Benthic Macroinvertebrate Response Monitoring in the Murray Mouth and Coorong, 2011/12

Final Report

for the Department of Environment and Natural Resources

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Justine Keuning, Eli Brown, Nat Navong, Kathleen Beyer, Stephanie Baggalley, Sabine Dittmann

Flinders University, School of Biological Sciences

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Contents

1. Executive Summary

- This report presents results from further monitoring of the response of macroinvertebrates to continued flow from barrages since spring 2010, which led to improved environmental conditions in the Murray Mouth and Coorong. The main objectives of this study were to monitor the macroinvertebrate communities, recruitment of individual macroinvertebrate species, environmental conditions and habitat quality of the sediments in the Murray Mouth and Coorong, in order to determine whether any recovery had occurred following a longer period of flow restoration. This project also measured the reef growth and occurrence of the tubeworm *Ficopomatus enigmaticus* in response to the changing environmental conditions. This study follows on from a previous water release study, which documented the first initial response of macroinvertebrates between December 2010 and April 2011.
- This project was conducted from October 2011 to May 2012 during the second year of restored and continuous river flow, following the first flood event to reach the estuary for over a decade. A total of eleven sites were sampled and included sites in the South Lagoon to determine if recovery of macroinvertebrates had extended further south, however, these sites were sampled less frequently. Samples were taken in both shallow intertidal and submerged subtidal locations of each site, with the intertidal locations being submerged at times as well, depending on the water levels. At all sites, samples were taken for macroinvertebrates, sediment and water characteristics, as described in the previous report (Dittmann et al. 2011a). Macroinvertebrates were sampled on five occasions between October 2011 and May 2012, while sediment characteristics were only sampled in October 2011 and March 2012, as it was expected that the rate of change in sediment characteristics would be minimal during the study period.
- Salinities measured during the current water release survey were substantially lower than during the extreme drought years (2008-10), but not as low as in 2010/11, after the initial first large flow over the barrages. Brackish conditions were recorded in the Murray Mouth in spring 2011 (1-22 ppt), but dropped again to near freshwater by May 2012 (0.1-0.5 ppt). Salinities varied across the sampling period at sites in the North Lagoon, dropping from hypersaline (31-69 ppt) to brackish conditions (0.7-10 ppt), which were recorded throughout the North Lagoon in March and May. Salinities in the South Lagoon continued to drop as well, from 70-80 ppt in early summer to 38-58 ppt by March 2012. Environmental conditions did not differ between subtidal and intertidal locations per site. Salinity was the primary environmental variable driving differences in macroinvertebrate assemblages communities between sites.
- Diversity and abundance of macroinvertebrates in the Murray Mouth and Coorong were higher during the current study in comparison to values recorded in 2010/11. The substantial increase in abundances and also an increase in diversity indicate recovery of macroinvertebrates, although not all species have re-established successfully. The polychaete *Capitella* sp., was still dominant in sediments at sites in the North Lagoon. Two mollusc species (*Notospisula* sp. and *Soletellina alba*) rarely encountered previously were also found.

- In comparison to 2010/11, the distribution of macroinvertebrates had shifted further into the North Lagoon as far as Noonameena, with the exception of *Nephtys australiensis*, which was confined to the Murray Mouth. Species specific patterns in shifts in distribution ranges were detected in the current study period. Species found in the Coorong in the initial survey months of October and December 2011 continued to increase in abundance at these sites, as well as extend their range back into the Murray Mouth. No range extension of macroinvertebrates further into the South Lagoon was observed.
- Intertidal and subtidal sediments contained comparable abundances, species numbers and diversity of macroinvertebrates in the current survey, compared to previous surveys where abundances were higher in subtidal than intertidal sediments. This indicated recolonisation of sediments in the mudflats of the Murray Mouth and Coorong.
- The presence of juvenile polychaetes, including *Simplisetia aequisetis* and *Australonereis ehlersi*, as well as egg carrying amphipods recorded throughout the study period indicate positive population responses to continued flow. Recolonisation could be possible by larval and adult life stages.
- To determine whether tubeworm reefs (*Ficopomatus enigmaticus*) showed renewed growth in the Coorong, and possibly in the Murray Mouth, reefs were measured in December 2011, but could not be remeasured due to high water levels. Samples of reef fragments from sites in the North Lagoon and Murray Mouth contained live *Ficopomatus enigmaticus*, some with mature gonads.
- This monitoring captured the increase in abundances of several macroinvertebrate species and the shifts in distribution ranges both further into the North Lagoon and into the Murray Mouth. Yet, diversity and abundances are still lower than about a decade ago, and several species have not yet been found again. The findings contribute to the understanding of flow-related responses and resilience of macroinvertebrates towards drought and flood events.

2. Introduction

Lack of freshwater flowing from the River Murray into the Lower Lakes and across the barrages into the Murray Mouth and Coorong during the extreme drought between 2007-2010 caused severe ecological degradation of the system (Lester & Fairweather 2009). This resulted in different stressors (salinity, acidity, reduced water levels) occurring in different parts of the system (Lester & Fairweather 2009; Kingsford et al. 2011), as well as an overall decline in macroinvertebrate communities inhabiting the sediments (Dittmann et al. 2010). Substantial winter rainfall in the catchment area of the River Murray in 2010, allowed restored flow to the Coorong, Lower Lakes and Murray Mouth in spring 2010 and opening of the barrages (Figure 1). Freshwater flows into estuaries are important regulators of macroinvertebrate communities, yet effects of flooding events are difficult to predict and heavily dependent on the intensity, frequency and timing of the flood (Moverley et al. 1986; Grilo et al. 2011), as well as the ecology and feeding habits of macroinvertebrate species (Miserendino 2009; Cardoso et al. 2008). Benthic macroinvertebrates are known to respond quickly to changes in water quality, sediment structure and other environmental factors (Boesch & Rosenberg 1981; Diaz & Rosenberg 1995; Ysebaert et al. 2002; Mackay et al. 2010). Geddes (1987) described substantial changes in the distribution of macrofauna and fish in the Murray Mouth and Coorong following extended river flow in the early 1980's, where typically estuarine macrofauna and fish shifted their distribution to the southern reaches of the North Lagoon. Smaller water releases over the barrages between 2003 and 2005/6 had limited immediate effects on the biota in the Murray Mouth and Coorong, (Geddes 2005a,b; Dittmann et al. 2006), but a positive effect on benthic densities on a longer time scale (Dittmann et al. 2010).

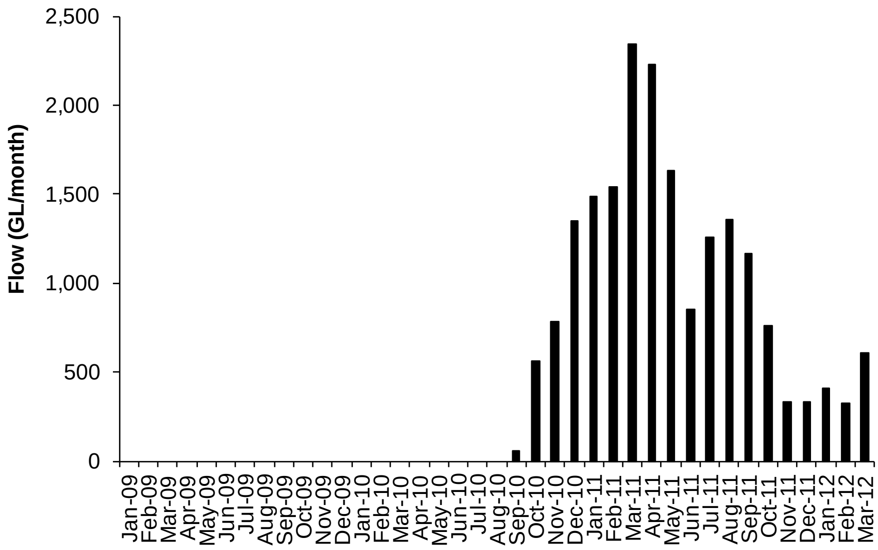


Figure 1: Monthly barrage flow from the Lower Lakes into the Murray Mouth and Coorong from January 2009 during the drought period up until March 2012 during the second consecutive year of recommenced flow. Based on data from the MDBA.

Following the recommencement of flow into the Coorong and Murray Mouth in spring 2010, investigations into the barrage release between December 2010 and April 2011 had identified a possible sequence consisting of an initial positive response with recruitment as flow commenced, followed by declines in abundances and diversity of estuarine benthos as freshwater conditions prevailed in the Murray Mouth, and a commencing shift in distribution of macroinvertebrates from the Murray Mouth into the Coorong where environmental conditions improved (Dittmann et al. 2011a). With continued flow over the barrages throughout 2011 and into 2012 (Figure 1), it was important to determine the effects on macroinvertebrate communities. This project investigated the response of benthic macroinvertebrates to the changing environmental conditions and evaluated the effects of the continued flow. This report presents data for the continued benthos sampling since October 2011 to investigate whether any recovery had occurred following the continued flow restoration across the last two consecutive years.

It was expected that the continued flow has brought further changes in the system, consisting of
(i) reduced salinity and improved water quality in the Coorong,
(ii) continued high water levels,
(iii) localised restoration of true estuarine character, and
(iv) intensified connectivity and possible influx from the southern ocean to facilitate recolonisation.

It was hypothesised that in response to these changes;

- Diversity and abundance of macroinvertebrates in the Murray Mouth region will be higher 2011/2012 in comparison to values recorded in 2010/2011.
- Estuarine macroinvertebrates will further extend their distribution range into the Coorong.
- Estuarine macroinvertebrates will recolonise the previously dried out mudflats, which have now been inundated for nearly a year.
- Estuarine macroinvertebrates will be recruiting in the system.
- Tubeworm reefs (*Ficopomatus enigmaticus*) show renewed growth in the Coorong, and possibly in the Murray Mouth.

To test these hypotheses, the sampling of macroinvertebrates was extended further into the Coorong, including the South Lagoon. These investigations will evaluate the benefit of the continued flow for macroinvertebrates, and ultimately the food webs in the Coorong and Murray Mouth.

3. Materials and Methods

3.1 Sampling sites and dates

Benthic macroinvertebrates were sampled in October and December 2011, and February, March and May 2012 at eleven sites between the Murray Mouth region and northern end of the South Lagoon (Figure 2, Table 1). Most of these sites were surveyed in previous studies (Dittmann et al. 2011a, Dittmann et al. 2011b), apart from additional sites included in the Coorong (Long Point, Noonameena, Parnka Point and Villa de Yumpa). The latter two sites were sampled less frequently, in an adaptive approach subject to the macroinvertebrate densities found (Table 1). The December 2011 survey overlapped with the annual "The Living Murray" (TLM) monitoring, and only supplementing sites (Sugars Beach, Mark Point, Long Point) and subtidal locations which are not part of the TLM, were sampled for this project. This synergy between the monitoring schemes had been approved by the respective Departments (Rolston and Frears, pers. comm.).

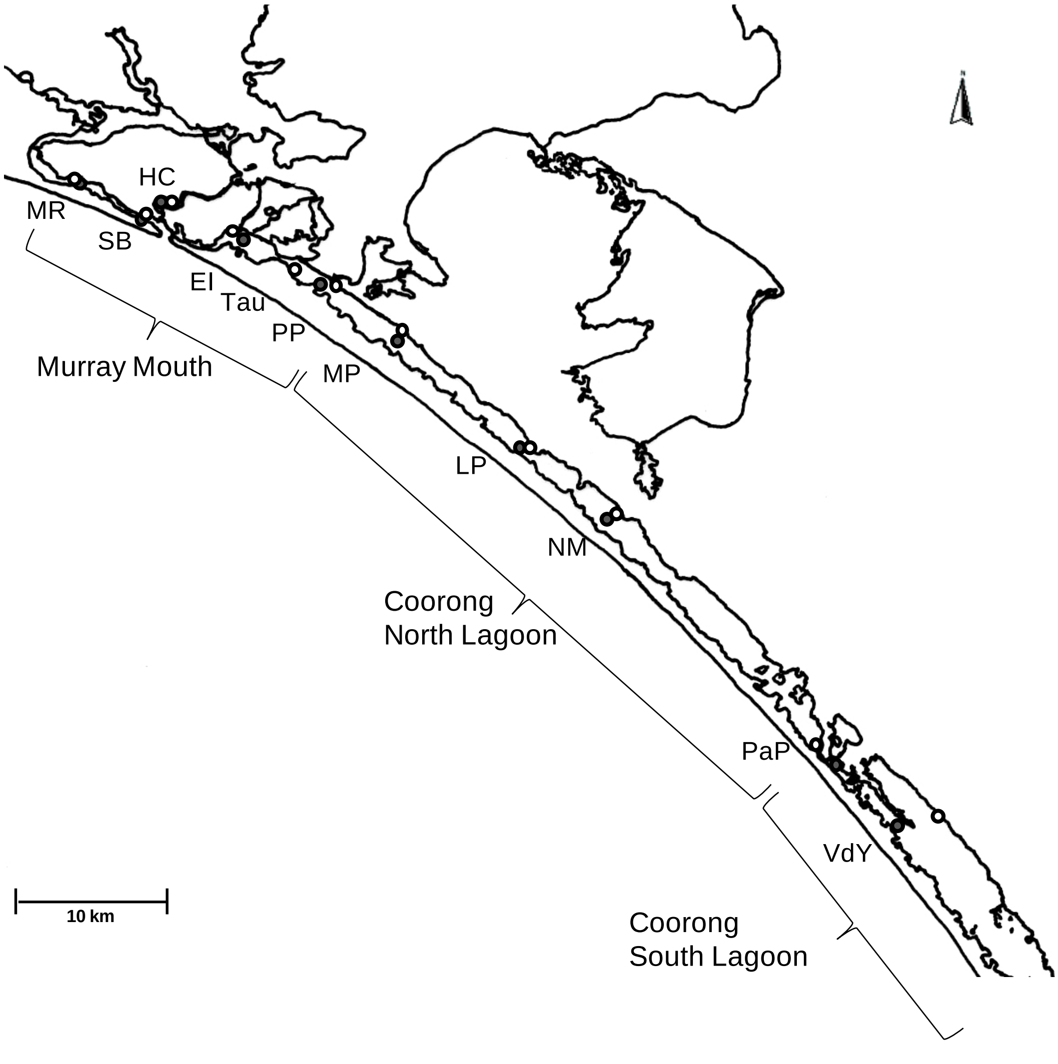


Figure 2: Study sites in the Murray Mouth and Coorong during the October 2011 – May 2012 monitoring period; Monument Road (MR), Sugars Beach (SB), Hunters Creek (HC), Ewe Island (EI), Tauwitchere (Tau), Pelican Point (PP), Mark Point (MP), Long Point (LP), Noonameena (NM), Parnka Point (PaP) and Villa de Yumpa (VdY). At each of these sites an 'intertidal' location (open circles) and a submerged location (closed circles) were sampled. The subtidal location for Pelican Point is located at the Tauwitchere subtidal location from the previous survey.

