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Family-level AMBI is valid for use in the north-eastern Atlantic but not for assessing the health of microtidal Australian estuaries

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Abstract

Analysis of benthic macroinvertebrate samples at a higher taxonomic level than species, e.g. family, potentially provides a more cost-effective protocol for environmental impact assessments and monitoring as it requires less time, funds and taxonomic expertise. Using the AMBI database, species ecological group scores are shown to be coherent within families. Faunal data from a wide range of environmental impact scenarios in the north-eastern Atlantic demonstrate that AMBI calculated from mean values for families, exhibits a strong linear relationship with species-level AMBI, the correlation improving markedly by using square-root transformed rather than untransformed abundances. In many regions of the world, however, the sensitivity of benthic macroinvertebrates to environmental perturbations is unknown, precluding the use of AMBI for environmental assessments. Yet the families are essentially the same as in the AMBI database. The utility of family-level AMBI is tested using data for four south-western Australian estuaries previously subjected to environmental quality assessments, but where only 17 species of the 144 taxa are included in the AMBI database. Although family-level AMBI scores reflect differences in environmental quality spatially and temporally within an estuary, they do not follow variations in environmental quality among estuaries. Indeed, south-western Australia estuaries are numerically dominated by families with high AMBI scores, probably due to the detrimental effects of natural accumulations of organic material in estuaries with long residence times. As taxonomic distinctness follows trends in environmental quality among estuaries, as well as temporally and spatially within a system, it provides an appropriate substitute for assessing the ‘health’ of microtidal estuaries.

1. Introduction

AMBI (AZTI’s Marine Biotic Index) was designed to assess the environmental quality of European coastal waters by classifying the benthic macroinvertebrate species present 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 from marine and transitional waters, supplemented by the consensus judgement of experts, with the index based on the relative abundances of species in each group (Borja et al., 2000; Teixeira et al., 2010). A total of 6435 species or higher taxa currently comprise the database, of which 469 (i.e. ~7%) have not been assigned to an ecological group. The index has become one of the mainstays 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 diversity (e.g. Muxika et al., 2007; Borja et al., 2007, 2009; Blanchet et al., 2008).

While the database has increasingly included species from outside the north-eastern Atlantic and Mediterranean regions, i.e. North and South America and Asia (Muniz et al., 2005; Borja and Tunberg, 2011; Cai et al., 2011; Teixeira et al., 2012; Wu et al., 2012), there remain many parts of the world where its species are not included in the database and whose sensitivity to environmental stress is not known. For example, only 17 species out of 144 taxa in the present study of estuaries in south-western Australia, i.e. 11.8%, are included in the AMBI database, representing just 30.1% of the total number of individuals recorded in these systems. This precludes the use of species-level AMBI for environmental assessment in these regions (Edgar and Barrett, 2002; Tweedley et al., 2012; Forde et al., 2013). Such assessments are important in microtidal estuaries, such as south-western Australia, because their long residence times make them extremely vulnerable to environmental degradation, particularly in those systems.
that are closed for protracted periods (Ranasinghe and Pataratchi, 1998; Wolanski, 2007). Indeed, in several cases, these estuaries have been subjected to extreme anthropogenic effects (see later).

Although most of the species in less well-studied regions differ from those in the database, many of the families are the same. For example, 73 of the 91 families, i.e. 80.2%, representing 97.3% of the individuals found in the estuaries of south-western Australia, are included in the AMBI database. Thus, if species within the families in the database have coherent ecological group scores, it should be possible to use the average scores for families to calculate AMBI. Furthermore, sample analysis at a higher taxonomic level, such as family, potentially provides a cost-effective protocol for environmental impact assessment, as it would require far less time and taxonomic expertise. Indeed, there is often a considerable degree of redundancy in species-level data (Clarke and Warwick, 1998) and changes in taxonomic composition in relation to environmental perturbations are often evident at higher taxonomic levels (Warwick, 1988; Ferraro and Cole, 1990; Olsgard et al., 1997), including those of estuaries in south-western Australia (Wildsmith et al., 2008, 2011; Tweedley et al., 2012).

Analysis at a higher level might also ameliorate the confounding effects of species-level responses to natural environmental variables and allow the effects of anthropogenic disturbance to be more clearly detected (Warwick, 1988). However, the recent study of Bevilacqua et al. (2012) is not encouraging in this respect. Following tests, based on random simulations to ascertain the ability of higher taxa to detect variation in multivariate structure, these authors concluded that “taxonomic ranks higher than species may not provide ecologically meaningful information, because higher taxa can behave as random groups of species unlikely to convey consistent responses to natural or human-driven environmental changes”. This implies that the species within families may not have any degree of coherence in their ecological group scores in AMBI, which, from both ecological and evolutionary standpoints, is very counter-intuitive.

