

The Benthic Macroinvertebrate Faunas of the Vasse-Wonnerup Estuary as Indicators of Environmental Degradation

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Photographs on the cover and inside cover taken by Jane Chambers

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1.0: Executive Summary

Benthic macroinvertebrates are a crucial component of estuarine ecosystems as they play an important role in nutrient cycling and also provide a food source for fish and birds (Kalejita & Hockey, 1991; Humphries & Potter, 1993; Penniford & Davis, 2001; Mermillod-Blondin, 2011). The limited mobility of these species and their predictable responses to natural and anthropogenic stressors has led to their extensive use in biotic indices of estuarine health and in other studies aimed at assessing the levels of degradation (*e.g.* Wiesberg *et al.*, 1997; Borja *et al.*, 2000; Tweedley *et al.*, submitted).

The Vasse-Wonnerup Estuary is a relatively small, shallow and nutrient-enriched system which undergoes pronounced changes in salinity. The estuary provides an important habitat and refuge area for birds and, as a result, has been awarded RAMSAR status. However, despite the recognized importance of this system it has become severely degraded through the input of nutrients. This study aims to (i) describe the spatial and temporal trends benthic invertebrate faunal composition within the system and (ii) compare this fauna to that found in other degraded estuarine environments such as the Peel-Harvey and Swan-Canning in both the 1980s and more recently (2000s).

The results of this study demonstrate that the benthic invertebrate fauna of the surface waters Vasse-Wonnerup Estuary comprises 62 species and are dominated by arthropods both in terms of the number of species (55) and percentage contribution to the total number of benthic invertebrates (97%), whereas the benthic invertebrate fauna of the sediments were dominated in terms of numbers by oligochaetes. Thus, the faunas collected using the different sampling methodologies differed markedly, an important consideration for future monitoring efforts. Many of the taxa found in relatively large numbers in both the sweep net and core samples *i.e.* oligochaetes and several chironomid, cladoceran, ostracod and copepod species are associated with eutrophication, while many species sensitive to this perturbation were absent and changes in the densities of these species along with taxonomic distinctness measures could be used to monitor the health of the benthic environment of the Vasse-Wonnerup. The data analysed here showed that while there was little variation among lagoons (*i.e.* Vasse or Wonnerup) there were marked temporal

changes in faunal composition and these factors should thus be incorporated into any future monitoring protocol.

The comparison of the benthic macroinvertebrate faunas of the Vasse-Wonnerup Estuary to the Peel-Harvey and Swan-Canning estuaries (in both the 1980s and 2000s), although tentative (due to differences in sampling methodology), indicate that the fauna of the Vasse-Wonnerup is very different to the other estuaries. This is primarily due to the fauna of the latter system being more typical of a wetland than an estuary. However, marked differences in density may reflect poor conditions in the sediments.

The benthic invertebrate data in this report, which was collected by Chambers *et al.* (2009, 2010, 2011), was measured at a range of sites where data on the water quality, water and sediment nutrient levels and the diversity, distribution and biomass of phytoplankton, macroalgae and macrophyte species were also recorded. These data collected in both February and November of each year could, along with those provided by Wilson *et al.* (2007, 2008), help determine the environmental preferences of key benthic invertebrate species and also provide benchmark data from which future changes in environmental conditions within the estuary can be detected. Due to the wide and successful use of benthic macroinvertebrates as indicators of ecosystem health it is recommended that the benthic macroinvertebrate fauna of the Vasse-Wonnerup be sampled at regular intervals along with other measures such as water quality and nutrient levels.

2.0: Introduction

The Vasse-Wonnerup Estuary provides an internationally important habitat for over 34,000 water birds comprising 90 species, a function that is recognised by its designation as a RAMSAR wetland (Lane *et al.*, 2007). This system thus contains an abundant source of food for these birds in the form of benthic macroinvertebrates, macrophytes and fish. However, although the importance of system is well recognised, the Vasse-Wonnerup Estuary has suffered in recent years from numerous detrimental effects, including increases in the prevalence of eutrophication, algal blooms, anoxia, fish kills, undesirable odours and mosquito problems (Department of Water, 2010). Although the importance of benthic macroinvertebrates in ecosystem functioning, as a food source for water birds and fish and as an indicator of the health of the benthic environment is widely recognised, there are no fully-quantitative data on the density and diversity of this crucial faunal component of the Vasse-Wonnerup Estuary. Furthermore, the data on species composition, while useful, is semi-quantitative and not derived using traditional estuarine methods for obtaining data that can be used to make rigorous comparisons with the results of studies conducted using such methods in other estuaries (*e.g.* Platell & Potter, 1996; Kanandjembo *et al.*, 2001; Tweedley, 2010).

Benthic macroinvertebrates are a crucial component of estuarine ecosystems. They are, for example, one of the major components of estuarine food webs and play an important role in nutrient cycling in those systems through their the effects of their burrowing and feeding activities (Hutchings, 1998; Constable, 1999). These fauna have been used in aquatic environments around the world as ecological indicators as they undergo well known changes following deleterious anthropogenic influences. For example, the benthic macroinvertebrate fauna is typically less diverse in eutrophic than pristine estuaries, whereas the reverse is often true of their densities (*e.g.* Gray *et al.*, 2002; Karlson *et al.*, 2002). Furthermore, when an estuary becomes excessively eutrophic, and thus experiences regular hypoxic events, its benthic macroinvertebrate fauna can become dominated by opportunistic species, such as capitellid and spionid polychaetes and oligochaetes, which are able to thrive under such conditions (Pearson and Rosenberg, 1978; Wilson *et al.*, 1998; Kemp *et al.*, 2005).

Recently, comparisons have been made between data obtained during 2003/4 for the benthic macroinvertebrate faunas in the basins of the Swan-Canning and Peel-Harvey estuaries and those

derived from the same sites in 1986/7. In the case of the Peel-Harvey Estuary, the following conclusions from these comparisons showed that the benthic macroinvertebrate faunas in this system had undergone deleterious changes in the intervening years. (i) There has been a decrease in the abundance and number of species of crustaceans, the most sensitive of the major benthic macroinvertebrate taxa to environmental stress, and an increase in the abundance and number of species of polychaetes, which are the less sensitive of those taxa to environmental disturbance. (ii) There has been a decline in the overall density of benthic macroinvertebrates and in taxonomic distinctness, a measure of diversity based on the taxonomic spread of species. (iii) There has been an increase in the variability among species composition within a site. Some idea of the pronounced extent of the change in faunal composition can be gauged from the fact that the compositions in the two periods differ even at the phylum level (Wildsmith *et al.*, 2009). The first of the above types of changes was also recorded in the Swan-Canning Estuary following a similar comparison of the benthic macroinvertebrate fauna (Wildsmith *et al.*, 2011).

In light of the above, the aims of this desktop study were as follows. (i) Collate and investigate the semi-quantitative data for the abundance of the components of the benthic macroinvertebrate fauna of the Vasse-Wonnerup Estuary using sweep net and core samples collected previously by Chambers *et al.* (2009, 2010, 2011). (ii) Carry out a very broad comparison of the compositions of the benthic macroinvertebrate fauna of the Vasse-Wonnerup Estuary with those determined for the Peel-Harvey and Swan-Canning in the 1980s and 2000s. It must be reiterated however, that the conclusions drawn from these comparisons between the Vasse-Wonnerup and the other two systems are tentative and must be treated with caution as the sampling methods and regimes differed.

3.0: Materials and Methods

3.1: Site description

The Vasse-Wonnerup Estuary is located immediately to the east of the town of Busselton in southwestern Australia in a low-lying coastal depression. The estuary itself covers an area of ~ 11 km² and comprises two basins (now essentially lagoons) the Vasse and Wonnerup which are 9 and 5 km long, respectively, around 600 m wide and typically < 1 m in depth (WRM, 2007). In its natural state, connection to the sea was via a channel which was seasonally closed from the ocean by the formation of a sand bar (Lane *et al.*, 1997). However, in 1908 floodgates were installed at the mouth of the estuary to regulate the inflow of seawater and to minimize flooding of the surrounding agricultural land.

The Vasse-Wonnerup Estuary has become highly nutrient-enriched due to run-off from agricultural land and unsewered areas of Busselton. The elevated nutrient levels are unsurprising as the Vasse-Wonnerup in the past experienced the greatest input of nutrients per m² of any estuary in Western Australia (McAlpine, 1989) and nutrients are still leaching from the estuarine sediment today (WRM, 2007).

Salinity within the estuary is highly dependent on groundwater and riverine flow in winter and spring and conversely evaporation in summer and autumn. Generally, salinity is < 5 between June and August, increasing to 15 in December/January and to full strength seawater (~ 35) by February (WRM, 2007). However, conditions can become hypersaline, for example, Chambers *et al.* (2009) noted that salinities reached between 40 and 113 in February 2009.

3.2: Collection of benthic invertebrate samples from the Vasse-Wonnerup Estuary

A full account of the sampling methodology and rationale for the collection of samples of the benthic invertebrate fauna of the Vasse-Wonnerup Estuary can be found in Chambers *et al.* (2009, 2010, 2011) but for convenience a brief summary is provided here.

Benthic invertebrates were collected at five sites in each of the Vasse and Wonnerup lagoons (Fig. 1) in November 2009 and 2010 and February 2010, while in February 2009 five sites were sampled. Sampling comprised two complementary methods namely sweep netting and sediment coring. The sweep net, which was 0.3 m wide and comprised 250 µm mesh, was moved through the waters of the site for two minutes. After this time the individuals collected along with

any macrophytes were transferred to the container and preserved in 70% ethanol. As at some sites the macrophytes were very numerous and thus the efficiency of the sweep netting was reduced core samples were also taken. A corer 9.4 cm in diameter which sampled to a depth of 5 cm was employed to collect sediment samples. These samples were placed into containers and preserved in 70% ethanol. Using a dissecting microscope, the invertebrates were removed from any sediment and/or macrophytes and identified to family and, on occasion, to species when there were multiple taxa from a single taxon. The abundance of each taxon was estimated as an order of magnitude *i.e.* 1-10, 11-100, 101-1000 and > 1,000 per m⁻².

3.3: Collection of benthic macroinvertebrate samples from the Swan-Canning and Peel-Harvey estuaries

A full account of the sampling methodology and rationale for the collection of samples of the benthic macroinvertebrate fauna of the Peel-Harvey and Swan-Canning estuaries can be found in Wildsmith *et al.* (2009) and Wildsmith *et al.* (2011), respectively, but for convenience a brief summary is provided here.

Benthic macroinvertebrates were sampled at four sites in the Swan-Canning Estuary (Dalkeith, Applecross, Matilda Bay and Deepwater Bay) and at four sites in the Peel-Harvey Estuary (Boodalan Island and Falcon Sticks in the Peel Inlet and at Dawesville and Grey's Beach in the Harvey Estuary) during the middle of each of five seasons between the winters of 1986 and 1987 and 2003 and 2004. At each site sampling was conducted in a rectangular area that measured 150 m along the shoreline and 10 m perpendicularly outwards from the shoreline and in water depths ranging from 0.5 to 1 m. Five randomly-located sediment cores were collected from each site during the day in each season using a corer. This corer, which was 11 cm in diameter and covered a surface area of 96 cm², sampled to a depth of 15 cm. The sediment samples were preserved in 5% formalin buffered in estuary water and subsequently wet sieved through a 500 µm mesh. Using a dissecting microscope the benthic macroinvertebrates were removed from any sediment retained on the mesh, identified to the lowest possible taxonomic level and stored in 70% ethanol. The number of each benthic macroinvertebrate taxon in each sample was converted to a density, *i.e.* number of individuals 0.1 m⁻².

