Benthic Macroinvertebrate survey

2020-2021 report

Coorong, Lower Lakes and Murray Mouth Icon Site

Report for the
Department for Environment and Water
and the Murray-Darling Basin Authority

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Cover photo: Filamentous algal mats covering the Ewe Island mudflat. Footprints of monitoring team reveal black anoxic sediment beneath the algal mat. Photo credit: S. Dittmann

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1. Executive summary

This report presents results from the annual macroinvertebrate and mudflat monitoring in the Lower Lakes, Coorong and Murray Mouth Icon site (LLCMM) as part of The Living Murray (TLM) Condition Monitoring Program. The data from the 2020/21 monitoring are compared against the long-term data series, which is now in its 16th year. The monitoring was carried out as per the TLM LLCMM Icon Site *Condition Monitoring Plan (Revised) 2017* (DEWNR). The results from the current monitoring investigations are assessed against the ecological objectives and specific targets of this plan, for macroinvertebrates and mudflats.

Field work occurred in December 2020 after above average rainfall in winter and spring, and flow releases of >200 GL in the months preceding the monitoring. The higher flow was reflected in reduced salinities in the North Coorong, while hypersaline conditions persisted in the South Coorong. The salinity gradient continues to define the environmental conditions in the Murray Mouth and Coorong. Dissolved oxygen levels were higher than in previous monitoring years. Macroinvertebrate populations, diversity, abundance and community compositions were similar to recent monitoring years, but biomass was reduced, and distribution ranges of several key species contracted.

The ecological targets for macroinvertebrates were either met, partially met or not met, similar to the 2019 monitoring.

The TLM condition monitoring ecological target 1- *Macroinvertebrate species richness increases throughout the Murray Mouth and Coorong w*as partially met. While macroinvertebrate communities in the North Coorong have become more diverse over the years with continuous flow, the high salinity of the South Coorong still inhibits the establishment of further macroinvertebrate species there. Species richness continued to decrease further south into the Coorong lagoons.

The target 2 - *Macroinvertebrate occurrence extends along the Coorong into the South Lagoon* was not met in 2020. Chironomid larvae continued to dominate the macroinvertebrate community in the South Lagoon, and few individuals of other species were recorded. The occurrence of almost all macroinvertebrates did not extend further south than Noonameena, located in the southern part of the North Lagoon.

The target 3 - *The area of occupation for typical estuarine and marine macroinvertebrate species exceeds 60% of the sites sampled* was partially met. Chironomid larvae, amphipods, *Arthritica* and dolichopodid larvae occurred at over 60% of the sites sampled, and some further key macroinvertebrate species (e.g., the polychaetes *Simplisetia* and *Capitella*, and hydrobiid snails) were present at >50% of the sites sampled. However, the area of occupation was slightly contracted for many macroinvertebrate species compared to the previous year. Several macroinvertebrate species, which were found with an increased area of occupation in 2019, had a rare occurrence again in 2020.

The target 4 - *Macroinvertebrate abundance is maintained at, or increases above, reference levels* was met in the 2020 monitoring, as abundances were maintained compared to recent years and the reference values. In the North Coorong, abundances were similar to other years with continuous flows.

An exception was the Ewe Island mudflat, where abundances had decreased further as macroalgal mats impacted on the habitat condition, which was already noted in 2019. In the South Coorong, total abundances were at the reference level due to chironomid larvae.

The target 5 - *Macroinvertebrate biomass is maintained at, or increases above, reference levels* was partially met. Biomass was lower than in the previous monitoring year, but not significantly different to the long-term reference value. In the North Coorong, biomass was still comparable to other years with flow. In the South Coorong, macroinvertebrate biomass was very low.

The target 6 - *Macroinvertebrate communities are similar to those occurring under intermediate continuous flows* was met, as communities in 2020 were very similar to those in previous years, for both the North and South Coorong. In the North Coorong, the macroinvertebrate community appears to have stabilised into a new state, distinct from the community present before the drought. The more diverse and abundant community present in the years with continuous flow was influenced by lower salinities, indicating beneficial effects of estuarine conditions with salinities below seawater. Once salinities exceed the threshold of 64 ppt, macroinvertebrates decline and only few salt-tolerant species such as chironomid larvae can occur.