On the other hand, Forde et al. (2013) found that the Multivariate-AZTI Marine Biotic Index (M-AMBI), using data from three European locations, produced a good agreement between the number of stations allocated to each of five Environmental Status (ES) categories (High, Good, Moderate, Poor, Bad) when the index was calculated at the species, genus and family levels. Family AMBI scores were determined as median (rather than average) values for species in the AMBI database to allocate each family to an AMBI ecological group (I to IV), which could then be used to calculate station scores using AMBI software (http://ambi.azi.t.es). However, M-AMBI is a combination of the AMBI score, the number of species and Shannon–Wiener diversity. The values for each of these metrics were normalized using the highest and lowest values in the datasets for each of the metrics to set a scale from 1 to 0, which was then divided into the five ES categories using standard intercalibrated class boundaries (Muxika et al., 2007), adjusted slightly for the family level to maximize the agreement with the species level. This was not, therefore, a test of the validity of the family-level AMBI score itself, because agreement between taxonomic levels may be due to the two diversity components which are given equal weight. Forde et al. (2013) went on to use family-level AMBI to demonstrate that there were seasonal differences in the appointment of M-AMBI environmental status categories for stations in Hong Kong Harbour, using the status boundaries adjusted for family data, but of course these categories are not equivalent to those determined from the European data because they would have been normalized over a different absolute range of values. The approach we have adopted below specifically addresses the performance of family-level AMBI alone, ascribes true averages for family ecological scores, rather than allocating them to four discretised ecological groups, and enables comparison to be made between assessments of ecological status in the north-eastern Atlantic and Australia.

The initial part of this study tests the hypothesis that AMBI ecological scores within the families of benthic macroinvertebrates are more coherent than would be expected under random aggregation into ‘pseudo-families’ of the same group sizes as the real family structures. As this hypothesis proved valid, the average ecological group scores within each family were used to calculate the AMBI scores at the family-level and the performance of this index was then compared with species-level AMBI using a dataset for predominantly marine waters in the north-eastern Atlantic, which represented a wide range of levels and types of environmental degradation.

Since there was shown to be a good relationship between family and species-level AMBI, the question of whether family-level AMBI could be reliably used for the same purpose was explored, employing data for benthic macroinvertebrates in four south-western Australian estuaries in which the characteristics ranged markedly. This second component of the paper is thus prefaced by a thorough account of the main features of each of these estuaries and how they reflect anthropogenic activities, followed by a comparison of the results of AMBI with the known environmental conditions in each estuary and the data for a range of other metrics.

2. Coherence of ecological group scores within families

Table 1 lists the average and standard deviation of the ecological group scores (1–5) for those species in the 147 families in the AMBI database that comprise ten or more species. The p₁% value in that table represents the results of a simulation test of whether the average score is lower than expected from the random selection of scores for the same number of species from the total database. They are one-sided p values, but this is logically a two-sided test, and thus values <2.5% indicate that the average observed score is less than expected under random assembly of species scores into families, and those >97.5% imply that the average is greater than expected for a two-sided test at the 5% significance level. The results show that the average score for 59 of the families was lower than would be expected from a random selection, and that 20 families have higher values (Table 1).

The p₁% value in Table 1 represents the results of the test of the standard deviation of observed species scores within a family against the random simulations. The test should be one-sided in this case and p values <5% thus indicate that 113 of the 147 species have species scores that are more tightly clustered within families than would be expected by chance. This provides very compelling evidence that, in the context of AMBI, the family structure is meaningful. As the tests are undertaken at the 5% level, only 5% of the 147 species, i.e. 7 or 8, would be expected to appear significantly non-random by this test, if species are being effectively grouped at random into families having no ecological coherence. However, since there is no information available on how ecological group scores have been assigned to individual species, the possibility that the consensus judgement of experts may have assumed coherence of scores of species within higher taxa cannot be excluded and, if truly independent data on each species were available, this coherence might not be so strong.

3. Calculation of family-level AMBI scores

The step from average ecological score to computation of an average AMBI value is straightforward, being the simple linear transformation:
Table 1

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Table 1 (continued)

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<td>16.3</td>
<td>0.65</td>
<td>Triphoridae</td>
<td>1.00</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Onuphiidae</td>
<td>1.71</td>
<td>4.1</td>
<td>0.46</td>
<td>Urothoidae</td>
<td>1.00</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Sabelidae</td>
<td>1.67</td>
<td>0.7</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AMBI = 1.5 (Average Ecological Score – 1)

The only exception here is that Borja et al. (2000) allocate an AMBI score of 7 to samples which are entirely defaunated, whereas we have chosen to work at a level of aggregation of samples and with data sets for which there are no empty samples, and thus this singularity does not arise.