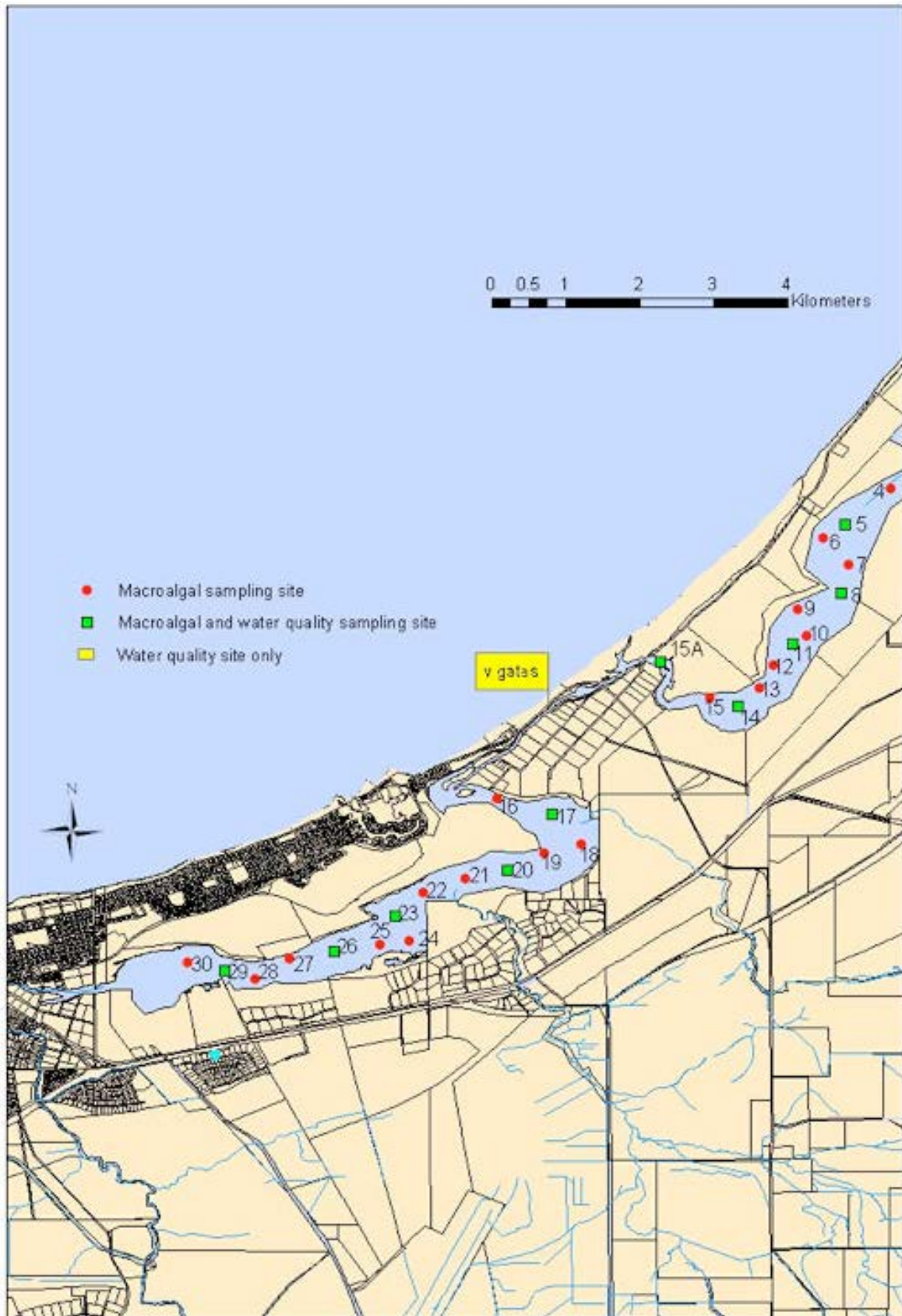


Figure 1: Map showing the location of the ten sites sampled in the Vasse-Wonnerup Estuary in February and November 2009 and 2010. Taken from Chambers *et al.* (2011).

3.4: Statistical analysis

Each of the following statistical analyses were performed using the PRIMER v6 multivariate software package (Clarke & Gorley, 2006) with the PERMANOVA+ add-on module (Anderson *et al.*, 2008).

3.4.1: Description of the benthic invertebrate faunas of the Vasse-Wonnerup Estuary

3.4.1.1: Preliminary analyses

In order to ascertain the appropriate statistical methodology that would be employed to describe the benthic invertebrate faunas of the Vasse-Wonnerup lagoons, the influence of the two different sampling methods had to be quantified. For this purpose, all the data collected by Chambers *et al.* (2009, 2010, 2011) were combined into a single data matrix. These data, which were semi-quantitative, were converted into presence/absence data in order to standardise any biases in the number of individuals recorded through the sampling of greater (or smaller) areas (*i.e.* water column *vs* sediment) and thus “leaving behind” only those differences relating to particular species being collected using either one or both of the sampling methods. This presence/absence data was then used to construct a Bray-Curtis similarity matrix which was, in turn, subjected to PERMANOVA (Anderson *et al.*, 2001). This test which was designed to primarily investigate differences in the species composition collected by the different methodologies also contained two other factors, sampling occasion (*e.g.* November 2010) and lagoon *i.e.* Vasse or Wonnerup. All factors were considered fixed. The null hypothesis of no significant differences in the species compositions of the benthic invertebrate faunas were rejected in the significant value (P) was < 0.05 . The influence of each term (factor) in the model was quantified using the estimates of the components of variation (COV), with higher values indicating that that term was more influential. As a strong sampling method effect was detected (see Preliminary results) the data collected using the two sampling methodologies were separated in subsequent analyses.

3.4.1.2: Univariate analyses

For each sample (core and sweep) collected during the studies by Chambers *et al.* (2009, 2010, 2011) a suite of diversity measures were calculated, namely species richness *i.e.* the number of species recorded and density (based upon the semi-quantities categories where 1-10 = 1, 11-100

= 10, 101-1,000 = 100 and > 1,000 = 1,000) and average taxonomic distinctness (Δ^+). The latter variable is a measure of species diversity that accounts for the extent to which individuals from different species are related based on their taxonomic separation through the hierarchical levels of the Linnaean tree (Warwick & Clarke, 1995, 2001). The values for all these diversity measures were calculated by the DIVERSE routine.

Prior to undertaking PERMANOVA to test for differences among *a priori* groups, the data for each of the dependent variables (*i.e.* species richness, density and Δ^+), was examined to ascertain the type of transformation required, if any, to meet the test assumptions of homogenous dispersions among groups (Anderson, 2001; McArdle & Anderson 2001). This was achieved by determining the extent of the linear relationship between the \log_e (mean) and the \log_e (standard deviation) of all groups of replicate samples, and then using the slope criteria provided by Clarke & Warwick (2001) to select an appropriate transformation. This methodology showed that for the sweep data the density per m^2 required a forth-root transformation while species richness and Δ^+ did not require any transformation. In the case of the core data only the density required transformation and was subjected to a square-root transformation.

Euclidean distance matrices containing all pairs of replicate samples were then constructed separately for each diversity measure. These matrices were then subjected to two-way PERMANOVA to test whether each dependent variable differed significantly among sampling occasions and/or lagoons. All factors were considered fixed. The main sources of any significant differences detected by PERMANOVA were identified by employing pairwise PERMANOVA. The trends were also displayed visually by displaying the means of the dependent variable, back transformed where necessary, with error bars constructed using the \pm one standard error.

3.4.1.3: Multivariate analyses

The semi-quantitative species abundance data recorded at each site in the Vasse-Wonnerup lagoons on each sampling occasion were first subjected to a square-root transformation to balance the contributions of highly abundant species with those that were less abundant. This pretreated data was then used to construct a Bray-Curtis similarity matrix, which was subjected to the same two-way PERMANOVA analyses as described above (see 3.4.1.2: Univariate analyses).

As the above PERMANOVA tests detected significant differences in main effects and not the interaction term in both the sweep net and core sample data to two-way and one-way Analysis of Similarities (ANOSIM) tests (Clarke & Green, 1988), respectively, were employed to elucidate, in more detail, the extent to which benthic invertebrate composition differed among lagoons and/or sampling occasions. In all ANOSIM tests, the null hypothesis that there were no significant differences in benthic invertebrate composition among factors was rejected if the significance level (P) was < 0.05 . The extent of any significant differences were determined by the magnitude of the test statistic (R), which typically ranges between 0 (*i.e.* no group differences) and 1 (*i.e.* the similarities between samples from different groups are all less than those between samples belonging to the same group). The same Bray-Curtis similarity matrix employed in the ANOSIM and PERMANOVA tests was also subjected to non-metric Multidimensional Scaling (nMDS) ordination in order to display visually the differences in the benthic invertebrate composition among lagoons and/or sampling occasions.

When ANOSIM detected a significant difference among habitats and the associated R -statistic was ≥ 0.2 , Similarity Percentages (SIMPER; Clarke, 1993) was then used to elucidate which species typified the assemblages at each group of samples (*e.g.* a sampling occasion) and those which contributed most to differences between each pair of groups. Focus was placed on those typifying and distinguishing species that had the highest similarity/standard deviation ratio and dissimilarity/standard deviation ratio, respectively, and those that were the most abundant.

3.4.2: Comparison of the benthic macroinvertebrate faunas of the Vasse-Wonnerup Estuary to the Peel-Harvey and Swan-Canning estuaries

3.4.2.1: Data compatibility

When comparing information from a number of studies it is important to standardise the data so that it is directly comparable. While the data collected by Rose (1994) in the Swan-Canning and Peel-Harvey estuaries in 1986/1987 is directly comparable to that recorded by Wildsmith *et al.* (2009, 2011) in 2003/2004 the methodology employed by Chambers *et al.*, (2009, 2010, 2011) differed in a number of key areas.

- i. The corers employed in the studies sampled different areas and depths. While, the samples from the Swan-Canning and Peel-Harvey estuaries were collected using a corer

15 cm in diameter and sampled to a depth of 15 cm, that employed in the Vasse-Wonnerup was 9.4 cm in diameter and sampled to a depth of 5 cm.

- ii. The data collected in the Swan-Canning and Peel-Harvey were fully quantitative (*i.e.* every organism retained on the sieve mesh was counted and converted to a density). In the Vasse-Wonnerup, however, the data collected were semi-quantitative (*i.e.* estimated as an order of magnitude *i.e.* 1-10, 11-100, 101-1000 and >1,000 per m⁻²).
- iii. The core samples in the Swan-Canning and Peel-Harvey estuaries were sieved through a 500 µm mesh and only organisms retained on this mesh were identified and counted, while the samples collected in the Vasse-Wonnerup were not subjected to sieving. Note that in marine and estuarine studies invertebrates > 500 µm are traditionally classified as benthic macroinvertebrates (Eleftheriou & Moore, 2005), while those between 500 and 63 µm are classified as meiofauna (Somerfield *et al.*, 2005).

To try and compensate for these differences in sampling methodology the following measures have been undertaken.

- i. No calculation of species richness will be employed in the comparison as this diversity measure is influenced heavily by the area sampled, for example, you would expect a larger number of species to be recorded in a larger area/sample (*e.g.* Escaravage *et al.*, 2009). Instead average taxonomic distinctness will be employed, which is independent of sample size and sampling effort (Warwick & Clarke, 1995).
- ii. The quantitative species data collected in the Swan-Canning and Peel-Harvey estuaries will be converted to the semi-quantitative scale employed by Chambers *et al.* (2009, 2010, 2011).
- iii. Organisms which are unlikely to have been retained on a 500 µm mesh will be removed from the Vasse-Wonnerup data for the purposes of the comparisons to the Swan-Canning and Peel-Harvey estuaries. This included all copepod, ostracod and cladocera (water flea) taxa (see Table 1). This approach is validated, somewhat, in the fact that while species belonging to these taxa were recorded in hyperbenthic samples collected in both the Swan-Canning and Peel-Harvey estuaries in 2003/4 they were not collected in benthic macroinvertebrate samples collected at the same sites and the same time (Valesini *et al.*, 2009). However, this methodology only works on a broad scale and it is

highly likely that the abundance of more taxa would be affected through the exclusion of juveniles of some species if sieved through a 500 μm mesh.

3.4.2.2: Multivariate analyses

The semi-quantitative data for the semi-quantitative density of each benthic macroinvertebrate taxa in each sample collected in spring and summer from each estuary were square-root transformed to down-weight the contributions of highly abundant species with those that were less common and used to construct a Bray-Curtis similarity matrix. This matrix was then subjected to a three-way PERMANOVA to test for differences in benthic macroinvertebrate composition among Estuary/Year (6 levels; Peel-Harvey1986/7, Peel-Harvey2003/4, Swan-Canning1986/7, Swan-Canning2003/4, Vasse-Wonnerup2009 and Vasse-Wonnerup2010) and Season (2 levels; spring [November] and summer [February]). Note that, as the estuaries were sampled in different years, year cannot be an independent factor and has thus been combined with estuary. The data for the Vasse and Wonnerup lagoons were combined as previous analysis of the core sample data indicated that there was no significant difference between the benthic macroinvertebrate compositions between the two lagoons (see subsection 4.4.1: Preliminary results). The null hypothesis and interpretations for this test PERMANOVA were the same as described above.

