic hydrocarbons [PAHs] and polychlorinated biphenyl [PCBs]) requires investigation.

The known toxicity of heavy metals was sufficient to elicit, in the concentrations recorded in the sediments sampled in this study, an impact on the benthic macroinvertebrate fauna. However, each species will have its own degree of sensitivity to each metal, and since both metals and species are found in the samples in different combinations, it is difficult to generalize regarding cause and effect relationships. Background Reference Concentrations (BRCs) for contaminants in seawater, sediment and biota were adopted by OSPAR in 1997 as tools for assessment in Quality Status Reports, and were developed by examining typical concentrations of both naturally occurring and man-made contaminants in the north-east Atlantic. For naturally occurring substances, the BRC is the range of concentrations that would be anticipated in the absence of any human activity. At the same time, Ecotoxicological Assessment Criteria (EACs) were also adopted by OSPAR, and are the concentrations of substances above which there may be impacts on biota. The BRCs and EACs for each metal in marine sediments both have a range of values which is relatively wide (Bignert et al., 2004). In the present study, median metal/aluminium ratios were elevated above the upper BRC at most sites, particularly in those of estuaries (CEFAS, 2004). Thus, of the 16 estuaries studied, arsenic concentrations exceeded the upper EAC in 15, lead in 10, mercury in 6, copper in 5, chromium in 5, cadmium in 3, nickel in 1 and zinc in none.

The contrasting environmental conditions present in estuaries and coastal areas naturally influence many attributes of the benthic macroinvertebrate fauna. The relative stability of coastal environments in terms of their consistent salinity and less physical disturbance of the sediment due to tidal and wave action leads to a higher species richness and diversity. In contrast, the higher productivity of estuaries (Schelske and Odum, 1961; Costanza et al., 2007) results in a greater faunal density, and their naturally high organic content and fluctuating salinity are reflected in the predominance of *r*-selected species that have high AMBI scores. Thus, in the present study, the percentage contributions of individuals belonging to AMBI ecological groups I, II and III are greater in coastal environments, whereas the reverse is true for those individuals in group V. Despite this, indicators utilising attributes of the structure and composition of benthic macroinvertebrate faunas are likely to remain at the forefront of methodologies for determining the biological quality of estuaries and coastal areas (Borja et al., 2009; 2013). In evaluating the suitability of the plethora of available indices, Dauvin (2007) makes the sound point that an increased frequency in using indices imposes an aura of respectability on those indices, which is self-perpetuating although they may not necessarily be the most valid or appropriate. This study has demonstrated that, in estuaries, it is important to consider why some indices may work and others do not. Indices, based on the relative sensitivities of taxa (i.e. AMBI, M-AMBI and BENTIX) may work well for species in marine situations where there are few natural stressors, but not in estuaries where the species are resilient to the natural variability in, for example, salinity, oxygen concentration, temperature and sediment dynamics, and thus score highly on scales of resilience. They can thus absorb anthropogenic stresses without adverse effects, which Elliott and Quintino (2007) term 'environmental homeostasis'. Such indices also suffer from the "more or less subjective, and sometimes arbitrary, aspect of the classification of species into different categories" (Dauvin, 2007) and a degree of circularity in such designations. Furthermore, more traditional species diversity indices are also strongly affected by natural environmental factors, which may confound the effects of any anthropogenic stressors.

The reason why taxonomic distinctness reflects anthropogenic rather than natural stress in estuaries must mean that, although species richness is lower in estuaries, the naturally stress-resistant species that are found there still belong to a wide range of higher taxa. Consequently, taxonomic distinctness does not differ between stressed areas and the unstressed areas often found in fully marine coastal environments. Anthropogenic stresses will then affect these taxa differently so that some become relatively more diverse (e.g. polychaetes) and others less so (e.g. crustaceans), thereby leading to a decrease in taxonomic distinctness and an increase in variation in taxonomic distinctness (Warwick and Clarke, 1993; Tweedley et al., 2012).

As so few indicators of anthropogenic perturbation seem to be applicable to estuaries, the search clearly needs to be widened. The debate on appropriate indicators in connection with the European Water Framework Directive, for example, seems to have narrowed to a few indicators for which no quantitative baselines can be established and which cannot distinguish between natural and anthropogenic perturbations. While almost all of the benthic indicators employed are based on abundance data of species or higher taxa, a few available indicators, which utilise both abundance and biomass data, might prove more appropriate. Once the fauna has been sorted into species and counted, biomass measurements for each species would represent a small extra step (simple blotted wet-weights would suffice). This would open more opportunities for assessing the extent of anthropogenic disturbance, for example the abundance/biomass comparison (ABC) method (Warwick, 1986; Warwick et al., 1987; Warwick and Clarke, 1994) or the phylum level meta-analysis (Warwick and Clarke, 1993; Savage et al., 2001; Somerfield et al., 2006). In the ABC method, separate *k*-dominance curves for species abundance and species biomass act as internal controls against each other, providing a snapshot of ecological condition that overcomes the need for reference samples in space or time. The phylum level meta-analysis compares the proportional ‘production’ of higher taxa (based on a combination of abundance and biomass) at a location with a training data set comprising a range of pollution/disturbance scenarios. Neither of these has been properly tested for estuaries, but intuitively it seems likely that, in both cases, the fauna from estuarine sediments that have not been subjected to anthropogenic perturbations will indicate a good ecological condition.

