The hypoxia that developed in a microtidal estuary following an extreme storm produced dramatic changes in the benthos

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Abstract.   Runoff from an extreme storm on 22 March 2010 led, during the next 3 months, to the formation of a pronounced halocline and underlying hypoxia in the upper reaches of the microtidal Swan–Canning Estuary. Benthic macroinvertebrates were sampled between January 2010 and October 2011 at five sites along 10 km of this region. By mid-April, the number of species, total density, Simpson's evenness index and taxonomic distinctness had declined markedly, crustaceans had disappeared and the densities of annelids and molluscs had declined slightly. These faunal attributes (except Simpson's index) and species composition did not recover until after the end of the hypoxia. The survival of annelids and loss of crustaceans in this period reflects different sensitivities of these taxa to severe environmental stress. The results emphasise that microtidal estuaries with long residence times are highly vulnerable to the effects of environmental perturbations, particularly during warmer periods of the year.

Additional keywords: crustaceans, estuarine, hydrology, invertebrates, tides.

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Introduction

Faunas in macrotidal estuaries (i.e. tidal range >4 m) are subjected to the effects produced by a strong input and output of water during each tidal cycle. These effects include marked diurnal changes in salinity, frequent cycles of immersion and emersion, strong tidal scour and high turbidity (McLusky and Elliott 2004; Savenije 2005; Uncles *et al.* 2006). In contrast, as tidal water movements in microtidal estuaries (i.e. tidal range <2 m) are far more restricted, the changes in salinity during a tidal cycle and amount of tidal scouring are far smaller and the residence time much greater, except during periods of heavy freshwater discharge (Ranasinghe and Pattiaratchi 1998; Wolanski 2007). Indeed, environmental conditions in these microtidal estuaries, which are found on every continent (Whitfield and Elliott 2011), are often regarded as being fairly stable when freshwater discharge is limited (Potter *et al.* 2015). For example, water movement is minimal in the permanently open estuaries of south-western Australia during the warm summer months. This is due to rainfall being typically low and, as during the rest of the year, tidal exchange with the ocean being limited. As a result, salinities and temperatures remain high and stable for long periods. These conditions are typical of other microtidal estuaries, which are prevalent in regions with Mediterranean climates. The absence of pronounced dramatic

tidal water movements and freshwater input leads, in turn, to increases in water clarity and the production of phytoplankton and the growth of seagrasses and macroalgae (Monbet 1992; Heip *et al.* 1995). This provides an ideal environment for the successful spawning and recruitment of biota during that period of the year (Glasby 1986; Potter *et al.* 1986*a*, 1986*b*, 2015).

The environment in microtidal estuaries can also be stressful at certain times of the year, for example, in south-western Australia, where rainfall is largely confined to the cooler months of the year, i.e. May to September (Hodgkin and Hesp 1998). Heavy precipitation in those months leads to a great increase in freshwater discharge and, thus, to scouring and a substantial decline in salinity (Spencer 1956; Stephens and Imberger 1996). It can also lead to the formation of a pronounced halocline, which, if particularly intense or persistent, can result in hypoxia in the deeper waters, i.e. >2 m (Douglas *et al.* 1997; Kurup and Hamilton 2002).

As microtidal estuaries in regions with a Mediterranean climate are not well flushed in the warm dry period and the residence time is thus long (Ranasinghe and Pattiaratchi 1998; Kalnejais *et al.* 1999; Uncles *et al.* 2002), they are also particularly susceptible to accumulating nutrients and thus to the proliferation of algal blooms, especially in areas where there is substantial runoff from surrounding agricultural land and

urban developments (Eyre 1998; Paerl *et al.* 1998; Buzzelli *et al.* 2002; Eldridge and Roelke 2011). Stratification of the water column caused by, for example, heavy freshwater discharge following very substantial rainfall, and the presence of an abundant source of nutrients below the halocline during periods of high water temperature generates a high biological oxygen demand. This can lead rapidly to the development of hypoxic conditions in deeper waters and, thereby, to massive mortalities of fishes (Whitfield 1995; Douglas *et al.* 1997; Paerl *et al.* 1998).

On 22 March 2010, an extreme storm passed over the Swan–Canning Estuary, which flows through the city of Perth in south-western Australia. The massive damage caused by hail, strong winds and heavy rain, with 44.2 mm falling in half an hour, which was the first rainfall in Perth for 144 days since the 3.9 mm on 20 November 2009 (Bureau of Meteorology 2010), made this the costliest natural disaster in Western Australian history, with an estimated damage bill of AU$1.05 billion (Buckley *et al.* 2010; Insurance Council of Australia 2014). The resultant inflows would inevitably have delivered additional inorganic nutrients, sediments and dissolved organic carbon to the Swan–Canning Estuary from the surrounding catchment. As shown in the results, it also led to the formation of a pronounced and enduring halocline, resulting in widespread and persistent deoxygenation of the bottom waters of the estuary.

The characteristics of the benthic macroinvertebrate fauna of a diverse range of aquatic environments have been used as indicators of the health of these systems (e.g. Reynoldson and Metcalfe-Smith 1992; Weisberg *et al.* 1997; Borja *et al.* 2009; Tweedley *et al.* 2015) and can thus, potentially, be used to highlight the ecological effects of perturbations, such as those described above. These indicators include the ATZI marine biotic index (AMBI), a range of species richness and evenness measures, including taxonomic distinctness measures, and the proportions of higher taxa (e.g. Warwick and Clarke 1993, 1995; Borja *et al.* 2000; Wildsmith *et al.* 2009). However, a recent study has shown that several of these indices are not useful for comparing the health of estuaries in south-western Australia (Tweedley *et al.* 2014). The latter authors concluded that AMBI, for example, was not useful in this regard, because the extremely long residence time of these microtidal estuaries in the extended dry periods, even in pristine estuaries, leads to the accumulation of nutrients and thus a high biological oxygen demand. Consequently, the resultant environment favours taxa that can tolerate naturally stressful conditions and have been assigned high AMBI scores (Tweedley *et al.* 2014).

However, two indices were found to be particularly reliable for comparing the health of microtidal estuaries in south-western Australia known to vary markedly in their levels of environmental degradation (Wildsmith *et al.* 2009, 2011; Tweedley *et al.* 2012, 2014). The first of these was the relative proportions of crustaceans, a group particularly sensitive to perturbation, and of annelids, which are far less sensitive to such changes (Reise 1982; Warwick and Clarke 1993; Dauvin and Ruellet 2007). The second was the extent of the taxonomic spread of species across higher taxa, measured as average taxonomic distinctness (e.g. Warwick and Clarke 1998, 2001; Leonard *et al.* 2006). The above changes in univariate indices were also inevitably exhibited by the results of multivariate

analyses of faunal composition (e.g. Wildsmith *et al.* 2009; Tweedley *et al.* 2012). In addition to inter-estuary comparisons, the proportions of higher taxa, taxonomic distinctness and faunal composition have shown intra-estuary variations between periods in those south-western Australian estuaries that have undergone substantial environmental changes over the past 30 years (Wildsmith *et al.* 2009, 2011).

