Evaluating the effects of artificial oxygenation and hypoxia on biota in the Upper Swan Estuary

Final report

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Executive Summary

In 2010, the Swan River Trust commissioned Murdoch University to evaluate the effects of artificial oxygenation on the benthic macroinvertebrate (BMI) fauna of the Upper Swan Estuary (USE). The project involved the collection of BMIs monthly between January 2010 and December 2011, at five locations in the USE between Ron Courtney Island and just upstream of Middle Swan Bridge (*i.e.* the Jane Brook confluence). Specifically, the project had two components; 1) to describe any spatial and/or temporal patterns in the BMI community during eight consecutive seasons in 2010 and 2011 and 2) to assess the ecological and management implications of the findings, with specific reference to the effects of hypoxia (low dissolved oxygen) and of the artificial oxygenation program.

The first component of the study recorded a total of ~47,000 individuals, belonging to 42 species, over eight seasons. The vast majority of the species belonged to one of four groups, with the polychaete worms containing the most species (16) and the crustaceans, molluscs and insects each represented by eight species. The total number of species declined progressively from 35 at the most upstream location to 22 at the most downstream location, while the density of benthic macroinvertebrates exhibited no consistent spatial trend. Mean densities of invertebrates ranged from 661 individuals 0.1 m^{-2} at Caversham Upstream to 1,732 at Caversham, with a mean density of 1,037 individuals 0.1 m^{-2} across the entire USE zone.

A small number of species dominated the abundances of invertebrates across the USE as a whole, with four species (the bivalve molluscs *Arthritica semen* and *Fluviolanus subortus*, and the polychaetes *Prionospio cirrifera* and *Desdemona ornata*) collectively accounting for ~78% of total numbers of invertebrates, and 10 species comprising almost 98% of total numbers. In contrast, the remaining 32 species were scarce, each accounting for <1% of the total abundance of invertebrates across the entire zone. Numerical dominance by a small number of taxa was also typically observed at each of the five locations, with suites of four species accounting for between ~69% and 97% of the total number of individuals per location. A common suite of eight to ten species were among the ten most abundant taxa at each of the five locations.

Overall, differences in the composition of the BMI community among locations and seasons were significantly related to patterns in salinities. Broad spatial trends in abundance were exhibited by several species. For example, densities of the bivalve mollusc *Arthritica semen* and the polychaete worms *Prionospio cirrifera* and

Desdemona ornata increased in a downstream direction, whereas the reverse was true for the polychaetes *Pseudopolydora kempi* and *Simplisetia aequisetis*, and the tube-dwelling amphipod crustacean *Paracorophium excavatum*.

The second component of this study was intended to determine the effects of the artificial oxygenation program on biota. However, several factors critically confounded the experimental design needed to address this question, thus preventing a valid analysis of the effects of the oxygenation plants on BMI communities. Nonetheless, during the study an extreme rainfall event occurred, which provided a unique opportunity to evaluate the effects of hypoxia on the benthic fauna. Precipitation associated with an extreme storm event during March 2010 exceeded the design specifications of the artificial oxygenation plants and led to the development of a large body of hypoxic water (*i.e.* water with a dissolved oxygen concentration of <2 mg/L) in the USE, which persisted for between 7 and 17 weeks, depending on the location within the USE.

The presence of these hypoxic conditions caused declines in the number of species, total density, evenness, taxonomic diversity and the densities of crustaceans and molluscs. Analysis of the faunal community composition indicated that, at all locations except Guildford Downstream, the overall composition of the BMI community was altered in response to hypoxia over the period April-July 2010. This was due mainly to the almost complete absence of crustacean species, which are known to be extremely sensitive to hypoxia. For example, the amphipod crustaceans *Paracorophium excavatum, Grandidierella propodentata* and *Melita matilda* were not recorded at any of the locations between March and July 2010, despite being recorded frequently on the other sampling occasions. Moreover, the hypoxic conditions also resulted in declines in abundance of the bivalve *Fluviolanatus subortus* and even the polychaetes *Pseudopolydora kempi*, *Leitoscoloplos normalis* and *Marphysa sanguinea,* which are regarded as being among the most tolerant group of BMIs. The changes described above were far less evident at the Guildford Downstream location, suggesting that this area appeared to be chronically disturbed throughout the course of the current study. It is hypothesized that this reflects the potential influence of flows from the Helena River and Bennett Brook as sources of fine sediments, particulate organic matter and possibly nutrient and non-nutrient contaminants, each of which might impact on the survival and/or recruitment success of more sensitive invertebrate species.

While oxygenation plants are present in the USE, only one of the two was operational at the time of the extreme rainfall event responsible for the hypoxic conditions, as the plant at Caversham only began regular operations in June 2011.

Furthermore, these plants were not designed to mitigate the effects of extreme storm events and so would not be expected to prevent the large scale hypoxia seen in the present study. Although the effects of artificial oxygenation on the BMI fauna could not be demonstrated directly during this study, it is likely that, under normal operating conditions, the oxygenation plants could help to bring about a more rapid return to ‘healthy’ oxygen levels (*i.e.* >4 mg/L) following such extreme events. Indeed, recent modeling approaches have demonstrated the capability of the oxygenation plants to improve near-bed oxygen status across large areas of the river (Hipsey *et al.,* 2014). The current study provides a suitable benchmark against which future changes in BMI communities might be assessed, including those that might be expected in response to ongoing, effective and large-scale oxygenation of the upper estuary.

Table of contents

1. Introduction

1.1. The Swan Canning Estuary

The Swan Canning Estuary (32.055 °S, 115.735 °E) is ~50 km long, covers a surface area of ~55 km^2 and comprises a narrow entrance channel, two central basins and the tidal portions of two main tributaries, the Swan and Canning rivers (Hodgkin and Hesp, 1998; Brearley, 2005; Fig.1). This permanently-open, microtidal system has been extensively modified through anthropogenic activities and climate change (Commonwealth of Australia, 2002), resulting in multiple stressors including increased delivery of sediments and nutrients, increasing salinisation and hydrological changes. In combination with the natural geomorphological, climatic and tidal characteristics of the system, these stressors have exacerbated the extent and persistence of the vertical stratification of the water column, which is typically observed in the upper reaches of this estuary at certain times (Hamilton *et al.*, 2001; Hamilton and Turner, 2001; Chan *et al.*, 2002). The system suffers from periodic deoxygenation of the bottom waters (Stephens and Imberger, 1996), most notably in the upper, estuarine reaches of the Swan and Canning Rivers. Such deoxygenation commonly leads to the development of hypoxic conditions, *i.e.* low dissolved oxygen (DO) concentrations of <2 mg/L (Rosenberg, 1980; Rabalais *et al.*, 2010), across much of the upper regions of the estuary, which occasionally become anoxic (*i.e.* 0 mg/L of DO; Douglas *et al.*, 1997; Kurup and Hamilton, 2002).

As in many other shallow, microtidal estuarine systems, hypoxia may develop within the Swan Canning Estuary as a consequence of stratification of the water column (Douglas *et al.*, 1997; Buzzelli *et al.*, 2002; Eldridge and Roelke, 2011), the occurrence of phytoplankton blooms (Hamilton *et al.*, 1999; Zammit *et al.*, 2005), microbial decomposition of dissolved organic carbon (DOC) and particulate organic matter (POM) inputs, *e.g.* following riverine flow events (Rabalais *et al.*, 2010; Eldridge and Roelke, 2011), or from a combination of the above factors. Hypoxic conditions can, in turn, lead to the production of ammonium, and in some cases hydrogen sulphide, and cause the release of sediment-bound nutrients (Middleburg and Levin, 2009; Rabalais *et al.*, 2010). The consequences of hypoxia and these accompanying biochemical processes are well-documented and can include algal blooms (Møller and Riisgård, 2007; Zhang *et al.*, 2010), effects on the distribution, feeding and growth of organisms (Pihl *et al.*, 1991; 1992; Burkholder *et al.*, 1999; Eby and Crowder, 2002; 2004; Eby *et al.*, 2005; Rabalais *et al.*, 2010), and significant mortalities of sessile invertebrates and even of highly mobile fish (Stachowitsch, 1984; Hosja and Deeley, 1994; Eby *et al.*, 2005; Diaz and Rosenberg, 2008). Many of these hypoxic effects are frequently observed in the Swan Canning Estuary.

1.2. Artificial oxygenation of the Swan Canning Estuary

In response to increasing concern over the occurrence and impacts of hypoxia in this system, various methods have been trialed in recent decades to directly address this issue. Trials using bubble curtains were initially conducted in 1997 in the vicinity of Belmont, in the Middle Swan Estuary zone, in an attempt to mix and artificially destratify part of the water column of the Swan River, thus enabling greater mixing of dissolved oxygen (Hamilton *et al.*, 2001). However, this method was judged to be inadequate to overcome the stratification in this region of the estuary, due to the limited extent of mixing observed.

Subsequent attempts to address the hypoxia and related nutrient issues have focused on artificial oxygenation of the denser, less oxygenated waters at the bottom of the stratified water column. Following a series of well-documented and increasingly successful trials of barge-based and ‘sidestream’ plant designs (Greenop *et al.*, 2001; Greenop and Robb, 2003), four oxygenation plants based on the latter design have been constructed and are now operational along the upper estuarine reaches of the Swan River and the Kent St Weir Pool in the Upper Canning River. These plants vaporize liquid oxygen from a supply on the river bank while drawing in deoxygenated water from the bottom of the water column. The resultant oxygen gas is added to the pressurized water and pumped back out into the estuary as a supersaturated solution. Two such plants have been operational on the Canning River since the late 1990s, whilst the Swan River is oxygenated by a plant at Guildford (operational since 2009) and another at Caversham that has been operating since mid-2011 (Fig. 1).

1.3. Study rationale and objectives

Prior to 2010, assessment of the oxygenation program focused on its ability to successfully add oxygen to the water column. However, the monitoring of biotic responses to oxygenation is part of a planned, longer term strategy to include broader ecosystem responses as measures of the success of the oxygenation program. Therefore, the SRT and Murdoch University entered into a collaborative arrangement in 2010 to monitor the effects on biota of artificial oxygenation in the Upper Swan Estuary (USE).

The oxygenation advisory group (OAG) of the SRT advised that benthic macroinvertebrates (BMI) form the basis of any sampling program aimed at quantifying ecosystem responses to oxygenation because of their; (1) broadly understood responses to low dissolved oxygen concentrations; (2) relative immobility (and hence ability to be affected by localized changes in dissolved oxygen concentrations) and (3) relative ease of sampling and identification in comparison with other biota, such as meiofauna.

Although benthic macroinvertebrate communities have previously been surveyed at various locations throughout one or more regions or zones of the Swan Canning Estuary (*e.g.* Chalmer *et al.*, 1976; Rose, 1994; Kanandjembo *et al.*, 2001; Wildsmith *et al.*, 2011), no studies have sampled the reaches of the Swan River influenced by the artificial oxygenation. The present study entailed two years of monthly monitoring of the BMI communities at numerous locations throughout the USE, including those in the vicinity of the oxygenation plants, and thus aimed to facilitate an evaluation of the impacts of the oxygenation program on the biota of the Swan Canning Estuary.

The current study had the following objectives:

- To conduct monthly monitoring during 2010 and 2011 of benthic macroinvertebrate communities at locations throughout the USE, including those in the vicinity of the artificial oxygenation plants at Guildford and Caversham.
- To interpret any spatial and/or temporal patterns observed in the macroinvertebrate fauna during the monitoring period (including, where possible, the effects of hypoxia and of artificial oxygenation).
- To put the findings of the study into the broader perspective of system ecology and management implications, with specific reference to the artificial oxygenation program.

It should be noted, however, that several extraneous factors have critically confounded the experimental design underlying the resulting sampling program. These include, but are not limited to, (i) the lack of any true ‘control’ sites in the USE or of any ‘before’ time period against which to make comparisons (and particularly so for the Guildford oxygenation plant), (ii) the sporadic use of the oxygenation plants in response to low oxygen levels (*i.e.* they are operated frequently yet irregularly in response to ambient oxygen levels, and the spatial and temporal extents of their oxygenation may vary with climatic and tidal conditions), and (iii) the effects of any stochastic environmental influences (algal blooms, high flow events etc.) which might occur during the study period. Consequently, the experimental design of the current study prevented a valid, direct assessment of the effects of oxygenation on BMI communities. In light of the above obstacles, it is considered that the effects of the artificial oxygenation plants on biota cannot be assessed through field studies and instead, would require controlled laboratory and mesocosm experiments (see section 4).

However, the sampling regime for this study coincided with the occurrence of a significant storm, the costliest natural disaster in Western Australia's history (Buckley *et al.,* 2010), which hit Perth and surrounding areas on 22 March 2010. The storm brought significant rainfall, with over 40 mm of rain (much of it falling in 30 minutes; Bureau of Meteorology, 2010), and ended a lengthy dry spell of 144 days (Appendix 1). Following the storm, persistent and widespread hypoxia occurred in the USE zone of the Swan Canning Estuary, which greatly exceeded the design specifications of the oxygenation plants, developed, and lasted for several weeks (Appendix 2). The occurrence of this hypoxic event during the course of sampling for the current study thus provided an opportunity to address an additional study objective:

- To investigate the effects of a notable period of hypoxia on the benthic macroinvertebrate fauna of the USE.

It is anticipated that the current study also will provide a suitable benchmark against which future changes in BMI communities in response to ongoing oxygenation might be assessed, in a manner similar to the inter-decadal comparisons of benthic community composition that have been conducted in the Lower Swan Canning Estuary (see Wildsmith *et al.*, 2011).

2. Materials and methods

2.1. Sampling regime and sample processing

Benthic macroinvertebrates (BMI) were sampled on a monthly basis between January 2010 and December 2011 at five locations, Caversham Upstream (CU), Caversham (C), Caversham-Guildford (CG), Guildford (G) and Guildford Downstream (GD), spread throughout the deeper (~1-4 m), offshore waters of the USE (Fig. 1). Five randomly-located samples of sediment were collected from each location on each sampling occasion in 2010 and 2011 using an Ekman grab (Wildco, Florida, USA), which collected substrata from an area of 225 cm^2 and sampled to a maximum depth of 15 cm. The sediment samples were preserved in 5% formalin buffered in estuary water and subsequently wet-sieved in the laboratory though a 500 µm mesh and stored in 70% ethanol. Using a dissecting microscope, the invertebrates were removed from any sediment retained on the mesh and identified to the lowest possible taxonomic level (usually species). The number (abundance) of each macroinvertebrate taxon in each replicate sample was determined for use in statistical analyses and, where appropriate, was also expressed as a density (*i.e.* number of individuals 0.1 m^{-2}) for presentation purposes.

Although salinity, water temperature and dissolved oxygen (DO) concentrations were recorded at each location on each sampling occasion using a Yellow Springs International Model 556 water quality meter (Ohio, USA), weekly measurements of these parameters at various water depths were recorded by the Swan River Trust and Department of Water (DoW, 2012) at a wider range of stations, encompassing the study locations. Thus, these more comprehensive data have been employed in this study as they provide a more effective and more relevant spatial and temporal resolution for interpreting the effects of water quality trends on BMI communities. As the sampling locations surveyed in this study were ~1.5 km long, they often encompassed several of the weekly water quality monitoring sites. Therefore, for each season, the weekly water quality data (from the bottom of the water column) that most closely matched the date on which the BMI fauna were collected were extracted. Where multiple water quality sites fell within the BMI sampling locations, the data from those sites were averaged to provide a single value for each of salinity, water temperature and dissolved oxygen concentration at each location in each season.

Five additional, randomly-located sediment samples, collected seasonally from each location using the same Ekman grab, were used to determine the % content of particulate organic matter (%POM) in the sediment and the grain size composition of the sediment. Any large benthic macroinvertebrates, particularly molluscs, were removed from the sediment samples, which were then dried for 24 h at 80 °C, weighed to the nearest 1 mg and then ashed for 2 h at 550 °C and re-weighed (Heiri *et al.*, 2001). The difference between the dry and ashed weight enabled the percentage contribution of POM in each sample to be calculated. Each ashed sample was then wet-sieved through a 63 µm sieve to remove the fine sediment particles, dried and re-weighed. The remaining material was wet-sieved through a stack of sieves with mesh sizes corresponding to the Wentworth Scale for grain size, *i.e.* 2,000, 1,000, 500, 250, 125 and 63 µm (Wentworth, 1922). After separation, the samples for each grain size were dried and weighed, enabling their percentage contributions by weight to be calculated.

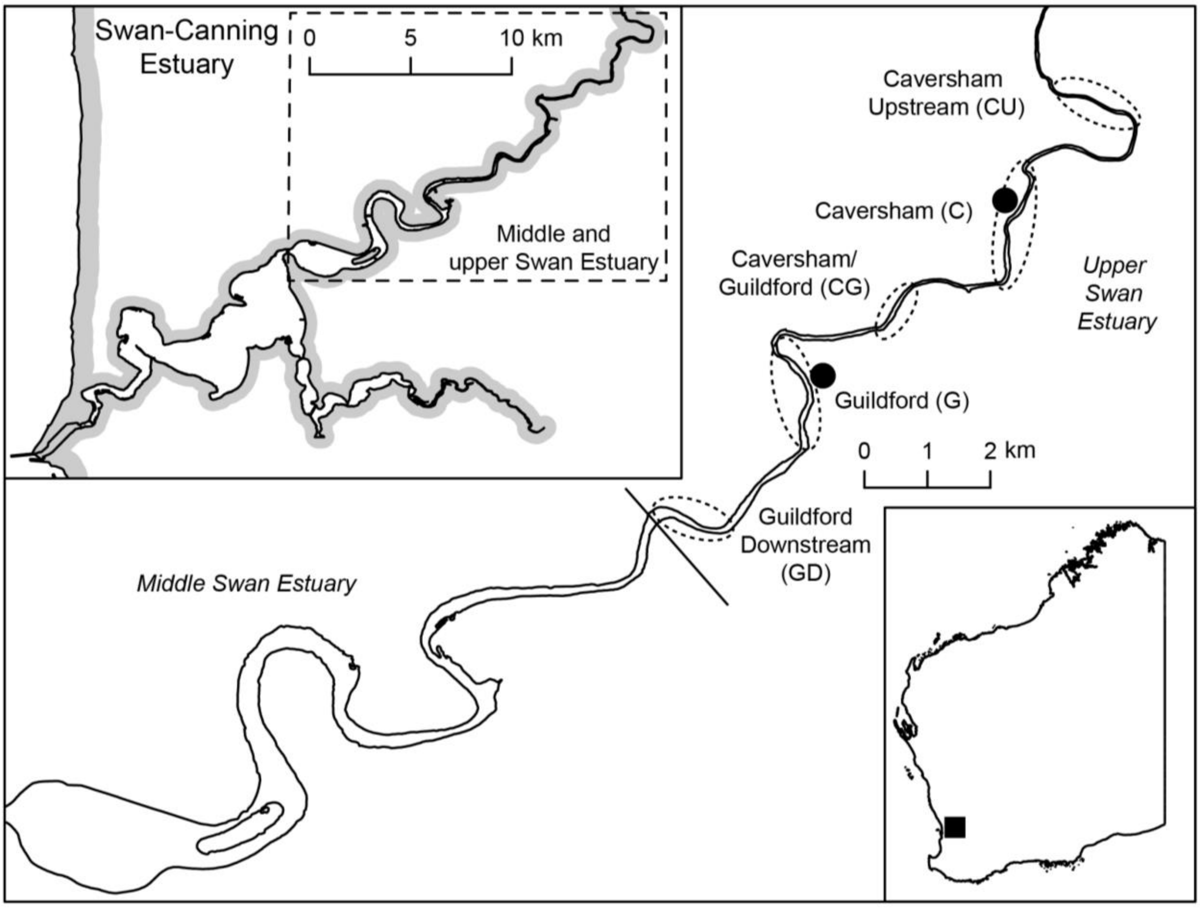


Figure 1. Map of the Swan Canning Estuary showing the sampling locations (dashed lines) and the locations of the oxygenation plants (●) in the Upper Swan Estuary (USE). ■ denotes location of the Swan Canning Estuary in Western Australia.

2.2. Statistical analysis

All statistical analyses were performed using the PRIMER v6 multivariate statistics software package (Clarke and Gorley, 2006) with the PERMANOVA+ add on (Anderson *et al.*, 2008), with the exception of the shade plots which were generated using an alpha test version of PRIMER v7.

2.2.1. Spatial and seasonal trends in benthic macroinvertebrate community characteristics

The abundances of the various BMI species in each of the five replicate Ekman grab samples collected from each location seasonally between January 2010 and October 2011 were initially fourth-root transformed to reduce the influence of any highly abundant species. These data were then employed to construct a Bray-Curtis resemblance matrix and subjected to a three-way permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001; Anderson *et al.*, 2008) to determine the extent to which the compositions of the invertebrate fauna were related to year (2 levels), season (4 levels) and location (5 levels) and whether there were any significant interactions between the factors. In this and all other tests, the spatial and temporal factors were considered fixed effects and the null hypothesis of no significant differences between *a priori* groups was rejected when the *p* value was <5%.

Analysis of Similarities (ANOSIM; Clarke and Green, 1988) tests were then employed to assess the relative influence of each of the factors. In this analysis the Bray-Curtis resemblance matrix was subjected to a series of two-way ANOSIM tests for each factor (year, season and location) versus each of the other two factors combined (*e.g.* location *vs* season_year). As the above PERMANOVA test detected significant interactions between all factors, including the year x season x location interaction, the Bray-Curtis resemblance matrix was separated for each level of the relevant temporal factors (*i.e.* each of the eight seasonal sampling occasions) in order to remove their confounding influence. The eight sub-matrices were then each subjected to one-way ANOSIM tests to elucidate, in more detail, the extent to which the composition of the BMI fauna differed among locations. The extents of any significant differences were determined by the magnitude of the test statistic (*R*), which typically ranges between ~0, *i.e.* no differences among groups and 1, *i.e.* the similarities between samples from different groups are all less than those between samples in the same group. The same Bray-Curtis sub-matrices were also subjected to non-metric Multidimensional Scaling (nMDS) ordination (Clarke, 1993), in order to display visually the differences in the BMI composition among locations.

When ANOSIM detected a significant difference in faunal composition among locations 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 location and those which contributed most to differences between each pair of locations. Focus was placed on those typifying and distinguishing species that had the highest similarity/standard deviation and dissimilarity/standard deviation ratios, respectively, and that were most abundant.

The Biota and Environment matching routine (BIOENV; Clarke and Ainsworth, 1993) was then employed to ascertain whether the relative differences among locations, as defined by their BMI faunal composition, were significantly correlated with a suite of water quality variables measured at the bottom of the water column by the Swan River Trust and the Department of Water (*i.e.* water temperature, DO concentration, salinity and pH) and the suite of sediment variables described above (*i.e.* organic matter content and each of the Wentworth grain sizes). This routine thus determined how similar the patterns of the rank orders of resemblance were between the Bray-Curtis resemblance matrix constructed from the fourth-root transformed and averaged BMI data and a complementary Euclidean distance matrix constructed from the water quality and sediment data.