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Figure 3. Non-metric multidimensional scaling ordination plot for the composition of benthic macroinvertebrate taxa in estuaries (●) and coastal areas (△), derived from fourth-root transformed densities for each taxon in each sites, using data averaged for 1999, 2000 and 2001.

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Figure 6. Shade plot of fourth-root transformed densities (no. m^{-2}) of the 67 species that represented ≥5% of the overall density at any site in an estuary, using data averaged for 1999, 2000 and 2001. Grey scale represents the fourth-root transformed densities of each species (no. m^{-2}). Species on the y axis are ordered by their AMBI ecological group (decreasing down the plot), while sites on x axis progress from left to right according to their PC1 scores, ranging from high to low metal enrichment. The AMBI disturbance classification for each site is also provided.

Figure 7. Dendrogram derived by subjecting to CLUSTER-SIMPROF a Whittaker's index of association resemblance matrix constructed from the standardised densities of the 67 species that represented ≥5% to the overall density at any estuarine site. Coherent groups of species are denoted by the thick black lines, i.e. have statistically indistinguishable patterns of abundance across the estuarine sites and are significantly different from those in all other groups. Arabic numerals refer to main species groups (see Results). The AMBI ecological group to which each species belongs is also provided.

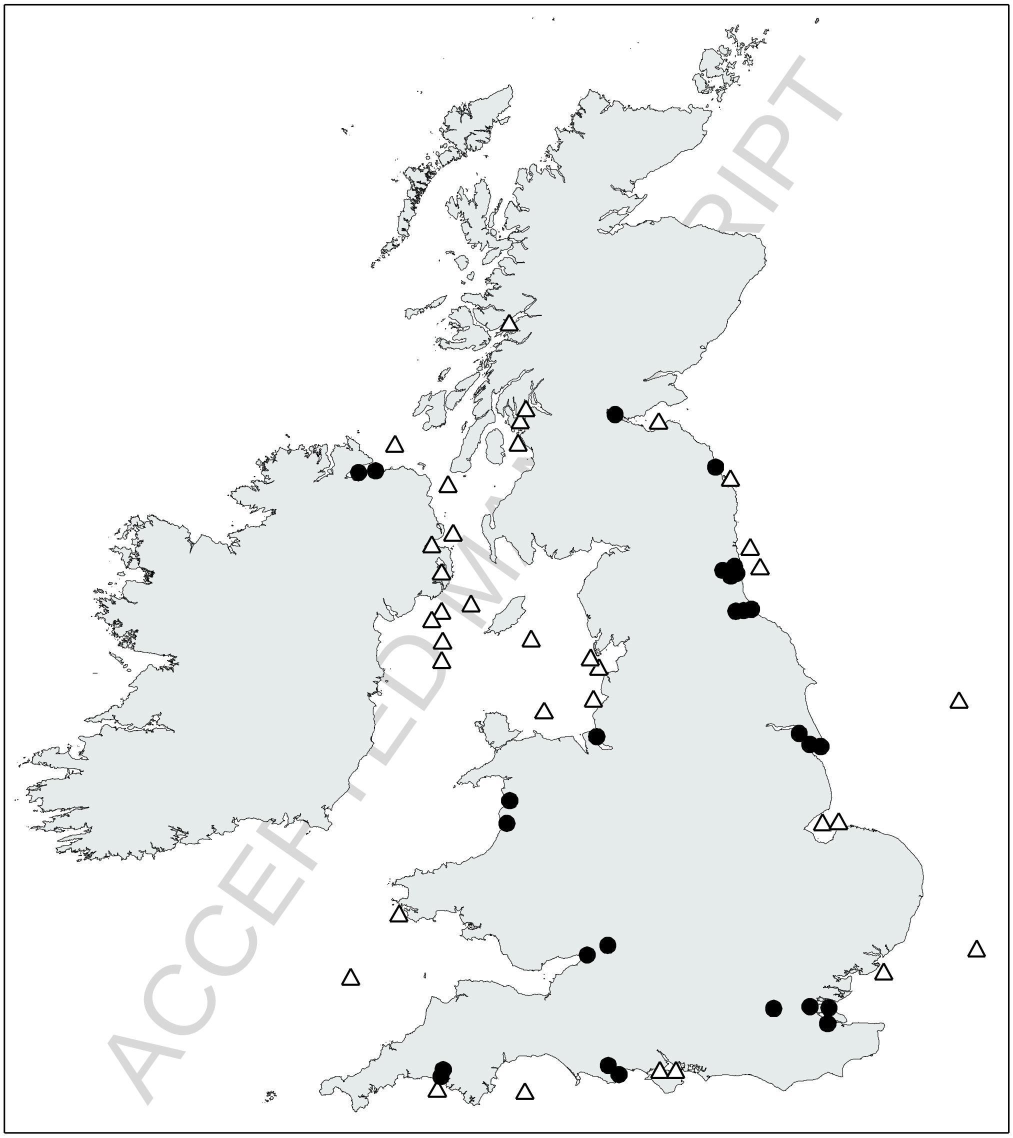
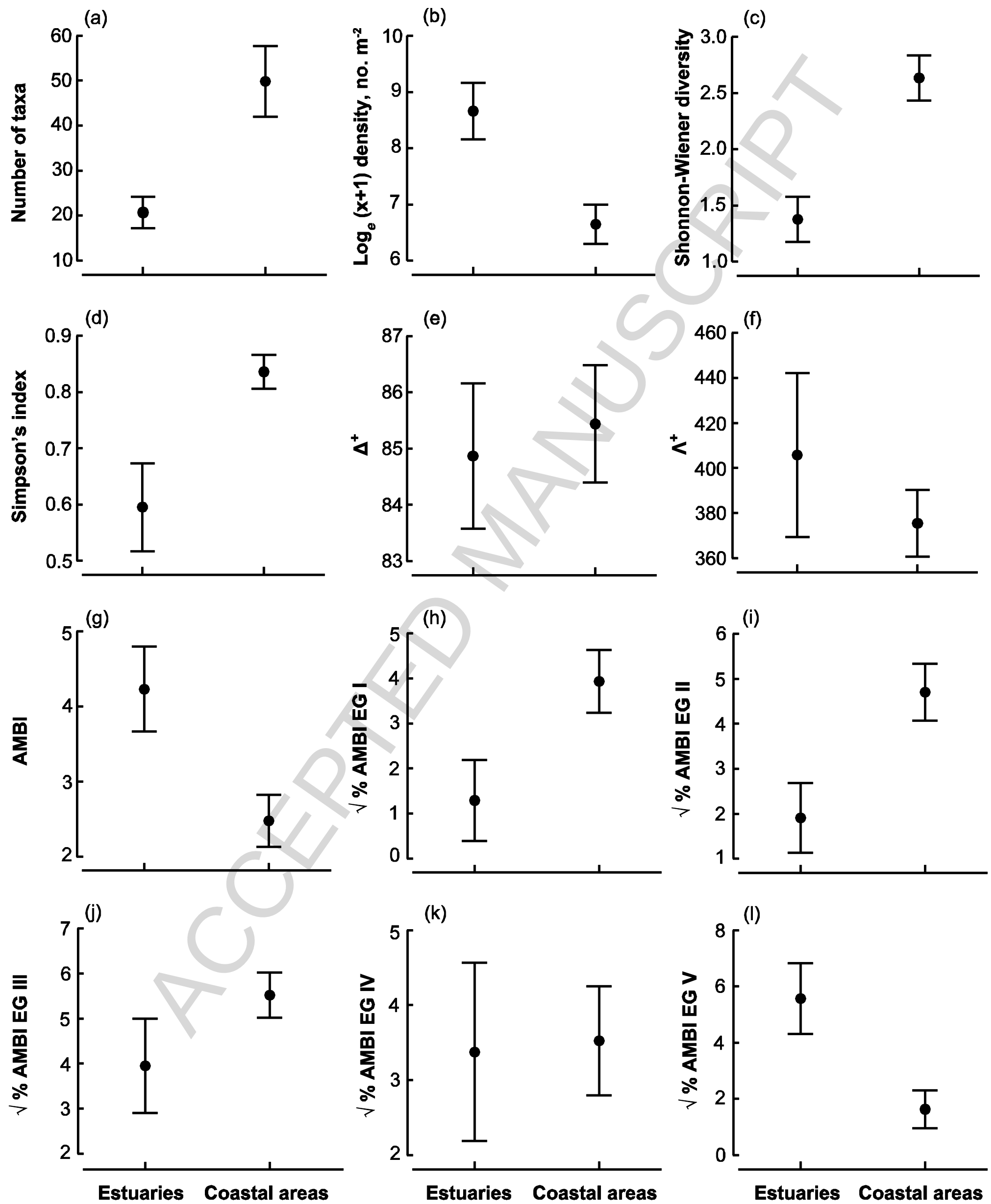


Figure 1.



(a)
Number of taxa
(b)
Loge (x+1) density, no. m-2
(c)
Shonnon-Wiener diversity
(d)
Simpson's index
(e)
Δ+
(f)
Λ+
(g)
AMBI
(h)
√ % AMBI EG I
(i)
√ % AMBI EG II
(j)
√ % AMBI EG III
(k)
√ % AMBI EG IV
(l)
√ % AMBI EG V
Estuaries
Coastal areas

Figure 2.

