Benthic Macroinvertebrate survey

2015-2016:

Coorong and Murray Mouth Icon Site

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

Sabine Dittmann & Ryan Baring
Flinders University, School of Biological Sciences
GPO Box 2100, Adelaide, SA 5001
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Cover Image: Waders feeding in the mudflats at Ewe Island, Murray Mouth, photo by S. Dittmann 2015

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Enquiries regarding the licence and any use of the document are welcome to: Adrienne Rumbelow LLCMM Icon Site Coordinator adrienne.rumbelow@sa.gov.au
Appendices provided electronically

The following Word and Excel data files were supplied:

Appendix 1 Sampling sites, dates and coordinates 2015-2016
Appendix 2 Murray Mouth and Coorong environmental parameters 2015-2016
Appendix 3 Murray Mouth and Coorong Sediment Characteristics 2015-2016
Appendix 4 Murray Mouth and Coorong Species Diversity 2015-2016
Appendix 5 Murray Mouth and Coorong macroinvertebrate abundances 2015-2016
Appendix 6 Murray Mouth and Coorong Biomass_2015-2016
1. Executive summary

The Living Murray (TLM) Condition Monitoring Program provides an assessment of the ecological condition of the Lower Lakes, Coorong and Murray Mouth (LLCMM) Icon Site. This report provides findings from the 11th survey of benthic macroinvertebrate monitoring in this Ramsar listed wetland addressing in particular the condition monitoring target I-1 “Maintain or improve invertebrate populations in mudflats”.

The response of benthic macroinvertebrates to environmental changes in the Murray Mouth and Coorong were assessed with measurements of diversity, abundance, biomass and community structure and various environmental parameters from sediments and overlying water. Identification of the influence of environmental conditions to changes in the benthic population structure addresses the TLM condition monitoring targets 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’, and also contributes to W-1 – ‘Assessment of estuarine conditions between Goolwa Barrage and Pelican Point’. The sampling regime for macroinvertebrates and environmental conditions in the November 2015 and April 2016 survey followed procedures from previous years and as described in The Living Murray LLCMM Icon Site Condition Monitoring Plan (Maunsell 2009).

Macroinvertebrate samples were taken in the Murray Mouth and Coorong at 11 sites with a hand-held PVC corer. The volume of water released over the barrages during the November 2015 (55 GL) and March 2016 (50 GL) period was lower than some recent years but consistent through time. Findings from the November 2015 survey indicate that macroinvertebrate populations are recovering in the system and providing food for shorebirds but they are reduced to sites in the Murray Mouth and northern reaches of the North Lagoon, specifically at site Mulbin Yerrok. Species numbers, diversity, abundances and biomass are similar to the recent survey in 2013/14 where flow has resumed. The micro-mollusc Arthritica helmsi, now has an increased range and highly abundant throughout the Murray Mouth and at Mulbin Yerrok, where it was once rare but now common in the system.

Macroinvertebrate community trajectories in the Murray Mouth appear to be shifting back to pre-drought conditions but it is difficult to determine and requires further investigation in future surveys. In comparison, the southern sites of the North Lagoon were much lower in abundance and less diverse than recent survey years and macroinvertebrates in the South Lagoon were very low or completely absent. The TLM icon site target I-1 “Maintain or improve invertebrate populations in mudflats” is only partially met and is restricted to the Murray Mouth and northern North Lagoon section of the Coorong. Comparisons between the November 2015 and April 2016 survey showed a decrease in macroinvertebrate abundances and biomass, and changes to community structure at sites sampled in the Murray Mouth and North Lagoon, which may be attributed to reduced flows and unusual high temperatures over the 2015/16 summer, or foraging by birds. Future surveys should investigate the macrobenthic communities and environmental conditions around the arrival and departure of shorebirds to better determine the influences of environmental change together with predation on macroinvertebrate communities.

Habitat conditions in the Murray Mouth and Coorong mudflats were within similar ranges to the 2013/14 survey. Salinities of overlying water in the mudflats followed the same pattern as previous years with low values in the Murray Mouth described as oligo- or mesohaline (<18 ppt) and hypersaline (>40 ppt) from the southern North Lagoon through to the most southern site of the South Lagoon. The sediment conditions have met TLM condition monitoring targets in the Murray Mouth and northern North Lagoon which have improved for the better. However, the facilitation of frequent changes in exposure and submergence of mudflats is not clear during warm summer periods as observed in 2015/16 where there was zero flow through the barrages for some time.

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2. Introduction

This report presents the findings from the 2015/16 benthic macroinvertebrate surveys undertaken as part of The Living Murray (TLM) Condition Monitoring Program (spring/summer) and Intervention Monitoring Program (April 2017). These monitoring programs provide an assessment of the Lower Lakes, Coorong and Murray Mouth Icon Site, its habitat values and state of recovery of key species (Maunsell 2009). In estuarine ecosystems such as the Murray Mouth and Coorong, macroinvertebrates are a key component, where they play an essential role in benthic functioning of sand/mudflats (Covich et al. 2004) and support aquatic food webs by providing food for higher trophic level consumers, such as birds (Botto et al. 1998). Benthic macroinvertebrates also respond quickly to change and are often used as indicator organisms for ecosystem health, particularly in estuarine environments (Cardoso et al. 2007; Wildsmith et al. 2011).

The Lower Lakes, Murray Mouth and Coorong is a Ramsar listed wetland of significant importance, which has undergone extreme environmental changes over the past decade (Wainwright and Christie 2008, Paton et al. 2009, Kingsford et al. 2011). Following the Millennium Drought, flow into the system resumed with large flows from spring 2010 to the end of 2012 and more modest but continuous flows in 2013 to 2014, albeit with some fluctuation with season and water management (Figure 1). Flow through the barrages continued during the 2015 year with a slight increase in the winter-spring period, which was then reduced back to very little or no flows during the 2015/16 summer period (Figure 1).

![Figure 1: Monthly barrage flow from the Lower Lakes into the Murray Mouth and Coorong during the years of macroinvertebrate and mudflat monitoring from 2004 to March 2016. Based on modelled monthly barrage outflow data from the MDBA.](image-url)

Long-term monitoring of the system since 2004 has provided insights into the loss of benthic habitat quality, macroinvertebrate species and populations during the drought years and the recovery of the macrobenthos after flows resumed in 2010. Signs of recovery in macroinvertebrate communities were delayed after the reintroduction of flows in 2010, which was only first noticeable in the 2011/12 and
2012/13 monitoring surveys (Dittmann et al. 2012, 2013a,b). Initially there was an increase in chironomids and amphipods during 2011/12, but since then there has been an increase in more macroinvertebrate species, particularly with larger bodied, deeper burrowing species that are important for bioturbation of sediments (Dittmann et al. 2012, 2013a, b, 2014, 2016). The delayed response of macroinvertebrate recolonisation into the benthos due to improving environmental conditions from re-introduced flows are some signs of recovery (Dittmann et al. 2016), however previous evidence shows that such processes can take several years or decades (Borja et al. 2010). Increases in climate variability and the associated availability of environmental flows will also add to the challenges for this system over the longer term.

This report presents the findings from the 11th year of condition monitoring of the Murray Mouth and Coorong to assess the food availability for migratory and native shorebirds and other higher trophic level organisms in the system. As in previous years, the same sampling sites were surveyed in November 2015 and a subset of four sites in April 2016 using the same methods for consistency and reliable comparison over time. Additional to the annual survey in late spring/early summer, the four sites re-surveyed in April 2016 were sampled due to concern of an immediately noticeable reduction in some larger-bodied species of polychaete worms in the November 2015 survey. All sites correspond with shorebird monitoring sites and most overlap with other components of TLM monitoring (e.g. fish). Findings from the current monitoring also included several environmental parameters for sediment condition and water quality, to assess changes across time and space at the sites with comparison to previous surveys. It was anticipated that this current survey would indicate further improvements of macroinvertebrate populations and communities and benthic condition since the re-introduction of flows in 2010/11. The continued monitoring in 2015/16 provides further evaluation of macroinvertebrate responses to improved environmental conditions of the system. Outcomes from this report of TLM condition monitoring in 2015/16 can inform decision making and future management of the Murray Mouth and Coorong.

The report aligns with a number of condition monitoring objectives for TLM, 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’ (Maunsell 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 analyses provided as supplementary material (table and figure reference prefix SM-).

