Benthic Macroinvertebrate survey 2012-13:

Lower Lakes, Coorong and Murray Mouth Icon Site

Report for the
Department of Environment, Water and Natural Resources
and the Murray-Darling Basin Authority

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Photo on cover page:
Bioturbation in mudflat sediment at Pelican Point, December 2012, with burrows of the polychaete *Simplisetia aequisetis* (one specimen visible)

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Appendices provided electronically

The following Word and Excel data files were supplied:

Appendix 1: Sampling sites, dates, times and coordinates
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1. Executive summary

- This report presents findings from the 9th year of Condition Monitoring for 'The Living Murray' program, with an assessment of the benthic macro-invertebrates in the Coorong, Lower Lakes and Murray Mouth. Aquatic macro-invertebrates were also assessed in the Lower Lakes. The main objectives were to evaluate the food availability for migratory waders and other higher trophic level organisms, assess the habitat value of mudflats for the entire ecosystem and migratory birds in particular, and identify whether changes in key species indicate or reflect environmental changes.
- The survey in summer 2012/13 was carried out during the third year of continuous river flow through the barrages since flows resumed in spring 2010. Sampling occurred between late November 2012 and January 2013. While water levels were still high in the Lakes and Coorong, larger mudflat areas were exposed in December. Samples were taken using hand-held corers, however an Ekman grab was used where water levels were high. Four of the sites around the Lower Lakes were discontinued. All methods applied were the same as in previous years and described in the Condition Monitoring Plan for macroinvertebrate and mudflat monitoring. Sediment horizons could not be differentiated for samples taken by the Ekman grab, and no horizons were separated for the Lower Lakes samples. These slight changes to the design for the Lower Lakes allowed the incorporation of dip net sampling for aquatic macroinvertebrates.
- Improvements in environmental conditions were detected in all study regions of the Lower Lakes, Murray Mouth and Coorong. While the gradient from brackish conditions in the Murray Mouth to hypersaline conditions in the Coorong continued, salinities were the lowest throughout the system since monitoring started in 2004. Salinities were also lower than in the previous five years at the sites around the Lower Lakes. Dissolved oxygen saturation was, however, still below the trigger value given by the ANZECC guidelines at several sites. In the sediments, grain size compositions and organic matter contents had remained largely unchanged over the years. Microphytobenthic biomass (indicated by chlorophyll-*a*) was higher than during the previous two years at the sites in the Murray Mouth and most of the North Lagoon, as well as several sites around the Lower Lakes.
- In the Murray Mouth and Coorong, diversity of benthic macroinvertebrates was highest between Monument Road and Noonameena, while remaining low at Parnka Point and sites in the South Lagoon. Diversity had increased especially at Pelican Point and Mark Point compared to the drought period, yet remained low at other sites because of the numerical dominance of amphipods and chironomids. Most of the key species of macroinvertebrates were more widely distributed, indicating that after the contraction of their distribution ranges during the drought, they are now spreading again throughout the area.
- Abundances and biomass were comparable or higher than in previous monitoring periods at most of the sites in the Murray Mouth and North Lagoon, but less so in the South Lagoon. Most of the benthic macroinvertebrates occurred in the surface layer of the sediment, accessible as food for short-billed waders. In the Murray Mouth, larger polychaete worms occurred again in higher numbers and biomass in deeper sediment layers, providing prey for shorebirds with longer beaks. Molluscs were, however, rarely encountered during this monitoring. The communities in the Murray Mouth, North and South Lagoon were distinct again from each other, and salinity, sediment organic matter and chlorophyll-a could explain some of this differentiation. Trajectories of change occurred in each region, and community differences during the drought and flow years were identified, yet communities had not fully returned to those present nearly a decade ago.
- In the Lower Lakes, species numbers of benthic macroinvertebrates had increased compared to the previous year, with the exception of sites around the central reaches of Lake Alexandrina (Milang, Tolderol, Poltalloch and Narrung). These four sites, which had coarser sediment with low organic matter content, also had very low abundances and biomass and were distinct from the community found at the other sites. Amphipods, chironomid larvae and oligochaetes accounted for most of the individuals in sediment samples around the Lower Lakes. Sites in the lower reaches of

Lake Alexandrina, but also at Boggy Lake, had higher abundances and biomass of macroinvertebrates than found in the last two years. Over longer timeframes, macroinvertebrate abundances and biomass were maintained within the range of values recorded previously. Site specific variability was high at the sampling sites around the Lower Lakes and no regional differentiation in diversity, abundances or biomass could be detected.

- Dip net samples obtained a high diversity and abundance of aquatic macroinvertebrates in the Lower Lakes, including at the sites scarcely populated by macroinvertebrates in the sediment. The use of corer and dip net sampling emerged as complementary. Amphipods, freshwater shrimp, chironomids and various larval or adult insect taxa were abundant components of aquatic macroinvertebrate communities, which were distinct between Lake Albert and Lake Alexandrina.
- The recovery of macroinvertebrates that started to become apparent in the 2011/12 monitoring was manifested by findings of this recent survey. This recovery has been particularly pronounced in the Murray Mouth and northern section of the North Lagoon, where benign environmental conditions prevailed. Yet, no full return to a possible reference condition after the small water release in 2005 has happened, indicating that recovery after a prolonged period of no flow is taking several years.

2. Introduction

As part of The Living Murray Program, the objectives of the Condition Monitoring include an assessment of the Lower Lakes, Murray Mouth and Coorong icon site, its habitat values and state or recovery of key species (SAMDBNRM 2009). Macroinvertebrates are a key component in the system, responding quickly to environmental changes and playing an important role in estuarine and aquatic food webs as prey items for higher trophic levels, such as birds. The Lower Lakes, Murray Mouth and Coorong are a Ramsar site, yet the extreme environmental changes over the last decade have affected this wetland of international importance (Wainwright and Christie 2008, Paton et al. 2009, Kingsford et al. 2011). Following the Millennium Drought (Leblanc et al. 2012), flow resumed in spring 2010 and has been continuous since, albeit with some fluctuation with season and water management (Figure 1).

This long-term monitoring, which commenced in 2004, has so far captured the loss of habitat quality and macroinvertebrate species and populations during the drought, and the delayed start of recovery. While no improvements in macroinvertebrate communities were seen in the first year since flows resumed (2010/11), signs of recovery were apparent in the monitoring data from the 2011/12 survey (Dittmann et al. 2012). Opportunistic and highly adaptable species such as chironomid larvae and amphipods had shown a strong response to the flow changes, occurring with very high densities, similar to responses after earlier water releases (Geddes 1987, Dittmann et al. 2006, 2012). Yet, the slow recovery of other macroinvertebrate species, which previously occurred in the system, raised concern that the prolonged drought could have affected the resilience of these taxa. Further monitoring was thus needed to assess the response and condition of the system under several years of continuous flow.

Flow variations in estuaries are common, subject to weather patterns and climatic variability, human land use or water regulation activities in the hinterland (Valiela 2006). Effects of flooding on estuarine macroinvertebrates are reported from many parts of the world, indicating that the intensity, frequency and timing of floods are important determinants of the macroinvertebrate response, as well as their life history characteristics (Moverley et al. 1986, Cardoso et al. 2008, Miserendino 2009, Grilo et al. 2011). Increasingly, restoration of riverine and estuarine ecosystems are carried out and macroinvertebrate responses measured, often showing quite complex and long recovery periods.

This survey documents macrobenthic response to the continued flow restoration in the Murray Mouth, Coorong and Lower Lakes. To allow comparisons over time, the same sampling sites were surveyed as in previous years, using the same methods. Some sites in the Lower Lakes were discontinued to add in aquatic macroinvertebrate monitoring. All sites correspond with ongoing shorebird monitoring and most overlap with sites for the assessment of further parameters (e.g. fish) for the TLM monitoring.

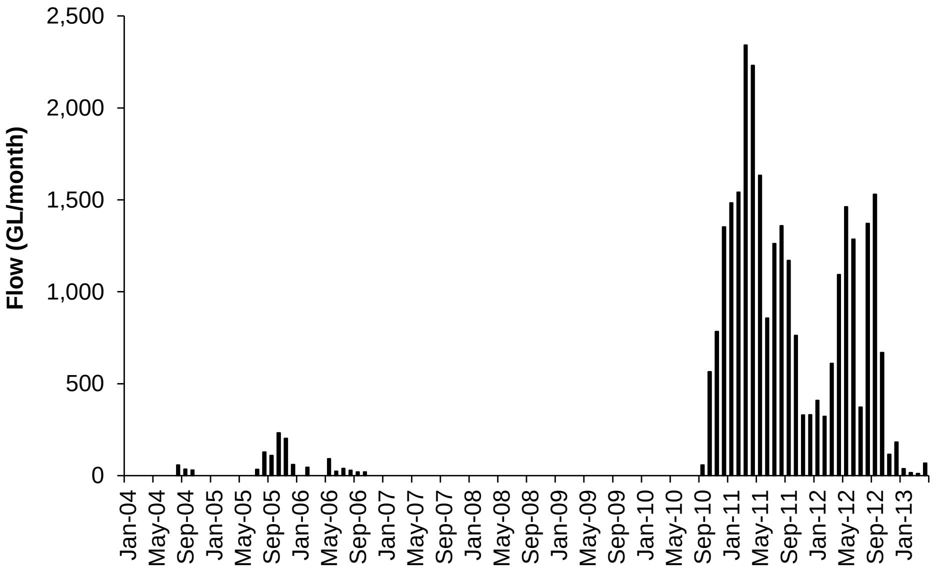

Figure 1: Monthly barrage flow from the Lower Lakes into the Murray Mouth and Coorong during the years of macroinvertebrate and mudflat monitoring. Based on data from the MDBA and SA Water (2013).

This report presents findings from the 9th year of condition monitoring for the assessment of macroinvertebrate food availability for shorebirds and other higher trophic level organisms prevalent in the Murray Mouth, Coorong and Lower Lakes. It contributes to a number of condition monitoring objectives for 'The Living Murray' program, which are; I-1 – 'Maintain or improve invertebrate populations in mudflats', M-1 – 'Facilitate frequent changes in exposure and submergence of mudflats', M-2 – 'Maintain sediment size range in mudflats', and M-3 – 'Maintain organic content for mudflats' (SAMDBNRM 2009). It also contributes to W-1 – 'Assessment of estuarine conditions between Goolwa Barrage and Pelican Point' with measurements of water quality taken at the time of invertebrate monitoring in late spring/early summer. The report is structured around the targets with detailed data analysis provided as supplementary material (table and figure reference prefix SM-).

To deliver the targets (I-1, M-1 to M-3) of 'The Living Murray' program, this report addresses the following questions for the spring/summer of 2012/13:

1) To describe the current environmental conditions of the Murray Mouth, Coorong and Lower Lakes
2) To determine the spatial and temporal distribution of macroinvertebrates, in terms of species composition, diversity, abundances and biomass in the Murray Mouth, Coorong and Lower Lakes since 2004.
3) To explore the relationship between environmental parameters and macroinvertebrate assemblages.

The findings will be analysed in comparison to investigations from previous years and with other estuarine systems. The analyses of several sedimentary and water quality parameters will assist in the interpretation of possible causes of temporal and spatial changes at the sites. It is expected that an improvement in benthic assemblages has occurred in this third summer since flows were restored.

3. Materials and Methods

3.1 Sampling sites and dates

The sampling design was continued from the previous monitoring and as described in the LLCMM Icon Site Condition Monitoring Plan (SAMDBNRM 2009), although in the Lower Lakes, five of the sites added in 2008 during the drought were discontinued to allow more in-depth sampling at the remaining sites. Benthic macroinvertebrate fauna was sampled at a total of 26 sites in the Murray Mouth region (5 sites), Coorong (6 sites) and Lower Lakes (15 sites) during the 2012/13 summer survey (Figure 2). Sampling occurred between the 27th November 2012 and the 22nd January 2013 (SM-Table 1), with the Murray Mouth and Coorong sampled in late 2012, and the Lower Lakes in January 2013.

Sites sampled in the Murray Mouth, between the Goolwa Barrage and the southern end of the Tauwitchere Barrage, included sites 1 (Monument Road), HC (Hunters Creek), 4 (Mundoo Channel), 6 (Ewe Island), and 20 (Pelican Point). Sites 22 (Mulbin Yerrok, near Long Point), 26 (Noonameena) and 24 (Parnka Point) are located in the North Lagoon of the Coorong. The South Lagoon sites were 19 (Villa de Jumpa), 16 (Jack Point), and 14 (Loop Road south of Salt Creek) (Figure 2).

Sampling sites in the Lower Lakes were differentiated by their location in Goolwa Channel (sites L1 inside of the Goolwa barrage, L7 opposite Currency Creek on Hindmarsh Island); Lake Alexandrina (10 sites) and Lake Albert (3 sites) (Figure 2). In the results, sites in Lake Alexandrina are depicted by increasing distance from the barrages; site L2 east of the regulator at Clayton, L16 inside Mundoo Channel barrage, L17 inside Ewe Island barrage, L6 inside Tauwitchere barrage near Pelican Point, L11 Loveday Bay, L9 Narrung, L4 Milang, L5 Poltalloch, L3 Tolderol, and L18 Mosquito Point at Boggy Lake. Sites in Lake Albert are L13 (Seacombes), L8 (Waltowa) and L12 (Vanderbrink). For analysis of longer term developments in the Lower Lakes, sites from previous monitoring (Currency Creek (CC1), at the confluence of the Tookayerta and Finniss (TF), Teringie (L10), Eckerts Road (L15) and Albert Station (L14)) were also considered.

Water levels in the Murray Mouth were lower in the 2012/13 survey period than in the previous survey, exposing mudflats both in the Murray Mouth and Coorong. At sites such as Ewe Island, about 200 m of mudflat were emerged at low tide. In the North Lagoon, the area of mudflats was narrower, with 1 to 10 m of sediments exposed from shore. Water levels in the North Lagoon were much more variable subject to inflow of water from barrages (DEWNR 2013), wind and tides (in the northern reaches). In the Lower Lakes, water levels had receded in January to below 0.65 m AHD, but no sediments were exposed.

3.2 Environmental parameters

Environmental characteristics (water temperature, salinity, oxygen content and saturation, sediment grain size, organic matter and chlorophyll-*a*) were recorded to analyse whether sediment size ranges and organic matter are maintained (objectives M-2 and M-3 of the Condition Monitoring), characterise the salinity regime at the time of sampling (objective W-1), and identify environmental parameters affecting the macrobenthic communities (SAMDBNRM 2009).

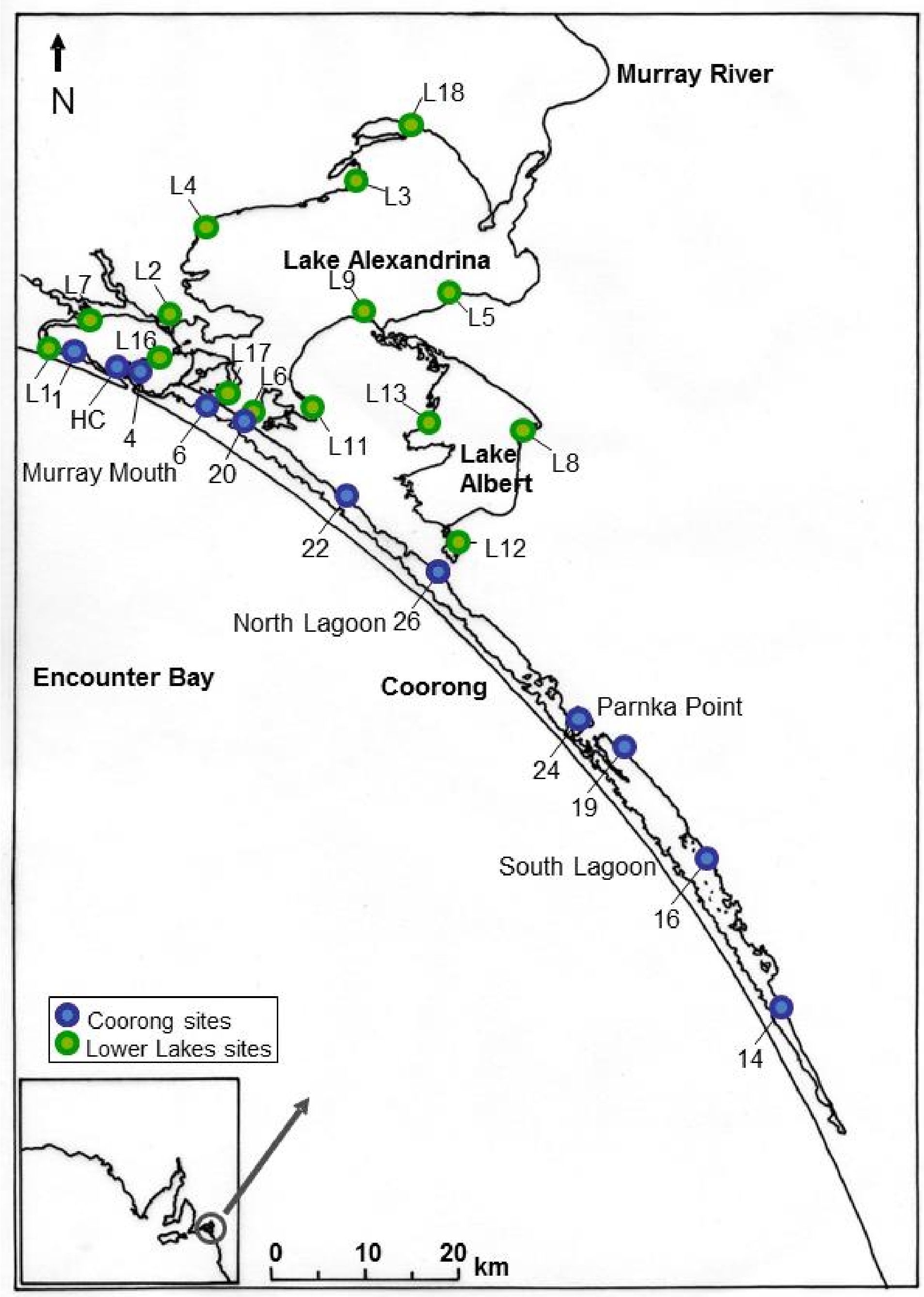

Figure 2: Location of study area and sampling sites for each region of the Murray Mouth, Coorong and Lower Lakes from the survey undertaken during the late spring/summer of 2012/13.

Water quality characteristics (temperature, salinity, dissolved oxygen concentration, oxygen saturation and pH) of the water overlying the sediments were measured *in situ* using hand held electrodes. Measurements were taken with a YSI 85 and/or a TPS WP-81 (for pH, temperature and salinity) and a WP-82Y (for dissolved oxygen concentration and saturation) electrode. Salinity was also measured using a refractometer at some sites. Wherever possible, two sets of electrodes were used to ensure correct measurements, given past experiences with faulty oxygen probes. However, due to instrument availability this was not possible at all sites. Three measurements were taken with either electrode for each parameter.

Sediment samples were obtained from each site for the analysis of grain size, organic matter content and chlorophyll-*a* (as a proxy for microphytobenthic biomass). All sediment samples were stored on ice and frozen upon return to the laboratory and until further analysis. Samples for sediment organic matter were extracted using a cut off 3 mL syringe (0.64 cm^2 surface area). To account for spatial variation, three replicate samples were taken and analysed separately, to calculate mean and standard error for organic matter. 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 ºC and then burnt in a muffle furnace at 450 ºC for 5 hrs.

For sediment grain size, samples were taken using a cut-off 60 mL syringe (surface area 6.6 cm^2). Three replicate samples were taken per site. Samples were stored on ice in the field and frozen until further analyses in the laboratory. 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 percentages of various particle sizes were obtained from the Mastersizer output. Sediment sorting was calculated according to $S_o = (P_{25}/P_{75})^{1/2}$, based on the metric scale.

For chlorophyll-*a*, three replicate samples were taken per site using a 5 mL vial inserted 1 cm into the sediment. Subsequently, 5 mL of methanol was added to extract the chlorophyll, and the vial was vigorously shaken before being wrapped in aluminium foil (Seuront and Leterme 2006). Upon return to the laboratory, vials were frozen for later analyses 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.

3.3 Macrofauna

To investigate macroinvertebrate species composition and abundance within the sediment, handheld PVC corers (83.32 cm^2 surface area) were used to sample the benthos in the littoral zone. At three sites in the Lower Lakes where the water level was too high to use handheld corers, samples were taken with a small benthic Ekman grab (225 cm^2 surface area) inserted approximately 10 cm into the sediment. The Ekman grab was deployed by a person wading into the water, at sites L7 (Hindmarsh Island, Goolwa Channel), L6 (Pelican Point, Lake Alexandrina) and L8 (Waltowa, Lake Albert).

Ten replicate samples were taken per site, scattered haphazardly between the mid to low shore levels around the respective water margin. Sediment cores were divided into two depth horizons to assess differences in food availability for birds with different bill lengths (Zwarts and Wanink 1993). To assess the vertical distribution of benthic fauna, sediment samples taken in the Murray Mouth and Coorong with the corer were split into two horizons (0-3 cm and 3-15 cm). Samples taken with the Ekman grab could not be split into horizons, however, this was not an issue for analysis as no horizons were separated for samples taken in the Lower Lakes in this survey.

At the Lower Lakes sites, additional dip net samples were taken, using a triangular net (surface area 0.11 m^2) with 0.5 mm mesh size. At all sites, dip net samples were taken within areas where reeds or other aquatic vegetation were present, over a distance of 2 m for 20 seconds. Five replicate dip net samples were taken per site and analysed for diversity and densities of aquatic macroinvertebrates. These dip net samples were preserved in the field in 70% ethanol prior to analysis.

All benthic samples were sieved through a 500 µm mesh size in the field before identification and counting of live organisms in the laboratory. Specimens were identified to the lowest possible taxonomic level and individual numbers of each species were counted. Amphipods were not differentiated to species, as shorebirds are unlikely to be selective towards particular amphipod species as prey. All polychaete specimens with a complete anterior region (prostomium) were considered for abundance counts, while polychaete fragments were included with complete specimens for biomass determination. For insects, larval and pupae stages were recorded, while all winged life stages were excluded, as they are highly motile and not part of benthic macrofauna.

All identified organisms were preserved in 70 % ethanol before further biomass determination. Biomass was analysed for the total benthos per replicate sample and not differentiated per phyla, given the understanding on the main taxa contributing to the biomass gained in previous monitoring. Each sampled was dried in an oven at 80 ºC until constant dry weight (d.w.) was achieved (at least 24 hours). Samples were then placed in a muffle furnace at 450 ºC for 5 hours. Samples were removed from the furnace and cooled in a desiccator before final weighing. The weight after burning was subtracted from the dry weight to obtain biomass as ash free dry weight (AFDW). No biomass was determined for the dip net samples.

3.4 Data Analysis

Data are presented separately for the Murray Mouth and Coorong, and the Lower Lakes, and compared according to their up and downstream locations of the barrages. The regions differentiated in the analysis designs correspond to the ‘sub-regions’ in the Condition Monitoring Plan. These are the Murray Mouth, North Lagoon and South Lagoon of the Coorong; and for the Lower Lakes the Goolwa Channel, Lake Alexandrina and Lake Albert. The Goolwa Channel was separated as a further sub-region for Lake Alexandrina because of its different history with the Goolwa Channel Water Level Management Project.

To assess whether LLCMM condition monitoring target parameters were maintained or improved, comparisons were carried out using all previous survey data since December 2004. With the extreme changes in environmental conditions over that time span and the lack of quantitative historic data, reference state or dynamics are difficult to define. The approach taken here was to divide the entire monitoring time span into three periods characterised by different flow conditions: 2004-2006 with no or small flow (in 2005), 2007-2009 being the years of the extreme drought without water releases from the barrages, and 2010-2012 as the period of flow, which commenced in spring 2010. Some parameters, such as Chl-*a*, or sites (e.g. around the Lower Lakes) were added later (2007 and 2008) and temporal comparisons respectively adjusted.

The design used for statistical analyses of environmental or biotic data was regions (fixed factor) and sites nested within regions (random factor), with the survey year as a further fixed factor for temporal comparisons. The analysis for temporal differences of environmental parameters was based on average values per site at each survey, due to a lower number of replicates in the data set prior to 2007, and a design using the survey year (fixed factor) and region (fixed factor), with sites as replicates for each respective region. This design was also used for testing diversity indices.

Tests were carried out using PERMANOVA (permutational analysis of variance) using the software PRIMER v6 with PERMANOVA add-on. Prior to analysis, environmental and biotic data were transformed as needed. Environmental data were square root or log(x+1) transformed as needed and normalised when parameters with different units were included in the analysis. Similarities of sampling sites based on environmental factors were explored with principle component analyses (PCA), with vector overlays for defining variables, or trajectories to display temporal change. Tests of homogeneity of dispersion (PERMDISP) were included for environmental and biotic data to assess variability within factor levels. For environmental data and univariate analysis of biotic data (e.g. tests for differences in total abundances or total biomass), Euclidean distance was used to create the resemblance matrix. In all multivariate analyses of biotic data, Bray-Curtis similarity was used, with a dummy value of 1 added when many zero values occurred in the data.

To explore differences in macroinvertebrate communities, principal coordinate analysis (PCO) plots were used with vector overlays (Spearman correlation) to illustrate species contributing to the differentiation of communities along the PCO axes. PERMANOVA test were carried out for community differences, following the designs explained above. SIMPER analyses revealed the species contributing most to the similarity within sites and those differentiating sites. ANOSIM (Analysis of similarity) tests were run between regions for each year to obtain the test statistic Global R, indicating community differences. To explore links between macroinvertebrate assemblages and environmental data, distance-based linear models (DISTLM) were calculated and visualised using distance-based redundancy analysis (dbRDA). Some sites or parameters had to be excluded from these analyses due to missing values for environmental factors (due e.g. to faulty oxygen probes or cases of autocorrelation). Detailed test outcomes are provided in the Supplementary Material.