Prior to use in BIOENV the water quality and sediment variables were initially examined using separate pairwise scatter plot (draftsman plot) to visually assess the extent to which the distribution of values for each variable were skewed and thus the type of transformation required to ameliorate any such effect. This analysis indicated that, while none of the water quality variables required transformation, each of the sediment variables required a square-root transformation. The separate matrices of water quality (containing two to five replicates per location per season) and sediment quality (containing five replicates per location per season) were then averaged to give a mean value for each variable at each location in each season, and combined to create a single matrix. This matrix was then used to create a draftsman plot to assess whether there was evidence of any co-linearity between each pair of variables (*i.e.* Pearson's correlation values ≥ 0.95; Clarke & Warwick, 2001). As this was not the case (see Appendix 3), all 12 of the water quality and sediment variables were retained in the BIOENV analysis. Lastly, as data for several of the environmental variables were not directly comparable due to their different units of measurement, the data for each variable was normalised to place all variables on a common scale, whilst retaining the variance within each variable (Clarke & Gorley, 2006).

Note that, although PERMANOVA showed that there was a significant year x season x location interaction and thus that the BIOENV analysis should be conducted separately for each sampling occasion (*e.g.* summer 2010), the five samples (*i.e.* each location) would not provide enough power for a robust analysis. Therefore, this analysis was done separately for each season (*i.e.* summer, autumn, winter and spring), thus giving 10 samples and enough power to reliably test for any similarity in the rank orders of resemblance between the faunal and environmental data. The test statistic, rho (ρ), was used to gauge the extent of any significant differences (*i.e.* p <5%), with ρ values ~ 0 reflecting little correlation in the rank order agreement and those ~ 1 reflecting a near perfect match.

nMDS ordination plots of the BMI faunal composition for each location in each of the four seasons were also constructed. Circles or ‘bubbles’ of proportionate sizes that represented the magnitude of a particular environmental variable at each location in that season were used to visually display any significant match identified by BIOENV.

2.2.2. Benthic macroinvertebrate community responses to a notable hypoxic ‘event’

A data matrix was constructed using the mean number, over five replicate Eckman grabs, of each species at each location on each sampling occasion and was, in turn, subjected to the DIVERSE routine to calculate the values for a number of diversity/abundance measures. These were: number of species, total density (per sample), Simpson’s evenness index (1-λ; Simpson, 1949; Somerfield *et al.*, 2008), and average quantitative taxonomic distinctness (Δ*; Warwick and Clarke, 1995). The densities of polychaetes, crustaceans and molluscs were each calculated, as was the percentage contribution of polychaetes to the combined polychaete and crustacean fauna. The latter measure provides a suitable modification of the widely-used polychaete to amphipod ratio (Dauvin and Ruellet, 2007), whereby values reflecting the relative dominance of more tolerant polychaete taxa over their more sensitive crustacean counterparts may indicate ecologically disturbed conditions (Marques *et al.,* 2009).

The diversity and abundance indices were separately subjected to univariate permutational ANOVA, by entering Euclidean distance between each pair of diversity/abundance values into the PERMANOVA routine. This was a two-way crossed ANOVA to determine whether each of the variables was related to location (5 levels) or sampling occasion (12 levels, corresponding to the 7 monthly sampling occasions from January to July 2010 and the five subsequent seasonal sampling occasions between October 2010 and October 2011). In order to provide a robust assessment of the diversity and abundance indices at each sampling location, the individual replicates were averaged prior to calculating the measures. The location x occasion interaction term was therefore employed as the ANOVA residual, providing a sound (and conservative) basis for assessing the statistical significance of the main effects of location and occasion. Prior to undertaking these analyses, draftsmans plots of the values recorded for each pair of diversity measures at each location were examined visually to assess whether the values for each variable were heavily skewed and, if so, which type of transformation could ameliorate that effect. These plots demonstrated that total density required a log(X+1) transformation, while the remaining variables did not require transformation.

For multivariate analyses of faunal composition, the abundances of each BMI species in each replicate sample from each of the five locations on each sampling occasion were converted purely to presence/absence data. This transformation was chosen as it maximised the information on turnover of species during the hypoxic event, rather than on changes in numerical abundance. This is because many of the most abundant species remained present in relatively large numbers throughout the time course at all locations and, unless heavily transformed, the interesting and important changes happening to less abundant species would be obscured by the influence of these large abundances. These data were then used to construct a Bray-Curtis resemblance matrix, which was subjected to the same two-way PERMANOVA design as described above, with the exception that as robust replicate data were available, the location x occasion interaction term was calculated rather than being used as the residual. As above (subsection 2.2.1), a two-way ANOSIM test was then employed to assess the relative influence of both factors, *i.e.* location and sampling occasion.

In order to visualise the time-course of change in community composition and assess which species may have declined after the hypoxic event, the above presence/absence matrix was averaged to create a ‘frequency matrix’. Thus, in the latter matrix, important information about the frequency with which individual species are found in replicate samples is maintained, whilst the species counts themselves are not influential. When, as in the current study, sufficient, consistently collected replicates are available, use of frequency data in this way is often a powerful and informative option for multivariate analysis in the presence of erratic fluctuations in counts. As the above PERMANOVA analysis indicated that there was an interaction between location and sampling occasion, further analyses were conducted on various subsets of the above matrix corresponding to a particular location.

While we were able to construct an *a priori* hypothesis stating that the BMI fauna in the Upper Swan Estuary would have changed as a result of the hypoxic event that followed heavy rains in March 2010, we were unable to predict when the hypothesised change in faunal composition would end and the fauna recover to that observed pre-hypoxia. Therefore, to identify those sampling occasions, at each location, that did not differ significantly in their BMI fauna composition the above ‘frequency matrix’ was separated for each location and, in turn, subjected to hierarchical agglomerative clustering with group-average linking (CLUSTER) and an associated Similarity Profiles (SIMPROF) test (Clarke *et al.*, 2008). A SIMPROF test was performed at each node of the dendrogram to ascertain whether the particular group of samples (*i.e.* sampling occasions) being subdivided contained any significant internal structure. This routine thus provided a sound and objective basis for ascertaining the points in the clustering procedure at which further subdivision of the samples was unwarranted. The null hypothesis that there was no significant faunal differences among sampling occasions was rejected if the significance level (p) associated with the test statistic (π) was <5%.

Finally, shade plots were produced in order to visualise the changes in the frequency of individual species occurrences over time at each location. The shade plot is a simple visualisation of the frequency matrix, with white and black rectangles indicating the respective absence or presence of a given species in all five replicates from that location on that sampling occasion, and grey-scale rectangles whose depth of shading is linearly proportional to the species being present in 20, 40, 60 and 80% of the replicates in that location on that sampling occasion (Clarke *et al.,* 2014; Valesini *et al.,* 2014).

3. Results

3.1. Spatial and seasonal trends in benthic macroinvertebrate community characteristics

3.1.1. Seasonal changes in environmental variables

Water temperatures at the bottom of the water column were relatively consistent among locations and underwent a progressive sinusoidal seasonal pattern with minimum values of ~11-15 °C during the two winters and maxima of ~27-29 °C during summer (Fig. 2a). The largest differences in temperatures between the five locations were observed during winter, when temperatures generally decreased in an upstream direction. Dissolved oxygen concentrations underwent a similarly sinusoidal pattern at the three locations furthest upstream (*i.e.* CU, C and CG), with minimum values of ≤2 mg/L during summer or autumn and maxima of ≥8 mg/L during winter. In contrast, dissolved oxygen levels at the G and GD locations generally remained lower, including during winter, and seldom exceeded 6 mg/L (Fig. 2b). Salinities at the bottom of the water column, whilst also demonstrating a sinusoidal pattern with autumnal maxima and minima in winter/spring, exhibited greater variability both temporally (*i.e.* higher maxima and lower minima in 2011 *vs* 2010) and spatially, with a notable upstream-downstream salinity gradient being present for the majority of the study period (Fig. 2c). The pH levels of the bottom waters at locations G and GD remained relatively constant at 7.2-7.3, whereas pH increased noticeably during winter of each year at the CG, C and CU locations (Fig. 2d).

The percentage contribution of organic matter in the sediments remained relatively consistent at most locations throughout the two years, with the exception of Caversham, where organic matter levels increased almost sequentially (Fig. 3). The sediments at the two most upstream locations (*i.e.* C and CU) contained relatively less organic matter, *i.e.* 1-6%, than those from CG, G and GD, *i.e.* 8-16% during 2010. In 2011, while the contribution of organic matter in the sediments at CU remained <5%, the levels recorded at C were more similar to those recorded further downstream at CG, G and GD, *i.e.* ~5-16%. In general, sediments from all locations in both years largely comprised grain sizes between 250 and 1,999 µm, (*i.e.* the 250 µm, 500 µm and 1,000 µm fractions), with these fractions comprising between 55 (CU in the summer of 2010) and 95% (C in the summer of 2011) of the total sediment (Fig. 4). In 2010 there was a clear spatial and temporal shift in the composition of the sediments, with greater contributions of finer grain sizes being recorded further downstream and during winter and spring compared to summer and autumn (Fig. 4a). These trends are less clear in 2011, mainly due to finer sediment being recorded at C than was previously observed in 2010 (*cf.* Figs. 4a & b).

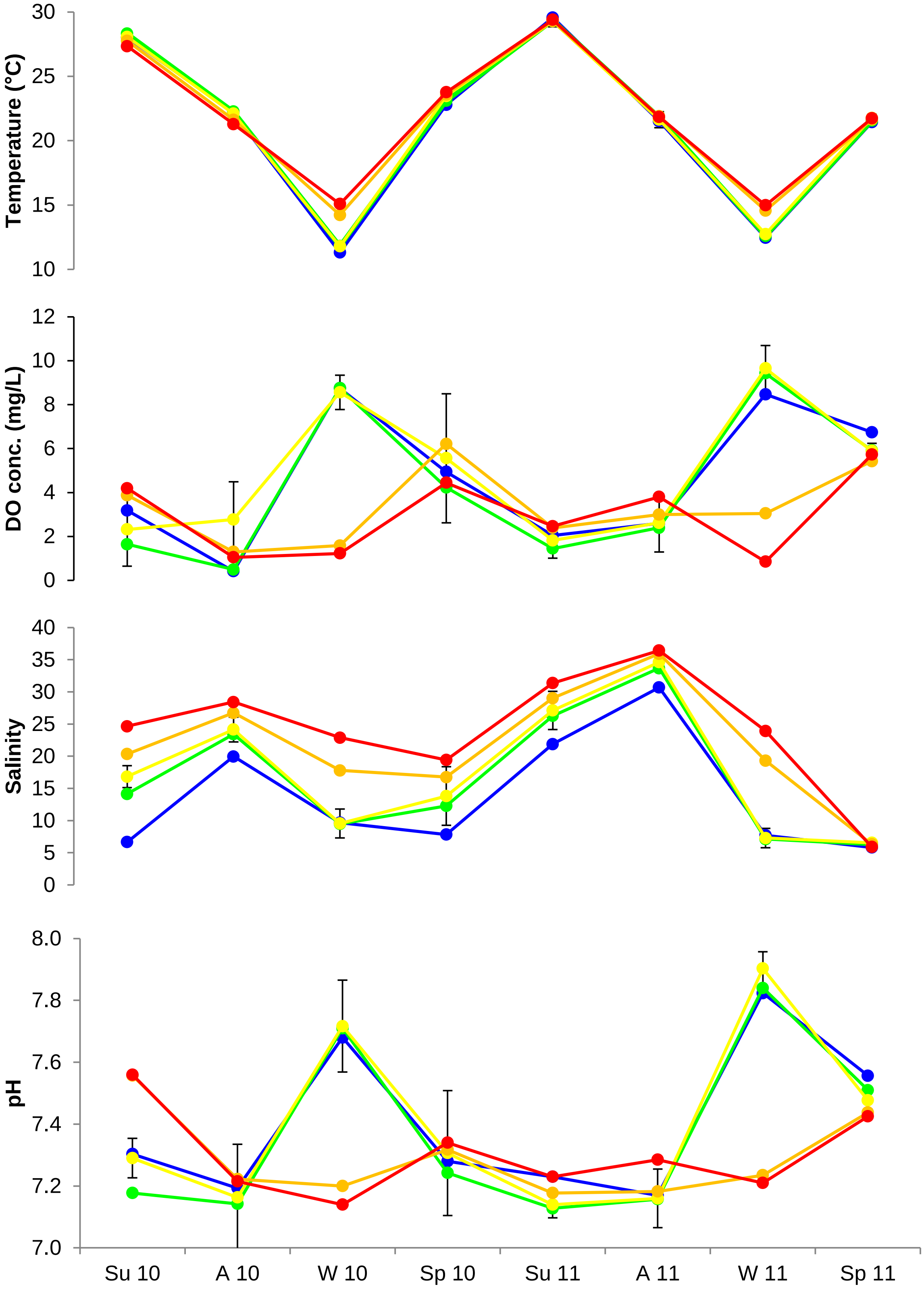


Figure 2. Average (a) water temperature, (b) dissolved oxygen concentration (DO conc.), (c) salinity and (d) pH of the bottom waters at the five locations in the Upper Swan Estuary over eight consecutive seasons throughout 2010 and 2011. 🟦, Caversham Upstream; 🟩, Caversham; 🟨,Caversham-Guildford; 🟧, Guildford; 🟥,Guildford Downstream. Su, Summer; A, Autumn; W, Winter; Sp, Spring; 10, 2010; 11, 2011. For clarity, error bars representing the mean ±95% confidence limits across all locations in each season are provided on the Caversham-Guildford line.

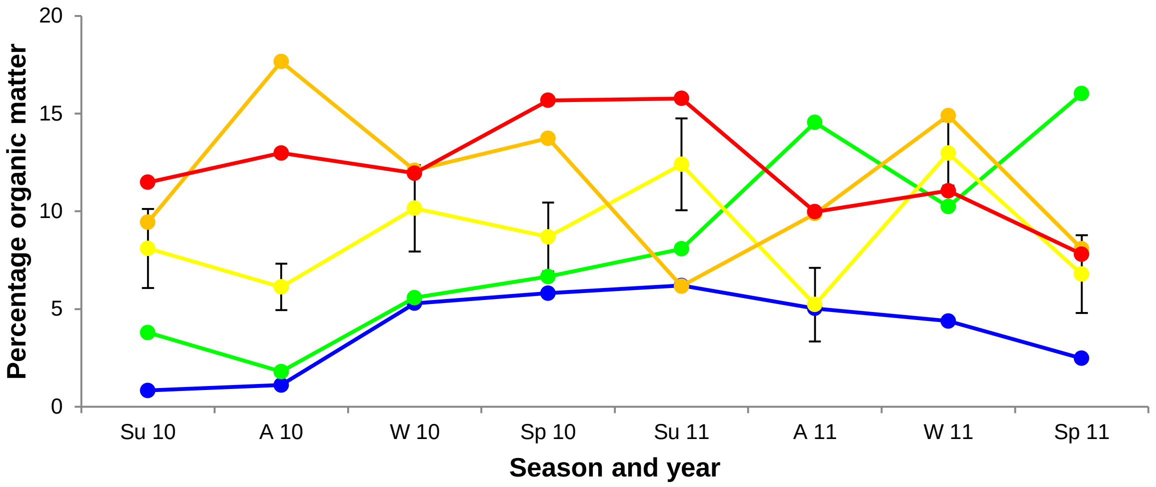


Figure 3. Average percentage sediment organic matter content at the five locations in the Upper Swan Estuary over eight consecutive seasons throughout 2010 and 2011. 🟦, Caversham Upstream; 🟩, Caversham; 🟨,Caversham-Guildford; 🟧, Guildford; 🟥,Guildford Downstream. Su, summer; A, autumn, W, winter; Sp, spring; 10, 2010; 11, 2011 For clarity, error bars representing the mean ±1 standard error for all locations in each season are provided on the Caversham-Guildford line.

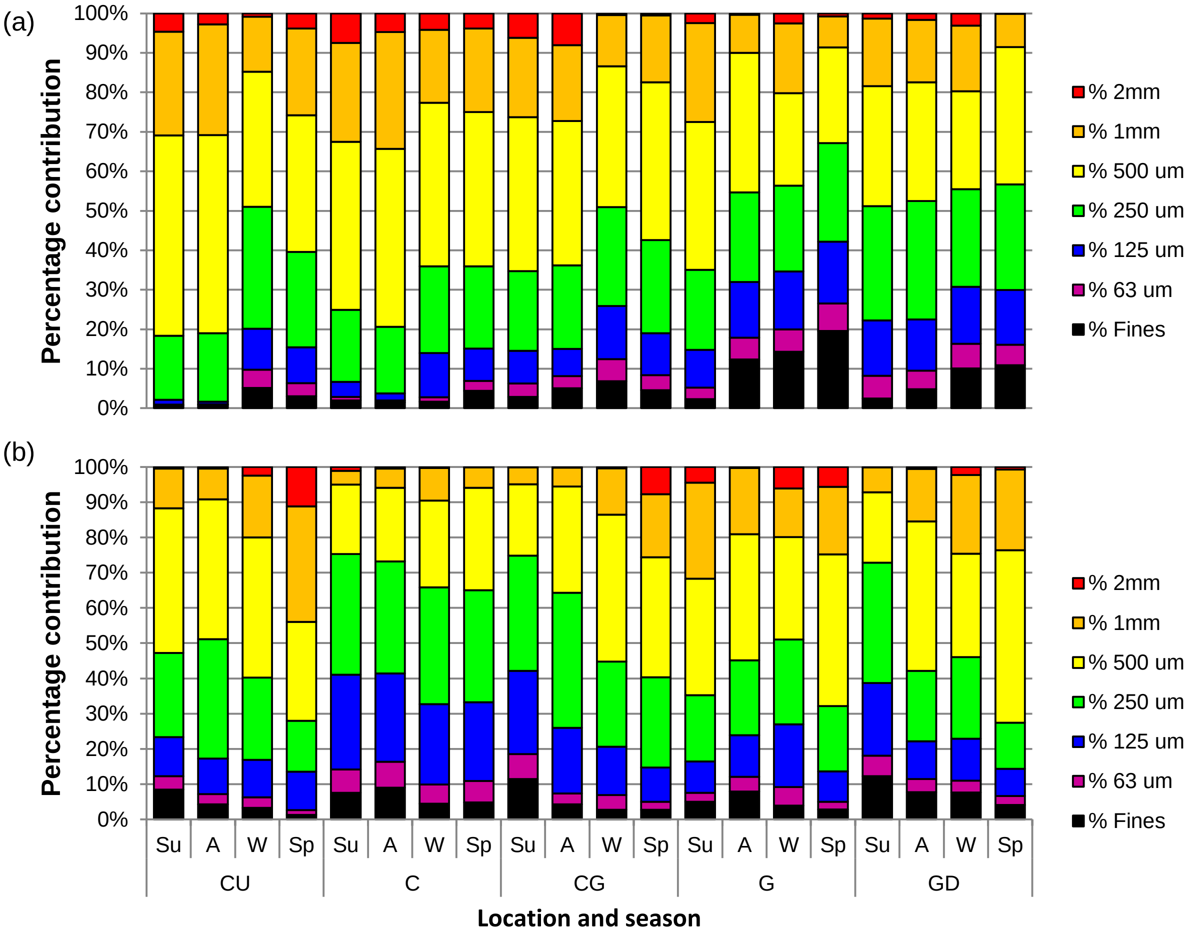


Figure 4. Seasonal variations in average sediment grain size at the five locations in the Upper Swan Estuary over eight consecutive seasons throughout (a) 2010 and (b) 2011. CU, Caversham Upstream; C, Caversham; CG, Caversham-Guildford; G, Guildford; GD, Guildford Downstream. Su, Summer; A, Autumn; W, Winter; Sp, Spring.

3.1.2. Abundance and diversity measures

A total of 46,680 individuals, representing 42 benthic macroinvertebrate species, were collected during seasonal sampling of the five locations in the USE zone. The most speciose subphylum was the polychaeta, with 16 species, whilst the mollusca, crustacea and insecta were each represented by eight species. The number of species collected per location decreased in a downstream direction from 35 species at CU to 22 species at GD (Table 1).

Mean densities of invertebrates ranged from 661 individuals 0.1 m^{-2} at CU to 1,732 at Caversham, with a mean density of 1,037 individuals 0.1 m^{-2} across the entire USE zone (Table 1). A small number of species dominated the abundances of invertebrates across the USE as a whole, with four species (the bivalve molluscs *Arthritica semen* and *Fluviolanus subortus*, and the polychaetes *Prionospio cirrifera* and *Desdemona ornata*) collectively accounting for ~78% of total numbers of invertebrates and 10 species comprising almost 98% of total numbers (Table 1). In contrast, the remaining 32 species were scarce, each accounting for <1% of the total abundance of invertebrates across the entire zone. Numerical dominance by a small number of taxa was also typically observed at each of the five locations, with suites of four species accounting for between 97% (at GD) and ~69% (at CG) of the total number of individuals. A common suite of eight to ten species were among the ten most abundant taxa at each of the five locations.

The densities of several species exhibited broad spatial trends in abundance. For example, densities of *A. semen*, *P. cirrifera* and *D. ornata* increased in a downstream direction, whereas the reverse was true for polychaetes *Pseudopolydora kempi* and *Simplisetia aequisetis*, and the tube-dwelling amphipod *Paracorophium excavatum* (Table 1).

Table 1. Mean ($\bar{x}$) and standard deviation (SD) of the density (0.1 m^{-2}), the percentage contribution to total abundance (%) and the rank abundance (R) of each benthic macroinvertebrate taxon in sediment samples collected at five locations in the Upper Swan Estuary zone, and collectively across the zone as a whole, in eight consecutive seasons between January 2010 and October 2011. Sp denotes subphylum to which each taxon belongs, *i.e.* M, Mollusca; P, Polychaeta; C, Crustacea; I, Insecta; N, Nematoda and Ch, Chelicerata. Abundant species at each location (*i.e.* those that contributed ≥5% to the catch) are highlighted in grey. Species are ranked by total abundance.