To deliver the TLM condition monitoring targets (I-1, M-1 to M-3), this report addresses the following questions for the spring/summer of 2015/16:

1) To describe the current environmental conditions of the Murray Mouth and Coorong
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.

Additional field monitoring across 4 sites (Ewe Island, Pelican Point, Mulbin Yerrok and Noonameena) was also undertaken in April 2016, as part of TLM Intervention Monitoring Program, to determine the following key objectives:

- ascertain whether spatial contraction of distribution ranges continued;
- assess whether abundances of macroinvertebrates have declined further since the start of summer; and
- assess whether biomass of macroinvertebrates has declined and thus food availability for migratory waders.
3. Materials and Methods

3.1 Sampling sites and dates

The same sampling design undertaken in previous years was continued in 2015/16 and is described in the LLCMM Icon Site Condition Monitoring Plan (Maunsell 2009). Benthic macroinvertebrate fauna was sampled at a total of 11 sites across the Murray Mouth (5 sites) and Coorong (6 sites) region during the 2015 late spring/summer survey (Figure: 2). Sampling occurred between the 17th and 24th November 2015 and a sub-set of four sites in autumn on April 4th 2016 (see also SM-Table 1).

In the November 2015 survey, sites sampled in the Murray Mouth, between the Goolwa Barrage and the southern end of the Tauwitchere Barrage included; sites 1 (Monument Road), 2 (Hunters Creek), 3 (Mundoo Channel), 4 (Ewe Island), and 5 (Pelican Point). Sites 6 (Mulbin Yerrok, near Long Point), 7 (Noonameena) and 8 (Parnka Point) are located in the North Lagoon of the Coorong. The South Lagoon sites were 9 (Villa dei Yumpa), 10 (Jack Point), and 11 (Loop Road south of Salt Creek) (Figure 2). In April 2016, two sites each in the Murray Mouth (sites 4 and 5) and North Lagoon (sites 6 and 7) were resampled for benthic macroinvertebrate fauna due to a noticeable decrease in fauna (particularly larger polychaete worms) from the November 2015 survey at sites 5 and 6.

During the November sampling, different sites along the Murray Mouth and Coorong had varying distances of sediments exposed ranging from approximately 10 to 200 metres, but the amount of sediment exposed was comparable to other recent macroinvertebrate sampling surveys conducted in the region during early 2015 (Dittmann et al. 2016). Some of the exposed sediment at Ewe Island was very disturbed with what appeared to be feeding pits (i.e. possibly from black swans) throughout the shoreline in the April 2016 survey. There was also a large dense expanse of filamentous green algae mats covering the benthos in the shallow subtidal region along the entire length of the Tauwitchere barrages during the April 2016 survey.
3.2 Environmental parameters

The same suite of environmental characteristics were sampled in 2015/16 as in previous years and included; water temperature, salinity, oxygen content and saturation, sediment grain size, organic matter and chlorophyll-a, which were recorded to establish environmental conditions at each site. Some of those environmental parameters are aligned with the condition monitoring objectives such as; whether sediment size ranges and organic matter were maintained (objectives M-2 and M-3), to
characterise the salinity regime at the time of sampling (objective W-1), and to identify environmental parameters that are influencing the macrobenthic communities (Maunsell 2009).

**Water Quality**

Various water quality characteristics were obtained (temperature, dissolved oxygen concentration, oxygen saturation, salinity and pH) at all of the sites during November 2015 and April 2016. Measurements for temperature, and oxygen concentration and saturation were taken with a YSI Pro2030 multi-parameter electrode at all sites. For the pH of water and sediment, pH indicator strips were used (Acilit 0-14) and a refractometer was used to measure salinity in the overlying water and additionally from the residual pore water of the core sampling, which showed comparable salinities to the overlying water. Three replicate measurements for each parameter were taken.

**Sediment analyses**

In November 2015 and April 2016, 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. Only the samples from the November 2015 survey were processed in the laboratory and data analysed, while the April 2016 sediment samples were kept frozen and will be processed at a later date.

Samples for sediment organic matter were extracted using a cut off 10 mL syringe (surface area 1.8cm²). To account for spatial variation, three replicate samples of each sediment parameter were taken and analysed separately. To obtain a bulk parameter of organic matter as % dry weight (d.w.), sediment samples were dried to constant weight using an Ohaus MB45 Moisture Balance. Sediment samples were homogenously distributed onto aluminium trays and dried using the standard drying protocol (controlling the temperature profile at 80 ºC). The profile burn was automatically completed after all moisture content was dried and remained stabilised for 30 seconds. Samples were then burnt in a muffle furnace at 450 ºC for 5 hrs.

For sediment grain size, three replicate samples per site were taken using a cut-off 60 mL syringe (surface area 6.6 cm²). 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. To correct for this procedure, the weight of this fraction and of the remaining sediment were determined and normalised in the data set. Median and quartiles as well as percentages of various particle sizes were obtained from the Mastersizer output. Sediment sorting was calculated using the formula $S_o = (P_{25}/P_{75})^{1/2}$, based on the logarithmic method of moments scale and categorised as sorting classes according to Gray (1981) and Blott and Pye (2001). At several sites at the Murray Mouth and North Lagoon, as well as at Villa de Yumpa (Site19), sediment grain size compositions had slightly bimodal distributions. For sediments composed of finer soft sediment and larger grain particles, the obscuration level of the Mastersizer is more sensitive to finer than larger particles.
For chlorophyll-

- \( a \) in the sediment surface, 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). Samples were placed on ice in the field and upon return to the laboratory, were frozen for later analysis with a fluorometer (Turner 450). After the initial reading for total chlorophyll, drops of 0.1 M HCl were added to the samples to correct for phaeophorbides.

### 3.3 Macrofauna

Benthic macroinvertebrates in the littoral zone were sampled for species composition and abundance within the sediment with the use of handheld PVC corers (83.32 cm\(^2\) surface area). Ten replicate samples were taken per site, scattered haphazardly between the mid to low shore levels around the respective water margin. All benthic samples were sieved through 500 µm mesh size in the field and sorting of live samples occurred in the laboratory within a few days of collection. Most of the macroinvertebrates were identified and counted in the laboratory straight after sorting. If time did not allow, due to large volume samples and/or high individual numbers of common species, then specimens were preserved in 70% ethanol. Specimens were identified to the lowest possible taxonomic level and the numbers of individuals 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 included in abundance counts. For measuring biomass, polychaete fragments were included with the complete specimens. The larval and pupae stages of insects were recorded, while all adult winged life stages were excluded as they are highly motile and not part of the benthic macrofauna. Further refinement of the dataset from 2004 to 2015/16 was undertaken to remove rare taxa that were only occasionally encountered or are generally not normally contributing to benthic macrofauna communities.

All identified organisms were preserved in 70 % ethanol until they were used for biomass determination. Biomass was analysed for the total benthos per replicate sample and not differentiated per phyla, given the understanding of the main taxa contributing to the biomass gained from previous monitoring. Each sample was dried to constant weight (d.w.) using an Ohaus MB45 Moisture Balance. Specimens were homogenously distributed onto aluminium trays and dried using the standard drying protocol (controlling the temperature profile at 80 °C). As samples with <0.5 g can give inaccurate results with the moisture balance, samples with very few specimens were dried in an oven at 80 °C until constant dry weight (d.w.) was achieved (at least 24 hours). Samples were then placed in a muffle furnace at 450 °C 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 the biomass measurement as grams of ash free dry weight (g AFDW). Large outliers or negative ash free dry weight values were deleted from the dataset to ensure that those values did not have influence on the rest of the dataset with erroneous results.
3.4 Data Analysis

The approach taken for data analysis follows previous monitoring reports (see Dittmann et al. 2013a, 2014). The three regions are differentiated in the analysis design and correspond to the 'sub-regions' in the LLCMM Condition Monitoring Plan, which are the Murray Mouth estuary, North Lagoon and South Lagoon of the Coorong. To assess whether TLM condition monitoring target parameters were maintained or improved, comparisons were carried out using the November 2015 and previous survey data from 2004 to 2013. 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 (see Figure 1): 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 2010-2013 as the period since the return of flows, which commenced in spring 2010. The November 2015 data was then compared to those three separate periods. Some parameters, such as Chl-a, were added later (2007 and 2008) and temporal comparisons respectively adjusted.