ANOSIM and SIMPER were then employed, as before, to elucidate the extent to which benthic macroinvertebrate composition differed among the significant main effects in the PERMANOVA model and which taxa were responsible for those differences. However, as the focus of this report is on comparing the benthic macroinvertebrate fauna of the Vasse-Wonnerup with the other estuaries only pairwise comparisons involving the Vasse-Wonneurp will be discussed. Non-metric MDS was then used to display visually the differences in benthic macroinvertebrate composition between main effects and any significant interactions. In the case of differences between main effects the nMDS was constructed using the same Bray-Curtis similarity matrix employed in the ANOSIM test, however, when showing any interactions a centroid nMDS plot was constructed from a distance among centroids matrix where each point represents the “central” location in multivariate space of all the points for a group of samples *e.g.* those from the Peel-Harvey in spring 1986/7 (see Lek *et al.*, 2011 for further details and examples of centroid

nMDS ordinations). To highlight any differences in benthic macroinvertebrate composition among estuaries and years at different taxonomic levels the data used to construct the nMDS plot were aggregated to the family, order, class and phyla level and used to construct a Bray-Curtis similarity matrix prior to being subjected to nMDS ordination.

The replicate data collected for each site (or lagoon in the case of the Vasse-Wonnerup) in each season (spring or summer) in each estuary were averaged, the average taxonomic distinctness (AvTD) calculated by TAXDTEST and used to produce a funnel plot. This graph shows the expected value for the average AvTD (dotted line) and ,any significant departure from this line *i.e.* a point falling outside the 95% probability intervals (solid lines), is considered to have an AvTD which departed significantly from expectation (Warwick & Clarke, 2001). This index has been shown to be very valuable in assessing degradation as taxonomic distinctness is not strongly affected by natural environmental factors, but retains its sensitivity to anthropogenic disturbances (*e.g.* Warwick & Clarke, 1995; Leonard *et al.*, 2006; Tweedley *et al.*, submitted).

4.0: Results

4.1: Broad characteristics of the benthic invertebrate fauna of the Vasse-Wonnerup Estuary

A total of 166,682 benthic invertebrates were collected in sweep samples, (*i.e.* after the number in each sample had been converted to that in one m⁻²) in the waters of the Vasse-Wonnerup Estuary in February and November 2009 and 2010. These invertebrates comprised 62 species from four phyla (Table 1). The Arthropoda were the most speciose, represented by 55 species and together accounted for 96.6% of all benthic invertebrates collected. The next most speciose phyla were the Mollusca which comprised five species and 3.3% of the benthic invertebrates recorded. The Nematoda and Annelida were also represented, however, both taxa made minor contributions to the total number of individuals.

Crustaceans were the most abundant class and taxa belonging to this group were ranked first to sixth in terms of abundance, together comprising ~75% of the total number of benthic invertebrates collected (Table 1). The species responsible for this dominance included cyclopoid (18%) and calanoid (10%) copepods, the amphipod *Ceinidae* sp. (12%), the ostracod *Mytilocypris tasmanica chapmani* (12%) and two species of water flea, *Daphnia* sp.1 and sp.2 (12 and 10%, respectively). Two types of insect were also relatively abundant namely, the non-biting midge larvae, Chironomidae spp. (7%) and backswimmer larvae, *Notonecta* sp. (5%). The most abundant mollusc, *Coxiella striatula*, was ranked ninth and contributed 3% to the total number of benthic invertebrates collected (Table 1).

A total of 8,148 benthic invertebrates were collected in the core samples (*i.e.* after the number in each sample had been converted to that in one m⁻²) comprising 32 species. As with the sweep samples the same four phyla were present, however, almost all individuals belonging to either the Annelida (50.8 %) or the Arthropoda (48.9 %). Oligochaeta spp. was the dominant taxa comprising 50.8 % of all individuals collected. The only other species which contributed more than 5% of the individuals were the cyclopoid copepods (27.6 %; Table 1).

Table 1: Total number of individuals collected (after the numbers in each sample had been converted to that in one m⁻²) (#), percentage contribution (%) and rank by number of individuals (Rank) of the benthic invertebrate taxa in samples collected from the Vasse-Wonnerup in sweep net and core samples by Chambers *et al.* (2009, 2010, 2011). Note these numbers of individuals have been estimated from the semi-quantitative data in the above publications, see material and methods.

Phylum/Class	Order	Species	Sweep samples			Core samples		
			#	%	Rank	#	%	Rank
Crustacea	Copepoda	Cyclopid Copepod	30,521	18.31	1	2,251	27.63	2
Crustacea	Amphipoda	<i>Ceinidae</i> sp.	20,544	12.33	2	117	1.44	8
Crustacea	Ostracoda	<i>Mytilocypris tasmanica chapmani</i>	20,527	12.32	3	212	2.60	6
Crustacea	Cladocera	<i>Daphnia</i> sp.1	19,322	11.59	4	259	3.18	5
Crustacea	Copepoda	Calanoid Copepod	17,040	10.22	5	284	3.49	4
Crustacea	Cladocera	<i>Daphnia</i> sp.2	16,711	10.03	6	113	1.39	9
Insecta	Diptera	Chironomidae spp. (larvae)	12,247	7.35	7	347	4.26	3
Insecta	Hemiptera	<i>Notonecta</i> sp.1 (larvae)	8,121	4.87	8	4	0.05	19
Mollusca	Pulmonata	<i>Coxiella striatula</i>	4,516	2.71	9			
Crustacea	Ostracoda	<i>Mytilocypris ambiguosa</i>	4,463	2.68	10	26	0.32	12
Crustacea	Copepoda	Harpacticoid Copepod	2,152	1.29	11	12	0.15	15
Crustacea	Cladocera	<i>Daphnia</i> sp.3	2,040	1.22	12	1	0.01	25
Crustacea	Ostracoda	Cyprididae sp.1	1,331	0.80	13	22	0.27	13
Crustacea	Ostracoda	Cyprididae sp.2	1,221	0.73	14	32	0.39	11
Crustacea	Ostracoda	<i>Platicypris baueri</i>	1,030	0.62	15	200	2.45	7
Crustacea	Ostracoda	Cyprididae sp.4	1,021	0.61	16	1	0.01	25
Mollusca	Pulmonata	<i>Potamopyrgus</i> sp.	882	0.53	17	1	0.01	25
Insecta	Hemiptera	Corixidae sp. (larvae)	643	0.39	18	2	0.02	21
Crustacea	Decapoda	Atyidae sp.	643	0.39	18	1	0.01	25
Insecta	Hemiptera	<i>Micronecta robusta</i>	269	0.16	20			
Insecta	Hemiptera	<i>Notonecta</i> sp.	212	0.13	21			
Crustacea	Amphipoda	<i>Perthidae</i> sp.	210	0.13	21			
Crustacea	Ostracoda	Cyprididae sp.3	110	0.07	23	10	0.12	18
Crustacea	Ostracoda	<i>Diacypris spinosa</i>	101	0.06	24	11	0.14	17
Nematoda	Nematoda	Nematoda spp.	100	0.06	24	12	0.15	15
Insecta	Zygotera	<i>Xanthagrion erythroneurum Selys</i>	86	0.05	26			
Insecta	Diptera	Tanyponidae sp. (larvae)	74	0.04	27	65	0.80	10
Insecta	Zygotera	<i>Austrolestes annulosus</i>	61	0.04	27			
Insecta	Coleoptera	<i>Berosus</i> larvae	52	0.03	29	13	0.16	14
Insecta	Coleoptera	<i>Megaporus</i> sp. larvae	48	0.03	29			
Insecta	Zygotera	<i>Austrolestes io</i>	48	0.03	29			
Insecta	Coleoptera	<i>Haliphus</i> sp. (larvae)	43	0.03	29	1	0.01	25
Crustacea	Ostracoda	<i>Bennelongia</i> sp.	42	0.03	29			
Insecta	Diptera	Ephydriidae sp. (larvae)	31	0.02	34	2	0.02	21
Crustacea	Decapoda	<i>Paleometes australis</i>	30	0.02	34	1	0.01	25
Insecta	Coleoptera	<i>Haliphus</i> sp.	28	0.02	34			
Insecta	Coleoptera	Dytiscidae sp. (larvae)	20	0.01	37			
Insecta	Anisoptera	Aeshnidae sp.	20	0.01	37			
Insecta	Coleoptera	<i>Berosus</i> sp.	17	0.01	37	2	0.02	21
Insecta	Diptera	Athericidae sp. (larvae)	16	0.01	37			
Insecta	Coleoptera	<i>Megaporus</i> sp.	11	0.01	37			
Insecta	Diptera	Orthoclanidae sp. (larvae)	10	0.01	37	3	0.04	20
Crustacea	Cladocera	<i>Daphniopsis</i> sp.	10	0.01	37			
Insecta	Zygotera	<i>Austrolestes analis</i>	7	> 0.01	44			
Polychaeta	Polychaeta	Polychaeta spp.	5	> 0.01	44	2	0.02	21
Arachnida	Trombidiformes	Eylais sp.	5	> 0.01	44			
Insecta	Coleoptera	Chostonectes sp. (larvae)	4	> 0.01	44	1	0.01	25
Arachnida	Trombidiformes	Hydrodromidae sp.	4	> 0.01	44			
Insecta	Coleoptera	<i>Necterosoma</i> sp.	4	> 0.01	44			
Insecta	Hemiptera	<i>Agraptocorixa</i> sp.	4	> 0.01	44			
Insecta	Hemiptera	<i>Sigara</i> sp.	4	> 0.01	44			
Oligochaeta	Oligochaeta	Oligochaeta spp.	3	> 0.01	44	4139	50.80	1
Insecta	Diptera	Culicidae sp. (larvae)	2	> 0.01	44			
Insecta	Diptera	Ceratopogonidae sp. (larvae)	2	> 0.01	44			
Insecta	Coleoptera	<i>Coleosoma</i> sp.	2	> 0.01	44			
Insecta	Coleoptera	<i>Rhantus</i> sp. (larvae)	2	> 0.01	44			
Insecta	Anisoptera	Corduliidae sp.	2	> 0.01	44			
Insecta	Trichoptera	<i>Acritoptila globosa</i>	2	> 0.01	44			
Mollusca	Pulmonata	<i>Planorbidae</i> sp.	2	> 0.01	44			
Mollusca	Pulmonata	<i>Lymnaeidae/Succinaeidae</i> sp.	2	> 0.01	44			
Insecta	Trichoptera	<i>Notalina spira</i>	1	> 0.01	44			
Crustacea	Ostracoda	<i>Ilyodromus</i> sp.	1	> 0.01	44			
Insecta	Diptera	Tipulidae sp. (larvae)				1	0.01	25
Number of individuals collected			166,682			8,148		
Number of species			62			32		
Number of samples			41			39		

4.2: Preliminary results: *is there a significant difference in species collected by the two sampling methodologies?*

A three-way crossed PERMANOVA based on the presence/absence of benthic invertebrate species collected in the Vasse and Wonnerup lagoons using two different sampling methodologies (sweep netting and core sampling), on different sampling occasions detected significant differences in the species composition of these samples among each of the main effects and in the interaction between sampling method and sampling occasion ($P = 0.001-0.021$; Table 2). As indicated by the associated components of variation (COV), differences between sampling methodologies exerted by far the greatest influence on the presence/absence of the species collected with the exception of sampling occasion. The influence of the former factor on the species composition is shown on the nMDS plot Fig. 2. In this plot, the samples collected employing either sampling method, *i.e.* sweep netting and core sampling are clearly separated.

Table 2: Mean squares (MS), pseudo F-ratios, components of variation (COV) and significance levels (p) from PERMANOVA tests on the data for the presence/absence of the various benthic invertebrate species in the Vasse-Wonnerup lagoons recorded in February and November 2009 and 2010. df = degrees of freedom. Significant results are highlighted in bold.