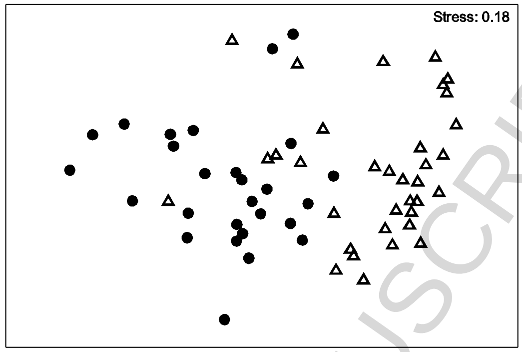
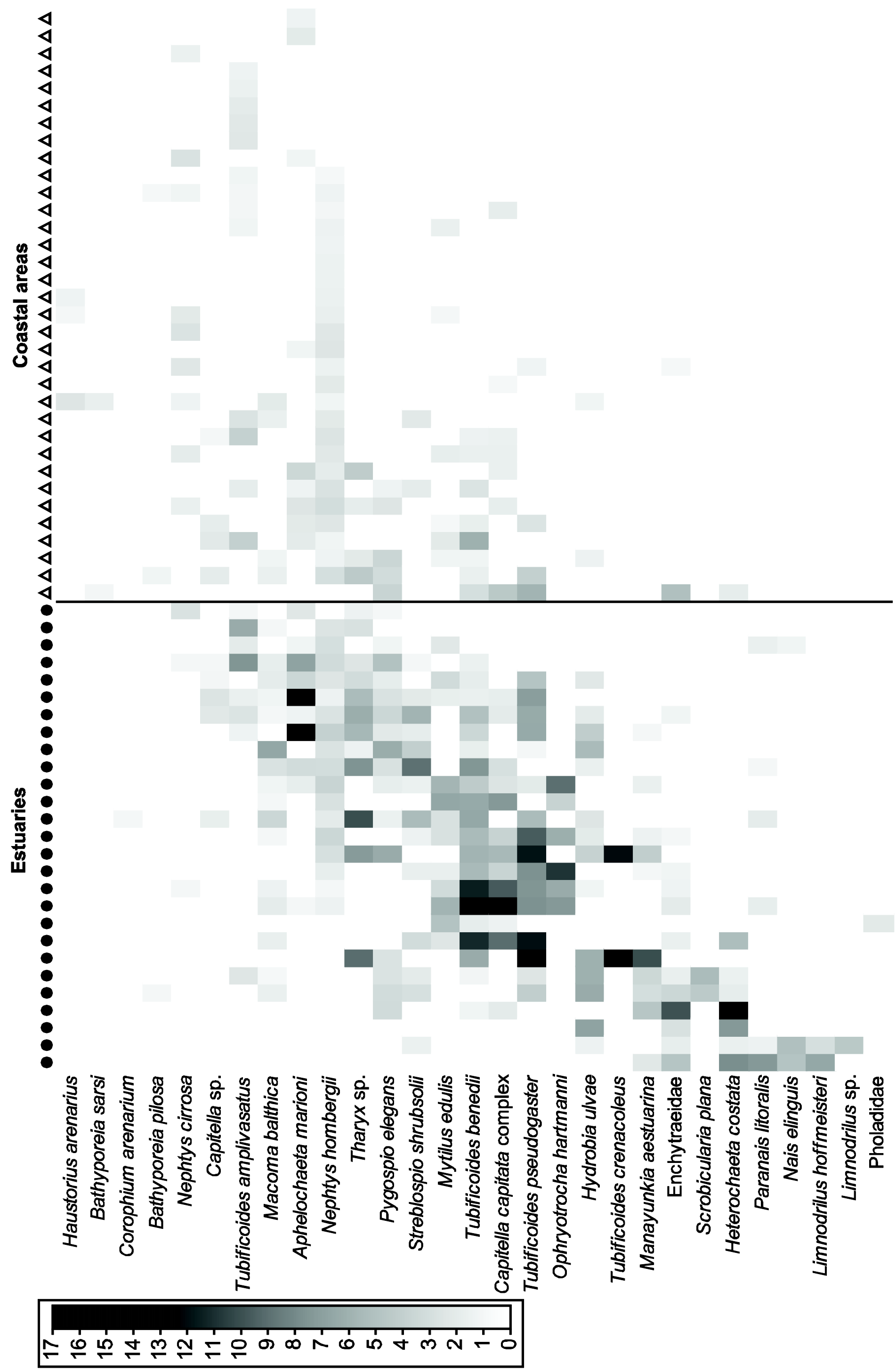


Figure 3.



Coastal areas
Estuaries
Haustorius arenarius
Bathyporeia sarsi
Corophium arenarium
Bathyporeia pilosa
Nephtys cirrosa
Capitella sp.
Tubificoides amplivasatus
Macoma balthica
Aphelochaeta marioni
Nephtys hombergii
Tharyx sp.
Pygospio elegans
Streblospio shrubsolii
Mytilus edulis
Tubificoides benedii
Capitella capitata complex
Tubificoides pseudogaster
Ophryotrocha hartmanni
Hydrobia ulvae
Tubificoides crenacoleus
Manayunkia aestuarina
Enchytraeidae
Scrobicularia plana
Heterochaeta costata
Paranais litoralis
Nais elinguis
Limnodrilus hoffmeisteri
Limnodrilus sp.
Pholadidae
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0

Figure 4.

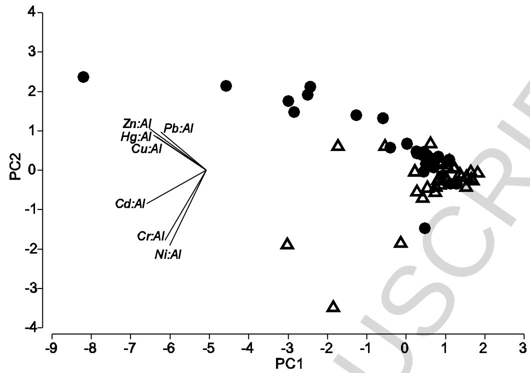


Figure 5.