Because the Swan–Canning Estuary has been subjected to the detrimental effects of periodic algal blooms and hypoxic episodes over several years, a study was initiated in January 2010, aiming at determining how the characteristics of the benthic macroinvertebrate fauna of this system change in response to such effects. The timing of this project was particularly fortuitous because it commenced just before the extreme storm, which occurred in March of that year, and extended into the following months when the fauna would be likely to recover. The aim of the present paper was to test, in particular, the hypothesis that the hypoxia produced as a result of the extreme storm led to a proportional decline in crustaceans and a proportional increase in annelids, a decline in taxonomic distinctness, a detectable shift in species composition from multivariate analyses, and a subsequent return towards the condition before the storm. Although microtidal estuaries occur throughout the world, they have been far less well studied than their macrotidal counterparts. Furthermore, because microtidal estuaries are inherently more susceptible to hypoxia than macrotidal estuaries (e.g. Hagy and Murrell 2007; Tweedley *et al.* 2014), the present case history can serve as an exemplar of the deleterious effects that can be produced in these types of systems when they become subjected to extreme climatic conditions.

Materials and methods

Sampling regime and processing

The Swan–Canning Estuary (32.055°S, 115.735°E), a permanently open, microtidal estuary in south-western Australia, is a drowned river valley system that is ∼50 km long and covers a surface area of ∼50 km². It comprises a narrow entrance channel, two central basins and the saline lower reaches of its two main tributaries, the Swan and Canning Rivers (Hodgkin and Hesp 1998; Brearley 2005; Fig. 1). The salinity in the upper Swan Estuary undergoes pronounced seasonal changes each year as a result of the highly seasonal rainfall (Hoeksema and Potter 2006).

Benthic macroinvertebrates were sampled at five sites situated at intervals along a 10-km stretch of the upper Swan Estuary in each month between January and July 2010 and, thereafter, seasonally, in the Austral spring (October) of 2010 and in the summer (January), autumn (April), winter (July) and spring (October) of 2011 (Fig. 1). Five randomly located samples of sediment were collected from the deeper (∼1–4 m) waters at each site on each sampling occasion, using an Ekman grab (Wildco, Yulee, FL, USA) that collected substrata from an area of 225 cm² and to a depth of 15 cm. The sediment samples were preserved in 5% formalin buffered in estuary water and subsequently wet sieved 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).
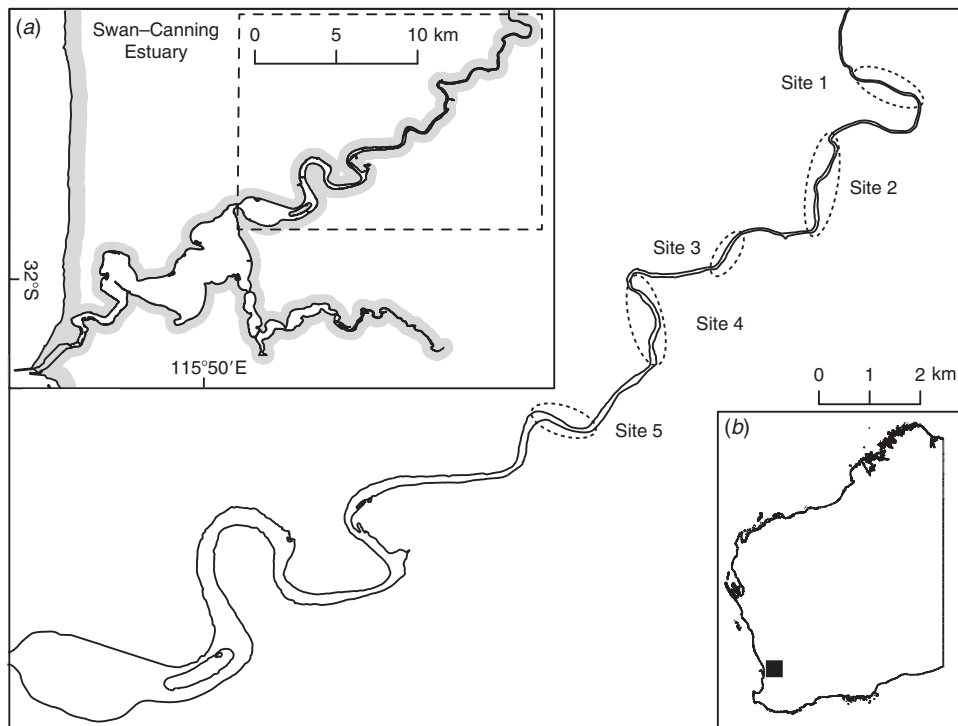
Fig. 1. Map showing the five sampling sites (dashed ellipses) in the upper Swan Estuary. Insets show (*a*) the study region within the Swan–Canning Estuary and (*b*) in Western Australia (solid square).

The abundance of each macroinvertebrate taxon in each replicate sample was converted to a density, i.e. number of individuals per 225 cm^2.

Salinity, water temperature (°C) and concentration of dissolved oxygen (mg L^{-1}) have been routinely recorded throughout the water column at regular intervals throughout the Swan–Canning Estuary since 1994 (Department of Water, unpubl. data) and, thus, encompassing the sites where benthic macroinvertebrates were sampled. These measurements were used to characterise the spatial and temporal extents of the hypoxia that followed the extreme storm.

Seasonal analyses of sediment from the five sites sampled during the 2 years of the study demonstrated that grain size decreased in a downstream direction, whereas the percentage contribution of particulate organic material increased (J. R. Tweedley, unpubl. data).

Statistical analyses

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), except for the shade plot, which was generated using an α test version of PRIMER v7.

A data matrix, constructed using the mean abundance of each species in the five replicate grabs at each site on each sampling occasion, was subjected to the DIVERSE routine to calculate reliable estimates of the following univariate measures of abundance and diversity: number of species, total density (individuals per 225 cm^2) and Simpson's evenness index (1–λ; Simpson 1949; Somerfield *et al.* 2008). The densities of

annelids, crustaceans and molluscs, and the percentage contribution of the density of annelids (a) to that of the collective annelid and crustacean (c) fauna, i.e. 100a/(a + c), were also calculated.

The above univariate measures were separately subjected to univariate permutational ANOVA (by entering Euclidean distances between each pair of values into the PERMANOVA routine). This was a two-way crossed ANOVA, to determine whether each variable was related to site (5 levels) or sampling occasion (12 levels, corresponding to the seven monthly sampling occasions from January to July 2010 and the five subsequent seasonal sampling occasions between October 2010 and October 2011). As noted above, robust univariate measures at each sampling site were obtained by, first, averaging the data matrix over replicate grabs. The occasion × site interaction term was therefore employed as the ANOVA residual, thus providing a sound and conservative basis for assessing the statistical significance of the main effects of site and sampling occasion. The null hypothesis that there were no significant faunal differences among sites or sampling occasions was rejected if the significance level (*P*) was <5%. Prior to undertaking these analyses, 'draftsman plots' of the values for each pair of univariate measures at each site were examined visually to assess whether the values for each variable were heavily skewed and, if so, which type of transformation would ameliorate that effect. These plots demonstrated that total density required a log-transformation and that the remaining variables did not require transformation.