Several indices were used to assess diversity, in addition to the species number found at each site; Shannon-Wiener diversity (H') using log_e, Margalef's Index (d) for species richness, Pielou’s index (J') for equitability and the Simpson index (an evenness index independent of sampling effort that is adjusted to small sample sizes).

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 added to the design (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. For comparisons between the November 2015 and April 2016 survey the same three factor design for all year comparisons was used, but the factor of region was dropped from the design when comparing species number and diversity indices due to a lack of replication on averaged data for that factor.

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 (square root, fourth root or log (x+1)). Environmental data were 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 some 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. For the tests between macroinvertebrate abundances
from four sites sampled in both November 2015 and April 2016 the possible permutations from PERMANOVA analyses were quite low and meaningless, so in those cases Monte Carlo sampling was used to obtain approximate but more interpretable P-values (Anderson et al. 2008).

The measure of occurrence was calculated for key taxa by counting the number of sites where a species occurred in previous monitoring years as the best achievable distribution range, which was then divided by the number of monitoring sites (11 sites). That calculation provides a historical measure of occurrence for key taxa that can then be used as a historical reference line to compare with the current survey measure of occurrence where the index has a number one if it occurred at all sites or zero if it was not found at all.

To explore differences in macroinvertebrate communities, principal coordinate analysis (PCO) plots were used with vector overlays (Pearson correlation) to illustrate species contributing to the differentiation of communities along the PCO axes. PERMANOVA tests 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 illustrate community changes over time, nMDS ordination plots were created with trajectories linking consecutive years, and significantly different clusters based on SIMPROF tests are indicated with circles around respective years.

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 some environmental factors in previous years 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

During the November 2015 survey, the water in the Murray Mouth and the most northern site of the North Lagoon (site 6) was fresh to brackish and all other sites further south (site 7 onwards) were all hypersaline (Figure 3). Compared to previous years since 2004, salinity levels in November 2015 were at the lower range across most sites throughout the Murray Mouth and Coorong Lagoons (Figure 3, SM-Figure 1). Some exceptions where salinity levels were showing an average range from previous surveys were Noonameena, Parnka Point and Villa dei Yumpa (sites 7 to 9). During the April 2016 survey, salinity levels at the two Murray Mouth sites (Ewe Island (site 4); Pelican Point (site 5) were higher than the November 2015 survey (Figure 3)). While the two sites in the North Lagoon had similar or lower salinity levels in April 2016 compared to November 2015 (Mulbin Yerrok (site 6); Noonameena (site 7); respectively) (Figure 3).

![Figure 3: Salinity (mean ppt ± S.E.) in the water overlying the mudflats of the Murray Mouth and Coorong during the survey at all eleven sites in November 2015 (black diamonds) and at four sites only in April 2016 (open diamonds). Minimum (blue dashed) and maximum (red dashed) lines are based on averages for salinities recorded in previous TLM surveys from 2004 to 2013/14. Grey line represents seawater salinity.](image)

Over the November 2015 and April 2016 sampling trips, the widths of the exposed mudflats varied. However, for condition monitoring target M-1: ‘Facilitate frequent changes in exposure and submergence of mudflats’, only observations from single days can be provided. The water level had decreased slightly between the sampling trips in November 2015 compared to April 2016 in the Coorong, due to very low or zero water releases over the barrages during the 2015/16 summer period (see Figure 1).
4.1.2 Water quality

Various other water quality parameters were measured throughout the Murray Mouth and Coorong sites in 2015/16. In November 2015, seven of the eleven sites had water temperatures at the upper end of the temperature range recorded from previous surveys since 2004, with some very warm water temperatures at Mundoo Channel (site 3) and Ewe Island (site 4) in the Murray Mouth (SM-Figure 2). Monument Road (site 1) had cooler water temperatures in November 2015, compared to the range in temperatures form previous years. During the April 2016 survey, water temperatures were cooler at the Murray Mouth sites (Ewe Island, site 4; Pelican Point, site 5) and slightly warmer at the North Lagoon sites (Mulbin Yerrok, site 6; Noonameena, site 7) compared to the November 2015 survey (SM-Figure 3).

In November 2015, dissolved oxygen concentration in the water column overlying mudflats throughout the Murray Mouth and Coorong was slightly lower than the previous 2013/14 survey (average 8.1 mg/L versus 9.9 mg/L) ranging from 6.1 to 9.9 mg/L (versus 6 to 14 mg/L 2013/14) (see Dittmann et al. 2014). However, some sites had high oxygen concentrations compared to previous surveys at those particular sites since 2004 (Hunters Creek, site 2; Mulbin Yerrok, site 6; Noonameena, site 7) (Figure SM-Figure 4). Dissolved oxygen saturation was above the ANZECC trigger value (90%) at 10 of the 11 sites, and 115% on average. The highest oversaturation of 124% was recorded in the water overlying the sediment at Hunters Creek (site 2) (SM-Figure 5). At most sites, saturation levels were within the upper range or sometimes higher than previously recorded values (SM-Figure 5). Both Oxygen concentration and saturation levels were quite low at Monument Road (site 1), (SM-Figure 5). Oxygen concentration and saturation levels were much higher in April 2016 compared to November 2015 at the four sites sampled, with saturation levels higher than previous years (SM-Figure 6; SM-Figure 7).

Water overlying sediments had a lower pH than previous years, with pH 6.2 on average (SM-Figure 8), which could have been due to the different method used (pH strips instead of electrode). The pH of water overlying sediments in April 2016 compared to November 2015 was slightly lower at Ewe Island (site 4) and Mulbin Yerrok (site 6) but within similar ranges (SM-Figure 9).

4.1.3 Sediment size ranges

During the November 2015 survey, sediments in the studied mudflats were sandy and mostly moderately sorted, with sites in the Murray Mouth and North Lagoon consisting of mainly fine sand (Table 1; SM-Figure 10). Two sites bordering the Murray Mouth and North Lagoon (Pelican Point, site 5; Mulbin Yerrok, site 6) were mainly medium sand. Sediments from sites in the South Lagoon were mainly medium sand with the exception of finer sands at Loop Road (site 11) (Table 1). Compared to the Murray Mouth region, there was a larger range in grain sizes from very fine to very coarse sands (SM-Figure 10) in the North and South Lagoons. Yet, the median grain size was only significantly different at the site level and not between regions (SM-Table 2).

In 2015, the median sediment grain size was comparable to previous years at most sites, with the exception of the lowest value ever recorded for the Monument Road site (site 1) (Figure 4). The
sediment grain size at Jack Point (site 10) was more comparable to other years, rather than the extremely large grain size value recorded in 2013 (Figure 4).

The sediment grain size composition at study sites in the Murray Mouth and North Lagoon appears to have become less mud and more very fine to fine sands compared to the 2012 and 2013 surveys (Figure 5). Yet, median grain sizes were not statistically different across the survey years, but differed by region (SM-Table 2). Thus, the condition monitoring target M-2 ‘Maintain sediment size range in mudflats’ is met.

Table 1: Sediment characteristics of mudflats in the Murray Mouth and Coorong region during summer 2015/16. 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 $S_o$, are provided as characteristics of mudflat sediment. The verbal description of sediment grain size and sorting follows Blott and Pye (2001).

<table>
<thead>
<tr>
<th>Region</th>
<th>Site</th>
<th>Organic Matter (% DW)</th>
<th>Grain size (µm)</th>
<th>Median description</th>
<th>Sorting</th>
<th>Sorting description</th>
</tr>
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<td>Fine sand</td>
<td>0.78</td>
<td>Moderately sorted</td>
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<tr>
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<td>156.61</td>
<td>Fine sand</td>
<td>0.80</td>
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<td>1.53</td>
<td>166.16</td>
<td>Fine sand</td>
<td>0.73</td>
<td>Moderately sorted</td>
</tr>
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<td>139.13</td>
<td>Fine sand</td>
<td>0.78</td>
<td>Moderately sorted</td>
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<td>Moderately sorted</td>
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<td>269.78</td>
<td>Medium sand</td>
<td>0.71</td>
<td>Moderately sorted</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.92</td>
<td>218.09</td>
<td>Fine sand</td>
<td>0.76</td>
<td>Moderately sorted</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.41</td>
<td>238.75</td>
<td>Fine sand</td>
<td>0.75</td>
<td>Moderately sorted</td>
</tr>
<tr>
<td>South Lagoon</td>
<td>9</td>
<td>4.08</td>
<td>272.46</td>
<td>Medium sand</td>
<td>0.64</td>
<td>Moderately well sorted</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.08</td>
<td>228.49</td>
<td>Fine sand</td>
<td>0.70</td>
<td>Moderately well sorted</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3.31</td>
<td>276.05</td>
<td>Medium sand</td>
<td>0.69</td>
<td>Moderately well sorted</td>
</tr>
</tbody>
</table>
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 (2) was included in 2005, and site 7 was not sampled that year. Note the different y-axes scales due to some outlying coarser sediment in the North Lagoon. See Figure 1 & 2 for site locations.
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 2015. Sites or regions are not shown in the figure. The same plot is shown for the survey years (left) and the regions Murray Mouth (MM), North Lagoon (NL) and South Lagoon (SL) (right). 2004 is not included as a different method was used for grain size analysis. The PCA axes explained 45.5 % (PC1) and 25.7 % (PC2) of the variation.