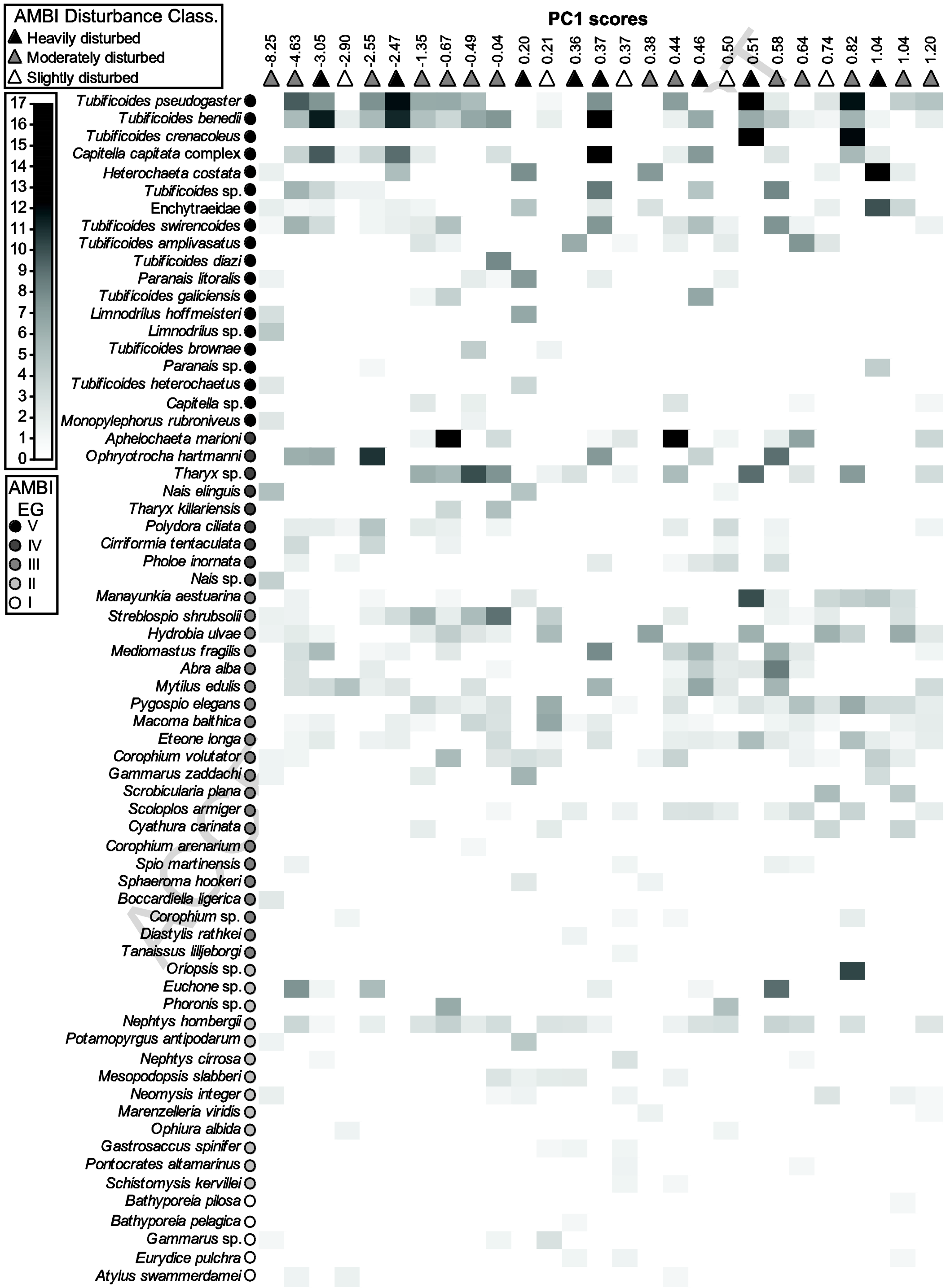
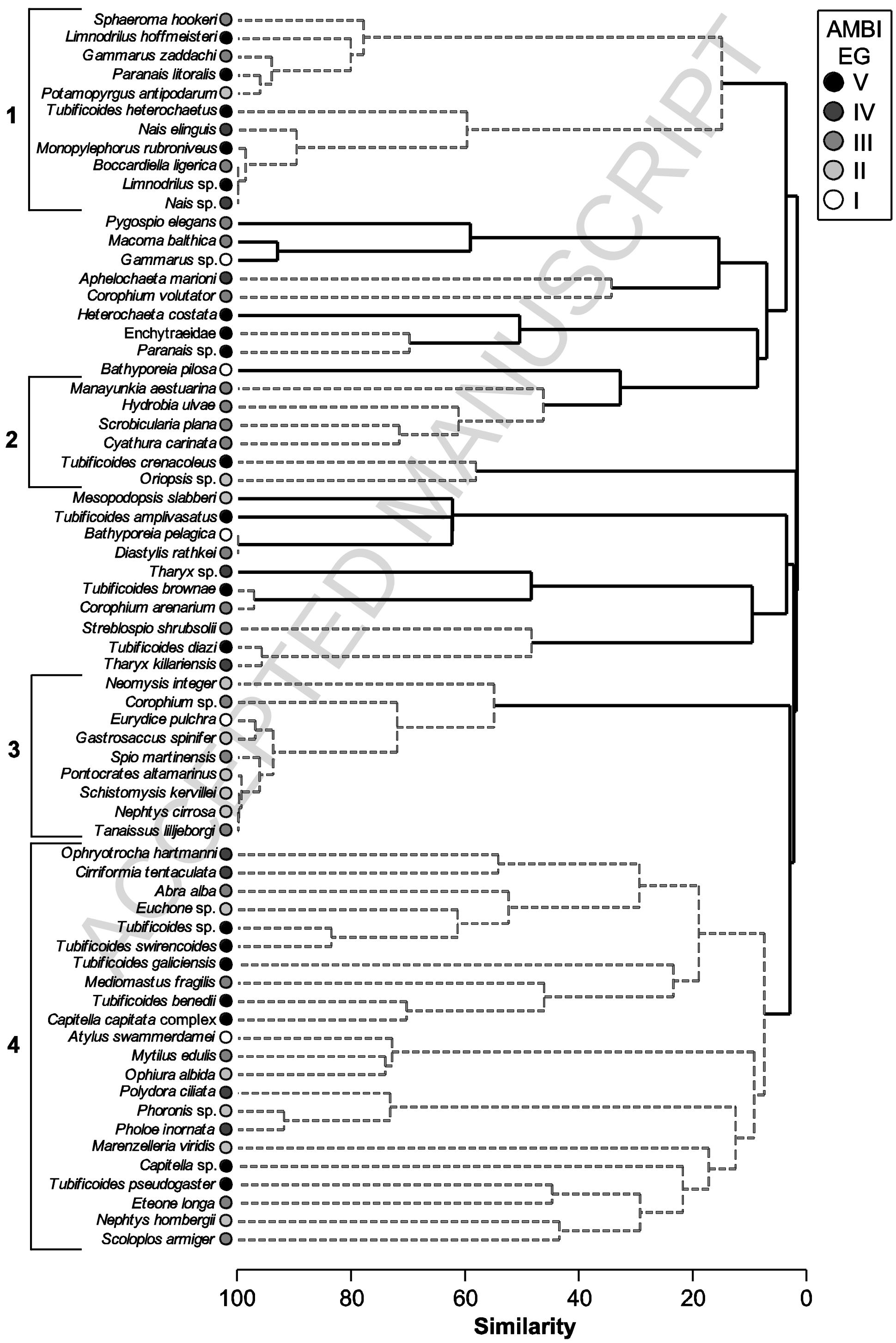