Average taxonomic distinctness (Δ^+) was used to explore the extent to which the taxonomic spread of the benthic

macroinvertebrate fauna changed from before to after the extreme storm. Δ^+ is a measure of the average spread of species across higher taxa, with a low Δ^+ indicating that the faunal assemblage is likely to have been impoverished (Warwick and Clarke 1995, 2001; Clarke and Warwick 2001). A matrix was constructed from the presence or absence of each species on each sampling occasion, averaged over all sites. This matrix was then subjected to TAXDTEST to determine the 'expected' value and 95% probability limits for Δ^+ in random subsamples of different numbers of species drawn from the full suite of 48 species (comprising 16 annelids, 9 molluscs and 10 crustaceans) collected during the study. These data were used to construct funnel plots onto which, for comparative purposes, the measured values of Δ^+ for each sampling occasion were superimposed and to test for any significant departures from expectation (Warwick and Clarke 2001). The values for Δ^+ and number of species (*S*) for each sampling occasion were also subjected to linear regression in Minitab v16 (Minitab Pty Ltd, State College, PA, USA), to determine whether there was a significant relationship between the two variables. As theory demonstrates that Δ^+ is independent of *S* (Clarke and Warwick 1998), an observed decline in Δ^+ and *S* together would indicate that species are not lost across all taxa equally, but differentially from particular higher taxonomic groups.

For the multivariate analyses of faunal composition, the numbers of the various benthic macroinvertebrate species in each replicate sample from the five separate sites on each sampling occasion were converted to presence (1) or absence (0) data. This transformation was chosen because it maximised the information on any turnover of species during the study period, rather than on changes in the numerical abundance of each species. This is because many of the most abundant species remained in relatively large numbers throughout the time course at all sites and, unless heavily transformed, the interesting and important changes to less abundant species would be obscured by the influence of these large abundances (Clarke *et al.* 2014). 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, except that, because robust replicate data were available, the occasion × site interaction term was calculated and not used as the residual. A two-way ANOSIM test was then employed, using the above Bray–Curtis resemblance matrix, to assess the relative influence of both factors, i.e. sampling occasion and site.

So as to visualise the time-course of any changes in community composition and to assess which species may have declined in response to the hypoxia, 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 replicates is maintained, whereas the abundances of each species are not influential. When sufficient and consistently collected replicates are available, the use of frequency data in this way is often a powerful and informative option for multivariate analysis in the presence of erratic fluctuations in abundance. Because the above PERMANOVA analysis indicated that there was an interaction between sampling occasion and site, further analyses were conducted on various subsets of the above matrix corresponding to a particular site.

Although an *a priori* hypothesis was able to be constructed, stating that the benthic macroinvertebrate fauna in the upper Swan Estuary would have changed as a result of the hypoxia in March 2010, it was not possible to predict when the hypothesised change in faunal composition would end and the fauna would thus have recovered to the pre-hypoxia state. Therefore, to identify those sampling occasions, at each site, on which benthic macroinvertebrate faunal composition did not differ significantly, the above frequency matrix was separated for each site and, in turn, subjected to hierarchical agglomerative clustering with group-average linking (CLUSTER) and associated similarity profile (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. Thus, this routine 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 were no significant faunal differences among sampling occasions was rejected if the significance level (*P*) associated with the test statistic (π) was <5%.

Finally, a shade plot was produced to visualise the changes in the frequency of occurrence of individual species over time at each site. The shade plot is a simple visualisation of the frequency matrix, where white space and black rectangles show whether a species was respectively absent or present in all replicates from that site on that sampling occasion, with the depth of the grey-scale rectangles for a species representing, on a linear scale, its presence in 20, 40, 60 and 80% of the replicates at that site on that sampling occasion (Clarke *et al.* 2014; Valesini *et al.* 2014).

Results

Salinities and concentrations of dissolved oxygen

Salinities within the sampling region on 22 February 2010, and thus before the extreme storm, increased progressively down the estuary, from 11 at Site 1 to 26 at Site 5, with the stratification being weak at all sites (Fig. 2*a*). In contrast, salinities at the top and bottom of the water column in April (soon after the storm) differed markedly and the water column remained highly stratified in May and July 2010 (Figs 2*b–d*). By October 2010, the water column had returned to a weakly stratified state (Fig. 2*e*).

On 22 February 2010, before the storm, the concentrations of dissolved oxygen at all sites were ≥ 5 mg L^{-1} throughout the water column, except in the troughs that characterise the topography of the substrate in the sampling region, where they were ~ 4 mg L^{-1} (Fig. 2*f*). In marked contrast, concentrations of dissolved oxygen in the bottom half of the water column at all sites (and also further upstream and downstream) in April, May and July 2010 were ≤ 2 mg L^{-1} (Fig. 2*g–i*). Indeed, concentrations of dissolved oxygen as low as 0.2–0.3 were frequently recorded in the troughs at all sites in April. By October, concentrations of dissolved oxygen at all sampling sites were ≥ 5 mg L^{-1} and low concentrations of oxygen were confined to the region immediately downstream of the sampling area (Fig. 2*j*).

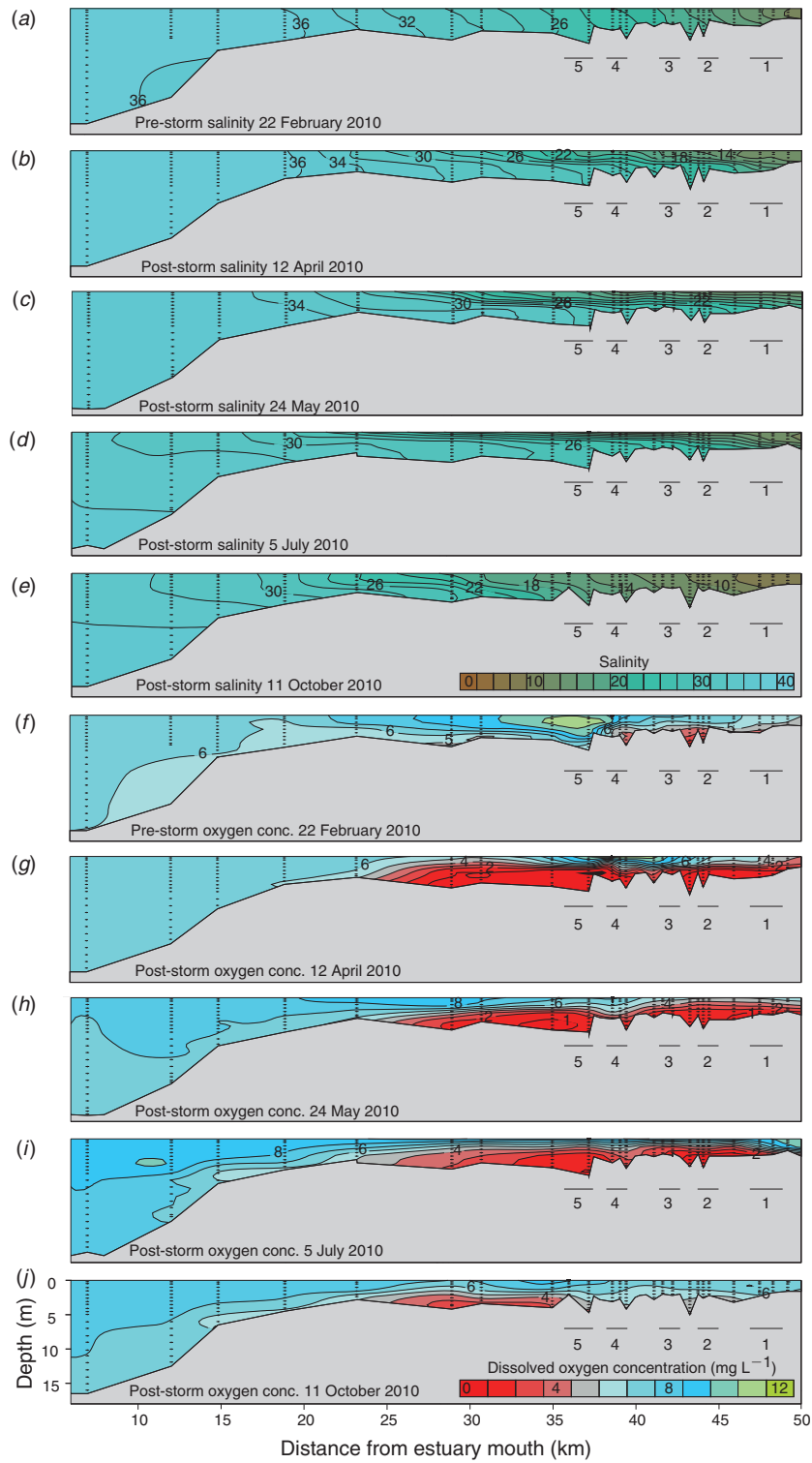**Fig. 2.** (*a–e*) Salinities and (*f–j*) concentrations of dissolved oxygen (mg L^{-1}) throughout the water column of the Swan Estuary, including at the five sampling sites (1–5). + denotes points in the water column where salinity and the concentration of dissolved oxygen were measured.