All four of the ecological targets for mudflat habitat conditions were partially met in the 2020 monitoring, as follows.

The target 1 - *Habitable sediments are occurring along the Coorong into the South Lagoon* was partially met. In the North Coorong, salinities and sediment characteristics were habitable, but where filamentous algae were present, most extensively at Ewe Island, the sediment became anoxic beneath the mats. An effect of these anoxic conditions were the decreased macroinvertebrate abundances. In the South Coorong, hypersaline porewater combined with high organic matter loads made sediments uninhabitable for most macroinvertebrates.

The target 2 - *Sediments are maintained as fine to medium sands and are mostly moderately well sorted* has been partially met. While sediments were mostly fine to medium sands, they were moderately or poorly sorted. Sediment composition was more heterogenous and contained coarser material at several sites.

The target 3 - *Sediment organic matter is maintained* was partially met, as sediments in the South Lagoon had higher organic matter content than in previous years. Sediment organic matter deviated significantly from long-term reference values reflecting the eutrophic conditions.

The target 4 - *Sediments provide microphytobenthic food for the benthic food web* was partially met, as chlorophyll-a concentrations were lower than in previous years and deviated significantly from the reference value for the South Coorong.

Overall, the macroinvertebrate and mudflat monitoring found that improved conditions which had developed with continuous flow stabilised in 2020, although not all targets were met. The condition monitoring objective I-1 *Maintain or improve mudflat invertebrate communities that are of high condition relative to southern Australian estuarine ecosystems* has been partially met. The condition monitoring objectives M-2 and M-3 combined *Maintain or improve habitable sediment conditions in mudflats* were partially met, with filamentous algal mats posing a threat to mudflat habitats and macroinvertebrate communities. The beneficial effects of continuous flows were detectable in the North Coorong, but did not reach the South Coorong which remained in a degraded state. Further flows and unregulated flow peaks are needed to improve the conditions in the entire Coorong.

2. Introduction

The Coorong and Lakes Alexandrina and Albert Ramsar wetland is of international significance (Phillips & Muller 2006). It is located at the terminus of the Murray-Darling river system in Australia, and one of the Icon sites of The Living Murray (TLM) initiative (Rumbelow 2018). Water regulation and management throughout the catchment is affecting the ecological health of the Coorong, Lower Lakes and Murray Mouth wetland, which is dependent on flows from the River Murray (Kingsford et al. 2011; Mosley et al. 2018; Thom et al. 2020). Environmental water planning in the Murray-Darling Basin is integral to the Basin Plan and informed by long-term ecological condition monitoring (DEWNR 2017; Rumbelow 2018). This report presents findings from monitoring of macroinvertebrates and mudflats in the Coorong, Lower Lakes and Murray Mouth (CLLMM) Icon site conducted in December 2020, the 16th year of monitoring.

The Coorong, Lower Lakes and Murray Mouth provides important habitat for migratory shorebirds, but populations of many species of migratory waders and other waterbirds have been in long-term decline (Prowse 2020). While processes outside of the CLLMM wetlands can affect migratory shorebird numbers (Clemens et al. 2016), the provisioning of sufficient food for these mostly benthivorous birds is important to meet their energy demands (Ye et al. 2020). Many of the fish species in the Coorong and Murray Mouth also rely on macroinvertebrate prey (Giatas et al. 2018; Ye et al. 2020). In addition to their relevance in the food web of the Coorong, macroinvertebrates are used as indicators around the world to detect changes in ecological character and measure success of river restoration schemes (Tweedley et al. 2015; Vinagre et al. 2015).