Ecological and AMBI scores for each family are therefore non-integral, but this does not change the essence of the AMBI calculation: abundances for each family are treated as frequencies of the AMBI weight for that family, the frequencies being cross-multiplied with their matching AMBI weights, summed and divided by the sum of the unweighted frequencies, to obtain an average AMBI score for each sample. This calculation is exactly the standard one that would be undertaken to compute a mean from a frequency diagram (albeit the AMBI scores for each family are not equally spaced, as they would be in a histogram). The use, however, of a family ecological score, which is the average of the species ecological scores in that family, inevitably shrinks the range of observed AMBI values for any study, so that previously postulated boundaries determining impact status (Borja and Muxika, 2005) need to be redrawn. This is achieved by regressing family scores on species scores for a data set in which both are defined.

4. Comparison of species-level and family-level AMBI using NE Atlantic data

We have chosen to compare the species and family-level AMBI outputs, using the results of an analysis of data for marine benthic macroinvertebrate communities, which assessed the severity of disturbance employing a very different approach from that of most other ecological indicators (Warwick and Clarke, 1993a). The data set comprises the following: (1) Clyde sewage-sludge dump-ground. A transect of 12 stations sampled in 1983 on a west–east transect across a sewage sludge dump-ground in the Firth of Clyde, Scotland (Pearson and Blackstock, 1984). Stations in the middle of the transect show clear signs of gross pollution (Pearson, 1987; Warwick et al., 1987). (2) Lochs Linne and Eil. A time series of samples from 1963 to 1973 at stations in two western Scottish sea lochs (Lochs Linne and Eil) covering the commissioning of a pulp mill (Pearson, 1975). The pollution effects on the macrofauna increased in later years, except in 1973 when a recovery was noted in Loch Linne following a decrease in pollution loading (Pearson, 1975; Warwick, 1988). (3) Oslofjord. Samples collected at six stations in Frierfjord/Langesundfjord (connecting to Oslofjord), Norway (Gray et al., 1988). The macrofauna at three of the stations in the deeper basins of the fjord were considered to be influenced by seasonal anoxia. (4) Bay of Morlaix. Macrofaunal species were sampled at station ‘Pierre Noire’ on 21 occasions between April 1977 and February 1982, spanning the period of the wreck of the ‘Amoco Cadiz’ in March 1978 (Dawin, 1984). Although the sampling site was ~40 km from the initial tanker disaster, it was still subjected to substantial coastal oil slicks. In view of the large number of observations, the 21 sampling occasions have been aggregated into years: 1977 = pre-spill, 1978 = immediate post-spill and 1979–1981 = recovery period. (5) Skagerrak. Two stations in the Skagerrak at depths of 100 and 300 m (Josefson, 1981). The 300 m station showed signs of disturbance attributable to the dominance of the sediment-reworing bivalve Abra nitida (Josefson, 1981; Warwick et al., 1987). (6) Northumberland. An undisturbed silty sediment station located off the coast of Northumberland, north-eastern England (Buckman and Warwick, 1979). (7) Carmarthen Bay. An undisturbed sandy sediment station situated in Carmarthen Bay, southern Wales (Warwick et al., 1978). (8) Kiel Bay. An undisturbed station in Kiel Bay, Germany; mean of 22 sets of samples at the control station for the experimental study of Arntz and Runnolv (1982). This provides 50 samples, the disturbance status of which has been assessed by a variety of different methods including univariate diversity indices, dominance plots, ABC curves and measured contaminant concentrations.

Species abundances and biomasses were aggregated to phylum level and merged, using an allometric equation to form a ‘production’ matrix. Non-metric Multi-Dimensional Scaling Ordination (Clarke, 1993) of these data, combined in a single meta-analysis, produced a configuration with disturbance as its major axis. The value of the PC1 score for a Principal Components Analysis of the same data can then be interpreted as a disturbance index for comparison with AMBI values. This is the same dataset that was used to experiment with a variety of manipulations of the raw input data for determining species-level AMBI (Warwick et al., 2010). As a mild transformation (square-root) of the input data on species abundances in that study improved slightly the correlation between AMBI scores and the PC1 score of the meta-analysis, this transformation was also applied here.

In the current study, there is a strong linear relationship between family-level AMBI scores and the PC1 scores of the meta-analysis, with the R² for this relationship being improved from 0.70 to 0.81 when calculating AMBI using abundances that were square-root transformed rather than untransformed (Fig. 1). There is also a strong linear relationship between family-level AMBI and species-level AMBI, with the R² improved from 0.81 to 0.90 when employing square-root transformed rather than untransformed abundances (Fig. 1). These improvements are not trivial and, in view of the very strong relationships between the square-root transformed abundances for calculating AMBI at the species and family-levels, we feel justified in using square-root transformed family abundances for determining whether AMBI is useful for evaluating the ecological status of estuaries in south-western Australia.