4.1.4 Sediment organic matter and chlorophyll-a

Sediment organic matter in the mudflats was high compared to previous years (overall average 2.4 % dry weight) at most sites but similar to previous years at Ewe Island (site 4), Noonameen (site 7) and the three South Lagoon sites (Figure 6). The sediment organic matter did not vary significantly between regions or sites in 2015 (SM-Table 2, SM-Figure 11).

Across all monitoring years, sediment organic matter varied significantly between regions and between the survey years (SM-Table-2). Variability was highest at Pelican Point (site 5), yet overall, values for sediment organic matter from November 2015 fell within the range of values recorded in previous years (Figure 6). Thus, the condition monitoring target M-3 ‘Maintain organic content for mudflats’ was met.

Microphytobenthic biomass in the sediments, as estimated by the Chlorophyll-a content was low across all regions (SM-Figure 12). The amount of Chl-a in sediments averaged 0.89 mg m⁻² and similar to values in the 2013 survey (see Dittmann et al. 2014) and were not significantly different between regions or sites (SM-Table 2). Microphytobenthic biomass have been consistently low since the 2010/11 survey and the Chl-a values from sediments in November 2015 were similarly low and in the lower boundaries compared to earlier years (Figure 7).
Figure 6: Sediment organic matter (as % dry weight) of mudflat sediments in the Murray Mouth and Coorong Lagoons during surveys from 2004 – November 2015 (boxplot) and during the survey in November 2015 (red triangles ▲; average ± SE).

Figure 7: Sediment chlorophyll-a content (in mg/m$^2$) of mudflats in the Murray Mouth and Coorong Lagoons during surveys from 2007 – 2015 (boxplot) and during the survey in November 2015 (red triangles ▲; average ± SE).

4.1.5 Trajectory for environmental conditions between years

In the November 2015 survey, there were similar groupings for the Murray Mouth sites of Hunters Creek and Ewe Island (sites 2 and 6), and particularly distinct environmental conditions for Monument Road (site 1) (SM-Figure 13). The North Lagoon sites and South Lagoon sites had large site to site variation and thus different habitat characteristics for those sites.
Figure 8: PCA (Principal component analysis) with 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. PC1 and PC2 are the first two PCA axes.

Similar to recent years there was a shift in environmental conditions for water and sediment characteristics in the Murray Mouth and Coorong compared to previous years (Figure 8). Trajectories for the Murray Mouth and North Lagoon were similar to the 2013 year for water conditions, but may be shifting back towards the sediment conditions recorded when monitoring began in 2004, particularly for the Murray Mouth region (Figure 8). In the South Lagoon, trajectories for water and sediment conditions may be heading back to conditions more similar to those recorded in the 2011 year (Figure 8).
4.2 Macroinvertebrate populations

This section of the report addresses whether the Condition Monitoring Target I-1: ‘Maintain or improve invertebrate populations in mudflats’ has been met. Comparisons of benthic macroinvertebrate populations are evaluated for diversity, abundance, distribution, biomass and community structure for the November 2015 survey versus previous years since 2004 and when flows resumed in 2010/11. Further evaluation and comparison of the four sites (sites 4 to 7) in the Murray Mouth and North Lagoon between November 2015 and April 2016 are also reported. More specific details of the findings from the November 2015 and April 2016 survey are presented in the supplementary material.

4.2.1 Macroinvertebrate species richness, diversity and distribution

The number of macroinvertebrate taxa totaled 24 in the November 2015 survey with the highest number of species recorded in the Murray Mouth (18 species), followed by the North Lagoon (17 species) and the South Lagoon (3 species) (Table 2, Figure 9). Compared to the 2013 survey, species numbers were similar in the Murray Mouth and higher in the North Lagoon (Figure 9). In comparison, species numbers were much lower in the South Lagoon and comparable to some of the drought years (Figure 9). Compared to previous years, at the site level species numbers were higher at all Murray Mouth sites and Mulbin Yerrok (site 6) in the North Lagoon and similar to other years at Noonameena, Parnka Point and Villa dei Yumpy (sites 7 to 9) (SM-Figure 14). The two most southern sites had no species found during the November 2015 survey (SM-Figure 14).

![Figure 9: Total number of macroinvertebrate species by region in the Murray Mouth (MM), North (NL) and South (SL) Lagoons of the Coorong in all monitoring years since 2004.](image-url)

The macroinvertebrate species found in November 2015 consisted mainly of molluscs (mostly Hydrobiid snails) and annelids in the Murray Mouth, insects (Hexapoda) and crustaceans in the North Lagoon and only one representative annelid, crustacean and insect species in the South Lagoon (SM-figure 15). Compared to the November 2015 survey the number of molluscs species in April 2016...
decreased at the two Murray Mouth sites (sites 4 and 5), while the number of insects species increased slightly (SM-Figure 15). Between the November 2015 and April 2016 survey, the number of crustacean and insect species decreased at both of the North Lagoon sites (sites 6 and 7) and the number of annelids species decreased by a larger amount at Noonameena (site 7) (SM-Figure 15).

Overall, most of the molluscs and polychaetes found in the November 2015 survey were found within the Murray Mouth and the northern sites of the North Lagoon (sites 1 to 7). During the November 2015 survey, there were large numbers of the snail Salinator fragilis on the surface of sediments at all Murray Mouth sites, which were not wholly represented in the core samples (pers. obs.). Also, there was a notable presence of Arthritica helmsi in the Murray Mouth and the northern sites of the Northern Lagoon, and the deeper burrowing bivalve Soletellina alba at Ewe Island and Mulbin Yerrok (Site 4 and 6) (Table 2).

Table 2: Occurrence of macrobenthic taxa and species numbers during the spring 2015 (November) and autumn 2016 (April) surveys (see Figure 2 for site location). The number of taxa is also indicated per site and region. Sampling sites were: Site 1 = Monument Road; 2 = Hunters Creek; Site 3 = Mundoo Channel; Site 4 = Ewe Island; Site 5 = Pelican Point; Site 6 = Mulbin Yerrok; Site 7 = Noonameena; Site 8 = Parnka Point; Site 9 = Villa dei Yumpa; Site 10 = Jack Point and Site 11 = Loop Road. Ticks indicate presence records from the November survey and o presence records from the survey in April 2016 at four of the sites.