		Caversham Upstream				**Caversham**				**Caversham-Guildford**				**Guildford**				**Guildford Downstream**				**Upper Swan Estuary**		
Species	**Sp**	$\bar{x}$	**SD**	**%**	**R**	$\bar{x}$	**SD**	**%**	**R**	$\bar{x}$	**SD**	**%**	**R**	$\bar{x}$	**SD**	**%**	**R**	$\bar{x}$	**SD**	**%**	**R**	$\bar{x}$	**%**	**R**
Arthritica semen	M	105.0	138.5	15.88	4	194.1	348.8	11.21	2	2,23.2	268.3	28.50	1	806.7	1279.8	69.72	1	383.9	450.5	45.02	1	**342.57**	**33.03**	**1**
Fluviolanatus subortus	M	117.1	375.7	17.71	3	1,051.0	4606.5	60.70	1	115.7	458.1	14.77	2	17.2	74.5	1.49	6	1.9	4.3	0.22	11	**260.57**	**25.13**	**2**
Prionospio cirrifera	P	9.9	23.1	1.50	9	83.7	135.5	4.83	4	101.2	126.9	12.92	3	96.4	92.1	8.33	3	230.1	166.8	26.99	2	**104.26**	**10.05**	**3**
Desdemona ornata	P	9.4	25.6	1.43	10	80.5	152.8	4.65	5	99.3	201.5	12.68	4	127.4	361.4	11.01	2	172.2	326.5	20.20	3	**97.79**	**9.43**	**4**
Pseudopolydora kempi	P	175.4	309.4	26.54	1	149.0	389.2	8.60	3	76.3	156.7	9.74	5	26.1	66.5	2.26	5	4.0	16.4	0.47	6	**86.17**	**8.31**	**5**
Paracorophium excavatum	C	124.0	231.6	18.76	2	52.2	120.8	3.02	6	23.1	45.9	2.95	8	13.8	28.9	1.19	8	2.7	6.3	0.31	7	**43.15**	**4.16**	**6**
Grandidierella propodentata	C	26.7	55.3	4.03	6	24.7	42.6	1.42	9	42.8	102.1	5.46	6	15.1	37.0	1.31	7	2.7	8.0	0.31	7	**22.38**	**2.16**	**7**
Capitella 'capitata'	P	5.4	12.2	0.82	11	1.8	5.0	0.10	14	19.2	25.7	2.45	10	30.4	51.5	2.63	4	41.6	60.3	4.87	4	**19.69**	**1.90**	**8**
Leitoscoloplos normalis	P	12.7	21.7	1.92	8	30.1	59.0	1.74	8	35.9	62.4	4.58	7	11.6	35.8	1.00	9	2.4	6.5	0.29	10	**18.53**	**1.79**	**9**
Simplisetia aequisetis	P	33.0	53.5	4.99	5	34.9	62.2	2.01	7	19.6	21.8	2.50	9	2.6	4.6	0.22	11	0.3	1.6	0.04	14	**18.06**	**1.74**	**10**
Corophium minor	C					11.1	33.6	0.64	10	12.1	32.4	1.55	11	2.3	5.8	0.20	12	0.2	1.4	0.03	16	**5.16**	**0.50**	**11**
Melita matilda	C	14.9	71.7	2.25	7	0.3	1.2	0.02	20	0.2	1.0	0.03	19	0.3	1.2	0.03	17	0.1	0.7	0.01	19	**3.18**	**0.31**	**12**
Paratanytrus grimmii	I	3.8	7.6	0.57	14	1.3	3.8	0.08	15	0.8	2.6	0.10	16	3.1	10.8	0.27	10	2.7	7.6	0.31	7	**2.33**	**0.22**	**13**
Boccardiella limnicola	P	4.4	11.5	0.67	12	2.0	6.3	0.12	13	4.4	19.2	0.57	12	0.1	0.7	0.01	21					**2.20**	**0.21**	**14**
Arcuatula senhausia	M	0.7	2.4	0.10	20	5.3	14.1	0.31	11	2.8	6.4	0.35	14	0.7	2.6	0.06	15	0.2	1.0	0.03	16	**1.93**	**0.19**	**15**
Nematoda spp.	N	1.0	2.6	0.15	18	0.4	1.7	0.03	19	0.8	4.3	0.10	16	0.1	0.7	0.01	21	4.9	14.6	0.57	5	**1.44**	**0.14**	**16**
Oligochaeta spp.	P	1.6	5.2	0.24	17	0.3	2.1	0.02	20	3.3	9.4	0.43	13	1.1	3.6	0.10	13	0.9	2.3	0.10	13	**1.44**	**0.14**	**16**
Marphysa sanguinea	P	3.1	9.2	0.47	15	0.7	1.6	0.04	18	1.4	4.2	0.18	15	0.8	1.7	0.07	14	0.1	0.7	0.01	19	**1.22**	**0.12**	**18**
Carazziella victoriensis	P	0.6	2.9	0.08	22	4.4	27.4	0.26	12									0.2	1.4	0.03	16	**1.04**	**0.10**	**19**
Syncassidina aestuarina	P	4.4	20.6	0.67	12																	**0.89**	**0.09**	**20**
Oniscidea sp	C	3.0	10.9	0.45	16	0.2	1.0	0.01	22	0.1	0.7	0.01	21									**0.67**	**0.06**	**21**
Ficopomatus enigmaticus	P	0.6	2.9	0.08	22	1.1	7.0	0.06	16	0.2	1.4	0.03	19									**0.38**	**0.04**	**22**
Chronomid sp.	I	0.4	2.0	0.07	25	1.1	7.0	0.06	16													**0.31**	**0.03**	**23**
Spisula trigonella	M					0.1	0.7	0.01	22					0.2	1.4	0.02	18	1.0	2.6	0.12	12	**0.27**	**0.03**	**24**
Culicidae sp.2	I	0.3	1.6	0.05	27	0.2	1.4	0.01	22	0.1	0.7	0.01	21	0.2	1.4	0.02	18	0.3	2.1	0.04	14	**0.24**	**0.02**	**25**
Heteromastus filiformis	P	0.9	3.9	0.13	19	0.1	0.7	0.01	22					0.1	0.7	0.01	21					**0.22**	**0.02**	**26**
Capitella sp.	P	0.2	1.0	0.03	28	0.2	1.4	0.01	22	0.1	0.7	0.01	21	0.2	1.0	0.02	18					**0.16**	**0.01**	**27**
Tatea preissii	M	0.6	2.9	0.08	22					0.1	0.7	0.01	21									**0.13**	**0.01**	**28**
Batillaria australis	M	0.7	2.6	0.10	20																	**0.13**	**0.01**	**28**
Limnoperna securis	M	0.1	0.7	0.02	30									0.4	2.2	0.04	16					**0.11**	**0.01**	**30**
Australospio trifida	P	0.1	0.7	0.02	30					0.3	1.2	0.04	18									**0.09**	**0.01**	**31**
Palaemonetes australis	C	0.4	2.8	0.07	25																	**0.09**	**0.01**	**31**
Melicertus latisulcatus	C	0.1	0.7	0.02	30													0.1	0.7	0.01	19	**0.04**	**0.00**	**33**
Collembola sp.	I					0.2	1.4	0.01	22													**0.04**	**0.00**	**33**
Aedes sp.	I	0.2	1.4	0.03	28																	**0.04**	**0.00**	**33**
Prionospio aff. *multipinnulata*	P																	0.1	0.7	0.01	19	**0.02**	**0.00**	**36**
Hydrococcus brazieri	M	0.1	0.7	0.02	30																	**0.02**	**0.00**	**36**
Aranea sp.	Ch									0.1	0.1	0.01	21									**0.02**	**0.00**	**36**
Diptera sp. (adult)	I	0.1	0.7	0.02	30																	**0.02**	**0.00**	**36**
Culicidae sp.1	I					0.1	0.7	0.01	22													**0.02**	**0.00**	**36**
Coquillettidia sp.	I					0.1	0.7	0.01	22													**0.02**	**0.00**	**36**
Gastrosaccus sp.	C	0.1	0.7	0.02	30																	**0.02**	**0.00**	**36**
Total number individuals*		**26,442**				**69,260**				**31,330**				**46,284**				**34,103**				**207,419**		
Total number of species		**35**				**29**				**25**				**23**				**22**				**42**		
Total density (no. /m^2)		**661**				**1,732**				**783**				**1,157**				**853**				**1,037**		
Total number of samples		**40**				**40**				**40**				**40**				**40**				**200**		

*This is the total number of individuals after the number of individuals in each sample had been adjusted to that in 0.1 m^2.

3.1.3. Benthic macroinvertebrate community composition

Three-way PERMANOVA demonstrated that the compositions of the BMI communities throughout the USE zone of the Swan Canning Estuary in 2010 and 2011 differed significantly among years, seasons and locations and that all two- and three-way interactions were significant (p = 0.1%; Table 2). Two-way crossed ANOSIMs using each factor *vs* the other two factors combined demonstrated that all three factors were significant (p = 0.1%) and that year had the greatest $\bar{R}$, *i.e.* 0.654 and was thus the most influential factor. This is reflected in the nMDS ordination plots constructed by calculating the distances between each pair of group centroids (*i.e.* an average in 'Bray-Curtis space') based on the fourth-root transformed data. Two broad clusters are evident containing the points representing each year, with those from 2010 tending to be distributed in the upper half of the plot and those from 2011 toward the lower half (Fig. 5a). The $\bar{R}$ values for season and location were very similar (*i.e.* 0.526 and 0.496, respectively). A more subtle pattern was observed among seasons, with the samples from summer and winter grouping to the right and left of the plot, respectively, and those from autumn and spring in the lower and upper portions of the plot, respectively (Fig. 5b). A clear spatial gradient was evident among locations, from the most upstream location (CU) on the left of the plot to the most downstream (GD) on the right (Fig. 5c). It is interesting to note that those points representing samples from Caversham were more widely dispersed on the plot than those for samples from other locations. On the basis of these results, subsequent analyses were then carried out separately for each of the eight consecutive seasons in order to remove the confounding influence of these temporal factors, and thus to more thoroughly investigate the differences among locations in BMI community composition.

One-way ANOSIM tests, comparing the fauna at each location separately for every season, illustrated significant differences in faunal composition between the locations during all of the eight seasons (all p = 0.1%; Fig. 6). The overall extent of those differences were moderate ($\bar{R}$ = 0.392–0.599), with the greatest overall differences among locations being observed in spring 2011 and the smallest in autumn 2011. In most seasons, the pairwise differences in BMI composition were generally greatest between locations that were geographically furthest away from each other (*e.g.* R ≥0.860 for the pairwise comparison between GD and CU in six of the eight seasons sampled). This is also reflected on the nMDS plots in the spatial separation of the upstream locations from those further downstream (Fig. 6).

Table 2. Mean squares (MS), coefficients of variation (COV), and significance levels (*p*) for three-way PERMANOVA tests on the Bray-Curtis resemblance matrix constructed from the fourth-root transformed abundance of each benthic macroinvertebrate species in each sample at the five locations in the Upper Swan Estuary zone of the Swan Canning Estuary in each season over two consecutive years between summer 2010 and spring 2011. *df* = degrees of freedom. Significant results are highlighted in bold.

	df	**MS**	**COV**	***p***
Main effects				
Year	1	**26161**	**253**	**0.1%**
Season	3	**16113**	**304**	**0.1%**
Location	4	**16882**	**399**	**0.1%**
Interactions	3			
Year x season	4	**9422**	**341**	**0.1%**
Year x location	23	**4324**	**171**	**0.1%**
Season x location	23	**2012**	**111**	**0.1%**
Year x season x location	160	**2216**	**262**	**0.1%**
Residual	199	904	904	

The bivalve mollusc *A. semen* characterised each of the five locations in almost all of the eight seasons sampled. Additionally, the polychaetes *P. cirrifera* and *P. kempi* tended to characterise the more downstream locations (GD, G, CG) and the more upstream locations (CU and C), respectively (Table 3). In general, locations were typified and/or distinguished from each other by differences in the abundances of small suites of species of polychaete (namely *P. kempi, P. cirrifera, Capitella 'capitata', D. ornata*, *S. aequisetis* and *Leitoscoloplos normalis*) and, to a lesser extent, molluscan (*i.e. A. semen, Fluviolanatus subortus, Arcuatula senhausia*) and crustacean (*i.e. P. excavatum, Grandidierella propodentata, Corophium minor*) species (Table 3). It is also notable that in the summer of 2010 the CU location was typified (and also distinguished from other locations) by relatively high and consistent abundances of the chironomid larva *Paratanytarsus grimmii*, whilst the GD location was similarly typified by the presence of nematodes in winter of 2010 (Table 3).

The spatial and temporal patterns in BMI faunal composition were next compared to those in a suite of water quality (*i.e.* water temperature, DO concentration, salinity and pH) and sediment variables (*i.e.* organic matter content and proportions of each of the Wentworth grain sizes) to elucidate whether there were any significant patterns in the rank order correlations. This BIOENV analysis indicated that in summer, autumn and spring a significant and moderately high correlation between the two complementary matrices was detected when the environmental matrix was constructed from various combinations of the environmental variables (Table 4).

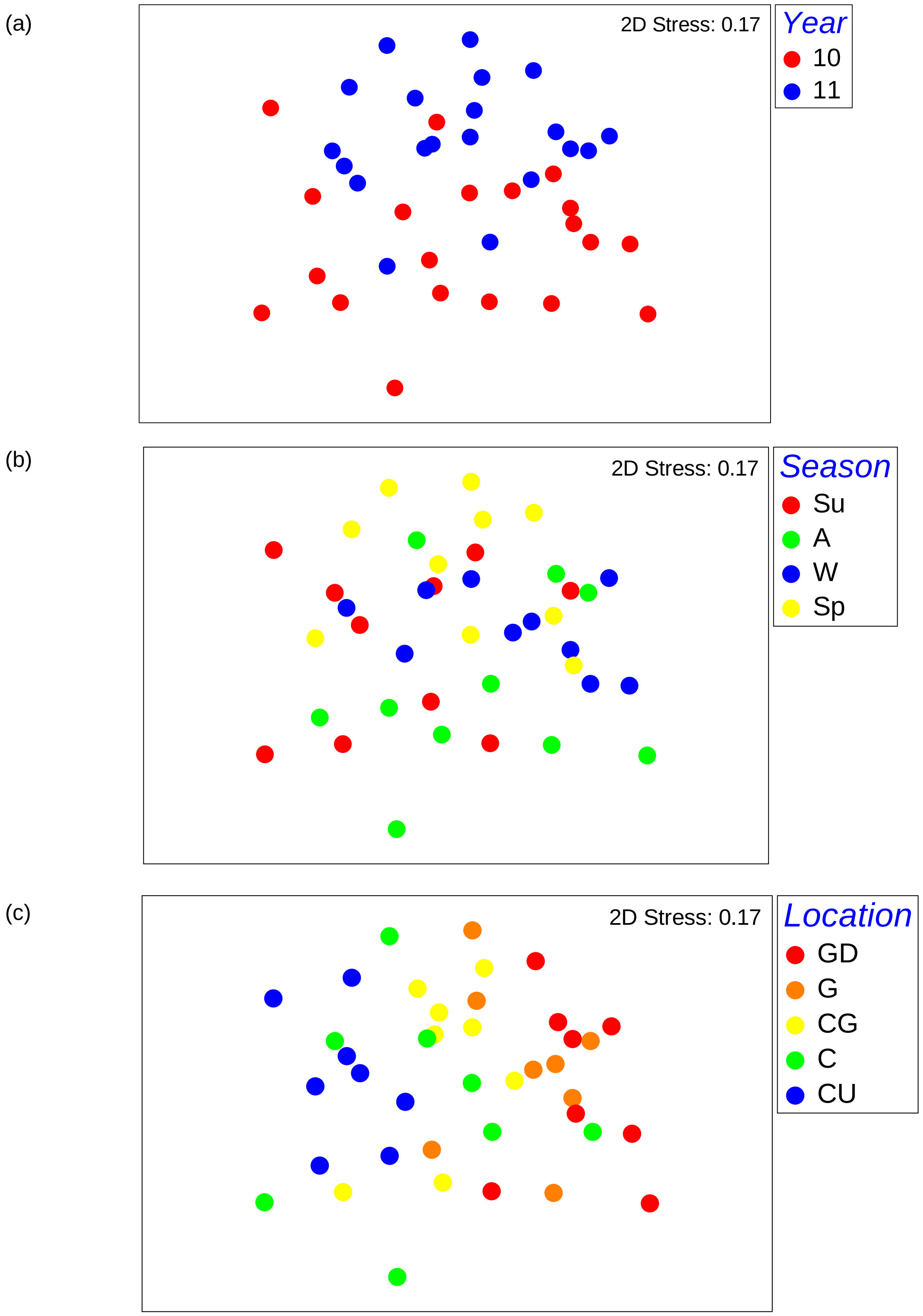
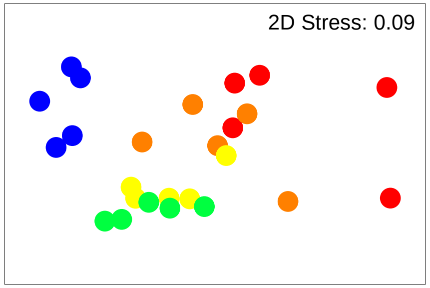


Figure 5. nMDS centroid ordination plots, constructed from a Bray-Curtis resemblance matrix derived from the fourth-root transformed abundances of the various benthic macroinvertebrate species recorded at five locations in the Upper Swan Estuary zone of the Swan Canning Estuary in each season over two consecutive years between summer 2010 and spring 2011. Data are coded for (a) year, (b) season and (c) location. 10, 2010; 11, 2011. Su, Summer; A, Autumn; W, Winter; Sp, Spring. GD, Guildford Downstream; G, Guildford; CG, Caversham-Guildford; C, Caversham; CU, Caversham Upstream.

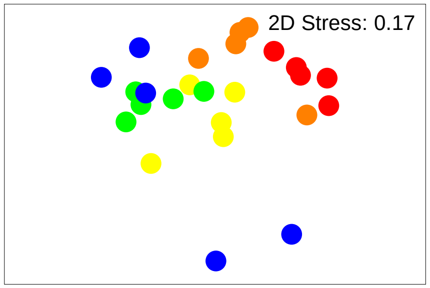
Summer 2010: $\bar{R}$ = 0.578, *p* = 0.1%

	GD	G	CG	C
G	0.080			
CG	0.596	0.268		
C	0.716	0.600	0.064	
CU	0.860	0.872	0.948	0.936



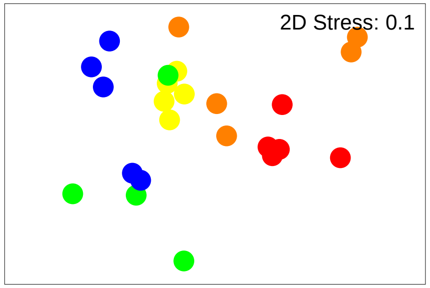
Summer 2011; $\bar{R}$ = 0.501, *p* = 0.1%

	GD	G	CG	C
G	0.560			
CG	0.736	0.340		
C	0.964	0.724	0.348	
CU	0.580	0.46	0.220	0.148



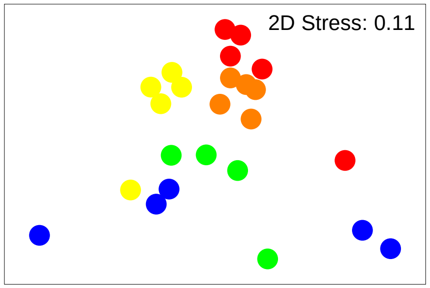
Autumn 2010; $\bar{R}$ = 0.429, *p* = 0.1%

	GD	G	CG	C
G	0.252			
CG	0.988	0.356		
C	0.556	0.270	0.258	
CU	0.976	0.484	0.468	0.074



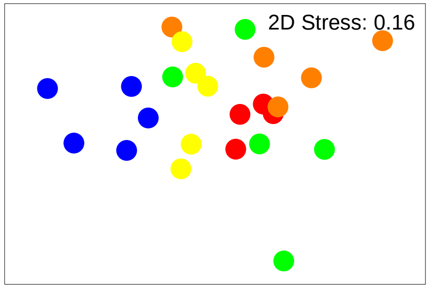
Autumn 2011; $\bar{R}$ = 0.392, *p* = 0.1%

	GD	G	CG	C
G	0.292			
CG	0.584	0.712		
C	0.292	0.448	0.392	
CU	0.472	0.512	0.472	0.040



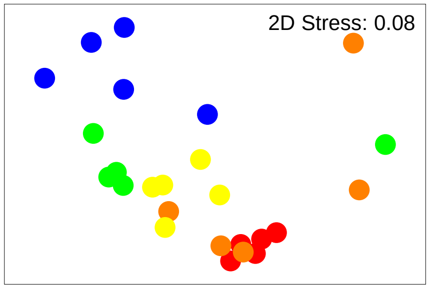
Winter 2010; $\bar{R}$ = 0.526, *p* = 0.1%

	GD	G	CG	C
G	0.368			
CG	0.752	0.524		
C	0.168	0.140	0.300	
CU	0.988	0.844	0.860	0.616



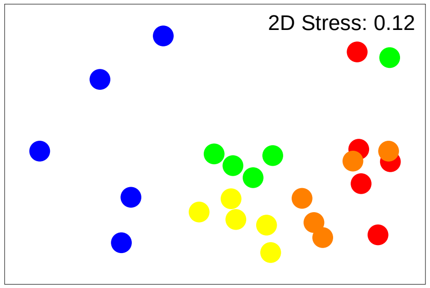
Winter 2011; $\bar{R}$ = 0.458, *p* = 0.1%

	GD	G	CG	C
G	0.088			
CG	0.864	0.284		
C	0.600	0.200	0.356	
CU	0.960	0.692	0.672	0.316



Spring 2010; $\bar{R}$ = 0.527 *p* = 0.1%

	GD	G	CG	C
G	0.072			
CG	0.896	0.564		
C	0.432	0.412	0.308	
CU	1.000	0.996	0.736	0.520



Spring 2011; $\bar{R}$ = 0.599 *p* = 0.1%

	GD	G	CG	C
G	0.460			
CG	0.564	0.188		
C	0.976	0.604	0.608	
CU	0.904	0.720	0.608	0.356

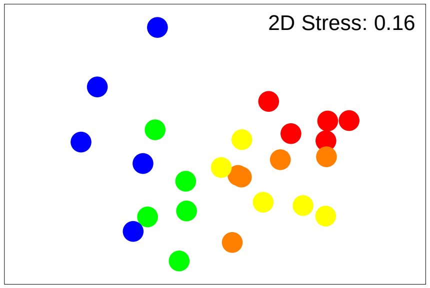


Figure 6. *R*-statistic and/or significance level (*p*) values derived from one-way ANOSIM tests of the benthic macroinvertebrate community composition at five locations in the Upper Swan Estuary zone of the Swan Canning Estuary during seasonal sampling conducted between summer 2010 and spring 2011. Insignificant pairwise comparisons are highlighted in grey. ● & CU, Caversham Upstream; ● & C, Caversham; ● & CG, Caversham-Guildford; ● & G, Guildford; ● & GD, Guildford Downstream.

Table 3. Species that consistently typified (provided along the diagonal) and distinguished (provided in the sub-directional) the benthic macroinvertebrate communities at each of five locations in the Upper Swan Estuary zone in each of eight consecutive seasons, as detected by one-way SIMPER. The location at which each species was most abundant is abbreviated for each pairwise comparison. Asterisks denote the relative consistency of each species in either typifying or distinguishing the faunal compositions at each location, as measured by the similarity to standard deviation ratio and dissimilarity to standard deviation ratio, respectively; 1.5-3*, 3.01-5**, >5*** . Grey shading represents insignificant pairwise comparisons. Coloured shading represents different taxa. 🟧, polychaete; 🟦, mollusc; 🟩, crustacean; 🟨, insect; 🟥, nematode. CU, Caversham Upstream; C, Caversham; CG, Caversham-Guildford; G, Guildford; GD, Guildford Downstream. Su, Summer; A, Autumn; W, Winter; Sp, Spring.