4. Results – Murray Mouth and Coorong

4.1 Mudflat habitats in the Murray Mouth and Coorong

4.1.1 Salinity regime and water level

In the third year after flow commenced, a regime shift in salinity was noticeable. The Murray Mouth and Coorong continued to be characterised by a steep salinity gradient during the 2012/13 survey (Figure 3, SM-Table 2), with fresh to brackish water in the Murray Mouth and northern reaches of the North Lagoon of the Coorong, and increasingly marine to hypersaline conditions into the South Lagoon. But, salinities recorded at the study sites in December and January 2012/13 were the lowest measured during the entire TLM monitoring period since 2004 (Figure 3). The condition monitoring target W-1: 'Establish and maintain variable salinity regime with >30% of area below sea water salinity concentrations in Murray Mouth Estuary and North Lagoon' has thus been exceeded with all of the Murray Mouth and about half of the North Lagoon area having salinities below sea water during the sampling period (Figure 3). Yet, this is based on spot measurements taken during the macroinvertebrate sampling, and short term variability in salinity does occur.

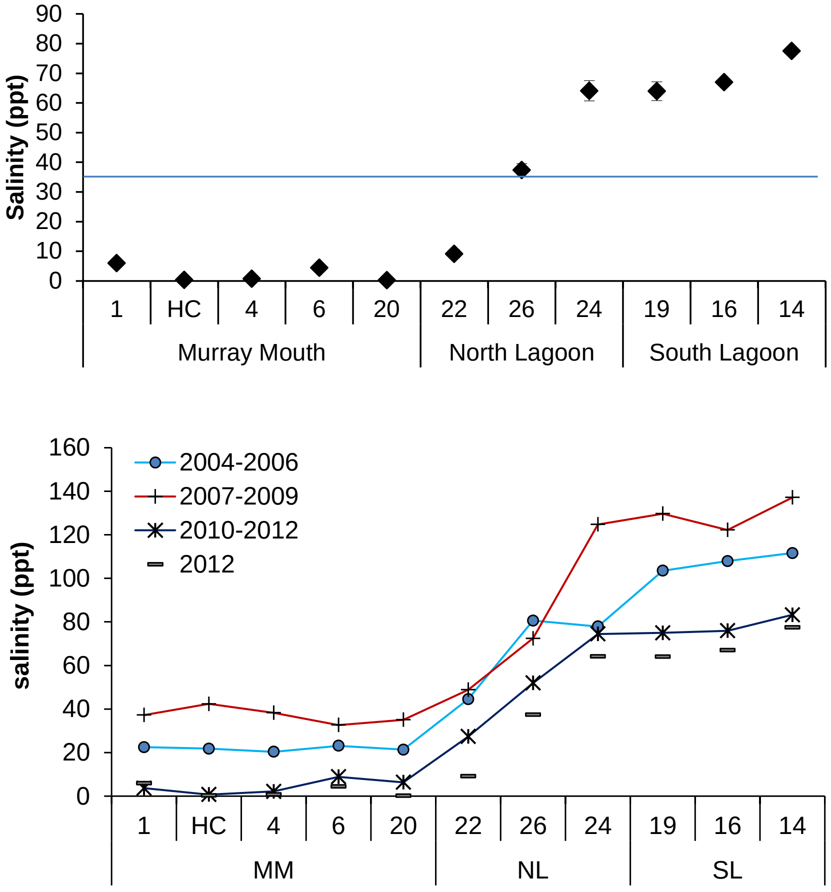

Figure 3: Salinity (mean ppt ± S.E.) in the water overlying the mudflats of the Murray Mouth and Coorong during the survey in 2012/13 (top figure), and in comparison to average salinities recorded in previous monitoring periods (bottom figure, mean values only), divided into the three year intervals for the early drought period (2004-2006), the severe drought years 2007-2009, and the three years since flow resumed (2010-2012) (see Figure 1). Salinities from the current monitoring year are also separately indicated in the bottom figure. Values are based on measurements with both electrodes and a refractometer. The blue line in the top figure indicates sea water salinity.

The reduction in salinities most likely resulted from the continued flow of water into the Murray Mouth and Coorong in recent years (Figure 1), and as the flow volume was reduced over summer 2012/13, water levels receded and larger areas of mudflats became exposed again. The Murray Mouth region is microtidal, yet with reduced flow over the barrages in December 2012, tidal water level variations were very noticeable. For condition monitoring target M-1: ‘Facilitate frequent changes in exposure and submergence of mudflats’, higher temporal resolution of data is needed, which are not recorded in this annual macroinvertebrate monitoring. Observations over consecutive sampling days indicate, however, a higher frequency of submergence and emergence of mudflats in the Murray Mouth and northern North Lagoon, thus providing accessible foraging ground with harvestable food for shorebirds.

4.1.2 Water quality

In addition to salinity several other water quality parameters were measured in waters overlying the mudflats. The average water temperature during the 2012/13 monitoring in December 2012 was 22 °C, comparable to previous years (SM-Figure 1). Differences in temperature between sites originate from sampling times (weather or day time). Dissolved oxygen concentrations in the water were around 5 mg/L in the South Lagoon, lower than in the 2011/12 monitoring. At other sites, dissolved oxygen concentrations were >6 mg/L, reaching 11 mg/L at Ewe Island, comparable to some of the highest values recorded in 2005 (SM-Figure 2). Dissolved oxygen saturation values showed well or over-saturated water at some sites in the Murray Mouth and Coorong, yet sites in either region had saturation levels below the trigger value of 90 % saturation of the ANZECC guideline (SM-Figure 3, SM-Table 2). These dissolved oxygen values indicate that water quality is still of concern and should receive more attention. The average pH in overlying waters was 8.42 and varied little across the sites in the Murray Mouth and Coorong, nor between the recent monitoring years (SM-Figure 4).

4.1.3 Sediment size ranges

Most of the sediments at the mudflats in the Murray Mouth and Coorong were classified as fine sands and very well sorted (Table 1, SM-Figure-5). Some sites in the Murray Mouth (Hunters Creek, Ewe Island and Pelican Point) had a higher proportion of finer particles. The samples from Pelican Point (site 20) indicate muddier sediments than in previous years (Figure 4), yet the site was fairly sandy and a measurement mistake cannot be ruled out. In the North Lagoon, sediments at Mulbin Yerrok (site 22) were much coarser than previously observed (Figure 4). Some variation in grain size can occur as it is never possible to resample the exact same location due to changes in water level between years, and sediment movements, silting or erosion. While some of these variations are apparent in a multivariate analysis of grain size compositions across all sites and years, the sediment size range remained mostly in the fine to medium size range (Figure 5, SM-Table 2). In general, median grain sizes at the study sites remained fairly similar over the years, thus meeting condition monitoring target M-2 ‘Maintain sediment size range in mudflats’.

Table 1: Sediment characteristics of mudflats in the Murray Mouth and Coorong region during summer 2012/13. Organic matter content (in per cent dry weight) within the sediment and the median grain size of sediment (in µm) along with the sorting coefficient, are provided as characteristics of mudflat sediment. The verbal description of sediment grain size and sorting follows Blott and Pye (2001).

Site		Organic matter (% dw)	Grain size			
			Median		Sorting	
Murray Mouth	1	1.27	164.1	Fine Sand	0.65	Moderately well sorted
	HC	0.99	96.4	Very fine sand	0.40	Well sorted
	4	0.99	148.2	Fine Sand	0.53	Moderately well sorted
	6	0.91	112.1	Very fine sand	0.42	Well sorted
	20	1.32	18.0	Coarse silt	0.39	Well sorted
North Lagoon	22	0.97	908.7	coarse sand	0.23	Very well sorted
	26	0.75	176.5	Fine Sand	0.66	Moderately well sorted
	24	1.47	171.0	Fine Sand	0.39	Well sorted
South Lagoon	19	2.22	173.6	Fine Sand	0.46	Well sorted
	16	1.51	234.0	Fine Sand	0.71	Moderately sorted
	14	2.27	247.9	Fine Sand	0.51	Moderately well sorted

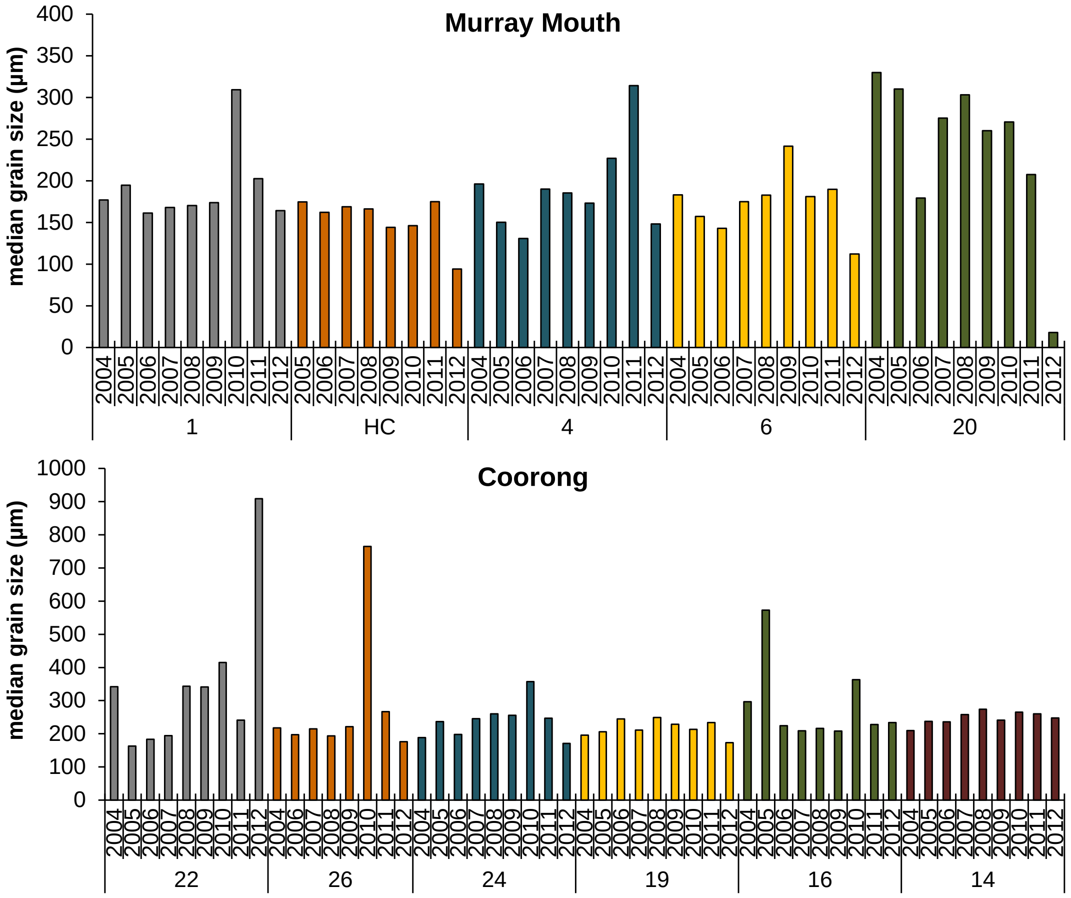

Figure 4: Median grain size values recorded in mudflats at each of the sites in the Murray Mouth and Coorong during monitoring surveys since 2004. Hunters Creek (HC) was included in 2005, and site 26 was not sampled that year. Note the different y-axes scales due to some outlying coarser sediment in the North Lagoon.

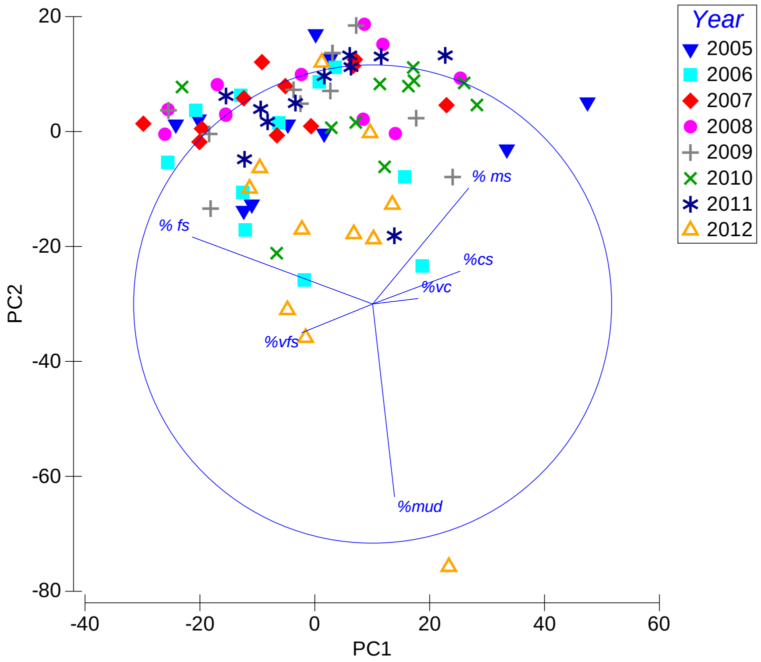

Figure 5: PCA (Principal component analysis) of sediment grain size compositions (% of major fractions, size in µm) in mudflats in the Murray Mouth and Coorong for the summer surveys from 2005 to 2012/13. Sites or regions are not shown in the figure. 2004 is not included as a different method was used for grain size analysis. The PCA axes explained 42 % (PC1) and 35 % (PC2) of the variation.

4.1.4 Sediment organic matter and chlorophyll-*a*

The content of sediment organic matter in mudflats of the Murray Mouth and the northern North Lagoon to Noonameena (site 26) was on average 1 ± 0.1 % dry weight, yet nearly doubled in the South Lagoon (1.9 ± 0.2) (Table 1, SM-Figure 6). Seen in comparison over the TLM monitoring years, the condition monitoring target M-3 ‘Maintain organic content for mudflats’ was met, as values for the 2012/13 monitoring fell well within the range of measurements from previous years (Figure 6, SM-Table 2).

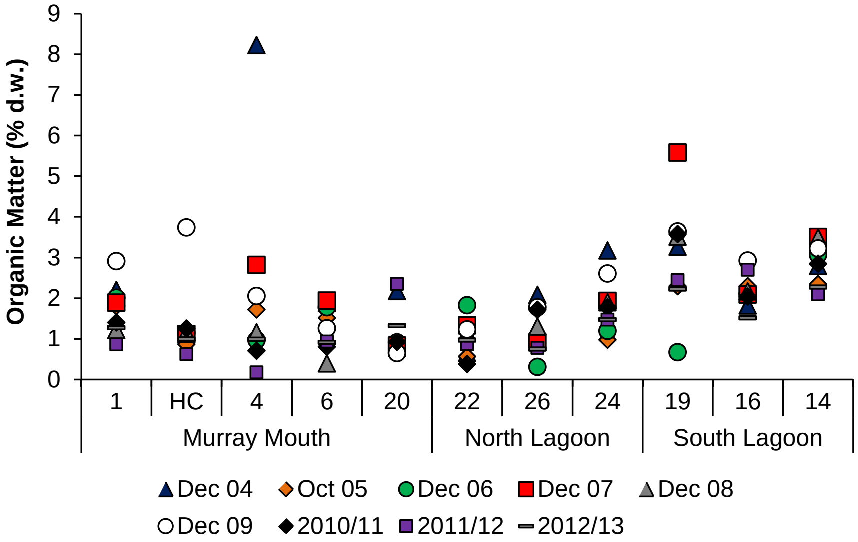

Figure 6: Sediment organic matter (as % dry weight, mean ± S.E.) of mudflat sediments in the Murray Mouth and Coorong sampled in late spring/early summer, since 2004.

The content of chlorophyll-*a*, a proxy for microphytobenthic biomass fuelling the food web in mudflat sediments, was higher at most sites than in the previous two years (Figure 7, SM-Table 2). Chl-*a* values were higher between Monument Road (site 1) and Noonameena (site 26) (on average 4.5 ± 0.9 mg m^{-2}) compared to much lower values (0.5 ± 0.2 mg m^{-2} on average) from Parnka Point (site 24) to Loop Road (site 14) in the South Lagoon. The highest Chl-*a* values were recorded at Hunters Creek (site HC) and Pelican Point (site 20). As for organic matter, there was a marked change in Chl-*a* values between Noonameena (site 26) and Parnka Point (site 24) (SM-Figure 7).

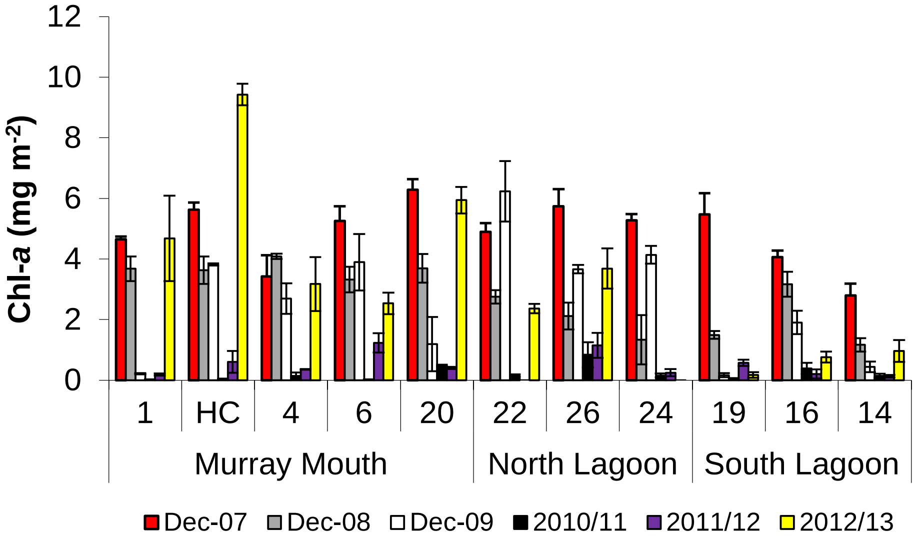

Figure 7: Sediment chlorophyll-*a* content (in mg m^{-2}; mean ± S.E.) at the study sites surveyed in summer 2012/13, in comparison to values from the previous surveys.

4.1.5 Trajectory changes in environmental conditions between years

In the current monitoring year, sites in the Murray Mouth and Coorong could be differentiated based on environmental variables measured (SM-Figure 8). The Coorong was more saline, and sediments coarser with more organic matter, whereas the Murray Mouth sites had higher chlorophyll-*a* content in sediments, and the overlying water a better dissolved oxygen saturation. To explore changes over time, water and sediment characteristics were analysed separately for each of the Murray Mouth, North and South Lagoon regions (Figure 8). All trajectories indicate ongoing change in the system with regards to the measured environmental parameters, based on measurements taken once a year (late spring/summer) during the annual monitoring.

For the water overlying mudflats in the Murray Mouth, salinity was the main contributor to the differentiation of years, with the hypersaline conditions of the intensive drought period from 2007-2009 on the right in the PCA plot (Figure 8). The years 2005, 2006, 2011 and 2012 were closer to each other, which could indicate similar salinity and dissolved oxygen saturation in periods after water releases, which occurred in 2005 and commenced in late 2010. The sediment characteristics in the mudflats of the Murray Mouth were defined by the mud and very fine sand grain size fractions (PC1-axis, explaining 53% of the variability) and organic matter and very coarse sand contributed to the PC2-axis (explaining 36.8%).

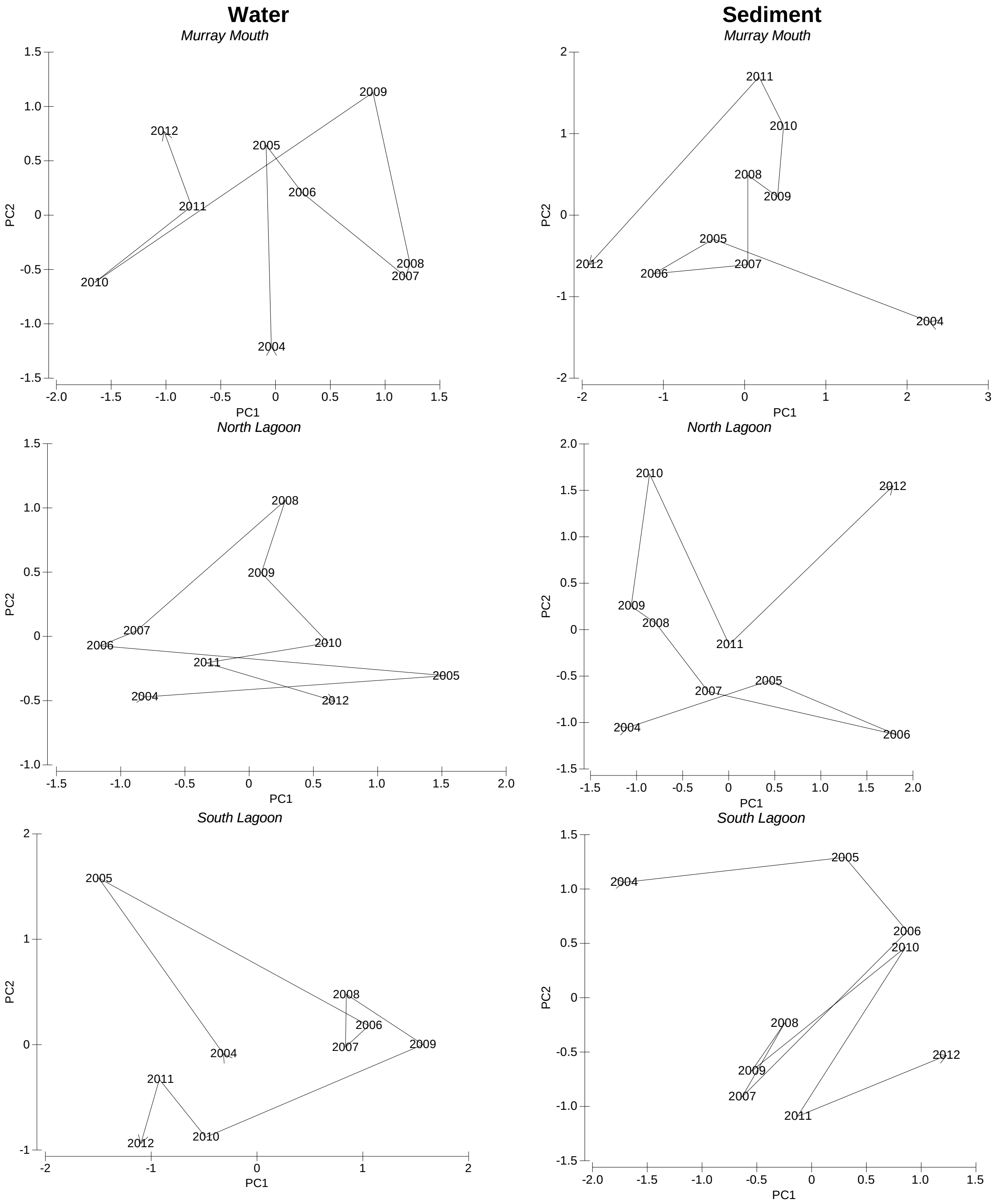

Figure 8: Trajectories of change in water parameters (salinity and dissolved oxygen saturation, left column), and sediment parameters (grain size fractions and organic matter, right column), for each of the regions of the Murray Mouth, North and South Lagoon since monitoring began in 2004.

For the North and South Lagoon of the Coorong, changes over time followed no clear paths (Figure 8). Salinity and dissolved oxygen saturation differentiated the years along the PC1-axis, explaining 75.6 and 68 % of the variability in the water respectively. In the North Lagoon, the years of water release (2005, 2010 and 2012) were grouped further to the right on PC1, yet 2011 deviated. The years with extreme hypersaline conditions in the North Lagoon (2008 and 2009) were separated from other years along the PC2 axis. For the South Lagoon, the years with no flow from 2006 to 2009, were set aside along the PC1 axis. Changes in the sediment characteristics of the Coorong lagoons over time were not aligned to any of the periods of flow or drought, and may be affected more by water level affecting sampling locations. Mud, fine and coarse sand fractions contributed to the variability along the axes. When sediment characteristics were analysed with inclusion of chlorophyll-a, which was measured since 2007 (plots not shown), it contributed most to the variability along the PC1 axis due to the low concentrations in 2010 and 2011 (SM-Figure 7).

All trajectories indicate that conditions in the water and sediments of the Murray Mouth and Coorong in the recent monitoring in December 2012 were distinct from the period of extreme drought (2007 to 2009). Yet, these improvements were not in all cases going along with a return to conditions at the start of the monitoring in 2004 (Figure 8). Macroinvertebrates inhabiting these regions had to be adaptable to these changes over the past years, and respective responses are seen in their assessment.

4.2 Macroinvertebrate populations

To report against Condition Monitoring Target I-1: ‘Maintain or improve invertebrate populations in mudflats’, findings from 2012/13 are presented in detail in the supplementary material, with a focus here on comparisons over time to assess whether improvements have occurred. The structure follows key parameters to assess improvements in invertebrate populations, namely diversity, abundances and distributions, biomass and community structures.

4.2.1 Macroinvertebrate diversity and distribution

Nineteen macroinvertebrate species were found in mudflat sediments of the Murray Mouth and Coorong in the 2012/13 monitoring, with annelids and hexapods (as insect larvae and pupae) contributing most species (6 taxa each) (Table 2). The species composition was similar to previous years, with a new find of a nemertean (ribbon worm) at Mulbin Yerrok (site 22). Several species of Crustacea occurred throughout the Murray Mouth and North Lagoon, with amphipods present at all sites, apart from Parnka Point (site 24) (Table 2, Figure SM-9). Polychaetes were also largely confined to sites between Monument Road (site 1) in the Murray Mouth and Noonameena (site 26) in the North Lagoon, yet Capitella sp. was also recorded at Villa de Yumpa (site 19). Of the molluscs, the two snail species occurred only at sites in the Murray Mouth region. The bivalves found last year were not recorded in the samples of this condition monitoring in 2012/13, yet present in the regions based on a concurrent project (Dittmann et al. 2013). Of the insect larvae, chironomids were omnipresent at all sites (Table 2), and hexapods contributed more species to the macroinvertebrates in the two Coorong lagoons (Table 2, Figure SM-9).

More species were found in the surface layers of the sediments than in deeper sediment horizons, only at Monument Road (site 1) and Noonameena (site 26) were species numbers similar in the two depth layers (SM-Table 3, SM-Figure 10). The polychaete *Australonereis ehlersi* and the nemertean were only found in the bottom horizon, whereas mysid shrimps, gastropods and several of the insect larvae were mostly recorded from the top layer of the sediment (SM-Table 3).

Table 2: Occurrence of macrobenthic taxa and species numbers during the summer 2012/13 survey (see Figure 2 for site location). The number of taxa is also indicated per site and region.