The monitoring in December 2020 occurred at the mark of a decade of continuous flows through the Lower Lakes into the Coorong and Murray Mouth (Figure 1). The long-term macroinvertebrate and mudflat monitoring spans periods of small flow after a short period of no flow in the early 2000s, three years of no flow (2007-2009), and the flood event at the end of 2010 which marked the end of the Millennium Drought. While flows have been continuous, they varied in intensity, and recent flows have not reached flood peak levels, the last of which occurred in 2016 (Figure 1). For the South Coorong, inflow from Salt Creek is also important, which has been highly variable as well (Mosley et al. 2020). The monitoring in December 2020 occurred after high flows which extended over several months in winter and spring. Freshwater inflows are essential for the ecosystem functions and services in hypersaline estuaries and lagoons (Tweedley et al. 2019; Stein et al. 2021), and flow volume and continuity can affect macroinvertebrate communities (Dittmann et al. 2015). Continuous condition monitoring of macroinvertebrates will supply data and knowledge for efficient water management.

Condition monitoring of Icon sites of The Living Murray (TLM) initiative provides information on changes in the ecology of the LLCMM Icon Site, and whether objectives and targets have been met in particular monitoring years (DEWNR 2017). For macroinvertebrates and mudflats, the objectives set out in the LLCMM Condition Monitoring Plan (Revised) (DEWNR 2017) are as follows:

a) Maintain or improve mudflat invertebrate communities that are of high condition relative to southern Australian estuarine ecosystems.

b) Maintain or improve habitable sediment conditions in mudflats.

Data from this monitoring are also informing progress against the following objective:

c) Support aquatic habitat by establishing and maintaining variable salinity regimes in the Murray Mouth Estuary, North Lagoon and South Lagoon.

To deliver on the aims of TLM condition monitoring, this report assessed the following ecological targets for macroinvertebrates:

1. *Macroinvertebrate species richness increases throughout the Murray Mouth and Coorong*
2. *Macroinvertebrate occurrence extends along the Coorong into the South Lagoon*
3. *The area of occupation for typical estuarine and marine macroinvertebrate species exceeds 60% of the sites sampled*
4. *Macroinvertebrate abundance is maintained at, or increases above, reference levels*
5. *Macroinvertebrate biomass is maintained at, or increases above, reference levels*
6. *Macroinvertebrate communities are similar to those occurring under intermediate continuous flows*

and for mudflats:

1. *Habitable sediments are occurring along the Coorong into the South Lagoon*
2. *Sediments are maintained as fine to medium sands and are mostly moderately well sorted*
3. *Sediment organic matter is maintained*
4. *Sediments provide microphytobenthic food for the benthic food web*

This report not only presents findings from condition monitoring carried out in December 2020, but also evaluates the findings with long-term data collected over 16 years of monitoring for macroinvertebrates and mudflats. Data are supplied electronically, and test outcomes and additional detailed figures and tables are provided as supplementary material (referred to with a prefix SM-).

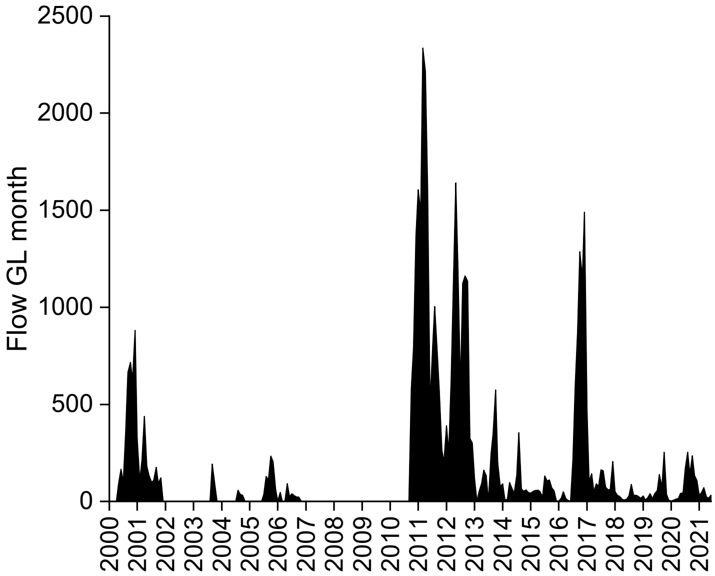


Figure 1: Monthly flow through the barrages from the Lower Lakes into the Murray Mouth and Coorong from 2000 to the end of June 2021, covering the years of macroinvertebrate and mudflat monitoring. Based on modelled monthly barrage outflow data from the MDBA and measured barrage flow data since 2017.