Samples were taken at two water depths, from the nearshore intertidal[1] (0-0.5 m water depth) and subtidal (~1 – 1.5 m water depth at time of sampling). As the subtidal sampling site at Pelican Point from the water release study 2010/11, was very rocky, the previous Tauwitchere subtidal site, which is located opposite the barrage and Pelican Point, was included in the continued monitoring. For comparisons with previous surveys, the subtidal site code 'TAU' was retained. Additional samples were taken at the Tauwitchere intertidal site in October 2011.

In the Coorong, water levels had dropped in October and December 2011, exposing larger areas of sediment. Reef sizes of *Ficopomatus enigmaticus* were measured at Long Point and Noonameena in October and again in December, when additional measurements and samples were taken at Mark Point. However, water levels rose again and measurements were unable to be taken in February, March and May 2012.

3.2 Environmental parameters

At each site in October and December 2011, and February, March and May 2012 water quality parameters including temperature (° C), salinity (ppt), oxygen concentration (O_2 mg/l) and saturation (O_2 %) and pH of the water overlying the mudflats (intertidal) or subtidal sediments were measured using both a TPS WP -81 (for pH, temperature and salinity) and WP-82Y (for dissolved oxygen) electrode, and a YSI 85 multi-parameter electrode. The use of two electrodes has proven useful in the past due to malfunctions experienced with either of the electrodes.

To characterise the sedimentary environment for benthic organisms, grain size, organic matter and chlorophyll-*a* were sampled in October 2011 and March 2012, taking three replicate samples for each parameter per site. Sediment samples for grain size and organic matter were taken with a 60 ml cut-off syringe (surface area 6.6 cm^2). Samples of the sediment parameters were stored on ice in the field, and frozen upon return to the lab until further analysis. Grain size was determined by laser diffraction using a particle size analyser (Malvern Mastersizer 2000). Sediment grain size samples were thawed and the fraction >1 mm sieved off manually to avoid blockage in the machine. The weights of this fraction and of the remaining sediment were determined for later normalisation of the data to correct for this procedure. Median and quartiles, as well as percentage of various particle sizes were obtained from the Mastersizer output. Sediment sorting (S_o) was calculated from the ratio of the quartiles (P_{25} and P_{75}) $S_o = (P_{25}/P_{75})^{1/2}$, based on the metric scale. The three sediment samples taken for sediment organic matter were analysed separately to account for spatial variation and to calculate means and standard error. To obtain a bulk parameter of organic matter as % dry weight (d.w), sediment samples were dried to constant weight (for 24 – 36 hours) at 80 °C and then burnt in a muffle furnace at 450 °C for 5 hours. For sediment chlorophyll-*a*, three replicate samples were taken per site by inserting a 5 ml vial about 1 cm into the sediment, 5 ml of methanol was added to extract the chlorophyll, and the vial was heavily shaken before being wrapped in aluminium foil (Seuront and Leterme 2006) and frozen for later analysis with a fluorometer (Turner 450). After the initial reading for total chlorophyll, drops of 0.1 M HCl were added to the samples to correct for phaeophorbides.

[1] Note the terminology is used although tides are only affecting the Murray Mouth region.

Table 1: Overview of the macroinvertebrate sampling in the Murray Mouth and Coorong to follow the water release effects in October and December 2011, and February, March and May 2012. See Figure 2 for site locations. The suffix -O indicates October 2011, D December 2011, F February 2012, M March 2012 and Ma May 2012 respectively. n/a indicates samples not taken.

Sites	Sampling Date					Water Characteristics	Chlorophyll-*a*	Sediment Characteristics	Abundance
	October '11	December '11	Jan/Feb '12	March '12	May '12				
Monument Rd (MR) - Intertidal	26/10/2011	12/12/2011	30/01/2012	19/03/2012	1/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Subtidal	26/10/2011	12/12/2011	1/02/2012	19/03/2012	1/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Sugars Beach (SB) - Intertidal	26/10/2011	12/12/2011	30/01/2012	19/03/2012	1/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Subtidal	26/10/2011	12/12/2011	30/01/2012	19/03/2012	1/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Hunters Creek (HC) - Intertidal	26/10/2011	12/12/2011	30/01/2012	27/03/2012	30/04/2012	O D F M Ma	O D M	O D M	O D F M Ma
Subtidal	26/10/2011	12/12/2011	30/01/2012	27/03/2012	30/04/2012	O D F M Ma	O D M	O D M	O D F M Ma
Ewe Island (EI) - Intertidal	26/10/2011	12/12/2011	1/02/2012	27/03/2012	30/04/2012	O D F M Ma	O D M	O D M	O D F M Ma
Subtidal	26/10/2011	12/12/2011	1/02/2012	27/03/2012	30/04/2012	O D F M Ma	O D M	O D M	O D F M Ma
Tauwitchere (TAU) - Intertidal	25/10/2011	n/a	n/a	n/a	n/a	O	O	O	O
Subtidal	25/10/2011	6/12/2011	31/01/2012	2/04/2012	7/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Pelican Point (PP) - Intertidal	24/10/2011	6/12/2011	31/01/2012	2/04/2012	7/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Mark Point (MP) - Intertidal	25/10/2011	6/12/2011	31/01/2012	22/03/2012	7/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Subtidal	25/10/2011	6/12/2011	31/01/2012	2/04/2012	8/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Long Point (LP) - Intertidal	24/10/2011	5/12/2011	31/01/2012	22/03/2012	8/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Subtidal	24/10/2011	7/12/2011	31/01/2012	2/04/2012	8/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Noonamenna (NM) - Intertidal	25/10/2011	6/12/2011	31/01/2012	22/03/2012	7/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Subtidal	25/10/2011	7/12/2011	31/01/2012	2/04/2012	8/05/2012	O D F M Ma	O D M	O D M	O D F M Ma
Parnka Point (PaP) - Intertidal	24/10/2011	5/12/2011	n/a	22/03/2012	n/a	O D M	O D M	O D M	O D M
Subtidal	24/10/2011	n/a	n/a	3/04/2012	n/a	O M	O M	O M	O M
Villa de Yumpa (VdY) - Intertidal	24/10/2011	5/12/2011	n/a	22/03/2012	n/a	O D M	O D M	O D M	O D M
Subtidal	24/10/2011	n/a	n/a	3/04/2012	n/a	O M	O M	O M	O M

3.3 Macrofauna

To investigate macroinvertebrate species composition and abundance within sediments, handheld PVC corers (83.32 cm^2 surface area) were used at each intertidal site. For each of the land-based sites that were intertidal or under shallow water, ten haphazardly placed replicate samples were taken. Each replicate core was inserted into the sediment to approximately 15 cm depth, sealed with a stopper to avoid disturbance of the sample and dug out with a shovel or by hand. For the water release study, subtidal locations within each site were located approximately 50 to 150 m towards the deeper channel from the intertidal location. Due to the changing water levels over time, the amount of water overlying subtidal locations varied between 50 cm to over 1.5 m in depth. When the overlying water was deeper than 1 m, submerged sites were sampled with a benthic Ekman grab (225 cm^2 surface area), deployed from a small boat. The grab penetrated approximately 10 cm into the sediment. The grab was only used at some subtidal sites in October 2011. When water levels were less than 1 m, subtidal locations were sampled using the PVC corers (83.32 cm^2 surface area) in the vicinity of the boat. All samples were sieved through a 500 µm mesh size *in situ*. Sorting of live samples was carried out within several days and organisms were identified to the lowest taxonomic level possible and individual numbers for each species counted. Amphipods were not differentiated into family or species. For insects, larval and pupae life stages encountered during sorting were noted in macrobenthic analyses, but not counted separately for chironomids. When samples could not be processed live in the given time period, due to the unexpected high number of macroinvertebrates encountered in the samples, they were preserved in 70 % ethanol.

To investigate whether recruitment of the four most abundant polychaete species (*Capitella* sp., *Australonereis ehlersi, Simplisetia aequisetis* and *Nephtys australiensis*) had occurred, the length of complete specimens of the four polychaete species were measured using graph paper to determine their size-frequency distribution in each survey month. A total of 30 individuals were measured per species per individual sample when possible.

Tubeworm reefs *(Ficopomatus enigmaticus)*

When tubeworm reefs were located in the North Lagoon, the dimensions of reefs and extent of new growth were quantified. In October, the widths of five reefs were measured at Noonameena and ten at Long Point. Unfortunately the marker pegs were lost, and new reefs marked and measured in December 2011, including an additional site (Mark Point). In December, measurements were further differentiated for three different reef sizes (< 1 m, 1 – 2 m, > 2 m in diameter) and ten reefs each marked out for future reference. Measurements included reef length, width and height, further separating old and new growth based on colour (new growth appearing white). However, due to raised water levels in February, March and May 2012, measurements could not be taken. To overcome this limitation, small sections of reef were collected in four of the five surveys to corroborate the presence of live tubeworms and length of individual tube growth. Sections of reefs were not sampled in May 2012 due to high water. Any associated fauna in these fragments was also recorded.

3.4 Data analysis

To explore spatial and temporal differences in environmental conditions, including salinity (ppt), temperature (°C), and oxygen saturation (O_2 %) and content (O_2 mg/L) in the Murray Mouth and Coorong, parameters were tested separately using PERMANOVA with two factors ‘site’ and ‘month’. Depth (intertidal and subtidal) was eliminated as a factor for analysis, as no stratification in water quality was observed with depth of the water column. Percentage sediment organic matter and chlorophyll-*a* (chl-*a*) were analysed using a three way PERMANOVA, for differences between sites, depth and survey months. Data were transformed as needed and tests based on Euclidean distance matrix.

The following diversity indices were calculated for each site; total species number (S), Shannon-Wiener diversity (H’) and Pielou’s index for equitability (J’). Differences between species number (S) and diversity (H’) across sites, depth and survey month were tested using a three-way PERMANOVA.

Differences in the macroinvertebrate abundances of total benthos, major phyla and individual species between monitoring sites, depth and survey months were tested using a three-way PERMANOVA. Data were fourth-root transformed prior to analysis and a dummy value of 1 added for Euclidean similarity when too many zero values occurred in the data set. Each analysis was run with 9999 permutations. The three factors tested in PERMANOVA were ‘sites’ (a fixed factor with eight levels, excluding intertidal Tauwitchere (TAU) in October 2011 as it was an additional site, and Parnka Point (PaP) and Villa de Yumpa (VdY) as they were irregularly sampled), ‘depth’ (a fixed factor with two levels: intertidal and subtidal) and ‘survey month’ (a fixed factor with five levels: October and December 2011, and February, March and May 2012). Differences in the abundance of total macroinvertebrates between current (October and December 2011, and February, March and May 2012) and previous water release surveys (December 2010, February, March and April 2011) were also tested using a three-way PERMANOVA using ‘site' (only those sites continued through both water release projects), ‘depth’ and ‘survey month’ as factors.

To investigate similarities of macroinvertebrate assemblages between sites, depth and survey month data were fourth-root transformed prior to analysis and a dummy value of 1 added for Bray-Curtis similarity, because of too many zero values in the data sets. Principle Coordinate plots (PCO) were used to display differences in macroinvertebrate assemblages across the monitoring sites and sampling times. Vector overlays depict species that are important for the assemblage patterns. Differences in the macroinvertebrate assemblage data between sites, survey months and depths were then tested using a three-way PERMANOVA, with ‘site’, ‘depth’ and ‘survey month’ as factors.

To explore links between macroinvertebrate assemblages and environmental variables, distance-based multivariate multiple regression linear models (DISTLM) were calculated using forward selection of the environmental variables and visualised using distance-based redundancy analysis (dbRDA). This method generates a prediction of the relative influence of each environmental variable in determining differences in macrozoobenthic community structure between sites and depth over time. The test was run for two months of data in October 2011 and March 2012, when all environmental variables including sediment characteristics were sampled.

All multivariate analyses and PERMANOVA testing was carried out using the software PRIMER v6 with PERMANOVA add-on.

4. Results

4.1 Environmental parameters

Water parameters

The water quality in the study period from October 2011 to May 2012 was reflective of the continued water release over the barrages (Figure 1). The salinity gradient along the length of the Murray Mouth and Coorong ranged from almost freshwater conditions in the Murray Mouth region to about 85 ppt in the northern reaches of the South Lagoon (Figure 3). However, while brackish to marine salinities prevailed in the Murray Mouth across all five sampling events, salinities in the North Lagoon and at the northern end of the South Lagoon dropped significantly between October 2011 and March/May 2012 from 45 to 5 ppt in the North Lagoon region between Mark Point and Noonameena, and from 70 to 41 ppt at Parnka Point and Villa de Yumpa, with the greatest drop at Parnka Point subtidal from 70 to 14 ppt. Salinities at sites between Mark Point (MP) and Villa de Yumpa (VdY) were highest in October and December 2011, with a marked decline recorded in March and May 2012 at both intertidal and subtidal locations (Figure 3; Table 2)., Hypersaline conditions were encountered during the early survey months (particularly at Noonameena (NM) and Parnka Point; PaP subtidal only), but dropped to marine conditions in both March and May 2012.

Water temperature at sites ranged between 12.2 – 26.7 °C, with an average of 19.1 °C, reflecting seasonal temperature variation (Figure 3, Table 2). Oxygen saturation was below the 90 % trigger value of the ANZECC guidelines at several sites in both the intertidal and subtidal zones, and oversaturation was only recorded in the North Lagoon and at single sites in the Murray Mouth (Figure 3). Oxygen concentrations varied between sites and surveys and were mostly within the range of ~4-12 mg/L (Figure 3). Across all sites, depths and survey months, there was significant variation in oxygen saturation and concentration (Figure 3, Table 2). The pH was around 8 at all sites across the five sampling events.

Table 2: Results from PERMANOVA on environmental parameters including water temperature (°C), salinity (ppt), oxygen saturation (O_2 %) and oxygen content (O_2 mg/l) recorded in samples of the eight sites (MR, SB, HC, EI, PP, MP, LP and NM) and across months (October and December 2011, and February, March and May 2012). Significant *P*-values are highlighted in bold.