Phyla/Class/Order		Family/Genus/Species	Murray Mouth					North Lagoon			South Lagoon		
			1	HC	4	6	20	22	26	24	19	16	14
Annelida	Oligochaeta			X		X	X	X	X	X			
	Polychaeta	*Aglaophamus (Nephtys) australiensis*	X		X	X							
		Australonereis ehlersi							X				
		Boccardiella limnicola	X		X		X						
		Capitella sp.						X	X		X		
		Simplisetia aequisetis	X		X	X	X	X					
Nemertea								X					
Crustacea	Amphipoda		X	X	X	X	X	X	X				
	Decapoda	*Paragrapsus gaimardii*	X										
	Ostracoda				X					X			
	Mysidacea		X	X		X							
Mollusca	Gastropoda	Hydrobiidae	X	X	X	X	X						
		Salinator fragilis				X							
Hexapoda	Diptera	Ceratopogonidae (larvae & pupae)								X			
		Chironomidae (larvae & pupae)	X	X	X	X	X	X	X	X	X	X	X
		Dolichopodidae (larvae & pupae)				X	X	X	X				
		Ephydridae (larvae & pupae)						X	X	X		X	X
		Sciomyzidae (larvae & pupae)						X					
	Coleoptera	Hydrophilidae (larvae & pupae)							X				
Total species number per site			8	5	7	9	7	9	8	5	2	2	2
Species number per region			12					13			3		

The number of macroinvertebrate species was about four times higher in the Murray Mouth (12 taxa) and North Lagoon (13 taxa) than in the South Lagoon (3 taxa) (Table 2) and species densities also differed significantly between regions and sites within regions (SM-Table 4). Yet, the number of species was highly variable between samples taken per site, and samples within regions (SM-Table 4). Diversity (Shannon-Wiener H') was highest in mudflats between Ewe Island (Site 6) and Parnka Point (site 24), while the high species numbers (S) at the other Murray Mouth sites were not matched by high diversity, as dominance of single taxa reduced eveness (J') (Figure 9).

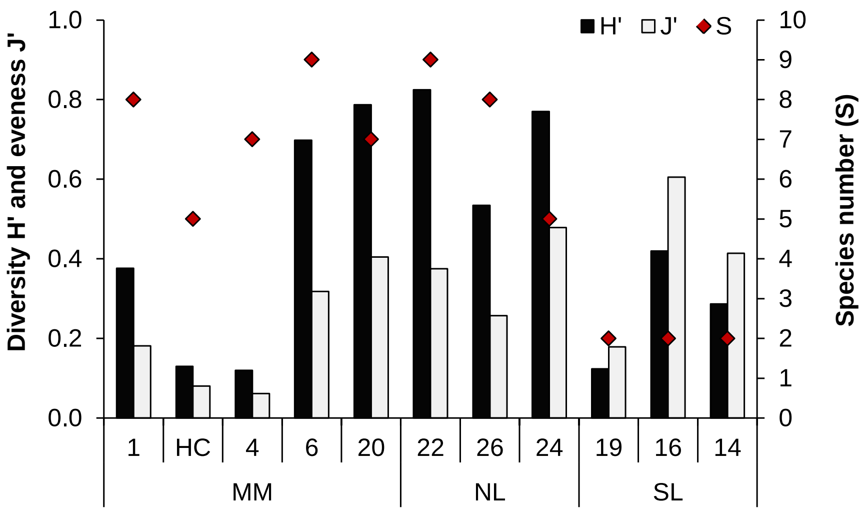

Figure 9: Total number of macroinvertebrate species (red symbols), Shannon-Wiener diversity H' (black bars, based on log$_e$) and eveness J' (white bars) at sites in the Murray Mouth (MM), North (NL) and South (SL) Lagoons of the Coorong in the 2012/13 monitoring. See Figure 2 for site codes and locations.

Diversity values were, however, low in comparison to previous monitoring years and periods, especially at sites 1 (Monument Road), HC (Hunters Creek) and 4 (Mundoo Channel) in the Murray Mouth and site 19 (Villa de Yumpa) in the Coorong (Figure 10, SM-Table 5). The low diversity was mainly caused by the dominance of single taxa (e.g. amphipods and chironomid larvae), as reflected in the low eveness values (Figure 10, SM-Table 5). Seen over the three year periods (Figure 10), the number of species fluctuated less per site than in an annual comparison (SM-Figure 11), and the recolonisation of mudflats between Pelican Point (site 20) and Noonameena (site 26) since flow commenced (period 2010-2012) was evident from higher species numbers and diversity compared to the drought period (2007-2009).

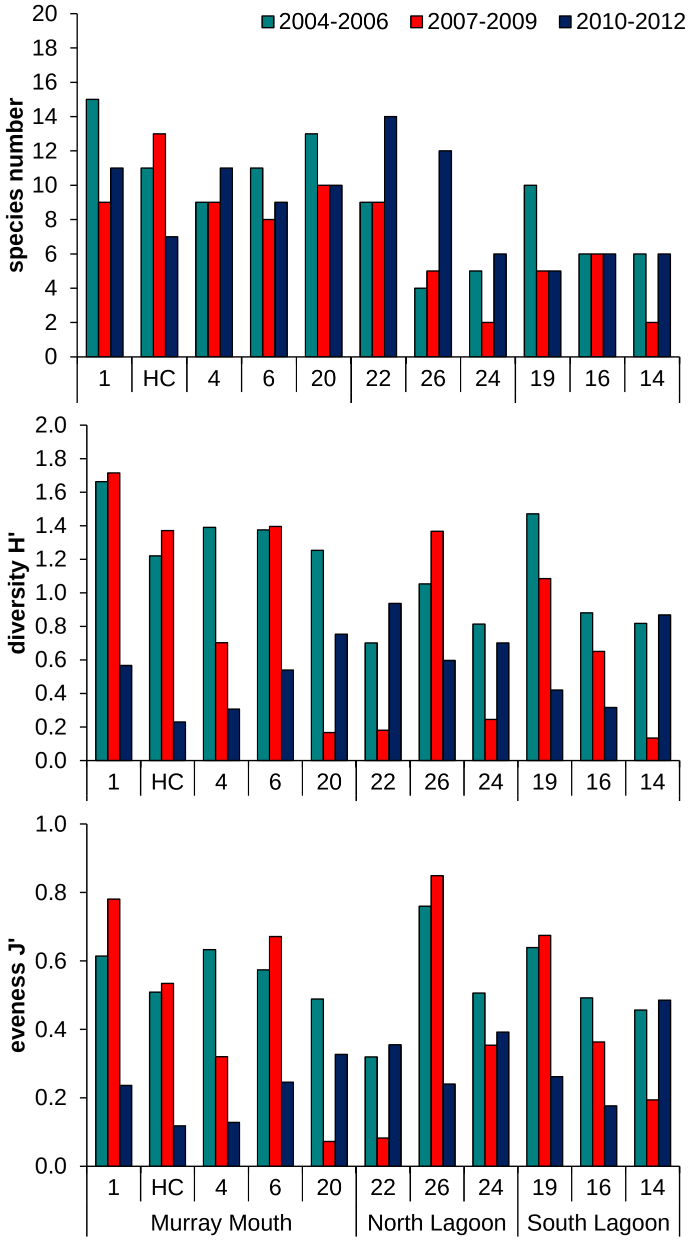

Figure 10: Changes in diversity (illustrated by species numbers, Shannon-Wiener index H' and Pielou's eveness index J') of benthic macroinvertebrates at sampling sites in the Murray Mouth and Coorong lagoons (see Figure 2 for site locations) over the monitoring time frame since 2004, divided into periods of early drought/small flow (2004-2006), severe drought (2007-2009) and restored flow (2010-2012).

The distribution range of macroinvertebrate species changed over the monitoring years, and species specific response patterns were apparent (Figure 11). During the extreme drought period (2007-2009), several species contracted their distribution to fewer sites and disappeared mostly from the Coorong (e.g. amphipods, *Arthritica helmsi*, *Simplisetia aequisetis*, oligochaetes), which they since recolonised in the flow period (2010-2012). Only the once widely distributed micro-mollusc *A. helmsi* was not found in this survey. The distribution range of *Nephtys australiensis* increased, similar to a response seen after the small water release in 2005. *Capitella* sp. contracted mostly into the Coorong after flow resumed, and *Australonereis ehlersi* shifted in distribution from the Murray Mouth into the North Lagoon. Chironomid larvae were more widespread in the regions now than in previous periods of pre-

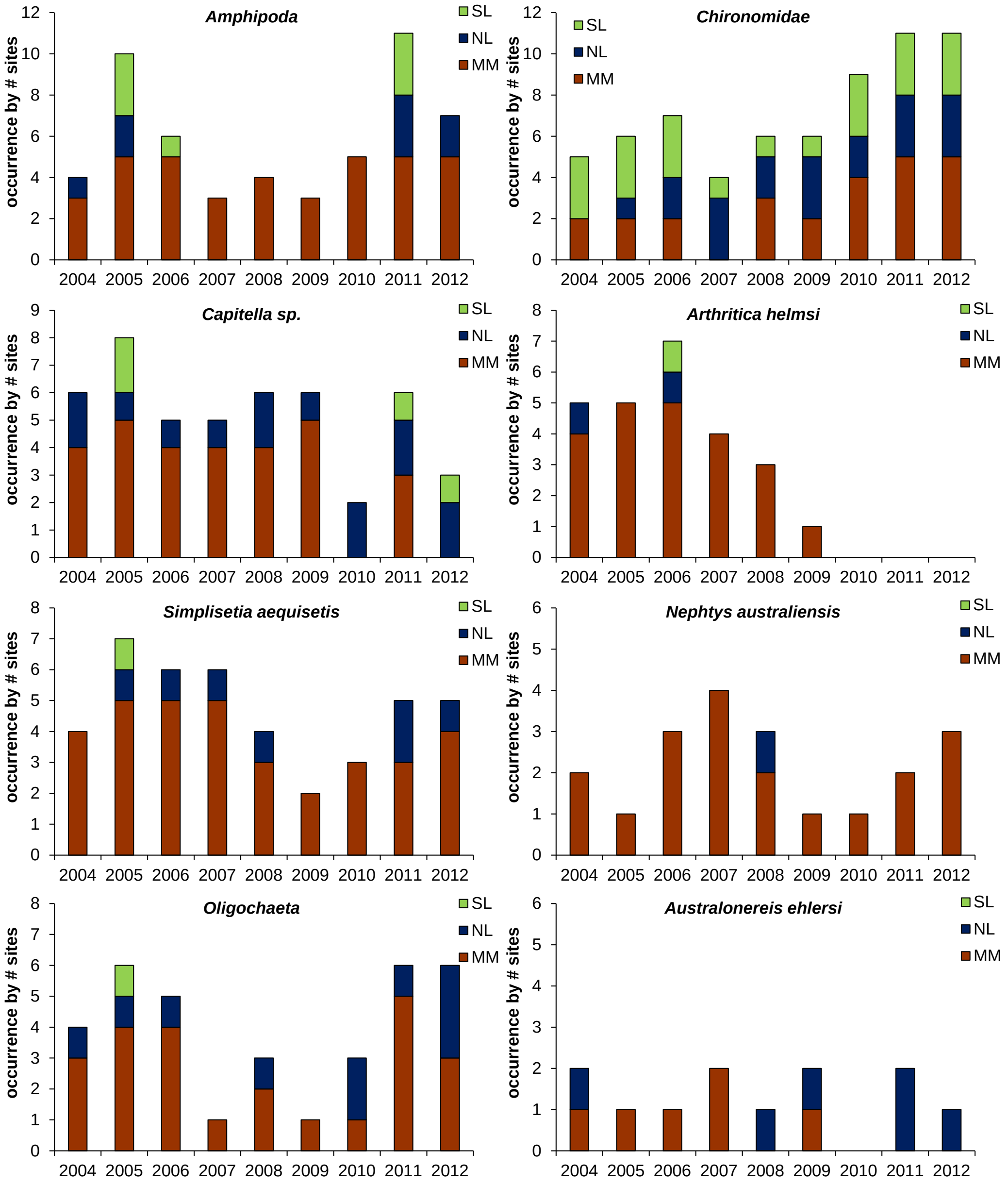

Figure 11: Changes in the distribution range of key macroinvertebrate taxa in the Murray Mouth (MM), North (NL) and South (SL) Lagoon of the Coorong across all monitoring years. The maximum number of sites across these study regions is 11. The number of sites at which each species/taxon occurred indicates how extensive or contracted its distribution range was. Note the different scales on the y-axes.

flow and drought (Figure 11). In general, most of the key macroinvertebrate species occupied more sites throughout the Murray Mouth and Coorong in 2011 and 2012 than during the drought years.

4.2.2 Macroinvertebrate abundances and distribution

In the 2012/13 monitoring, abundances of macroinvertebrates were high in mudflats throughout the Murray Mouth and into the North Lagoon, whereas few specimens were found in sediments at the South Lagoon sites (Figure 12). Compared by region, abundances of all benthic organisms were on average about six times higher in the Murray Mouth than in the North Lagoon, and nearly 80 times higher than in the South Lagoon (SM-Table 6). The total abundances were significantly different between the three regions, and also between sites nested in each region, yet also highly variable within sites and regions (SM-Table7). Significant site specific differences in abundances were pronounced for the tested species and higher taxa, while few of these showed significant differences in abundances between regions as well (SM-Table 7). As occupancy and abundances of macroinvertebrates were more similar at sites in the Murray Mouth to Noonameena, they bridged the geographic sub-region distinction.

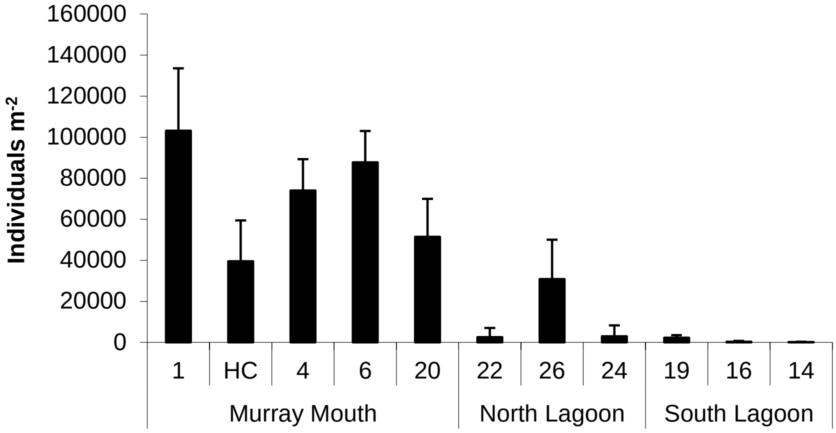

Figure 12: Mean abundance (ind. m^{-2}) and standard deviation (±S.D.) (*n* = 10) of benthic macrofauna recorded at sampling sites in the Murray Mouth and Coorong during the 2012/13 summer survey.

The pattern of abundances was mainly driven by the high abundances of amphipods between sites 1 (Monument Road) and 6 (Ewe Island) in the Murray Mouth and chironomid larvae, which were most abundant at Ewe Island and Pelican Point (site 20) (SM-Figures 12 and 13). While many of the macroinvertebrate taxa were found at multiple sites in the study regions (Table 2), they occurred in higher abundances only in samples at single sites (SM-Figure 13). *Capitella* sp. and *Australonereis ehlersi* were most abundant at Noonameena (site 26) and *Boccardiella limnicola* at Monument Road (site 1), where several crabs (*Paragrapsus gaimardii*) were found as well. *Nephtys australiensis* and *Simplisetia aequisetis* were recorded from sites in the Murray Mouth, and most abundant at Ewe Island (site 6) and Pelican Point (site 20) respectively. Oligochaetes were recorded in higher numbers between Ewe Island and Noonameena, yet were very patchy in their occurrence (SM-Figure 13). Snails grazing on the mudflat surface were encountered at all sites in the Murray Mouth in moderate numbers (SM-Figure 12).

While several species and taxa thus contributed to the high abundances in the Murray Mouth, abundances in the North Lagoon were dominated by *Capitella* sp. and oligochaetes, and chironomid insect larvae accounted mostly for the abundances in the South Lagoon, especially at Villa de Yumpa (Site 19) (SM-Figure 13).

Over time, total macroinvertebrate abundances fluctuated, with a decrease during the extreme drought years of 2007-2009 and first big flow impulse in 2010 and a consolidated recovery with the second year of higher abundances recorded in the 2012 monitoring (SM-Figure 14). Abundances recorded in the current monitoring year (2012/13) were above the average of the past three years in the period since flow resumed (Figure 13). This was most pronounced at the sites in the Murray Mouth, and at Noonameena (site 26) in the Coorong (Figure 13). Total macroinvertebrate abundances were significantly different across the surveys and sites (nested in regions) (SM-Table 7).

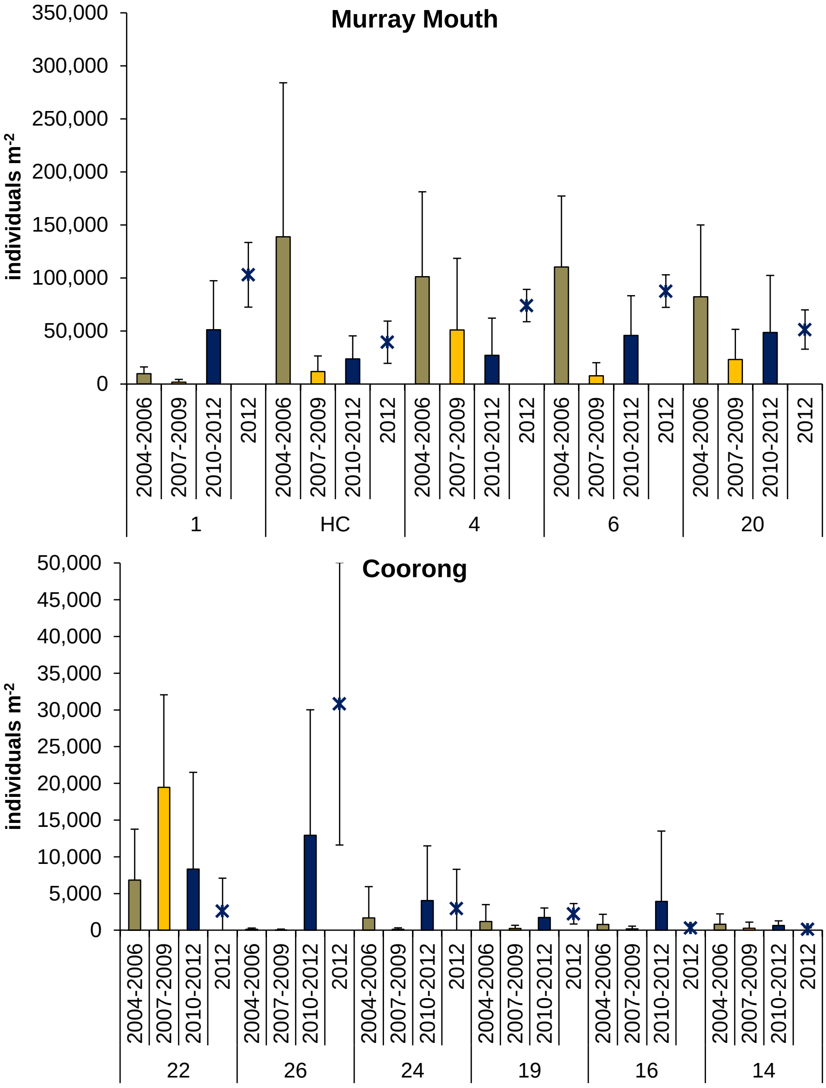

Figure 13: Mean abundances (ind. m^{-2}) and standard deviation (±S.D.) (*n* = 10) of benthic macrofauna recorded at sampling sites in the Murray Mouth (top figure) and Coorong (bottom figure) over the monitoring time frame since 2004, divided into periods of early drought/small flow (2004-2006), severe drought (2007-2009) and restored flow (2010-2012). Abundances from the current monitoring in 2012/13 are also separately indicated with asterisks. Note the difference in y-axes scales.

Different groups of benthic organisms contributed to the total macroinvertebrate counts over the years (SM-Figure 15). Annelids and molluscs were the most abundant organisms, especially between 2005 and 2007, whereas in the period since flow resumed in 2010, crustaceans (mainly amphipods) and hexapods (insects, as larvae and pupae) accounted for most of the total benthos. While annelids have increased in numbers in the recent monitoring timeframe, no recovery of mollusc numbers was recorded in December 2012 (SM-Figure 15). Abundances of all of these major macroinvertebrate taxa, as well as single species, were significantly different between survey years, subject to sites within regions (SM-Table 7). Although small-scale variability occurs (see also high standard deviation bars in figures), changes in temporal and spatial distribution patterns are apparent.

Of the key species that have indicator value and are important food sources for shorebirds and fish, only the polychaete *Capitella* sp. has contracted its distribution range to the North Lagoon since flow resumed, where it occurred in high abundance at Noonameena (site 26), with abundances comparable to those previously found at the Murray Mouth sites (Figure 14). As this species[1] is worldwide renowned as a pollution indicator, this change in distribution and abundance can be seen as a sign of improvement.

Simplisetia aequisetis occurs more widespread again in the Murray Mouth since flow resumed (Figure 11) and numbers have increased at Pelican Point (site 20), yet abundances are still far lower than in 2005 and 2006, after a small water release (Figure 14). Similarly, *Nephtys australiensis* was found at more sites in the Murray Mouth than in the last few years (Figure 11), yet abundances are well below the high numbers of individuals encountered around 2006 at Monument Road (site 1) and Hunters Creek (site HC) (Figure 14). Only at Ewe Island (site 6), abundances were comparable to previous monitoring years. A further key species, the reef building tube worm *Ficopomatus enigmaticus*, was not quantitatively assessed using core samples in this monitoring, yet live tube worms were seen at Monument Road and Long Point.

The micro-mollusc *Arthritica helmsi* has been declining in abundances since the start of the drought, and has been absent from the Murray Mouth and Coorong for a few years (Figures 11 and 14). While no specimens were found in the samples for this monitoring, this species was recorded in sediments from deeper water in recent months from a separate monitoring project (Dittmann et al. 2013).

Amphipods were the numerical winner of the restored flow, with a large increase in abundance at all Murray Mouth sites, similar to a response seen in 2005 after a small water release (Figure 14). These species are also highly mobile, and have extended their distribution range further into the Coorong in recent years (Figure 11). Similarly, chironomids were recorded at almost all sites in the Murray Mouth and Coorong during the survey in 2012/13, with some shifts between recent years as to where peaks in abundance occur (Figures 11 and 14).

Give these very different spatial and temporal responses of the key species, no one species alone can indicate the changes in environmental condition the system has experienced over the last few years. Yet, the data and knowledge gained allows to specify habitat requirements, which are useful for

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[1] *Capitella capitata* is a species complex, with similar ecology (Grassle & Grassle 1976; Mendez *et al.* 2000).

developing future response curves. The links between abundances and salinity (Figure 15), derived from the entire monitoring period since 2004, already explain most of the pattern described above, and links between macroinvertebrate assemblages and environmental conditions are further explored in chapter 4.2.6.