(a) Su 2010	**GD**	**G**	**CG**	**C**	**CU**
GD	*Arthritica semen* * *Prionospio cirrifera*				
G	▨	*Arthritica semen* ** *Pseudopolydora kempi* ** *Prionospio cirrifera*			
CG	*Fluviolanatus suborta* CG* *Pseudopolydora kempi* CG *Desdemona ornata* CG* *Prionospio cirrifera* GD*	▨	*Fluviolanatus suborta* *** *Pseudopolydora kempi* *** *Arthritica semen**** *Desdemona ornata*		
C	*Fluviolanatus suborta* C* *Pseudopolydora kempi* C* *Prionospio cirrifera* GD* *Arthritica semen* C*	*Fluviolanatus suborta* C* *Pseudopolydora kempi* C* *Prionospio cirrifera* G*	▨	*Fluviolanatus suborta* ** *Pseudopolydora kempi* *** *Arthritica semen***	
CU	*Paracorophium excavatum* CU* *Pseudopolydora kempi* CU* *Fluviolanatus suborta* CU* *Boccardiella limnicola* CU*** *Paratanytarsus grimmii* CU*** *Prionospio cirrifera* GD*	*Paracorophium excavatum* CU* *Fluviolanatus suborta* CU* *Paratanytarsus grimmii* CU*** *Leitoscoloplos normalis* CU* *Simplisetia aequisetis* CU** *Prionospio cirrifera* G*	*Paracorophium excavatum* CU* *Boccardiella limnicola* CU*** *Leitoscoloplos normalis* CU** *Desdemona ornata* CG* *Paratanytarsus grimmii* CU*** *Simplisetia aequisetis* CU**	*Fluviolanatus suborta* C *Paracorophium excavatum* CU* *Boccardiella limnicola* CU*** *Syncassidina aestuarina* CU* *Paratanytarsus grimmii* CU*** *Simplisetia aequisetis* CU**	*Pseudopolydora kempi* *** *Arthritica semen* *** *Fluviolanatus suborta*** *Paracorophium excavatum* *Boccardiella limnicola* *** *Paratanytarsus grimmii* ***

(b) A 2010	**GD**	**G**	**CG**	**C**	**CU**
GD	*Prionospio cirrifera* *** *Arthritica semen*				
G	*Prionospio cirrifera* GD *Arthritica semen* G *Pseudopolydora kempi* G *Capitella ‘capitata’* G	*Prionospio cirrifera* *Arthritica semen* *Pseudopolydora kempi* *Capitella ‘capitata’*			
CG	*Pseudopolydora kempi* CG** *Prionospio cirrifera* GD* *Arthritica semen* CG* *Fluviolanatus suborta* CG*	*Arthritica semen* CG *Pseudopolydora kempi* CG *Fluviolanatus suborta* CG* *Capitella ‘capitata’* G	*Arthritica semen* *** *Pseudopolydora kempi* ** *Prionospio cirrifera* *** *Fluviolanatus suborta*		
C	*Prionospio cirrifera* GD* *Pseudopolydora kempi* C	*Prionospio cirrifera* G *Arthritica semen* G	*Arthritica semen* CG* *Pseudopolydora kempi* CG *Prionospio cirrifera* CG	*Pseudopolydora kempi* *Arthritica semen*	
CU	*Prionospio cirrifera* GD* *Pseudopolydora kempi* CU** *Arthritica semen* CU *Leitoscoloplos normalis* CU *Simplisetia aequisetis* CU	*Pseudopolydora kempi* CU *Prionospio cirrifera* G* *Leitoscoloplos normalis* CU *Simplisetia aequisetis* CU *Capitella ‘capitata’* G	*Prionospio cirrifera* CG* *Desdemona ornata* CG *Leitoscoloplos normalis* CU *Simplisetia aequisetis* CU	▨	*Pseudopolydora kempi* * *Arthritica semen* *

(c) W 2010	GD	G	CG	C	CU
GD	Prionospio cirrifera *** Desdemona ornata *** Arthritica semen ** Nematoda spp.				
G	Nematoda spp. GD* Desdemona ornata GD* Capitella ‘capitata’ G* Prionospio cirrifera GD Arthritica semen G	Prionospio cirrifera ** Arthritica semen ** Desdemona ornate Capitella ‘capitata’			
CG	Nematoda spp. GD* Simplisetia aequisetis CU* Fluviolanatus suborta CG* Arthritica semen CG*	Desdemona ornata CG Fluviolanatus suborta CG* Simplisetia aequisetis CG Capitella ‘capitata’ G*	Prionospio cirrifera *** Arthritica semen *** Desdemona ornata ** Fluviolanatus suborta ***		
C	Nematoda spp. GD* Arthritica semen GD Paratanytarsus grimmii C		Arthritica semen CG Fluviolanatus suborta CG* Simplisetia aequisetis CG Paratanytarsus grimmii C	Prionospio cirrifera ** Desdemona ornata ***	
CU	Pseudopolydora kempi CU*** Fluviolanatus suborta CU* Paracorophium excavatum CU* Prionospio cirrifera GD Desdemona ornata GD	Pseudopolydora kempi CU* Fluviolanatus suborta CU* Paracorophium excavatum CU*	Pseudopolydora kempi CU*** Desdemona ornata CG* Prionospio cirrifera CG Paracorophium excavatum CU*	Pseudopolydora kempi CU* Fluviolanatus suborta CU* Arthritica semen CU Paratanytarsus grimmii C	Pseudopolydora kempi *** Arthritica semen *** Fluviolanatus suborta *** Paracorophium excavatum *** Prionospio cirrifera ***

(d) Sp 2010	GD	G	CG	C	CU
GD	Prionospio cirrifera ** Arthritica semen *** Desdemona ornata				
G		Prionospio cirrifera *** Arthritica semen *** Desdemona ornate Grandidierella propodentata Paracorophium excavatum			
CG	Grandidierella propodentata CG* Simplisetia aequisetis CG*** Desdemona ornata GD Leitoscoloplos normalis CG*	Simplisetia aequisetis CG* G. propodentata CG Leitoscoloplos normalis CG* Desdemona ornata GD	Arthritica semen *** Grandidierella propodentata ** Prionospio cirrifera *** Paracorophium excavatum *** Simplisetia aequisetis ***		
C	Grandidierella propodentata C* Pseudopolydora kempi C Simplisetia aequisetis C* Paracorophium excavatum C	Pseudopolydora kempi C* Arthritica semen G Fluviolanatus suborta C	Desdemona ornata C* Grandidierella propodentata C Paracorophium excavatum CG Arthritica semen CG	Prionospio cirrifera *** Arthritica semen *** Grandidierella propodentata Desdemona ornate	
CU	Pseudopolydora kempi CU* Fluviolanatus suborta CU** Prionospio cirrifera GD* Desdemona ornata GD Paracorophium excavatum CU*	Fluviolanatus suborta CU** Pseudopolydora kempi CU* Prionospio cirrifera G* Desdemona ornata G Grandidierella propodentata G	G. propodentata CG* Pseudopolydora kempi CU* Prionospio cirrifera CG* Fluviolanatus suborta CU*	Prionospio cirrifera C* Desdemona ornata C* Fluviolanatus suborta CU* Grandidierella propodentata C*	Fluviolanatus suborta ** Arthritica semen ** Pseudopolydora kempi ** Paracorophium excavatum **

(e) Su 2011	**GD**	**G**	**CG**	**C**	**CU**
GD	*Arthritica semen* *** *Prionospio cirrifera* *** *Grandidierella propodentata* ***				
G	*Arthritica semen* G* *Corophium minor* G** *Paracorophium excavatum* G* *Capitella ‘capitata’* GD	*Arthritica semen* *** *Prionospio cirrifera* *** *Grandidierella propodentata* *** *Corophium minor* ***			
CG	*Simplisetia aequisetis* CG** *Arthritica semen* GD*	*Arthritica semen* G* *Paracorophium excavatum* G *Simplisetia aequisetis* CG *Leitoscoloplos normalis* CG*	*Arthritica semen* *** *Prionospio cirrifera* ** *Simplisetia aequisetis* *** *Grandidierella propodentata*		
C	*Paracorophium excavatum* C* *Arthritica semen* GD** *Arcuatula senhausia* C** *Pseudopolydora kempi* C* *Fluviolanatus suborta* C* *Prionospio cirrifera* GD*	*Arthitica semen* G** *Fluviolanatus suborta* C* *Arcuatula senhausia* C*	*Paracorophium excavatum* C *Arcuatula senhausia* C* *Fluviolanatus suborta* C *Prionospio cirrifera* CG	*Grandidierella propodentata* ** *Paracorophium excavatum* *** *Arthritica semen* *** *Fluviolanatus suborta* *** *Arcuatula senhausia* ** *Pseudopolydora kempi* ***	
CU	*Prionospio cirrifera* GD** *Arthritica semen* GD* *Pseudopolydora kempi* CU* *Paracorophium excavatum* CU	*Arthritica semen* G* *Prionospio cirrifera* G** *Corophium minor* G* *Pseudopolydora kempi* CU	*Prionospio cirrifera* CG* *Grandidierella propodentata* CU *Pseudopolydora kempi* CU	*Arcuatula senhausia* C* *Grandidierella propodentata* C *Paracorophium excavatum* C *Prionospio cirrifera* C	*Arthritica semen* * *Paracorophium excavatum* *Pseudopolydora kempi*

(f) A 2011	**GD**	**G**	**CG**	**C**	**CU**
GD	*Arthritica semen* *** *Capitella ‘capitata’* *** *Prionospio cirrifera*				
G	*Arthritica semen* GD *Corophium minor* G *Desdemona ornata* GD	*Arthritica semen* *** *Prionospio cirrifera* *** *Capitella ‘capitata’*			
CG	*Corophium minor* CG* *Simplisetia aequisetis* CG** *Leitoscoloplos normalis* CG* *Pseudopolydora kempi* CG* *Arthritica semen* GD*	*Leitoscoloplos normalis* CG*** *Corophium minor* CG* *Simplisetia aequisetis* CG* *Pseudopolydora kempi* CG*	*Arthritica semen* *** *Corophium minor* *** *Leitoscoloplos normalis* *** *Simplisetia aequisetis* *** *Prionospio cirrifera* *Pseudopolydora kempi* ***		
C	*Arthritica semen* GD *Capitella ‘capitata’* GD* *Simplisetia aequisetis* C *Desdemona ornata* GD	*Prionospio cirrifera* G *Arthritica semen* G *Capitella ‘capitata’* G*	*Corophium minor* CG* *Leitoscoloplos normalis* CG* *Capitella ‘capitata’* CG* *Pseudopolydora kempi* CG*	*Arthritica semen* *Prionospio cirrifera* *Simplisetia aequisetis*	
CU	*Arthritica semen* GD* *Capitella ‘capitata’* GD* *Prionospio cirrifera* GD *Pseudopolydora kempi* CU *Desdemona ornata* GD	*Prionospio cirrifera* G* *Arthritica semen* G* *Capitella ‘capitata’* G** *Pseudopolydora kempi* CU *Corophium minor* G	*Corophium minor* CG** *Leitoscoloplos normalis* CG** *Prionospio cirrifera* CG *Capitella ‘capitata’* CG* *Arthritica semen* G		*Arthritica semen* *Pseudopolydora kempi* *Simplisetia aequisetis*

(g) W 2011	**GD**	**G**	**CG**	**C**	**CU**
GD	*Prionospio cirrifera* *** *Arthritica semen* *** *Desdemona ornata* *** *Capitella ‘capitata’* ***				
G		*Arthritica semen* * *Desdemona ornate* *Prionospio cirrifera*			
CG	*Leitoscoloplos normalis* CG* *Pseudopolydora kempi* CG*** *Simplisetia aequisetis* CG* *Paracorophium excavatum* CG*	*Leitoscoloplos normalis* CG* *Pseudopolydora kempi* CG* *Prionospio cirrifera* CG *Simplisetia aequisetis* CG	*Prionospio cirrifera* *** *Arthritica semen* *** *Desdemona ornata* ** *Leitoscoloplos normalis* *** *Capitella ‘capitata’* ** *Pseudopolydora kempi* ***		
C	*Capitella ‘capitata’* GD *Prionospio cirrifera* GD *Paracorophium excavatum* C* *Pseudopolydora kempi* C* *Leitoscoloplos normalis* C*		*Capitella ‘capitata’* CG *Corophium minor* C *Prionospio cirrifera* CG	*Arthritica semen* ** *Leitoscoloplos normalis* *Paracorophium excavatum* *Desdemona ornate*	
CU	*Desdemona ornata* GD** *Prionospio cirrifera* GD* *Arthritica semen* GD* *Pseudopolydora kempi* CU** *Simplisetia aequisetis* CU**	*Desdemona ornata* G* *Arthritica semen* G* *Pseudopolydora kempi* CU* *Simplisetia aequisetis* CU* *Grandidierella propodentata* CU	*Desdemona ornata* CG*** *Prionospio cirrifera* CG* *Leitoscoloplos normalis* CG* *Arthritica semen* CG* *Grandidierella propodentata* CU	*Desdemona ornata* C* *Arthritica semen* C* *Corophium minor* C	*Pseudopolydora kempi* ** *Simplisetia aequisetis* ** *Grandidierella propodentata* *Paracorophium excavatum*

(h) Sp 2011	**GD**	**G**	**CG**	**C**	**CU**
GD	*Arthritica semen* *** *Desdemona ornata* *** *Capitella ‘capitata’* *** *Prionospio cirrifera* *Paratanytarsus grimmii* ***				
G	*Prionospio cirrifera* GD* *Desdemona ornata* GD *Fluviolanatus suborta* GD* *Leitoscoloplos normalis* G	*Arthritica semen* *** *Capitella ‘capitata’* *** *Leitoscoloplos normalis* *** *Desdemona ornate* *Paratanytarsus grimmii* ***			
CG	*Desdemona ornata* GD* *Prionospio cirrifera* GD* *Leitoscoloplos normalis* CG* *Fluviolanatus suborta* GD*		*Arthritica semen* *** *Leitoscoloplos normalis* *** *Desdemona ornata* ** *Capitella ‘capitata’* ***		
C	*Prionospio cirrifera* GD* *Desdemona ornata* GD* *Capitella ‘capitata’* GD** *Simplisetia aequisetis* C* *Paracorophium excavatum* C	*Capitella ‘capitata’* G** *Simplisetia aequisetis* C* *Paracorophium excavatum* C	*Capitella ‘capitata’* CG** *Prionospio cirrifera* CG* *Simplisetia aequisetis* C *Paracorophium excavatum* C	*Arthritica semen* *** *Simplisetia aequisetis* *** *Leitoscoloplos normalis* *** *Desdemona ornata* **	
CU	*Desdemona ornata* GD** *Prionospio cirrifera* GD* *Arthritica semen* GD* *Capitella ‘capitata’* GD* *Simplisetia aequisetis* CU*	*Arthritica semen* G* *Capitella ‘capitata’* G* *Desdemona ornata* G* *Paracorophium excavatum* CU *Simplisetia aequisetis* CU*	*Desdemona ornata* CG* *Arthritica semen* CG* *Prionospio cirrifera* CG* *Capitella ‘capitata’* CG*	*Arthritica semen* C* *Desdemona ornata* C* *Grandidierella propodentata* CU *Fluviolanatus suborta* CU*	*Simplisetia aequisetis* *** *Arthritica semen* ** *Leitoscoloplos normalis* ***

The BMI faunas in the summers of 2010 and 2011 were best correlated to trends in the values for salinity, water temperature and dissolved oxygen concentration. Changes in salinity among the locations were responsible for ~80% of the correlation. This is illustrated in the associated bubble plots where, in both years, the locations are arranged in order from lowest to highest salinity along the *x*-axis of the ordination plot, corresponding to increasing salinities, *i.e.* ~7-24 in 2010 and ~22-31 in 2011 (Fig. 7a). The notable split between the two years on the ordination plot, with those points representing locations in 2010 lying above those from 2011, is also matched by salinity, with higher values recorded at each location in 2011 than in 2010. A similar trend was present in water temperature with the values recorded in 2010 being less than those recorded at the same locations in 2011 (Fig. 7b). While there was no clear overall trend in the values for dissolved oxygen concentration, the points representing CU, C, CG and G in 2011 formed a small cluster and had similar values, *i.e.* 1.4-2.4 mg/L (Fig. 7c).

In autumn a moderately high correlation was observed between the BMI fauna and a combination of salinity and pH (ρ = 0.715). Again salinity was the main driver, with a correlation of 0.712 between the fauna and salinity alone. As was the case in summer, salinities increased progressively downstream and were greater in 2011 than 2010 (Fig 8a). pH followed a similar spatial trend, being slightly greater at G and GD (~7.2-7.3) than at CU, C and CG (~7.15; Fig. 8b).

Salinity and pH also were identified as being related to the patterns observed in the BMI fauna recorded in spring (Table 4). Once again, salinity increased progressively downstream in 2010, although this trend was far less pronounced in 2011. In contrast to summer and autumn, salinities were higher in 2010 than 2011 (Fig. 9a). The separation between the two years was also matched by pH with values <7.4 recorded in 2010 and values >7.6 recorded in 2011 (Fig. 9b).

Table 4. Rho statistic (ρ) and significance values (p) for the correlation between a resemblance matrix constructed from the average benthic macroinvertebrate data in each location in each season (of both years) and complementary matrices constructed from a suite of water quality and sediment variables. Significant correlations are highlighted in bold.

Season	ρ	p	Variables selected
Summer	**0.616**	**0.8%**	**Salinity, water temperature and dissolved oxygen concentration**
Autumn	**0.715**	**0.1%**	**Salinity and pH**
Winter	0.435	24.7%	Salinity and 63-124 um grain size
Spring	**0.6388**	**0.6%**	**Salinity and pH**

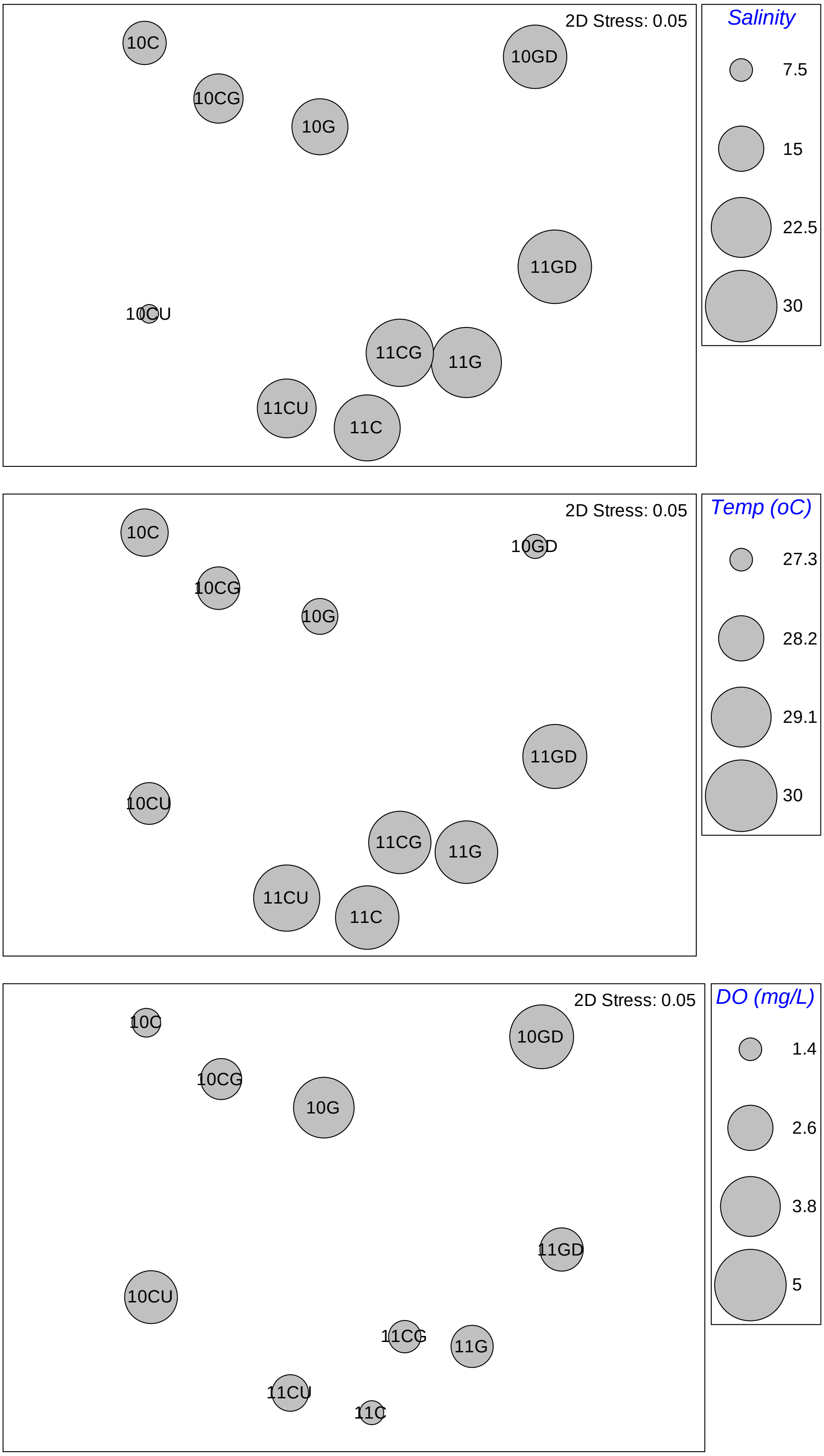


Figure 7. nMDS ordination plots of the average benthic macroinvertebrate faunal composition recorded at each location in the summers of 2010 and 2011. The corresponding values for (a) salinity, (b) water temperature (°C) and (c) dissolved oxygen concentration (mg/L) are displayed as circles of proportionate sizes

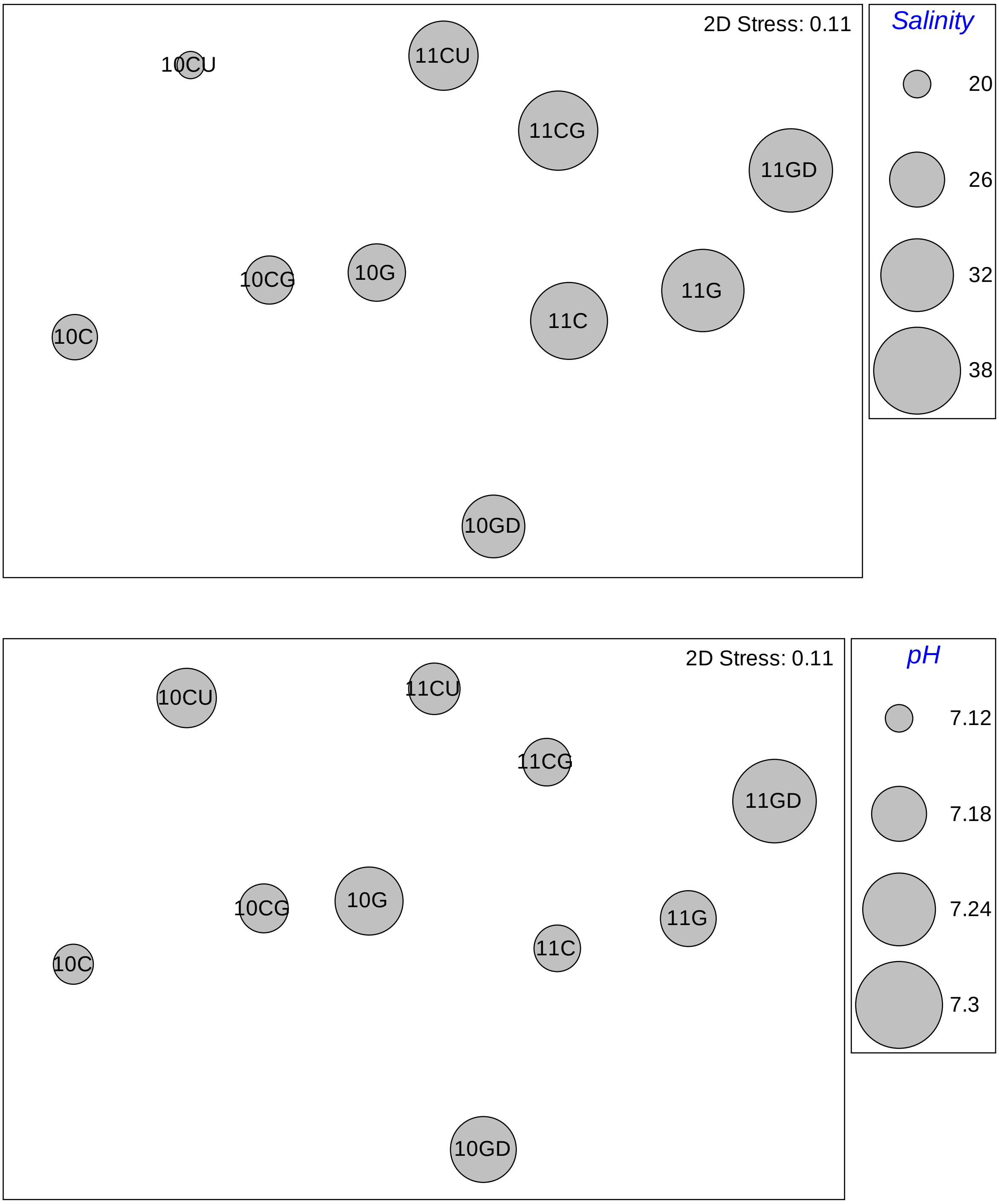


Figure 8. nMDS ordination plots of the average benthic macroinvertebrate faunal composition recorded at each location in the autumns of 2010 and 2011. The corresponding values for (a) salinity and (b) pH are displayed as circles of proportionate sizes.