AMBI Disturbance Class.
Heavily disturbed
Moderately disturbed
Slightly disturbed
PC1 scores
-8.25 -4.63 -3.05 -2.90 -2.55 -2.47 -1.35 -0.67 -0.49 -0.04 0.20 0.21 0.36 0.37 0.37 0.38 0.44 0.46 0.50 0.51 0.58 0.64 0.74 0.82 1.04 1.04 1.20
17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
AMBI EG
V
IV
III
II
I
Tubificoides pseudogaster
Tubificoides benedii
Tubificoides crenacoleus
Capitella capitata complex
Heterochaeta costata
Tubificoides sp.
Enchytraeidae
Tubificoides swirencoides
Tubificoides amplivasatus
Tubificoides diazi
Paranais litoralis
Tubificoides galiciensis
Limnodrilus hoffmeisteri
Limnodrilus sp.
Tubificoides brownae
Paranais sp.
Tubificoides heterochaetus
Capitella sp.
Monopylephorus rubroniveus
Aphelochaeta marioni
Ophryotrocha hartmanni
Tharyx sp.
Nais elinguis
Tharyx killariensis
Polydora ciliata
Cirriformia tentaculata
Pholoe inornata
Nais sp.
Manayunkia aestuarina
Streblospio shrubsolii
Hydrobia ulvae
Mediomastus fragilis
Abra alba
Mytilus edulis
Pygospio elegans
Macoma balthica
Eteone longa
Corophium volutator
Gammarus zaddachi
Scrobicularia plana
Scoloplos armiger
Cyathura carinata
Corophium arenarium
Spio martinensis
Sphaeroma hookeri
Boccardiella ligerica
Corophium sp.
Diastylis rathkei
Tanaissus lilljeborgi
Oriopsis sp.
Euchone sp.
Phoronis sp.
Nephtys hombergii
Potamopyrgus antipodarum
Nephtys cirrosa
Mesopodopsis slabberi
Neomysis integer
Marenzelleria viridis
Ophiura albida
Gastrosaccus spinifer
Pontocrates altamarinus
Schistomysis kervillei
Bathyporeia pilosa
Bathyporeia pelagica
Gammarus sp.
Eurydice pulchra
Atylus swammerdamei

Figure 6.