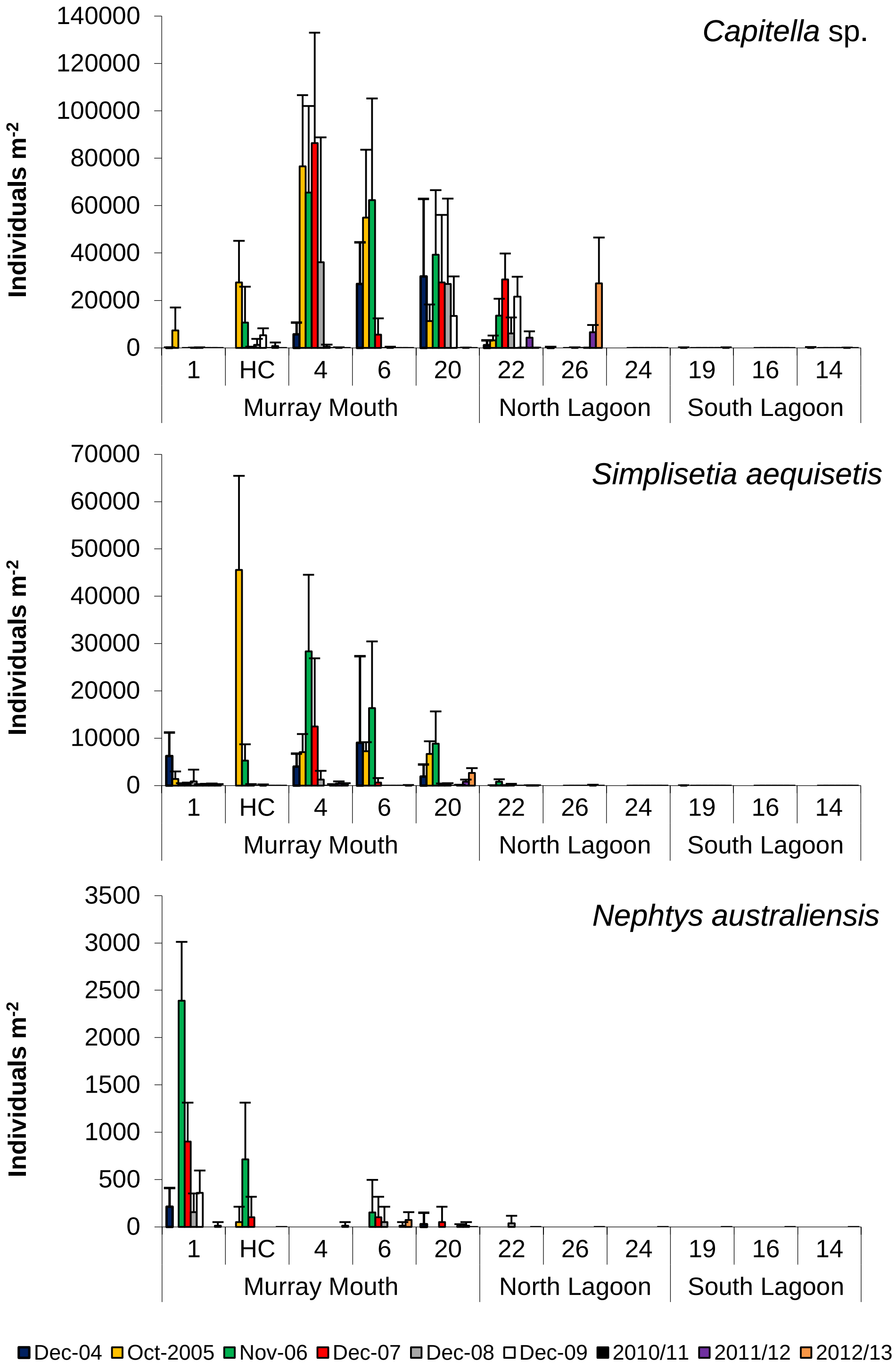

Figure 14, continued…

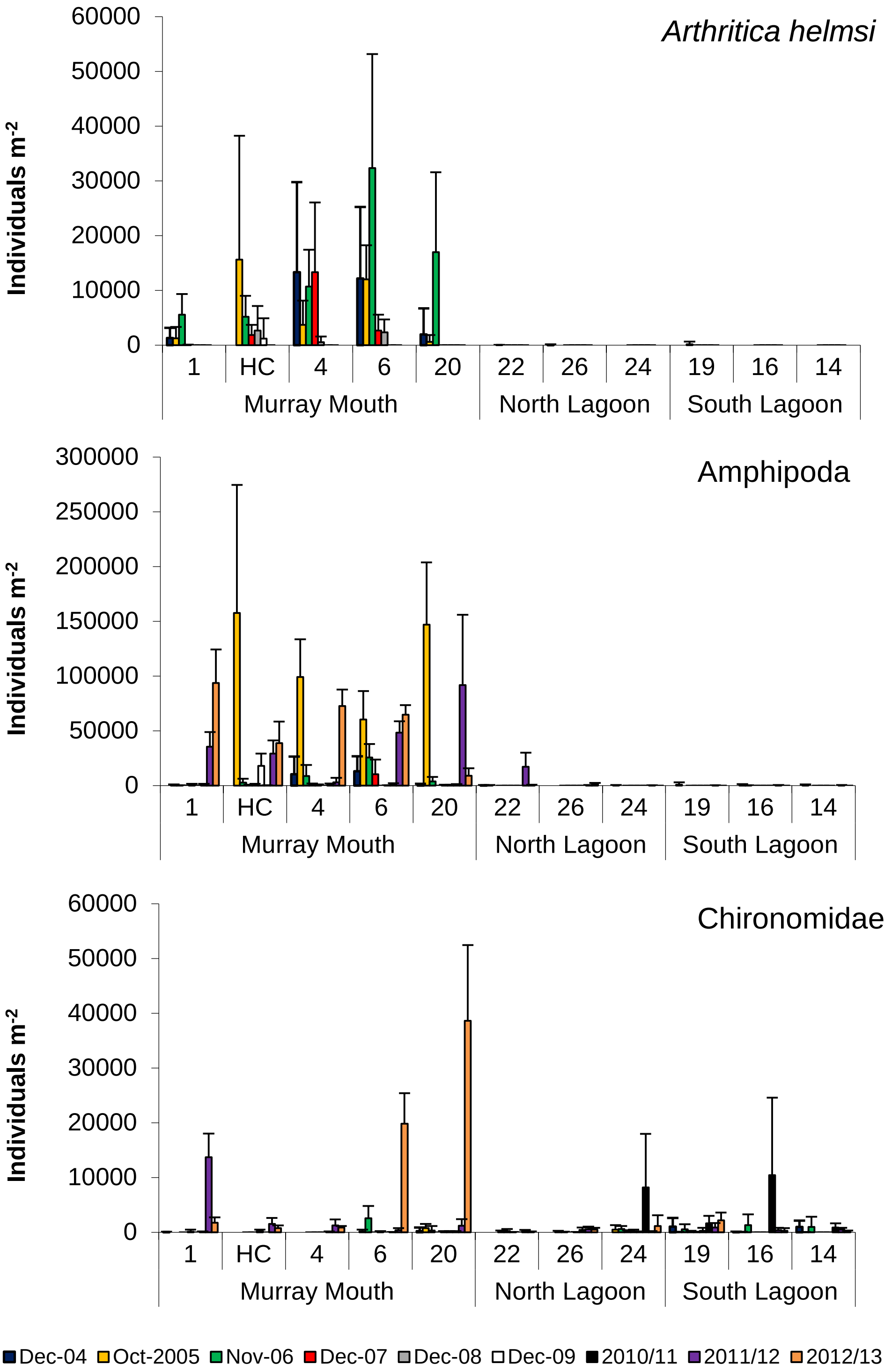

Figure 14. Mean abundances (ind. m^{-2}) and standard deviation (± S.D.) (*n* = 10) of key species and taxa with potential indicator value, recorded at sites around the Murray Mouth and Coorong since 2004. Note not all sites were sampled during each survey and the different scales of the y-axis.

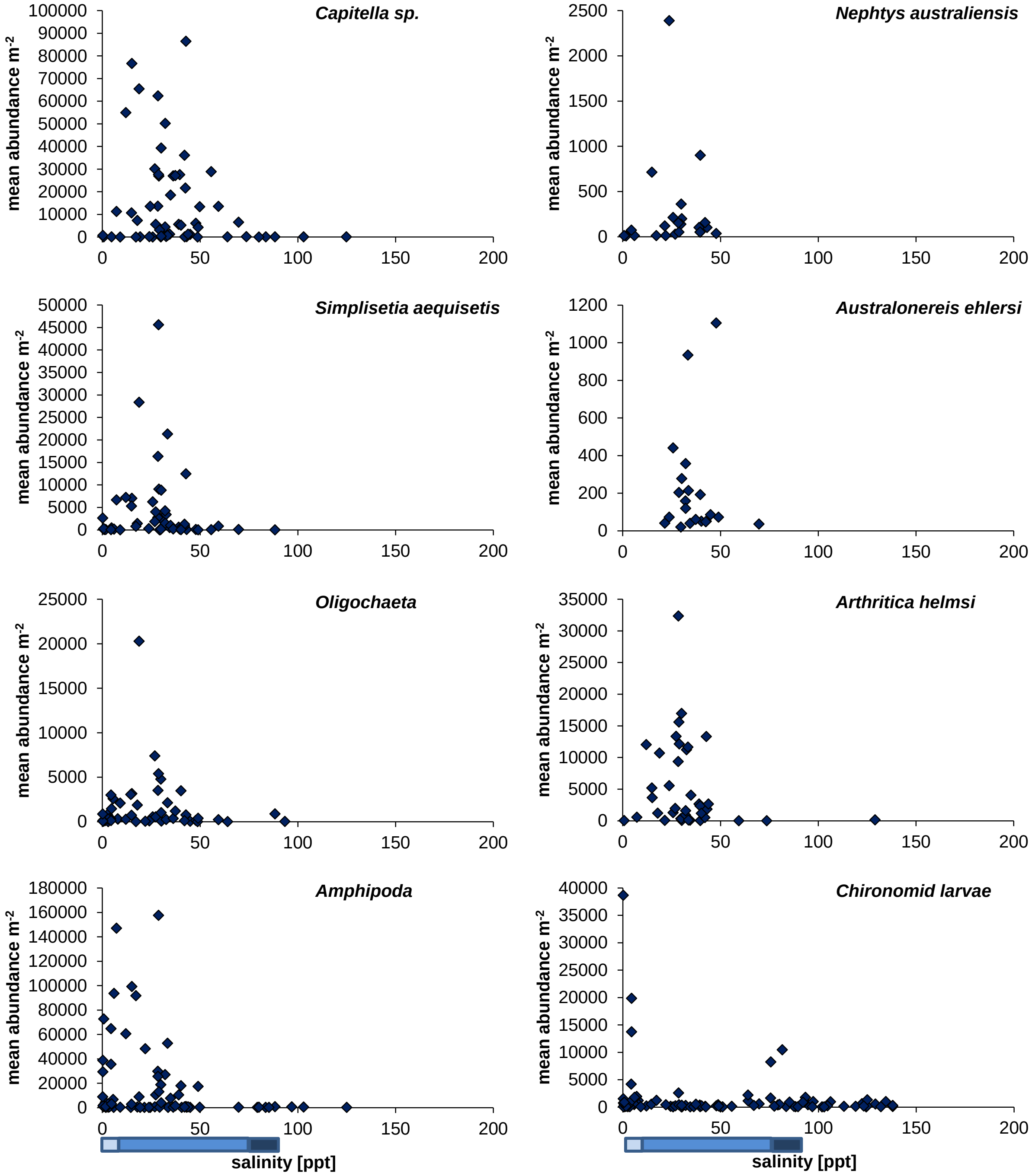

Figure 15. Mean abundances (ind. m^{-2}) of key species and taxa, based on all records from the Murray Mouth and Coorong over the entire monitoring time span from 2004 to 2012/13, in relation to the salinity at the sites and time of sampling. The salinity range recorded in the study regions in the 2012/13 monitoring is indicated in bottom bars with colours from light blue (Murray Mouth, 0.3-6 ppt) to blue (North Lagoon ,9-64 ppt) and dark blue for the South Lagoon (64-78 ppt). See Figure 3 for changes in salinities over the monitoring periods.

4.2.3 Vertical distributions

Most of the macroinvertebrates occurred in the surface sediment layer, as abundances were higher in the top than in the deeper sediment horizons (Figure 16). Over 80% and up to 100% of the total abundance recorded per site were obtained from the surface sediment layer at almost all sites (Figure 16). This concentration of organisms in the top layer reflects the high abundances of shallow dwelling amphipods and chironomids (SM-Figure 16). Other taxa, such as oligochaetes and hydrobiid snails, were also concentrated near the sediment surface. Food resources for birds with longer beaks were available only at Monument Road (site 1), Mundoo Channel (site 4), Ewe Island (site 6) and Pelican Point (site 20), mostly as worms (*Simplisetia aequisetis, Nepthys australiensis,Boccardiella limnicola*) (SM-Figure 16).

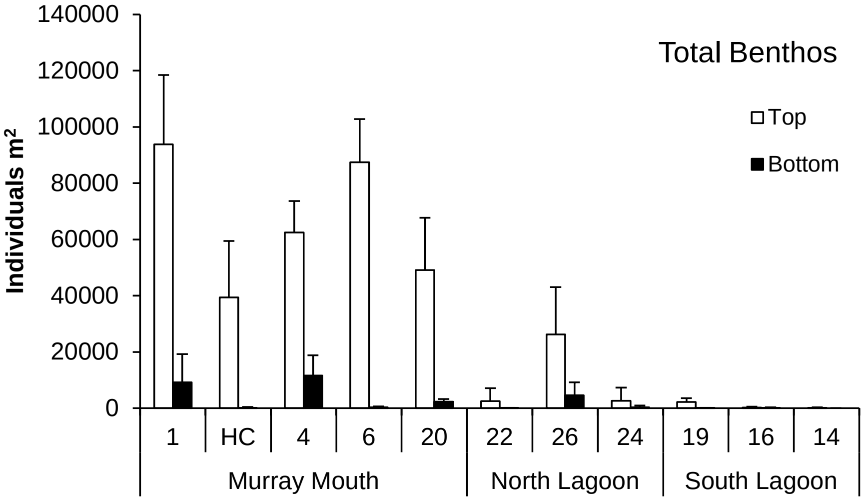

Figure 16: Vertical distribution of total benthic macrofauna (mean abundances (ind. m^{-2}) and standard deviation (± S.D.) (*n* = 10) recorded during the 2012/13 mudflat survey. Top horizon = 0 – 3 cm, bottom horizon = 3 – 15 cm sediment depth.

4.2.4 Macroinvertebrate biomass

Biomass of macroinvertebrates followed a similar pattern as abundances, being highest at sites throughout the Murray Mouth and at Noonameena (site 26) in the North Lagoon (Figure 17). Biomass was about four times higher in the Murray Mouth (nearly 5 g AFDW m^{-2}) than in the Coorong lagoons (<1 g AFDW m^{-2}) (SM-Table 6). The variation between regions as well as between sites within each region was significantly different, and also characterised by smaller scale spatial variability (SM-Table 7). Different organisms contributed to the biomass at the sites and sediment depths, when seen in comparison with abundance data of various taxa (see chapters 4.2.2 and 4.2.3). At Monument Road (site 1), high numbers of amphipods in the surface sediment accounted for the high biomass in the top horizon (SM-Figure 16, Figure 17), whereas chironomids dominated in the surface layer at Pelican Point (site 20) and contributed to the biomass there. At Pelican Point, the large polychaete worm *Simplisetia aequisetis* occurred in moderate abundances compared to earlier monitoring years, but constituted a high biomass in the bottom horizon and thus food availability for birds with medium and longer bill lengths (Figures 14 and 17).

Seen over the periods during the monitoring time frame characterised by different flow conditions, biomass in 2012/13 was comparable to or a little higher than in previous periods at most Murray Mouth sites, apart from high values in the 2004-2006 period at some sites (Figure 18). In the Coorong, more variability is apparent in the generally low biomass, yet at most sites biomass appeared higher in the 2010-2012 period than in the previous drought period (Figure 18). Seen by year, biomass was higher in 2012/13 than in the previous three to four years at all of the Murray Mouth sites, while not reaching the high biomass values from 2005 and 2006 after a small water release (SM-Figure 17). In the Coorong, the overall low biomass was fluctuating in recent years (SM-Figure 17). Biomass was higher in 2010/11 than in the current monitoring year at sites between Parnka Point (site 24) and Loop Road (site 14) in the South Lagoon (SM-Figure 17), where chironomid abundances were high (Figure 14). Yet at Mulbin Yerrok (site 22) and Noonameena (site 26), biomass had increased in in 2011/12, a year after flow resumed, and was as high as last year's biomass at Noonameena. Biomass was significantly different between years, subject to significant spatial variation within regions (SM-Table 7).

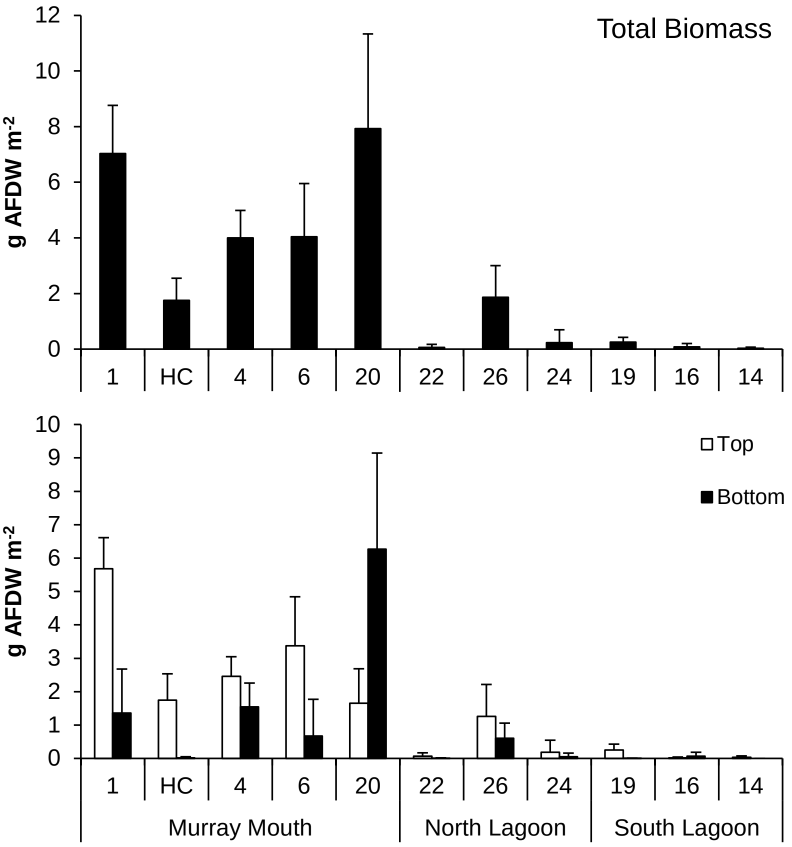

Figure 17: Mean biomass (g AFDW m^{-2}) and standard deviation (± S.D.) (*n* = 10) of benthic macrofauna recorded at sampling sites during the 2012/13 summer survey. Biomass is shown in total over the entire sediment sample depth (top figure), and for the two depths horizons (top = 0-3 cm, bottom = 3 – 15 cm) (bottom figure).

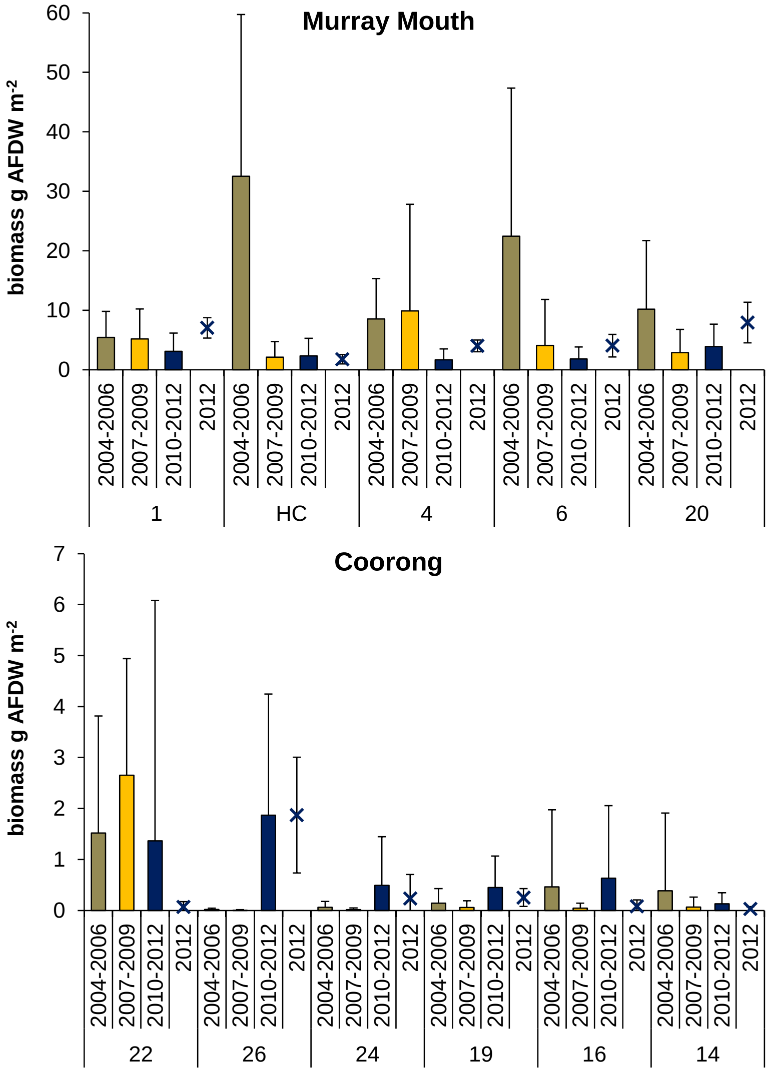

Figure 18: Biomass of benthic macrofauna (g AFDW m^{-2}) (mean and standard deviation (± S.D.) at sites sampled in the Murray Mouth (top figure) and Coorong (bottom figure) over the monitoring time frame since 2004, divided into periods of early drought/small flow (2004-2006), severe drought (2007-2009) and restored flow (2010-2012). Biomass from the current monitoring in 2012/13 is also separately indicated with asterisks. Note the order of magnitude difference in the y-axes scales.

4.2.5 Macroinvertebrate communities

Distinct benthic communities occurred in the 2012/13 monitoring, with the Murray Mouth sites forming a well-defined community characterised by several polychaete species, chironomids and amphipods (Figure 19). The northern most site in the North Lagoon, Mulbin Yerrok (Site 22) was characterised by oligochaetes and dolichopodid larvae, unlike assemblages found at other sites (Figure 19, SM-Table 8). The macroinvertebrate community at Noonameena (site 26) shared some similarity with sites further north (Figure 19), and was defined by capitellid polychaetes, amphipods and oligochaetes (SM-Table 8). The southern reaches of the Coorong, from Parnka Point (site 24) to Loop Road (site 14), shared a similar community characterised by the dominance of chironomids. The macroinvertebrate communities were significantly different between regions (Pseudo-F = 6.238, $P_{(perm)}$ = 0.0009) and sites nested in regions (Pseudo-F = 11.216, $P_{(perm)}$ = 0.0001).

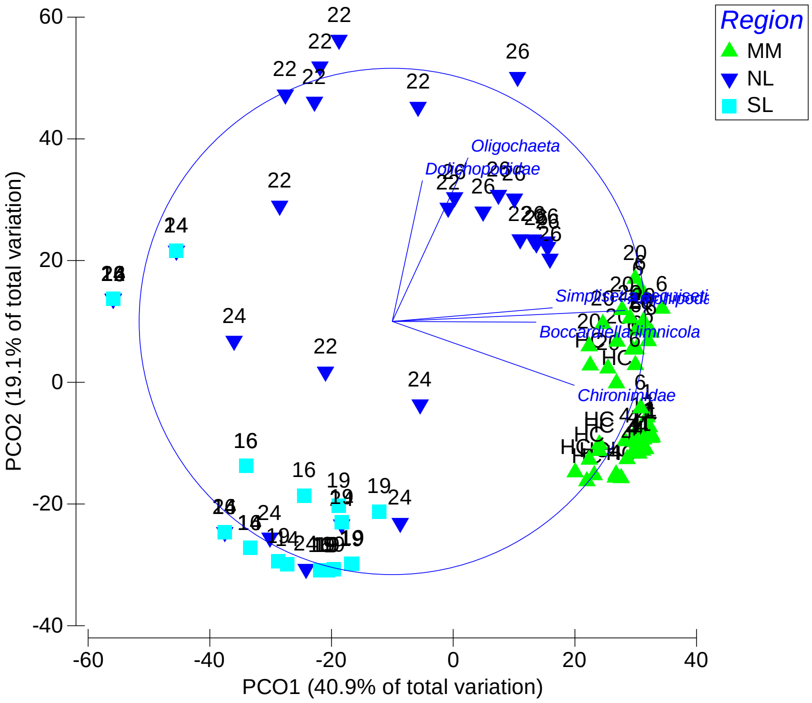

Figure 19: Principal coordinate analysis (PCO) of macroinvertebrate data from the mudflat survey in summer 2012/13, with the regions MM=Murray Mouth (sites 1, HC, 4, 6 and 20), NL=North Lagoon (sites 22, 26 and 24) and SL=South Lagoon (sites 19, 16 and 14). The circle represents a vector overlay (Spearman correlation) illustrating the contribution of the respective species to the PCO axes.

Regional differences in the macroinvertebrate community structures were also apparent over the entire monitoring time frame since 2004 (SM-Figure 18), and communities were significantly different across factors and their interactions (years, regions and sites nested in these regions) (SM-Table 8). To explore further how communities changed in each of the regions, analyses were carried out per region. Trajectories of change were apparent in each region (Figure 20).

For the Murray Mouth, a clear path of change was evident, with communities changing from the early monitoring period 2004-2006, which included a small water release in 2005, to increasing deviation as the drought progressed from 2007 to 2009, and the very distinct community in 2010 when the major water release occurred, followed by the development of a community in 2011 and 2012 which was different from previous years (Figure 20 top right). The changes along the PCO1 axis explained a very high part (51.6%) of the variation between communities in the years before (2004 to 2009) and after flow resumed (2010 to 2012).

In the North Lagoon, the trajectory is taking a more complex path, which indicates a recovery, as the community in 2011 and 2012 was more similar to the one in 2005. The community was distinct in the years between 2006 and 2009, which were separated along the PCO1 axis explaining 36.5 % of the variation. The year flow recommenced (2010) was characterised by a different community deviating from the ones found in other years (Figure 20 top left). Changes in the macroinvertebrate community in the South Lagoon over the years were not revealing any clear pattern in relation to drought and flow periods, and the PCO axes explained less of the variation than for the other two regions (Figure 20 lower right). However, the community in 2012 was more similar to the one in 2004.

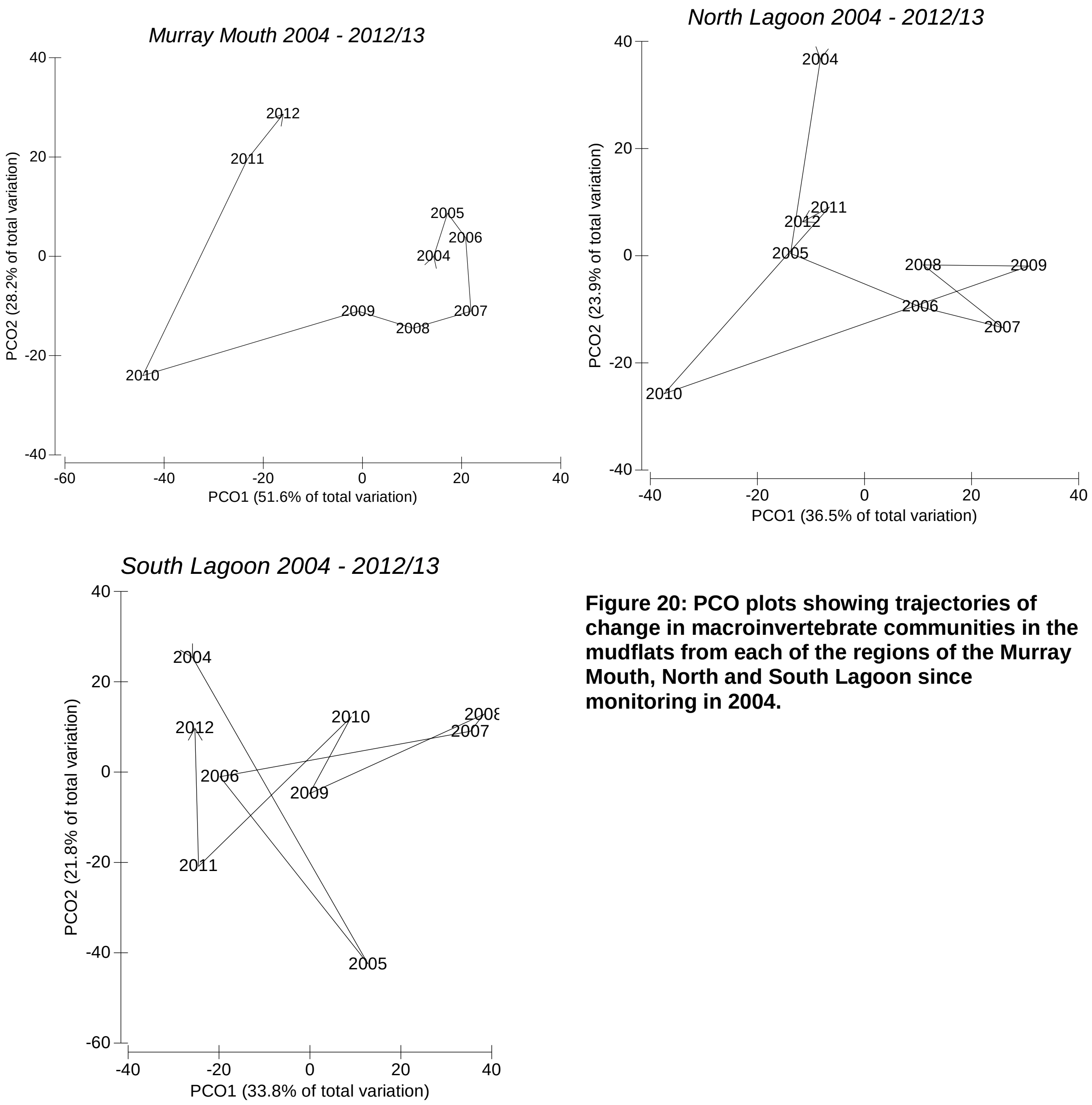

Figure 20: PCO plots showing trajectories of change in macroinvertebrate communities in the mudflats from each of the regions of the Murray Mouth, North and South Lagoon since monitoring in 2004.