Main effects	df	MS	Pseudo-F	COV	P
(sampling) Method	1	13110	9.344	512.19	0.001
(sampling) Occasion	4	14620	10.420	940.05	0.001
Lagoon	1	2926	2.086	66.65	0.047
Interactions					
Method x Occasion	3	6306	4.494	697.44	0.001
Method x Lagoon	1	1298	0.925	-9.17	0.511
Occasion x Lagoon	3	2090	1.490	97.73	0.084
Method x Occasion x Lagoon	3	1169	0.833	-66.58	0.675
Residual	45	1403		1403.10	

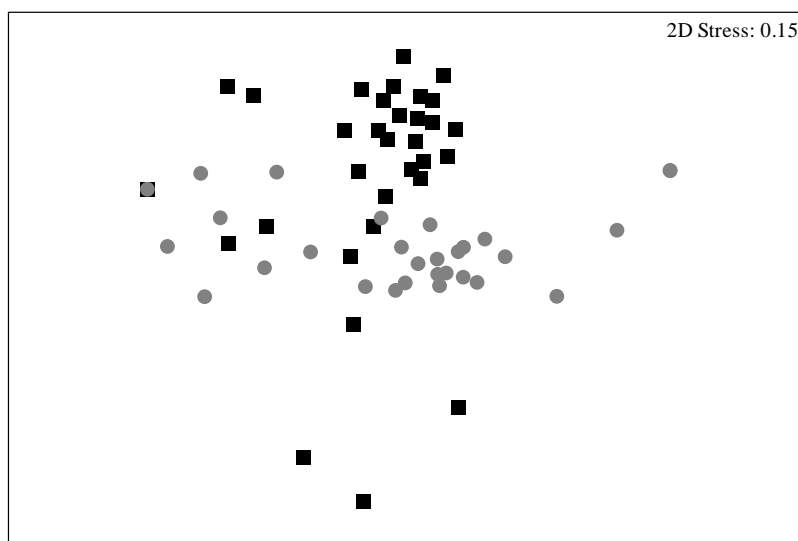


Figure 2: nMDS ordination plots constructed from the presence/absence of each benthic invertebrate species collected in core (●) and sweep (■) samples from the Vasse and Wonnerup lagoons during February and November 2009 and 2010.

4.3: Sweep net data

4.3.1: Diversity measures

Mean species richness differed significant among sampling occasions ($P = 0.001$), but not among lagoons or the interaction term between these main effects (Table 3a). A pairwise PERMANOVA identified a significant difference in species richness among all pairs of sampling occasions with the exception of February 2009 vs February 2010. Mean species richness was greatest in November 2009 (~ 19) and least in February 2009 and 2010 (~ 4 and 4, respectively), with intermediate values recorded in November 2010 (~ 15; Figure 3a).

The mean density (individuals per m^{-2}) of benthic invertebrates collected in sweep nets was also found to differ significantly but among only sampling occasions and not between lagoons (Table 3b). Analysis using pairwise PERMANOVA showed that density was significantly different in each sampling occasion. The highest numbers of individuals per m^2 was recorded in the samples collected in November 2010 (~ 1,110), while the lowest, only ~ 100 were found samples from February 2009 (Figure 3b).

Average taxonomic distinctness was found to not differ significantly among sampling occasions, lagoons or the interaction between these main effects ($P > 0.05$; Table 3c). This is most likely due to the wide variation in taxonomic distinctness (58 - 90) found among replicates in comparison to the main effects (Table 3c).

Table 3: Mean squares (MS), pseudo F-ratios (pF), components of variation (COV) and significance levels (P) from PERMANOVA tests on the data for mean (a) mean species richness (b) mean density (individuals per m^{-2}) and (c) mean average taxonomic distinctness (AvTD) derived from the composition of the various benthic invertebrate species collected in sweep nets in the Vasse-Wonnerup lagoons recorded in February and November 2009 and 2010. df = degrees of freedom. Significant results are highlighted in bold.

Main effects	(a) Mean species richness					(b) Mean density				
	df	MS	pF	COV	P	df	MS	pF	COV	P
(sampling) Occasion	3	381.34	38.77	48.19	0.001	3	46.29	23.34	5.75	0.001
Lagoon	1	12.27	1.25	0.17	0.285	1	0.12	0.06	-0.13	0.803
Interactions										
Occasion x Lagoon	3	2.87	0.29	-1.81	0.824	3	0.24	0.12	-0.45	0.951
Residual	24	9.83		9.84		24	1.98		1.41	

Main effects	(c) Mean AvTD				
	df	MS	pF	COV	P
(sampling) Occasion	3	281.35	1.26	7.51	0.285
Lagoon	1	11.67	0.05	-14.67	0.846
Interactions					
Occasion x Lagoon	3	352.99	1.58	33.60	0.219
Residual	24			14.95	

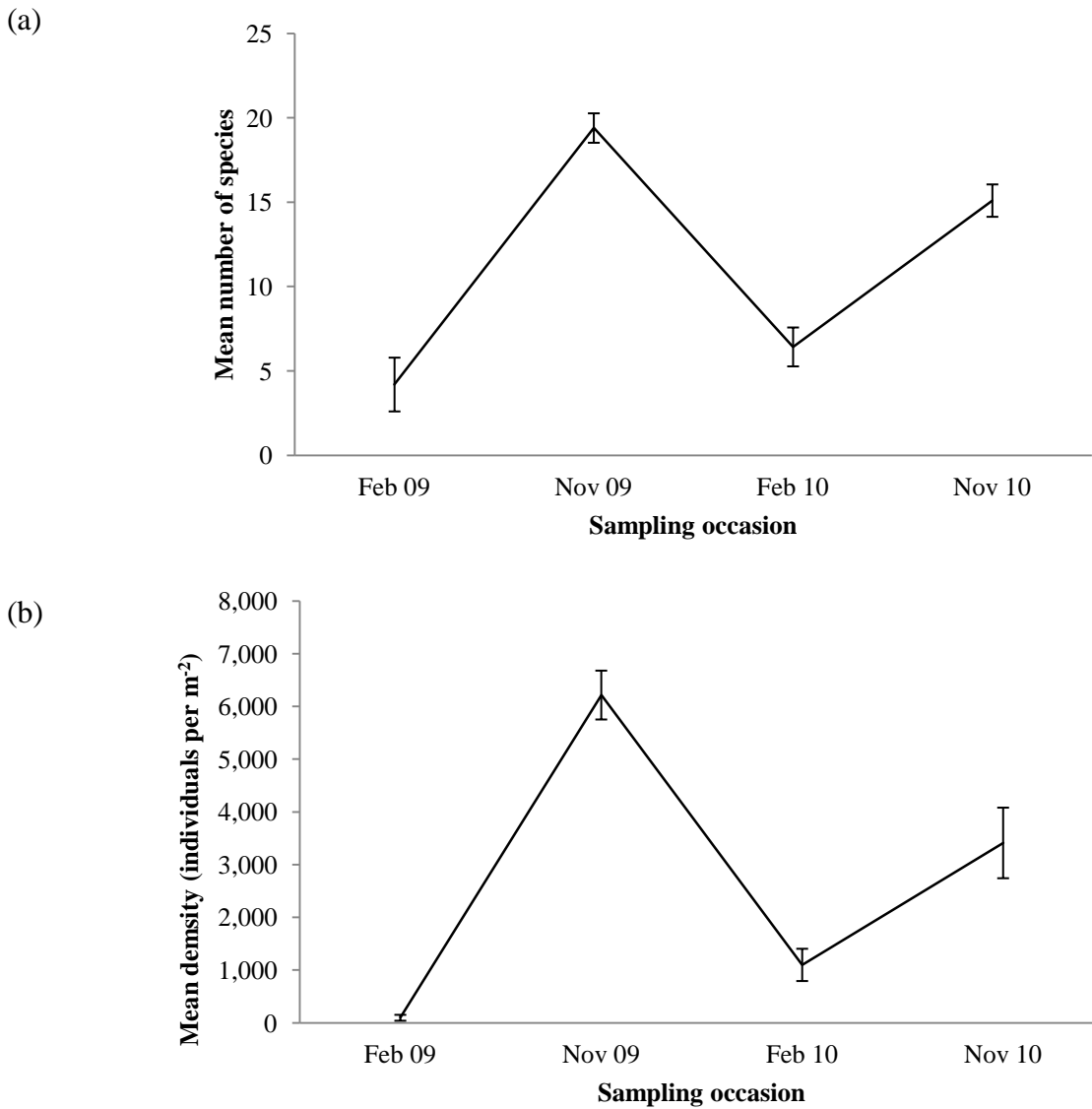


Figure 3: Mean (a) species richness and (b) density (individuals per m⁻²) from sweep net samples from the Vasse-Wonnerup in February and November 2009 and 2010. The error bar represents ± 1 standard error.

4.3.2: Benthic invertebrate assemblage composition

A two-way crossed PERMANOVA demonstrated that the species composition of the benthic invertebrate faunas of the Vasse-Wonnerup Estuary differed significantly between sampling occasions and lagoons and that there was no interaction between those main effects (Table 4). The components of variation associated with the main effects showed that the time of sampling (sampling occasion) explained almost ten times the amount of variance than lagoon and was thus by far the most influential of the main effects.

As no interaction was present between the two main effects, One-way ANOSIM was then employed to elucidate the extent of the temporal and/or spatial differences in benthic invertebrate composition without the confounding influence of the other main effect. The small influence of

Table 4: Mean squares (MS), pseudo F-ratios, components of variation (COV) and significance levels (*P*) from PERMANOVA tests on the data for the composition of the various benthic invertebrate species collected in sweep nets in the Vasse-Wonnerup lagoons recorded in February and November 2009 and 2010. df = degrees of freedom. Significant results are highlighted in bold.

Main effects	df	MS	Pseudo-F	COV	<i>P</i>
Sampling occasion	3	11667	8.31	1131	0.001
Lagoon	1	3526	2.51	147	0.003
Interactions					
Occasion x Lagoon	3	2151	1.53	194	0.035
Residual	24	1404		1404	

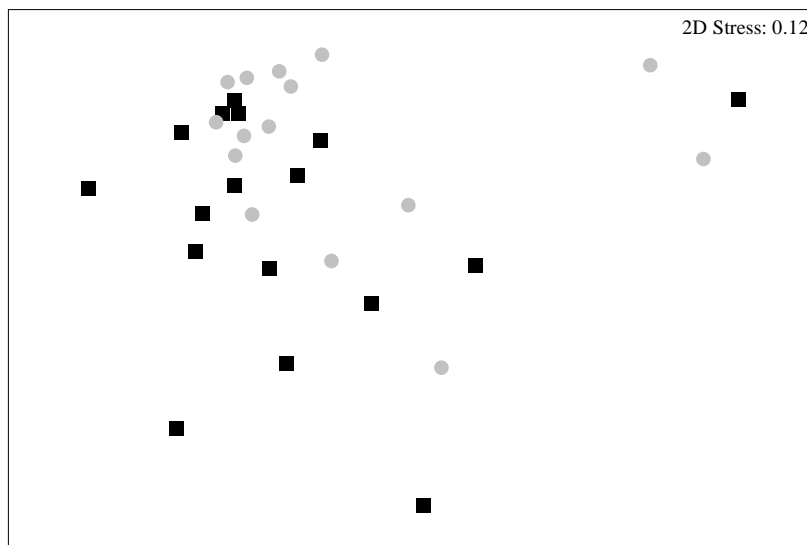
lagoon ($R = 0.154$; Table 5) is illustrated by the fact that none of the samples for either lagoon formed discrete groups on the nMDS ordination plot shown in Figure 4a. Because the extent of the differences in benthic invertebrate faunal composition among lagoons, although significant, were negligible (*i.e.* $R < 0.2$) SIMPER was not used to identify which species best typified and/or distinguished between those samples.

One-way ANOSIM detected significant differences in benthic invertebrate composition between sampling occasions, with the extent of those differences being moderate (Table 5). The largest differences were between pairs of sampling occasions were generally those involving February 2009 and 2010 and reflect the relatively distinct benthic invertebrate compositions in samples collected from those months. This is shown on Figure 4b where the points representing those samples form groups that are well separated from each other and from those points representing the other sampling occasions. In the case of February 2009 the samples were relatively depauperate and were typified by presence of cyclopoid copepods, the fly ephydriidae sp. and ostracod cypridiidae spp. This sampling occasion was distinguished from all others by the higher abundances of cypridiidae spp. and lower abundances of cyclopoid and calanoid copepods (Table 6). The samples collected in February 2010 were also fairly sparse and were typified by cyclopoid copepods and the gastropod *Potamopyrgus* sp.. The depauperate nature of the samples collected in this month was reflected in the fact that it was the lack taxa which distinguished the fauna in February 2010 from the other months. Moderate significant differences in benthic invertebrate composition were detected in comparisons involving samples collected in November 2010. These samples contained cyclopoid copepods, the water flea *Daphnia* sp.1, the ostracod *Mytilocypris tasmanica chapmani* and amphipod *Ceinidae* sp. and was distinguished from those collected in November 2009 by lower abundances of Chironomidae sp. and *Notonecta* sp. 1 (Table 6).

Table 5: *R*-statistic and/or significance level (*P*) values derived from two-way crossed sampling occasion x lagoon ANOSIM tests on the benthic invertebrate composition data recorded sweep nets. Insignificant pairwise comparisons are highlighted in grey.