The AMBI score is often discretised into a small number of status categories; High, Good, Moderate, Poor and Bad (Borja and Muxika, 2005). The boundaries for such classifications need to be set appropriately at different points on the AMBI scale, depending on the formulation of the index. Comparing boundaries between status categories for AMBI based on raw species abundances (the usual formulation) with family-level AMBI, based on square–root transformed abundances (Fig. 2), changes the accepted values between High and Good from 1.2 to 1.4, from Good to Moderate from 3.3 to 2.2, from Moderate to Poor from 4.3 to 2.8 and from Poor to Bad from 5.5 to 3.6. Note that, in this case, an exponential regression provides a better fit to the data than a linear regression. Having demonstrated that family-level AMBI is a reliable surrogate for species-level AMBI in the north-eastern Atlantic, attention then...
focused on determining whether a family-level AMBI approach is also appropriate using data for the benthic macroinvertebrates in estuaries in south-western Australia.

5. Benthic macroinvertebrate faunas of estuaries in south-western Australia

The quantitative data for benthic macroinvertebrate species in four estuaries in south-western Australia (Fig. 3) have been used as indicators of environmental deterioration in these systems (Platell and Potter, 1996; Wildsmith et al., 2009, 2011; Tweedley et al., 2012). Two indicators, i.e. taxonomic distinctness and the proportions of certain higher taxa, proved to be particularly reliable for comparing the environmental conditions within an estuary in different decades, with their values being consistent with other indicators of environmental degradation, such as multivariate metrics of temporal and spatial variability in species composition.

In the case of the proportions of higher taxa, the contributions to the total fauna by crustaceans, a group particularly susceptible to environmental stress and by the more tolerant polychaetes (Reise, 1982; Warwick and Clarke, 1993a; Dauvin and Ruellet, 2007) were especially important. Differences between the values for these...
biotic indicators reflect differences in nutrient input from surrounding farmland and urban development, and thus of eutrophication, and in the multiple influences of greatly increased urbanization, the relative effects of which vary among estuaries (see later). The latter pronounced variations in degradation among estuaries is emphasised by the comparisons between Broke Inlet and the Peel-Harvey Estuary. Thus, Broke Inlet and its catchment are located in a national park and this estuary is therefore regarded as pristine, whereas the Peel-Harvey Estuary became so eutrophic that it stimulated a multimillion dollar investment into firstly understanding the problem and then constructing a large entrance channel to ameliorate those deleterious effects.

5.1. Peel-Harvey Estuary

A major artificial channel was constructed between the large microtidal Peel–Harvey Estuary on the lower west coast of Australia and the Indian Ocean to greatly increase tidal exchange and thereby ameliorate the problems of extreme eutrophication (McComb and Lukatelich, 1995). The opening of this channel in 1994 led to a reduction in macroalgal biomass, and thus of particulate organic matter, and essentially eliminated the prolific blooms of Nodularia spumigena by elevating salinities in the spring to levels that were not conducive to the germination of seeds of this cyanobacterium (Lukatelich and McComb, 1986; Hearn and Robson, 2000).

Comparisons of data for benthic macroinvertebrates collected at the same four sites in 1986/7 and 2003/4, and thus eight years before and ten years after the above extreme modification of the Peel-Harvey Estuary, strongly indicated that, contrary to managerial expectations, the benthic environment had deteriorated since the construction of the channel (Wildsmith et al., 2009). Thus, although species richness increased due to an influx of marine species, taxonomic distinctness declined at each sampling site in each season. Furthermore, the variability in species composition among replicate samples at each site increased, a trend regarded as
symptomatic of environmental stress (Warwick and Clarke, 1993b).
Moreover, temporal (seasonal) variability in species composition
also increased at each sampling site, reflecting a decrease in community
stability, which is likewise considered indicative of such stress (Warwick et al., 2002).

The abundance and number of species of crustaceans declined
between 1986/7 and 2003/4, which, at the phylum level, was the
most important feature that distinguished between the faunal
compositions in these two periods. The Annelida, predominantly
represented by polychaetes, was the only phylum whose density and
number of species increased. The average number of polychaete
species at each site in each season also increased between 1986/7
and 2003/4. Molluscs, predominantly represented by bivalves, which
are moderately sensitive to environmental stress (Warwick and
Clarke, 1993a), declined in density and species richness between
the two periods. In combination, the above comparisons provide
compelling evidence of environmental deterioration. The over-
whelming evidence that the benthos of the Peel–Harvey Estuary has
become more stressed is presumably due to the multiple effects
produced by a massive expansion in the population of the city of
Mandurah, through which this estuary flows, and a greatly increased
use of this system (Wildsmith et al., 2009).