A comparison of the sediment at the five sites in January and April 2010, i.e. before and after the extreme storm, demonstrated that the percentage contribution of particulate organic matter to the sediment and the sediment grain-size composition at the corresponding sites did not differ between these two months ($P \geq 5\%$).

Abundance and diversity measures

Two-way univariate PERMANOVA demonstrated that the number of species, mean total density (individuals per 225 cm^2) and Simpson's evenness index differed significantly among sampling occasions and the same was true for the number of species and Simpson's index among sites (Table 1). At all sites, the number of species declined between March 2010, before the extreme storm, and April 2010, soon after the storm, to fewer than eight species and, at all but one site (Site 1), remained low until June 2010 and then increased in the ensuing months, before tending to level off (Fig. 3*a*). It should also be noted that, exceptionally, the number of species at Site 1 was far greater than at any other site at the beginning of sampling and exhibited a particularly sharp peak in October 2010.

The total density of invertebrates declined markedly at most sites between March and April 2010 and, at all sites, was lower than ~200 individuals per 225 cm^2 in April and May 2010 and tended to increase gradually over the subsequent period (Fig. 3*b*). Although total density was far greater at Site 2 in January 2010 and at Site 4 in January 2011, these elevated values were entirely due to one of the five grabs from Sites 2 and 4 containing very large densities of the bivalves *Fluviolanatus subortus* and *Arthritica semen* respectively. The values for Simpson's evenness index declined at every site between March and April 2010 and then increased in the ensuing months until October 2010, after which the trend was not so consistent (Fig. 3*c*).

At the commencement of sampling in January 2010, the densities of crustaceans exhibited very low values at all sites except Site 1, where there were ~140 individuals per 225 cm^2 (Fig. 3*d*). Small numbers of crustaceans were collected from four of the five sites in February 2010 and at all sites in March 2010, whereas only a single individual crustacean was recorded in the total of 75 samples collected in April, May and June 2010. Crustaceans at all sites started to recover in July 2010 and were represented by appreciable, but fluctuating, densities at four of the sites on subsequent sampling occasions. However, the densities of crustaceans never exceeded five individuals per 225 cm^2 at Site 5. The densities of annelids varied markedly among sites in January to March 2010, but were lower than ~70 individuals per 225 cm^2 at all sites in April 2010 and typically ranged between 30 and 90 individuals per 225 cm^2 between May 2010 and April 2011 and rose to produce a pronounced peak in July 2011 (Fig. 3*f*). The densities of molluscs typically declined between March and April 2010 and were very low (i.e. <60 individuals per 225 cm^2) until July 2010, after which they showed variable tendencies to increase (Fig. 3*e*).

Although the contribution of annelids to the collective annelid and crustacean fauna varied markedly at the different sites before the extreme storm, they contributed ~94–100% at all sites in the following 3 months between April and June 2010 (Fig. 3*g*). The proportion of annelids declined precipitously to

Table 1. Significance levels for two-way crossed ANOVA employing Euclidean distance matrices constructed from the values for a suite of abundance and diversity measures derived from the average faunal composition at each site on each sampling occasion, tested by permutation

n.s., not significant

Abundance and diversity measure	Occasion (%)	Site (%)
Number of species	<1	<1
Total density (per 225 cm^2)	<5	n.s.
Simpson's evenness index	<5	<1
Density of annelids (per 225 cm^2)	<5	n.s.
Density of molluscs (per 225 cm^2)	<5	n.s.
Density of crustaceans (per 225 cm^2)	<1	<1
Contribution of annelids (%)	<1	<1

far lower levels in October 2010 and January 2011 and then increased to variable extents.

The funnel plot shown in Fig. 4 demonstrates that the values for Δ^+ and number of species were lowest immediately after the storm in April, May and June 2010, but remained within the 95% probability limits. Collectively, however, linear regression of the values for Δ^+ against the number of species indicated that there was a significant and relatively strong relationship between the two variables (Fig. 4), with a decline in the number of species being mirrored by a reduction in Δ^+.

Composition of the benthic macroinvertebrate community

Two-way crossed multivariate PERMANOVA demonstrated that the compositions of the benthic macroinvertebrate communities throughout the upper Swan Estuary in 2010 and 2011 differed significantly among occasions and sites and that the interaction between these two effects was significant ($P = 0.1\%$; Table 2). Two-way crossed ANOSIM indicated that the changes in faunal composition were related more to sampling occasion ($\overline{R} = 0.494$, $P = 0.1\%$) than to site ($\overline{R} = 0.375$; $P = 0.1\%$).

CLUSTER–SIMPROF demonstrated that, on the basis of their species compositions, the samples from Sites 1, 2 and 3 in April, May and June 2010 formed groups which, together with those from March 2010 at Site 1, from July 2010 at Site 2 and from January and July 2010 at Site 3, were significantly different from those on all other occasions (Fig. 5). Other samples from Sites 1, 2 and 3, except for those from Site 1 in April 2011 and from Site 2 in January 2010, formed a second statistically discrete group. The samples from Site 4 in April, May and June 2010 also formed a group that was statistically discrete from those on all other sampling occasions (Fig. 5). The compositions of the samples collected at Site 5 on the various occasions were not significantly different.