Lagoon: <i>P</i> = 0.029, Global <i>R</i> = 0.154			
Sampling occasion: <i>P</i> = 0.001, Global <i>R</i> = 0.670			
	Feb 2009	Nov 2009	Feb 2010
Nov 2009	0.885		
Feb 2010	0.634	0.887	
Nov 2010	0.890	0.426	0.672

(a)



(b)

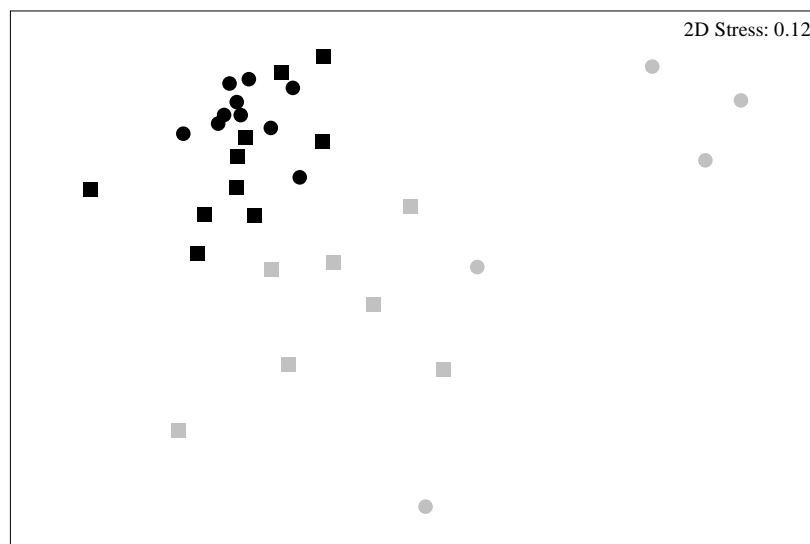


Figure 4: nMDS ordination plots constructed from the forth-root transformed benthic invertebrate composition data collected from sweep net samples in the Vasse- and Wonnerup lagoons in 2009 and 2010. Points are coded for a) lagoon *i.e.* Vasse (●) and Wonnerup (■) and b) sampling occasion *i.e.* February 2009 (●), November 2009 (●), February 2010 (■) and November 2010 (■).

Table 6: Species that consistently typified (provided along the diagonal in light grey boxes) and distinguished (provided in the sub-diagonal) the benthic invertebrates collected in sweep nets in the Vasse and Wonnerup lagoons as detected by one-way SIMPER. The sampling occasion in which each species was most abundant is given in superscript for each pairwise comparison. Insignificant pairwise comparisons are highlighted in dark grey. Asterisks denote the relative consistency of each species in either typifying or distinguishing the faunal composition in that season or year, as measured by the similarity to standard deviation ratio and dissimilarity to standard deviation ratio, respectively; > 1.5-3*, > 3-5**, > 5***.

	Feb 2009	Nov 2009	Feb 2010	Nov 2010
Feb 2009	Ephydriidae sp. Cypridiadae spp. Cyclopoida spp.			
Nov 2009	<i>Ceinidae</i> sp. ^{Nov 09 ***} <i>M. tasmanica chapmani</i> ^{Nov 09 **} Cyclopoida spp. ^{Nov 09 *} Cypridiadae spp. ^{Feb 09}	Cyclopoida spp. ^{***} <i>Ceinidae</i> sp. ^{***} <i>M. tasmanica chapmani</i> <i>Daphnia</i> sp.2		
Feb 2010	Cyclopoida spp. ^{Feb 10 *} Calanoida spp. ^{Feb 10} Cypridiadae spp. ^{Feb 09} Ephydriidae sp. ^{Feb 09}	<i>Ceinidae</i> sp. ^{Nov 09 **} <i>Daphnia</i> sp.1 ^{Nov 09 *} <i>M. tasmanica chapmani</i> ^{Nov 09 *} Chironomidae spp. ^{Nov 09 *}	Cyclopoida spp.* Potamopyrgus sp.	
Nov 2010	<i>Daphnia</i> sp.2 ^{Nov 09 *} Cyclopoida spp. ^{Feb 10 *} Calanoida spp. ^{Feb 10 *} Cypridiadae spp. ^{Feb 09}	Chironomidae sp. ^{Nov 09 *} <i>Notonecta</i> sp.1 ^{Nov 09 *} <i>M. ambiguosa</i> ^{Nov 10}	<i>Daphnia</i> sp.2 ^{Nov 10 *} <i>Daphnia</i> sp.1 ^{Nov 10} <i>Ceinidae</i> sp. ^{Nov 10} <i>M. tasmanica chapmani</i> ^{Nov 10}	Cyclopoida spp.* <i>Daphnia</i> sp.2 <i>M. tasmanica chapmani</i> <i>Ceinidae</i> sp.

4.4: Core data

4.4.1: Diversity measures

Mean species richness in the core samples was found to differ significantly among sampling occasions ($P = 0.001$), but not among lagoons and there was no significant interactions between these main effects (Table 7a). Mean species richness was highest in November 2009 and 2010 (~ 7) and lowest in February 2009 (~ 1; Fig. 5a). Significant differences were detected between all pairs of sampling occasion with the exception of both November 2009 vs November 2010 and February 2009 vs February 2010.

PERMANOVA detected no significant differences in the mean density (individuals per m^{-2}) recorded in the core samples (Table 7b), with the values ranging from 2 to 1,243 (data not shown). This non-significant difference is likely due to the large amount of variance in this dependent variable.

Mean average taxonomic distinctness (AvTD) was identified by PERMANOVA as differing significantly among only sampling occasions ($P = 0.003$; Table 7c). This difference was due to AvTD being 0 in the samples collected in February 2009 and ranging between 65 and 80 on all other sampling occasions (Fig. 5b). This was reflected in the pairwise PERMANOVA where differences between pairs of sampling occasions were only detecting in comparisons involving February 2009.

Table 7: Mean squares (MS), pseudo F-ratios (pF), components of variation (COV) and significance levels (P) from PERMANOVA tests on the data for mean (a) mean species richness (b) mean density (individuals per m^{-2}) and (c) mean average taxonomic distinctness (AvTD) derived from the composition of the various benthic invertebrate species collected in sweep nets in the Vasse-Wonnerup lagoons recorded in February and November 2009 and 2010. df = degrees of freedom. Significant results are highlighted in bold.

Main effects	(a) Mean species richness					(b) Mean density				
	df	MS	pF	COV	P	df	MS	pF	COV	P
(sampling) Occasion	3	48.61	12.72	6.67	0.001	3	41.72	0.38	-10.08	0.784
Lagoon	1	0.01	0.01	-0.40	0.978	1	231.99	2.12	12.96	0.143
Interactions										
Occasion x Lagoon	3	4.58	1.20	0.23	0.365	3	25.05	0.23	-25.13	0.864
Residual	21	3.82		1.96		21	109.46		109.46	

Main effects	(c) Mean AvTD				
	df	MS	pF	COV	P
(sampling) Occasion	3	4279.60	14.66	593.44	0.003
Lagoon	1	57.69	0.20	-24.77	0.622
Interactions					
Occasion x Lagoon	3	0.05	0.05	-82.40	0.974
Residual	21			291.96	

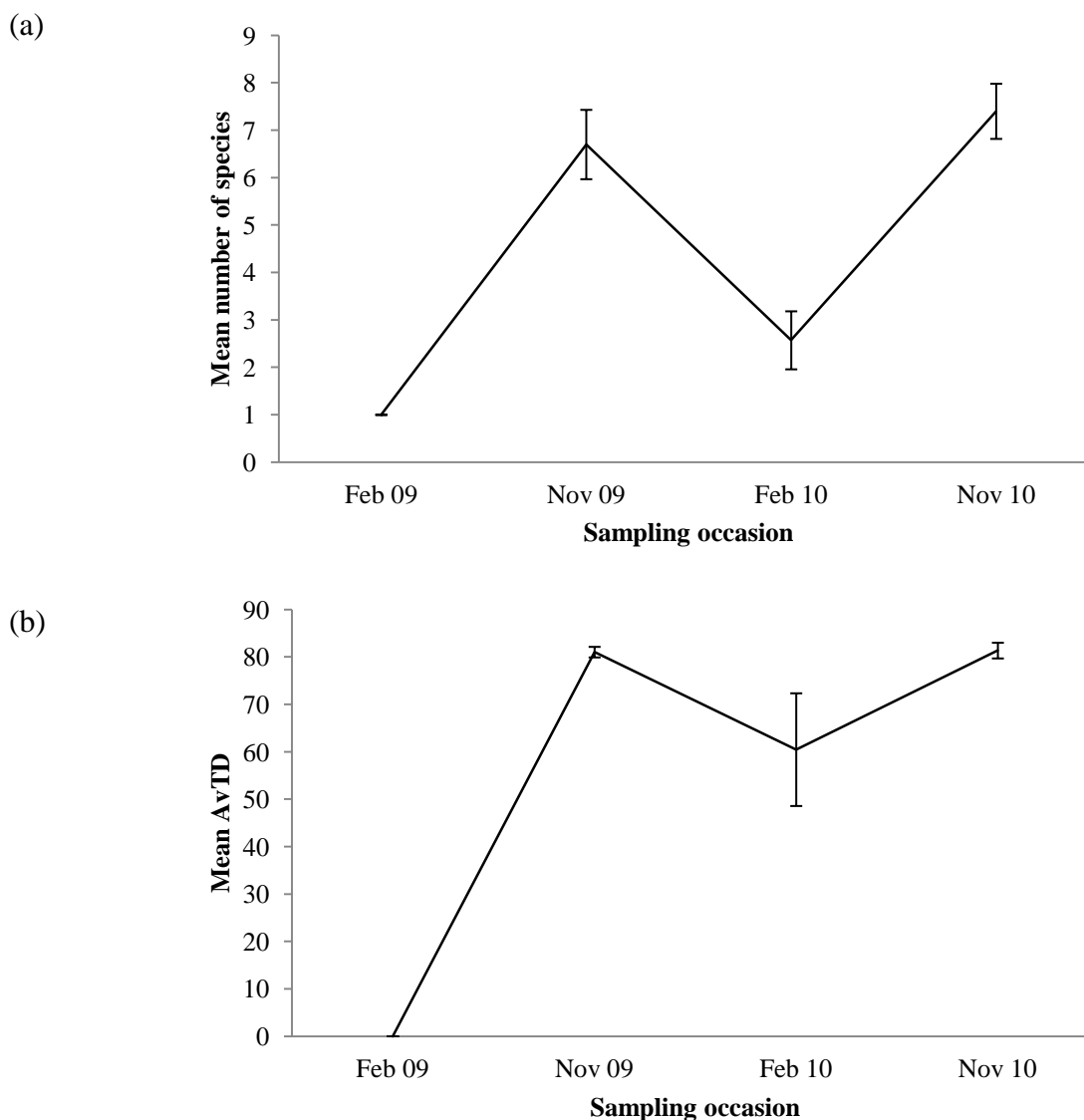


Figure 5: Mean (a) species richness and (b) average taxonomic distinctness (AvTD) collected from sweep net samples from the Vasse-Wonnerup in February and November 2009 and 2010. The error bar represents ± 1 standard error.

4.4.2: Benthic invertebrate assemblage composition

A two-way crossed PERMANOVA demonstrated that the benthic invertebrate composition of the Vasse-Wonnerup differed among sampling occasions ($P = 0.001$) but not among lagoons or the interaction between these two main effects (Table 8). This is reflected in the nMDS ordination plots where the points representing the different lagoons do not form discrete groups (Fig. 6a), whereas those representing the different sampling occasions do (Fig. 6b).

One-way ANOSIM was then employed to elucidate which pairs of sampling occasions were responsible for the significant differences. This test showed that the composition of the benthic invertebrate fauna differed in all sampling occasions, with the biggest differences involving

Table 8: Mean squares (MS), pseudo F-ratios, components of variation (COV) and significance levels (*P*) from PERMANOVA tests on the data for the composition of the various benthic invertebrate species in core samples from the Vasse-Wonnerup lagoons collected in February and November 2009 and 2010. df = degrees of freedom. Significant results are highlighted in bold.