5.2. Swan-Canning Estuary

The benthic macroinvertebrate fauna of the large central basin
region of the Swan-Canning Estuary, just to the north of the Peel-
Harvey Estuary (Fig. 3), changed between 1986/7 and 2003/4 in
ways consistent with a deterioration in environmental conditions
as this estuary became more eutrophic, contaminated and
and disturbed (Wildsmith et al., 2011). Thus, the densities and species
richness of crustaceans declined, whereas those of polychaetes
increased. However, taxonomic distinctness declined consistently
only at one of the four widely-spaced sampling sites, suggesting
that the benthic fauna had undergone less extreme changes than in
the nearby Peel-Harvey Estuary.

5.3. Broke and Wilson Inlets

The composition of the benthic macroinvertebrate faunas in the
shallow (<1 m) and deeper (1–6 m) waters of the essentially prist-
tine, seasonally-open Broke Inlet on the south coast of Western
Australia differ radically at all taxonomic levels from those in cor-
responding waters of the nearby eutrophic, seasonally-open Wilson
Inlet (Tweedley et al., 2012). Crustaceans, the most sensitive of all of
the main taxa to environmental stress (Reise, 1982; Warwick and
Clarke, 1993a), thus contributed more to the total numbers of
benthic macroinvertebrates in the Broke than Wilson inlets, whereas
the opposite was true with the less sensitive polychaetes. Further-
more, average taxonomic distinctness was greater in Broke Inlet than
in Wilson Inlet and 16 other estuaries in temperate areas elsewhere
in the southern hemisphere, whereas the reverse pertained with
variation in taxonomic distinctness (Tweedley et al., 2012). This is
consistent with the conclusion that Broke Inlet is essentially pristine
(Commonwealth of Australia, 2002). In contrast, Wilson Inlet has
become increasingly eutrophic over the last 40 years and is charac-
terised by the presence of very substantial growths of the seagrass
Ruppia megacarpa (Dudley et al., 2001; Department of Environment,
Western Australia, 2003; Wilson Inlet Catchment Committee, 2013).

6. Metrics employed for analysing data from south-western
Australian estuaries

Values for eight univariate metrics and family-level AMBI scores
were calculated for the benthic macroinvertebrate faunas in the
Peel-Harvey and Swan-Canning estuaries and the Broke and Wilson
inlets (Table 2). Shallow waters of the Peel-Harvey and Swan-
Canning estuaries were each sampled seasonally in two periods,
i.e. 1986/7 and 2003/4 (Wildsmith et al., 2009, 2011), whereas the
shallow and deeper waters of the Broke and Wilson inlets were
sampled seasonally in a single period, i.e. 2007/8 and 1989/90,
respectively (Platell and Potter, 1996; Tweedley, 2011; Tweedley
et al., 2012).

Average taxonomic distinctness ($\Delta^*$), variation in taxonomic
distinctness ($\Delta^\ast$), Shannon diversity and Simpson’s index for the
benthic macroinvertebrates of each estuary water depth/period
combination were calculated using the DIVERSE routine in PRIMER
v6. Because the number of species was low in many replicate
samples, it was not sensible to calculate the above taxonomic
distinctness indices at the replicate level. These three indices, and
also total number of species, Shannon diversity and Simpson’s Index,
have thus been computed from the mean abundances of species for each estuary water depth/period combination.

Following recommendations by Borja and Muxika (2005)
regarding calculation of species-level AMBI, the family-level AMBI
scores have been calculated as the mean for the replicate scores for
each estuary water depth/period combination, rather than as the
scores for the mean abundance of each family for each combination.
However, one other recommendation in that paper is not followed,
i.e. removal of Insecta from samples where salinity is >10, because
insects were consistently collected in even the more saline regions
of these estuaries (Kanandjembo et al., 2001; Tweedley et al., 2012).

The mean densities of each species for each water depth/period
combination in each estuary were subjected to TAXDTEST (Clarke
and Gorley, 2006) to determine the ‘expected’ mean value and
95% probability limits for $\Delta^*$ and $\Delta^\ast$ in random subsamples of
different numbers of species drawn from the full suite of species
across all estuaries. The expected values and 95% probability limits
were then used to construct funnel plots, onto which the measured
values of $\Delta^*$ and $\Delta^\ast$ for each estuary were superimposed to identify
any significant departures from expectation (Warwick and Clarke,
2001). $\Delta^*$ is a measure of the average spread of species across
higher taxa, while $\Delta^\ast$ is a measure of the lack of evenness of the
spread of species across higher taxa. An increase in $\Delta^\ast$ and/or
decrease in $\Delta^*$ usually indicate that the fauna has responded to
environmental perturbation (Warwick and Clarke, 1995, 2001;
Clarke and Warwick, 2001).