3. Materials and Methods

3.1 Sampling sites and dates

The monitoring for macroinvertebrates and mudflats was carried out in early December 2020, at the eleven sites identified in the TLM LLCMM Condition Monitoring Plan (Revised) (DEWNR 2017). Sites 1 to 7 are located in the North Coorong: site 1 (Monument Road, near the Goolwa Barrage) to site 5 (Pelican Point, at the southern end of the Tauwitchere Barrage) in the Murray Mouth region, and sites 6 (Mulbin Yerrok) and 7 (Noonameena) in the North Lagoon. As the ecological division along the Coorong occurs between Noonameena and Parnka Point (site 8) (Dittmann 2017), four sampling sites are located in the South Coorong, including Parnka Point and three sites from the South Lagoon: sites 9 (Villa de Yumpa) to 11 (Loop Road) (Figure 2, Table 1).

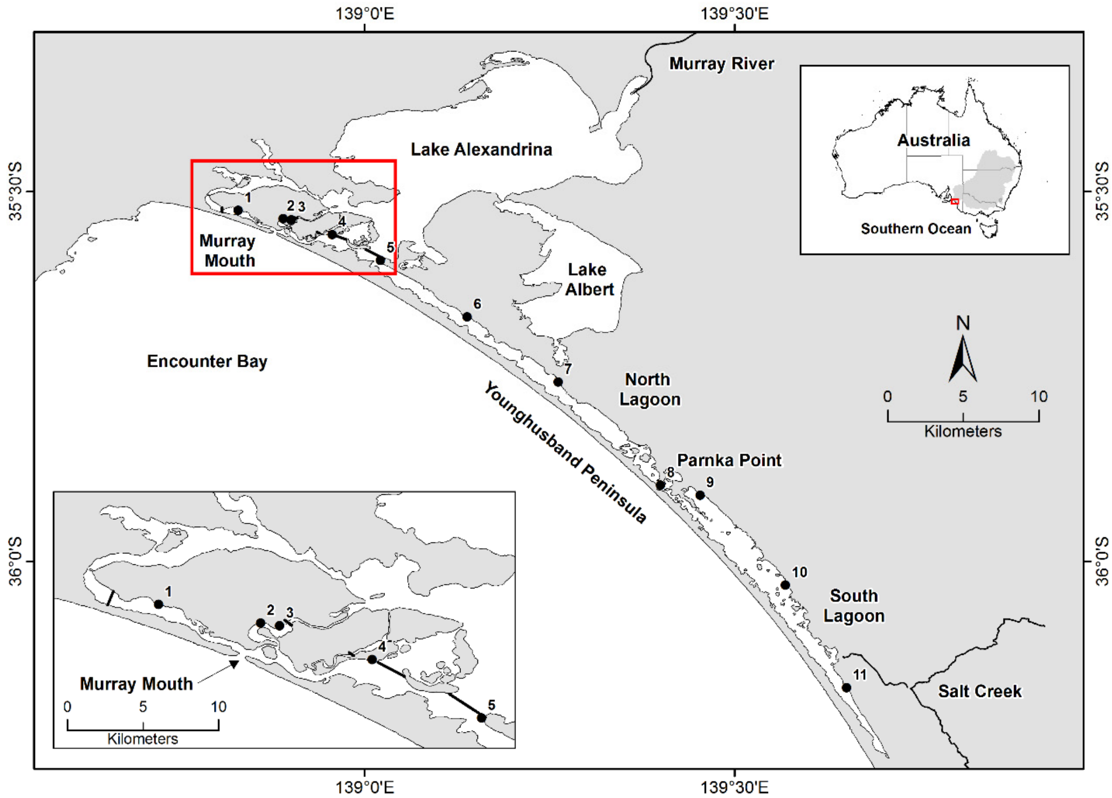


Figure 2: Location of study area and sampling sites for macroinvertebrate and mudflat monitoring in the Murray Mouth and Coorong. See Table 1 for the site names.