The shade plot shown in Fig. 6, representing the frequency of occurrence of species, demonstrates very clearly that nine of the 10 crustacean species recorded in this study were not found in samples from the five sites in the three months following the extreme storm. Indeed, in this period, crustaceans were represented in total only by a single *Paracorophium excavatum*. However, amphipods such as *P. excavatum* and *Grandidierella propodentata* were frequently found in the three most upstream sites before the storm in late March 2010 and at all sites on
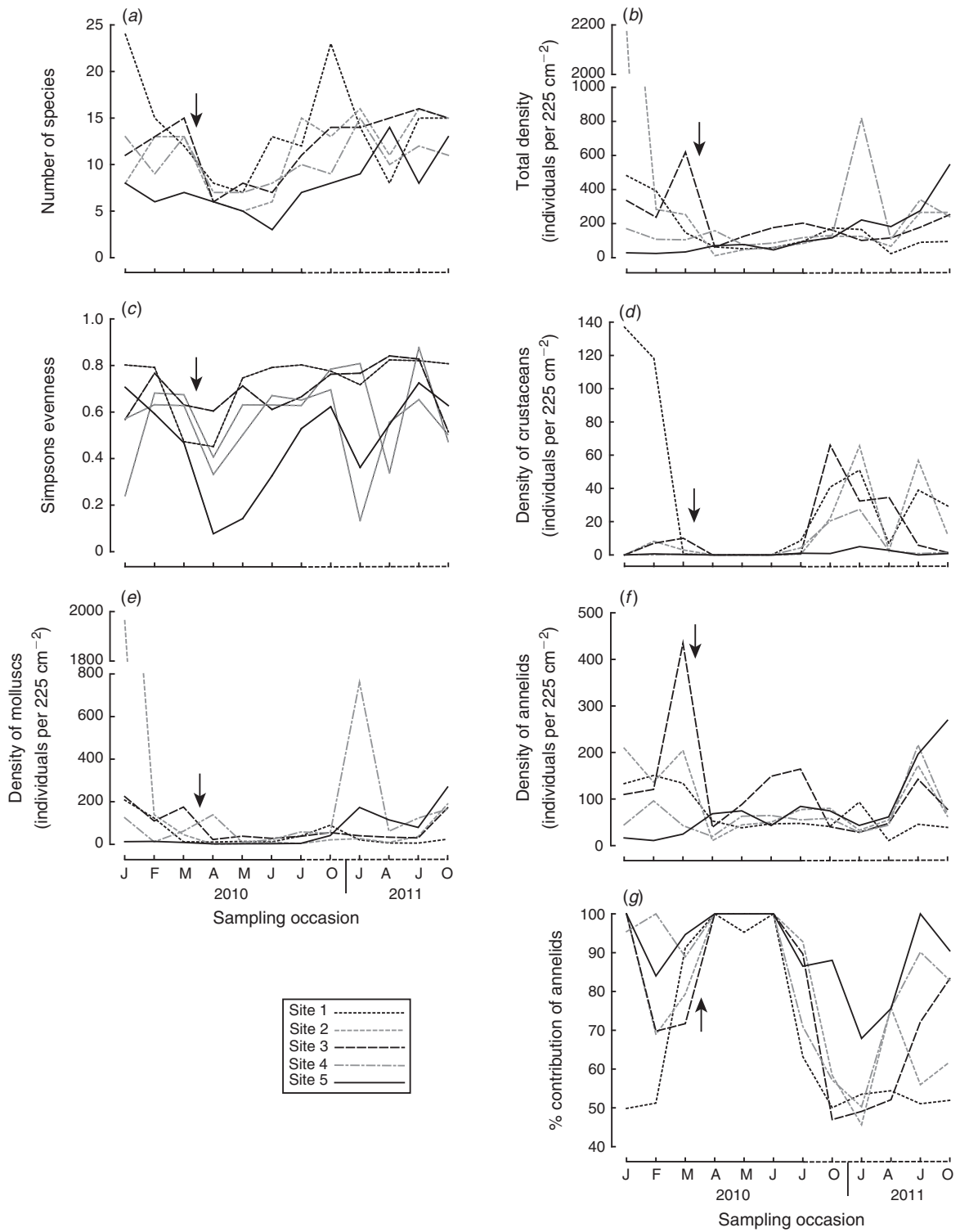
Fig. 3. Line graphs showing data on benthic macroinvertebrates on the different sampling occasions at the five sampling sites. (*a*) Number of species, (*b*) total density, (*c*) Simpson's evenness index, (*d*) density of crustaceans, (*e*) density of molluscs, (*f*) density of annelids and (*g*) percentage contribution of annelids to the collective annelid and crustacean fauna. Arrow indicates the date of the storm. The solid line on the *x*-axis denotes monthly sampling, and the dashed line denotes seasonal sampling.
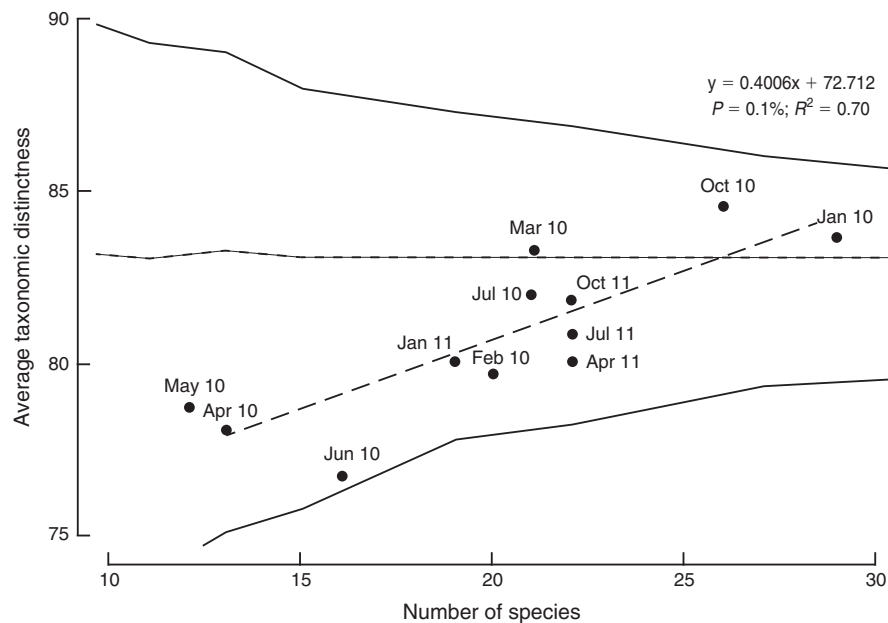
Fig. 4. Funnel plot showing the 'expected' mean (horizontal dashed line) for average taxonomic distinctness (Δ^+) derived from data for random subsamples from the suite of 48 benthic macroinvertebrate species recorded on the 12 sampling occasions in the upper Swan Estuary, together with their upper and lower 95% probability limits (solid lines). The Δ^+ values, based on the species present across all sites, have been plotted against the number of species recorded on each sampling occasion. The linear regression of the relationship between Δ^+ and the number of species is also shown (diagonal dashed line).