A shade plot was produced to detect any differences in the
relative abundance of the various families in each estuary water
depth/period combination. This shade plot is a simple visualization
of the frequency matrix, where a white space for a family demonstrates
that the family was never collected, while the depth of shading from
grey to black is linearly proportional to the abundance of that family (Clarke et al., 2014; Valesini et al., in press).

7. Results and discussion of data from south-western
Australian estuaries

7.1. Taxonomic distinctness

The estuaries and their water depth/period combinations have
been aligned in descending order in Table 2 according to their
values for $\Delta^*$, a measure of the average spread of species across
higher taxa (see above). The rank order for the values of $\Delta^*$ in
descending order are as follows; Broke shallow waters 2008/09,
Broke deep waters 2008/09, Swan shallow waters 1986/7, Swan
shallow waters 2003/4, Wilson shallow waters 1989/90, Peel
shallow waters 1989/90 and Peel shallow waters 2003/4 (Table 2).
The value for $\Delta^*$ in shallow waters of Broke Inlet is the only one that lies above the mean in the funnel

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plot in Fig. 4 and the value for shallow waters of the Peel-Harvey in 2003/04 is the only one outside the 95% probability limits. The rank order for \( \Delta^+ \) largely follows the opposite pattern (Table 2), with the value for shallow waters of Broke Inlet lying below the mean, while the value for shallow waters in the Peel-Harvey in 2003/04 is the only one above the 95% probability limits of the funnel (Fig. 4). The values for \( \Delta^- \) and \( \Delta^+ \) for the deeper waters in Broke Inlet lie very close to the mean in their respective plots (Fig. 4a,b). The trends exhibited by the rank order for the measures of taxonomic diversity \( \Delta^- \) and \( \Delta^+ \) are consistent with the consensus view that the environmental quality of the estuaries range from Broke Inlet as the most pristine to the Peel-Harvey in 2003/4 as the most impacted and that the environmental quality of the Swan-Canning Estuary, and more particularly the Peel-Harvey Estuary, deteriorated between 1986/7 and 2003/4 (Table 2; Fig. 4).

7.2. Proportions of higher taxa

While the percentage contributions of crustaceans (\( %C \)) were high in both shallow and deep waters of Broke Inlet, further supporting the view that this estuary is pristine, they were similarly high in the less pristine Swan-Canning Estuary in 1986/7 (Table 2). However, the \( %C \) in the Swan-Canning declined appreciably between 1986/7 and 2003/4, which is consistent with the conclusion that the health of this estuary declined between these two periods (Wildsmith et al., 2011). The contribution of crustaceans in both the shallow and deeper waters of Wilson Inlet was exceptionally low and a small fraction of those recorded in all other estuaries (Table 2). The trends exhibited by the contribution of crustaceans across the four estuaries thus only partially follow those of \( \Delta^- \) and \( \Delta^+ \) and the values for the eutrophic Wilson Inlet are atypically low, as has been recorded for estuaries in eastern Australia that are closed from the ocean for considerable periods (Dye and Barros, 2005). As expected, the trends exhibited by the percentage contributions of annelids (\( %A \)) to the total fauna, and of course also to annelids and crustaceans collectively, \( %A/(A+C) \), are the reverse of that for the contribution of crustaceans (Table 2). While, as anticipated, both of these metrics were lowest for the pristine Broke Inlet and greater for the Swan-Canning and Peel-Harvey in 1986/7 than in 2003/4, they did not otherwise follow the trends exhibited by \( \Delta^- \) and \( \Delta^+ \), particularly in the case of Wilson Inlet.

7.3. Species diversity

The total number of species recorded in the Peel-Harvey in 2003/4, i.e. 63, was far greater than when using the same sampling regime in that estuary in 1986/7, i.e. 28 (Table 2), which is due to the presence of a greater number of marine species. These probably entered this estuary as planktonic larvae following the construction of the large artificial entrance channel in 1994 (Wildsmith et al., 2005). Otherwise, there was no consistent trend in numbers of species across the estuaries. This suggests that, when making broad comparisons between the benthic macroinvertebrate fauna of estuaries, species richness measures are not particularly useful in evaluating the extent of environmental degradation because they are confounded by the number of individuals sampled, the characteristics of the estuary, i.e. size and extent of environmental...
heterogeneity and connectivity with the ocean, and faunal biogeography (Dye and Barros, 2005; Dye, 2006; Tweedley et al., 2012). This is also true for temporal changes and spatial differences within individual estuarine system.