The monitoring occurred during a La Niña year, where above average spring rainfall in the catchment of the Murray-Darling river system enabled higher flow of environmental water. During the flow year from July 2020 to June 2021, 1260 GL of water was released over the barrages, with highest monthly flow (>200 GL/month) in August and October before the time of sampling.

Despite La Niña conditions, heat waves occurred in November, interrupted by storm fronts. Temperatures had reached over 40 ºC in late November, but a storm front passed through several days before sampling occurred.

Because of the water releases, water levels were higher than during monitoring in recent years, but mudflats could still be accessed by foot. The replicate samples taken at each site were scattered across the same distances from shore as when mudflats were exposed.

Filamentous green algae were present at most sites, especially in the North Coorong. At the Ewe Island mudflat (Site 4), the cover of filamentous macroalgae had intensified from 2019. Large sections of this mudflat were smothered by algal mats, fresh as well as decaying, and patches of what appeared to be purple sulphur bacteria were seen. The filamentous algae created anoxic conditions in the sediment (see SM-Figure 1 for site photos). As was noticed in 2019, the shell of snails (*Salinator fragilis*) provided substrate for some of the filamentous green algae.

Small flocks of shorebirds were seen during the field sampling in December 2020, mostly Sharp-tailed sandpiper, Red-necked stints, Red-capped plover, Red-necked avocets, and Curlew sandpiper. The waders were either foraging along the water edge or resting on the shore or rocky outcrops. Several species of terns, seagulls, pelican, swans, ducks, ibis, and Cape Barren geese were also noted.

Table 1: Sampling sites, site numbering, and dates for macroinvertebrate monitoring in December 2020. Both old and new regional terminologies are included in the table. See SM-Table 1 for coordinates and daytime of sampling.

Region		Site		Sampling dates
Old	New		Name	
Murray Mouth	North Coorong	1	Monument Road	4/12/20
		2	Hunters Creek	4/12/20
		3	Mundoo Channel	4/12/20
		4	Ewe Island	4/12/20
		5	Pelican Point	4/12/20
North Lagoon		6	Mulbin Yerrok	4/12/20
		7	Noonameena	3/12/20
	South Coorong	8	Parnka Point	3/12/20
South Lagoon		9	Villa de Yumpa	3/12/20
		10	Jack Point	3/12/20
		11	Loop Road	3/12/20

3.2 Environmental parameters

Sediment and water conditions can influence the macroinvertebrate communities at the study sites. Several environmental variables were thus measured to evaluate the objective '*Maintain or improve habitable sediment conditions in mudflats*'. Water quality was recorded in the field at the time of sampling, and sediment samples were taken for later analysis in the laboratory.

Water Quality

The water quality variables recorded during sampling in December 2020 included temperature, salinity and dissolved oxygen (DO). Measurements were taken with handheld devices from the water column at the edge of the mudflat. Water temperature, salinity (PSU) and pH were measured with a Hannah

HI98194 Multiparameter Meter. Dissolved Oxygen (DO) concentration and saturation were measured using an Oxiguard meter. Three replicate measurements were taken for each parameter per site. For long term comparability and measurements in extreme hypersaline waters, salinity was measured with a refractometer, and salinity data presented in this report are based on the refractometer readings. To measure the pH of sediment, pH indicator strips were used (Acilit 0-14).

Sediment analyses

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). To account for spatial variation, three replicate samples of each sediment parameter were taken per site and analysed separately. All sediment samples were stored in a portable freezer (-4°C) in the field and frozen (-20°C) upon return to the laboratory until further analysis.

For sediment organic matter, samples were extracted using a cut off 10 mL syringe (surface area 1.8 cm^2). 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 a 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 10 seconds of drying time. Samples were 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). Grain size was determined by laser diffraction using a particle size analyser (Malvern Mastersizer Model: 2000). After frozen samples were thawed, the fraction >1 mm was 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 (emulated) in the data set. Average values for grain size fractions for each site were entered into the *Gradistat* program v8 (Blott and Pye 2001), to obtain the median (D_{50}) and sorting (σ_G). For the sorting coefficient, the geometric (modified) Folk and Ward graphical measures method and verbal descriptors were used as per Blott and Pye (2001).