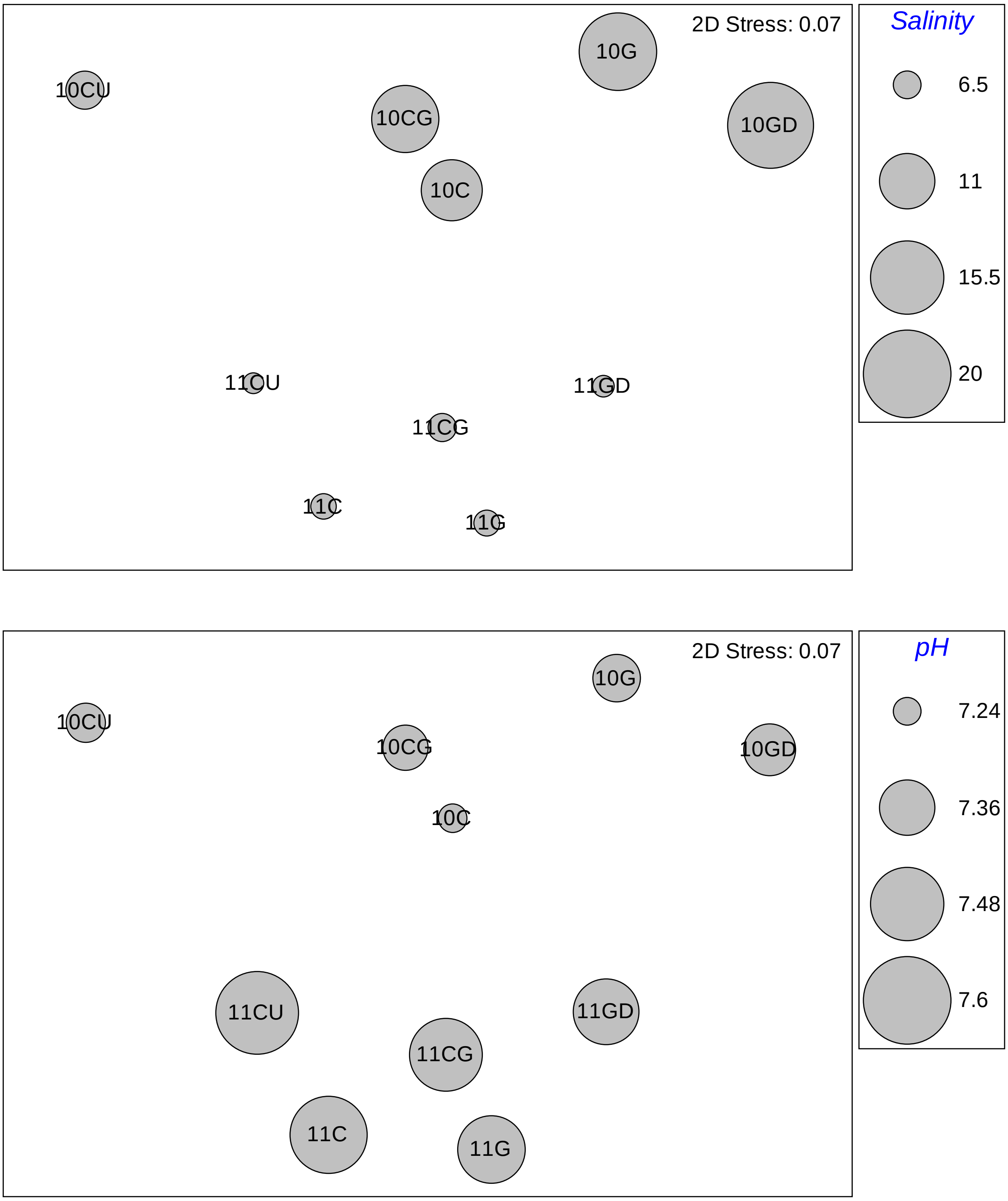


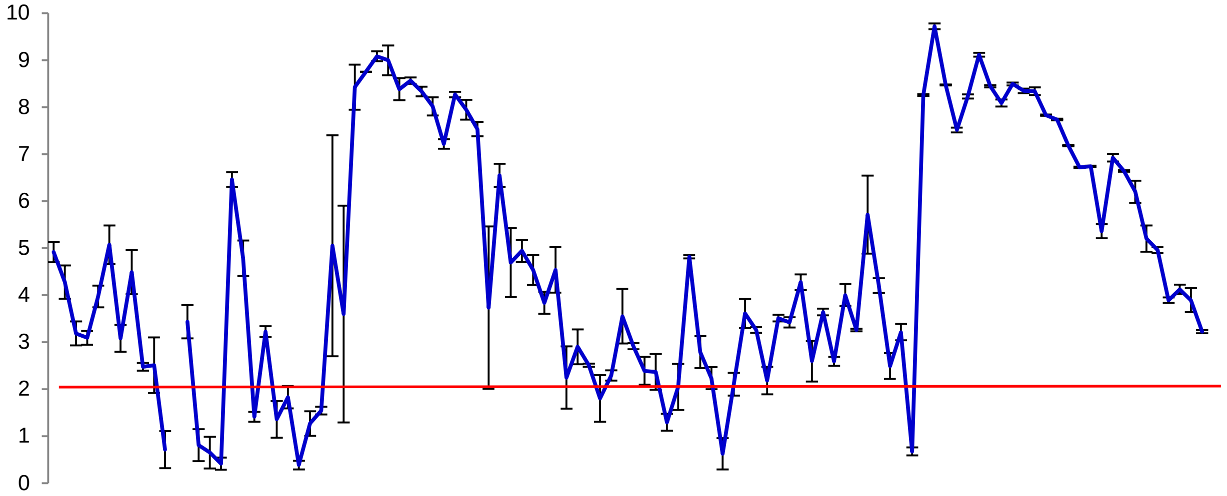
Figure 9. nMDS ordination plots of the average benthic macroinvertebrate faunal composition recorded at each location in the springs of 2010 and 2011. The corresponding values for (a) salinity and (b) pH are displayed as circles of proportionate sizes.

3.2. Benthic macroinvertebrate community responses to a notable hypoxic ‘event’

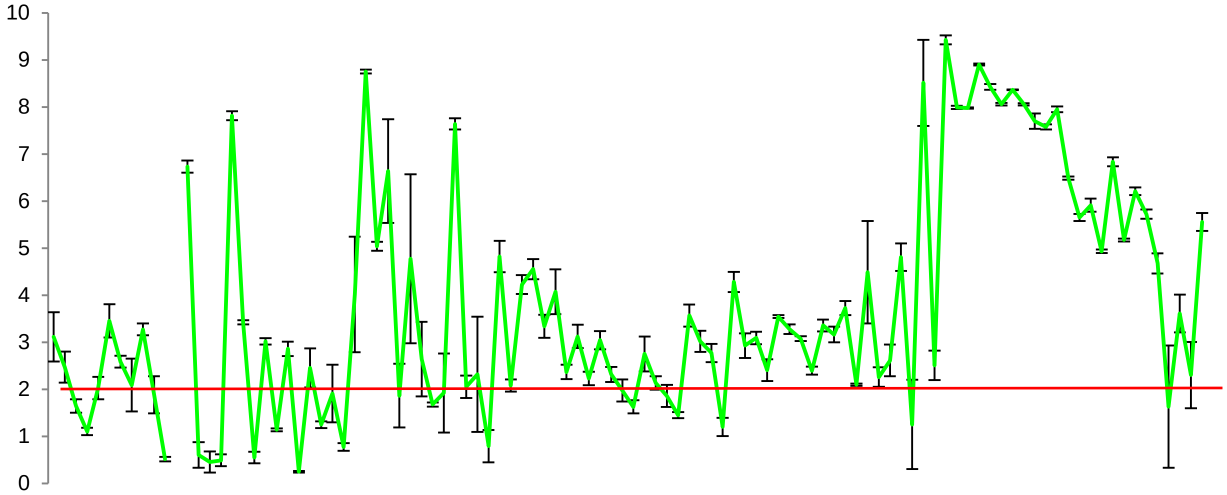
3.2.1. Changes in environmental conditions with particular emphasis around the hypoxic ‘event’

In general, dissolved oxygen concentrations in the bottom waters at the five locations in the USE largely remained normoxic (*i.e.* >2 mg/L) across the two years of the study, with the notable exception of a protracted period of hypoxic conditions that commenced in late March 2010 and persisted until late July or early October 2010, depending on the location in question. For example, dissolved oxygen concentrations in the bottom waters at CU dropped below 2 mg/L around 15th March 2010 and largely remained below 1 mg/L for five weeks until ~19th April 2010, with a subsequent second period of hypoxia lasting seven weeks between ~2nd May and ~25th June 2010 (Fig. 10a). A similar temporal pattern of hypoxic conditions was observed at Caversham (Fig. 10b), whilst at CG dissolved oxygen fell below 2 mg/L for two weeks in early April 2010, with a second period of highly variable and sometimes hypoxic conditions occurring between ~2nd May and ~25th June 2010 (Fig. 10c). At both Guildford (Fig. 10d) and GD (Fig. 10e), hypoxic conditions were more persistent, lasting from ~12 April to ~3rd October 2010, albeit interspersed with brief periods of normoxia.

(a) Caversham Upstream



(b) Caversham



(c) Caversham/Guildford

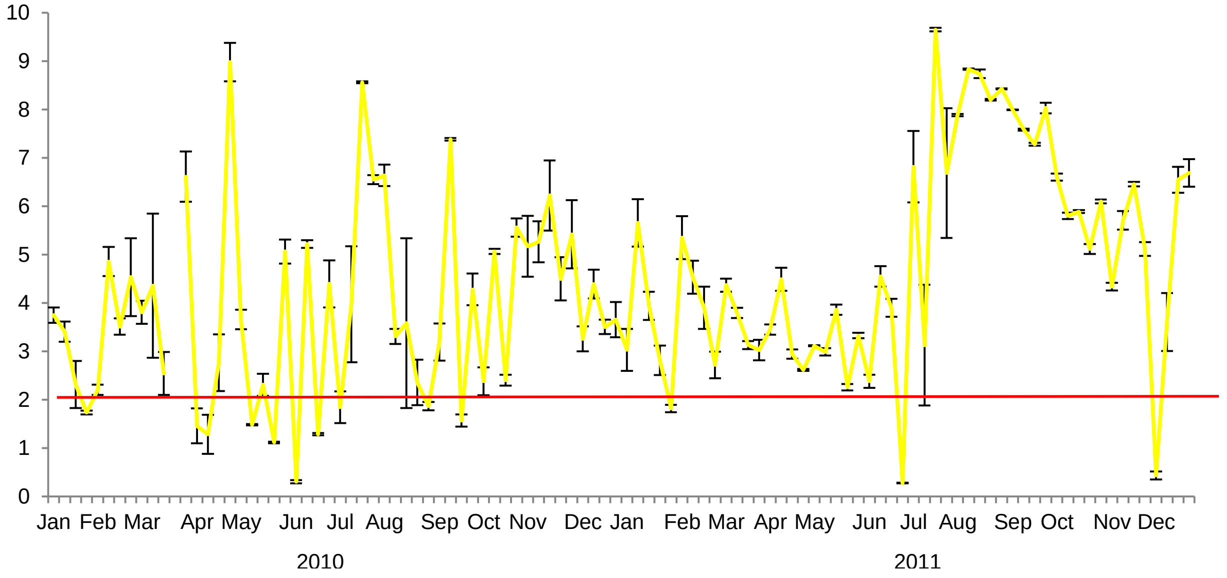
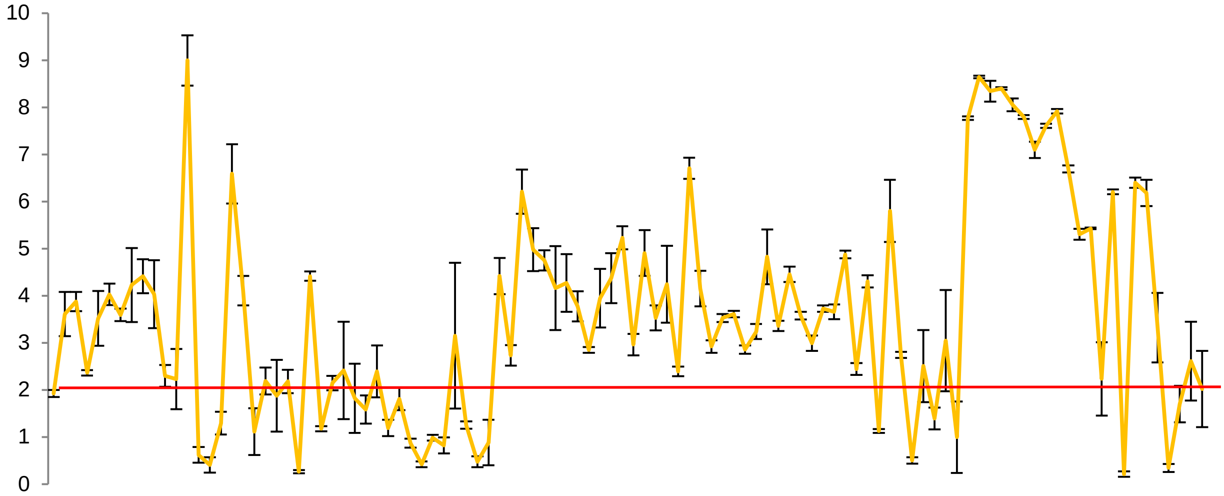


Figure 10. Mean dissolved oxygen concentrations (mg/L) measured weekly in the bottom waters at five locations in the Upper Swan River between January 2010 and December 2011. The line at 2 mg/L indicates hypoxic conditions (Rosenberg, 1980). Error bars represent ± one standard error.

(d) Guildford



(e) Guildford downstream

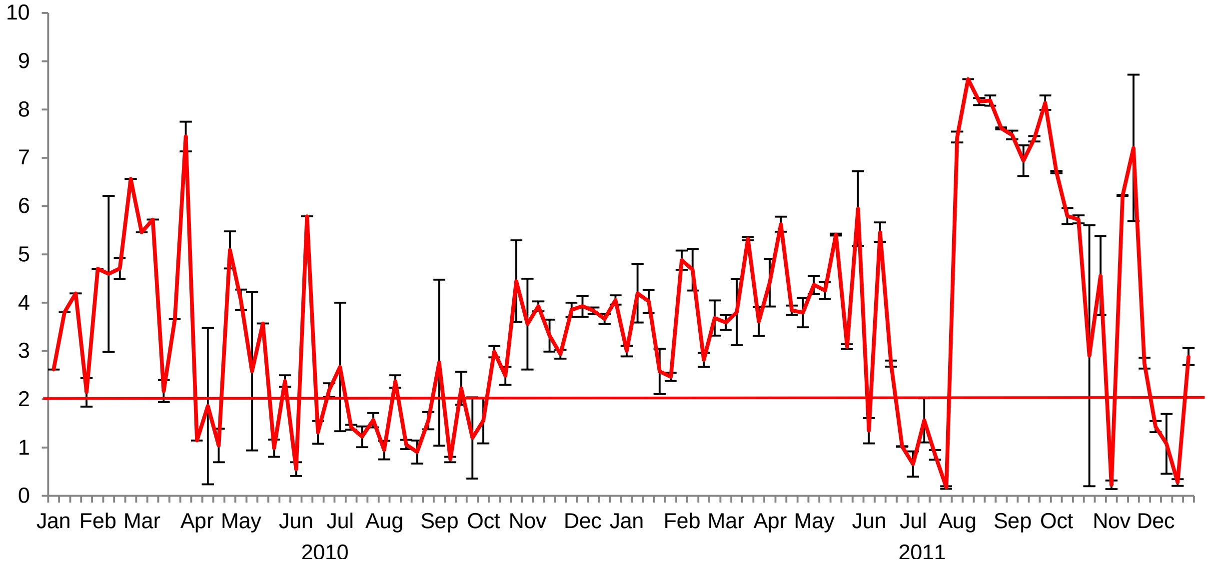


Figure 10 (continued). Mean dissolved oxygen concentrations (mg/L) measured weekly in the bottom waters at five locations in the Upper Swan River between January 2010 and December 2011. The line at 2 mg/L indicates hypoxic conditions (Rosenberg, 1980). Error bars represent ± one standard error.

3.2.2. Abundance and diversity measures

Two-way univariate permutational ANOVA demonstrated that the number of species, mean total density (individuals 0.1 m^{-2}), Simpson's evenness index and average quantitative taxonomic distinctness (Δ*) differed significantly among sampling occasions. The same was true among locations for the number of species and Simpson's evenness index, but not for mean density or Δ* (Table 5). The number of species declined between March and April 2010 at all locations and usually remained low until June 2010, after which it increased gradually (Fig.11a). However, it should also be noted that the number of species had already declined markedly at CU between January and March 2010.

The mean total density of invertebrates underwent a marked decline at most locations in April 2010 and remained low until October 2010, before increasing, and markedly so at some locations (Fig. 11b). Again, it is also interesting to note that total densities had exhibited marked declines at C and CU between January and March 2010, prior to the storm event in March of that year, and remained low after the event. In contrast, total invertebrate densities at the GD location were already at their minima in January 2010 and did not increase until July of that year.

The values for Simpson's evenness index declined at every location in April 2010 before recovering between May and July, depending on the location (Fig. 11c). The values for Δ* at most locations also formed a conspicuous trough between April and July or October 2010 (Fig. 11d).

In general, similar, consistent declines in the above abundance and diversity indices were not observed across the five locations during 2011, although Δ* consistently decreased at all locations in winter of 2011 (Fig. 11d), corresponding to another, albeit briefer, hypoxic event that occurred in June/July of that year (Fig. 10).

Table 5. Significance levels derived using PERMANOVA and employing Euclidean distance matrices constructed from the values of a suite of abundance and diversity measures derived from the average faunal composition at each location on each sampling occasion. Ns = no significant difference.

Abundance/diversity measure	Occasion	Location
Number of species	<1%	<1%
Total density	<5%	Ns
Simpson's evenness index	<5%	<1%
Average quantitative taxonomic distinctness (Δ*)	<1%	Ns
Density of polychaetes	Ns	Ns
Density of molluscs	<5%	Ns
Density of crustaceans	<1%	<1%
Contribution of polychaetes	<1%	<1%

The total density of crustaceans and the percentage contribution of polychaetes to the combined polychaete and crustacean fauna each differed significantly among both occasions and locations, whilst the densities of molluscs exhibited significant differences among sampling occasions, but not among locations (Table 5). Although ANOVA did not detect a significant difference in the density of polychaetes among either sampling occasions or locations, the densities of this subphylum typically fell to very low levels in April 2010 before increasing again in subsequent months (Fig. 11e). Similarly, the densities of molluscs at most locations exhibited declines between March and April 2010, with the exception of Guildford, where abundances increased in April and declined immediately thereafter (Fig. 11f). In January 2010, crustaceans were present in relatively high densities at CU, but were essentially absent from all other locations situated further downstream. Very few crustaceans were recorded at any location in any month between April and July 2010, but their numbers showed a pronounced increase thereafter at all locations except GD (Fig. 11g). Once again, it is also noteworthy that at this latter location, densities of polychaetes, molluscs and crustaceans were already very low in January 2010, prior to the storm-induced hypoxic event in March-April of that year.

Polychaetes made a variable contribution across locations to the combined polychaete and crustacean fauna during January to March of 2010. Subsequently, the virtual absence of crustaceans from all locations during April to June resulted in an overwhelming dominance (94-100%) of the combined polychaete and crustacean fauna by polychaetes during this period (Fig. 11h). The relative contribution of polychaetes to this index decreased after July 2010, yet once again remained spatially variable, with polychaetes representing ~65-100% of the combined fauna at GD, but only~50-60% at CU.