Source	df	Temperature (° C) *P* (perm)	df	Salinity (ppt) *P* (perm)	df	O_2 % *P* (perm)	df	O_2 mg/l *P* (perm)
Survey	4	**0.0001**	4	**0.0001**	4	**0.0001**	4	**0.0001**
Site	7	**0.0001**	7	**0.0001**	7	**0.0001**	7	**0.0001**
Survey * Site	28	**0.0001**	28	**0.0001**	28	**0.0001**	28	**0.0001**
Residual	266		377		266		260	

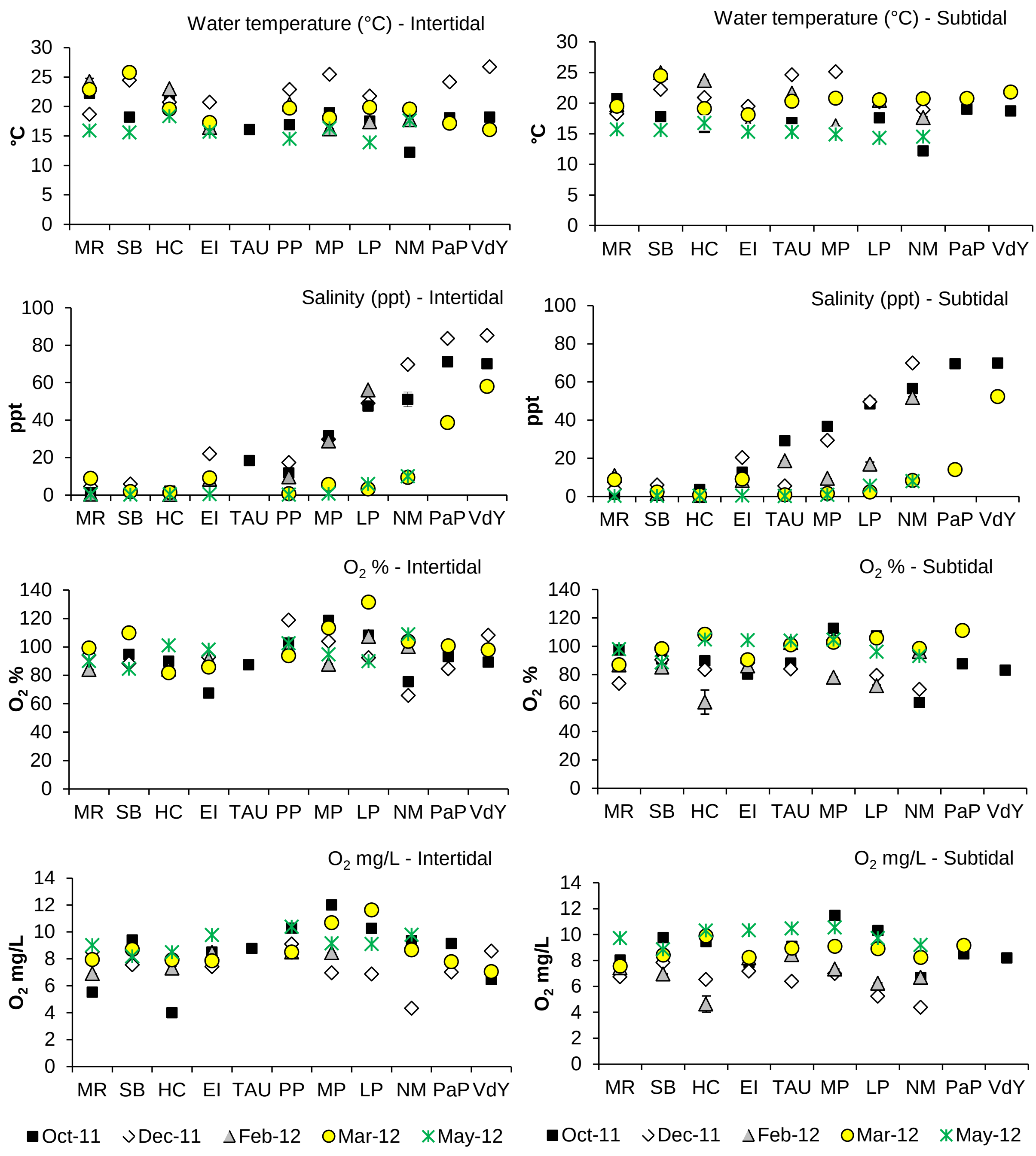


Figure 3: Water parameters (average ± S.E.) including temperature (°C), salinity (ppt), oxygen saturation (O_2 %) and content (O_2 mg/L) in intertidal and subtidal sampling locations at the water release monitoring sites in October and December 2011, and February, March and May 2012.

Sediment characteristics

Sediments in the Murray Mouth consisted of fine to medium sands, with the find sand fraction (125 – 250 µm) being prominent across most sites. Grain composition at sites in the Murray Mouth varied from moderately well to very well sorted in October 2011 and March 2012 (Figure 4, Table 3), except at Pelican Point (PP) at the southern end of the Murray Mouth, where a larger median grain size (250-500 µm) was recorded in March 2012 in comparison to October 2011 (Figure 4; Table 3). Sediments at intertidal and subtidal sites in the Coorong contained similar proportions of fine to medium sands at most sites in October 2011 and March 2012 (Figure 4), with the exception of intertidal locations at Mark Point (MP), Long Point (LP) and Villa de Yumpa (VdY), where a more prominent medium or coarse particle fractions was recorded in either survey month.

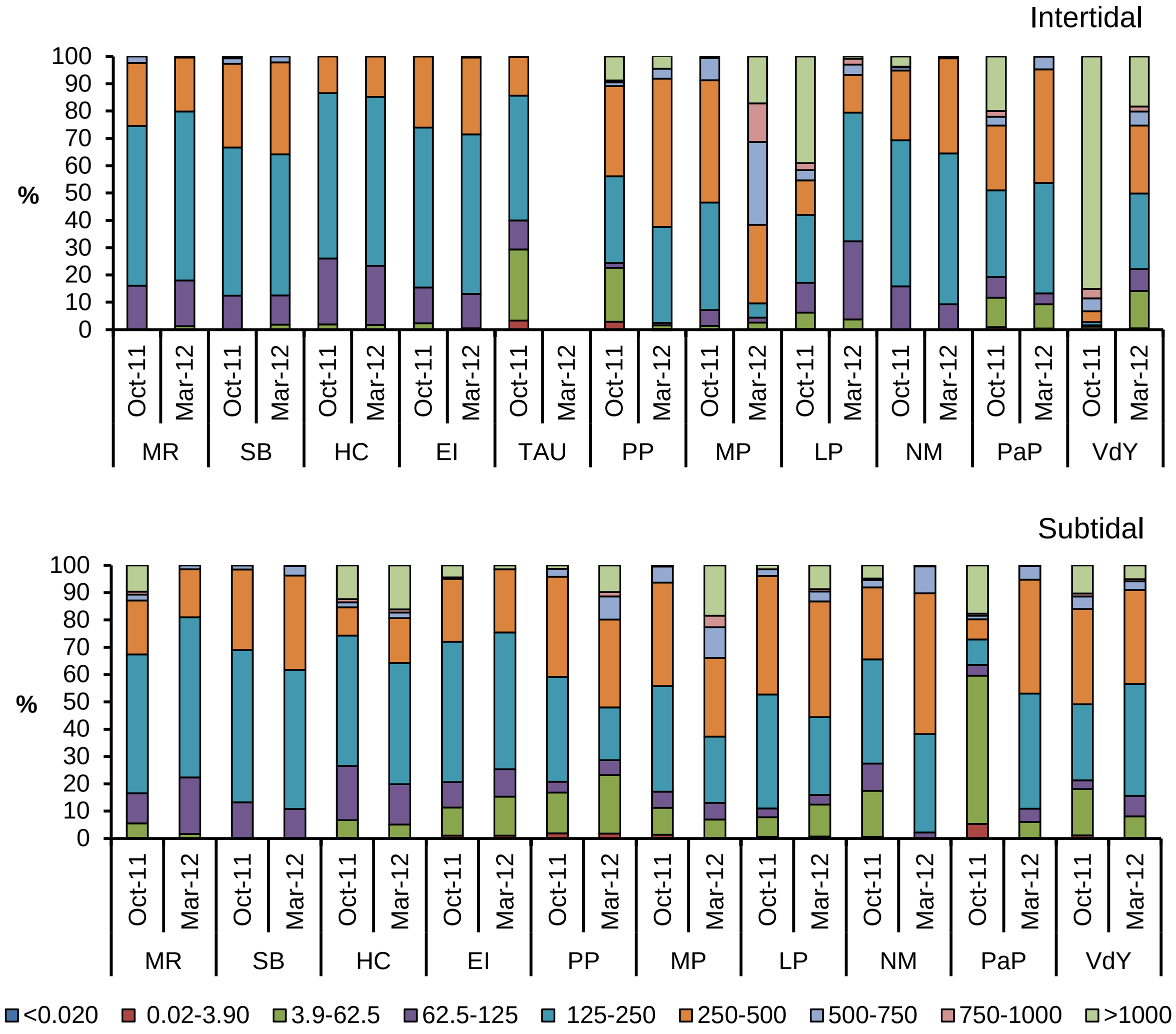


Figure 4: Sediment grain size composition (% of major fractions, size in µm) at the monitoring sites in the water release project during the October 2011 and March 2012 surveys.

Table 3: Sediment characteristics of the Murray Mouth and Coorong lagoons during the sampling months of October 2011 and March 2012 in the a) intertidal and b) subtidal locations. The median grain size of sediment is represented in µm along with the sorting coefficient. The verbal description of sediment grain size and sorting follows (Blott & Pye 2001).

a) Intertidal

		October 2011				March 2012			
Region	**Site**		**Median**		**Sorting**		**Median**		**Sorting**
Murray Mouth	**MR**	186.54	Fine Sand	0.58	Moderately Well Sorted	181.75	Fine Sand	0.60	Moderately Well Sorted
	SB	208.76	Fine Sand	0.57	Moderately Well Sorted	213.95	Fine Sand	0.56	Moderately Well Sorted
	HC	166.66	Fine Sand	0.58	Moderately Well Sorted	170.66	Fine Sand	0.59	Moderately Well Sorted
	EI	193.02	Fine Sand	0.59	Moderately Well Sorted	198.45	Fine Sand	0.59	Moderately Well Sorted
	TAU	153.10	Fine Sand	0.24	Very Well Sorted	Not Sampled			
	PP	227.50	Fine Sand	0.33	Very Well Sorted	284.95	Medium Sand	0.58	Moderately Well Sorted
North Lagoon	**MP**	261.17	Medium Sand	0.52	Moderately Well Sorted	584.72	Coarse Sand	0.45	Well Sorted
	LP	579.42	Coarse Sand	0.10	Very Well Sorted	159.35	Fine Sand	0.49	Well Sorted
	NM	199.12	Fine Sand	0.55	Moderately Well Sorted	215.53	Fine Sand	0.59	Moderately Well Sorted
South Lagoon	**PaP**	247.38	Fine Sand	0.29	Very Well Sorted	239.05	Fine Sand	0.52	Moderately Well Sorted
	VdY	2549.7	Granules	0.36	Well Sorted	250.89	Medium Sand	0.26	Very Well Sorted

b) Subtidal

		October 2011				March 2012			
Region	**Site**		**Median**		**Sorting**		**Median**		**Sorting**
Murray Mouth	**MR**	202.62	Fine Sand	0.51	Moderately Well Sorted	174.38	Fine Sand	0.58	Moderately Well Sorted
	SB	204.10	Fine Sand	0.58	Moderately Well Sorted	220.20	Fine Sand	0.55	Moderately Well Sorted
	HC	174.55	Fine Sand	0.46	Well Sorted	206.70	Fine Sand	0.37	Well Sorted
	EI	194.90	Fine Sand	0.54	Moderately Well Sorted	181.83	Fine Sand	0.44	Well Sorted
	PP	219.65	Fine Sand	0.39	Moderately Well Sorted	250.65	Medium Sand	0.25	Very Well Sorted
North Lagoon	**MP**	230.93	Fine Sand	0.47	Well Sorted	369.39	Medium Sand	0.30	Very Well Sorted
	LP	242.16	Fine Sand	0.54	Moderately Well Sorted	264.95	Medium Sand	0.41	Well Sorted
	NM	190.95	Fine Sand	0.36	Well Sorted	286.27	Medium Sand	0.55	Moderately Well Sorted
South Lagoon	**PaP**	46.83	Very Coarse Silt	0.06	Very Well Sorted	240.97	Fine Sand	0.53	Moderately Well Sorted
	VdY	245.04	Fine Sand	0.33	Very Well Sorted	229.08	Fine Sand	0.48	Well Sorted

The amount of sediment organic matter at sites in the Murray Mouth and Coorong ranged from 0.39 % at Mark Point (MP) in the North Lagoon to 9.76 % at Villa de Yumpa (VdY) in the northern end of the South Lagoon, with an overall mean value of 1.42 % dry weight (Figure 5). Organic matter was highest in October 2011 at several sites, especially Parnka Point in the subtidal and Villa de Yumpa in the intertidal (Figure 5), supported by a significant interaction between the amount of sediment organic matter between surveys, sites and depth ($F_{9,80}$ = 8.908, P<0.001). However, *post-hoc* pairwise tests revealed that at an individual site and depth location level, the amount of sediment organic matter did not differ significantly between the two surveys.

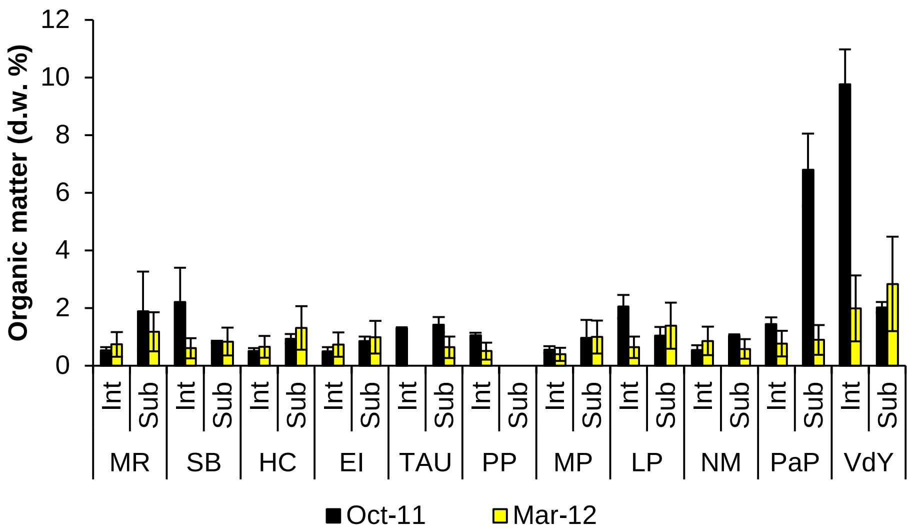


Figure 5: Sediment organic matter (as % dry weight, ± S.E., *n*=3) at intertidal and subtidal locations of water release study sites during October 2011 and March 2012.