For chlorophyll-*a*, the sediment surface was sampled with a 5 mL plastic 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 later analysed with a fluorometer (Turner 450). After the initial reading for total chlorophyll, drops of 0.1 M HCl were added to take a reading to correct for phaeophorbides.

3.3 Macrofauna

To analyse whether the objective '*Maintain or improve mudflat invertebrate communities that are of high condition relative to southern Australian estuarine ecosystems*' has been met, diversity, abundance, biomass and community composition of benthic macroinvertebrates were assessed and compared over time.

Sediment samples for macroinvertebrates were taken using handheld PVC corers (83.32 cm^2 surface area) and sieved through 500 µm mesh size in the field. The corer was pushed into the sediment up to 20 cm depth. Where underlying rock occurred, as at Mulbin Yerrok (site 6) and Jack Point (site 10), the corer could only be pushed to depths of 5 to 10 cm into the sediment. Ten replicate samples were taken haphazardly per site, scattered across the widths of the mudflat to the water edge and into knee-deep water. As water levels were high at several sites, sampling occurred over the widths of the mudflats as known from surveys in previous years.

Some samples were sorted live within a few days of collection, but most samples were preserved in 70% ethanol until processing. After sorting, specimens were identified to the lowest possible taxonomic level and individual numbers of each species counted. Amphipods and chironomid larvae were not differentiated to species, as shorebirds are unlikely to prey selectively on particular species of these taxa. It is common in benthic macroinvertebrate studies to identify organisms to different taxonomic levels (Gerwing et al. 2020). While identification occurred to operational taxonomic units appropriate for the objectives of the monitoring, the terminology ‘species’ or ‘taxa’ is used throughout the report, regardless of specific taxonomic level. Where recent taxonomic revision has changed the genus or species name, both new and old name are given in the species list, but for long-term comparisons, we still refer to formerly used names. In the results, we mostly refer to the genus name only in the text (e.g. for *Capitella capitata*, which can be a complex of species). All polychaete specimens with a complete anterior region (prostomium) were included in abundance counts. For biomass determination, polychaete fragments were also included. The larval and pupae stages of insects were recorded, but all adult winged life stages were excluded as they are highly motile and not part of the benthic macrofauna. All macroinvertebrates were further preserved in 70% ethanol until biomass determination.

Biomass was analysed for all macroinvertebrate taxa per replicate sample and combined as total biomass per replicate for the report. For large bivalves (>0.5 mm length), like *Hiatula alba*, shells were removed before biomass determination. 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 a 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 10 seconds of drying time. 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 the biomass measurement as grams of ash free dry weight (g AFDW).

3.4 Data analysis

The analysis follows procedures used in previous monitoring reports (e.g. Dittmann et al. 2020) and outlined in the TLM Condition Monitoring Plan for macroinvertebrates and mudflats (Dittmann 2017). For long-term comparisons, data from previous monitoring since 2004 were included, yet no TLM spring/early summer monitoring occurred in 2014. As per recommendation in the TLM LLCMM

Condition Monitoring Plan (Revised) (DEWNR 2017), reference values had been recalculated in the 2018 monitoring report (Dittmann et al. 2019) to include further years of flow. This report is using values for reference conditions based on monitoring data from 2004 to 2017 (see tables in supplementary material and Dittmann et al. 2020). For environmental variables, some analyses and plots (e.g. Index of Relative Change) use the differentiation into three regions (Murray Mouth, North and South Lagoon) from earlier monitoring which reflects the spatial changes in environmental conditions. All other regions used in figures, tables and for tests refers to the differentiation between the North and South Coorong.

Diversity is presented as species richness (number of species S), and several indices: Shannon-Wiener diversity (H') using $\log_e$, Margalef's Index (d) for species richness and Pielou's Evenness Index (J'). The higher the values, the higher the diversity. For Evenness, a higher value indicates that the abundances of co-occurring species are more similar. For comparison over time, a tolerance range is included for species numbers for data from 2004-2017 (SM-Table 7).