While the trajectories are indicating deviations in community compositions between drought and flow years, they only show signs of return to a similar macroinvertebrate community as found in the early monitoring years for the Coorong (Figure 20). Another way of exploring recovery is presented in Figure 21, where the Global R index from a test statistic revealed the increasing differentiation in macroinvetebrate communities between the three regions (Figure 21). The estuarine/marine Murray Mouth community was very different to the one found in the hypersaline North Lagoon, and especially South Lagoon in the period 2004 to 2006. Yet, this difference was reduced as environmental conditions deteriorated in the Murray Mouth during the extreme drought, and in 2008 and 2009, communities in all three regions were quite similar. Since flow resumed in 2010, the distinction has returned, driven by the recovery in diversity and abundances in the Murray Mouth described in the chapters above.

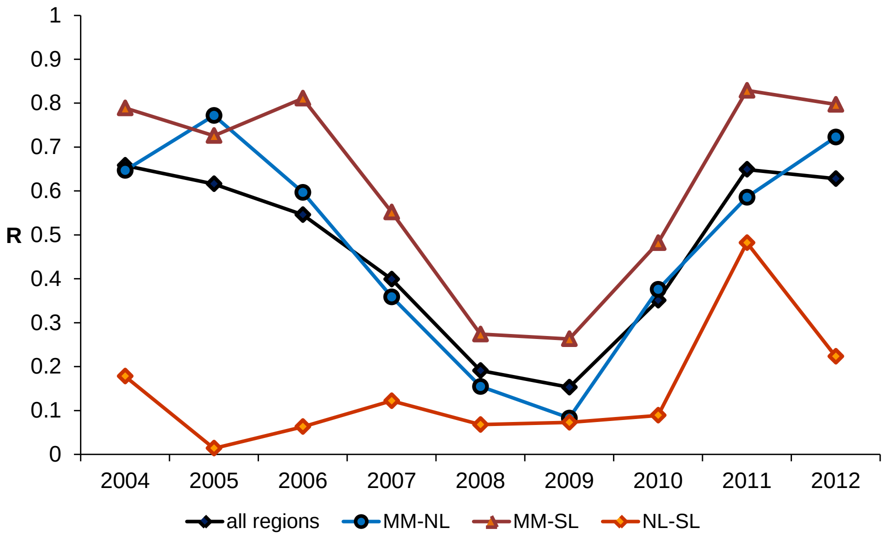

Figure 21: Differences in the similarity of macroinvertebrate communities between the three regions in the Murray Mouth (MM) and Coorong (North Lagoon = NL, South Lagoon = SL) over the monitoring years, based on the Global R statistic from ANOSIM tests/ R indicates the degree of separation, with R-values closer to 1 indicating greater differences, and R-values closer to 0 indicating greater similarity between regions. Differences are shown for any combination between regions, and the black line indicates differences between all regions.

4.2.6 Environmental conditions as predictor variables for macroinvertebrate communities

The pattern of macroinvertebrate communities in the Murray Mouth and Coorong was driven by environmental variables, with nearly 50% of the total variation explained by the considered water and sediment parameters (Figure 22). Salinity was the almost sole determinant, explaining 45% of the variation. Sediment organic matter and chlorophyll-a contributed to the explanation of the community differentiation as well, whereas all other variables had no statistically significant contribution.

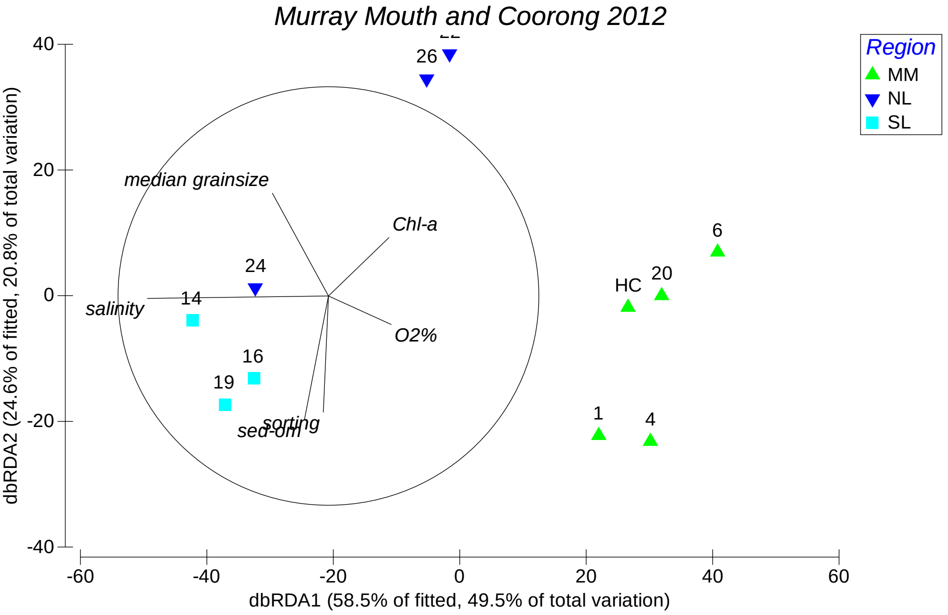

Figure 22: dbRDA (distance based redundancy analysis) illustrating relationships between environmental parameters and the benthic community at the study sites in summer 2012/13. The vector overlay uses base variables of environmental data. The site codes are 1, HC, 4, 6 and 20 for the Murray Mouth, 22, 26 and 24 for the North Lagoon, and 19, 16 and 14 for the South Lagoon (see Figure 2).

For each of the three regions, the relationship between environmental predictor variables and macroinvertebrate communities over time also identified salinity as the predominant variable affecting the patterns (SM-Table 10). For the Murray Mouth and South Lagoon, the community in the flow period (2001 to 2012) was distinct in the dbRDA plots from earlier years), while this was not as clear

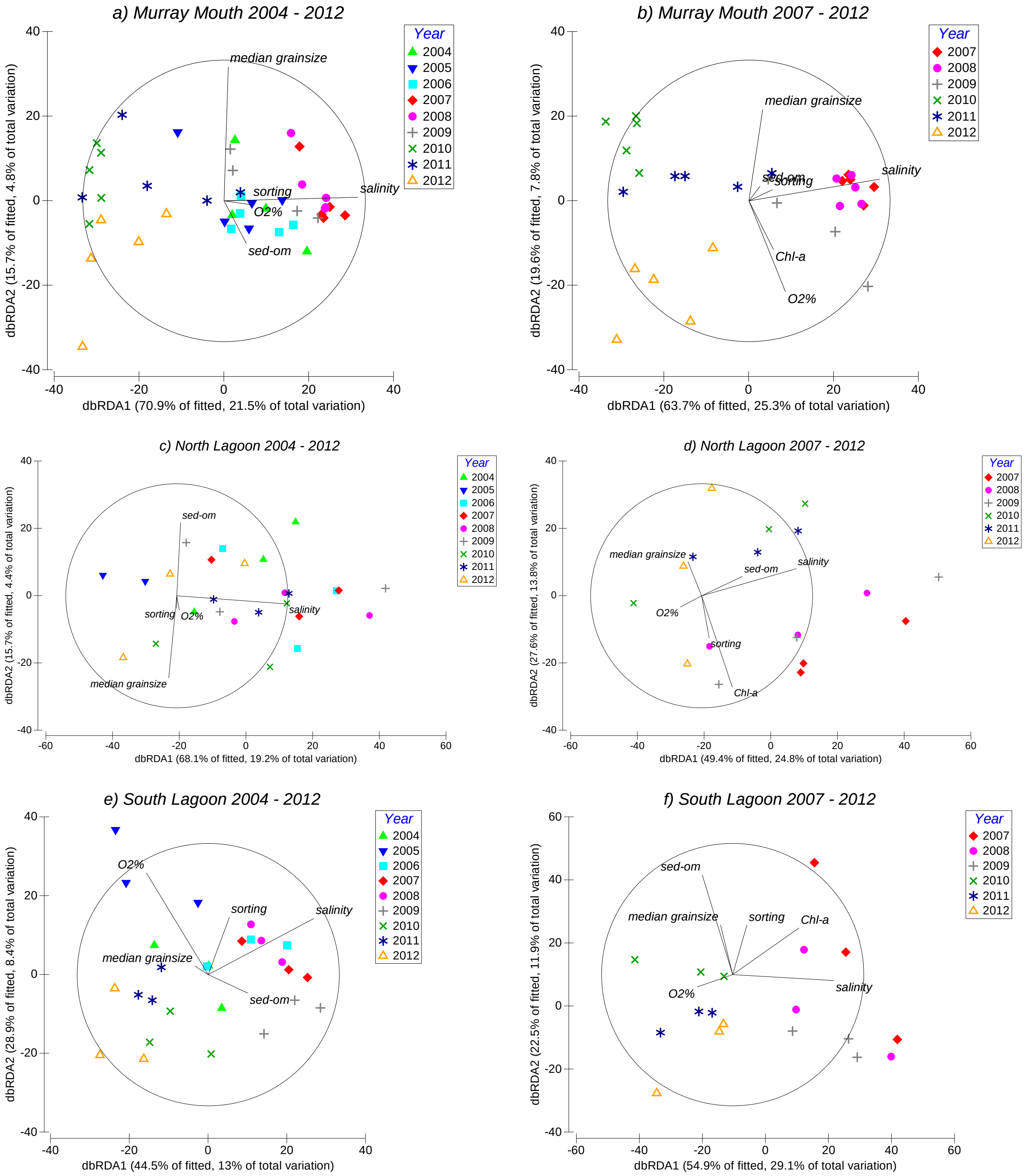

Figure 23: dbRDA (distance based redundancy analysis) plots for the three separate regions Murray Mouth, North and South Lagoon of the Coorong, illustrating relationships between environmental parameters and the benthic community at the study sites in the surveys from December 2004 to December 2012 (right graphs) and from December 2007 to December 2012 for the time frame that also included chlorophyll-*a* analyses in sediments. The vector overlay uses base variables of environmental data that explain the patterns in macroinvertebrate communities.

for the North Lagoon (Figure 23). However, the measured parameters explained only a small percentage (13 to 29%) of the total variation in the community patterns (Figure 23). According to the DISTLM analyses over the time frame from 2004 to 2012, all five variables explained only 30% of the variation, while in the time period from 2007 to 2012, when Chl-*a* was also measured in sediments, the five variables together explained 40% of the variation in macroinvertebrate communities in the Murray Mouth and over 50% in the two Coorong lagoons.

5. Results – Goolwa Channel and Lower Lakes

5.1 Environmental conditions

5.1.1 Salinity regime and water level

Salinities in the Lower Lakes were fresh (0.17 ppt on average) in the Goolwa Channel and Lake Alexandrina, to slightly brackish (1.21 ppt on average) and more variable in Lake Albert (Figure 24, SM-Table 11). These were some of the lowest salinity values recorded over the entire monitoring timeframe, indicative of a change in the salinity regime after the drought (Figure 24, SM-Figure 20). The significant differences in salinity between the survey years were driven by the differences of any previous year to the values recorded in 2012 (SM-Table 11). Freshwater conditions also occurred on the lake side of the barrages, despite barrages being open, indicating that no influx of seawater

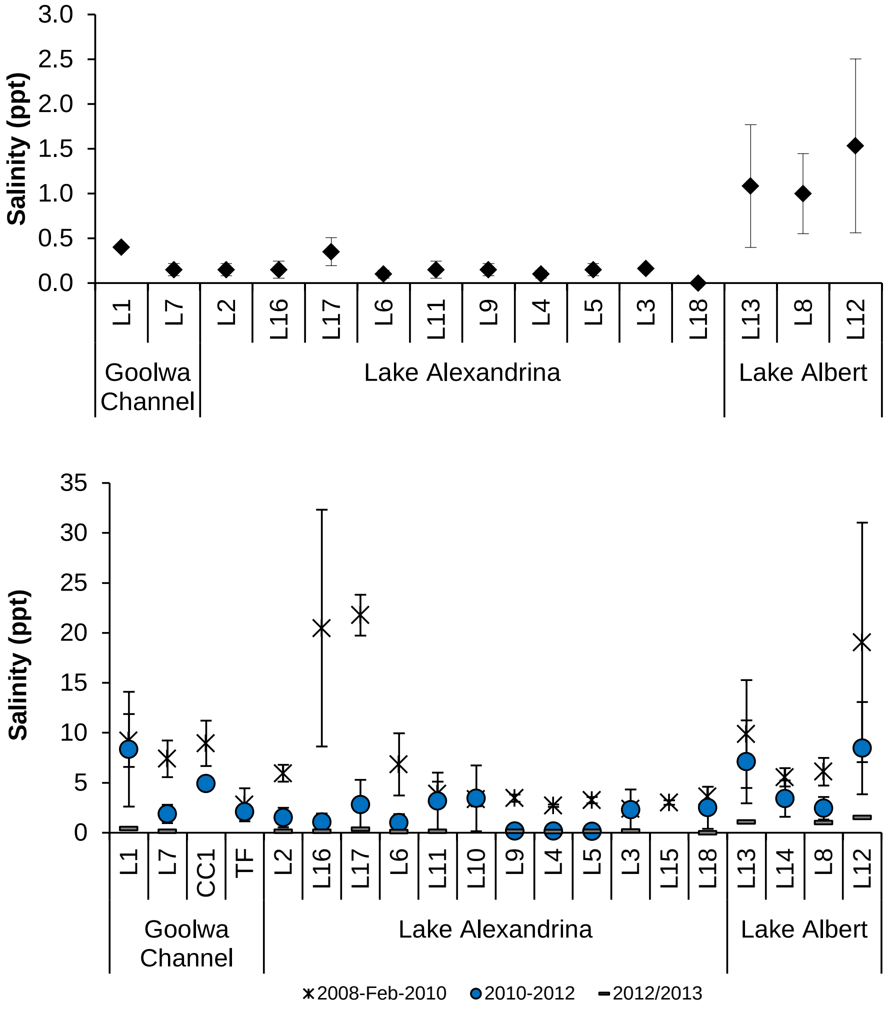

Figure 24: Salinity (mean ppt ±S.E.) of waters overlying sediments in the Goolwa Channel, Lake Alexandrina and Lake Albert during the survey in summer 2012/13 (top figure) and during surveys since December 2008, averaged across three years of severe drought (2008-February 2010) and since river flows resumed (2010-2012) (bottom figure). Salinities from the current monitoring year are also separately displayed in the bottom figure. Sites were L1=Goolwa Channel, L7=Hindmarsh Island, L2=Clayton, L16=Mundoo Channel (Lake), L17=Ewe Island (Lake), L6=Pelican Point (Lake), L11=Loveday, L9=Narrung, L4=Milang, L5=Poltalloch, L3=Tolderol, L18=Boggy Lake, L13=Seacombes, L8=Waltowa, L12=Vanderbrink (see Figure 2). Note that not all sites were included in the monitoring each year, and the large difference in scale of the y-axis between plots.

occurred during the survey time and the restoration of estuarine characteristics was confined to the seaward side of the barrages (see Figure 3).

The condition monitoring target M-1 ‘Facilitate frequent changes in exposure and submergence of mudflats’ can be indirectly addressed through the salinity and water level changes in the Lower Lakes (DEWNR 2013). The continued river flow since spring 2010 led to an increase in the water level, thus inundating the large areas of mudflats which had been exposed when the water level had dropped far below sea level. While water level and salinity data from DEWNR (2013) reveal fluctuations on smaller timeframes in the recent years as well (monthly or seasonal, also subject to water management measures), the sediments around the lake shores have now remained permanently submerged and are thus not accessible as foraging habitat for waders. Yet, the submergence will allow recolonisation of sediments by aquatic invertebrates.

5.1.2 Water Quality

Water temperatures in the Lower Lakes were 22 ºC on average, with little variation over the sampling days for the 2012/13 survey (SM-Figure 19). Further water quality parameters measured around the Lower Lakes corroborate the recovery of the lakes, as already seen with the decreasing salinity values (see 5.1.1). The pH in the water was 8.8 on average, with little variation across the sites, and as high, or higher, at most sites than during previous monitoring since 2008 (SM-Figure 21). There was little variation in dissolved oxygen in the water across the lake regions during the 2012/13 survey, with dissolved oxygen concentration on average 8.3 mg/L, and saturation levels around 97% (SM-Figures 22 and 23, SM-Table 11). Dissolved oxygen saturation was a little lower than the ANZECC guidelines at some sites, yet in comparison to previous monitoring years, 2012/13 fell well within the range of records (SM-Figure 23) which varied across the survey years (SM-Table 11).

5.1.3 Sediment size ranges

Sediments were mainly classed as fine sands, and were only coarser around the central region of Lake Alexandrina, at Milang (site L4), Poltalloch (site L5), Narrung (site L9) and Loveday (site L11) (Table 3), which was also noted in the field. Sediment at sites in Lake Albert (Seacombes (site L13), Waltowa (site L8) and Vanderbrink (site L12)) as well as Boggy Lake (site L18) and Clayton (site L2) had a higher contribution of clay and mud in the sediment than other sites (SM-Figure 24). Yet, most sediments classified as fine or very fine sand and well to moderately well sorted (Table 3). Differences in sediment were site specific (SM-Table 11).

A multivariate analysis of changes in grain size composition over the years indicated some shift between years, although no significant differences were detected (Figure 25, SM-Table 11). When water levels were low (2008 and 2009), sediments were mainly medium to coarse sand, and became finer since the lakes refilled with water. Median grain sizes of sediments, however, varied little over the years at sites in Goolwa Channel and closer to the barrages, while some more variation occurred near Lake Albert (sites L13, L8 and L12) and at Narrung (site L9) (Figure 26). The coarse sediment encountered at Loveday (site L11) and Milang (site L4) could be due to some variation in location of sampling sites compared to previous years following water level changes and access logistics (Figure 26).

Table 3: Sediment characteristics of sampling sites in the Lower Lakes survey in summer 2012/13. Organic matter content (per cent dry weight) within the sediment and the median grain size of sediment (in µm) with the sorting coefficient, are provided as characteristics of sediment. The verbal description of sediment grain size and sorting follows Blott & Pye (2001).

Site		Organic matter (% dw)	Grain size			
			Median		Sorting	
Goolwa Channel	**L1**	0.53	178.82	Fine Sand	0.69	Moderately well sorted
	L7	0.67	159.32	Fine Sand	0.40	Well sorted
Lake Alexandrina	**L2**	5.09	188.87	Fine Sand	0.30	Very well sorted
	L16	0.46	192.36	Fine Sand	0.58	Moderately well sorted
	L17	0.70	176.93	Fine Sand	0.61	Moderately well sorted
	L6	1.56	221.85	Fine Sand	0.35	Well sorted
	L11	0.40	1489.95	Very coarse sand	0.31	Very well sorted
	L9	0.44	668.52	Coarse Sand	0.41	Well sorted
	L4	0.17	1560.97	Coarse Sand	0.43	Well sorted
	L5	0.51	410.57	Medium Sand	0.46	Well sorted
	L3	0.29	166.16	Fine Sand	0.78	Moderately sorted
	L18	1.81	103.88	Fine Sand	0.41	Well sorted
Lake Albert	**L13**	0.48	117.01	Very fine sand	0.40	Well sorted
	L8	7.65	180.78	Fine Sand	0.32	Very well sorted
	L12	0.98	120.64	Very fine sand	0.31	Very well sorted

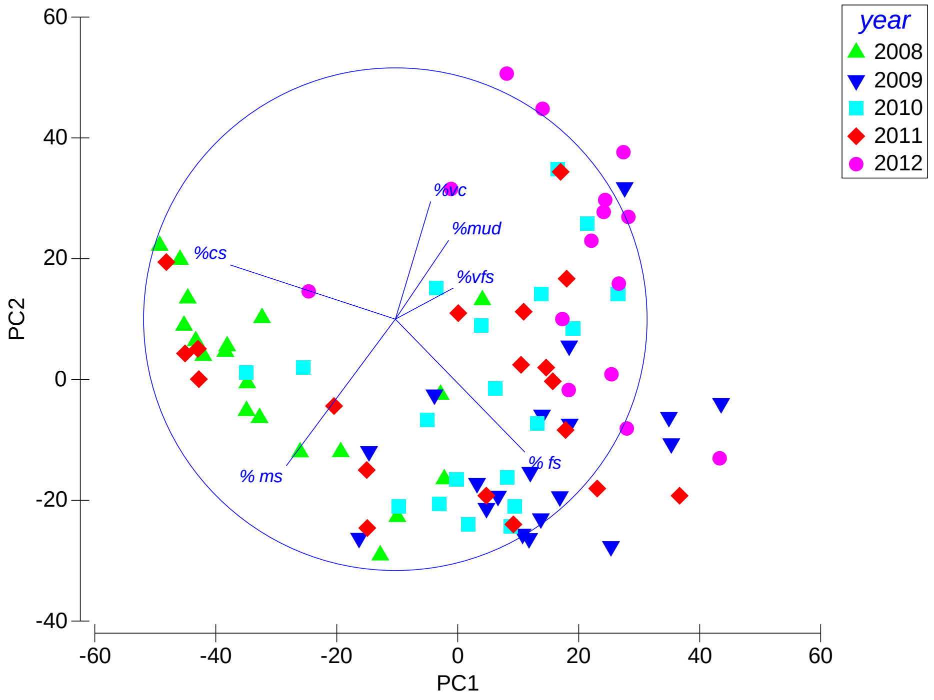

Figure 25: PCA (Principal component analysis) of sediment grain size compositions (% of major fractions, size in µm) in sediments around the Lower Lakes for the summer surveys from 2008 to 2012/13, when most sites were sampled annually. Sites or regions are not shown in the figure. The PCA axes explained 51 % (PC1) and 29 % (PC2) of the variation.

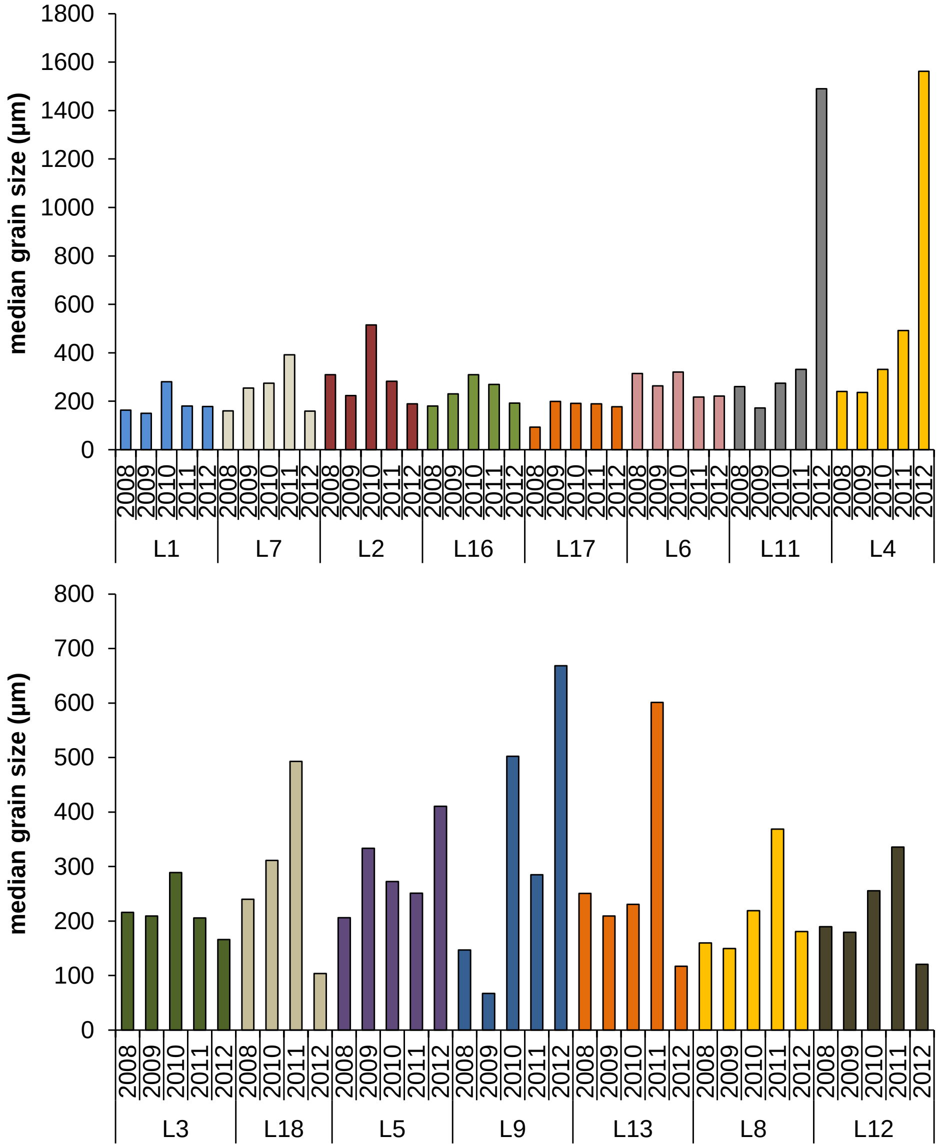

Figure 26: Median grain size values recorded in sediments around the Lower Lakes, sampled since 2008. Note the different y-axes labels due to some outlying coarser sediment in the North Lagoon. Sites were L1=Goolwa Channel, L7=Hindmarsh Island, L2=Clayton, L16=Mundoo Channel (Lake), L17=Ewe Island (Lake), L6=Pelican Point (Lake), L11=Loveday, L9=Narrung, L4=Milang, L5=Poltalloch, L3=Tolderol, L18=Boggy Lake, L13=Seacombes, L8=Waltowa, L12=Vanderbrink (see also Figure 2).