Table 2. 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 site on each sampling occasion between January 2010 and October 2011

d.f., degrees of freedom. Significant results are highlighted in bold

Parameter	d.f.	MS	COV	*P*
Main effects				
Occasion	11	**12378**	**466**	**0.1%**
Site	4	**20816**	**335**	**0.1%**
Interactions				
Occasion × site	44	**1683**	**193**	**0.1%**
Residual	240	719	719	

almost all sampling occasions after July 2010. Among the polychaetes, *Prionospio* sp. and *Desdemona ornata* were frequently recorded throughout the sampling period and even tended to increase in the 3 months following the storm. Conversely, the frequency of occurrence of *Simplisetia aequisetis*, *Capitella capitata* (species complex), *Leitoscoloplos normalis* and *Marphysa* sp. declined during those same 3 months, but did subsequently increase (Fig. 6). Although *Arthritica semen* was present in samples from each site on each sampling occasion, there were indications that the frequency of occurrence of this bivalve mollusc did decline at three of the sites between April and June 2010. The frequency of occurrence of *Fluviolanatus subortus* at the three upper most sites declined between April

and June 2010, noting that it also declined in a downstream direction (see Discussion).

Discussion

The establishment in January 2010 of a regular sampling regime for the benthic macroinvertebrate fauna of the upper Swan Estuary provided a unique opportunity to track the changes in the characteristics of that component of the benthic fauna from before, during and then after the extreme storm. The severity of the resultant marked and protracted period of hypoxia can be gauged by the fact that, by 3 weeks after the storm, the concentrations of dissolved oxygen in the bottom waters were frequently ≤ 2 mg L^{-1} and remained so for 3 months. That oxygen concentration is the cut-off point often designated for hypoxia (Rosenberg 1980; Diaz and Rosenberg 1995). The severity of the effects of this hypoxia on the fauna is reflected, in particular, by very pronounced changes in species richness, the relative proportions of crustaceans and annelids, the values for taxonomic distinctness and the composition of the fauna. The reduction in the number of species was due, in particular, to a loss of the crustaceans *P. excavatum*, *G. propodentata* and *Melita matilda*, the molluscs *F. subortus* and *Arcuatula senhausia* and the polychaetes *C. capitata* (species complex) and *L. normalis*, but noting that the polychaetes *Prionspio* sp. and *D. ornata* and the mollusc *A. semen* survived throughout this period.

Development of hypoxia

The increase in freshwater discharge that followed the storm on 22 March 2010 led to pronounced stratification of the water
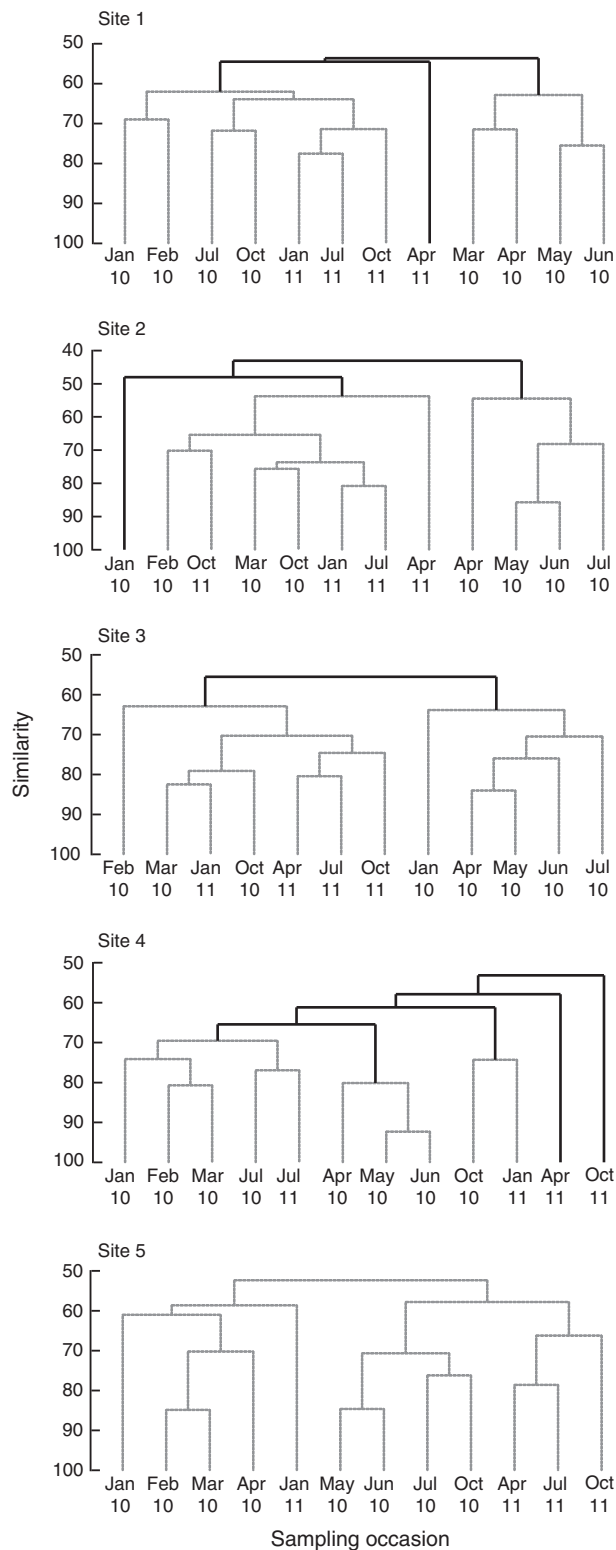
Fig. 5. Dendrograms derived by subjecting to the CLUSTER and SIM-PROF utilising Bray–Curtis similarities, the frequency of occurrence of each species across the replicate samples collected from each site on each sampling occasion between January 2010 and October 2011. The groups in which the species compositions are significantly different from those in all other groups are denoted by thick black lines.

column in the upper Swan Estuary. The production of a very conspicuous halocline meant that there would have been restricted vertical mixing within the water column and, thus, replenishment of oxygen in the bottom waters. Moreover, this occurred at a time when water temperatures were near their annual maxima (Kurup and Hamilton 2002; Hoeksema and Potter 2006) and biological oxygen demand would, thus, have been very high (Price and Warwick 1980; Lin *et al.* 2006). The halocline in the narrow upper reaches of the Swan Estuary (typically <50 m in width) was maintained for 3 months as a result of continuing freshwater input resulting from the storm and subsequent periodic rainfall over the large catchment, i.e. 121 000 km[2] (see Bureau of Meteorology, Climate and past weather, http://www.bom.gov.au/climate/, accessed 30 October 2014). In this context, it is relevant that the extent of hypoxia was greater in 2010 than in the preceding years (Cottingham *et al.* 2014).

Any consideration of hypoxic conditions in the upper Swan Estuary should take into account the very protracted residence time in microtidal estuaries (Wolanski 2007), which, during the summer of 1996 was estimated as lasting for 235 days in the upper Swan Estuary (Kalnejais *et al.* 1999). Although the large amount of rainfall and thus of freshwater discharge in the summer of 2010 was atypical, the presence of very pronounced stratification implied that, although residence time became low in the surface waters, it remained high in the bottom part of the water column. The hydrological conditions were, therefore, not as extreme as can occur in winter, when the majority of rainfall occurs and can lead to large volumes of freshwater discharge, thus resulting in low salinities throughout the water column, and to a residence time of only a day (Kalnejais *et al.* 1999; Kanandjembo *et al.* 2001).