Shannon (H') and Simpson diversity indices (1−λ) remained essentially unchanged in the Swan-Canning Estuary between 1986/7 and 2003/4. In contrast, they both increased in the Peel-Harvey Estuary between these two periods, which reflected the very pronounced increase in number of species, presumably due to a greatly increased tidal exchange with the ocean following the opening of the large artificial entrance channel. The values for H' and 1−λ fall within narrow ranges of 2.23−2.44 and 0.83−0.88, respectively, in all but the shallows of the Peel-Harvey Estuary in 1986/7 and the deeper waters of Wilson Inlet in 1989/90 (Table 2). Thus, like %C, %A and %A/(A − C), the trends exhibited by H' and 1−λ do not follow those exhibited by Δ+ and Δ−. These last two metrics are the only ones that consistently reflect the known differences in environmental conditions among estuaries, and in the temporal and water depth differences within estuaries.

7.4. Family-level AMBI

Although the four estuaries (and their time periods) vary greatly in the extent to which they have been subjected to anthropogenic influences, such as those resulting from eutrophication, the AMBI scores ranged only from 2.11 to 2.89 (Table 2). Indeed, five of the eight AMBI scores corresponded to an AMBI status of Moderate. Moreover, the scores of 2.11 for shallow waters in the Swan-Canning in 2003/4 are on the borderline of Good/Moderate, i.e. 2.2, and those of 2.82 and 2.89 for shallow waters in the Peel-Harvey in 2003/4 and deeper waters of Wilson Inlet in 1989/90 are on the border of Moderate/Poor, i.e. 2.8. The narrowness of the range of AMBI scores for the estuaries of south-western Australia is emphasized by the fact that, when the north-eastern Atlantic meta-analysis data were subjected to the same family-level approach, the scores ranged widely from 1.2 to 4.8 and thus spanned all categories, i.e. High, Good, Moderate, Poor and Bad (Fig. 2).

The AMBI scores were lowest for the shallow waters of the Swan-Canning Estuary in 1986/7 and 2003/4, lying just below and above the borderline between Good and Moderate, respectively (Table 2), even though the region around this estuary is highly urbanized and particularly so in the more recent period. In contrast, the AMBI scores were greater for the shallow and deeper waters of Broke Inlet than the Swan-Canning Estuary and corresponded to only a Moderate status (Table 2), despite this estuary being essentially pristine (Commonwealth of Australia, 2002), with its entire catchment situated within the D’Entrecasteaux and Shannon National Parks and therefore not exposed to the effects of urban, industrial or agricultural activity (Breachley, 2005). Thus, on the basis of these comparative data, the environmental quality of Broke Inlet would appear to be less than that of the Swan-Canning Estuary, which is clearly not the case. The AMBI scores thus failed to reflect the known differences between the environmental quality among estuaries, and the environmental status categories indicated by these scores are not commensurate with the known characteristics of the fauna and catchments of these estuaries.

In the case of the north-eastern Atlantic meta-analysis data, an AMBI status of Moderate, i.e. equivalent to that of Broke Inlet, is found at sites associated with the effluent of paper-pulp mills in two Scottish sea-locks, at stations subjected to offshore sewage sludge dumping in the Firth of Clyde and at sites suffering from seasonal anoxia in Oslobor (Warwick et al., 2010). Clearly, in terms of anthropogenic environmental impacts, these detrimental effects far exceed those experienced by Broke Inlet, the most pristine of the 18 southern hemisphere microtidal estuaries compared by Tweedley et al. (2012).

It is relevant, however, that the AMBI scores within both the Swan-Canning and Peel-Harvey estuaries increased between 1986/7 and 2003/4, indicating that environmental quality in these two systems declined between those periods. This conclusion is consistent with the decrease in Δ+ and percentage contribution of crustaceans, and the increase in Δ− and percentage contribution of annelids. Furthermore, in the Broke and Wilson inlets, AMBI, Δ+ and percentage contribution of annelids increased between shallow and deep waters, whereas Δ− declined. Although the percentage contribution of crustaceans increased between shallow and deep waters in Broke Inlet, the trends exhibited by all of the other above metrics strongly suggest that environmental quality was less in the deeper than shallow waters of these systems. Such a conclusion is consistent with the marked tendency for organic material to accumulate in the deeper waters of these systems (Plattell and Potter, 1996; Tweedley, 2011).

A shade plot, in which the families are ordered by their AMBI scores, demonstrates that families with high AMBI scores are spread rather evenly across all sites and periods (Fig. 5). As the grey-scale key identifies, the abundances are first strongly transformed (by a fourth-root power) so that the plot allows the presence (shaded) or absence (white space) of all families to be instantly identified. A strong correlation on the relative abundance of the families present (Clarke et al., 2014). In this plot, the sites/years (across the plot) are ordered from left to right in line with their decreasing Δ+ scores, an indication of increasing levels of environmental stress, from the shallow waters of Broke Inlet on the left to those of the Peel-Harvey Estuary in 2003/4 on the right, and the families (down the plot) ordered by decreasing AMBI score.