<table>
<thead>
<tr>
<th>Phyla/Class/Order</th>
<th>Family/Genus/Species</th>
<th>Murray Mouth</th>
<th>North Lagoon</th>
<th>South Lagoon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nov</td>
<td>Nov</td>
<td>Apr</td>
</tr>
<tr>
<td>Annelida Oligochaeta</td>
<td>Capitella spp.</td>
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<td>o</td>
<td>✓</td>
</tr>
<tr>
<td>Polychaeta</td>
<td>Simplicia aoquisitis</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Australoneires ehlersi</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Aglaophamus (Nephtys) australiensis</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Boccardiella limnicola</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td>Crustacea</td>
<td>Amphipoda</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Isopoda</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Ostracoda</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Mysidacea</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td>Mollusca</td>
<td>Bivalvia</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Arthritica helmsi</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Soletellina alba</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Gastropoda</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Hydrobiidae sp. 2</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
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<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Hydrobiidae sp. 4</td>
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<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Hydrobiidae sp. 5</td>
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<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Hydrobiidae sp. 6</td>
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<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Salinator fragilis</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Hexapoda</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Diptera</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Chironomidae (larvae or pupae)</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Diptera (larvae or pupae)</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Dolichopodidae (pupae)</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Staphylinae</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Scolymizidae (larvae)</td>
<td>✓✓✓</td>
<td>o</td>
<td>✓</td>
</tr>
<tr>
<td>Total species number per site</td>
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<td>14</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Species number per region</td>
<td>20</td>
<td>19</td>
<td>19</td>
<td>18</td>
</tr>
</tbody>
</table>

Diversity values (Shannon-Wiener index H') were highest throughout the Murray Mouth region and at the northern section of the North Lagoon (Mulbin Yerrok, site 6) and low at southern sites (site 7 to site 9) (Figure 10, SM-Tables 3, 4). Evenness values (J') were highest at the more southern sites of the Coorong at Parnka Point and Villa dei Yumpa (site 8 and 9) due to the lower number of species with similar numbers of few individuals contributing to species diversity (Figure 10, SM-Table 4).
Throughout the Murray Mouth and northern sites of the North Lagoon, the evenness values at sites were generally higher than the 2013/14 survey, indicating less dominance of particular species as seen in previous years (Figure 10 & 11, SM-Table 4). Both species numbers and the diversity index $H'$ were significantly different between the three regions (SM-Table 5). Species numbers ($S$) and the diversity index $H'$ varied significantly over all surveys and regions (SM-Table 5).

Compared to November 2015, two of the sites sampled in April 2016 (sites 5 and 7) increased in diversity values and were influenced by higher contributions from a few species (site 5, oligochaetes and *Arthritica helmsi*; site 7 by insect larvae) (Figure 10, SM-Tables 3, 4). The Ewe Island site (site 4) was lower in species diversity and evenness in April 2016 versus November 2015, while Mulbin Yerrok (site 6) was similar between surveys (Figure 10). Between the November 2015 and April 2016 surveys, species numbers and diversity values were not significantly different, but there was a difference at the site level for diversity values (SM-Table 5).

![Figure 10: Total number of macroinvertebrate species (red symbols), Shannon-Wiener diversity $H'$ (black bars, based on log$_e$) and evenness $J'$ (white bars) at sites in the Murray Mouth (MM), North (NL) and South (SL) Lagoons of the Coorong in the (a) November 2015 and (b) April 2016 surveys. Only two of each sites were sampled in the Murray Mouth (sites 4 & 5) and North Lagoon (sites 6 & 7) during the April 2016 survey. See Figure 2 for site locations.](image-url)
In November 2015, species numbers were mostly lower than the 2010-2013 restored flow period but higher than the drought period from 2004-2009. Some exceptions were the reduced species numbers below drought years at Monument Road (site 1), Parnka Point (site 8) and Villa dei Yumpa (site 9) (Figure 11). Diversity values were mostly higher than all previous drought and restored flow years.

Figure 11: Changes in diversity of benthic macroinvertebrates over time, illustrated by species numbers, Shannon-Wiener index $H'$ and Pielou's evenness index $J'$ at sampling sites in the Murray Mouth and Coorong lagoons (see Figure 2 for site locations). Diversity from the sampling in November 2015 is shown against previous periods in the monitoring since 2004, divided into three year intervals of early drought/small flow (2004-2006), severe drought (2007-2009) and restored flow (2010-2013).
(2004-2013), except they were similar or lower than previous years at Parnka Point (site 8), and Noonameena (site 7) and Villa dei Yumpa (site 9), respectively (Figure 11). Evenness values were similar to or exceeded those of drought years (2004-2009) at most sites, except at Noonameena, which was lower than during the drought (site 7) (Figure 11). Diversity and evenness values were both similar to drought years (2004-2009) at Monument Road (site 1).

4.2.2 Macroinvertebrate abundances and distribution

During November 2015, the abundances of benthic macroinvertebrates were high throughout sites in the Murray Mouth region and at one site in the North Lagoon at Mulbin Yerrok (site 6) (Figure 12). In comparison, sites further south in the North Lagoon and South Lagoon had very low macroinvertebrate abundances, with no specimens found at the most southern sites of Jack Point and Loop Road (sites 10 and 11). Average abundances per region were three times higher in the Murray Mouth versus the North Lagoon and extremely low in the South Lagoon (SM-Table 6). There were significant differences in total abundances and most major phyla or separate taxa between regions and sites within regions (SM-Table 7). Some exceptions were oligochaetes, Capitella spp., Nephtys australiensis and Austalonereis ehlersi which were only significantly different at the site level (SM-Table 7).

In April 2016 macroinvertebrate abundances were slightly higher at Ewe Island (site 4) and lower at the other three sites (sites 5 to 7) compared to the 2015 survey (Figure 12). Yet, there were only a few significant differences for some of the phyla and separate taxa between the two surveys and regions which were for gastropods, crustaceans, Capitella spp. and amphipods (SM-Table 7). The total benthos, major phyla and most of the separate taxa were significantly different at the site level (SM-Table 7).

![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 November 2015 survey (black bars); and at two of each sites in the Murray Mouth and North Lagoon during the April 2016 survey (blue bars).](image)
Macroinvertebrate abundances in the Murray Mouth and Mulbin Yerrok (site 6) in the North Lagoon were similar to or higher in some cases to previous years or periods of reintroduced flows (Figure 13, SM-Figure 16). This was not the case for the more southern sites (sites 7 to 11) where total abundances were lower compared to recent years and more similar to pre-flood years (Figure 13, SM-figure 16). There is some fluctuation in macroinvertebrate abundances in recent years since flow resumed at the Murray Mouth sites and Mulbin Yerrok (site 6), but there is a general trend of recovery of the system for that particular region (Figure 13, SM-Figure 16). However, this does not apply to the North Lagoon and the entire South Lagoon where macroinvertebrate abundances have declined in November 2015 compared to recent years of returned flows (Figure 13, SM-Figure 16).

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 and northern section of the North Lagoon (top figure) and remaining 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 (2010-2013). Abundances from the current monitoring in 2015 are separately indicated with asterisks. Note the difference in y-axes scales.
Some macroinvertebrate populations also showed further signs of recovery in the Murray Mouth and at Mulbin Yerrok (site 6) with a similar expanded range to what was found in the 2013/14 survey (Dittmann et al. 2014). Macroinvertebrate abundances in the Murray Mouth and northern North Lagoon are still largely being driven by high abundances of amphipods, chironomid larvae and *Simplisetia aequisetis* (SM-Figure 17). The increase in abundances in *Arthritica helmsi* is now also largely adding to the total benthos throughout the Murray Mouth and Mulbin Yerrok (site 6) region (SM-Figure 17). Key species with indicator value (e.g. amphipods, *C. capitata*, *S. aequisetis* and chironomid larvae) and functioning as an important food source for shorebirds and fish were common again in the Murray Mouth and northern section of the North Lagoon.

Crustaceans mainly consisted of amphipods in November 2015 throughout the Murray Mouth and at Mulbin Yerrok (site 6) in the North Lagoon (SM-Figure 17 and 18). Amphipod abundances decreased in November 2015 compared to the 2013/14 survey and was more comparable to previous years when flows resumed (Figure 14, SM-Figure 19). In April 2016, amphipod abundances declined at Ewe island and Pelican Point (sites 4 and 5) and were similar to the November 2015 survey at Mulbin Yerrok and Noonameena (sites 5 and 6) (SM-Figure 17).

Annelids were abundant throughout the Murray Mouth and at Mulbin Yerrok (site 6) in the North Lagoon (SM-Figure 18) and mainly consisted of *Simplisetia aequisetis* and *Capitella* spp. (SM-Figure 17). Abundances of *Boccardiella limnicola* also contributed to annelid numbers but they were mainly restricted to the Murray Mouth sites (SM-Figure 17). Compared to 2013/14, *Capitella* spp. declined slightly at some sites, but *S. aequisetis* abundances were similar to recent years (Figure 14). April 2016 versus November 2015 saw *S. aequisetis* abundances similar at Pelican Point and Mulbin Yerrok (sites 5 and 6) but lower in abundance at Ewe Island (site 4) (SM-Figure 17). During April 2016, abundances of *Capitella* spp. increased at Ewe Island (site 4) and oligochaetes increased with large numbers at Ewe island and Pelican point (sites 4 and 5) (SM-Figure 17). Other polychaete species such as *Nephtys australiensis*, which was rare in recent years (Figure 14), and *Australonereis ehlersi* were found in November 2015 and April 2016 at one or two sites only (SM-Figure 17).