Abundance and diversity measures

The pronounced declines in the number of species and total density of benthic macroinvertebrates in the period following the extreme storm of 22 March 2010 parallel the changes that have been described for that type of fauna following the development of similar extreme expressions of hypoxia in estuaries, fjords and coastal environments (e.g. Josefson and Widbom 1988; Rakocinski *et al.* 1997; Gray *et al.* 2002; Karlson *et al.* 2002; Steckbauer *et al.* 2011). Those declines reflect reductions in the number of species and density, particularly of crustaceans and to a lesser extent of molluscs and polychaetes (see below). Although Simpson's evenness index also declined in April 2010, it recovered more rapidly than did the number of species, which is mainly due to a reduction in the dominance of certain polychaete species such as *S. aequisetis*, *C. capitata* (species complex) and *L. normalis*.

Crustaceans

The almost total absence of crustaceans among the 75 samples collected in April, May and June 2010 contrasts markedly with the substantial numbers of this group often recorded subsequently throughout the study area between July 2010 and October 2011. This is due to the frequency of occurrence of the dominant crustacean species, which are all amphipods (i.e. *P. excavatum*, *G. propodentata* and *Corophium minor*),
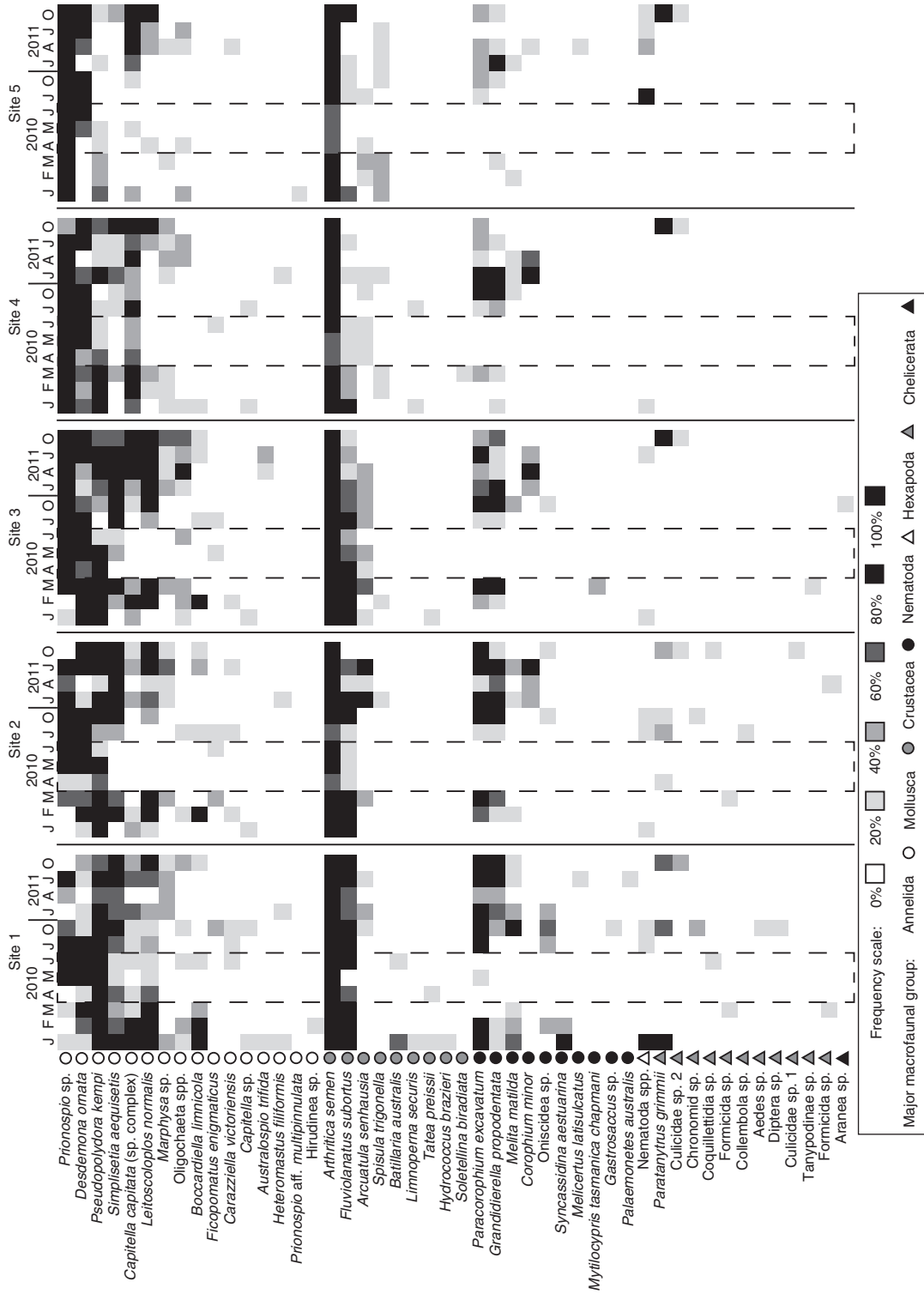
Fig. 6. Shade plot based on the frequency of occurrence of species across the five replicate samples from each site on each sampling occasion. Species are arranged by major macrofaunal group and ordered within this taxonomic rank by decreasing total frequency of occurrence across all samples. Dashed box denotes the 3 months after the storm.

each exhibiting that trend (Fig. 6). Although the densities of crustaceans also increased at Site 5 during the latter part of the study period, they were always low and generally far lower than at other sites and were very low before the storm. The shade plot demonstrated that the frequency of occurrence of the dominant crustacean species also declined gradually in a downstream direction from Site 1 to Site 5. It is, therefore, relevant that, in comparison with other sampling sites, the sediment at this latter site was thixotropic, comprised larger percentage contributions from Wentworth grain sizes <250 μm and contained the greatest amount of particulate organic matter (i.e. 12%; J. R. Tweedley, unpubl. data), conditions that would not have provided an environment conducive for habitation by the main species of crustacean found in the upper estuary (e.g. Teske and Wooldridge 2003).

Annelids

Although the density of annelids (predominantly polychaetes), which are generally considered to be the most tolerant to hypoxia of the major macrofaunal groups (Reise 1982; Warwick and Clarke 1993), did decline immediately after the storm and remained low at four of the five sites, the trend was not as strong as with crustaceans. The trends exhibited in the shade plot (Fig. 6) demonstrated that polychaete species, such as *Marphysa* sp., *Simplisetia aequisetis*, *L. normalis* and *Pseudopolydora kempi*, tended to occur less frequently in samples between April and July 2010 than in those before and after these months. In contrast, the spionid polychaete *Prionospio* sp., whose congenerics feed on detritus and are tolerant of polluted, eutrophic and other disturbed conditions (Pearson and Rosenberg 1978; Borja *et al.* 2000; Shen *et al.* 2010), became more frequently present in the samples collected from most sites between April and July 2010 than was the case during the previous 3 months. *Desdemona ornata*, a small sabellid polychaete, also occurred consistently between April and July. This species is morphologically similar to and lives in a habitat similar to that of *Manayunkia aestuarina*, which is found in fine organic sediments of northern hemisphere estuaries (Bell 1982; Bick 1996). Thus, among polychaetes in general, the frequency of occurrence of the largest species, i.e. *Marphysa* sp., *S. aequisetis* and *L. normalis*, clearly declined immediately after the storm, whereas the opposite trend was exhibited by smaller species such as *Prionospio* sp. and *D. ornata*.