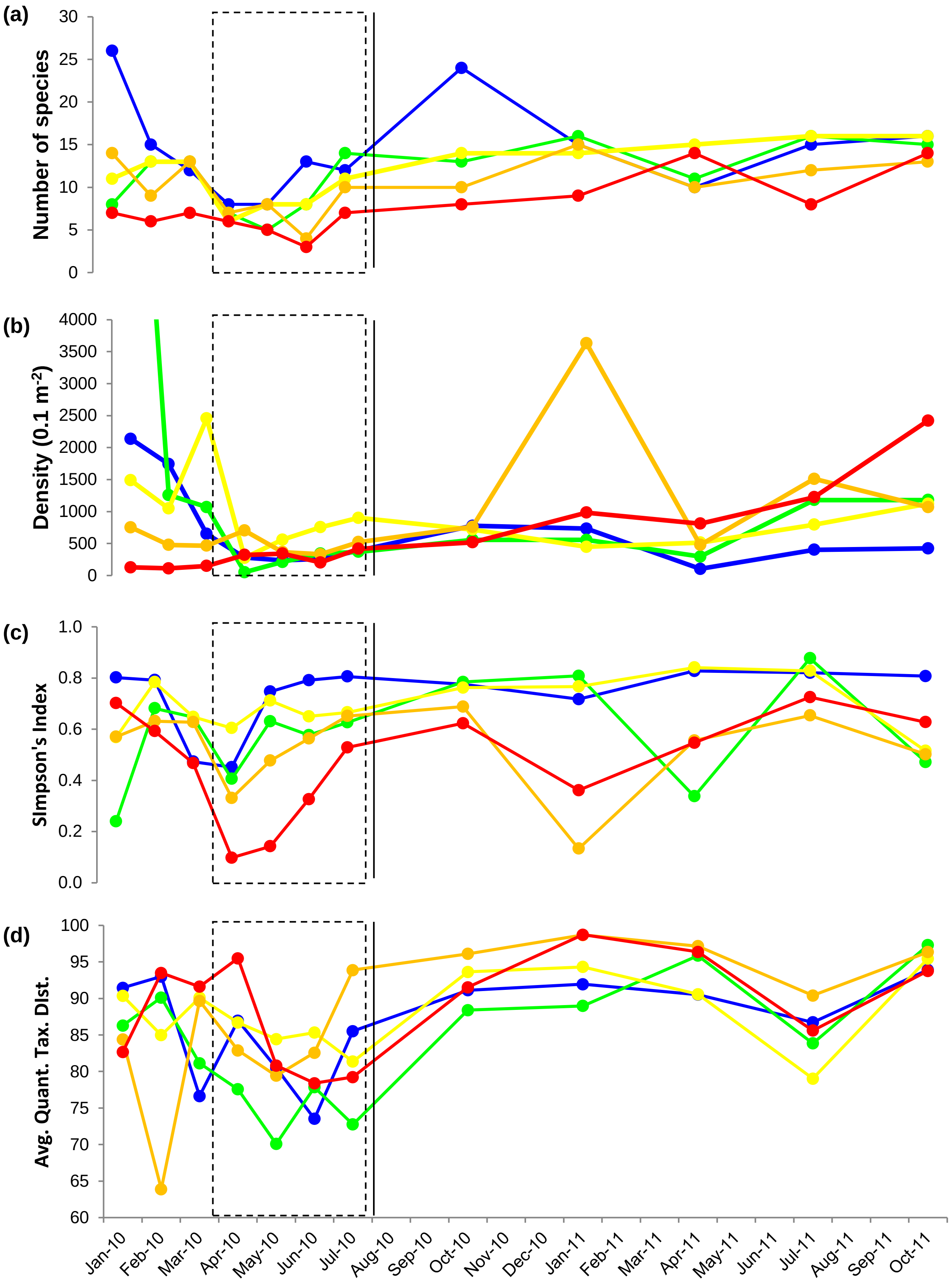


Figure 11. Variations in the (a) number of species (b) density of benthic macroinvertebrates 0.1 m^{-2} (c) Simpson's evenness index and (d) average quantitative taxonomic distinctness across locations and sampling occasions. Vertical line denotes the end of monthly sampling and the start of seasonal sampling. The dashed box shows the period during which parts of the Upper Swan Estuary became hypoxic. 🟦, Caversham Upstream; 🟩, Caversham; 🟨, Caversham-Guildford; 🟧, Guildford; 🟥, Guildford Downstream.

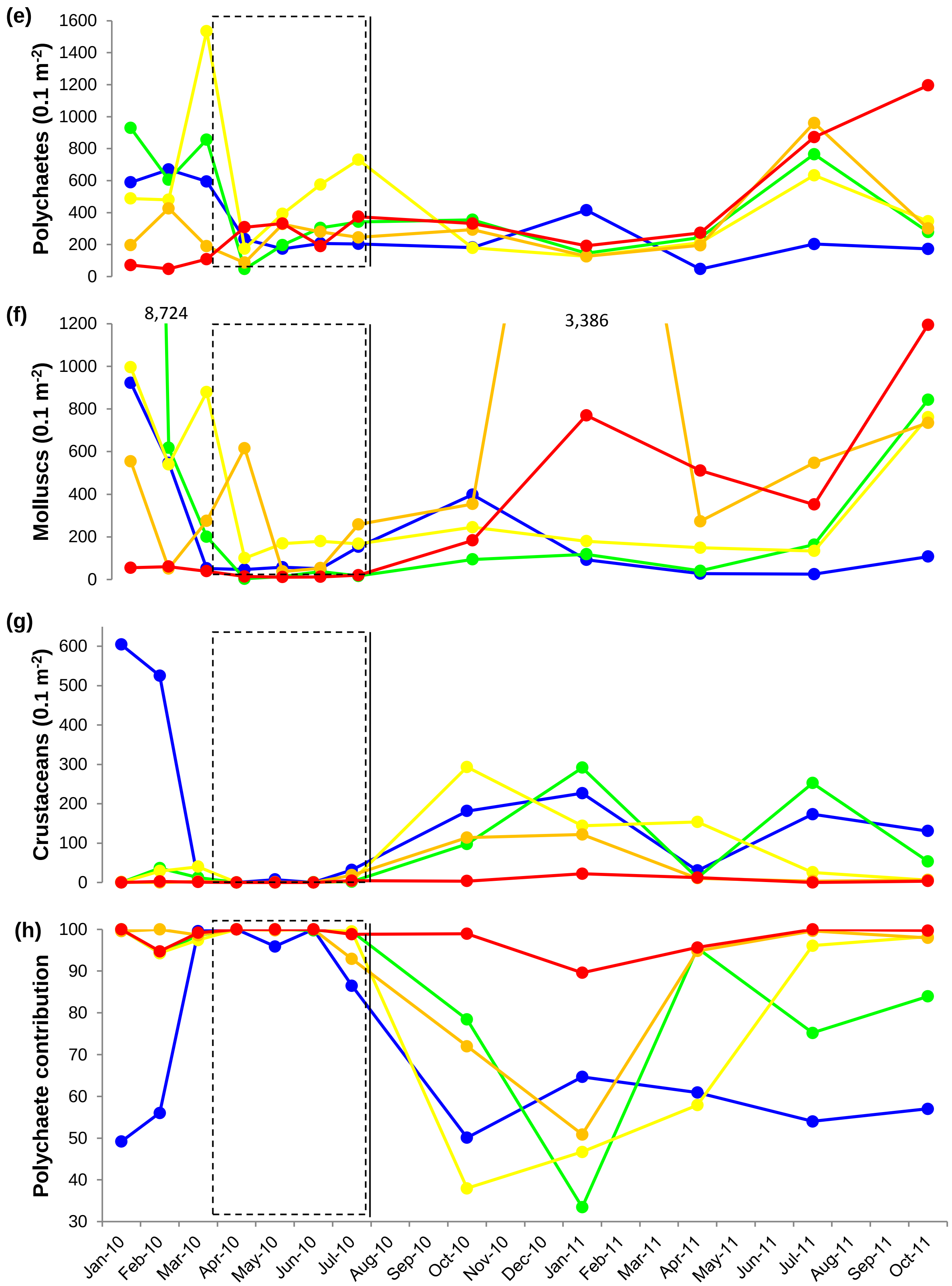


Figure 11 continued. Variations in the density (individuals 0.1 m^{-2}) of (e) polychaetes (f) molluscs (g) crustaceans and (h) the percentage contribution of polychaetes to the total polychaete and crustacean fauna across locations and sampling occasions. Vertical line denotes the end of monthly sampling and the start of seasonal sampling. The dashed box shows the period during which parts of the Upper Swan Estuary became hypoxic. 🟦, Caversham Upstream; 🟩, Caversham; 🟨, Caversham-Guildford; 🟧, Guildford; 🟥, Guildford Downstream.

3.2.3. Benthic macroinvertebrate community composition

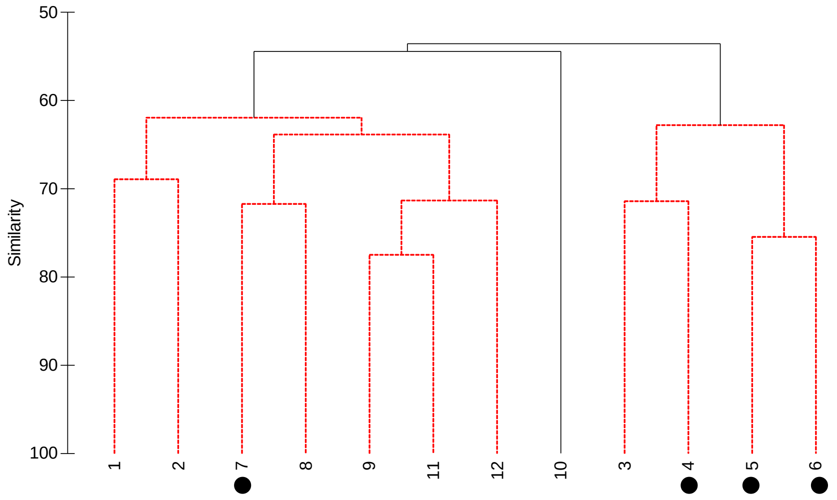
Two-way crossed PERMANOVA demonstrated that the compositions of the BMI communities throughout the USE zone of the Swan Canning Estuary in 2010 and 2011 differed significantly among occasions and locations and that the two-way interaction between occasion and location was significant (p = 0.1%; Table 6). Two-way crossed ANOSIM indicated that, although both factors were significant, the changes in the composition of the fauna were related slightly more to the sampling occasion ($\bar{R}$ = 0.494) than to the sampling location ($\bar{R}$ = 0.375).

Table 6. Mean squares (MS), components of variation (COV) and significance levels (*p*) derived using PERMANOVA and employing the Bray–Curtis resemblance matrix constructed from presence or absence of the various benthic macroinvertebrate species in Ekman grab samples from each location on each sampling occasion between January 2010 and October 2011. df = degrees of freedom. Significant results are highlighted in bold.

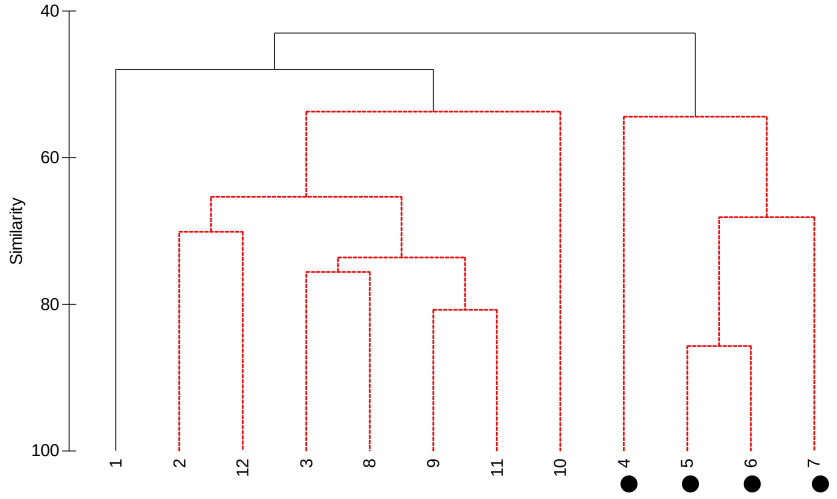
	df	MS	COV	*p*
Main effects				
Occasion	11	**12378**	**466**	**0.1%**
Location	4	**20816**	**335**	**0.1%**
Interactions				
Occasion x location	44	**1683**	**193**	**0.1%**
Residual	240		719	

Clustering of the samples that represented each of the twelve sampling occasions was performed, employing a Bray-Curtis resemblance matrix calculated from the frequency of occurrence of each species in replicates samples collected from each location on each sampling occasion. In the cases of the four most upstream locations (*i.e.* CU, C, CG and G; Fig. 12a-d), samples from the monthly sampling occasions 4, 5 and 6 (*i.e.* April-June, the three months in 2010 following the storm and onset of hypoxic conditions) formed a cluster. Thus, the faunal communities recorded during each of these three months were statistically similar and also significantly different from those on the other sampling occasions. In the case of the cluster dendrogram for CU, this ‘post-storm’ cluster also included occasion 3, *i.e.* March 2010, whilst those for Caversham and CG both comprised occasions 4-7 (and also occasion 1 in the case of CG). The same degree of clustering was not observed at GD, however, where samples from occasions 1-12 could not be separated on the basis of significant differences in their faunal composition. These sampling occasions formed a single cluster and thus the fauna recorded on all of the sampling occasions was deemed to be similar (Fig. 12e).

(a) Caversham Upstream



(b) Caversham



(c) Caversham-Guildford

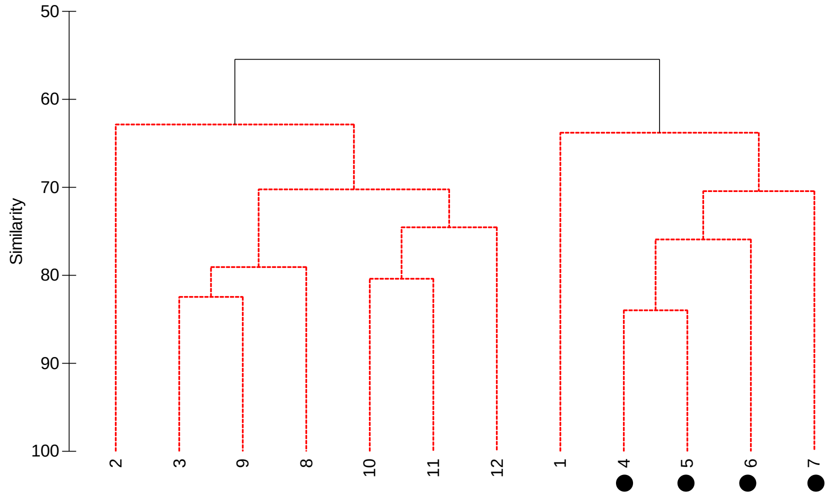
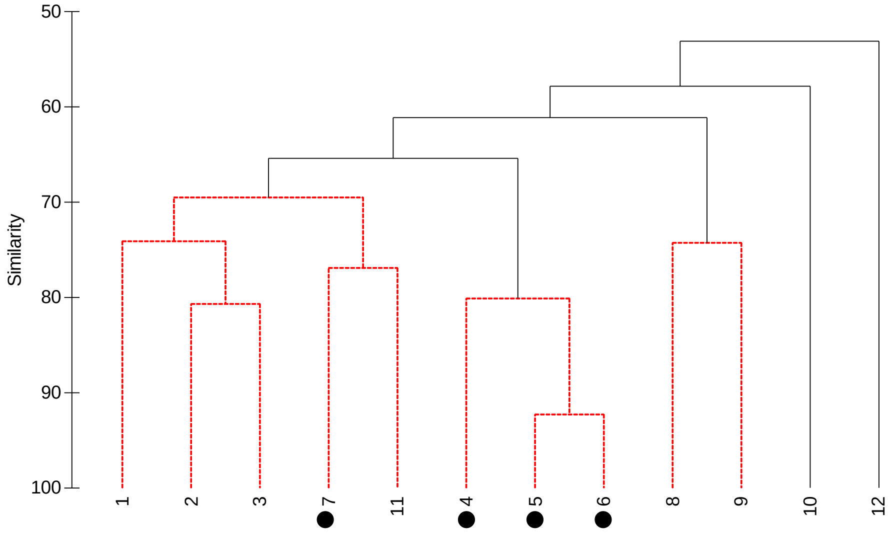


Figure 12. Dendrogram derived by subjecting the frequency of occurrence of each species on each sampling occasion during 2010-2011 at a given location to the CLUSTER and SIMPROF routine. Groups of sampling occasion(s) denoted by red lines do not contain significant faunal differences. Occasions 1-7 represent monthly sampling in January-July 2010, whilst occasions 8-12 represent subsequent seasonal sampling between October 2010 and October 2011. The black circles show the sampling occasions during which parts of the Upper Swan Estuary became hypoxic.

(d) Guildford



(e) Guildford Downstream

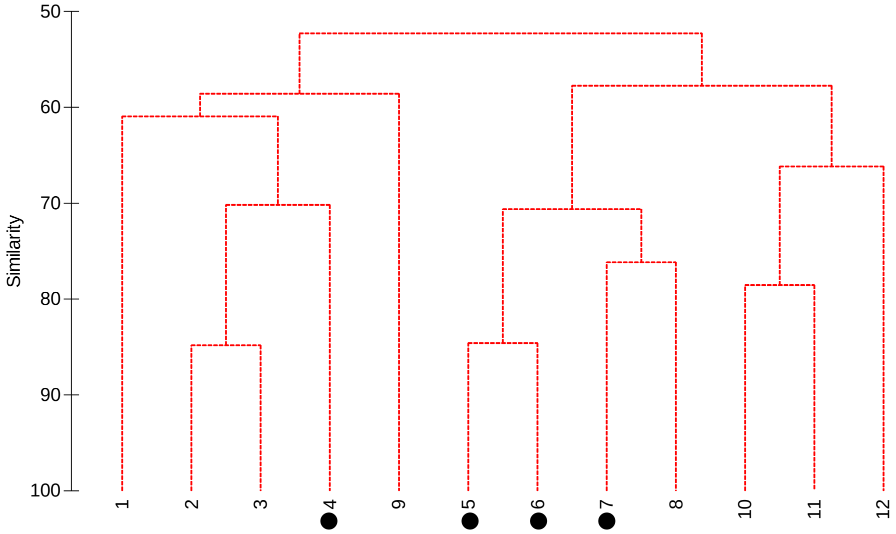


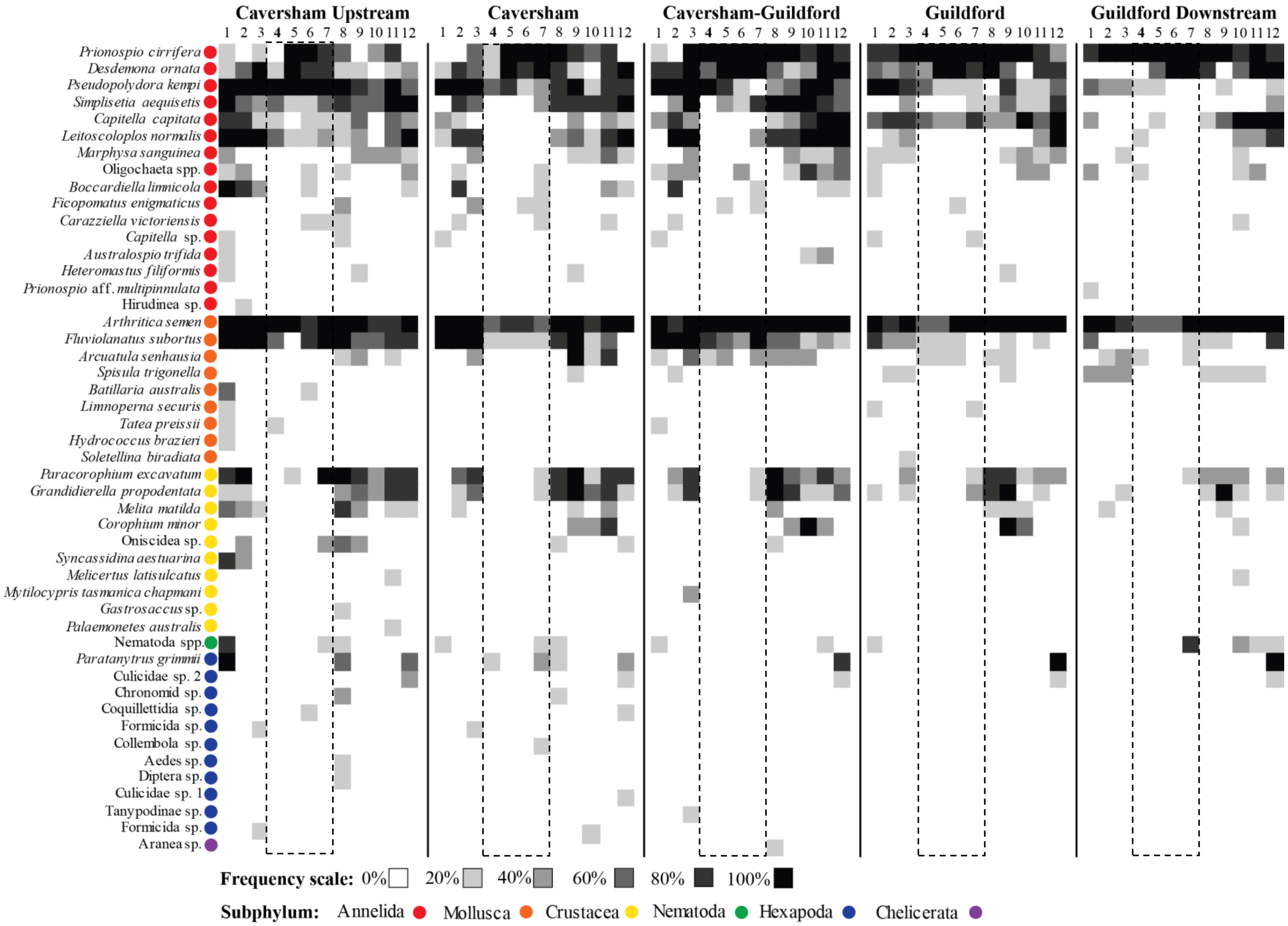
Figure 12 (Continued). Dendrogram derived by subjecting the frequency of occurrence of each species on each sampling occasion during 2010-2011 at a given location to the CLUSTER and SIMPROF routine. Groups of sampling occasion(s) denoted by red lines do not contain significant faunal differences. Occasions 1-7 represent monthly sampling in January-July 2010, whilst occasions 8-12 represent subsequent seasonal sampling between October 2010 and October 2011. The black circles show the sampling occasions during which parts of the Upper Swan Estuary became hypoxic.

Notable effects of the hypoxic event on the benthic invertebrate fauna of the Upper Swan Estuary are also evident from the shade plot representing changes in the frequency of occurrence of individual taxa across the five replicate samples collected from each location on each sampling occasion. Consistent decreases in the occurrence of numerous polychaete species, including *L. normalis, P. kempi* and *S. aequisetis*, were observed between sampling occasions 4 and 7, *i.e.* April-July 2010, presumably as a consequence of the hypoxic event (Fig. 13).

The frequency of occurrence of the bivalve molluscs *F. subortus* and, at C, G and GD, *A. semen*, similarly declined over the same period. In contrast, the surface deposit-feeding polychaete *P cirrifera* was observed more commonly from sediment samples collected between March/April and July. With few exceptions, crustacean taxa were conspicuously absent from all five locations between April and July 2010 (*i.e.* sampling occasions 4-7), despite numerous species, including the amphipods *P. excavatum, G. propodentata* and *M. matilda*, being commonly observed among samples collected both before and after this period. Moreover, crustaceans were also largely absent from the four most downstream locations (*i.e.* GD, G, CG and C) during January 2010, perhaps reflecting the effects of a disturbance, which may have occurred prior to the commencement of the current study.

In addition to illustrating the above effects of the hypoxic event on the BMI fauna, the shade plot also effectively highlights and reinforces the broader spatial patterns in the frequency of occurrence of numerous taxa over the course of the study. The polychaete *P. cirrifera* and the bivalve mollusc *Spisula trigonella* were generally more commonly encountered among the more downstream locations, whereas the mollusc *F. subortus*, the polychaetes *P. kempi, Boccardiella limnicola, S. aequisetis* and *L. normalis* were more prevalent upstream. The crustaceans *P. excavatum, G. propodentata* and *M. matilda,* and a number of insect species were largely restricted to the two uppermost locations, namely C and CU.