The reef building tubeworm *Ficopomatus enigmaticus*, was not quantitatively assessed using core samples in the November 2015 survey, but live tube worms were found at Monument Road (site 1) and Mundoo Channel (site 3).

Molluscs were dominated by the small bivalve *Arthritica helmsi* in November 2015 throughout the Murray Mouth and at Mulbin Yerrok (site 6) in the North Lagoon (SM-Figures 17 and 18). After declining in abundance during the drought years, *A. helmsi* has increased in abundance in recent years and had some of the highest abundances in recent years for this species in November 2015 at all sites where it was found (Figure 14). The larger-bodied and deeper burrowing bivalve *Soletellina alba* was found at Ewe Island (site 4) and Mulbin Yerrok (site 6), but not in high abundances (SM-Figure 17). Both *A. helmsi* and *S. alba* were lower in abundance at the sites where they found in April 2016 compared to November 2015 (SM-Figure 17). During November 2015, there were also large
numbers of grazing snails (*Salinator fragilis*) on the sediment surface at sites in the Murray Mouth but they appeared to be lower in numbers by April 2016 (pers. obs.).

Insect larvae which mainly consisted of Chironomid larvae were found in high abundances throughout sites in the Murray Mouth and less abundant throughout the North Lagoon and at the northern South Lagoon site (SM-Figures 17 and 18). Chironomid larvae abundances have fluctuated over recent years but they have been found in similar numbers in the current November 2015 survey and in 2013/14 (Figure 14, SM-Figure 19).

Total abundances, major macroinvertebrate phyla and single taxa were significantly different between the 2004 to 2015 surveys, with exception of a few species (oligochaetes, *N. australiensis* and *A. ehlersi*) (SM-Table 7).

The constancy index of key taxa is identified by the presence of each taxon at sites within the November 2015 survey divided by all possible sites. The constancy value compared to previous surveys as a historical baseline provides an understanding over the years of the occurrence of taxa based on ranges of rare to very common or constantly present (Figure 15). Based on the occupancy index, since the return of flows in 2010 there have been improvements in the occurrences of various key taxa. Examples of this are where *Capitella* spp. went from not very common to common; *S. aequisetis* from common to very common; amphipods from common to constant; *A. helmsi* from rare to very common; and chironomid larvae have been constant (Figure 15).
Figure 14, continued
Figure 14. Mean abundances (ind. m$^{-2}$) and standard deviation (± S.D.) ($n = 10$) of key species and taxa identified in the TLM condition monitoring plan (Maunsell 2009), 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$^{-2}$) and standard deviation (± S.D.) ($n = 10$) of key species and taxa identified in the TLM condition monitoring plan (Maunsell 2009), recorded at sites around the Murray Mouth and Coorong since 2004 (left hand column). The constancy index ranges are: <12% = rare; 13-24% = not very common; 25-49% = common; 50-74% = very common; 75-100% = constant. Note not all sites were sampled during each survey and the different scales of the y-axis.
Similar to recent years, there are fluctuations in macroinvertebrate abundances but there is a clear pattern of recovery in the recent monitoring survey. There was still no clear uniform response to changes in environmental conditions in the Murray Mouth and Coorong during November 2015. Compared to previous years, there were still high abundances found within the salinity range measured in November 2015 and abundances fit within the same range (Figure 16, SM-Figure 20).

Figure 16. Mean abundances (ind. m$^{-2}$) of total macroinvertebrates, based on all records from the Murray Mouth and Coorong over the entire monitoring time span from 2004 to 2015, in relation to the salinity at the sites and time of sampling. Abundances from the monitoring in November 2015 are highlighted by red circles and the salinity range recorded in the study regions during this recent sampling indicated in bottom bars with colours from light blue (Murray Mouth, 3-10 ppt) to blue (North Lagoon ,20-96 ppt) and dark blue for the South Lagoon (78-90 ppt). See Figure 3 for changes in salinities over the monitoring periods. The vertical line indicates the salinity of seawater.

4.2.4 Macroinvertebrate biomass
Biomass corresponded with the higher abundances of macroinvertebrates throughout the Murray Mouth and Mulbin Yerrok (site 6) in the North Lagoon (Figure 17). Average biomass was highly variable within and between sites but significantly different for regions and sites nested within regions (SM-Table 7). Compared to the November 2015 survey, macroinvertebrate biomass was lower but highly variable at all of the four sites sampled in April 2016 and there was no significant difference between surveys or regions (Figure 17, SM-Table 7).

The current survey in 2015 had higher biomass at Murray Mouth and northern North Lagoon sites compared to recent years of restored flow (2010-2013) and severe drought years (2004-2006), and were more comparable to pre-drought levels in some cases (Figure 18, SM-Figure 21). The more southern sites in the North Lagoon and South Lagoon (sites 8 to 11) contributed very little to biomass, with the lowest values in 2015 compared to the early drought (2004-2006), severe drought (2007-2009) and restored flow periods (2010-2013) (Figure 18). Across all monitoring years, biomass was significantly different between surveys, regions and sites nested 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 November 2015 (sites 1 to 11; black bars) and April 2016 survey (sites 4 to 7 only; blue bars) survey.

Figure 18: Biomass of benthic macrofauna (g AFDW m$^{-2}$) (mean and standard deviation, $n=10$) at sites sampled in the Murray Mouth and northern section of the North Lagoon (top figure) and remaining 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 (2010-2013). Biomass from the current monitoring in November 2015 is indicated by the asterisks. Note the order of magnitude difference in the y-axes scales.
Overall, abundance and biomass in November 2015 was comparable to recent years in the Murray Mouth and northern North Lagoon, indicating that there is still recovery of macroinvertebrate populations in that particular section of the system. Based on those findings, there is food available for shorebirds and fish in the northern section of the system (sites 1 to 6), but little food available in the southern end of the North Lagoon and the entire South Lagoon (sites 7 to 11).

4.2.5 Macroinvertebrate communities

Benthic communities at the Murray Mouth sites clustered closely together, while Mulbin Yerrok (site 6) and Noonameena (site 7) in the North Lagoon clustered loosely with some cross-over between those two sites (Figure 19). Some samples from Parnka Point and Villa dei Yumpa (sites 8 and 9) had similar macroinvertebrate communities, but most of the samples from those sites and the two most southern sites (sites 10 and 11) clustered together due to the large number of zeros in the dataset (Figure 19). In November 2015, macroinvertebrate communities were significantly different between regions (Pseudo-$F = 11.003$, $P_{(perm)} = 0.0004$) and sites nested in regions (Pseudo-$F = 15.06$, $P_{(perm)} = 0.0001$). The most common characteristic species for the Murray Mouth sites (sites 1 to 5) were *Boccardiella limnicola*, *Simplisetia aequisetis* and *Arthritica helmsi* (SM-Table 7, Figure 19). The characteristic species for Mulbin Yerrok (site 6) were *Capitella* spp., *S. aequisetis* and *A. helmsi*, and insect larvae were characteristic for more southern sites (sites 7 to 9) (SM-Table 7, Figure 19).

![Figure 19](image-url)
Benthic communities showed some separation for the two Murray Mouth sites (sites 4 and 5) between November 2015 and April 2016 (Figure 20). Mulbin Yerrok communities had some crossover between the 2015 and 2016 surveys, while Noonameena loosely clustered away from other sites, but with large overlap between surveys (Figure 20). There was a significant interaction between surveys and regions, but most of the significant differences were at the site level within the 2015/16 surveys (SM-Table 8). Characteristic species at Ewe Island and Pelican Point (sites 4 and 5) in the November 2015 survey were *S. aequisetis* and *A. helmsi* (Figure 20). Dolichopodidae larvae were most characteristic of benthic communities at site 7 for both the 2015/16 surveys (Figure 20).

![Figure 20: Principal coordinate analysis (PCO) of macroinvertebrate data from the mudflat survey in November 2015 and April 2016 at two of each sites within the Murray Mouth and North Lagoon regions (sites 4, 5, 6 and 7). The circle represents a vector overlay (Pearson correlation) illustrating the contribution of the respective species to the PCO axes.](image)

Across all monitoring years, there were distinct regional differences in benthic communities with the Murray Mouth distinct from each other. In comparison the North Lagoon was less distinct and crossed over with the Murray Mouth and South Lagoon sites throughout the years (SM-Figure 22). The benthic communities were significantly different between surveys, regions and sites nested within regions (SM-Table 10).