Even the pristine sites to the left of the shade plot are dominated by families with high AMBI scores, and there is a similar vertical gradation in dominance from high to low scoring families for shallow and deeper waters in the Broke and Wilson inlets and for the two periods in the Swan-Canning and Peel-Harvey estuaries.

The reason why the four estuaries sampled in south-western Australia are each numerically dominated by high AMBI scoring families of benthic macroinvertebrates can be related largely to the particular characteristics of these systems. As they are microtidal and have narrow entrance channels, the amount of tidal water movement in the main body of the estuary is far less than in macrotidal systems. Furthermore, rainfall in this region is highly seasonal, typically occurring mainly from mid-autumn to mid-spring (Spencer, 1956). Residence time is thus strongly influenced by freshwater discharge and therefore longest in the dry late spring to early autumn months. It thus follows that residence times in these poorly-flushed estuaries are far longer than those of macrotidal systems, which are generally funnel-shaped and subjected to strong flushing during each tidal cycle (cf. Ranasinghe and Pattiaratchi, 1998; Uncle et al., 2002; Wolanski, 2007). Moreover, in seasonally-open estuaries, such as the Broke and Wilson inlets, there is no exchange with the ocean during those months when their mouths are closed.

Estuaries with long residence times are intrinsically less robust than more well-flushed estuaries because they facilitate the accumulation of contaminants. Furthermore, even when there are no anthropogenic inputs, naturally-occurring dissolved and particulate organic material from terrestrial sources accumulate in these systems and this can lead to a marked depletion in dissolved oxygen (Nixon et al., 1996; Josephson and Rasmussen, 2000; Wolanski, 2007). This problem is exacerbated in south-western Australian estuaries because residence times are longest in the warmer months when rainfall is low, and the problem will be particularly marked in those estuaries in which the mouth closes and thereby prevents flushing, such as in Wilson Inlet (Davis and Koop, 2006). High temperatures lead to an increase in the rates at which
particulate organic matter decomposes and dissolved nutrients are utilized, sometimes giving rise to phytoplankton blooms (Stoltenberg and Sobel, 1965). These naturally high levels of organic matter and oxygen depletion will encourage the success of families characterized by high AMBI scores and thus occur even in the essentially pristine Broke Inlet (Pearson and Rosenberg, 1978; Dauer et al., 1992). Such high AMBI scores elsewhere would be found in locations polluted by anthropogenic sources of organic matter. However, as in the north-eastern Atlantic, family-level AMBI might be useful for comparing the severity of environmental degradation in open coastal regions in Australia, where the particular characteristics of microtidal estuaries are not present.

In summary, the present study demonstrates that family-level AMBI is not useful for comparing the health of south-western Australian estuaries because it is unable to distinguish between the effects of anthropogenic changes and those due to natural

**Fig. 5.** South-western Australia. Shade plot of fourth-root transformed abundances of the families of benthic macroinvertebrates in the four estuaries sampled (see grey-scale key), with the y axis ordered by the AMBI score for each family (decreasing down the plot) and the water depth/period combinations of estuaries on the x axis ordered by their taxonomic distinctness score ($\Delta$, decreasing left to right). SW, shallow waters (<1 m deep); DW, deeper waters (1–6 m deep).
environmental influences. This is because, in particular, the characteristics of microtidal estuaries, i.e. retention of naturally-derived organic material for long periods, produces an environment that is not conducive to the success of families with high AMBI scores and consequently the variation in AMBI scores across estuaries is limited. Family-level AMBI is, to some extent, effective, however, in detecting temporal changes and spatial differences in environmental quality within an estuary, because the confounding effects of natural environmental variation are effectively standardized. This point is especially important since comparisons between the AMBI scores for estuaries in south-western Australia are complicated as the natural environmental characteristics of these systems vary greatly, irrespective of any anthropogenic effects. It is particularly encouraging therefore that the trends exhibited by taxonomic distinctness across estuaries, as well as within estuaries, followed precisely those expected on the basis of known differences in environmental perturbations. This is due to taxonomic distinctness differing from AMBI, and also the number of species, Shannon and Simpson diversity, in not being affected by natural stressors, such as salinity fluctuations, sediment instability and whether the estuary is closed for a period, while still being sensitive to anthropogenic effects. Compared to other measures, the value of using taxonomic distinctness for assessing anthropogenic effects, as it overcomes any confounding environmental differences, was emphasised in the study by Leonard et al. (2006). Those authors also pointed out that this index can be calculated retrospectively from quite low grade information, which adds to its cost effectiveness.

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