AMBI EG
V
IV
III
II
I
1
Sphaeroma hookeri
Limnodrilus hoffmeisteri
Gammarus zaddachi
Paranais litoralis
Potamopyrgus antipodarum
Tubificoides heterochaetus
Nais elinguis
Monopylephorus rubroniveus
Boccardiella ligerica
Limnodrilus sp.
Nais sp.
Pygospio elegans
Macoma balthica
Gammarus sp.
Aphelochaeta marioni
Corophium volutator
Heterochaeta costata
Enchytraeidae
Paranais sp.
Bathyporeia pilosa
2
Manayunkia aestuarina
Hydrobia ulvae
Scrobicularia plana
Cyathura carinata
Tubificoides crenacoleus
Oriopsis sp.
Mesopodopsis slabberi
Tubificoides amplivasatus
Bathyporeia pelagica
Diastylis rathkei
Tharyx sp.
Tubificoides brownae
Corophium arenarium
Streblospio shrubsolii
Tubificoides diazi
Tharyx killariensis
3
Neomysis integer
Corophium sp.
Eurydice pulchra
Gastrosaccus spinifer
Spio martinensis
Pontocrates altamarinus
Schistomysis kervillei
Nephtys cirrosa
Tanaissus lilljeborgi
4
Ophryotrocha hartmanni
Cirriformia tentaculata
Abra alba
Euchone sp.
Tubificoides sp.
Tubificoides swirencoides
Tubificoides galiciensis
Mediomastus fragilis
Tubificoides benedii
Capitella capitata complex
Atylus swammerdamei
Mytilus edulis
Ophiura albida
Polydora ciliata
Phoronis sp.
Pholoe inornata
Marenzelleria viridis
Capitella sp.
Tubificoides pseudogaster
Eteone longa
Nephtys hombergii
Scoloplos armiger
100
80
60
40
20
0
Similarity

Figure 7.

Table 1. Number of taxa in each phylum (#) and their percentage contribution (%) and cumulative percentage contribution (C%) to each phylum in estuaries and coastal areas and in both environments collectively. Phyla contributing >5% of the number of taxa are shaded in grey. Blank spaces denote that a phylum was not represented.

Phylum	Total			Estuaries			Coastal areas			Both		
	#	%	C%	#	%	C%	#	%	C%	#	%	C%
Annelida	382	44.3	44.3	155	52.4	52.4	349	44.5	44.5	122	56.0	56.0
Crustacea	224	26.0	70.3	68	23.0	75.3	199	25.4	69.9	43	19.7	75.7
Mollusca	168	19.5	89.8	53	17.9	93.2	152	19.4	89.3	37	17.0	92.7
Echinodermata	33	3.8	93.6	4	1.4	94.6	33	4.2	93.5	4	1.8	94.5
Cnidaria	14	1.6	95.2	2	0.7	95.3	12	1.5	95.0	0	0.0	94.5
Chelicerata	12	1.4	96.6	5	1.7	97.0	10	1.3	96.3	3	1.4	95.9
Sipuncula	8	0.9	97.6	2	0.7	97.6	8	1.0	97.3	2	0.9	96.8
Chordata	8	0.9	98.5				8	1.0	98.3			
Nemertea	5	0.6	99.1	3	1.0	98.6	5	0.6	99.0	3	1.4	98.2
Priapulida	2	0.2	99.3	1	0.3	99.0	2	0.3	99.2	1	0.5	98.6
Phoronida	2	0.2	99.5	2	0.7	99.7	2	0.3	99.5	2	0.9	99.5
Hemichordata	2	0.2	99.8				2	0.3	99.7			
Platyhelminthes	1	0.1	99.9	1	0.3	100.0	1	0.1	99.9	1	0.5	100.0
Echiura	1	0.1	100				1	0.1	100			
Total		**862**			**296**			**784**			**218**	

Table 2. Mean squares (MS), Pseudo-*F* (p*F*) values and significance levels (*P*) for two-way PERMANOVA tests, employing separate Euclidean distance resemblance matrices constructed from the (pre-treated) data for each of the 12 univariate biotic indicators, calculated from the densities of the various benthic macroinvertebrate taxa in the five samples collected from each of the 27 estuarine sites and 34 coastal areas in 1999, 2000 and 2001. The number of individuals belonging to each of the five AMBI ecological groups (I-V) is given as a percentage of the total number of individuals. df = degrees of freedom. Significant differences (P = <0·05) are highlighted in bold.