The chlorophyll-*a* content in the sediments, as a proxy of microphytobenthic biomass, varied across October 2011 and March 2012, ranging from 0.01 mg m^{-2} at Parnka Point (PaP) subtidal in October 2011 to 7.01 mg m^{-2} at Sugars Beach (SB) intertidal in March 2012, with an overall mean value of 2.48 mg m^{-2} (Figure 6). Chl-*a* concentrations in the sediment were higher in March 2012 across most sites and depths, supported by a significant site, depth and survey month interaction ($F_{9, 78}$ = 4.176, P<0.001) (Figure 6). There was no obvious difference between concentrations of chl-*a* between intertidal and subtidal locations in both survey months (P>0.05).

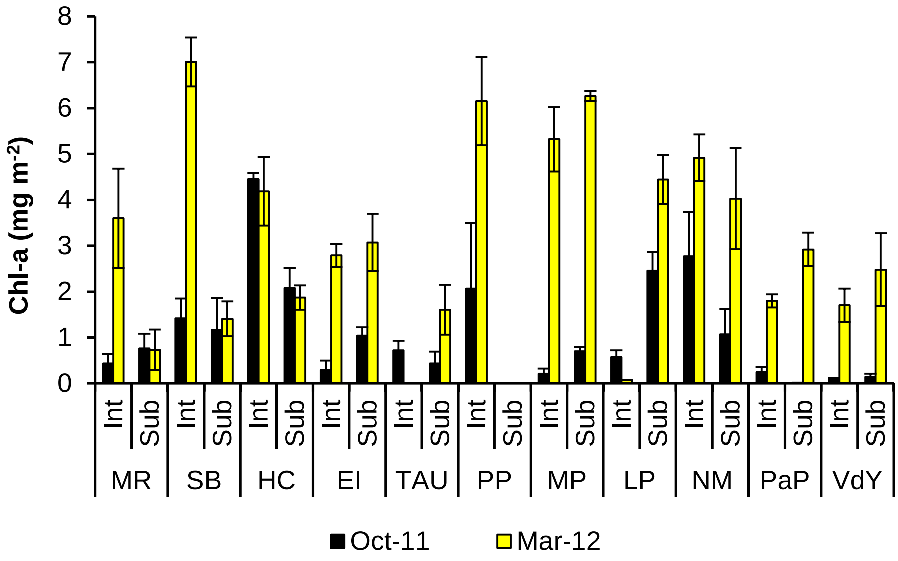


Figure 6: Sediment chlorophyll-*a* content (mg m^{-2}, average ± S.E., *n*=3) as a proxy for microphytobenthic biomass at the water release study sites surveyed in October 2011 and March 2012.

4.2 Macroinvertebrate diversity

A total of 22 macroinvertebrate taxa were recorded across the eleven monitoring sites during the five sampling events between October 2011 and May 2012. Annelids were most diverse and numerous, represented by seven taxa. Six taxa of insects, five molluscs, which included two species (*Soletellina alba, Notospisula* sp.) rarely encountered before, and only four crustacean taxa were found within the samples. Species numbers at single sites varied between one and nine, with an average of three species per individual core sample and five species per site (Figure 7).

Annelids occurred in the Murray Mouth and Coorong up to Noonameena (NM) in the North Lagoon, with only one individual of an oligochaete found in the South Lagoon at Villa de Yumpa (VdY) subtidal in March 2012 (Figure 7, Table 4). The pollution indicator *Capitella* sp. was present in both the intertidal and subtidal locations predominately at sites in the Coorong, especially Long Point (LP) and Noonameena (NM) (Figure 7, Table 4). Specimens of *Simplisetia aequisetis*, amphipods and chironomid larvae had the highest frequency of occurrence by being present at almost every site across depths and sampling months (Table 4). *Australonereis ehlersi* was only recorded at two sites in the North Lagoon, at Long Point and Noonameena, throughout this study. The distribution of *Nephtys australiensis* was mainly confined to the Murray Mouth, with low levels of occurrence at sites in the Coorong, mainly Mark Point, where this species was not recorded in the final survey month. Oligochaetes showed some range expansion into the North Lagoon in March and May 2012.

The diversity and number of species found in the sediments at sites and depth locations throughout the study were typically higher within the Coorong, and during March and May 2012 (Figure 7, Table 6). Diversity also significantly increased at sites throughout the study (Table 6). Despite this increase, diversity indices revealed a low diversity with the dominance of few species at most sites in October and December 2011originating from the numerical abundance of amphipods, *Capitella* sp., *Simplisetia aequisetis* and chironomid larvae (Figure 8 and 12; Table 4), and supported by low evenness (Table

5). Later surveys showed higher diversity at most sites between Ewe Island (EI) to Noonameena (NM) at both intertidal and subtidal locations (Figure 8). This increase in diversity was caused by reduced dominance of single taxa, including amphipods.

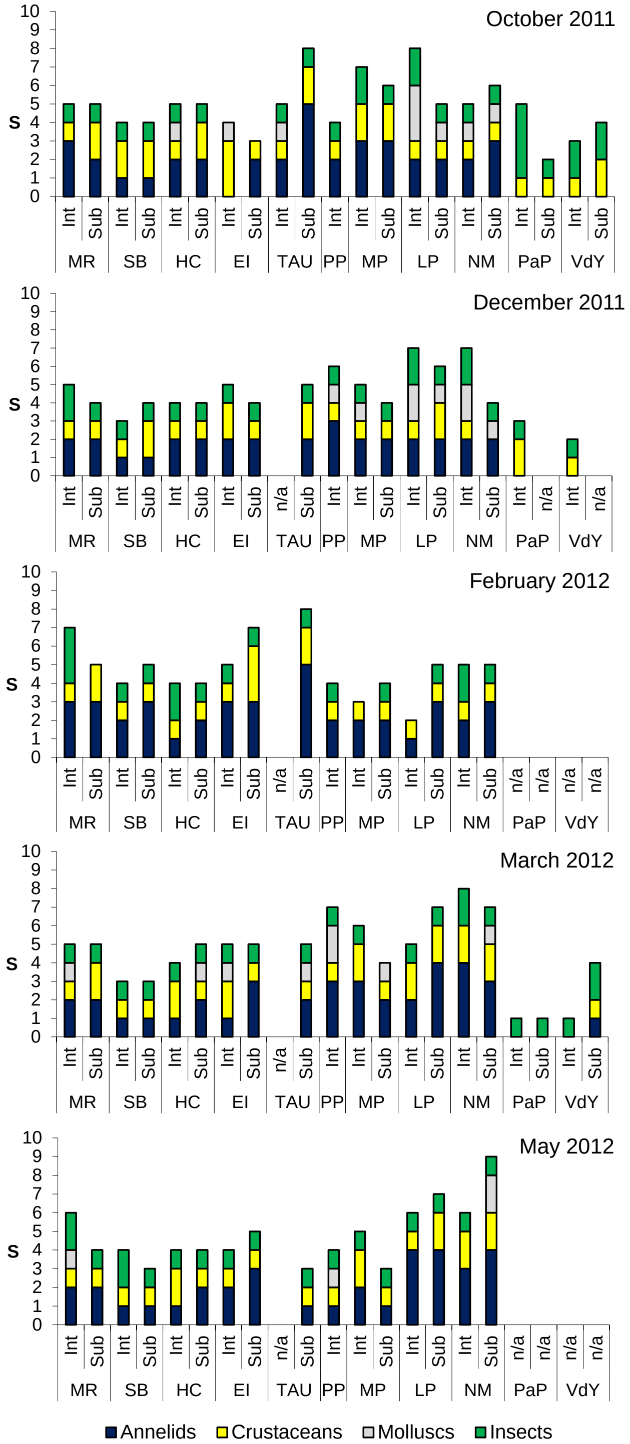


Figure 7: Numbers of macroinvertebrate species (S) and their major taxonomic composition found in the intertidal and subtidal locations at the water release sites in October and December 2011, and February, March and May 2012 (see Figure 2 for site acronyms). n/a indicates samples not taken. Samples were taken at TAU intertidal only in October 2011, and PaP and VdY subtidal locations in December 2011. Values for PaP and VdY intertidal sites in December 2011 are taken from TLM monitoring.

Table 4: Occurrence of macrobenthic taxa and species during the water release sampling period for the October (O) and December 2011 (D), and February (F), March (M) and May (Ma) 2012 sampling events. The presence of taxa is indicated for the intertidal (I) and subtidal (S) sampling locations. n/a refers to site not sampled.

PHYLA	SPECIES	Monument Road					Sugars Beach					Hunters Creek					Ewe Island					Tauwitcheree					Pelican Point					Mark Point					Long Point					Noonameena					Parnkae Point					Villa de Yumpa				
		O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma	O	D	F	M	Ma
Annelida	*Capitella* sp.			I								I	I									S	S	S			I	I				IS					IS	IS	S	S	IS	IS	IS	IS	IS	IS										
	Simplisetia aequisetis	IS	IS	IS	IS	IS			IS			S	S	IS	I	S		S	IS		IS	IS	S	S	S	S		I	I	I	I	IS	IS	IS	IS	IS			IS	IS	IS	I	I	IS	I	S										
	Australonereis ehlersi			I					S										IS	S				S													S	I	S		IS	S	S	S	IS	IS										
	Nephtys australiensis	IS		S	I		IS	IS	IS	IS	S	S		S	S		S		IS	IS	IS	IS		S				I				S	IS	S	I			S																		
	Boccardiella limincola				S										S							S								I																										
	Ficopomatus enigmaticus																																				I			S		S														
	Oligochaeta	I	IS	S		IS					I	I	IS			IS	S	IS		S	S	S		S	S		I		I	I		I		I	IS	I				IS	IS				IS	IS									S	
Crustacea	Amphipoda	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS		IS	IS	IS	IS	S	S	S	S	I	I	I	I	I	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	I	IS	IS	IS	S	I				IS	I		S	
	Ostracoda														I	I	I		S																												I	I				S				
	Mysidacea	S		S	S		IS	S				S					I	I	S	I		S	S	S								IS			I	I		S		IS	S				IS	IS										
	Paragrapsus gaimardii																											I																												
Mollusca	*Arthritica helmsi*																I																																							
	Notospisula																					I											I				IS	IS				IS	IS		S	S										
	Soletellina alba																			I																	I	I					I													
	Salinator fragilis																													I							I									S										
	Hydrobiidae				I	I						I			S										S					I	I				S																					
Insecta	Chironomid (Larvae + pupae)	IS	IS	I	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS	IS		IS	IS	IS	IS	IS	S	S	S	S	I	I	I	I	I	IS	IS	S	I	IS	IS	IS	S	IS	IS	IS	IS	IS	IS	IS	IS	I		IS		IS	I		IS	
	Culicidae (pupae/adult)																																										I									S				
	Diptera adult (Nematocera)																															I																								
	Unidentified diptera pupae/larvae			I																																		I														I			S	
	Ceratopogonidae pupae													I																																	I									
	Dolichopodidae larvae			I		I	I				I																																	I	I		I									
Species number per sampling event		6	4	10	7	6	5	4	5	3	5	8	5	5	7	5	6	4	7	7	5	9	5	8	5	3	4	6	4	7	4	8	5	5	7	5	8	9	5	7	7	7	8	6	9	9	5	3	n/a	1	n/a	5	2	n/a	4	n/a
Species number per site		12					8					11					10					11					10					10					13					13					5					6				

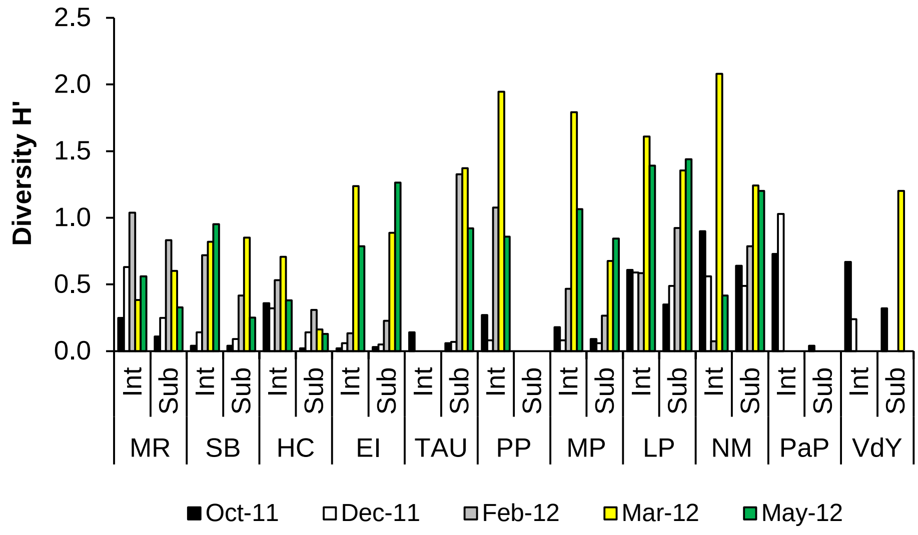


Figure 8: Diversity (H’ ($\log_e$) = Shannon–Wiener diversity index) of macroinvertebrates found in the intertidal and subtidal locations at the water release sites in October and December 2011, February, March and May 2012 (see Figure 2 for site acronyms). Samples were taken at TAU intertidal only in October 2011, and various months for PaP and VdY.

Table 5: Pielou's evenness index (J) of macroinvertebrates in October and December 2011, and February, March and May 2012 in the intertidal and subtidal sampling stations at all eleven monitoring sites. Samples at TAU intertidal were taken only in October 2011 and varied for PaP and VdY intertidal and subtidal locations.