5.1.3 Sediment organic matter and chlorophyll-*a*

The sediment organic matter content was low (average of 0.7 ± 0.1 % dry weight) for most of the lake sites, with outliers of >5 % dry weight at Clayton (site L2) and Waltowa (site L8) (SM-Figure 25), causing significant differences between sites (SM-Table 11). However, seen in the longer term comparison of sediment organic matter in the lake sediments, the values were within the range, or at the lower end, of sediment organic matter determined in previous monitoring years (Figure 27, SM-Table 11). Yet, given the frequent outliers, which often come together with high variation, further analysis into small scale patchiness and biogeochemistry would be needed to differentiate whether these are artefacts or true patterns. Overall, as in the Murray Mouth and Coorong, the condition monitoring target M-3 ‘Maintain organic content for mudflats’ was met.

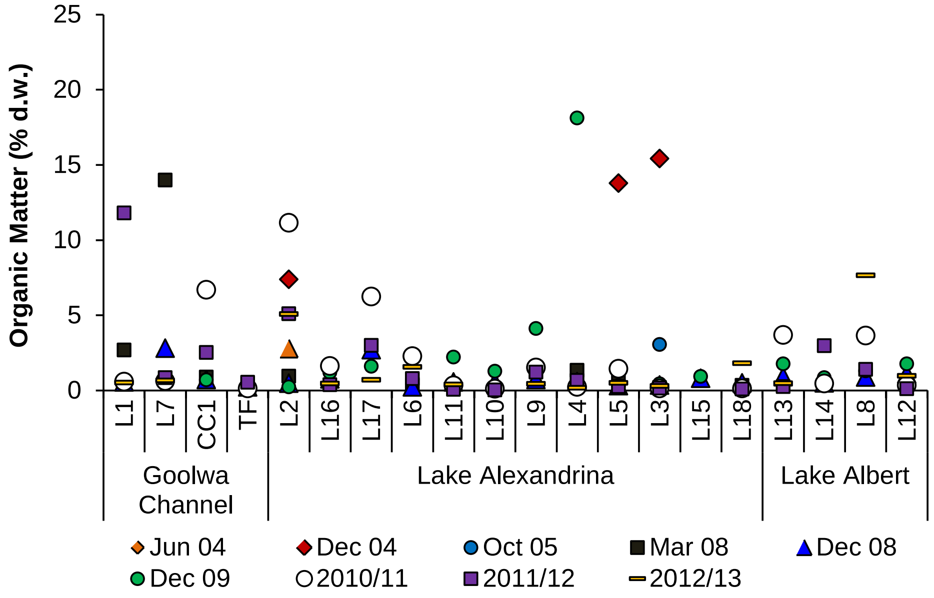

Figure 27: Sediment organic matter (as % dry weight) at sites sampled from 2004 to 2012/13 in the Lower Lakes. Note that not all sites were sampled each year. An outlying value of 55 % organic matter from site L3 in June 2004 is omitted from this figure for clarity.

The chlorophyll-*a* content of sediments around the Lower Lakes was variable between sites, and ranged from 0.3 – 5 mg m^{-2} (SM-Figure 26, SM-Table 11). Values for chlorophyll-*a* were around 1 mg m^{-2} at most sites, but more than twice as high at sites near the inside of the barrages (Sites L6, L17, L6 and L11, see map in Figure 2) as well as Milang (site L4) and Seacombes (site L13). Chlorophyll-*a* values were significantly different between years and in comparison to previous years, the values from 2012/13 were higher than in the last two monitoring years, and more similar to records from 2008 (Figure 28, SM-Table 11).

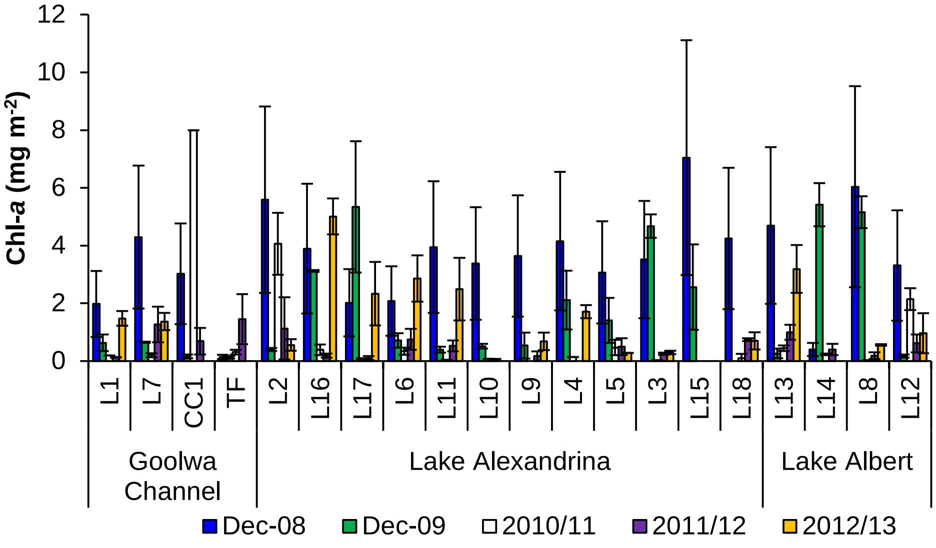

Figure 28: Sediment chlorophyll-*a* content (in mg m^{-2}) at study sites around the Lower Lakes surveyed in summer 2012/13, in comparison to previous years. Note that not all sites were sampled in all years.

5.1.5 Changes in environmental conditions between years

The environmental variables measured in the 2012/13 monitoring accounted for a differentiation of sites around the Lower Lakes, with two sites in Lake Albert (Waltowa (site 8) and Vanderbrink (site 12)) deviating because of salinity and, together with sediments at Clayton (site L2), also higher sediment organic matter (SM-Figure 27). Yet, site specific differences in environmental conditions prevailed over regional ones (SM-Table 1). A shift in environmental conditions was obvious over time, as all samples from 2012/13 were very distinct from those taken in 2008, regardless of sites or regions (Figure 29). This deviation between years was mainly driven by a reduction in salinity, dissolved organic matter, and single grain size fractions. This shift was obvious in each of the regions of the Lower Lakes, where environmental conditions from 2008 to 2012 changed along a path from higher salinity and coarser sediments in 2008, to finer sediments with more organic matter in the recent survey (SM-Figure 28). These changes could be related to the distance from shore of sampling locations with rising water levels in the lakes.

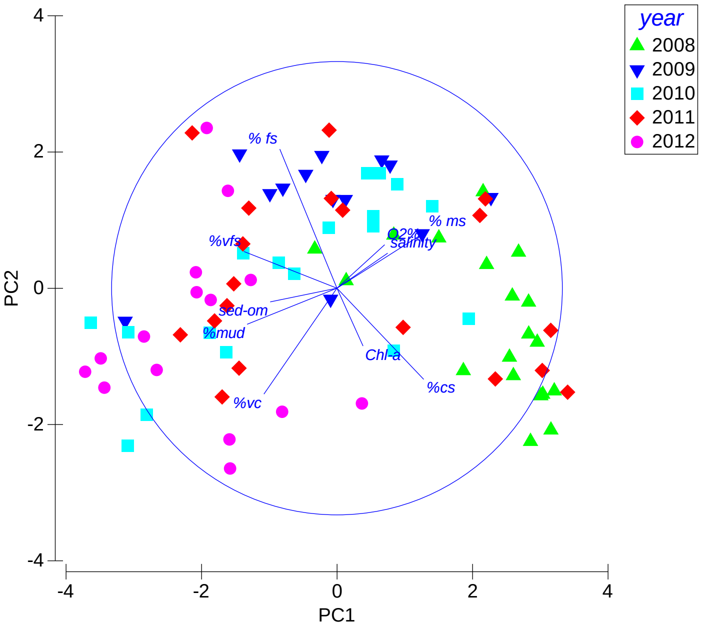

Figure 29: Principle component analysis (PCA) plot of environmental variables for each site sampled in the Lower Lakes from annual summer surveys since 2008 to 2012/13. Environmental variables included were salinity and dissolved oxygen content (O2%) from the water column, and from the sediment single grain size fractions, organic matter and chlorophyll-*a* (Chl-*a*). PC1 explains 41.7%, and PC2 16.8% of the variation.

5.2 Macroinvertebrate populations

The Condition Monitoring Target I-1: 'Maintain or improve invertebrate populations in mudflats' is evaluated for the Lower Lakes based on continued sampling of sediments for benthic macro-invertebrates, which will be assessed for temporal changes, and dip net samples for aquatic invertebrates, which were added in this 2012/13 monitoring. Detailed findings and outcomes from tests are presented in the Supplementary Material. The structure follows key parameters to assess improvements in invertebrate populations, namely diversity, abundances and distributions, biomass and community structures over time.

5.2.1 Macroinvertebrate diversity and distribution

A diverse macroinvertebrate community was encountered in the Lower Lakes in the 2012/13 survey. Based on the core/grab (benthic) samples, 21 different taxa were recorded living in or near the sediment (SM-Table 12). The additional dip net samples taken in this year's monitoring obtained 36 different species living in the water column, usually associated with vegetation along the shore (SM-Table 13). In total, 41 macroinvertebrate taxa were thus found in the Lower Lakes, whereby five were recorded in benthic samples only, and 21 taxa occurred exclusively in dip net samples. These included several molluscs, crustaceans and in particular insects in both larval and adult life stages (Table 4). Annelids and several other mollusc species were mainly found in benthic samples (Table 4, Figure 30). The significant differences in species numbers recorded at the sites around the regions of the Lower Lakes were thus mainly driven by additional insect species found exclusively in dip nets (Figure 30, SM-Figure 29, SM-Table 15).

The overall species number was highest in Lake Alexandrina with 32 taxa, compared to 27 taxa in Lake Albert and 15 in the Goolwa Channel, which shared all species with Lake Alexandrina apart from a spionid polychaete which occurred in the Goolwa Channel region only (Table 4). A similar

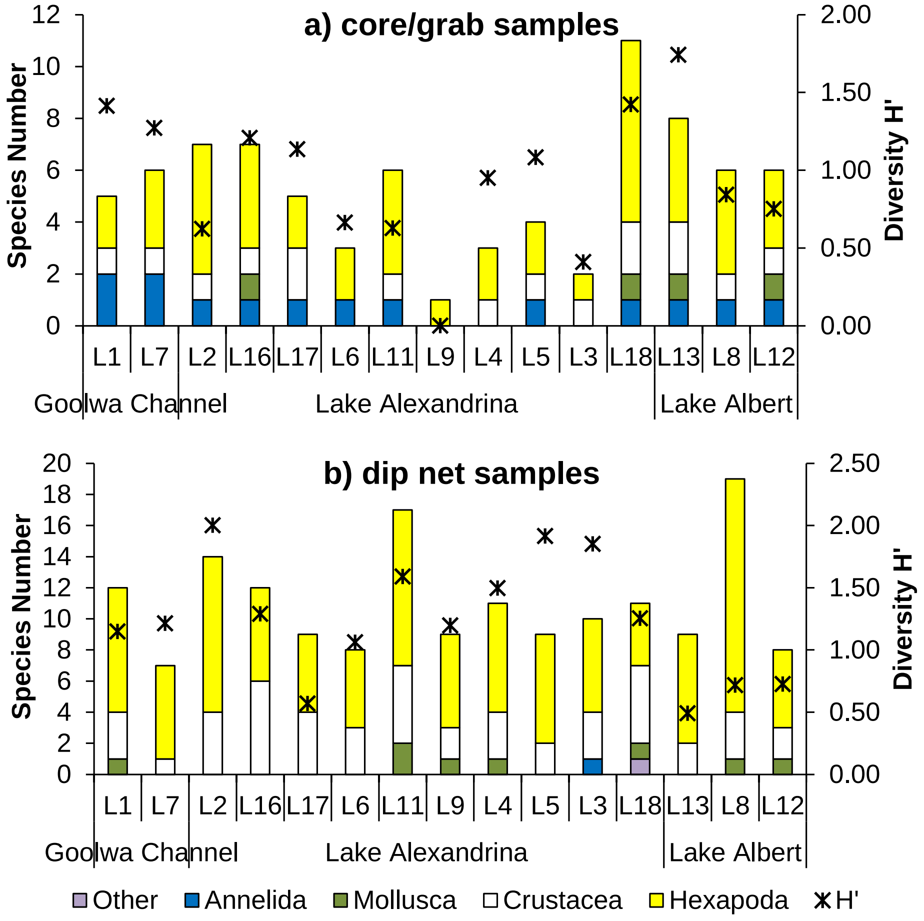

Figure 30: Total number of macroinvertebrate species recorded at the sampling sites around the Lower Lakes in January 2013, based on samples taken using a) sediment corer or grab, or b) dip nets. The major phyla or taxa constituting the total species count are indicated by colours, with 'other' being an arachnid. Asterisks are the Shannon-Wiener diversity index H' (base $\log_e$). Note the different scales on the y-axes.

relationship of highest species numbers in Lake Alexandrina compared to the adjacent regions was also apparent for the sediment core samples as well as dip net samples alone (Table 4). The average number of species per site was, however, more similar between the three regions (Table 4).

Table 4: Occurrence of macroinvertebrate taxa during the 2012/13 summer survey around the Lower Lakes, based on samples obtained by sediment core or grab samples (indicated as X) and dip net samples (indicated as D). Note that some rare species identifications are still to be cross-checked with experts. The total species number per site and region is also provided based on each method, and for a combined assessment of macroinvertebrate diversity per site and region. As the number of sites per region differs, the average number of taxa (± standard error) based on both methods is provided as well.

Phyla/Class/Order		**Family/Genus/Species**	**Goolwa Channel**		**Lake Alexandrina**										**Lake Albert**		
			L1	**L7**	**L2**	**L16**	**L17**	**L6**	**L11**	**L9**	**L4**	**L5**	**L3**	**L18**	**L13**	**L8**	**L12**
Annelida	Oligochaeta		X	X	X	X	X	X	X			X	D	X	X	X	X
	Polychaeta	Spionidae	X	X													
Mollusca	Bivalvia	Corbiculidae												X			
	Gastropoda	*Physa acuta*	D			X			D						X	D	
		Hydrobiidae															X D
		Ferrissia sp.							D	D	D			D			
Crustacea	Amphipoda		X D	X	X D	X D	X D	D	X D	D	X D	X D	X D	X D	X D	X D	X D
	Cladocera		D		D	D			D					D			
	Copepoda					D	X D							D			
	Ostracoda					D	D	D	D					X D	X D	D	
	Decapoda	Palaemonidae				D			D		D		D				
		Parastacidae			D												
		Paratya australiensis	D	D	D	D	D	D	D	D	D	D	D	D		D	D
Arachnida	Mesostigmata													D			
Hexapoda	Collembola	Isotomidae										X				X	
		Sminthuridae															D
	Diptera	Chironomidae	X D	X D	X D	X D	X D	X D	X D	X D	X D	X D	D	X D	X D	X D	X D
		Culicidae														D	
		Dolichopodidae				X			X					X	X	X	
		Muscidae														D	
		Stratiomyidae							D							D	
	Coleoptera	Dytiscidae			D								D				
		Heteroceridae														D	
		Hydrophilidae												X		D	
		Scirtidae				D											
	Hempitera	Belostomatidae														D	
		Corixidae	X D		X D	X	D	D	XD			D		X	X D	X D	X D
		Notonectidae	D	X D	D	D	D	D	D	D	D	D	D		D	D	D
		Veliidae														D	
	Ephemeroptera	Baetidae			D												
		Caenidae	D	D	X D				D	D	D	D	D	X D			
	Trichoptera	Conoesucidae					X										
		Ecnomidae	D	X D	X D	D	D		X D	D	X D	D	X D	X D			
		Hydroptilidae	D	D	D	D		D	D		D	D	D	D	D		
		Leptoceridae	D		X D	X		D	D		D	D		X	D	D	
	Odonata	Aeshnidae														D	
		Coenagrionidae	D		D	D	D		D	D					X D	D	X D
		Corduliidae														D	
		Libellulidae							D								
	Lepidoptera	Crambidae								D					D		
	Unidentified insect larvae			D				X			D					D	
Core/grab samples (X):		Number of taxa per site	**5**	**6**	**7**	**7**	**5**	**3**	**6**	**1**	**3**	**4**	**2**	**11**	**8**	**6**	**6**
		Number of taxa per region	**7**		**16**										**10**		
Dip net samples (D):		Number of taxa per site	**12**	**7**	**14**	**12**	**9**	**8**	**17**	**9**	**11**	**9**	**10**	**11**	**9**	**19**	**8**
		Number of taxa per region	**13**		**26**										**23**		
Total (all samples):		Number of taxa per site	**14**	**10**	**15**	**17**	**11**	**10**	**19**	**9**	**11**	**10**	**10**	**17**	**12**	**22**	**9**
		Number of taxa per region	**15**		**32**										**27**		
	average number of taxa per region ± SE		**12 ± 2**		**13 ± 1**										**14 ± 5**		

Diversity values (Shannon-Wiener H') were higher at most sites for samples obtained by dip nets, in particular at sites L2 (Clayton), L9 (Narrung), L4 (Milang), L5 (Poltalloch) and L3 (Tolderol), than those obtained by sediment core or grab samples (Figure 30). Yet, at sites L17 (Ewe Island, lake side), L11 (Loveday) and L13 (Seacombes), diversity of macroinvertebrates was higher in the sediments (Figure 30). Both methods capture different components of the macroinvertebrate fauna in the aquatic environment of the Lower Lakes, and are clearly complementary to each other.

The diversity values and species numbers recorded in the recent monitoring fell within the range of values recorded since 2008 (SM-Table 14), especially when seen on a regional level (Figure 31). Looking at single study sites, species numbers had increased or were comparable to those recorded in the previous two years, with the exception of Narrung (site L9, which could not be sampled at exactly the same location) and Poltalloch (site L5) (Figure 32, see SM-Figure 30 for inclusion of sites sampled since 2004). However, the differences in species density between years and sites nested in region were significantly different (SM-Table 15).

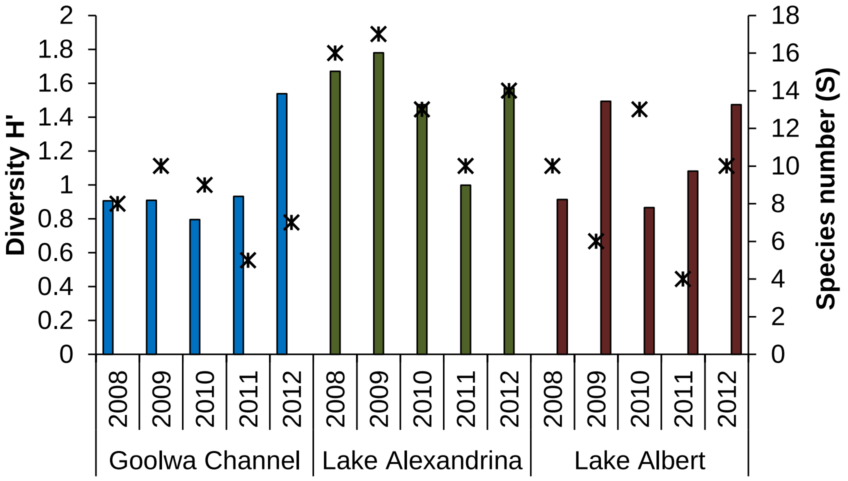

Figure 31: Changes in diversity in each of the three regions of the Lower Lakes in the monitoring periods from 2008 to 2012/13, based on samples taken with a sediment corer or grab. Bars indicate Shannon-Wiener diversity index H' (base log$_e$) and asterisks are the species number. Note that the number of sites sampled per region differed between years.

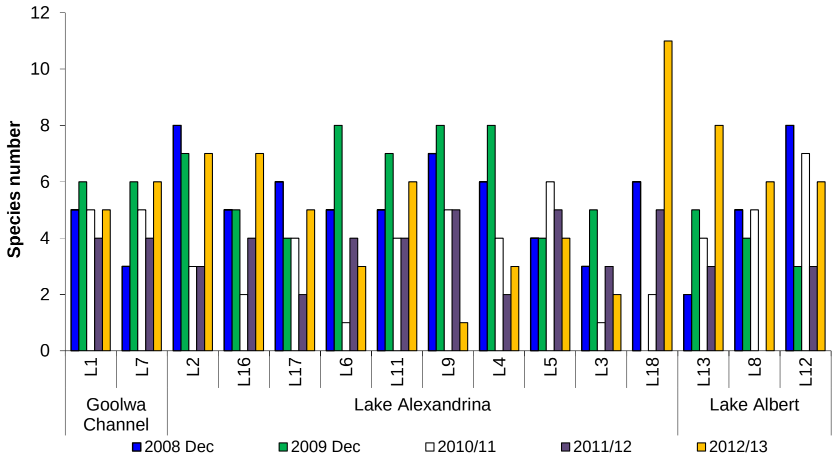

Figure 32: Total number of benthic macroinvertebrate species recorded at those sites sampled annually around the Lower Lakes since 2008, using a corer or grab.

5.2.2 Macroinvertebrate abundances and distribution

5.2.2.1 Benthic macroinvertebrates

Abundances of macroinvertebrates collected in sediments around the Lower Lakes were low, on average 1245 individuals m^{-2}, with the exception of much higher individual numbers at Boggy Lake (site L18) (Figure 33). Variability was high between sites within regions, especially in Lake Alexandrina (Figure 33). The shores around the central region of Lake Alexandrina (Narrung (site L9), Poltalloch (site L5), Milang (site L4) and Tolderol (site L3)) were scarcely populated by macroinvertebrates in the sediments (Figure 33). The significant site specific variability (patchiness) obscured any regional differentiation in macroinvertebrate abundances around the Lower Lakes and, apart from spionid polychaetes, which were only found in the Goolwa Channel, no significant regional differences in abundance occurred (SM-Tables 16 and 17).

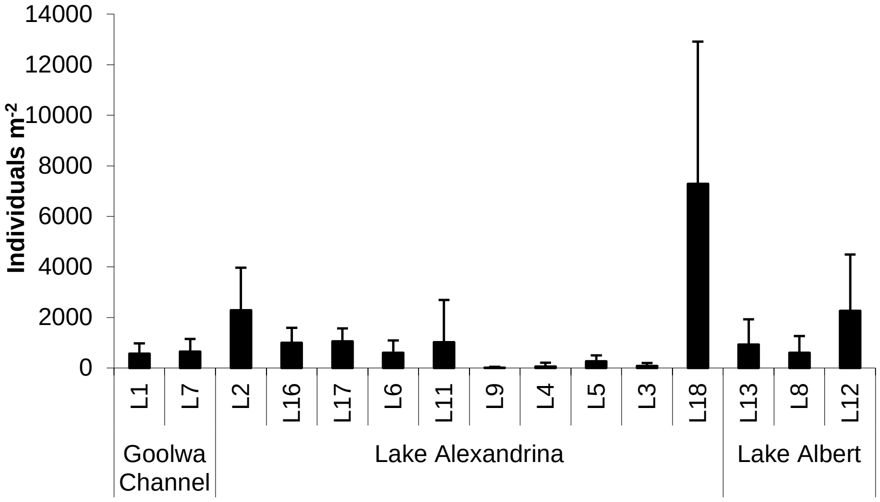

Figure 33: Mean abundances (ind. m^{-2}) and standard deviation (± S.D.) (*n* = 10) of benthic macroinvertebrates recorded at sites sampled around the Lower Lakes during the 2012/13 summer survey.

Amphipods, chironomid larvae, and oligochaetes accounted for the highest numbers and were widespread around the lakes, with amphipods dominating at Boggy Lake (SM-Figure 31 and 32). Chironomids accounted for the high abundances at Clayton (site L2) and Vanderbrink (site L12). Several other insect larvae also occurred around the lakes, yet other species (for example, ostracods) were recorded in samples from single sites only (SM-Figure 32). Molluscs were rare with only few individuals of snails (*Physa acuta* and Hydrobiidae) and several bivalve (Corbiculidae) specimens found at single sites each.

Changes in abundances of all macroinvertebrates found in the samples from sites monitored since 2008 to 2012/13 were significant between years subject to sites (SM-Table 17), with no clear pattern being apparent because of localised high variability in particular years (Figure 34a). Yet, average abundances were higher in 2012/13 compared to the previous two survey years at sites in the southern parts of Lake Alexandrina, between Clayton (site L2) and Loveday (site L11), as well as Boggy Lake (site L18) (Figure 34a). This increase occurred for the three major taxa (chironomids, amphipods and oligochaetes) inhabiting the sediments around the lakes (Figure 35), who all had significant variations in abundances per site and survey year (SM-Table 17).

For sites monitored since 2004, the total abundances encountered in 2012/13 were low compared to earlier surveys (Figure 34b). Yet at Boggy Lake (site L18), abundances had noticeably increased to be comparable to abundances seen at this site in 2004 (Figure 34b). Insect larvae (hexapods) and oligochaetes, as the main annelids, also occurred in higher abundances at Boggy Lake, whereas abundances of all major taxa were within the lower range of values recorded over the years at the other sites (SM-Figure 33). While there was some improvement, macroinvertebrate abundances in sediments around the Lower Lakes were mainly maintained in the 2012/13 survey (Condition Monitoring Target I-1).