Figure 13. Shade plot based on the frequency of occurrence of a species across the five replicate samples collected at each location on each sampling occasion. Species are grouped by subphylum and ordered within this taxonomic rank by decreasing total frequency of occurrence across all samples. Occasions 1-7 represent monthly sampling in January-July 2010, whilst occasions 8-12 represent subsequent seasonal sampling between October 2010 and October 2011. The dashed box shows the period during which parts of the Upper Swan Estuary became hypoxic.



4. Discussion

4.1. Spatial and seasonal trends in benthic macroinvertebrate community characteristics

The total of 42 benthic macroinvertebrate (BMI) species recorded from sediment samples obtained seasonally from deeper waters throughout the Upper Swan Estuary (USE) zone during the present study is broadly similar to the 35 obtained during roughly equivalent, seasonal sampling conducted between 1995 and 1997 by Kanandjembo *et al*. (2001) in the deeper waters of the Middle Swan Estuary (MSE) zone. Polychaete worms, including those belonging to the families Spionidae, Nereidae, Capitellidae and Orbiniidae, were the most speciose subphylum among the BMI fauna of the USE. This is consistent with previous investigations reported in the Swan Estuary (Rose, 1994; Kanandjembo *et al.*, 2001; Wildsmith *et al.*, 2011) and also in other estuaries in south-western Australia (Wildsmith *et al.*, 2009; Tweedley *et al.*, 2012).

Interestingly, the number of species collected at each location decreased in a downstream direction from a maximum of 35 species recorded at CU to a minimum of 22 species at GD. This finding contradicts the classical Remane curve, where values for species richness decline from freshwater to a minimum in salinities of 5-8 and then rise progressively to their maxima in marine waters (Remane and Schlieper, 1971), and also studies on the Hawkesbury Estuary in New South Wales and the Schelde Estuary in the Netherlands and Belgium (Jones *et al.*, 1986; Ysebaert *et al.*, 1993). One possible reason for this is differences in the composition of the BMI fauna among these estuaries. Notably, none of the 133 and 35 respective species recorded in the Hawkesbury and Schelde estuaries belonged to the subphylum Insecta. By comparison, eight of the 42 species recorded in the current study (*i.e.* 19%) were insects and furthermore, seven of the eight were recorded exclusively from the two most upstream locations. Typically, insects comprise the majority of species in rivers and wetlands and are very poorly represented in estuarine environments (Batzer and Sharitz, 2006). Their exclusion from the lower three locations in the Swan Estuary, however, cannot be attributed solely to salinity as these species dominate the fauna of saline wetlands, where salinities often far exceed those in estuaries (*e.g.* Batzer and Sharitz, 2006; Tweedley *et al.*, 2011). Thus, other workers have suggested that competition may play a role (*e.g.* Daly *et al.*, 1998).

The composition of the sediment is widely regarded as a key determinant in the composition of benthic macroinvertebrate faunas (*e.g.* Gray, 1974; Snelgrove and Butman, 1994). It is thus relevant that, during 2010, the sediments from G and GD contained greater proportions of both fine particles and organic matter than did those from CG, C and CU. It is hypothesised that these findings reflect the potential influence of flows from the Helena River and Bennett Brook as sources of fine sediments, particulate organic matter (POM) and possibly nutrient and non-nutrient contaminants, each of which might impact on the survival and/or recruitment success of more sensitive invertebrate species (see below).

The mean total density of BMI across all locations in the USE recorded in this study, *i.e.* 1,037 individuals/0.1m^2, was ~50% greater than the 777 individuals/0.1m^2 recorded in the deeper waters of the MSE by Kanandjembo *et al*. (2001). Increased densities of BMI, and particularly those of opportunistic polychaete species, have traditionally been regarded as a sign of eutrophication (Gray *et al.*, 2002; Karlson *et al.*, 2002). For example, the average BMI density in the deeper waters of the essentially-pristine Broke Inlet, on the south coast of Western Australia, was 178 individuals/0.1m^2 and was about a fifth of the 817 individuals/0.1m^2 recorded in the nearby and eutrophic Wilson Inlet (Tweedley *et al.*, 2012).

A small suite of species dominated the fauna of the USE in terms of numerical abundance. The most abundant species was the bivalve mollusc, *Arthritica semen,* which represented ~33% of total BMI abundance across the zone as a whole. Kanandjembo *et al*. (2001) similarly reported this species as being the most abundant and comprising >25% of total invertebrate numbers in both deep and shallow waters of the MSE. Together with *A. semen*, the bivalve *Fluviolanus subortus* and the polychaetes *Prionospio cirrifera* and *Desdemona ornata* accounted for ~78% of total numbers of invertebrates during the current study, and just ten species comprised almost 98% of total abundance across the USE. Similarly, three of the same four species, plus *Pseudopolydora* sp. comprised ~75%, and 10 species accounted for >90%, of total invertebrate abundances during a former study of the estuarine reaches of the Swan River (Kanandjembo *et al.*, 2001).

Such a high degree of numerical dominance by a few species is often interpreted as a signal of degraded ecosystem condition, resulting from organic pollution and hypoxia (Gray *et al.*, 2002; Karlson *et al.*, 2002) or the more general effects of urbanisation and population growth (Edgar and Barrett, 2000; Wildsmith *et al.*, 2009). However, high dominance and low species diversity/evenness are also natural characteristics of estuarine macroinvertebrate faunas and can occur in relatively undisturbed estuaries (Tweedley *et al.*, 2012), and such indices thus require further exploration. Nonetheless, the overwhelming collective contribution of *A. semen, P. cirrifera, D. ornata* and *Capitella 'capitata'* to invertebrate densities at GD resulted in dominance being particularly high in this location, with these four species comprising ~97% of invertebrate numbers.

Furthermore, these species are all relatively tolerant of degraded and/or eutrophic environments.

Both *C. 'capitata'* and *P. cirrifera* are regarded in Europe as indicator species of such conditions in the widely used AZTI Marine Biotic Index (AMBI) (Borja *et al.*, 2000; Tweedley *et al.*, 2014), while *A. semen* dominated the molluscan fauna in the Peel-Harvey Estuary during the late 1970s when eutrophication was high, with densities of this species averaging and peaking at 810 and 4,591 individuals/0.1m^2, respectively (Wells and Threlfall, 1982; Whisson *et al.*, 2004). Moreover, *D. ornata* is a secondary deposit feeder whose abundances are correlated with the nutritional content of the sediment (Fauchald and Jumars, 1979; Rossi, 2002; 2003). Therefore, the relatively high densities and the high degree of dominance by these species provide some evidence that the benthic environment at GD suffers from chronic stress/degradation (see below).

Broad spatial patterns in the abundance of individual species were also evident from the seasonal sampling of the USE. Densities of *A. semen, P. cirrifera, D. ornata* and the bivalve *Spisula trigonella* increased in a downstream direction. Such a trend may be due to the fact that the first three species are able to switch between deposit and suspension feeding and could thus take advantage of the higher levels of POM in the sediments further downstream, while *S. trigonella* has generally been recorded in the lower and middle regions of the estuary (Chalmer *et al.*, 1976) and thus may prefer higher salinities. In contrast, the polychaetes *Pseudopolydora kempi, Simplisetia aequisetis*, *Leitoscoloplos normalis* and *Boccardiella limnicola,* and the amphipods *Paracorophium excavatum, Grandidierella propodentata* and *Mellita matilda* became more abundant further upstream. A similar trend was observed with *S. aequisetis, B. limnicola* and *Paracorophium* sp. in the Duck and Montagu estuaries in Tasmania (Beard, 2012), and Kanandjembo (1998) and Rose (1994) have suggested that the first of those species prefers coarser, shallower sediments, which are less prone to hypoxia. It was also notable that the presence of insects, including the larvae of the chironomid *Paratanytarsus grimmi*, was largely restricted to the uppermost two locations (see above for explanation).

The above broad spatial differences, both in the numbers of species and the abundances of individual species, resulted in significant differences in the composition of BMI communities between locations, with a clear spatial gradient evident on the nMDS ordination (Fig. 5c and Fig. 6). These spatial differences in faunal composition were observed in all of the eight seasons sampled, and were shown by BIOENV to be consistently related to salinity and other important water quality variables.

Although seasonal differences in macrofaunal composition were also evident and have been demonstrated in previous studies of many other permanently-open estuarine systems (*e.g.* Jones, 1987; Chainho *et al.*, 2006), the greatest differences were observed between years, with a clear separation of the points representing samples from each of the two years (Fig. 5a). It is likely that this marked difference between years reflects the influence of the storm that occurred in March 2010, resulting in unseasonal rainfall, river flows and environmental conditions which deleteriously impacted the macrofaunal communities during 2010, but were not replicated in the subsequent year of more typical flow conditions (see subsections 3.2 and 4.2). Kanandjembo *et al*. (2001) noted a similar inter-annual difference in faunal composition among shallow-water locations, which they attributed to the effects of heavy freshwater discharges that occurred in the winter of 1996, but not during the winter of the previous year.

4.2. Benthic macroinvertebrate community responses to a notable hypoxic ‘event’

The storm of March 2010 resulted in the development of hypoxic conditions (<2 mg/L; Rosenberg, 1980) that affected much of the bottom waters of the USE and persisted for several months. The persistence of this hypoxic ‘event’ varied across the five locations within this zone, with dissolved oxygen concentrations of <2 mg/L being recorded over ~7 weeks and lasting to the end of June at CG, over 11 to 12 weeks at both C and CU, and for as long as 17 weeks at G and GD, with consistent normoxia being restored only around the end of October at these two most downstream sites (Fig. 10).

The present study has identified numerous lines of evidence that this period of notable and sustained hypoxia impacted the benthic macroinvertebrate communities of the USE. PERMANOVA detected significant differences in community composition between sampling occasions, with the influence of this temporal factor ($\bar{R}$ = 0.494) exceeding that due to sampling location ($\bar{R}$ = 0.375). The presence of a significant interaction also indicates that the effects of the hypoxic event on community composition differed among locations. The samples representing the three or four sampling occasions following the storm (*i.e.* April to June/July 2010) formed clear clusters on the dendrograms for all locations except GD. The presence of these clusters signifies that the faunal communities recorded at these locations during each of these months were statistically similar, and also significantly different from those on the other sampling occasions. This ‘post-storm cluster’ thus indicates that the extreme storm event of March 2010, and the associated hypoxia that persisted over subsequent months, brought about significant changes in the composition of the BMI communities over a large area of the USE. The fact that no such cluster of samples representing sampling occasions following the storm could be identified in the case of GD may be explained by the hypothesis that this location was under chronic stress (see below), and thus that any impact of the hypoxic event could not be determined there. With the exception of GD, the clustering of occasions after June/July 2010, and sometimes also including January and February 2010, indicated a return toward a pre-storm state, and thus a degree of recovery of the fauna at each location.

At a broad level, whilst a relatively diverse range of taxa were present across the five locations in January 2010, there was a subsequent shift to far fewer species in April 2010. The suggestion that this reflects the loss of some species following the storm and subsequent onset of hypoxia is further supported by numerous lines of evidence. The number of species declined between March and April 2010 at all locations, and did not recover until June at the earliest. Moreover, total invertebrate densities decreased at most locations in March or April of that year, and remained low for several months. Numerous studies have demonstrated such effects of hypoxia on species richness and invertebrate densities (Josefson and Widbom, 1988; Gray *et al.*, 2002; Karlson *et al.*, 2002). Similarly, the hypoxic ‘event’ brought about clear changes in the diversity, dominance and evenness characteristics of the benthic community. Simpson’s evenness index decreased in March-April 2010 at all locations, exhibiting a recovery in May-July. Low values for this index are indicative of an assemblage that is highly dominated by a small number of species (*i.e.* the numerical distribution of total abundance across the component species is highly uneven; Somerfield *et al.*, 2008). Relatively low values for average taxonomic distinctness were also observed at most locations between April and July, signifying that the species present were closely related and thus that the BMI community had also become taxonomically less biodiverse. This measure, which is not influenced by natural variation, such as seasonal trends in the abundance of various species, has been widely used to detect degradation of benthic environments (*e.g.* Warwick and Clarke, 2001; Leonard *et al.*, 2006; Tweedley *et al.*, 2012; 2014). Such changes in the structural, numeric and taxonomic diversity of BMI communities are typical responses to degradation of the benthic environment resulting from stressors such as hypoxia, organic enrichment or chemical pollution (Pearson and Rosenberg, 1978; Boesch and Rosenberg, 1981; Gaston, 1985; Warwick and Clarke, 1995; Gaston *et al.*, 1998; Wilson *et al.*, 1998; Kemp *et al.*, 2005; Levin *et al.*, 2009).

The identities of the taxa whose relative abundance and diversity either declined or increased following the onset of hypoxia are also highly informative. Crustaceans are generally regarded as being less tolerant of degraded conditions, including low dissolved oxygen concentrations, than the other major groups of invertebrates recorded from these estuarine communities (Theede, 1973; Josefson and Widbom, 1988; Gray *et al.*, 2002; Levin *et al.*, 2009). It is thus pertinent that crustacean taxa were virtually absent from all locations between April and July 2010. Indeed, crustacean populations at the four most downstream locations appear also to have been impacted prior to the commencement of the study (see below), and their sensitivity to hypoxia resulted in their numbers being dramatically depressed until after July 2010.

Decreases in the densities of molluscs also occurred between March and April 2010 at most locations and the shade plot (Fig. 13) revealed that *F. subortus* was generally scarcer among samples from April to July than among those from earlier or later periods. Similarly, *A. semen* was less frequently encountered among samples from C, G and GD on occasions coinciding with hypoxic conditions. Numerous bivalve species have been identified as exhibiting behavioural and physiological mechanisms for tolerating hypoxic conditions and so, by closing their shells and reducing their metabolism, can survive for up to two months in anoxic water (Theede, 1973). The severity of the impacts of the hypoxic event on the macroinfaunal communities can thus be gauged by the fact that densities of molluscs (which are widely regarded as being more tolerant of hypoxia than crustaceans) declined at most locations.

The densities of polychaetes, which are generally considered to be the most tolerant of the major macrofaunal taxa to hypoxia (Reise, 1982; Warwick and Clarke, 1993), also decreased between March and April 2010 but remained higher than those of crustaceans or molluscs. *Leitoscoloplos normalis, P. kempi* and *S. aequisetis* all tended to occur less frequently during April-July than during prior or subsequent sampling occasions. However, *P. cirrifera,* a spionid polychaete that feeds on detritus and is tolerant of polluted, eutrophic or otherwise disturbed conditions (Pearson and Rosenberg, 1978; Borja *et al.*, 2000; Shen *et al.*, 2010), was observed more commonly among sediment samples collected between March/April and July at most locations. These results thus provide further indication of the degree to which the hypoxic event impacted the benthic fauna of the USE. In addition, the percentage contribution of polychaetes to the combined polychaete and crustacean fauna was almost total between April and June at all locations, reflecting both the virtual loss of crustaceans and the fact that numerous polychaete species remained relatively numerous and widespread, despite the hypoxic event. Wildsmith *et al*. (2009) interpreted such an increase in the abundance of polychaetes relative to crustaceans as evidence of deterioration in the benthic environment of the Peel-Harvey Estuary between 1986/7 and 2003/4.

It should also be noted that all locations exhibited signs of having suffered from a previous perturbation or stress prior to the onset of the storm-induced hypoxic ‘event’. For example, the number of species recorded from CU had already exhibited a marked decline from January to March 2010, whilst similar declines in both total invertebrate density and the density of molluscs were observed at C and CU over the same period. Most notably, at all locations other than CU, the crustacean fauna appears to have undergone a decline due to a perturbation that occurred prior to the commencement of the study. This can clearly be seen in the almost total lack of crustaceans at these locations during January 2010 (Fig. 11g and 13). Crucially, water quality data collected by the Swan River Trust indicate that this region of the Swan River experienced a period of low dissolved oxygen conditions between mid-December 2009 and mid-January 2010 (Appendix 4). This situation was caused by the intrusion of the salt wedge into the study area as river flows declined, and exacerbated by necessary repairs and other issues that prevented the Guildford oxygenation plant from operating effectively at times during this period (Alex Hams, SRT, personal communication). Together, these findings suggest that the BMI community of the USE had been impacted by the development of hypoxic conditions immediately prior to the first sampling occasion of the current study, causing not only the loss of the sensitive crustacean taxa from all locations within the zone of hypoxia (*i.e.* downstream of CU), but also declines in the densities of some of the relatively more tolerant molluscan and polychaete species. The subsequent development and persistence of hypoxic conditions following the storm in March 2010 is likely to have further exacerbated environmental conditions and prevented the recolonisation of these impacted locations by crustaceans and molluscs, until consistent normoxic conditions had been restored around July of that year.

The most obvious signs of previous (and even ongoing, chronic) impacts on BMI communities were seen at GD. The total density of invertebrates recorded from GD was already at its minimum in January 2010, and number of species was also low. Indeed, the densities of all three major subphyla (including those of the relatively tolerant polychaetes) were already very low prior to the storm, indicative of a considerable impact on the benthic community. Accordingly, it is unsurprising that the CLUSTER-SIMPROF analysis for GD did not identify occasions whose faunal composition differed from that observed during the hypoxic event of 2010 (noting that intermittent hypoxia lasted for many months in this location). Given that the samples collected from this location on all 12 sampling occasions demonstrated statistically similar BMI community composition and were thus grouped as a single cluster, we can conclude that the benthic macroinvertebrate fauna at GD appears to be characteristic of a chronically stressed community over the course of this two-year study. These stresses clearly included a hypoxic event that occurred in December 2009 and a second, lengthier period of hypoxia that persisted from March to October 2010. Yet continuing, unknown stressors also appear to have maintained the benthic community at GD in an impacted or degraded state through to October 2011. As outlined in section 4.1, we hypothesise that flows from the Helena River and/or Bennett Brook, whose confluences are situated upstream from the GD location, may be the underlying cause of this chronic stress. The exact nature of these stressors is unknown, but may relate to these tributaries being a source of POM into the Swan River. For example, the increase in sediment organic matter content at Guildford in autumn of 2010 may reflect storm input from the adjacent confluence.

4.3. Conclusions and implications

The present study has documented the broad spatial and temporal patterns in the composition of the macroinvertebrate fauna of the Upper Swan Estuary during a two-year monitoring period. The first component of the study, which was conducted at five locations over eight consecutive seasons, recorded ~47,000 individuals belonging to 42 taxa. The total number of species recorded at each location declined in a downstream direction, primarily due to the absence of insects in the lower locations, while the density of benthic macroinvertebrates exhibited no consistent spatial trend. Differences in faunal composition among locations and seasons were significantly related to patterns in salinities.

The second component of this study was to determine the effects of the artificial oxygenation program on biota. However, as detailed in the introduction, the experimental design was critically confounded by the lack of a Before- After-Control-Impact (BACI) set-up (*e.g.* see Downes, 2010). This prevented a valid, direct analysis of the effects of the oxygenation plants on BMI communities. However, during the study an extreme rainfall event occurred which caused a sustained (2-3 month) period of hypoxia in the USE, and provided an opportunity to evaluate the effects of hypoxia on the benthic biota. The benthic macroinvertebrate fauna exhibited classic responses to this degradation. For example, for a three to four month period following the March rainfall event, the number of species, total density, evenness, taxonomic diversity and the density of crustaceans and molluscs all declined markedly. CLUSTER-SIMPROF analysis indicated that, at all locations except GD, the sampling occasions fell into one of two clusters, namely 1) hypoxia [~April-July 2010] or 2) before the hypoxic event and during the subsequent recovery [~January-March 2010 and October 2010-October 2011]. Analysis of the frequency of occurrence of all species using shade plots indicated that the changes in faunal composition detected between April and July 2010 were due largely to the almost complete absence of crustacean species, which are known to be extremely sensitive to hypoxia, but also to declines in the bivalve *Fluviolanatus subortus* and even the polychaetes *Pseudopolydora kempi*, *Leitoscoloplos normalis* and *Marphysa sanguinea,* which are regarded as being among the most tolerant group of macrofauna. The changes described above were far less evident at GD, to the extent that CLUSTER-SIMPROF could not separate the faunas based on their frequency of occurrence, thus indicating that this location appeared to be chronically disturbed, at least throughout the course of the study.

While oxygenation plants are present in the USE, only one of the two was operational at the time of the extreme rainfall event responsible for the hypoxic conditions, as the plant at Caversham only came online in April 2011 and only began regular operations in June 2011. Furthermore, as these plants were not designed to mitigate the effects of extreme storm events, they would not be expected to prevent the large scale hypoxia seen in the present study. In the case of the hypoxic event of March/April 2010, recolonisation of the impacted benthic communities occurred within six months, though, as was evident from the hypoxia that occurred in December 2009 (prior to the start of this study), recovery can only occur if sufficient oxygen is available. Although the effects of artificial oxygenation on biota could not be demonstrated directly during this study for reasons outlined above, it is likely that under normal operating conditions the oxygenation program would help to bring about a more rapid return to normoxic conditions following such extreme events. Close attention should therefore be paid to the oxygen levels of the bottom waters of the USE, to determine the extent and duration of hypoxic conditions under normal flow conditions and following extreme events.

The current study provides a suitable benchmark against which future changes in BMI communities might be assessed, in a manner similar to the inter-decadal comparisons of benthic community composition that have been conducted in the Lower Swan Canning Estuary (Wildsmith *et al.*, 2011). It is possible that such a comparison would illuminate any longer-term responses of the BMI community to ongoing oxygenation of the upper estuary, as recent investigations and modeling have demonstrated the capability of the oxygenation plants to improve near-bed oxygen status across large areas of the river (Hipsey *et al.,* 2014),

In order to explicitly and directly demonstrate that artificial oxygenation is effective in maintaining benthic macroinvertebrate communities across the USE, rather than via inference alone (as in this report), an extensive BACI-type experiment would need to be conducted. Such a study would require multiple ‘treatment’ (*i.e.* sites with artificial oxygenation) and control sites and each site would be surveyed regularly over an extended period of time, both before and after artificial oxygenation is supplied. Moreover, the design of the experiment should enable it to be shown conclusively that (i) ‘treatment’ sites would be hypoxic if it were not for the oxygenation and (ii) that artificial oxygenation generated and maintained normoxic conditions over a sustained period of time. Data on dissolved oxygen levels in both the water and particularly the water-sediment interface (or ideally sediment oxygen penetration) would need to be recorded at fine spatial and temporal (*e.g.* 15 minutes) scales. An alternative approach, however, would be the establishment of a mesocosm experiment (see below).

It is worth noting that estuaries in south-western Australia may be naturally pre-disposed to periods of hypoxia due to the fact that residence times in these estuaries are far higher than in other systems (*cf*. Ranasinghe and Pattiaratchi, 1998; Kalnejais *et al.*, 1999; Uncles *et al*., 2002; Borja *et al.,* 2006). This is due, in part, to the geomorphology of estuaries in south-western Australia, which not only experience microtidal conditions but also have a narrow entrance channel which attenuates large proportions of the tidal force (Hodgkin and Hesp, 1998; Potter and Hyndes, 1999; Potter *et al.,* 2010). Moreover, as rainfall in this region is highly seasonal (Hodgkin and Hesp, 1998) residence time also varies seasonally. For example, Kalnejais *et al.* (1999) estimated that the residence times in the Upper Swan Estuary (in this case all waters upstream of Heirisson Island), which contains 11 million m^{-3} of water, are one day in winter, but 235 days in summer. Such a phenomenon means that any plug of hypoxic water is likely to persist in the Upper Swan Estuary at certain times of the year, unless alleviated through the use of the artificial oxygenation plants.