	J (evenness)									
Site	**Oct-11**		**Dec-11**		**Feb-12**		**Mar-12**		**May-12**	
	Intertidal	**Subtidal**	**Intertidal**	**Subtidal**	**Intertidal**	**Subtidal**	**Intertidal**	**Subtidal**	**Intertidal**	**Subtidal**
MR	0.15	0.06	0.39	0.15	0.53	0.52	0.24	0.37	0.31	0.24
SB	0.03	0.03	0.13	0.06	0.52	0.26	0.75	0.78	0.69	0.23
HC	0.22	0.01	0.23	0.09	0.38	0.22	0.51	0.10	0.28	0.09
EI	0.01	0.02	0.03	0.03	0.08	0.12	0.77	0.55	0.57	0.79
TAU	0.08	0.03	n/a	0.04	n/a	0.64	n/a	0.85	n/a	0.84
PP	0.19	n/a	0.05	n/a	0.78	n/a	0.64	n/a	0.62	n/a
MP	0.09	0.05	0.05	0.04	0.43	0.19	0.30	0.49	0.66	0.77
LP	0.31	0.22	0.31	0.27	0.85	0.57	0.62	0.70	0.78	0.74
NM	0.56	0.36	0.29	0.14	0.05	0.49	0.44	0.64	0.23	0.55
PaP	0.52	0.06	0.94	n/a	n/a	n/a			n/a	n/a
YdY	0.61	0.23	0.35	n/a	n/a	n/a		0.87	n/a	n/a

Table 6: Three-way PERMANOVA test for species number (S) and Shannon-Wiener (H’) diversity with survey month, site and depth as factors. Significant *P*-values are in bold.

Source	**df**	**Species Number (S)** ***P* (perm)**	**Shannon-Wiener (H’)** ***P* (perm)**
Survey	4	**0.0001**	**0.0001**
Site	7	**0.0001**	**0.0001**
Depth	1	**0.0013**	0.8661
Survey * Site	28	**0.0001**	**0.0001**
Survey * Depth	4	**0.0001**	**0.0001**
Site * Depth	7	**0.0001**	**0.0001**
Survey * Site * Depth	28	**0.0001**	**0.0001**
Residual	720		

4.3 Macroinvertebrate abundances and distribution

Sediments in the Murray Mouth and North Lagoon contained significantly higher macroinvertebrate densities between October 2011 and May 2012 than in the few months after flows were first restored in 2010 ($F_{59,\ 1467} = 13.72$, $P<0.001$), indicating a recovery of macroinvertebrate populations with continued water release (Figures 9 and 10). Within the current water release survey period October 2011 to May 2012, high variability of macroinvertebrate abundances was observed across sites and over sampling months (Figure 9, Table 7). The high densities of total benthos at most sites in October and December 2011, in particular at Pelican Point, were due to amphipods (Figures 9 and 11). One individual core sample taken during these two survey months contained 2520 amphipods (83 cm^2, raw data value). A drop in amphipod abundances across all sites accounted for a decline in overall benthic abundances at both inter- and subtidal locations in February and March 2012 (Figures 9 and 12). In May 2012, a small increase in total benthic abundances was apparent at several sites in the Murray Mouth (Figure 9).

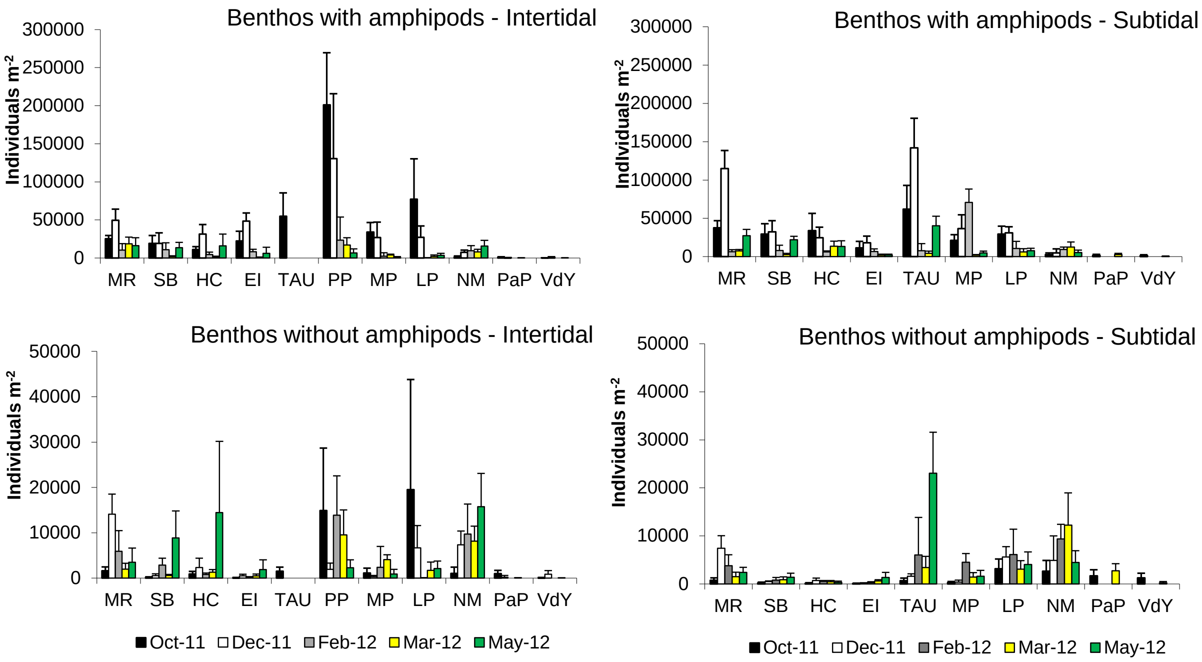


Figure 9: Average abundances (individuals m^{-2}, ± S.D., *n*=10) of macroinvertebrates with and without amphipods, recorded in the intertidal and subtidal sampling locations of water release study sites surveyed in October and December 2011, and February, March and May 2012. Samples were not taken at TAU intertidal, and in particular months for PaP and VdY. Note the different y-axis scales.

Table 7: Three-way PERMANOVA results for effects of survey months, sites, and intertidal and subtidal depth on abundances of total macrobenthos and major phyla (Annelida, Crustacea, Mollusca and Insecta). Significant *P*-values are in bold.

Source	df	Total Benthos *P* (perm)	Annelida *P* (perm)	Crustacea *P* (perm)	Mollusca *P* (perm)	Insecta *P* (perm)
Survey	4	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Site	7	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Depth	1	**0.0001**	0.0872	**0.0001**	**0.0113**	**0.0001**
Survey * Site	28	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Survey * Depth	4	**0.0001**	**0.0104**	**0.0001**	0.2054	**0.0001**
Site * Depth	7	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Survey * Site * Depth	28	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Residual	720					

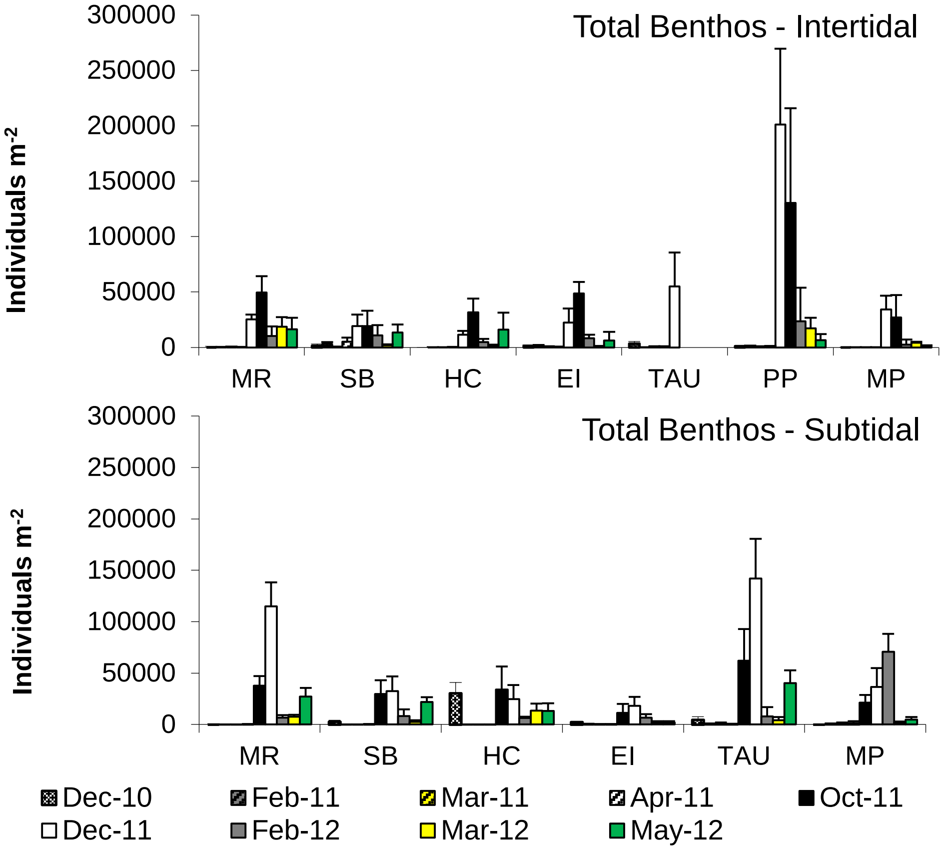


Figure 10: Comparison of average abundances (individuals m^{-2}, ± S.D., *n*=10) of total macroinvertebrates recorded in the previous water release study between December 2010 – April 2011 and the current water release study between October 2011 and May 2012. Only sites continued from 2010/11 into the current study are shown.

Abundances of most of the major macroinvertebrate phyla and individual species changed significantly over the sampling period and in their distribution (Figures 9 and 12, Tables 7 and 8). Annelids were most abundant between Pelican Point (PP) and Noonameena (NM) in the North Lagoon, with abundances increasing at the latter site in March and May 2012 (Figure 11). Differences observed in annelid abundances between sampling survey and sites were irrespective of depth location (Table 7). The pollution indicator *Capitella sp.,* which was abundant at Long Point in October, decreased in abundance at this site over time and increased in later survey months at Noonameena, a site further south. Abundances of other polychaete species, including *Simplisetia aequisetis* and *Australonereis ehlersi*, also increased at sites in the North Lagoon between February and May 2012, with a notable peak in abundance of *A. ehlersi* at Noonameena in March 2012 (Figures 11 and 12). The predatory *Nephtys australiensis,* was found in consistently low abundances across the survey sites, mainly in the Murray Mouth (Figure 12, Table 8). Higher abundances of oligochaetes occurred in the Coorong in the later survey months, in comparison to earlier surveys where they were mainly confined to intertidal Murray Mouth locations in low abundances (Figure 12, Table 8).

Abundances of insect larvae (mainly chironomids) contributed to the overall high abundances of macroinvertebrates observed during the October and December 2011 surveys, especially at Pelican Point and Monument Road in the Murray Mouth (Figures 11 and 12). Abundances of chironomids dropped at most sites throughout the survey, apart from Sugars Beach (SB) and Hunters Creek (HC) intertidal sites and Tauwitchere (TAU) subtidal in May 2012, where a peak in chironomid larval abundance contributed to the observed overall increase in total benthos (Figures 9 and 12, Table 8).

Intertidal and subtidal sediments contained on average comparable abundances of macroinvertebrates in the current survey between October 2011 and May 2012 (Figure 9), whereas in previous surveys abundances were higher in subtidal than intertidal sediments at some sites (Figure 10). Abundances of *Capitella* sp. and oligochaetes did not differ significantly between intertidal and subtidal locations over the study period, whereas *S. aequisetis, N. australiensis, A. ehlersi,* amphipods and chironomid larvae all had significantly different abundances in either sampling location, however this varied over time. *Simplisetia aequisetis* was significantly more abundant in the subtidal in February and May (*post-hoc* pairwise tests, $P<0.05$), similar to *A. ehlersi* which had significantly higher abundances in subtidal sediments in October, February and March ($P<0.05$). Only *N. australiensis* had significantly higher abundances in the subtidal in all sampling months, except for December 2011 (*post-hoc* pairwise tests, $P<0.05$). Amphipods were significantly more abundant at intertidal locations in October, yet in subtidal locations from February to May 2012 ($P<0.05$). Chironomid abundances were higher in intertidal sediments in October, December and March ($P<0.05$).