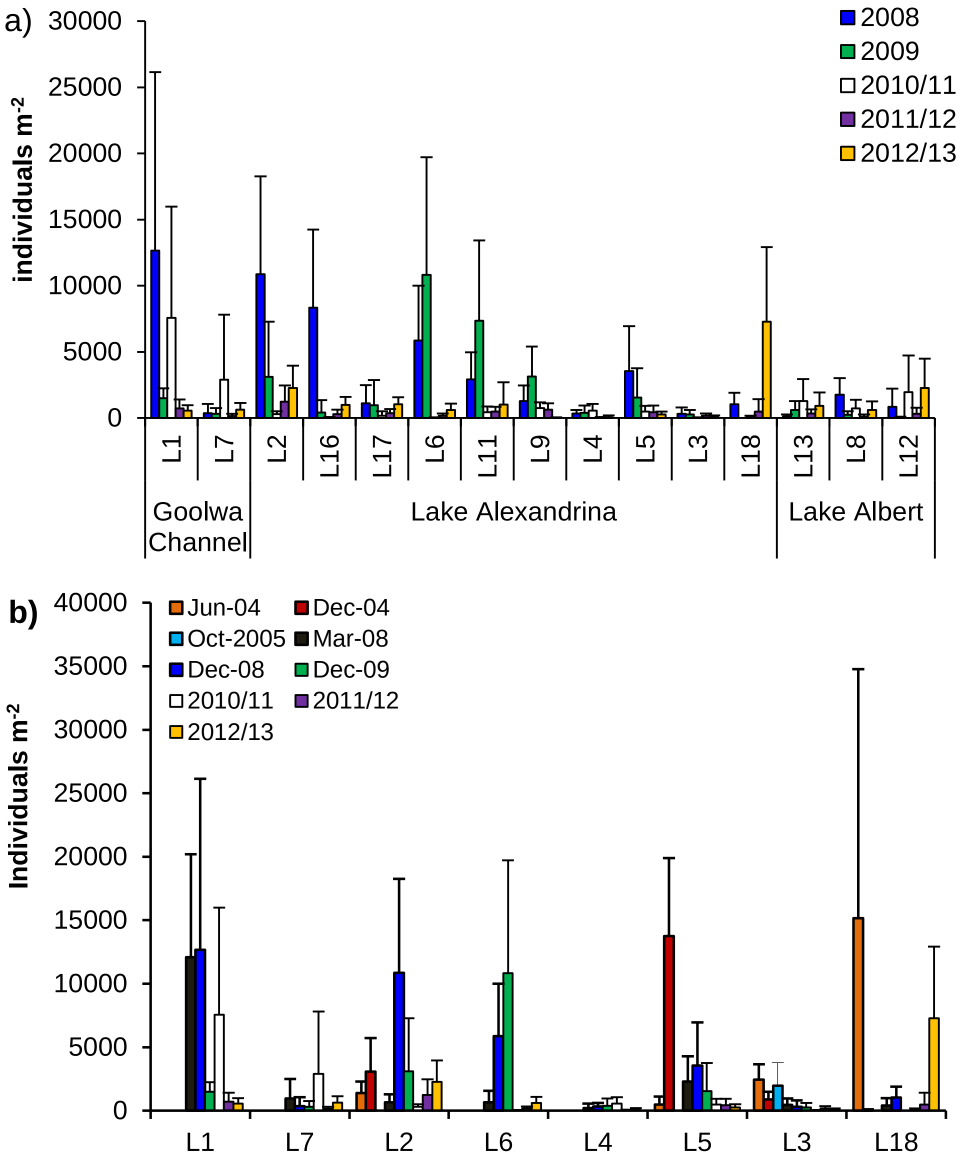

Figure 34: Mean abundances (ind. m^{-2}) and standard deviation (S.D.) of all benthic macroinvertebrates recorded at the sampling sites around the Lower Lakes a) compared at sites sampled annually in spring/summer from 2008 to 2012/13, and b) compared between sites sampled during various surveys from 2004 until 2012/13. Note that not all sites were sampled in each year. Also note the different y-axes scales.

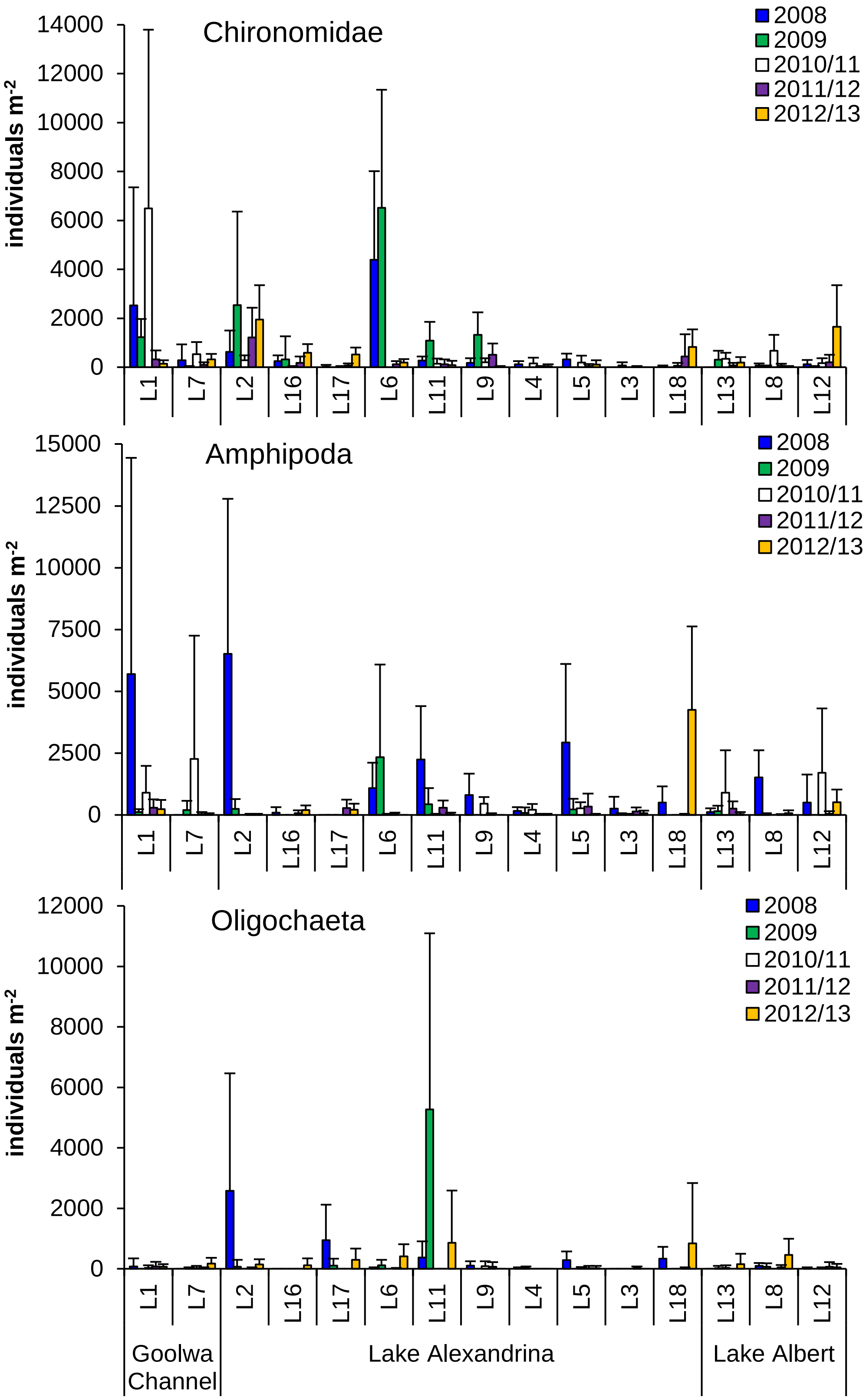

Figure 35: Mean abundances and standard deviation (*n* = 10) of macroinvertebrate taxa considered to be of indicator value for the Lower Lakes, recorded at the sampling sites continued to be surveyed in early spring/summer since 2008 until 2012/13. Note variations in scale of the y-axis.

5.2.2.2 Aquatic invertebrates

Abundances of aquatic macroinvertebrates caught with dip nets were high, but also variable within and between sites, with 99 ± 94 individuals per dip net on average (Figure 36). Site specific differences in abundance occurred for total abundances as well as major organism groups and key taxa, apart from hexapods (insects), which were prevalent throughout the lakes (SM-Table 18, Figure 37). Crustaceans and insects contributed most to the high abundances of aquatic invertebrates (Figure 37).

Aquatic macroinvertebrate abundances were high at Seacombes (site L13) and Waltowa (site L8) in Lake Albert, driven mainly by amphipods, but also chironomid larvae and several other insects, such as backswimmers (Notonectidae) and damselfly larvae (Coenagrionidae) (Figures 36 and 38). Within Lake Alexandrina, abundances were highest at the lake side of Ewe Island (site L17), driven by amphipods (Figures 34 and 38). Another abundant crustacean was the freshwater shrimp (*Paratya australiensis*), which occurred in high numbers in samples taken on the lake side of Mundoo Channel (site L16), Milang (site L4) and Boggy Lake (site L18) (Figure 38). Amongst the insect species, caught as either or both larval and adult life stages, Notonectidae (backswimmers), Ecnomidae (free-living caddis) and Hydroptilidae (micro caddis) occurred wide spread around the Lower Lakes, yet were absent at particular sites (Figure 38). Other aquatic macroinvertebrates were even more site specific in their occurrence and abundance (Figure 38, Table 4).

High abundances of macroinvertebrates in the water column were not always matched by high abundances in sediments, nor *vice versa*, as several sites with low benthic abundances of macroinvertebrates had higher numbers of aquatic insects and crustaceans (SM-Figure 34, Figures 37 and 33). The use of dip nets is thus not only complimentary for the assessment of biodiversity of macroinvertebrates in the Lower Lakes, but also their abundance pattern.

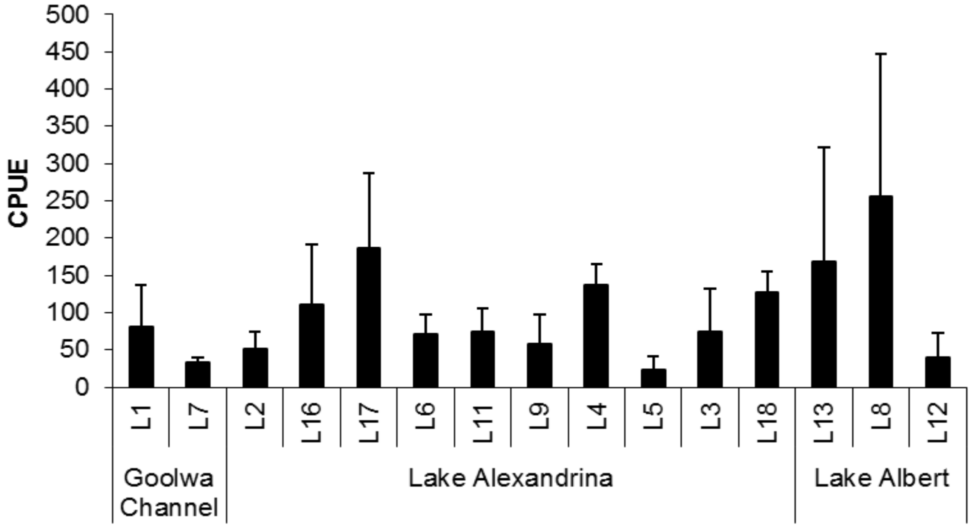

Figure 36: Mean abundances and standard deviation (*n* = 5) of all aquatic macroinvertebrates caught with dip nets at the sampling sites around the Lower Lakes in January 2013. CPUE=Catch per Unit Effort.

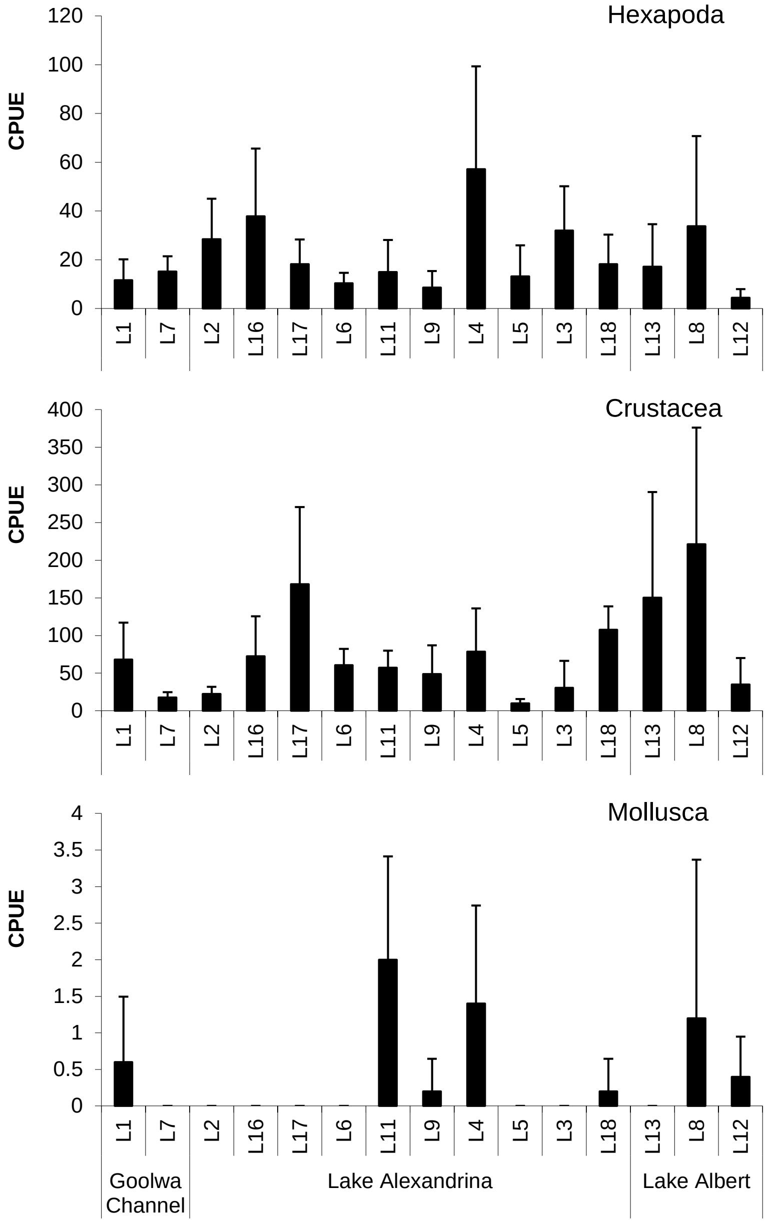

Figure 37: Mean abundances and standard deviation (*n* = 5) of major taxonomic groups found in dip net samples for aquatic macroinvertebrates at the sampling sites around the Lower Lakes in January 2013. CPUE=Catch per Unit Effort.

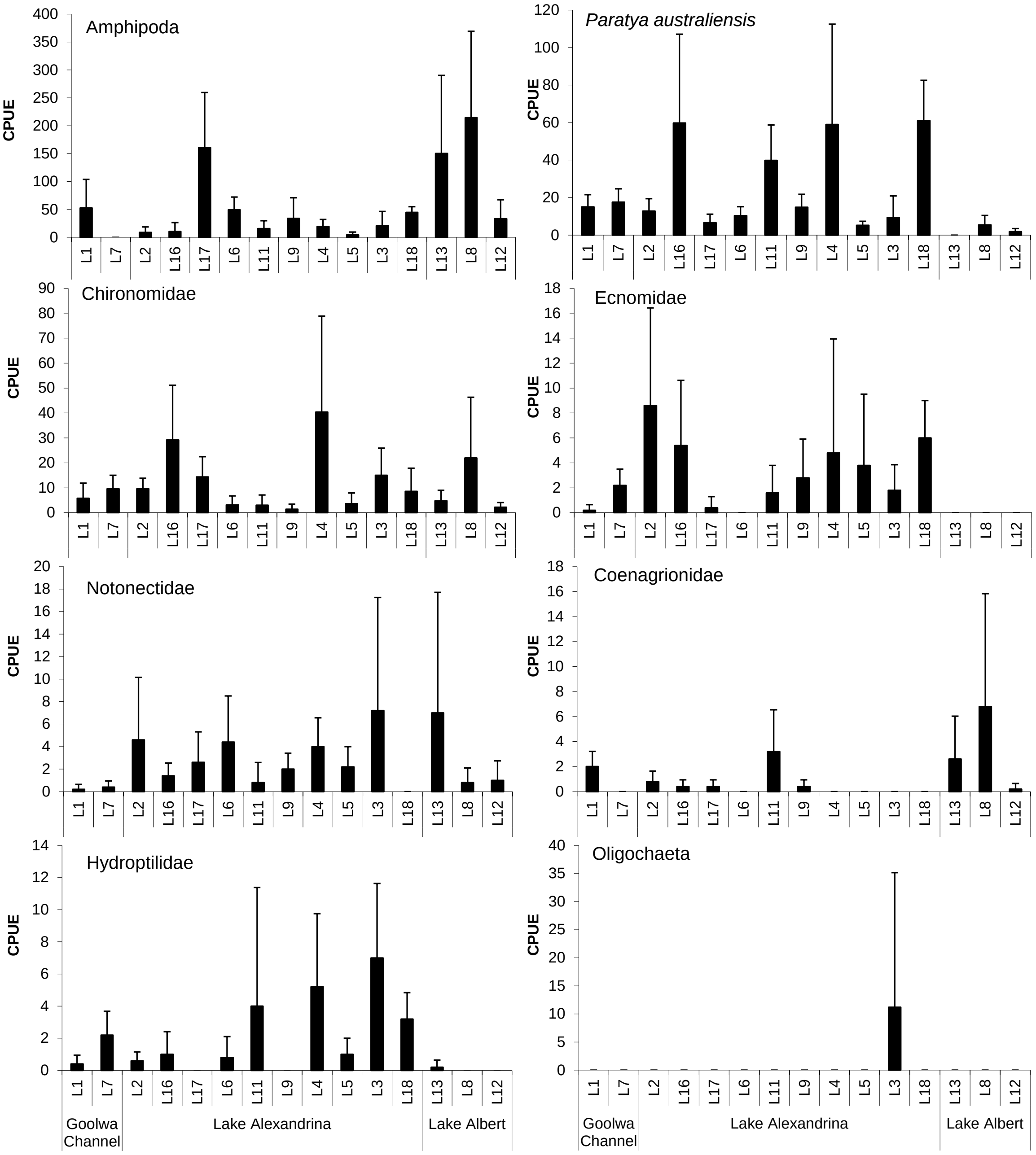

Figure 38: Mean abundances and standard deviation (*n* = 5) of the most abundant species or taxa found in dip net samples for aquatic macroinvertebrates at the sampling sites around the Lower Lakes in January 2013. CPUE=Catch per Unit Effort.

5.2.3 Macroinvertebrate biomass

Biomass of macroinvertebrates in sediments around the Lower Lakes was low, but variable between sites, with 0.24 ± 0.74 g AFDW m^{-2} on average (Figure 39, SM-Table 16). Biomass was highest at sites where abundances of amphipods, chironomids and oligochaetes were high as well, especially at Boggy Lake (Site L18) (Figures 39 and 33). At Hindmarsh Island (site L2) and Mundoo Channel (lake side (site L16)), various insect larvae that were not numerically abundant contributed to the biomass (compare Figure 39 and SM-Figure 32). The high variability within sites and between sites within each region of the Lower Lakes resulted in no significant biomass differences by region, but significant differences among sites (nested in regions) (SM-Table 17).

Compared over the years since 2008, biomass of macroinvertebrates varied significantly between surveys and sites (SM-Table 17), but had increased over values recorded in the previous year (Figure 40). While biomass was still lower than several years ago, an improvement could be seen at most sites over the time span of monitoring since 2008 (Figure 40), as well as when sites sampled since 2004 were considered (SM-Figure 35).

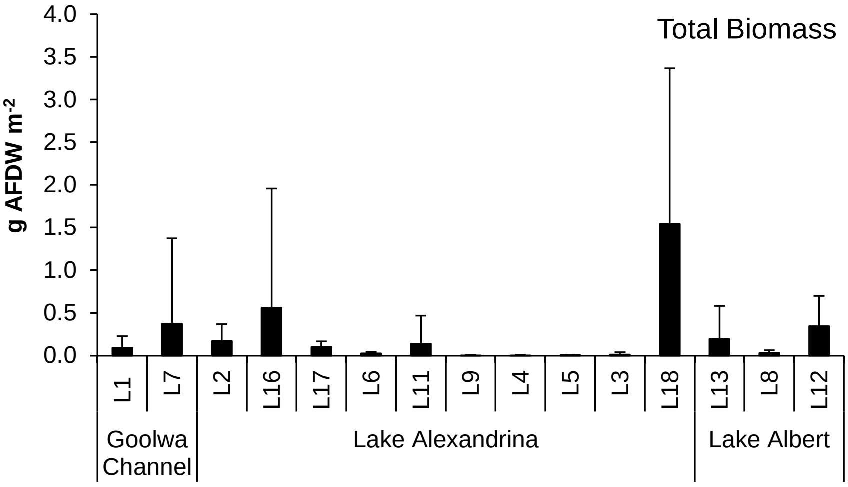

Figure 39: Average biomass of benthic macrofauna (g AFDW m^{-2} with standard deviation, *n* = 10) at sites sampled around the Lower Lakes during the survey in summer 2012/13.

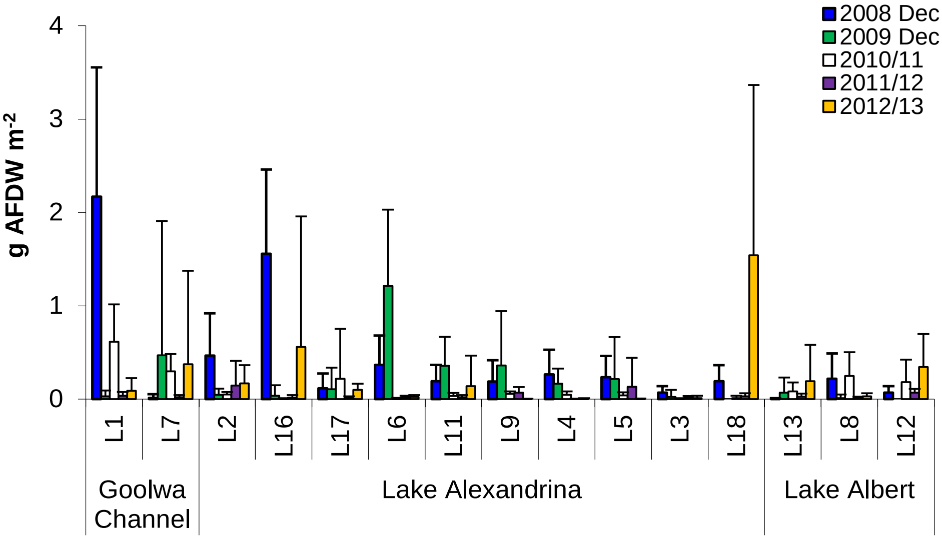

Figure 40: Biomass of benthic macroinvertebrates (mean and standard deviation, *n* = 10) at sites sampled around the Lower Lakes compared between early spring/summer surveys from 2008 to 2012/13.

5.2.4 Macroinvertebrate communities

5.2.4.1 Benthic macroinvertebrate communities

For benthic macroinvertebrates, only site specific differences in community structure were detected (SM-Table 19). Sites inhabited by very few macroinvertebrates over summer 2012/13 (sites L9 (Narrung), L4 (Milang), L3 (Tolderol) and L5 (Poltalloch) (Figure 33) were separated from the remaining sites, which shared greater similarity (Figure 41).

SIMPER analysis indicated very low similarities within each site (SM-Table 21), corresponding with the high variability on site level for abundances of single taxa (chapter 5.2.2.1). SIMPER indicated further that communities of benthic macroinvertebrates at sites in the southern reaches of Lake Alexandrina and the Goolwa Channel were more similar to each other, as dissimilarity was higher compared to the sites located in the northern reaches of the lake (SM-Table 21). These were mainly those four sites mentioned above that were split in the PCO plot because of relatively low abundances (Figure 41). Chironomids, amphipods and oligochaetes were the main characterising taxa throughout the Lower Lakes macroinvertebrate community in summer 2012/13 (SM-Table 21).

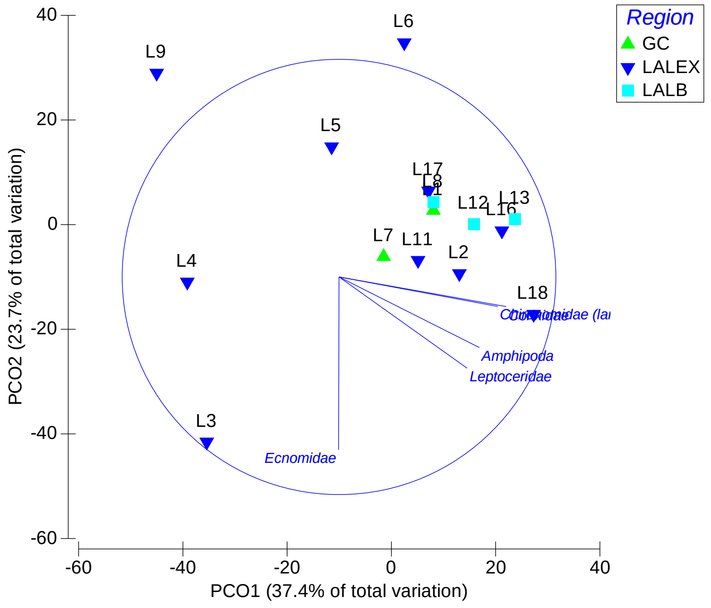

Figure 41: PCO (Principal Coordinates Analysis) plot of benthic macroinvertebrates from the survey in the Lower Lakes in summer 2012/13. The circle represents a vector overlay (Spearman correlation) illustrating the contribution of the respective species to the PCO axes.

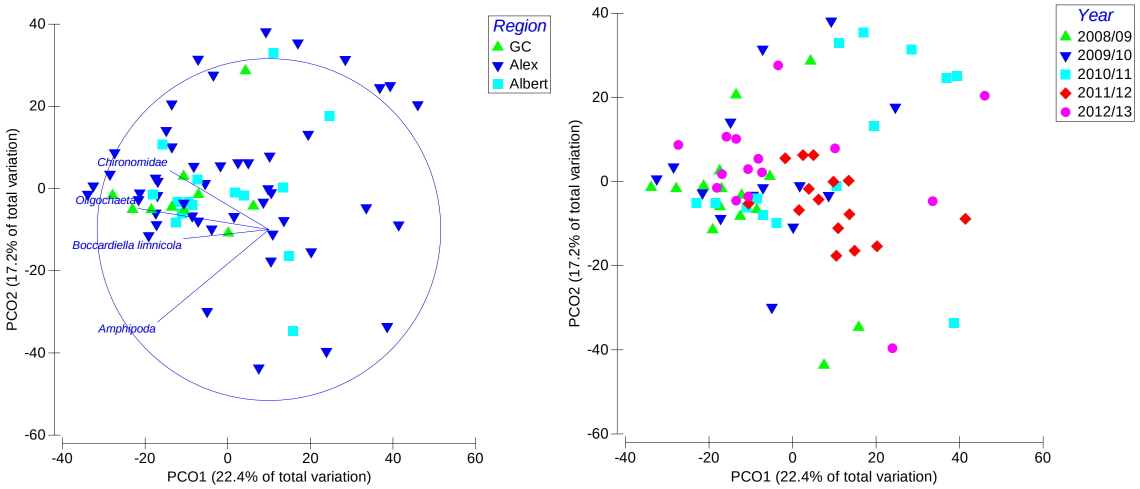

Figure 42: PCO plot of benthic macroinvertebrates around the Lower Lakes from the summer surveys since December 2008 to 2012/13, considering only those sites continued into the recent survey (yet site L18 was not sampled in 2009 because of acidity in sediments). The PCO is shown with highlighting the regions (left) or survey years (left). The circle represents a vector overlay (Spearman correlation) illustrating the contribution of the respective species to the PCO axes.