The BMI community of the deeper, offshore waters of the USE, especially during hypoxic events, seems to be characteristic of early successional communities in being dominated by small-bodied, surface deposit feeders. This is indicative of numerous, perhaps constant disturbances, which result in communities that seldom progress beyond the early stages of benthic-community succession (*sensu* Pearson and Rosenberg, 1978; Warwick and Clarke, 1993; Gaston *et al.*, 1998). Several authors have identified potentially severe implications of these disturbance effects for fisheries, particularly as a result of the decreased trophic diversity and reduced benthic-pelagic flows of nutrients and energy which accompany the shift towards small-bodied, surface deposit feeding BMI taxa (Rakocinski *et al.*, 1997; Gaston *et al.*, 1998). Moreover, evidence from the York River, which flows into Chesapeake Bay (USA), indicates that frequent hypoxia can strongly influence the benthic community (Boesch *et al.*, 1976) and there is growing evidence to suggest that this can lead to the fauna exhibiting resistance and resilience to short- term hypoxia

(Pihl *et al.*, 1991; Dauer *et al.*, 1992; Sagasti *et al.*, 2001). To understand what these impacts on the benthic macrofauna mean for the Swan Canning Estuary and other systems in Western Australia that exhibit similar patterns (particularly the Peel-Harvey Estuary), further work is needed in a few areas:

- Controlled laboratory and mesocosm experiments to determine the short- and long-term oxygen tolerances of key benthic macroinvertebrate species, and also of other taxa such as fish and prawns. These studies would provide an indication of how resilient the estuarine fauna are to hypoxia, and thus what oxygen levels are required to maintain a viable benthic community and appropriate ecosystem functioning. The information from such studies could also inform modeling of the responses of fauna to artificial oxygenation (*e.g.* Hipsey *et al.,* 2014).

- Ecological and biological studies on the key benthic macroinvertebrate species are vital, as much of our understanding of the species present in the Swan Canning Estuary is based on work on those relatively few species which occur both here and in polluted systems in the northern hemisphere, such as the species complex *Capitella 'capitata'*.

- Assessments of the role of the sediments in the shallow, nearshore waters of the USE should be undertaken as some benthic macroinvertebrate species are known to migrate further up into the water column and also laterally, to areas in which dissolved oxygen concentrations may be higher (Diaz and Rosenberg, 1995; Hagerman, 1998; Sagasti *et al*., 2001). Moreover, as many of the species in the deep waters of the MSE and the Broke and Wilson Inlets are also present, to some extent, in adjacent shallow waters (Kanandjembo *et al*., 2001; Tweedley *et al*., 2012), these nearshore waters may also act as a crucial source for recolonisation, particularly when hypoxia in the deeper waters occurs over wide areas (*e.g.* as was observed during April-May 2010).

- The pronounced response of the benthic macroinvertebrate fauna of the USE to hypoxia, and as shown by a plethora of studies in estuaries throughout the world, showcase the ability of this fauna to respond to environmental perturbations. Thus, it would be incredibly beneficial to develop an index of ecosystem health derived using benthic macroinvertebrate data. Such an index would complement that currently in operation in this system based on fish community characteristics.

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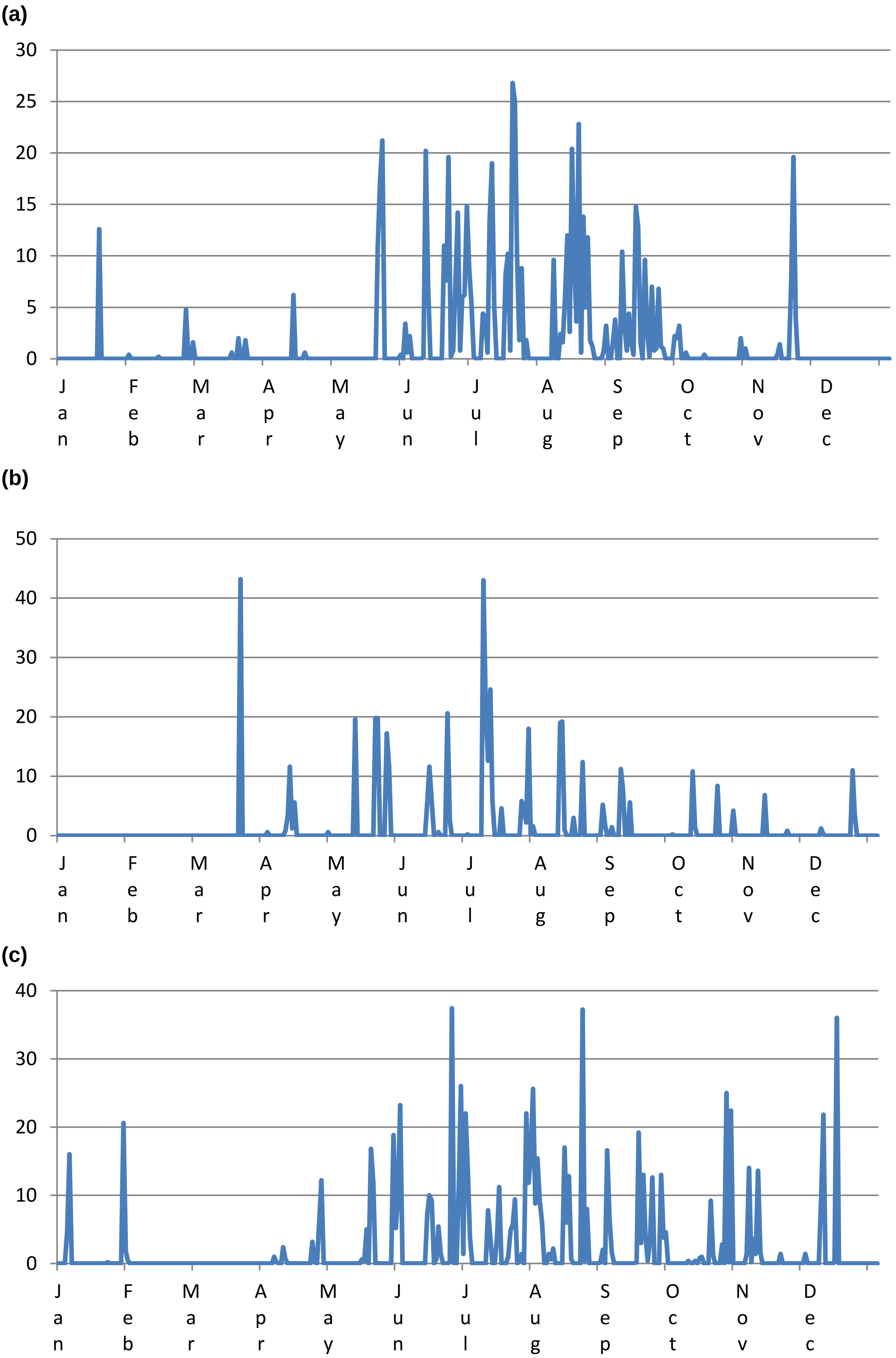
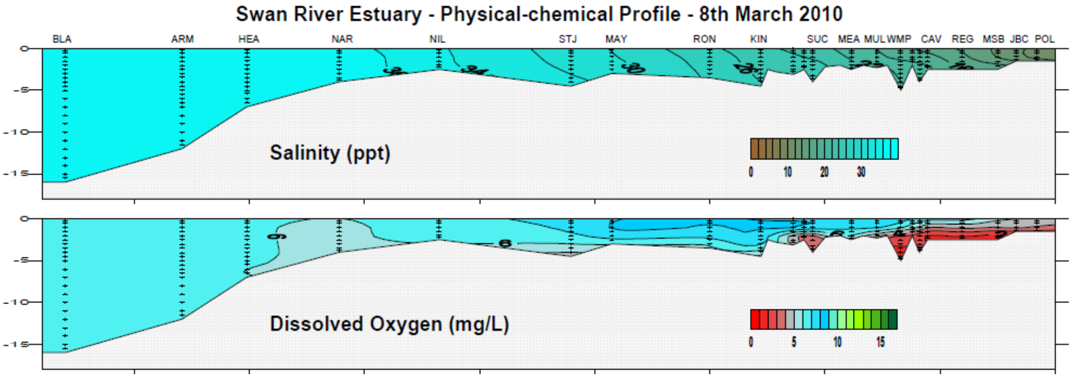
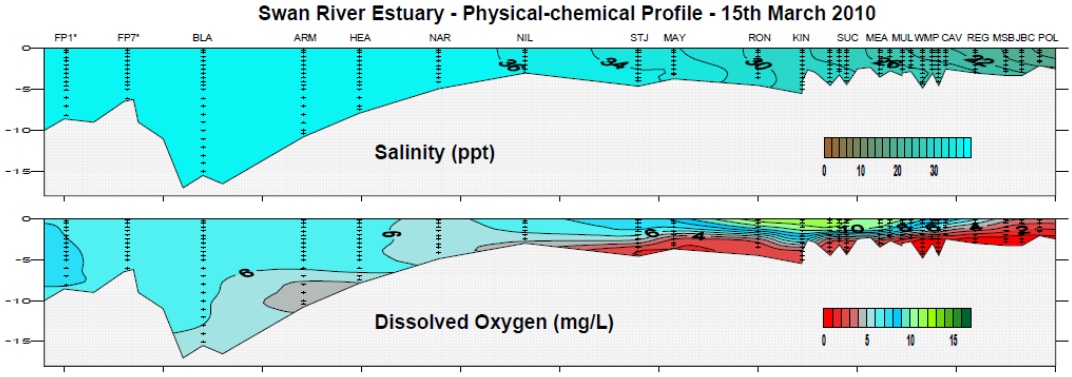
6. Appendices

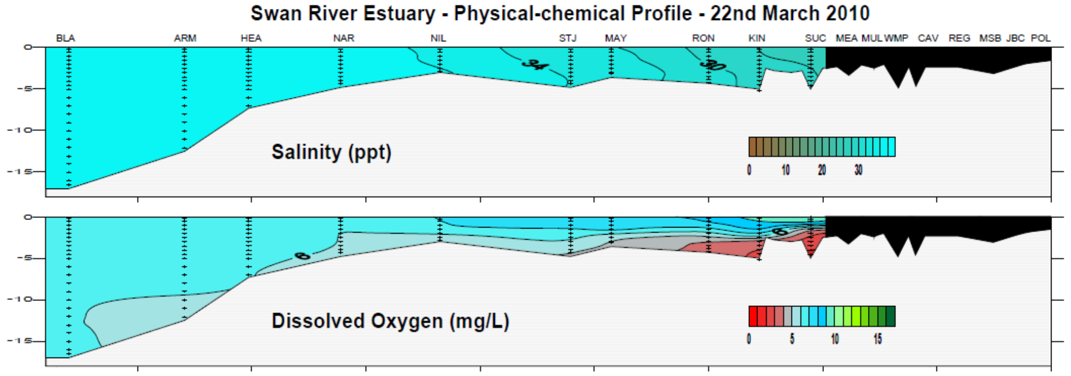
Appendix 1. Daily record for rainfall (mm) at Perth airport during (a) 2009, (b) 2010 and (c) 2011.

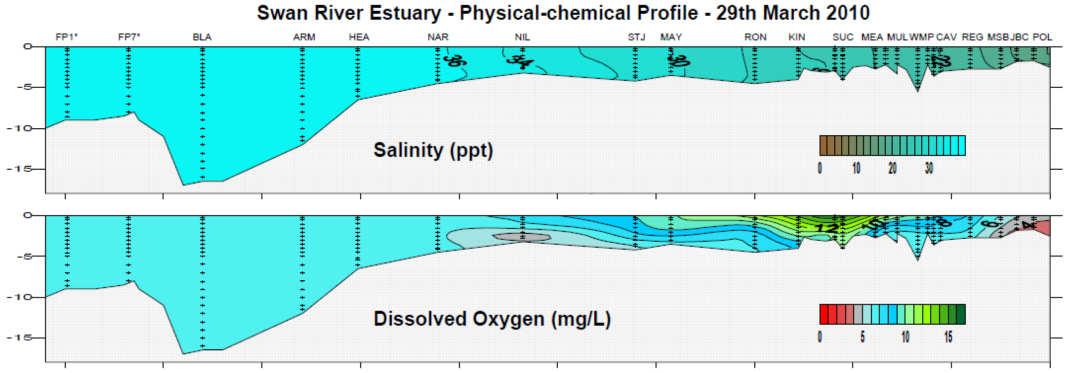


Appendix 2. Weekly vertical contour plots of salinity and dissolved oxygen concentrations measured at monitoring stations along the length of the Swan Estuary in March and April 2010, during which time a notable storm event occurred (22nd March 2010).



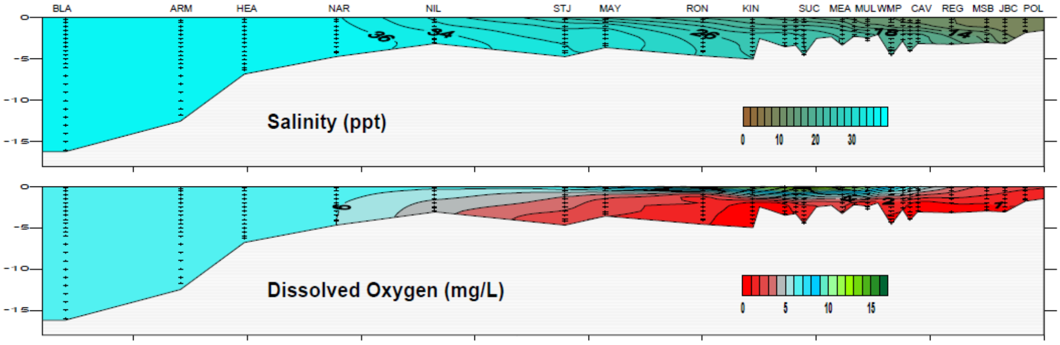




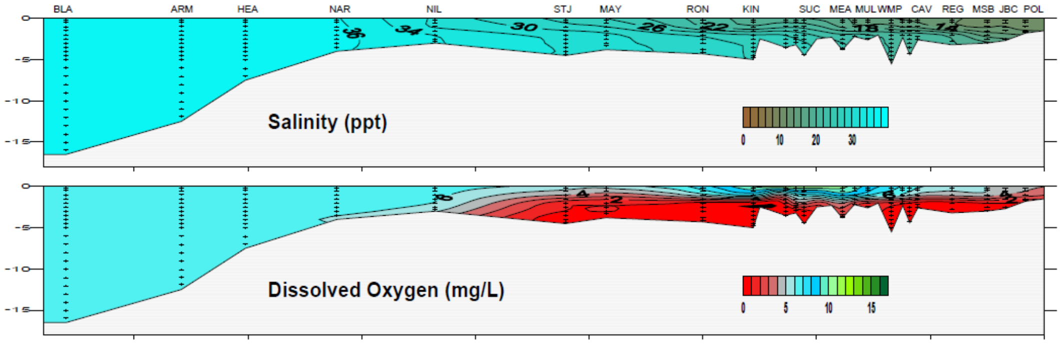


Appendix 2 (continued). Weekly vertical contour plots of salinity and dissolved oxygen concentrations measured at monitoring stations along the length of the Swan Estuary in March and April 2010, during which time a notable storm event occurred (22nd March 2010).

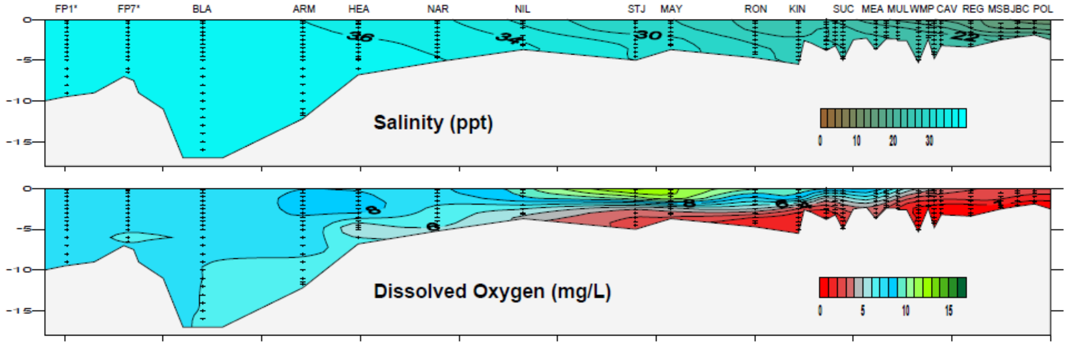
Swan River Estuary - Physical-chemical Profile - 6th April 2010



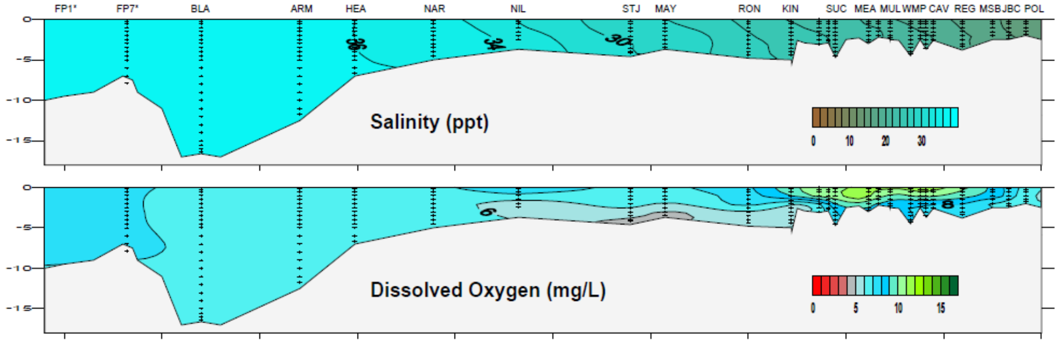
Swan River Estuary - Physical-chemical Profile - 12th April 2010



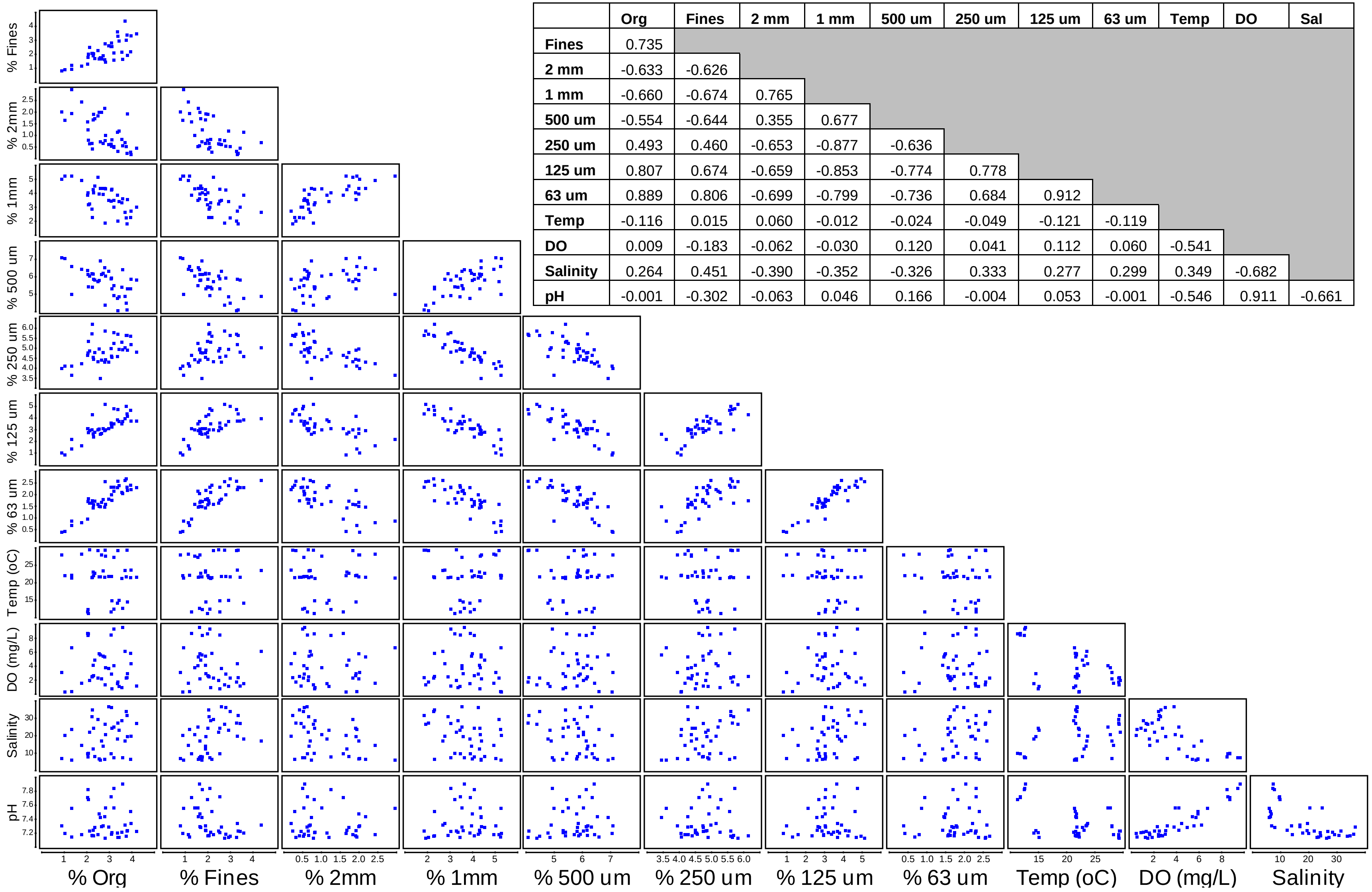
Swan River Estuary - Physical-chemical Profile - 19th April 2010



Swan River Estuary - Physical-chemical Profile - 27th April 2010



Appendix 3. Draftsman plot (*i.e.* pairwise scatter plots between all water quality and sediment variables) of the various environmental variables to be employed in the BIOENV analyses. The Pearson correlation coefficients between each pair of variables are also provided.



	Org	Fines	2 mm	1 mm	500 um	250 um	125 um	63 um	Temp	DO	Sal
Fines	0.735										
2 mm	-0.633	-0.626									
1 mm	-0.660	-0.674	0.765								
500 um	-0.554	-0.644	0.355	0.677							
250 um	0.493	0.460	-0.653	-0.877	-0.636						
125 um	0.807	0.674	-0.659	-0.853	-0.774	0.778					
63 um	0.889	0.806	-0.699	-0.799	-0.736	0.684	0.912				
Temp	-0.116	0.015	0.060	-0.012	-0.024	-0.049	-0.121	-0.119			
DO	0.009	-0.183	-0.062	-0.030	0.120	0.041	0.112	0.060	-0.541		
Salinity	0.264	0.451	-0.390	-0.352	-0.326	0.333	0.277	0.299	0.349	-0.682	
pH	-0.001	-0.302	-0.063	0.046	0.166	-0.004	0.053	-0.001	-0.546	0.911	-0.661

Appendix 4. Weekly vertical contour plots of salinity and dissolved oxygen concentrations measured at monitoring stations along the length of the Swan Estuary, for the period immediately preceding the commencement of sampling under the current study.