Proportions of crustaceans and annelids

It is highly relevant that, in terms of abundance, the percentage contribution made by annelids to the collective annelid and crustacean fauna increased dramatically immediately following the storm, to the point where it was 100% at all sites and remained thus, or at very high levels, for the next 2 months, before declining precipitously. These clear trends support our hypothesis that extreme hypoxia in the upper Swan Estuary led to a pronounced decline in the proportion of crustaceans and to an overwhelming increase in the proportion of annelids. These trends parallel those described for hypoxia and other detrimental effects in other estuaries and coastal waters (e.g. Gray *et al.* 2002) and are consistent with other evidence that crustaceans are far more susceptible than annelids to deleterious environmental

changes (Vaquer-Sunyer and Duarte 2008; Steckbauer *et al.* 2011). Furthermore, the virtual absence of crustaceans in the 3 months following the storm undoubtedly made an important contribution to the reduction in taxonomic distinctness during this period and which is typical of stressful environmental conditions (Reise 1982; Warwick and Clarke 1993; Tweedley *et al.* 2012). Wildsmith *et al.* (2009) considered that a similar increase in the abundance of annelids relative to crustaceans provided evidence that the quality of the benthic environment of the large Peel–Harvey Estuary, just to the south of the Swan–Canning Estuary, had deteriorated between 1986–1987 and 2003–2004.

Molluscs

Although the densities of molluscs declined immediately after the storm and later showed a marked recovery, thereby paralleling the trends with crustaceans, the molluscs differed from the crustaceans in that they did not completely disappear in the months that immediately followed the storm. These comparisons suggest that, although molluscs are slightly more tolerant of hypoxia than are crustaceans (Vaquer-Sunyer and Duarte 2008), they still respond negatively to low oxygen concentrations. It is, thus, relevant that numerous bivalve species possess behavioural and physiological mechanisms that enable them to tolerate hypoxic conditions, for example, by closing their shells, reducing their burial depth, extending their siphons and decreasing their metabolism or feeding rates (Theede 1973; Jørgensen 1980; Brooks *et al.* 1991; Tamai 1993; Nilsson and Rosenberg 1994). Nevertheless, the trends in the shade plot (Fig. 6) showed that the bivalve *Fluviolanatus subortus* was generally found less frequently in samples taken during the hypoxic period, i.e. April to June, than in those from earlier or later periods. Similarly, another bivalve, *Arthritica semen*, was less frequently present in samples from Sites 3, 4 and 5 during the hypoxic period. Therefore, this suggests that, despite these potential tolerance mechanisms, anoxic conditions were sufficiently severe to affect these two dominant species. However, habitat 'preferences' can also influence the frequency of occurrence of certain mollusc species along the estuary. For example, the amount of hard substrata, such as wood and stones, to which the false mussel *F. subortus* attaches (J. R. Tweedley, unpubl. data), declined in a downstream direction within the sampling region and, thus, accounts for the frequency of occurrence of this species also declining along this axis. In contrast, the presence of extensive soft sediments along the sampling region explains why the small bivalve *A. semen*, which lives buried in such sediments (Wells 1984), occurs at high frequencies throughout the upper estuary (Kanandjembo *et al.* 2001) and was largely unaffected by the hypoxia.

Broader implications

The Swan–Canning Estuary periodically experiences hypoxic conditions, which tend to be most persistent and extensive in the bottom waters of the upper estuary (Douglas *et al.* 1997; Kurup and Hamilton 2002; Cottingham *et al.* 2014). Numerous authors have identified severe implications of the effects of hypoxia on the benthos for secondary production, higher trophic levels and even fisheries. Responses of benthic community to disturbances,

such as those described in the present study, are frequently likewise characterised by a shift towards small-bodied, surface deposit-feeding benthic macroinvertebrate taxa (Gaston *et al.* 1998). These changes in faunal composition may lead to decreased trophic diversity, inhibited benthic–pelagic flows of nutrients and energy, shortened food chains, altered sediment biogeochemistry and nutrient fluxes as a result of reduced bioturbation, and other impacts on ecosystem function and health (Rakocinski *et al.* 1997; Gaston *et al.* 1998; Middelburg and Levin 2009). As a result, sustained or regular hypoxia can cause dramatic declines in macrobenthic production (Sturdivant *et al.* 2014), causing potentially severe implications for higher-order consumers, including estuarine fishes and the fisheries that exploit them (Breitburg 2002).

In addition, many species of fish in estuaries, including those in south-western Australia, can respond to detrimental changes in a region of these systems by moving to sites not likewise affected. For example, increases in hypoxia in the deeper waters of the upper Swan Estuary over the past 20 years have led to the black bream, *Acanthropagrus butcheri*, tending to move from these waters to the better oxygenated near-shore, shallow areas (Cottingham *et al.* 2014). Similarly, when confronted with deoxygenation and other problems posed by massive seasonal growth of *Nodularia spumigena* in a region of the Peel–Harvey Estuary, immediately to the south of the Swan–Canning Estuary, mobile fish species moved to other regions of this large system where conditions were not suitable for the growth of this cyanobacterium (Potter *et al.* 1983). It is, therefore, assumed that species such as black bream would have moved either in to shallower waters or upstream and or downstream of the area in the Swan that was severely affected by hypoxia in 2010.

It is difficult to develop management schemes to counteract extreme and unpredictable climatic events, which are likely to become more prevalent (Easterling *et al.* 2000; Huntington 2006). As a result, it is essential that the on-going efforts to reduce the inputs of organic material and nutrients to the Swan–Canning Estuary are continued so that the severity and extent of the hypoxia affecting this system are minimised.

Conclusions

The dramatic changes in the characteristics of the benthic macroinvertebrate fauna of the upper Swan Estuary, which occurred in response to prolonged hypoxia, serve as a model for faunal responses to such events, which frequently occur in microtidal estuaries elsewhere in the world. This study also highlighted the vulnerability of systems with long residence times to the effects of environmental perturbations, whether from anthropogenic or natural causes. As the majority of microtidal estuaries occur in Mediterranean climates, the development of hypoxia and its effect on the fauna will be particularly pronounced during the warm summer months when the biological oxygen demand is greatest and the metabolic rates of the members of the fauna are high. The narrowness of the upper Swan Estuary and of many other microtidal estuaries clearly exacerbates the situation, a point emphasised by the fact that the runoff from the storm did not result in salinity stratification or hypoxia in the bottom waters in the wide basins further down the estuary. Such a storm would not

have led to such a severe effect in a macrotidal well flushed estuary with a short residence time.

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