Considering benthic macroinvertebrate data for sites sampled around the Lower Lakes annually since 2008, significant differences in communities occurred between the surveys, subject to the sampling site (SM-Table 20). Yet, apart from some closer grouping of samples from particular years, no distinct pattern emerged for a change in macroinvertebrate communities in the Lower Lakes over the last five years. The PCO plot also explained only a small percentage of the variability within the data, and thus not capturing any pattern in the data well, but appears to separate sites and years with higher abundances of key taxa for the macroinvertebrates in the lakes.

5.2.4.2 Aquatic macroinvertebrate communities

The aquatic invertebrate community was significantly different between regions and sites within regions (SM-Table 19), as the sites in Lake Albert were set aside in the community composition from the remaining lake sites. This distinction was largely driven by amphipods, Corixidae (waterboatmen) and Coenagrionidae (damselfly larvae) which were more abundant in Lake Albert than Lake Alexandrina (Figures 38 and 43). This community distinction occurred despite the high variability in abundances for single taxa across sites (chapter 5.2.2.2). SIMPER analysis showed that the three most abundant taxa, amphipods, chironomids and the freshwater shrimp, *Paratya australiensis,* contributed together around 70% or more to the similarity within each region and, together with Ecnomidae, Notonectidae and some further insect taxa, also to the dissimilarity between sites.

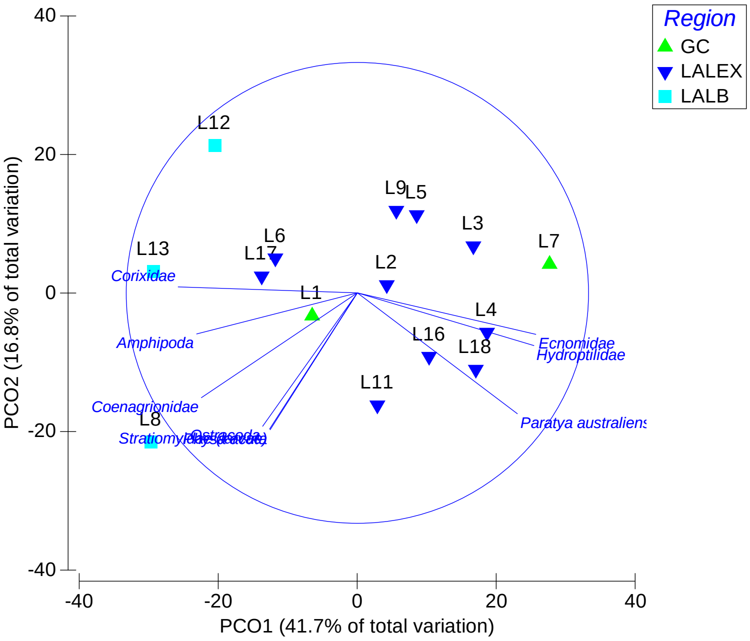

Figure 43: PCO plot of aquatic macroinvertebrates around the Lower Lakes, differentiated by regions, based on dip net samples taken in January 2013. See Figure 2 and SM-Table 1 for site codes. The circle represents a vector overlay (Spearman correlation) illustrating the contribution of the respective species to the PCO axes.

5.2.5 Macroinvertebrate communities and environmental conditions

Community patterns of benthic macroinvertebrates were not well defined in sediments around the Lower Lakes, yet those sites that were distinct to other sites in the PCO plot (Figure 41) were also distinct in the dbRDA plot (Figure 44). These sites (L3, L4, L5 and L9) shared coarser sediments and low organic matter content, which can explain their low abundances of benthic macroinvertebrates. Median grain size was the only variable that significantly contributed to the pattern and, taken altogether, the considered environmental variables explained only a small percentage of the variation in macroinvertebrate data (Figure 44).

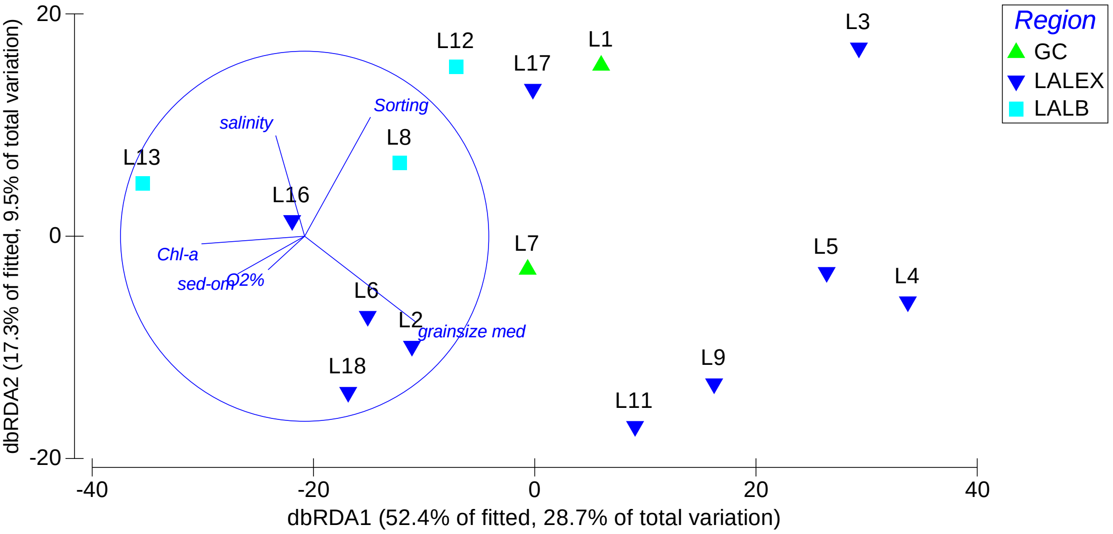

Figure 44: dbRDA (distance based redundancy analysis) plot illustrating relationships between environmental variables and benthic macro-invertebrates around the Lower Lakes in summer 2012/13, see Figure 2 for site details. The regions within the lakes are GC (Goolwa Channel) LALEX= Lake Alexandrina, LALB=Lake Albert. Parameters were transformed prior to analysis. The vector overlay uses base variables of environmental data, Spearman rank correlation.

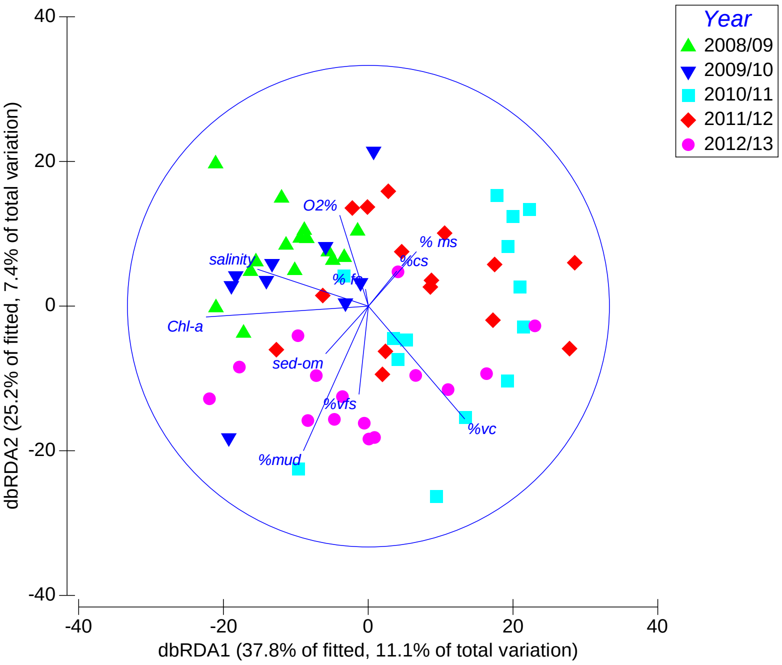

Figure 45: dbRDA (distance based redundancy analysis) plot illustrating relationships between environmental variables and benthic macro-invertebrates around the Lower Lakes for the annual surveys in summer 2008 to 2012/13. Note that some sites in 2009 and one site in 2010 had to be omitted because of some missing environmental variables. Parameters were transformed prior to analysis. The vector overlay uses base variables of environmental data, Spearman rank correlation.

The differences in benthic macroinvertebrate communities over the last five years were not very pronounced (Figure 42), but a greater similarity in particular years driven by environmental variables was also apparent in a dbRDA plot, however explaining a small percent of the total variation in the benthic data only (Figure 45). Apart from some single grain size fractions (fine and coarse sand), all environmental variables considered had a significant relationship with the macroinvertebrate community, but together explained only 30% of the total variation.

For aquatic macroinvertebrates, the regional differentiation of communities with a separation of sites located in Lake Albert (Figure 43) was driven by salinity, which contributed significantly to the pattern (Figure 46). Only three environmental variables measured in the water were considered in this case, which together accounted for only a small part of the variation in the aquatic macroinvertebrate pattern. Other variables which were not quantified in this survey, such as vegetation type and density are further important habitat parameters for these organisms, and may need consideration in future surveys.

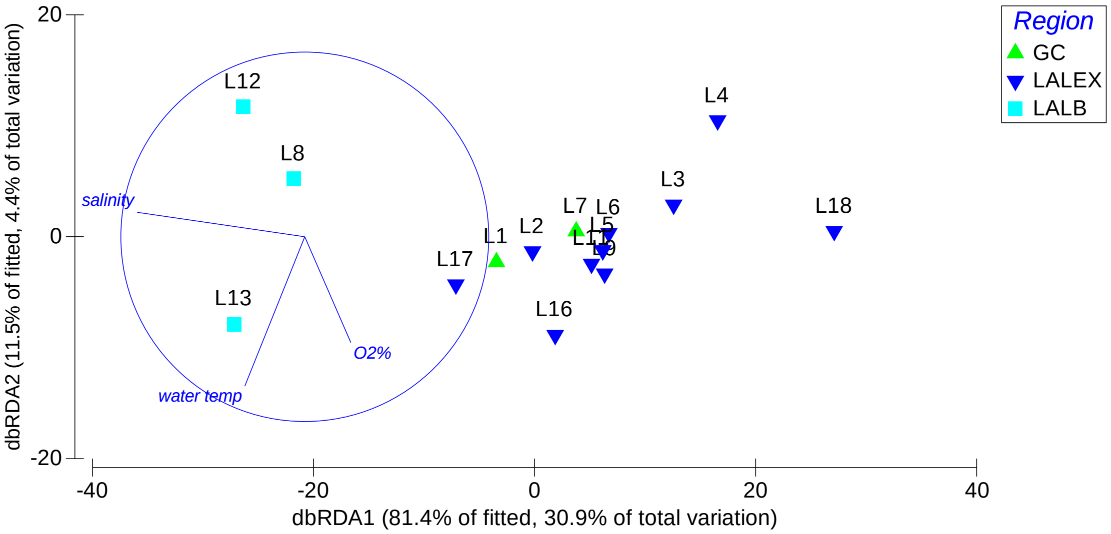

Figure 46: dbRDA (distance based redundancy analysis) plot illustrating relationships between environmental variables in the water column and aquatic macro-invertebrates around the Lower Lakes in summer 2012/13, see Figure 2 for site details. The regions within the lakes are GC (Goolwa Channel) LALEX= Lake Alexandrina, LALB=Lake Albert. Parameters were transformed prior to analysis. The vector overlay uses base variables of environmental data, Spearman rank correlation.

5.3 Benthic macroinvertebrates on either side of the barrages

In the third year since barrages were opened, differences in benthic macroinvertebrates on either side of the barrages continued, especially as the recovery in the Murray Mouth region led to increased abundances and biomass at all barrage sites in the 2012/13 survey (Figures 47 and 48).

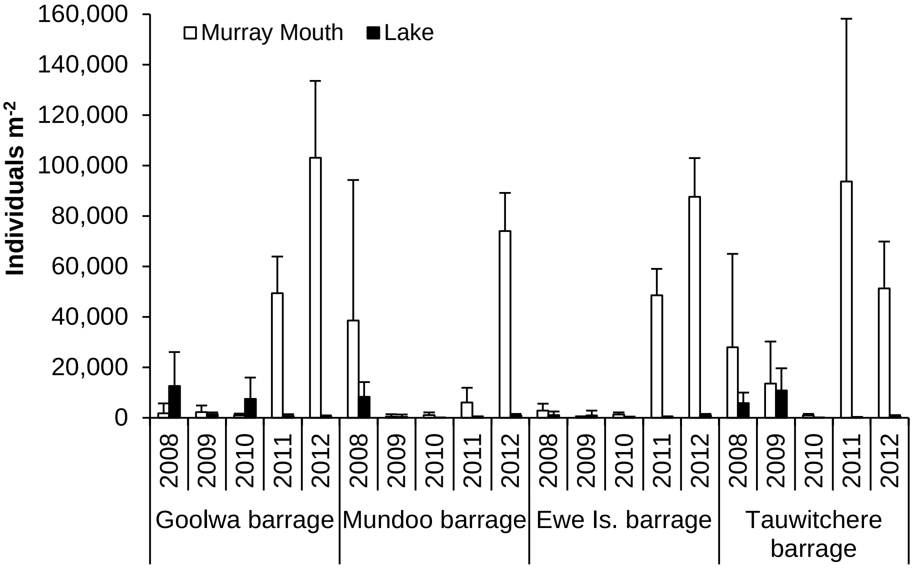

Figure 47: Comparison of abundances of total benthos (mean values with standard deviation, *n* = 10) between the annual surveys from 2008 to 2012/13 from Murray Mouth (white bars) and Lake Alexandrina (black bars) sides of four barrages.

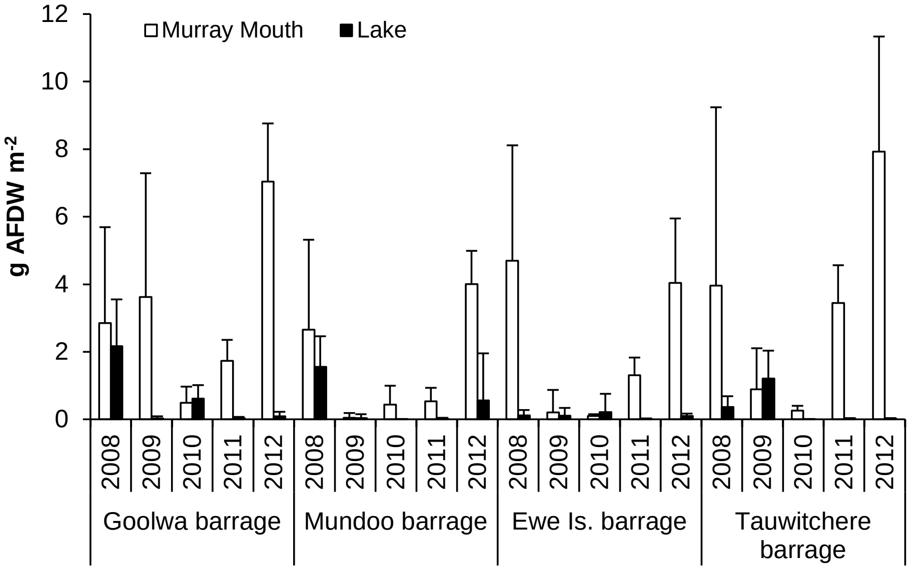

Figure 48: Comparison of total biomass of benthos (mean values with standard deviation, *n* = 10) between the annual surveys from 2008 to 2012/13 from Murray Mouth (white bars) and Lake Alexandrina (black bars) sides of four barrages.

Considering all regions on either side of the barrages and over all monitoring years since 2004, the clear region specific distinction of macroinvertebrate assemblages was apparent, whereby the three regions in the Murray Mouth and Coorong lagoons shared less similarity than the regions in the Lower Lakes (Figure 49). Patterns of change over time are less clear when considering the freshwater, estuarine and hypersaline regions together (Figure 49).

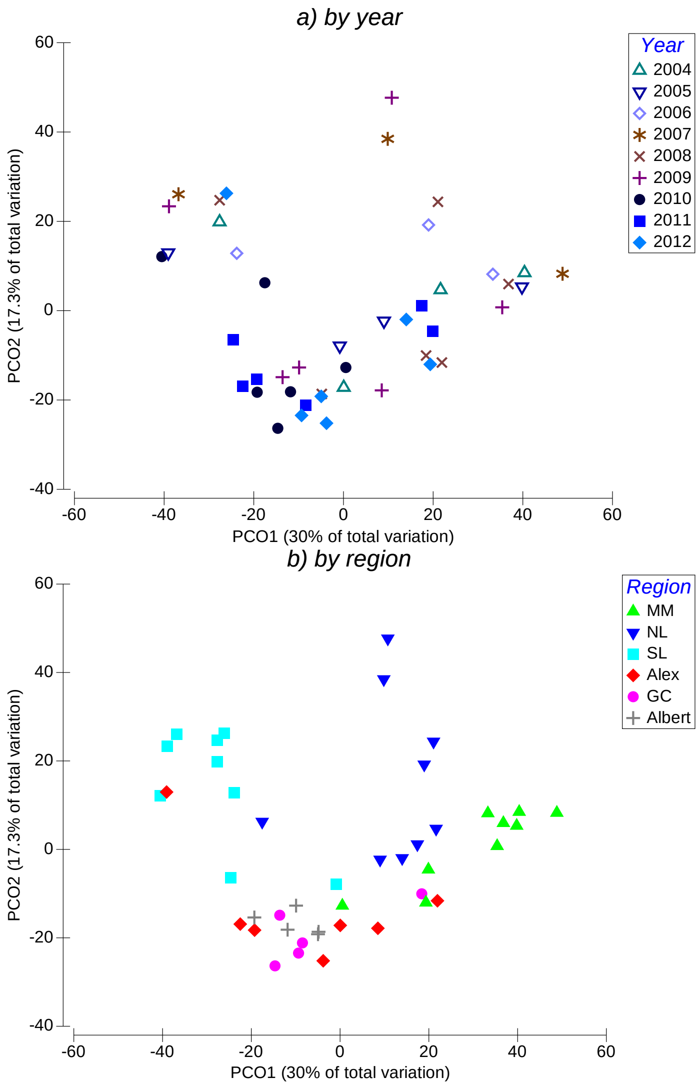

Figure 49: PCO plot of macroinvertebrates around the Coorong, Murray Mouth and Lower Lakes over all TLM surveys since December 2004 to 2012/13. Note that not all sites were sampled every year. For clarity, the analysis was carried out on average values per region and year. The figure is displayed twice, illustrating a) the years and b) the regions of the study area.

6. Discussion and Conclusions

Long-term monitoring of macroinvertebrates in the Lower Lakes, Murray Mouth and Coorong Icon Site has proved essential for assessing the changes after restored freshwater flow. The system experienced two consecutive major events, the severe Millennium Drought (Leblanc et al. 2012), and the freshwater pulse during the initial flood peak passing through the estuary in 2010/11. The monitoring time frame also included a small water release in late 2005 (Dittmann et al 2006), which provides an opportunity for developing a reference dynamic for the Murray Mouth and Coorong. However, without quantitative historical data, caution needs to be applied to refer to any condition at the onset of the TLM monitoring as a baseline for the natural state of the ecosystem. Developments recorded in the 9th year of condition monitoring allow the following conclusions against the condition monitoring objectives for 'The Living Murray' program (SAMDBNRM 2009).

M-1 – 'Facilitate frequent changes in exposure and submergence of mudflats'

Higher resolution data on water levels and tides within the Murray Mouth are not available from our annual monitoring, hence only observational records can be considered. Following continued water release, the wider Murray Mouth allowed greater tidal and wind driven changes in submergence and exposure of mudflats in the Murray Mouth, which could have facilitated the recolonisation of sediments by macroinvertebrates. Frequent changes in water level also provide suitable foraging grounds for shorebirds, especially in the overwintering period of shorebirds over the summer months. In the Lower Lakes, the sediments around the shores remained submerged with the restored higher water levels, and thus inaccessible for foraging shorebirds.

M-2 – 'Maintain sediment size range in mudflats'

The comparison of grain size compositions over the years revealed great similarity between all survey years for the Murray Mouth and Coorong sites, where sediment size ranges were thus maintained. Slight variation between years or sites could be due to sampling artefacts, if the exact location varied subject to water level, or to genuine silt or sand depositions. These possible reasons could also explain some of the variation in grain size compositions around the Lower Lakes. Sediment size is affecting porewater space and biogeochemistry in sediments, and thus the living conditions for macroinvertebrates (Snelgrove & Butman 1994). Grain size fractions emerged as variables that were contributing to the explanation of some of the patterns in macroinvertebrate communities seen in this monitoring.

M-3 – 'Maintain organic content for mudflats'

Organic matter content is indicative of nutrient loading and food supply for lower trophic levels, and important for further biogeochemistry in sediments (Krull et al. 2009). Localised patchiness and large fluctuations in organic matter occurred in all study regions of this monitoring over the years, yet values recorded this year fell within previously recorded ranges.

W-1 – 'Assessment of estuarine conditions between Goolwa Barrage and Pelican Point'

The target of a variable salinity regime with >30% of the area below sea water salinities has been exceeded, with salinity values recorded in this monitoring (summer 2012/13) showing freshwater or brackish conditions throughout the Murray Mouth and into the North Lagoon. This project carries out spot measurements during the annual sampling, yet compared over the monitoring years, it emerged that salinities in all regions of the Murray Mouth and Coorong were lower than in previous years, and have now been below seawater in the Murray Mouth for three years. As known from other estuaries (Hirst 2004, Whitfield et al. 2012) and seen from correlations between abundances of key macroinvertebrate species and salinity in this monitoring, salinity affects the distribution and occurrence of macroinvertebrates. Estuarine conditions imply frequent changes of freshwater or sea water dominated water quality characteristics (Potter et al. 2010, Elliott & Whitfield 1011), yet in the Murray Mouth and Coorong, the time frame of these changes is unlike in any other estuary.

I-1 – 'Maintain or improve invertebrate populations in mudflats'

The 2012/13 monitoring has revealed improvements in invertebrate populations in mudflats throughout most of the Murray Mouth and North Lagoon of the Coorong compared to the drought years. For several macroinvertebrate parameters, including similarities in community structure, the drought years emerged as outliers and recoveries in diversity, abundances and biomass were detected within the Murray Mouth and Coorong over the last three years. Improvements in diversity and abundances were also seen at several sites around the Lower Lakes. While the system continues to be poor in macroinvertebrate species compared to other estuaries around southern Australia (Hirst 2004, Dye & Barros 2005, Wildsmith et al 2005), species numbers had increased again at many sites in the Murray Mouth region. Previously abundant species like the micro-mollusc *Arthritica helmsi* were recorded from subtidal samples in a concurrent project on effects of water release (Dittmann et al 2013), illustrating that the TLM macroinvertebrate monitoring is directed specifically at the condition of nearshore mudflats and that data from both monitoring schemes should be evaluated together for a comprehensive assessment and understanding of the changes in the system.

The pollution indicator species *Capitella capitata* had contracted its distribution and was no longer found in the Murray Mouth, but is extending its occurrence into the South Lagoon. *Australonereis ehlersi* shifted into the North Lagoon, whereas the other nereidid species *Simplisetia equisetis* became more abundant in the Murray Mouth. Such changes in the occupancy and distribution ranges of macroinvertebrates indicated improved habitat conditions, and variability was also lower than in previous years. Similar changes in distribution ranges were described for the flood and drought striken St Lucia estuary in South Africa (Cyrus et al. 2010). Recovery is known to occur in different stages, subject to the type of pressure and its duration, and requires a restoration of habitat conditions before community structure and associated functioning can re-develop (Borja et al. 2010). Recovery can take less than 5 years or several decades in coastal and estuarine ecosystems after a century of degradataion (Borja et al. 2010). As there was no consistent return yet to the characteristics of the macroinvertebrate communites seen in the early period of monitoring from 2004 to 2006, further development should be monitored to understand whether a complete recovery occurs or the system develops into a new state or dynamic.

Changes in the macroinvertebrate community compositions over the drought and flow periods would have affected the foraging of shorebirds, as macroinvertebrate prey items differ in their energy content. The polychaetes and molluscs that were present in high numbers before the drought provided a high energy content as food for shorebirds (Alerstam *et al.* 1992; Zwarts & Wanink 1993). Crustaceans with exoskeleton contain less energy and the high abundances of amphipods occurring after the flow may have been of lower nutritional value for migratory shorebirds, althought the assimilation efficiency for amphipods can be high (Zwarts & Wanink 1993). The energy content of the highly abundant chironomids is, however, similar to that of annelids, especially oligochaetes (Farmer & Wiens 1999; Smith *et al.* 2012). Small prey items may contribute less to the net energetic intakes of shorebirds (Turpie & Hockey 1997), and the migratory shorebirds are still encountering reduced nutritional resources in the Coorong. Continued flow and appropriate water levels in the Murray Mouth and Coorong are required to assure the further recovery of macroinvertebrates and habitat conditions to support overwintering shorebird populations.

7. Acknowledgements

Funding for condition monitoring has been provided by the Murray-Darling Basin Authority through The Living Murray initiative. This project has been managed by the Department of Environment, Water and Natural Resources, through the Lower Lakes, Coorong and Murray Mouth Icon Site staff. Several land owners and the Ngarrindjeri people provided access through their properties to reach the lake shore sites for sampling. We greatly acknowledge the helping hands with field and lab work, from George Giatas, Michael Drew, Shea Cameron, David Rudd and further volunteers.

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