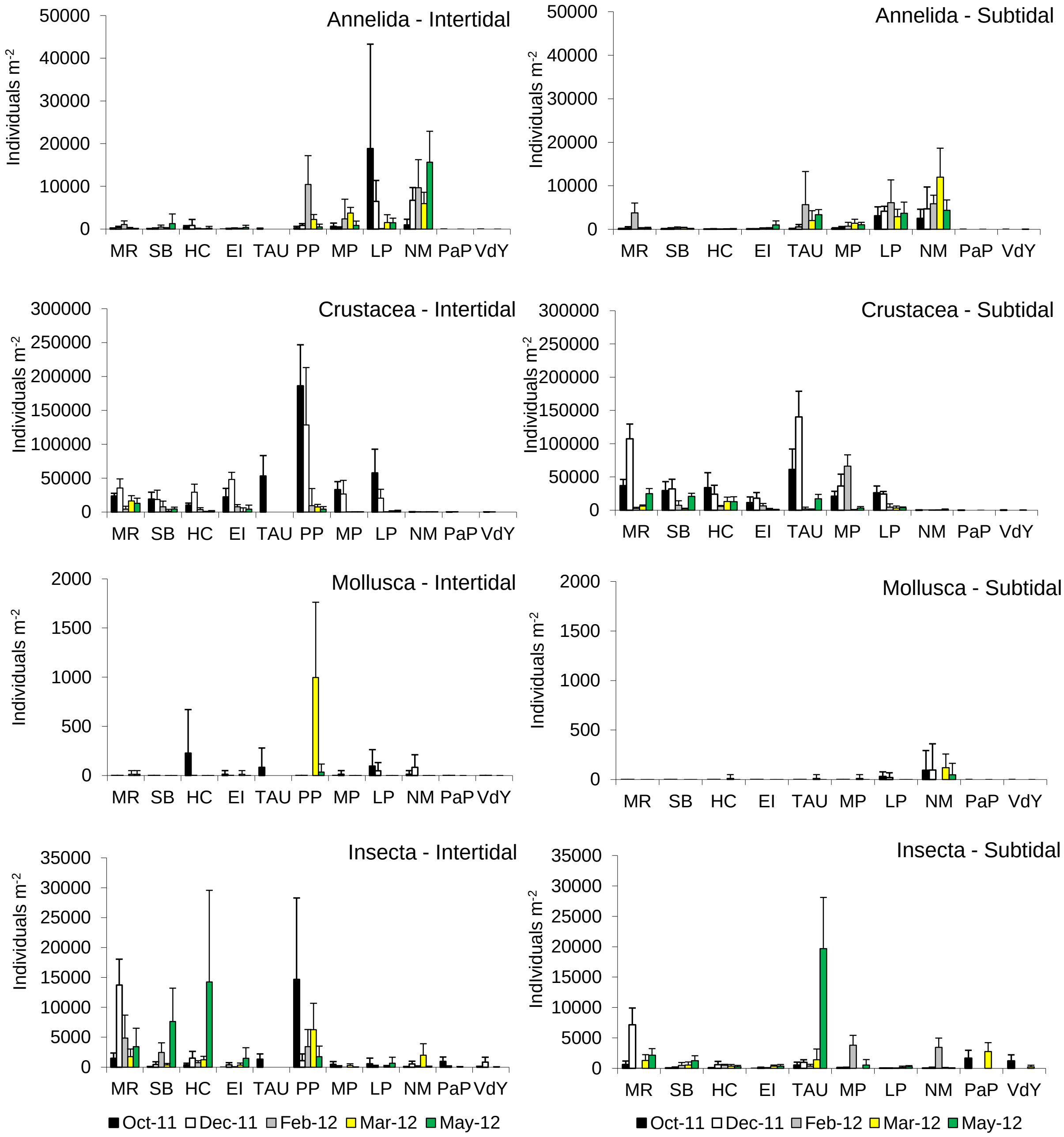
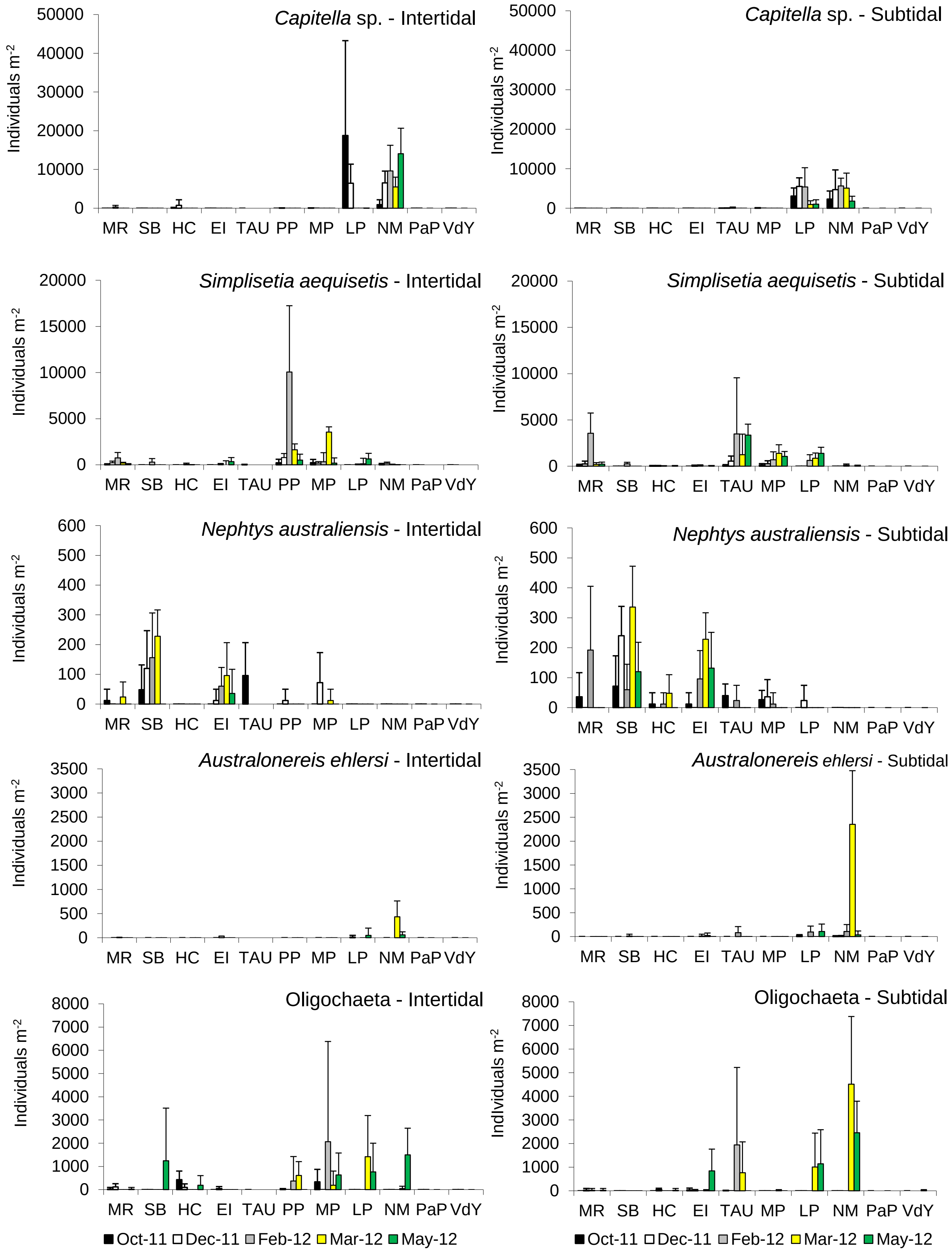


Figure 11: Average abundances (individuals m^{-2}, ± S.D., *n*=10) of major taxa (Annelida, Crustacea, Mollusca and Insecta) recorded at sampling locations in October 2011 (black bars), December 2011 (white bars, February 2012 (grey bars), March 2012 (yellow bars) and May 2012 (green bars) in both intertidal and subtidal locations at the water release sites. See Figure 2 for site location. Note that the scale of the y-axis varies substantially between taxa.


Capitella sp. - Intertidal
Capitella sp. - Subtidal
Simplisetia aequisetis - Intertidal
Simplisetia aequisetis - Subtidal
Nephtys australiensis - Intertidal
Nephtys australiensis - Subtidal
Australonereis ehlersi - Intertidal
Australonereis ehlersi - Subtidal
Oligochaeta - Intertidal
Oligochaeta - Subtidal
Individuals m-2
MR SB HC EI TAU PP MP LP NM PaP VdY
MR SB HC EI TAU MP LP NM PaP VdY
Oct-11 Dec-11 Feb-12 Mar-12 May-12

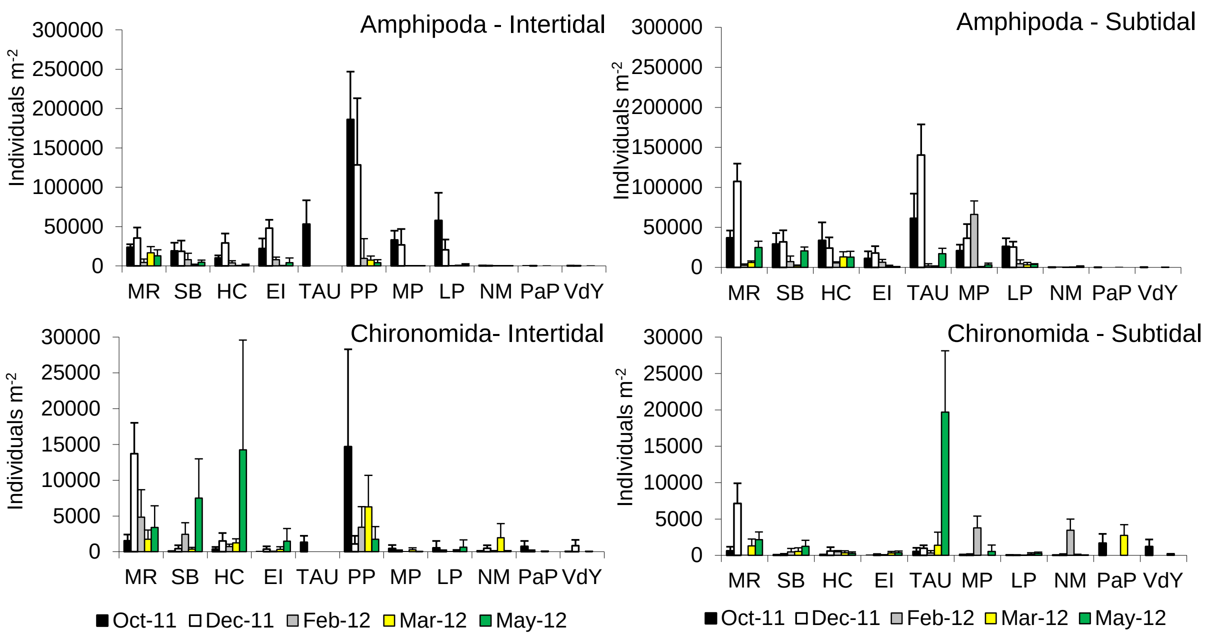


Figure 12: Average abundances (individuals m^{-2}, ± S.D., *n*=10) of *Capitella* sp., *Simplisetia aequisetis, Nephtys australiensis, Australonereis ehlersi*, Oligochaeta, Amphipoda and Chironomida recorded at sampling locations in October and December 2011, February, March and May 2012 in both intertidal and subtidal locations at the water release sites. Note that the scale of the y-axis varies substantially between taxa.

Table 8: Three-way PERMANOVA test results for effects of survey months, sites and depth on abundances of single macroinvertebrate species. Significant *P*-values are in bold.

Source	df	*Capitella* sp. *P* (perm)	*S.aequisetis* *P* (perm)	*N.australiensis* *P* (perm)	*A. ehlersi* *P* (perm)	Oligochaeta *P* (perm)
Survey	4	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Site	7	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Depth	1	0.6841	**0.0016**	**0.0001**	**0.0001**	0.1325
Survey * Site	28	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Survey * Depth	4	**0.0003**	**0.0001**	0.2306	**0.0008**	**0.0016**
Site * Depth	7	**0.0001**	**0.0001**	**0.0255**	**0.0001**	**0.0001**
Survey * Site * Depth	28	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Residual	720					

Source	df	Amphipoda *P* (perm)	Chironomida *P* (perm)
Survey	4	**0.0001**	**0.0001**
Site	7	**0.0001**	**0.0001**
Depth	1	**0.0001**	**0.0001**
Survey * Site	28	**0.0001**	**0.0001**
Survey * Depth	4	**0.0001**	**0.0001**
Site * Depth	7	**0.0001**	**0.0001**
Survey * Site * Depth	28	**0.0001**	**0.0001**
Residual	720		

4.4 Size frequency

The size frequency distributions of key polychaete species (*Capitella* sp., *Australonereis ehlersi, Simplisetia aequisetis* and *Nephtys australiensis*) revealed species specific recruitment patterns (Figure 13). *Capitella* sp. were found in similar sizes across all five sampling occasions and reached a maximum length of about 30 mm. For the nereid *A. ehlersi*, only few specimens of particular sizes were found, such as smaller worms in October and two specimens of large worms in December. However, given the sample size of this annelid, it is difficult to interpret these patterns as recruitment and could instead be a reflection of the sampling and burrowing depth of this species during the earlier survey months. High numbers of smaller sized *A. ehlersi*, were recorded in March and May 2012 and could indicate a possible recruitment event for this species in autumn (Figure 13). The nereid *S. aequisetis* occurred in a range of sizes from juveniles (< 6 mm length) to large worms (110 mm length) and had undergone a spawning event in the earlier surveying months, as indicated by the presence of small juvenile worms in October 2011. However, similarly small size classes were present for the remainder of survey months indicating that this species underwent a continuous recruitment across the entire study (Figure 13). The size range of the predatory polychaete *N. australiensis* was 5 mm to 75 mm, with larger worms found mainly in October and December 2011, while juvenile/smaller worms were found in February and March, but not May 2012, indicating a possible summer recruitment event (Figure 13).

Amphipods displayed a positive response to the continued water release, being present in several size classes, and frequently including egg-carrying females in October and December 2011, as well as in the May 2012 samples, showing that reproduction of these crustaceans had occurred prior to and during the survey project.

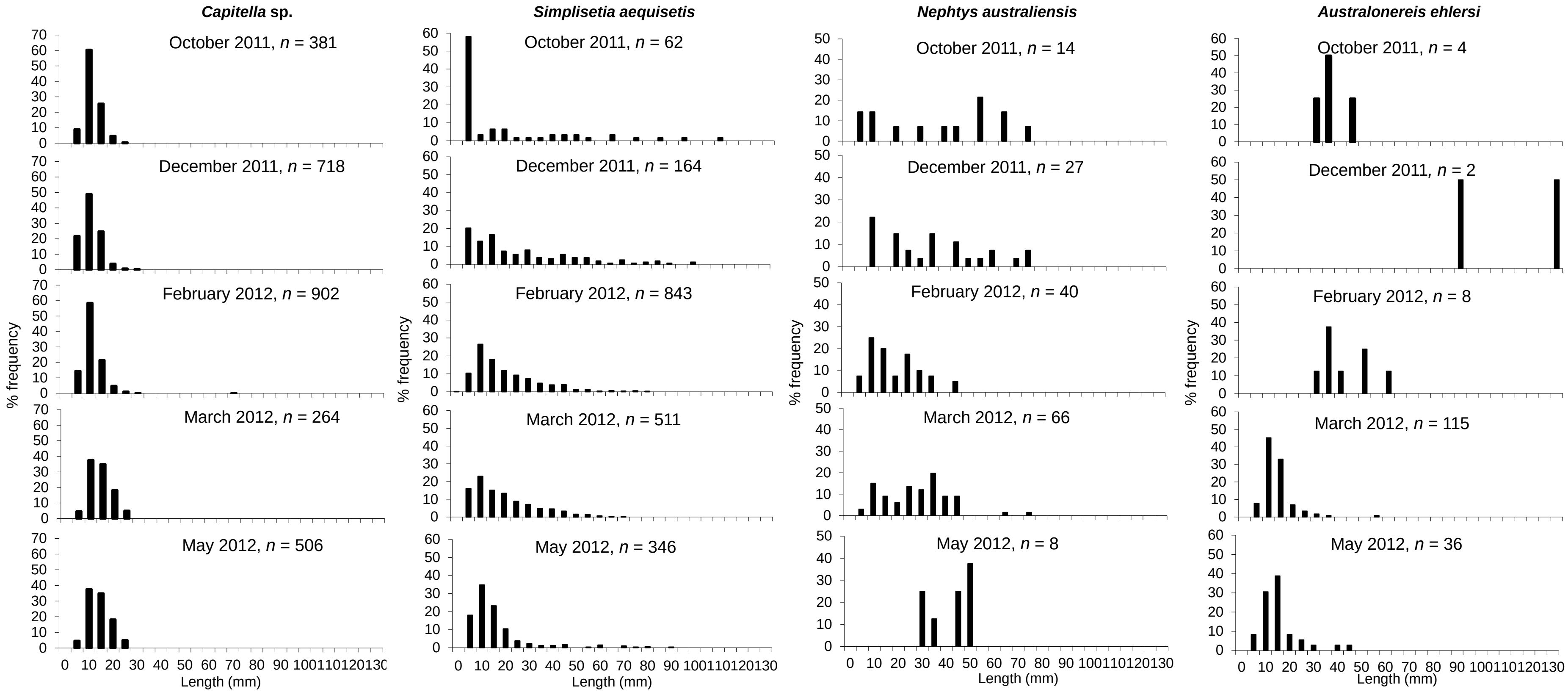


Figure 13: Percentage size frequencies (%) of length (mm) of the four most abundant polychaete species *Capitella* sp., *Simplisetia aequisetis, Nephtys australiensis* and *Australonereis ehlersi,* combined across all monitoring sites in the Murray Mouth and Coorong in October and December 2011, and February, March and May 2012.