A greater distinction between benthic communities of the three regions becomes apparent from plotting the Global R value from a test statistic (Figure 21). When monitoring started in 2004 to 2006 the Murray Mouth region was distinct from the other two regions, as shown by a high R value (Figure 21). During the peak of the drought, benthic communities were more similar between all three regions. The restored flows in 2010/11 promoted a shift to more distinct benthic communities between regions, particularly between the Murray Mouth and the other two regions (Figure 21). In 2015, the Murray Mouth was still distinct from the other two regions and even completely distinct from the South Lagoon.
Lagoon as identified by the highest R value recorded across all years (Figure 21). The North Lagoon and South Lagoon are still the regions that have the most similar benthic communities.

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 from 2004 to 2015, 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.

To explore the benthic community change over time and to determine if there was a shift in direction back towards the years before drought, trajectories were plotted across all survey years from 2004 to 2015 (Figure 22). Across all regions the system was most similar during drought years (2004 to 2009), initial flows (2010), large but variable flows (2011-2012) and currently small but consistent flows (2013-2015) (Figure 22). That particular pattern has largely been driven by the patterns of benthic community structure in the Murray Mouth region over the years (Figure 22). The Murray Mouth and North Lagoon are both currently showing a shift in trajectory towards the early drought benthic community structure (e.g. 2004 to 2006). However, it is not a complete return to benthic community structure as found in early drought years, and only further monitoring in coming years will determine if that might happen. The South Lagoon trajectory is not as consistent over the years but has been more similar in recent years (2011 to 2013). There has been a shift in the South Lagoon trajectory in 2015 with benthic communities becoming more similar to drought years (2008 -2009) and very depauperate of benthic fauna (Figure 22).
Figure 2: nMDS ordination plots showing trajectories of change in macroinvertebrate communities in the mudflats from each of the regions of the Murray Mouth, North and South Lagoon, as well as for the entire Murray Mouth and Coorong, in the monitoring from 2004 to 2015. The lines are connecting consecutive years. The circles are delineating significantly different clusters, based on SIMPROF tests, showing 50% (blue line) and 70% (red line) similarity for the Murray Mouth and Coorong, and clusters with 70% (purple line) similarity for the Murray Mouth.

4.2.6 Environmental conditions as predictor variables for macroinvertebrate communities

In 2015, the only environmental variable that contributed significantly to benthic community patterns in the Murray Mouth and Coorong was salinity, which explained 51% of the variation in the macroinvertebrate data. Overall 64% of the total variation in the macroinvertebrate community data was explained by the set of considered water and sediment variables (Figure 23). All of the other environmental parameters considered had no statistically significant contribution (e.g. sediment organic matter, sediment Chl-a, sediment grain size, sorting or dissolved oxygen saturation). The range of environmental parameters that were contributing to differences in benthic communities, particularly between the Murray Mouth and North Lagoon sites versus the more southern sites (sites 8 to 11) was mainly driven by salinity (Figure 23).
Figure 23: dbRDA (distance based redundancy analysis) illustrating relationships between environmental parameters and the benthic community at the study sites in November 2015. The vector overlay uses base variables of environmental data. The site codes are 1 to 5 for the Murray Mouth, 6 to 8 for the North Lagoon, and 9 to 11 for the South Lagoon (see Figure 2).

Throughout the survey years (2004 to 2015), salinity was the main environmental parameter attributed to changes in benthic communities which explained between 9 % and 23 % of the data (SM-Table 11). Other environmental variables contributed less than 10 % during the 2004 to 2015 survey period. If the survey period from 2007 to 2015 is taken into account when Chlorophyll-a was included, then sediment sorting (Murray Mouth, 12 %; South Lagoon 12 %), organic matter (North Lagoon, 15 %) and Chlorophyll-a (North Lagoon, 11 %; South Lagoon, 12 %) also contributed to benthic community patterns on a regional level (SM-Table 11).

The dbRDA plots for environmental conditions and benthic communities there is a separation between drought and resumed flow years, which is less distinct for the North Lagoon (Figure 24). Some of those distinctions in the Murray Mouth and North Lagoon are clearer in the 2007-2015 dataset when Chlorophyll-a was included (SM-Figure 23). In the South Lagoon the recent resumed flow years were separate to previous drought years, but the 2015 data are more similar to the early drought years (Figure 24).

Across the years from 2004 to 2015, all five variables explained only 20-30 % of the variation in the Murray Mouth and Coorong (Figure 24). In the years from 2007 to 2015 when Chlorophyll-a was added, the environmental variables explained 32-39 % of the variation across the regions (SM-Figure 23). There may be other environmental or biotic factors that were not assessed across the survey years that must be affecting the benthic communities throughout the Murray Mouth and Coorong.
Figure 24: 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 November 2015. The vector overlay uses base variables of environmental data that explain the patterns in macroinvertebrate communities.
Current assessment of the benthic macroinvertebrate populations throughout the Murray Mouth and Coorong in the recent survey, and in comparison to the previous surveys since 2004, complies with the condition monitoring target I-1 ‘*Maintain or improve invertebrate populations in mudflats*’. There are still signs of recovery in macroinvertebrate populations since flow returned in 2010/11. It appears that macroinvertebrate communities may be shifting back to early drought conditions in the 2015 survey for the Murray Mouth and Mulbin Yerok (site 6) in the North Lagoon only, but this requires further assessment over the coming years to determine if that will be reached.
6. Discussion

The findings of the 2015/16 macroinvertebrate monitoring are discussed below against the condition monitoring objectives for 'The Living Murray' program (Maunsell 2009).

6.1 Macroinvertebrate populations

I-1 – ‘Maintain or improve invertebrate populations in mudflats’

Over the previous 11 years of monitoring from 2004 to 2015/16, macroinvertebrate populations have become re-established in the Murray Mouth and northern North Lagoon, but are now very depleted in the southern Coorong. There are signs of macroinvertebrate populations shifting back towards pre-drought conditions according to the data obtained since 2004, but that would require further assessment in coming years. However, without historic baselines for comparison it is difficult to determine how comparable future macroinvertebrate populations might be to pre-drought years.

Since the flows resumed into the Murray Mouth and Coorong in 2010/11 after the Millennium Drought (Leblanc et al. 2012), there has been slow recovery of the system. The slow response may be attributed to an unnatural disturbance history, which is known to have lasting effects on estuarine systems where cumulative disturbances such as climate, eutrophication and pollution may impact the resilience of macroinvertebrates to stressors (Kennish 2002, Dolbeth 2007, Neto et al. 2010, Dolbeth et al. 2011, Grilo et al. 2011). The current survey revealed that macroinvertebrate populations were continuing to recover in the Murray Mouth and northern North Lagoon (restricted to Mulbin Yerrok, site 6), but were declining again in the southern Coorong. Combination of factors may have influenced the reduction of macroinvertebrates in the southern Coorong. Flows through the barrages throughout the 2015/16 survey period were consistent yet lower than previous years, particularly over the 2015/16 summer. Also, the shallow waters of the Coorong, particularly in the southern region, were subjected to the record breaking average high air temperatures in Australia over the 2015/16 spring/summer (NOAA 2016). Those warmer, low-flow conditions were favourable for the rapid growth of dense filamentous green algae observed throughout the shallow subtidal region of the southern Murray Mouth barrages in November 2015 and April 2016.

The initial increase in dominant macroinvertebrate taxa soon after flows resumed in 2010/11 (e.g. amphipods and chironomids) has been followed by a slow increase in benthic community complexity (e.g. multiple polychaete and mollusc species) in the Murray Mouth and northern North Lagoon. The high abundances of amphipods during the period when flow resumed in 2010/11 may be due to their high reproductive output and the more favourable hydrodynamic and organic loading conditions available (Ford et al. 2001). The polychaete Simplisetia aequisetis was still persistent throughout the Murray Mouth and northern sites of the North Lagoon in the current survey. That particular species and other Nereididae have the advantage of flexibility in early life history, where they can switch between pelagic or benthic larvae depending on current estuarine conditions (Sato 1999). Capitella spp. were lower in abundance, but their range expanded to more sites in the Murray Mouth in the current survey. This group of worms are an indicator species for eutrophication and pollution (Grassle
and Grassle 1976), but it is too early to determine if their range expansion is a reflection of declining environmental conditions which should be investigated further in future monitoring surveys.