The Index of Occurrence was calculated based on the number of sites where a species was found across all sites sampled in the survey, and compared with reference values from 2004 – 2017 (SM-Table 6). Values for the Index of Occurrence were converted to percent to obtain the Constancy Index (Rabaoui et al. 2009), which has set ranges for index classifications from rare to constant.

Due to the large spread in data for some environmental variables and macroinvertebrate abundances within and between sites, data were mostly treated as non-parametric and are displayed as box and whisker plots, showing the median, the 25th and 75th percentiles, and outliers (1.5 IQR = interquartile range). All boxplots were constructed in Origin Pro 2020.

Prior to statistical tests and further multivariate community analyses in PRIMER Version 7/PERMANOVA+ add-on, environmental data were transformed as needed (mostly square root, fourth-root transformation for sediment median grain size and organic matter). All macroinvertebrate data were log-transformed (log(x+1)) and because of the large number of 0 values, the Bray-Curtis similarity was calculated with a dummy value of 1 added. For test of single taxa, no dummy value was added for chironomid larvae and Hexapoda, as they were found at all sites and had few 0 values. For single variable tests, Euclidean distance was used for resemblance.

Tests for significant differences between environmental data, abundances and also between communities were carried out using PERMutational ANalysis of VAriance (PERMANOVA). PERMANOVA were run with 9999 permutations. If the number of unique permutations was <100, a Monte Carlo test was carried out (Anderson et al. 2008). Pairwise tests were carried out in several cases where the interaction terms were significant. In tests for the December 2020 survey, the factors were region (fixed) and site nested in region (random). For tests over the long-term monitoring, the factors were year (fixed), region (fixed) and site nested in region (random). For testing long term differences of environmental data, the factors were year (fixed) and region (fixed), including only the years for which particular environmental variables were measured.

Patterns in environmental variables measured from water and sediment at the mudflats were explored with a Principal Component Analysis (PCA) and plotted. Where variables could be auto-correlated (e.g. dissolved oxygen (DO) saturation and concentration, sediment grain size and sorting) only one of the variables was used in the ordination.

For benthic community analysis of the current monitoring year 2020, all species were included in analyses carried out for the 11 sampling sites of the TLM monitoring. For comparisons of macroinvertebrate communities over long-term datasets, rare species were excluded when they met the combined criteria of (i) having been found in only one of the 16 monitoring years, (ii) occurring in <1% of all samples taken, and (iii) contributing <0.005% to the total count of individuals found. Snails of the family Hydrobiidae were combined for long-term comparisons. To detect differences between benthic communities, data were analysed through PERMANOVA with *a priori* defined sites and regions (and years for long term data), and multivariate cluster analysis with *a posteriori* testing for significant differences between sites using SIMPROF tests. Community configurations are displayed as nMDS plots with overlays of significantly different clusters as identified by SIMPROF tests. Segmented bubble plot overlays illustrate the species accounting for community differences.

To illustrate community patterns further, shade plots were prepared in combination with cluster analysis. Using Matrix display under the Wizard function in PRIMER v7, shade plots were created in combination with a cluster analysis on species utilising Whittaker's Index of Association (Clarke et al. 2014). The grouping of samples in the shade plot was constrained by a cluster analysis on samples with Type 1 SIMPROF tests. Dendrograms for species and samples were rotated to maximise the illustration of community differences. The average similarity of communities within each region and monitoring year was obtained from SIMPER analysis. The RELATE analysis in PRIMERv7 was used for comparisons between two similarity matrices and Spearman's rank correlation coefficient δ plotted to test relatedness of community patterns between years.

To explore relationships between macroinvertebrate communities and predictor variables from environmental data (water quality and sediment), distance-based linear models (DISTLM) were carried out using the forward selection procedure and displayed with distance-based redundancy analysis (dbRDA) plots (Anderson et al. 2008). DISTLM was carried out for the years from 2005, when sediment grain size analysis commenced with laser diffraction. LINKTREE analyses gave dendrograms by divisive clustering showing splits in macroinvertebrate community data explained by environmental data. LINKTREE was used with SIMPROF tests to show significantly different groupings.