Main effects	Df	MS	Pseudo-F	COV	<i>P</i>
Sampling occasion	3	11641	6.66	1472	0.001
Lagoon	1	939	0.54	-85	0.793
Interactions					
Sampling occasion x Lagoon	3	2070	1.18	96	0.264
Residual	28	1749		1749	

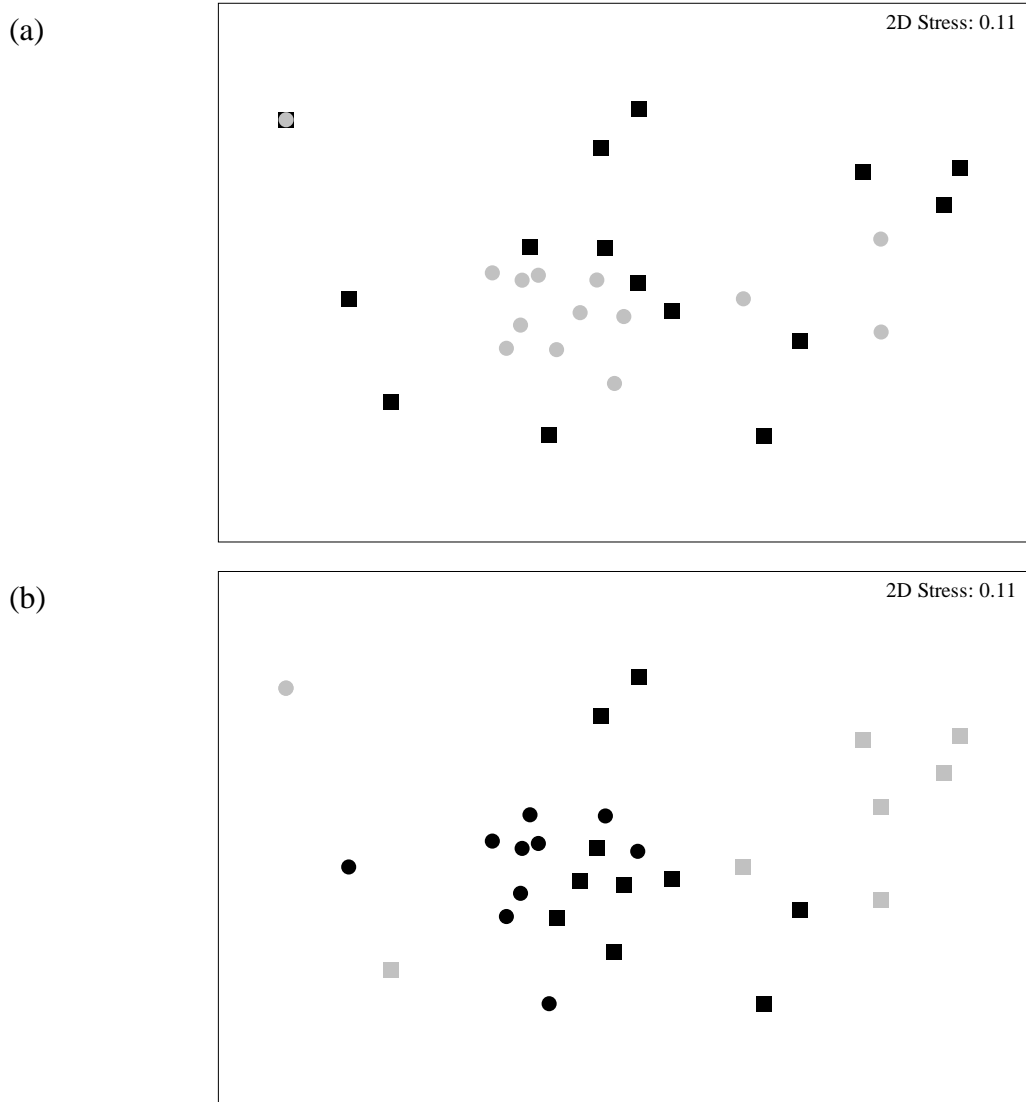


Figure 6: nMDS ordination plots constructed from the forth-root transformed benthic invertebrate composition data collected from core samples in the Vasse- and Wonnerup lagoons in 2009 and 2010. Points are coded for a) lagoon *i.e.* Vasse (●) and Wonnerup (■) and b) sampling occasion *i.e.* February 2009 (●), November 2009 (●), February 2010 (■) and November 2010 (■)

February 2009 (Table 9). The distinctness of this sampling occasion was attributable to the presence of *Daphnia* sp.1 which was the only typifying species and the depauperate nature of the benthic invertebrate fauna at this time in comparison to other sampling occasions (Table 10). February 2010 was the next most distinct sampling occasion and was also depauperate being typified by a single taxa, Cyclopoida spp. (Table 10). The fauna collected in this sampling occasion were distinguished from those in the others by relatively higher densities of Cyclopoida spp. and lower densities of Oligochaeta spp. Chironomidae spp., and *M. tasmanica chapmani*, which were all more abundant in November 2009 and 2010. These last three species were responsible for the relatively small difference in faunal composition between the two sampling occasion in November with Oligochaeta spp. and *M. tasmanica chapmani* being more abundant in 2009, while the reverse was true with Chironomidae spp. (Table 10).

Table 9: *R*-statistic and/or significance level (*P*) values derived from two-way crossed sampling occasion x lagoon ANOSIM tests on the benthic invertebrate composition data recorded in core samples. Insignificant pairwise comparisons are highlighted in grey.

Sampling occasion: <i>P</i> = 0.001, Global <i>R</i> = 0.548			
	Feb 2009	Nov 2009	Feb 2010
Nov 2009	0.926		
Feb 2010	0.727	0.752	
Nov 2010	0.983	0.154	0.581

Table 10: Species that consistently typified (provided along the diagonal in light grey boxes) and distinguished (provided in the sub-diagonal) the benthic invertebrates collected in core samples from the Vasse and Wonnerup lagoons as detected by one-way SIMPER. The sampling occasion in which each species was most abundant is given in superscript for each pairwise comparison. Insignificant pairwise comparisons are highlighted in dark grey. Asterisks denote the relative consistency of each species in either typifying or distinguishing the faunal composition in that season or year, as measured by the similarity to standard deviation ratio and dissimilarity to standard deviation ratio, respectively; > 1.5-3*, > 3-5**, > 5***.

	Feb 2009	Nov 2009	Feb 2010	Nov 2010
Feb 2009	<i>Daphnia</i> sp.1			
Nov 2009	<i>Daphnia</i> sp.1 ^{Feb 09} * Chironomidae spp. ^{Nov 09} * Oligochaeta spp. ^{Nov 09} * <i>Ceinidae</i> sp. ^{Nov 09} *	Chironomidae spp. * <i>Ceinidae</i> sp. * Oligochaeta spp.		
Feb 2010	<i>Daphnia</i> sp.1 ^{Feb 09} * Cyclopoida spp. ^{Feb 10} * Calanoida spp. ^{Feb 10}	Cyclopoida spp. ^{Feb 10} Chironomidae spp. ^{Nov 09} * Oligochaeta spp. ^{Nov 09} <i>Ceinidae</i> sp. ^{Nov 09} *	Cyclopoida spp.	
Nov 2010	<i>Daphnia</i> sp.1 ^{Feb 09} * <i>M. tasmanica chapmani</i> ^{Nov 10} * Oligochaeta spp. ^{Nov 10} * Tanyponidae sp. ^{Nov 10} *	Oligochaeta spp. ^{Nov 10} Chironomidae spp. ^{Nov 09} <i>M. tasmanica chapmani</i> ^{Nov 10}	Cyclopoida spp. ^{Feb 10} Oligochaeta spp. ^{Nov 10} <i>M. tasmanica chapmani</i> ^{Nov 10} Tanyponidae sp. ^{Nov 10} *	<i>M. tasmanica chapmani</i> * Oligochaeta spp. Tanyponidae sp. * <i>Ceinidae</i> sp.

4.3: Comparison of the benthic macroinvertebrate faunas of the Vasse-Wonnerup Estuary to the Peel-Harvey and Swan-Canning estuaries

4.3.1: Benthic macroinvertebrate assemblage composition

A two-way crossed PERMANOVA identified significant differences in the composition of the benthic macroinvertebrate faunas of the Peel-Harvey, Swan-Canning and Vasse-Wonnerup estuaries in the years in which they were sampled (*i.e.* Estuary/Year) and also among seasons (spring and summer) and the interaction term between these two main effects ($P = 0.001$; Table 11). The levels of the components of variation, which show how much of the variance can be attributed to each term in the model and thus determine how influential that term is, were by far the highest for estuary/year, followed by the interaction between estuary/year and season. As estuary/year explained the greatest amount of the variance in the data this term was explored further using a two-way ANOSIM, while the interaction term was explored visually using a centroid nMDS plot.

Table 11: Mean squares (MS), pseudo F-ratios, components of variation (COV) and significance levels (P) from PERMANOVA tests on the data for the composition of the various benthic macroinvertebrate species in core samples from the Peel-Harvey (1986/7 and 2003/4), Swan-Canning (1986/7 and 2003/4) and Vasse-Wonnerup (2009 and 2010) estuaries. df = degrees of freedom. Significant results are highlighted in bold.

Main effects	df	MS	Pseudo-F	COV	P
Estuary/Year	5	12415	13.012	1747	0.001
Season	1	5097	5.342	230	0.001
Interactions					
Estuary/Year x Season	5	2252	2.360	396	0.001
Residual	28	954		954	

A two-way crossed ANOSIM showed that there were considerable differences in the composition of the benthic macroinvertebrates in the different estuaries in the different years ($R = 0.746$; $P = 0.001$). Among the various estuaries and years the pairwise differences involving the Vasse-Wonnerup were typically very large (*i.e.* all $R = 1.000$), thereby reflecting the very distinct benthic macroinvertebrate faunal composition in this system (Table 12). This is illustrated on the nMDS plot where those samples from the Vasse-Wonnerup in 2009 and 2010 are widely separated from those representing the Peel-Harvey and Swan-Canning estuaries in both 1986/7 and 2003/4 and relatively dispersed (Fig. 7).

SIMPER, showed that the main cause of the differences between the Vasse-Wonnerup and the Peel-Harvey and Scan-Canning estuaries was the absence of a number of key polychaete species namely, *Capitella* spp., *Ceratonereis aquisetus* and *Leitoscolopos normalis*, several crustaceans

Table 12: *R*-statistic and/or significance level (*P*) values derived from two-way crossed ANOSIM tests on the benthic macroinvertebrate composition data recorded in core samples from the Peel-Harvey 1986/7 (PH 80s) and in 2003/4 (PH 00s), the Swan-Canning in 1986/7 (SC 80s) and in 2003/4 (SC 00s) and in the Vasse-Wonnerup in 2009 (VW09) and 2010 (VW10). Insignificant pairwise comparisons are highlighted in grey.

Estuary/Year: <i>P</i> = 0.001, Global <i>R</i> = 0.746					
	PH 80s	PH 00s	SC 80s	SC 00s	VW 09
PH 00s	0.531				
SC 80s	0.479	0.521			
SC 00s	0.953	0.490	0.896		
VW 09	1.000	1.000	1.000	1.000	
VW 10	1.000	1.000	1.000	1.000	0.000



Figure 7: nMDS ordination plots constructed from the square-root transformed benthic macroinvertebrate composition data collected from core samples in the Peel-Harvey in 1986/7 (▼) and 2003/4 (▼), Swan-Canning in 1986/7 (■) and in 2003/4 (■) and in the Vasse-Wonnerup in 2009 (○) and 2010 (●).

species, such as the amphipods, *Grandidierella propdentata* and *Corophium minor* and the bivalve molluscs, *Arthritica semen* and *Sanguinolaria biradiata* all of which were abundant in the Peel-Harvey and Swan-Canning estuaries in both the 1980s and 2000s (Table 12). Instead the fauna of the Vasse-Wonnerup was relatively depauperate and mainly comprised oligochaetes and midge larvae (chironominae spp. and tanypodinae sp.) with the first taxa helping to distinguish the benthic macroinvertebrate fauna of the Vasse-Wonnerup from the other two estuaries (Table 12). The fundamental nature of the differences in benthic macroinvertebrate faunal composition among estuaries are emphasised by the retention of a marked distinction between the Vasse-Wonnerup and other estuaries, even when the data were aggregated to the family, order, class and even phyla level (Fig. 8).

Table 12: Species that consistently typified (provided along the diagonal in light grey) and distinguished (provided in the sub-diagonal) the benthic macroinvertebrates collected in core samples from the Peel-Harvey in 1986/7 (PH 80s) and 2003/4 (PH 00s), Swan-Canning in 1986/7 (SC 80s) and in 2003/4 (SC 00s) and in the Vasse-Wonnerup in 2009 (VW 09) and 2010 (VW 10) as detected by one-way SIMPER. The sampling occasion in which each species was most abundant is given in superscript for each pairwise comparison. Insignificant pairwise comparisons are highlighted in dark grey. Asterisks denote the relative consistency of each species in either typifying or distinguishing the faunal composition in that season or year, as measured by the similarity to standard deviation ratio and dissimilarity to standard deviation ratio, respectively; > 1.5-3*, > 3-5**, > 5***. Note to save space species spelt out in full in typifying species are written in short hand in the distinguishing species.