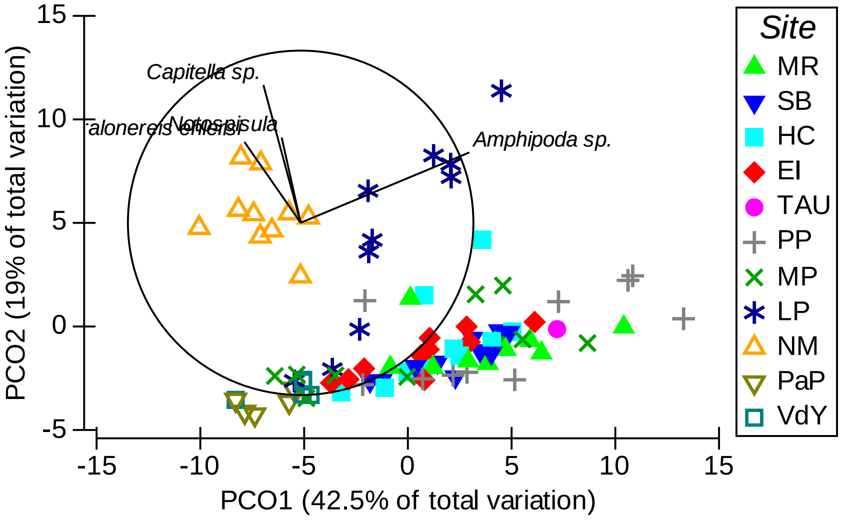
4.5 Macroinvertebrate assemblages

Over the study period between October 2011 and May 2012, the macroinvertebrate assemblages were significantly different between sites, water depth of sampling location and the five sampling months (Table 9). This was driven by a distinct difference between the benthic assemblages found at sites in the Murray Mouth and Coorong, especially Long Point (LP) and Noonameena (NM) in the North Lagoon (Figure 14). The species responsible for the assemblage differentiation between sites and depth over time included amphipods, which occurred at all sites, although in varying abundances, the polychaetes *Capitella* sp. and *A. ehlersi* and the mollusc *Notospisula* (Figure 14). The latter three species drove the distinction of the community assemblages at Long Point and Noonameena, due to their increasing abundances and current restricted distribution range at these sites (Figures 12 and 14, Table 4). The PCO plots also indicated similar macroinvertebrate assemblages at sites Parnka Point (PaP) and Villa de Yumpa (VdY) and sites in the Murray Mouth, however, this pattern was difficult to interpret as sampling at Parnka Point and Villa de Yumpa occurred only in October 2011 and March 2012. No clear separation of assemblages by depth location was apparent for any of the five sampling occasions in the PCO plot (Figure 14), with *post-hoc* pair wise tests revealing high variability in macroinvertebrate assemblages at intertidal and subtidal locations across sites and survey month ($P<0.001$).

Table 9: Result from PERMANOVA on the macroinvertebrate assemblages for the factors site (MR, SB, HC, EI, PP, MP, LP, NM), depth (inter- and subtidal) and month (October and December 2011, and February, March and May 2012). Significant P-values are highlighted in bold.

Source	*df*	MS	*Pseudo-F*	*P (perm)*
Survey	4	36339	79.228	**0.0001**
Site	7	60732	132.41	**0.0001**
Depth	1	18229	39.744	**0.0001**
Survey * Site	28	7298.2	15.912	**0.0001**
Survey * Depth	4	3139.4	6.844	**0.0001**
Site * Depth	7	5848.6	12.751	**0.0001**
Survey * Site * Depth	28	3537.2	7.711	**0.0001**
Residual	720	458.67		

a)



b)

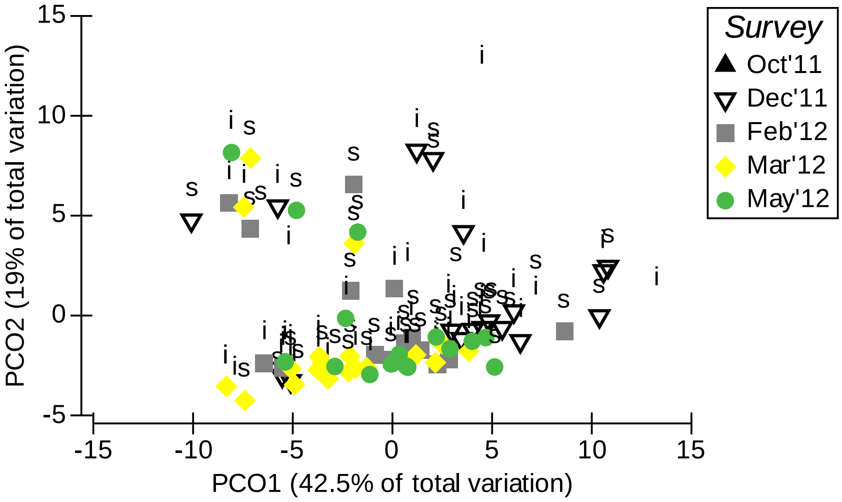
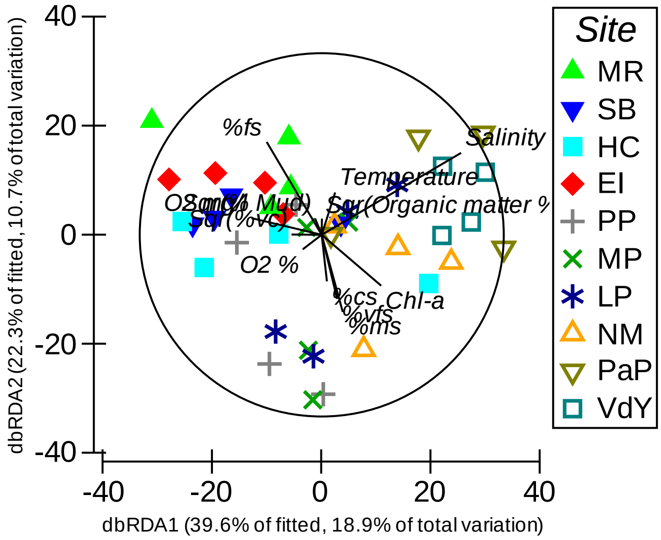


Figure 14: Principle coordinate analysis (PCO) of macroinvertebrate data showing macroinvertebrate assemblages in October and December 2011, and February, March and May 2012 for each of the sites (top figure) and the intertidal (i) and subtidal (s) location (bottom figure). The contribution of respective species (*Capitella* sp., *Australonereis* ehlersi, *Notospisula* and *Amphipoda* sp.) to the PCO axes is indicated by the vector overlay. See Figure 2 for site codes.

4.6 Patterns in community structure and environmental conditions

Changes in the macroinvertebrate assemblages between the Murray Mouth and Coorong in October 2011 and March 2012 were only partly explained by salinity (13 %, distance-based linear model DISTLM, Pseudo-F = 6.037, P = 0.0005) (Figure 15). Sediment organic matter (DISTLM, Pseudo-F = 2.615, P = 0.0202) and the sediment fraction of fine sands (DISTLM, Pseudo-F = 2.306, P = 0.0424) also significantly contributed to the observed patterns in macroinvertebrate assemblages during the water release, however explaining only 6 and 5 % respectively. Finer sediment was prominent in the Murray Mouth region, where insect larvae, amphipods and *Nephtys australiensis* occurred, yet their presence is unlikely to be determined by sediment properties alone. The higher sediment organic matter load at Noonameena fell together with the presence of *Capitella* sp. The first two axes together accounted for 61.9 % of the total variation that is explained by the model, with a moderate overall fit (R^2=0.47). A distinction of the benthic communities at sites in the Murray Mouth and Coorong, with some overlap with Pelican Point (PP) and Mark Point (MP), subject to environmental variables is clearly apparent (Figure 15).

a)



b)

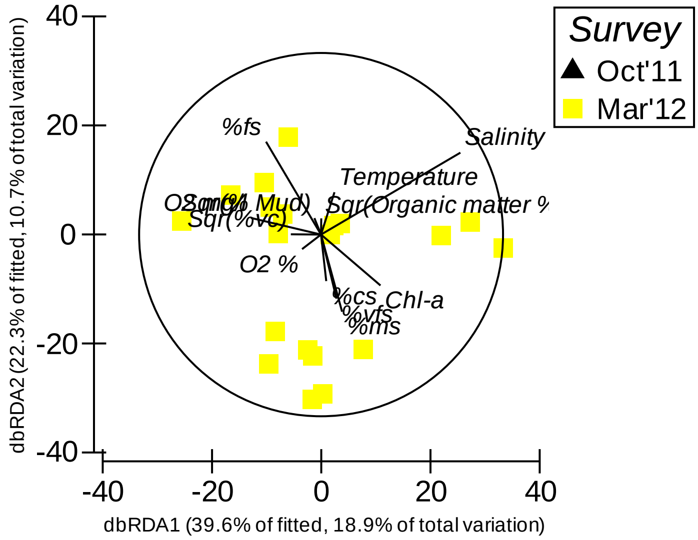


Figure 15: dbRDA (distance based redundancy analysis) illustrating relationships between environmental parameters and the benthic community a) across the study sites and b) between surveys October 2011 and March 2012. The vector overlay uses base variables of environmental data.

4.7 Tubeworm reefs

In December 2011, and February and March 2012, live tubeworms (*Ficopomatus enigmaticus*) were found in samples taken from reefs at Noonameena, Mark Point and Long Point, indicating that tubeworms have recovered in the North Lagoon. No samples were taken in May 2012, due to high water. Between December and March, tubeworms with mature gonads visible through the body wall, were present at sites, but currently no juvenile worms have been found. Some signs of new tube growth (increased width of white rim) was recorded throughout the study, with new reef growth being approximately 1 – 5 mm. Reef size parameters, measured only in December 2011 due to water levels, were found to vary in overall length 30 – 375 cm and height 10 – 54 cm. In February and March 2012, samples of reef structures located at Monument Road in the Murray Mouth were taken and live *F. enigmaticus* were found, although without eggs. Several other macroinvertebrate species were found amongst the reef fragments, including those commonly found in sediment samples (amphipods, *Capitella* sp., *Simplisetia aequisetis*, oligochaetes and chironomid larvae), and other species rarely encountered in our monitoring (e.g. bryozoans, *Paragrapsus gaimardii*).

5. Discussion

This report presents the response and recovery of macroinvertebrate assemblages following the significant high flows across the barrages from the Lower Lakes into the Murray Mouth since spring 2010. This report follows on from the previous water release survey, which documented the initial response of macroinvertebrates to restored flow between December 2010 and April 2011 (Dittmann et al. 2011a). The continued availability of water was beneficial for the restoration of macroinvertebrate populations, and the objectives of the monitoring were met (Table 10). However, no full recovery of macroinvertebrates has occurred yet, as several species that used to be found in the system were still missing, and abundances were still lower than in the past. The findings will be discussed in detail with regards to the objectives and hypotheses for this study.

Environmental conditions

Salinities were hypothesised to be not as reduced in the Murray Mouth with the continued, but lower flow volume of the water release as in 2010/11, but were expected to be lower in the Coorong than during the drought. The salinities recorded during this monitoring corroborate this hypothesised pattern, and also resemble the fall in salinities after a water release in 1983 (Geddes 1987). In the Murray Mouth, the salinity range recorded increased from 0.1-5 ppt in the study period from December 2012 to April 2011, to 0.2 – 29 ppt in the current survey from October 2011 to May 2012, thus changing from predominantly freshwater conditions when the peak flood volume passed through to more brackish conditions(Dittmann et al. 2011a). The wider mouth, naturally opened by the flood waters, could facilitate influx of oceanic waters from Encounter Bay, restoring some estuarine mixing near the Murray Mouth. In the Coorong, salinities continued to increase from brackish to hypersaline with distance to the Murray Mouth, as observed in the past (Dittmann et al. 2011b), yet had dropped by about 30 ppt or more, especially in the South Lagoon. This drop in salinities in the Coorong indicates that a natural reduction of the ultrahaline conditions in the South Lagoon may be possible.

Further improvements of environmental conditions since April 2011 were evident in the dissolved oxygen saturation, although some DO values were still around the trigger value for water quality set by the ANZECC guidelines. Microphytobenthic biomass, measured as chlorophyll-*a*, increased substantially across the system during the latter months of this monitoring, and can further indicate improved food availability for grazing macrobenthic organisms, as microphytobenthos and algal enriched suspended matter are primary food sources for intertidal benthic animals (Magni et al. 2006). However, higher chlorophyll-*a* values were recorded in March 2012, when macroinvertebrate abundances were lower than in spring 2011.

Sediment properties (grain sizes and organic matter content) varied a bit across the current monitoring period, similar to changes observed after floods elsewhere (Hermand et al. 2008), and with possible effects on macrobenthic communities (Norkko et al. 2002). However, the changes in sediment properties between months at the South Lagoon sites were more likely due to a slight deviation in sampling location between the first and the remainder of the surveys.

The monitoring of environmental changes with higher frequency of surveys is yielding a more detailed assessment of the effects of the water releases than those detected by annual surveys carried out for The Living Murray (TLM) program (Dittmann et al. 2012).