		Number of taxa			**Overall density (no. m-2)**			**Shannon-Wiener diversity (H′)**		
	df	MS	p*F*	*P*	MS	p*F*	*P*	MS	p*F*	*P*
Environment	1	**11965.00**	**15.690**	**0.002**	**26.58**	**9.296**	**0.002**	**19.62**	**66.418**	**0.001**
Year	2	375.77	0.493	0.605	6.57	2.298	0.109	0.06	0.215	0.818
Environment x year	2	1.12	0.001	0.996	5.15	1.803	0.158	0.35	1.180	0.307
Residual	130	762.58			2.86			0.30		

		Simpson's index			**Average taxonomic distinctness (Δ^+)**			**Variation in taxonomic distinctness (Λ^+)**		
	df	MS	p*F*	*P*	MS	p*F*	*P*	MS	p*F*	*P*
Environment	1	**0.78**	**31.757**	**0.001**	0.12	0.004	0.945	1697.40	0.210	0.573
Year	2	0.01	0.408	0.660	29.97	1.019	0.279	2100.90	0.260	0.713
Environment x year	2	0.05	1.937	0.162	28.20	0.959	0.332	881.50	0.109	0.884
Residual	130	0.02			29.40			8081.70		

		AMBI			**AMBI Group I (% contribution)**			**AMBI Group II (% contribution)**		
	df	MS	p*F*	*P*	MS	p*F*	*P*	MS	p*F*	*P*
Environment	1	**51.39**	**44.953**	**0.001**	**92.29**	**35.484**	**0.001**	**172.25**	**42.799**	**0.001**
Year	2	0.13	0.111	0.887	0.10	0.039	0.965	0.39	0.096	0.895
Environment x year	1	0.69	0.601	0.438	0.67	0.259	0.659	4.06	1.008	0.322
Residual	67	1.14			2.60			4.02		

		AMBI Group III (% contribution)			**AMBI Group IV (% contribution)**			**AMBI Group V (% contribution)**		
	df	MS	p*F*	*P*	MS	p*F*	*P*	MS	p*F*	*P*
Environment	1	**35.06**	**7.414**	**0.008**	1.58	0.205	0.667	**293.96**	**39.845**	**0.001**
Year	2	3.37	0.713	0.492	0.13	0.016	0.981	2.59	0.351	0.709
Environment x year	1	6.18	1.306	0.247	2.17	0.281	0.593	8.82	1.195	0.297
Residual	67	4.73			7.72			7.38		

Table 3. Mean ($\bar{x}$), standard error (SE), minimum (Min) and maximum (Max) concentrations of the seven heavy metals studied and of aluminium in the sediments of estuaries and coastal areas. Concentrations are expressed as mg kg^{-1} dry weight in the sediment.

	Estuaries				**Coastal areas**			
	$\bar{x}$	**SE**	**Min**	**Max**	$\bar{x}$	**SE**	**Min**	**Max**
Cr	101.10	10.38	41.98	265.80	93.06	9.25	20.59	281.00
Ni	38.13	2.75	17.05	77.90	33.76	2.45	9.55	75.63
Cu	69.58	8.12	19.60	233.01	26.43	7.23	3.28	70.80
Zn	256.57	23.80	98.08	757.70	113.87	21.21	22.88	212.67
Cd	0.84	0.18	0.14	5.91	0.50	0.16	0.06	1.60
Hg	0.65	0.14	0.04	5.01	0.21	0.12	0.03	0.76
Pb	130.24	18.31	22.27	536.10	47.59	16.32	11.04	132.40
Al	5.50	0.28	2.10	7.91	4.63	0.25	0.75	6.74

Table 4. Pearson correlation coefficients (*r*), and associated significance values (*P*), for the relationships between each of Shannon-Wiener diversity (H′), AMBI, average taxonomic distinctness (Δ^+) and variation in taxonomic distinctness (Λ^+) and both the PC1 scores and the ratios of seven heavy metals to aluminium in samples from estuaries. Significant correlations (P = <0.05) are highlighted in bold and shaded in grey.

	H′		**AMBI**		**Δ^+**		**Λ^+**	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
PC1	0.083	0.681	-0.173	0.389	**0.580**	**0.002**	**-0.547**	**0.003**
Cr:Al	-0.081	0.688	-0.037	0.854	-0.341	0.082	**0.392**	**0.043**
Ni:Al	-0.299	0.129	0.152	0.451	-0.344	0.079	**0.538**	**0.004**
Cu:Al	0.044	0.828	0.120	0.552	**-0.379**	**0.049**	**0.481**	**0.011**
Zn:Al	-0.187	0.350	0.191	0.339	**-0.388**	**0.046**	0.315	0.109
Cd:Al	-0.022	0.914	0.209	0.296	**-0.502**	**0.008**	**0.609**	**0.001**
Hg:Al	0.019	0.924	-0.027	0.894	**-0.454**	**0.017**	**0.516**	**0.006**
Pb:Al	-0.135	0.502	0.276	0.163	**-0.649**	**0.001**	0.252	0.205

Table 5. Number of key species belonging to each AMBI ecological group assigned to each of the four main coherent species groups and the groups comprising one or two species (Others) as identified by CLUSTER-SIMPROF (see Fig. 7).

	Coherent species group				
AMBI EG	**1**	**2**	**3**	**4**	**Others**
I	0	0	1	1	3
II	1	1	5	5	1
III	3	4	3	5	6
IV	2	0	0	4	3
V	5	1	0	7	6
Total	**11**	**6**	**9**	**22**	**19**

Highlights

- Faunal composition in estuaries and coastal areas differ markedly
- Taxonomic distinctness indices were similar in the two environments
- Shannon diversity and AMBI were not correlated with heavy metal contamination
- Indices of taxonomic distinctness were significantly correlated with heavy metals
- Taxonomic distinctness valid indicator of anthropogenic disturbance in estuaries