	PH 80s	PH 00s	SC 80s	SC 00s	VW 09	VW 10
PH 80s	<i>Capitella</i> spp. * <i>Arthritica semen</i> *** <i>Ceratonereis aquisetus</i> <i>Tanais dulongi</i>					
PH 00s	<i>A. semen</i> ^{PH 80s *} <i>Capitella</i> spp. ^{PH 80s} <i>T. dulongi</i> ^{PH 80s *} <i>C. aquisetus</i> ^{PH 80s}	<i>Ceratonereis aquisetus</i> <i>Grandidierella propdentata</i> * <i>Capitella</i> spp. <i>Corophium minor</i> *				
SC 80s	<i>Capitella</i> spp. ^{PH 80s *} <i>G. propdentata</i> ^{SC 80s *} <i>Corophium minor</i> ^{SC 80s *} <i>Boccardiella limnicola</i> ^{SC 80s} <i>Melita zylanica</i> ^{PH 80s}	<i>G. propdentata</i> ^{SC 80s *} <i>A. semen</i> ^{SC 80s *} <i>Boccardiella limnicola</i> ^{SC 80s} <i>T. dulongi</i> ^{SC 80s} <i>C. minor</i> ^{SC 80s}	<i>Grandidierella propdentata</i> *** <i>Ceratonereis aquisetus</i> * <i>Arthritica semen</i> * <i>Corophium minor</i> * <i>Leitoscolopos normalis</i> *			
SC 00s	<i>S. biradiata</i> ^{SC 00s **} <i>Pseudopolydora</i> sp. 2 ^{SC 00s **} <i>A. semen</i> ^{PH 80s *} <i>L. normalis</i> ^{SC 00s *} <i>T. dulongi</i> ^{PH 80s}	<i>Pseudopolydora</i> sp. 2 ^{SC 00s *} <i>S. biradiata</i> ^{SC 00s **} <i>L. normalis</i> ^{SC 00s *} <i>Capitella</i> spp. ^{SC 00s *} <i>Heteromastus</i> sp. ^{PH 00}	<i>Pseudopolydora</i> sp. 2 ^{SC 00s **} <i>S. biradiata</i> ^{SC 00s *} <i>Boccardiella limnicola</i> ^{SC 80s} <i>C. minor</i> ^{SC 80s} <i>Capitella</i> spp. ^{SC 00s *}	<i>Capitella</i> spp. *** <i>Ceratonereis aquisetus</i> ** <i>Leitoscolopos normalis</i> *** <i>Sanguinolaria biradiata</i> ** <i>Grandidierella propdentata</i> **		
VW 09	<i>Capitella</i> spp. ^{PH 80s *} <i>A. semen</i> ^{PH 80s ***} <i>C. aquisetus</i> ^{PH 80s *} <i>T. dulongii</i> ^{PH 80s *} Oligochaete spp. ^{VW 09}	<i>Capitella</i> spp. ^{PH 00s} <i>C. aquisetus</i> ^{PH 00s} <i>C. minor</i> ^{PH 00s *} <i>G. propdentata</i> ^{PH 00s *} Oligochaete spp. ^{VW 09}	<i>C. aquisetus</i> ^{SC 80s **} <i>G. propdentata</i> ^{SC 80s ***} <i>A. semen</i> ^{SC 80s *} <i>C. minor</i> ^{SC 80s *} Oligochaete spp. ^{VW 09}	<i>Capitella</i> spp. ^{SC 00s **} <i>L. normalis</i> ^{SC 00s *} <i>C. aquisetus</i> ^{SC 00s **} <i>S. biradiata</i> ^{SC 00s **} Oligochaete spp. ^{VW 09}	Oligochaete spp. Chrionomidae spp. <i>Ceinidae</i> sp. Tanyponidae sp. <i>Berosus</i> sp.	
VW 10	<i>Capitella</i> spp. ^{PH 80s *} <i>A. semen</i> ^{PH 80s ***} <i>C. aquisetus</i> ^{PH 80s *} <i>T. dulongii</i> ^{PH 80s *} Oligochaete spp. ^{VW 09}	<i>Capitella</i> spp. ^{PH 00s} <i>C. aquisetus</i> ^{PH 00s} <i>C. minor</i> ^{PH 00s *} <i>G. propdentata</i> ^{PH 00s *} Oligochaete spp. ^{VW 09}	<i>C. aquisetus</i> ^{SC 80s **} <i>G. propdentata</i> ^{SC 80s ***} <i>A. semen</i> ^{SC 80s *} <i>C. minor</i> ^{SC 80s *} Oligochaete spp. ^{VW 09}	<i>Capitella</i> spp. ^{SC 00s **} <i>L. normalis</i> ^{SC 00s **} <i>C. aquisetus</i> ^{SC 00s **} <i>S. biradiata</i> ^{SC 00s **} Oligochaete spp. ^{VW 09}		Oligochaete spp. Tanyponidae sp. <i>Ceinidae</i> sp. <i>Berosus</i> sp.

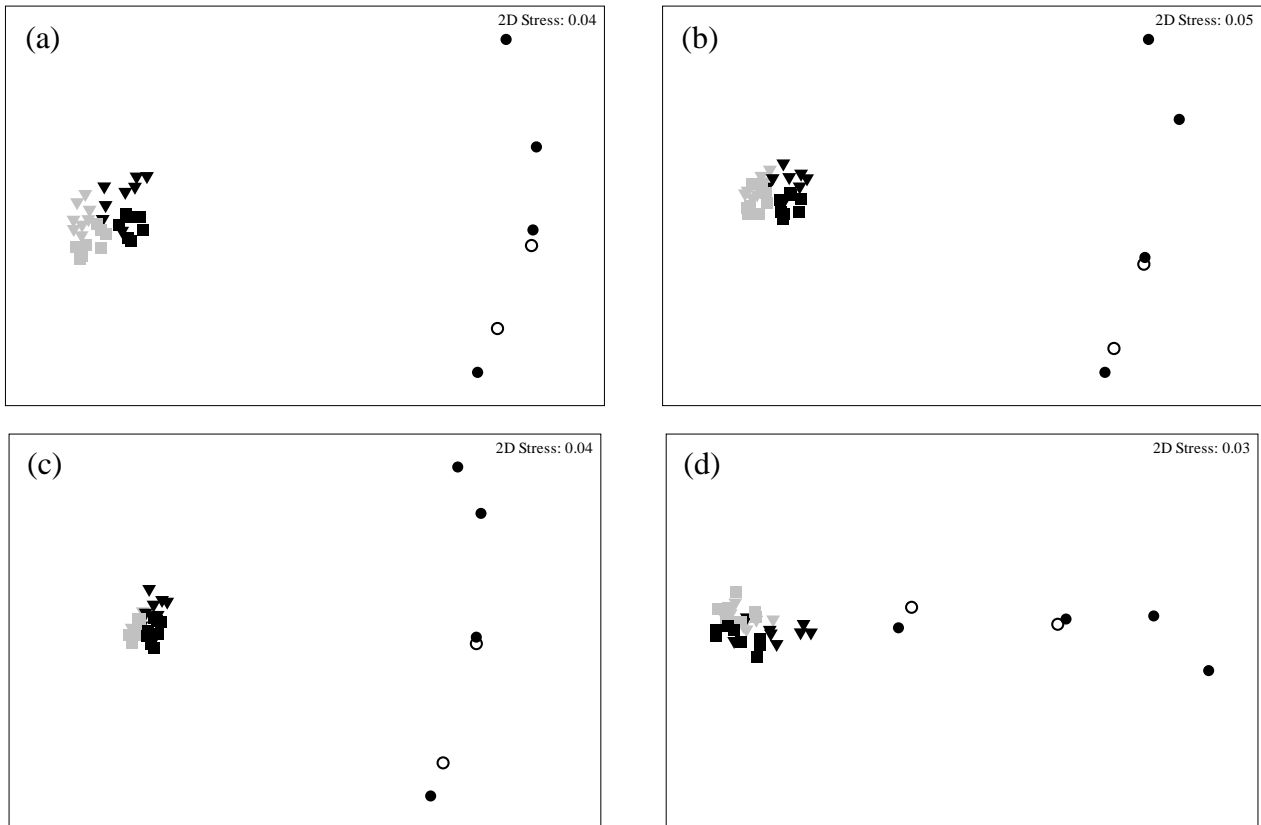


Figure 8: nMDS ordination plots constructed from the square-root transformed benthic macroinvertebrate composition data collected from core samples in the Peel-Harvey in 1986/7 (∇) and 2003/4 (\blacktriangledown), Swan-Canning in 1986/7 (\blacksquare) and in 2003/4 (\blacksquare) and in the Vasse-Wonnerup in 2009 (\circ) and 2010 (\bullet) aggregated to the (a) family, (b) order, (c) class and (d) phyla level.

The interaction between estuary/year and season identified by PERMANOVA was due to the fact that while for the Peel-Harvey and Swan-Canning estuaries differences between seasons relative to differences among estuary/year were small the reverse was true for the Vasse-Wonnerup (Fig. 9).

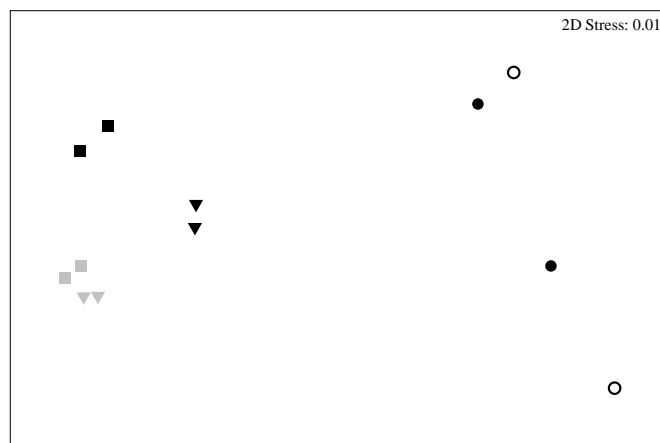


Figure 9: centroid nMDS ordination plots derived from a distance among centroids matrix constructed from the square-root transformed benthic macroinvertebrate composition data collected from core samples in the Peel-Harvey in 1986/7 (∇) and 2003/4 (\blacktriangledown), Swan-Canning in 1986/7 (\blacksquare) and in 2003/4 (\blacksquare) and in the Vasse-Wonnerup in 2009 (\circ) and 2010 (\bullet).

4.3.2: Taxonomic distinctness

In Fig. 10, the AvTD funnel plot is based on the total list of all 106 species from the three estuaries. Although the values for AvTD for most of the sites (lagoons) in each estuary in each season fall below the expected mean, they generally are all within the 95% confidence limits of the funnel, with the exception of several points from the Peel-Harvey and five of the eight points representing the Vasse-Wonnerup. Note that one point from the Vasse-Wonnerup had a AvTD score of zero and was removed from the figure to increase the resolution. This indicates that, in these terms and compared with expectation, the biodiversity in the Vasse-Wonnerup and to a lesser extent the Peel-Harvey is significantly lower than would be expected.