Flow related changes in macroinvertebrate communities

Worldwide, macroinvertebrates in estuaries are affected by drought and flood events (Cardoso et al. 2008; Mackay et al. 2010; Dolbeth et al. 2011). It had been therefore hypothesised that, in response to continued flow of water over the barrages in the Murray River estuary, the diversity and abundance of macroinvertebrates will be higher in comparison to values recorded in 2010-2011. The survey results did indeed reveal a substantial increase in diversity and abundances in 2011/12 compared to the surveys up to April 2011 (Dittmann et al. 2011a). A renewed decline in abundances in early 2012 could be due to environmental conditions or to effects from predation over the summer months by migratory shorebirds and benthic-feeding fish, for which macroinvertebrates are an important part of their diet (Wilson 1991; Kalejta 1993; Hilton et al. 2002; Earl, J. pers. comm.).

Direct effects from floods on macroinvertebrates in estuaries are known to include an initial reduction in macroinvertebrate diversity and abundance, as was observed in the Murray Mouth and Coorong (Grilo et al. 2011; Dittmann et al. 2011a). Yet, at all monitoring sites in the Murray Mouth and North Lagoon in 2011/12, a substantial increase in diversity and abundances of macroinvertebrates occurred, with a time lag of about one year after the commencement of the flow. An earlier study in the Murray Mouth and Coorong on a small release of freshwater (122 GL) in 2005 also indicated little immediate effect on the benthic community (Dittmann et al. 2006), but higher abundances and diversity over the following years (Dittmann & Nelson 2007). Time lags in the recovery of benthic communities after floods have been reported from estuaries around the world (Moverley et al. 1986; Currie & Small 2005). Yet, abundances of macroinvertebrates remained negligible in the South Lagoon in the second year after the flow resumed. A recent review by Borja et al. (2010) includes several examples where macroinvertebrate recovery was incomplete several years after a lagoon isolation was broken. While findings from the water release monitoring are indicating that a recovery is happening in parts of the system, a complete recovery may still rely on a longer time period of improved estuarine conditions.

Salinity is the main driver for patterns in the distribution and abundances of estuarine communities (Joyce et al. 2005; Pech et al. 2007), and continued to be the variable that best explained the pattern in macroinvertebrate communities detected in this monitoring. While estuarine macroinvertebrate species are usually tolerant of a wide range of salinities, extreme changes in salinities induced by drought and flood conditions can affect species specific patterns, as documented for the St Lucia estuary, South Africa, where comparable extremes occurred to those in the Murray Mouth and Coorong (Boltt 1975).

Following the overall reduction in diversity and abundances in 2010/11 (Dittmann et al. 2011a), several species have not yet successfully returned to the system a year after flows recommenced, including the micro-mollusc *Arthritica helmsi,* recorded with only one individual at Ewe Island in

October 2011. *A. helmsi* were abundant prior to drought years (Dittmann & Nelson 2007). On the other hand, the improved conditions within the system have lead to the occurrence of species rarely encountered previously, including the molluscs *Notospisula* sp. and *Soletellina alba*. These molluscs are common in other estuaries in southern Australia (Matthews 2006) and have been reported in the Coorong in the past (Geddes & Butler 1984; Geddes 1987). Flood related patterns in bivalve abundances are also known from the Mondego estuary in Portugal (Grilo et al. 2011).

Capitella sp. was one of the numerically dominant species in the current 2011/12 monitoring, yet largely confined to sites in the North Lagoon, where it was also found in the 2010/11 TLM monitoring (Dittmann et al. 2011b). Capitellids have been driving the succession after floods elsewhere, mainly in response to organic matter quality (Cardoso et al. 2007, Hermand et al. 2008), however in the current study *Capitella* sp. were not found at sites with exceptionally high organic matter content.

Changes in distribution ranges

Changes in distribution ranges of benthic species subject to drought and flood events were known from the St Lucia estuarine system in South Africa (Boltt 1975, Pillay & Perissinotto 2008, Cyrus et al. 2010) and it was hypothesised that the distribution of macroinvertebrates in the Murray Mouth and Coorong will change with the continued water release. Changes in the extent of the distribution ranges were detected for most of the macroinvertebrate species. This included both a spread in distribution further south into the North Lagoon, as well as a recolonisation of the Murray Mouth, obscuring the proposed refuge function which was observed in 2010/11 (Dittmann et al. 2011a). Furthermore, overall abundances were higher compared to the previous monitoring period and highest between the southern end of the Murray Mouth and the northern end of the North Lagoon. As the further sampling sites in the Coorong were only added for this monitoring period, analysis of distribution expansion into the North Lagoon relies on the current monitoring and information from TLM monitoring (Dittmann et al. 2011b).

Species previously found mainly in the North Lagoon continued to increase in abundance and spread to further sites such as Long Point and Noonameena (e.g. *Capitella,* oligochaetes). Other species found mostly in the Murray Mouth in the past now occurred in the North Lagoon (e.g. *Australonereis ehlersi*). Some species (e.g. *Simplisetia aequisetis*) extended their range into the North Lagoon as well as back into the Murray Mouth. *Nephtys australiensis* re-established at most sites in the Murray Mouth during the study period. Such species specific patterns in distribution ranges are reflective of the environmental tolerance of estuarine macroinvertebrates (Hutchings & Glasby 1985; Nanami et al. 2005; Dittmann et al. 2011a). Amphipods and chironomids are known to be tolerant towards a wide range of salinities (Kangas & Geddes 1984; Kokkin 1986) and continued to dominate the abundances at most sites, similar to the previous monitoring study (Dittmann et al. 2011a). High abundances of amphipods, as recorded in our monitoring, were also observed after earlier water releases in the Coorong in the 1980s (Geddes 1987). No further distribution range extension of macroinvertebrates into the South Lagoon was found.

Recolonisation of mudflats

During the extreme drought years (2007-2010), the low water level had led to long term exposure of nearshore sediments which were otherwise periodically submerged by tidal water movements or wind seiching. Prolonged periods of submergence and emergence as induced by a storm surge barrier had affected benthic abundances (Hummel et al. 1986; Hummel et al. 1988), and similar declines had been recorded over the TLM monitoring (Dittmann et al. 2010). It was thus hypothesised that the restored flow with more frequent inundation of the mudflats will lead to a recolonisation by estuarine macroinvertebrates. In the current survey between October 2011 and May 2012, intertidal and subtidal sediments contained comparable abundances of macroinvertebrates, whereas abundances were higher in subtidal sediments in 2010/11 (Dittmann et al. 2011a). This recolonisation of the previously desiccated mudflats is a further indication for recovery in the system.

Recolonisation of sediments which become hospitable again, can occur by adult migration or larval dispersal (Thrush & Whitlatch 2001). Earlier translocation experiments in the Murray Mouth and Coorong had shown increased abundances and diversity in sediment cores moved from high to low salinity, and from low to high submergence conditions (Rolston & Dittmann 2009). It was hypothesised that increased recruitment of macroinvertebrates will occur in response to the environmental changes induced by the water releases, and contribute to the recolonisation. The presence of juvenile specimens of several polychaete species had been observed in the previous water release 2010/11 (Dittmann et al. 2011a) and the more detailed size frequency analyses carried out in this monitoring period gave further and stronger evidence for continued recruitment as well as to the frequency or timing of recruitment. The information from size-frequencies can be further explored with the abundance and occurrence pattern of particular polychaetes species at particular sites, to understand the mechanisms of resilience in the system. Estuarine species can have flexibility in their life histories to take advantage of benign environmental changes (Sato 1999). The strong response to flow by amphipods detected in this monitoring and earlier studies (Kangas & Geddes 1984; Geddes 1987), appears to be supported by their high reproductive output, as egg carrying amphipods were recorded throughout the study period.

Response of tube worms

Renewed growth of tubeworms (*Ficopomatus enigmatus*) in the North Lagoon had been observed a year after the 2005 barrage release (Dittmann & Nelson 2007), and it was postulated that a similar response might occur after the current water release. While no live tubeworms had been found immediately after the flows commenced in 2010 (Dittmann et al. 2011a), renewed reef growth and live and reproducing worms were found throughout the current monitoring at multiple sites in the North Lagoon as well as at Monument Road. Observations also showed that the reef patches were inhabited by other macroinvertebrates, and distinct benthic assemblages in association with these reefs have been described before (Goldschmidt 2010). High water levels prevented continued assessment of live reefs or their occurrence at further locations like Mundoo Channel, and it is unknown whether the tubeworms could spread back into the Lower Lakes, where they created ecological and economic concern in the past (Dittmann et al. 2009).

Conclusions and recommendations for future monitoring

This monitoring captured the progressing recovery of macroinvertebrate populations and communities in the Murray Mouth and Coorong, following past declines induced by a prolonged drought and a flood event. The continued water release improved environmental conditions and facilitated a substantial increase in abundances and spread in distribution of several key macroinvertebrate species in the system. The high sampling frequency was essential to detect the patterns and changes in the course of the ongoing water release. Sediments throughout the North Lagoon and Murray Mouth are being colonised again, including the previously dried out mudflats, supported by recruitment of several species. However, diversity remained very low and several species present in the past were not or rarely found again. The positive responses to water release also included a regrowth of tubeworm reefs in the North Lagoon. The South Lagoon remains depauperate of macroinvertebrates, yet the substantial drop in salinities and range extension of several species further south into the North Lagoon, can facilitate a future recolonisation.

Several patterns detected in the current monitoring require further analysis. This includes the recorded decrease in macroinvertebrate abundances over summer, which can be due to environmental conditions or predation pressure from migratory birds and fish. Predator exclusion experiments are a possible approach to determine the driving process. The current sampling approach excludes an assessment of larger and mobile crabs or species inhabiting sediment in deeper water, which can be addressed by different equipment and strategies added to the monitoring. More attention to possible interactions between biotic components, such as macroalgal mats and macroinvertebrate densities, needs to be considered in the future, and links with further biophysical and biogeochemical data can improve the detection of causal relationships further. We found juvenile and mature specimens of several species and recruitment is an essential process for resilience in estuarine and coastal ecosystems (Dittmann et al. 1999). Experimental investigations into pathways for recolonisation (larval dispersal versus bedload transport of juveniles and adults) can elucidate the further capability of recovery and support future modelling of response mechanisms.

Table 10. Key findings (column highlighted) from macroinvertebrate monitoring in relation to the original objectives and key questions of the monitoring program. Note that only those objectives and questions pertaining to the macroinvertebrate monitoring are listed in the table below.

Monitoring Objective	Key Questions	Related Hypotheses	Key Findings
To assess the response of benthic macroinvertebrates to a minimum of 1000 GL being released over the barrages in 2011-2012, and to the continued water availability following the recent drought.	Are there indications of system recovery in 2011-2012 following the significant flows of 2010-2011 and further flows in 2011-2012?		• The substantial increases in abundances and also an increase in diversity indicate recovery of macroinvertebrates, although not all species have returned. A renewed small decrease of abundances occurred in the course of summer, which could be due to environmental conditions or shorebird/fish predation pressure.
	Will the reduced flows in 2011-2012 (compared to 2010-2011) result in salinity stratification being observed throughout the Murray Mouth and Coorong Lagoons?	Salinity in the Murray Mouth in 2011-2012 will not be reduced as significantly as observed in 2010-2011. Salinity in the North and South Lagoons will be maintained at levels lower than measured from 2008-2010.	• Salinities in the Murray Mouth (MM) were not as low as in 2010/2011 after the large flow. Brackish conditions were recorded in spring 2011, but dropped again to near freshwater by May 2012. • Salinities in the North Lagoon (NL) changed with distance to the MM. At Mark Point, salinities were brackish to marine in spring/summer 2011/2012, while still being hypersaline at the sites further south in the NL. Yet, in March and May, brackish conditions were recorded throughout the NL. • Salinities in the South Lagoon (SL) continued to drop, from 70-80 ppt in October/December 2011 to 38-58 ppt by March and May 2012. • There was little indication for stratification of salinities based on our measurements.
	Have macroinvertebrate diversity and abundances increased at all sampling sites following the 2010-2011 barrage flows into the Coorong?	Benthic macroinvertebrates will recolonise tidal mudflats in the Murray Mouth region. Benthic macroinvertebrates will continue to maintain refuge in the North Lagoon but will remain absent (apart from insect larvae) in the South Lagoon.	• Species numbers and diversity values, which were very low in the February-April 2011 surveys, had increased by spring 2011. • Diversity was similar between inter- and sub-tidal locations and increased over the survey time 2011-2012. • All sites in the MM and NL showed a substantial increase in macroinvertebrate abundances in October and December 2011 compared to summer 2010/2011, while abundances remained negligible in the SL. • This increase in spring 2011 was followed by a renewed decline in abundances in early 2012, which was observed at all sites. • As abundances in MM were higher compared to 2010/2011, the potential refuge function of the NL was not as pronounced.

	Have species further increased their ranges beyond that observed in 2011-2012?	Benthic macroinvertebrates will continue to maintain refuge in the North Lagoon but will remain absent (apart from insect larvae) in the South Lagoon.	• There were species specific patterns in distribution ranges; • *Capitella* remained largely confined to the NL (mainly Long Point and Noonameena). • *Simplisetia* had a disjunct distribution occurring at Monument Road and then again in higher abundances from Pelican Point into the NL, with signs of further spreading into the NL. • *Australonereis* is spreading further into the NL. • *Nephtys* shows some spread into the NL, but is mainly re-establishing in the MM. • Amphipoda have re-established throughout the MM and NL to Long Point. • Chironomid larvae were abundant at sites in the MM, but occurred throughout the system.
	Will benthic macroinvertebrates increase abundance and diversity in the tidal mudflats of the Murray Mouth and North Lagoon?	Benthic macroinvertebrates will recolonise tidal mudflats in the Murray Mouth region.	• Species numbers of macroinvertebrates had increased in spring 2011, particularly at the intertidal sites, when compared to autumn 2011. • Average macroinvertebrate abundances were often similar, if not higher at the intertidal than subtidal locations, indicating that the previously desiccated mudflats have been recolonised.
	Do *Ficopomatus engimaticus* reefs in the North Lagoon exhibit new growth?	*Ficopomatus engimaticus* reefs show renewed growth in the Coorong, and possibly in the Murray Mouth region.	• *Ficopomatus enigmaticus* occurred throughout the North Lagoon and live specimens were also found at Monument Road in February and March 2012. • Specimens collected from reef fragments contained sexually mature worms. • New growth was recorded on tubeworm reefs at all three sites in the North Lagoon in December 2011.

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