Bivalves and gastropods persisted during the current survey, with the substantial increase in numbers of *Arthritica helmsi* through the Murray Mouth and northern section of the North Lagoon. This species was dominant in the Murray Mouth region historically (Wells and Threlfall 1981) and in the early years of the monitoring surveys. The brooding rather than pelagic larvae reproductive mode of *A. helmsi* may account for the slow recolonisation and rarity of this species in recent years since flows resumed in 2010/11 (Wells and Threlfall 1982).

Responses by specific species to drought and freshwater releases require further investigation into the life history strategies and other biological traits. Biological traits for the Murray Mouth and Coorong have been investigated in another project that assessed the response of benthic macroinvertebrates to water release from 2010 to 2015 (Dittmann et al. 2016). Since the water release in 2010/11 life history strategies have shifted from small-bodied, short-lived, benthic larve to large-bodied, long-lived, pelagic larvae and more diverse biological traits (Dittmann et al. 2016). However, further investigations should focus on life history strategies and specific tolerances to changing conditions for key taxa to better understand recolonisation of the Murray Mouth and Coorong as investigated in other regions (Cañedo-Argüelles and Rieradevall 2010, 2011, Dunlop et al. 2008).

Recolonisation of benthic macroinvertebrates can be affected by sediment properties (Pearson and Rosenberg 1978, Kanaya 2014). Succession of diverse macroinvertebrate taxa into sediments can be facilitated by the sedimentary bioturbation of early colonisers (Snelgrove and Butman 1994, Rosenberg 2001). Bioturbation of sediments through the building of burrows or tubes is undertaken by various taxa including chironomids, oligochaetes, amphipods and polychaetes (Nogaro et al. 2006, O'Brien et al. 2009). The depth of bioturbation can improve sediment conditions by oxygenating the sediment and reducing the anoxic layer, which is currently seen at several sites in the Murray Mouth and Mulbin Yerrok in the North Lagoon. Since 2010 there has been a shift from dominant species with only shallow burrowing capabilities (e.g. amphipods) to species that have larger bodies and deeper burrowing characteristics (*S.aequisetis* and *Soletellina alba*) (Dittmann et al. 2016), which was also observed in the current 2015/16 survey.

The abundances and biomass of macroinvertebrate taxa was higher in the November 2015 survey and comparable to recent years for many species in the Murray Mouth and North Lagoon. However, there was a decrease in abundance and biomass of macroinvertebrates at some sites in the Murray Mouth and North Lagoon during the April 2016 survey. At Ewe Island, the exposed intertidal sediments were very disturbed across the entire site with large pits, possibly created by foraging birds. Previous monitoring in the Murray Mouth and Coorong has identified similar decreases in abundances that could be related to bird foraging (Dittmann et al. 2014). However it cannot be fully determined if the macrobenthos was affected by bird foraging or environmental influences without further investigation in the future, before and after the overwintering period of migratory shorebirds.
The current survey has documented further improvements of benthic macroinvertebrate populations, however this is restricted to the Murray Mouth and the most northern site in the North Lagoon at Mulbin Yerrok (site 6). Other sites in the southern region of the North Lagoon and more so at all sites in the South Lagoon, macroinvertebrate communities are either scarce or completely depauperate. Therefore the condition monitoring target I-1 – ‘Maintain or improve invertebrate populations in mudflats’ has only been met in the most northern section of the Murray Mouth and Coorong. Further improvement of habitat conditions is essential for recolonisation of more diverse macroinvertebrate taxa back into the sediments in the southern North Lagoon and the entire South Lagoon of the Coorong.

6.2 Habitat conditions


Macroinvertebrates in the Murray Mouth and Coorong are mainly influenced by environmental conditions such as exposure and submergence, organic content, sediment and water conditions, similar to other estuarine environments elsewhere (Gimenez et al. 2006, Correia et al. 2012, Rodrigues et al. 2012). The more frequent patterns of exposure and submergence with increased flow conditions and opening of the Murray Mouth have provided more estuarine conditions. Sediment conditions have changed slightly for the better in recent years, including the current survey and are still well within normal ranges across the monitoring surveys.

6.3 Intervention monitoring

Although the sampling for the intervention monitoring in April 2016 occurred at four sites only at the interface of the Murray Mouth and North Lagoon, it gave information on the contraction and expansion of distribution ranges of macroinvertebrate species. Changes in distribution ranges can be assessed based on a comparison of species occurrence at sites in April versus their occurrence in November 2015 and previous monitoring. Yet an index for constancy in distribution could not be calculated as it would have required all 11 sites. In April, *Arthritica helmsi*, *Boccardiella limnicola* and *Salinator fragilis* were found at Noonameena and Mulbin Yerrok in the North Lagoon where they were not recorded in November 2015, and where they had rarely been found in the past. These findings indicate extended distribution ranges of some estuarine/marine macroinvertebrates into the North Lagoon. However, *B. limnicola*, and some other species which occur in low numbers (*Australonereis ehlersi*, *Soletellina alba*), were missing in the April samples from some sites where they had been found in November. Most other species were recorded in November and April at the four sites sampled on both surveys, thus no further spatial contraction in distribution was detected.

The April survey found significantly lower abundances for several macroinvertebrate species (e.g. *Simplisetia aequisetis*, *A. helmsi*, *B. limnicola*, amphipods and chironomids), and this further decline in abundance occurred mostly at Ewe Island and Pelican Point. Causes for a decline in abundances over summer could result from foraging by shorebirds and fish (Salem et al. 2014, Earl 2014, Souza et al.)
2015). However, reduced flow in combination with unusually high temperatures as seen in summer 2015/16 and higher salinity in the Murray Mouth could also contribute to reduced individual numbers (Jones, 1987; Lucero et al. 2006, Meerhoff et al 2013).

Abundances of Oligochaeta had significantly increased in April at three of the four sites (Ewe Island, Pelican Point and Mulbin Yerrok) and Capitella capitata occurred in higher densities at Ewe Island as well, yet with lower density at Noonameena. Shorebirds have been found to selectively avoid oligochaetes, which could explain their higher abundance at the end of the over-wintering season (Smith et al. 2012). Yet, both oligochaetes and capitellid polychaetes respond positively to organic matter load in sediments (Tsutsumi 1990, Attrill et al. 2009, Coelho et al. 2015), which can give an indication to further environmental changes in sediments that could have contributed to the decline in abundance of macroinvertebrate species less tolerant to organic matter load.

Corresponding with the decreased abundances of macrofauna in April, biomass was lower than in November at all of the four sites surveyed. Similar seasonal reductions in biomass over summer have been recorded in other estuaries (Kalejta and Hockey 1991). The lower biomass could be a result of prey depletion from foraging shorebirds or changing environmental conditions, as discussed above. However, reduction in prey biomass in late summer could affect shorebirds still needing to build up energy reserves for their return flight to the breeding grounds in the northern hemisphere (Schneider and Harrington, 1981, Goss-Custard, 1984).

6.4 Conclusion

The condition monitoring of benthic macroinvertebrates has assessed the long-term effects of prolonged drought and subsequent restoration of flows in the Murray Mouth and Coorong from 2010 to 2015/16. Through time populations have slowly recovered but only in the Murray Mouth and northern section of the North Lagoon. In comparison, the southern section of the North Lagoon and the entire South Lagoon is scarcely populated by macroinvertebrates where they are in fact non-existent at the most southern South Lagoon sites. Macroinvertebrate communities appear to be following a trajectory back towards pre-drought conditions but that is difficult to determine without further sampling into the future. Therefore, TLM icon site objective for maintaining or improving macroinvertebrate populations has only partially been met and is restricted to the Murray Mouth and northern section of the North Lagoon. The TLM icon site objective for habitat conditions have been partially met with sediment conditions improving throughout the Murray Mouth and northern North Lagoon in the Coorong. The “facilitation of frequent changes in exposure and submergence of mudflats” is less defined, particularly during the hotter summer months during 2015/16 where flow was reduced altogether for some time which requires further investigation of possible impacts to benthic habitat conditions during such warm summer events.
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8. References
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