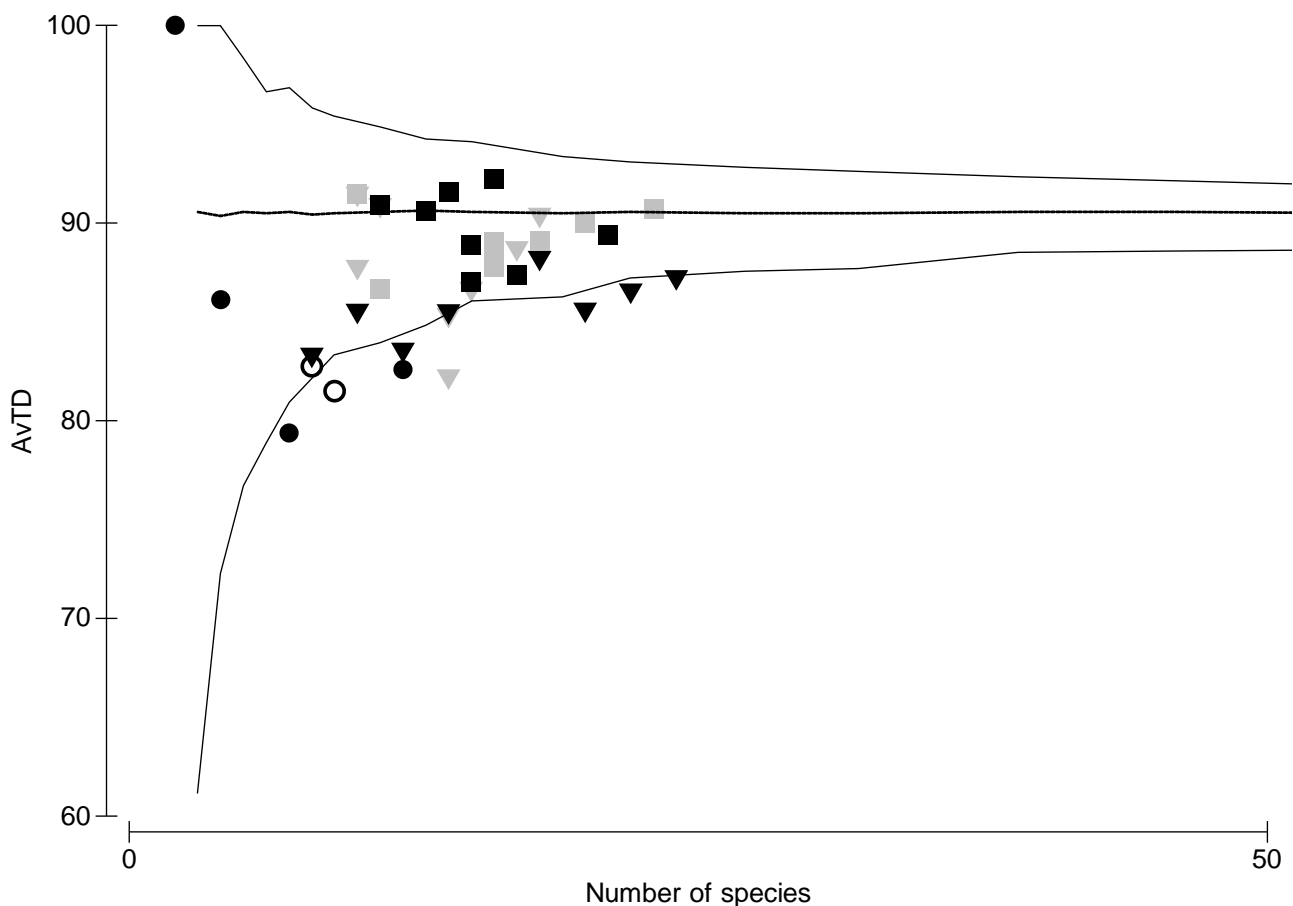


Figure 10: Average taxonomic distinctness (AvTD) against number of species, for the benthic macroinvertebrate species lists compiled from each season in the Peel-Harvey in 1986/7 (▼) and 2003/4 (▼), Swan-Canning in 1986/7 (■) and in 2003/4 (■) and in the Vasse-Wonnerup in 2009 (○) and 2010 (●). The 'expected' value AvTD, in a random subsamples of the 106 benthic macroinvertebrate species included in the study (solid line) and the upper and lower 95% probability limits for a single AvTD value (dashed line) are shown.

5.0: Conclusions

5.1: Description of the benthic invertebrate faunas of the Vasse-Wonnerup Estuary

- Despite the presence of high salinities at certain times of the year in the Vasse-Wonnerup and a seasonal connection with the ocean, the composition of the benthic invertebrate fauna is more similar to that of a wetland than an estuary. This is particularly interesting as the majority of fish species known to have been caught or been subjected to fish kills have been estuarine species (Lane *et al.*, 1997).
- The 62 species of invertebrate recorded in the sweep nets by Chambers *et al.* (2009, 2010, 2011) is slightly greater than the average of 53 species recorded by Grouns *et al.* (1993) for wetlands in the Swan Coastal Plain. The implications of such comparisons, however, must be approached with extreme caution due to differences in the details of the sampling regime (see Azovsky, 2011).
- The number of species and mean density of species recorded in the sweep net and core samples in the Vasse-Wonnerup differed markedly. Thus, whereas 62 species were recorded in sweep nets, only 32 were recorded in core samples. Furthermore, on average, 4,065 individuals per m² were recorded in sweep net samples compared with 209 individuals per m² in the core samples, a difference of approximately 20 times.
- The fauna in sweep net samples comprised mainly arthropods (55 out of 62 species and 97% of the individuals) and only < 0.01% of oligochaetes, while the former group constituted 49% of the total number of individuals and oligochaetes as much as 51%. The prevalence of the latter group of annelids is generally recognized as an indicator of eutrophication and pollution (Lafont, 1984; Verdonschot, 1996; Lin & Yo, 2008).
- Large densities of many of the arthropod taxa, which were highly abundant in the Vasse-Wonnerup, such as chironomids (which contributed 7 and 4% respectively of the individuals recorded in the sweep and core samples), cladocerans (*Daphnia* spp., which contributed 23 and 5% respectively of the individuals recorded in the sweep and core samples), and some ostracod and cyclopoid copepod species are associated with eutrophication (*e.g.* Campbell, 1978; Saether, 1979; Crosser, 1988; Cheal *et al.*, 1993). In contrast, other taxa that are typically present and often in reasonable densities in more pristine environments,

e.g. chydorids, odonates, ephemeropteran larvae (Cheal *et al.*, 1993), were not found in the Vasse-Wonnerup.

- Although the compositions of the benthic invertebrates in the Vasse and Wonnerup lagoons are similar, they undergo marked seasonal and inter-annual changes in both of these water bodies. Any detailed study of the benthic macroinvertebrate faunas of the Vasse-Wonnerup should, therefore, be undertaken in, at least, each season of the year.

5.2: Comparison of the benthic macroinvertebrate faunas of the Vasse-Wonnerup Estuary with the Peel-Harvey and Swan-Canning estuaries

- It must be recognized that, due to differences in sampling methodology, comparisons between the data recorded for the benthic macroinvertebrates in the Vasse-Wonnerup Estuary and those for the Peel-Harvey and Swan-Canning estuaries have to be treated with the upmost caution. However, the data demonstrate that there are some marked differences between these faunas.
- The benthic macroinvertebrate fauna of the Vasse-Wonnerup (those individuals [species] > 500 μm in diameter) is very different to that recorded in the Peel-Harvey and Swan-Canning in the 1980s and 2000s.
- The Vasse-Wonnerup is highly atypical of an estuarine environment in that it contains essentially no polychaetes, which are a major component of the benthic macroinvertebrate fauna of estuaries elsewhere throughout the world. This is despite the fact that invertebrates could be recruited from the estuary during periods when the estuary is open to the ocean and would be able to survive in the range of salinities found in the Vasse-Wonnerup.
- The extreme differences in faunal composition between the Vasse-Wonnerup and Peel-Harvey and Swan-Canning estuaries are also seen at the family, order, class and phylum levels.
- The benthic macroinvertebrate fauna of the Vasse-Wonnerup Estuary is far more depauperate than those of the Peel-Harvey and Swan-Canning estuaries. This point is emphasized by the core samples from the Vasse-Wonnerup yielding only 32 species compared with 63 and 42 species in the Peel-Harvey and Swan-Canning respectively, (Wildsmith *et al.*, 2009, 2011). Note that the above 32 species also contained some species

that would be smaller than 500 μm and would thus not have been retained during the sampling procedure adopted in the Peel-Harvey and Swan-Canning estuaries.

- The mean density (individuals per m^{-2}) of 209 in the Vasse-Wonnerup is radically lower than the 31,950 and 9,590 recorded in the Peel-Harvey in 1986/7 and 2003/4 respectively (Wildsmith *et al.*, 2009) and the 12,340 and 12,140 recorded in the Swan-Canning in 1986/7 and 2003/4 respectively (Wildsmith *et al.*, 2011). Such a dramatic difference may reflect the poor condition of the sediment and possibly a lack of oxygen.
- The average taxonomic distinctness of samples collected from the Vasse-Wonnerup indicated that in 60% of cases the fauna in that lagoon in that season contained a lower taxonomic distinctness than would be expected from the regional species pool (*i.e.* the species which could potentially occur in that system). This measure has been shown to be a good indicator of environmental degradation and thus indicates that the benthic environment of the Vasse-Wonnerup has undergone substantial degradation.

6.0: Recommendations for management

The conservation importance of the Vasse-Wonnerup Estuary is widely recognised as are the environmental perturbations from which it suffers. Annual surveys of the water quality, water and sediment nutrient levels and the diversity, distribution and biomass of phytoplankton, macroalgae and macrophyte species have provided very useful information and should continue to be conducted, particularly with assessing the effectiveness of efforts to decrease nutrient loads into the system and re-vegetate areas. As such, funding has been obtained by Murdoch University through SWCC to conduct another benthic invertebrate survey in 2011 to add to this valuable data set. However, it is vital that the benthic invertebrate fauna continue to be monitored. Such data if continued to be collected in conjunction with environmental data would allow spatial and temporal trends in benthic invertebrate composition to be matched (and correlated) with environmental conditions. This would allow the effect of future changes in the environmental conditions (*e.g.* a decrease in rainfall and/or nutrient levels) on the benthic invertebrate fauna and the knock on effects on the water bird population to be predicted.

The benthic invertebrate data provided here is suitable to be used as benchmark data from which future changes can be detected. As the magnitude of the temporal changes in benthic invertebrate composition greatly exceeded any broad spatial (*i.e.* inter lagoon) differences in this study, any future monitoring regime should incorporate temporal variation. This is particularly important as the salinity changes dramatically throughout the year in this system and less pronounced changes in the salinity of the Swan-Canning Estuary have been shown to markedly alter the composition of the benthic macroinvertebrate fauna (Kanadjembo *et al.*, 2001). It should be noted, however, that although there was no significant differences in faunal composition among lagoons, the sites sampled by Chambers *et al.* (2009, 2010, 2011) were all located in either the Vasse or Wonnerup lagoons. It is thus recommended that in the future benthic invertebrate sampling also occur in the Wonnerup Inlet (the areas between the two sets of flood gates) and the Deadwater as these areas are important fish habitats and undergo different hydrological regimes to the lagoons. Data collected in these regions would greatly increase our understanding of the Vasse-Wonnerup Estuary.

Numerous indicators of estuarine health employing benthic invertebrates have been developed, however, many are complex and require detailed knowledge of the biology, ecology and

environmental/pollution tolerances of all key (*i.e.* abundant) species as well as extensive water and sediment quality data, for example the very successful AMBI index (see Borja *et al.*, 2001). As we know relatively little about the benthic invertebrate fauna of south-western Australia and do not for example have regular quantitative data on heavy metal levels, these more complex indices are inappropriate at the current time. Therefore, we recommend the use of simple matrixes and indicator species/ taxa. These include; 1) taxonomic distinctness (see Wildsmith *et al.*, 2009; Tweedley *et al.*, submitted), which has been successful in south-western Australian estuaries (and throughout the Europe) and was employed in this report, 2) the ratio between oligochaetes and chironomids (see Saether *et al.*, 1979), which is widely used to assess levels of eutrophication in Northern Hemisphere lakes and 3) the densities of key indicators taxa such as oligochaetes, *Daphnia* spp, ostracod and cyclopid species which are associated with eutrophication and other taxa namely chydorids, odonates, ephemeropteran larvae which are indicators of a relatively pristine environment (*e.g.* Crosser, 1988; Cheal *et al.*, 1993) and 4) if the Wonnerup Inlet and Deadwater, when sampled, yielded a more estuarine fauna due to their connection to the ocean, then the relative percentage contribution of polychaetes (which are resilient to environmental perturbation) to crustaceans and to a lesser extent molluscs (which are more sensitive) could prove to be a good indicator (see Wildsmith *et al.*, 2009, 2011; Tweedley *et al.*, submitted).

With the exception of taxonomic distinctness which requires the full taxonomic information for a species and a particular statistical software package (*i.e.* PRIMER, see Materials and Methods) the other indices described above only require basic identification skills and are simple to calculate. These methods, if taught by adequately trained personnel, could, after scientific validation, be passed on the local community and form the basis of a local/community monitoring scheme.

In summary, benthic invertebrates have been shown in a plethora of studies from all around the world to provide a clear indication as to the relative health of the benthic environment. Furthermore, the data analysed in this study is the only data on the benthic invertebrate fauna of the Vasse-Wonnerup Estuary and could provide a baseline in assessing the extent of environmental perturbation against which future changes can be compared.

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