4. Results – Murray Mouth and Coorong

4.1 Mudflat habitats in the Murray Mouth and Coorong

4.1.1 Water quality

The water quality recorded during the survey in December 2020 reflected the higher flow which had occurred in winter and spring (Figure 1). The North Coorong was thus characterised by low salinities, and high dissolved oxygen (DO). The salinity gradient continues to define the environmental conditions throughout the Murray Mouth and Coorong. The flow had not reached the South Coorong where salinities were within the upper ranges of long-term measurements and remained extremely hypersaline. The DO saturation levels were particularly high at sites in the North Coorong which were covered in filamentous algae. Both DO saturation and concentration were in the upper ranges or higher than values from the long-term monitoring.

4.1.1.1 Salinity

Following the higher flow over the barrages in winter and spring 2020, salinities were brackish (average 17 ppt) in most of the North Coorong, and increased to hypersaline (average 105 ppt) in the South Coorong (Figure 3). While the lower salinities in the water overlying the mudflats in the North Coorong reflected the higher freshwater inflow, salinities in porewater were higher than in overlying water at Hunters Creek (site 2) and Noonameena (site 7). In the South Coorong, salinities in porewater exceeded water column salinities at Parnka Point (site 8). At Jack Point (site 10) porewater salinities were almost marine, indicating possible groundwater seepage. Salinities were significantly different between regions and sites within regions (SM-Table 2).

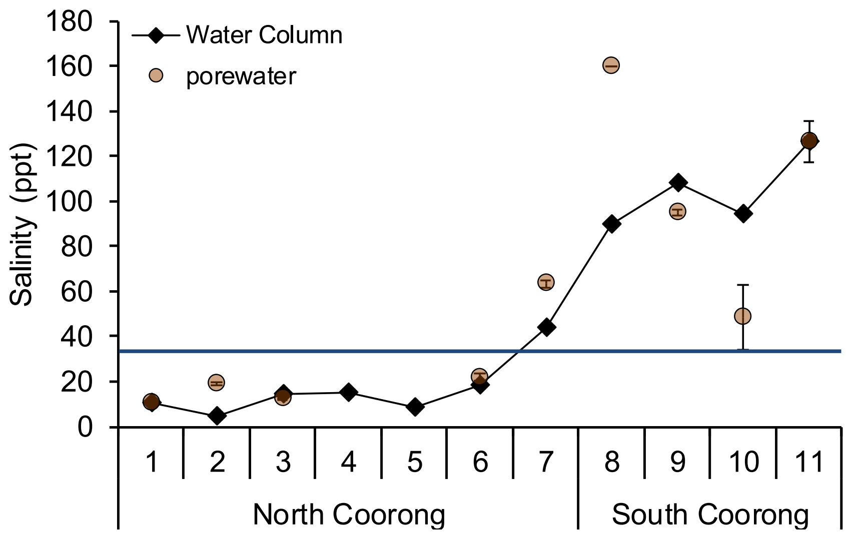


Figure 3: Salinity (average ± SE, n=3) in the water overlying the mudflats (black diamonds) and in porewater (brown circles) at the 11 sampling sites in the North and South Coorong during the survey in December 2020. Salinity in sediment porewater could not be measured at sites 4 (Ewe Island) and 4 (Pelican Point) because of overlying water. The blue line indicates the level of seawater salinity.

In long-term comparison, the lower salinities at the North Coorong sites between the Goolwa barrage (site 1, Monument Road) and Pelican Point (site 5, at the southern end of the Tauwitchere barrage) were within the range of values recorded over the previous 15 years (Figure 4). For Mulbin Yerrok (site 6) and Noonameena (site 7), salinities in December 2020 were lower than during most of the previous monitoring. For the South Coorong sites, salinities in December 2020 fell within the upper ranges of salinities recorded over the previous 15 years. Salinities were significantly different between years and between the regions, as the salinity gradient throughout the Coorong was persistent over all monitoring years (Figure 4, SM-Table 2).

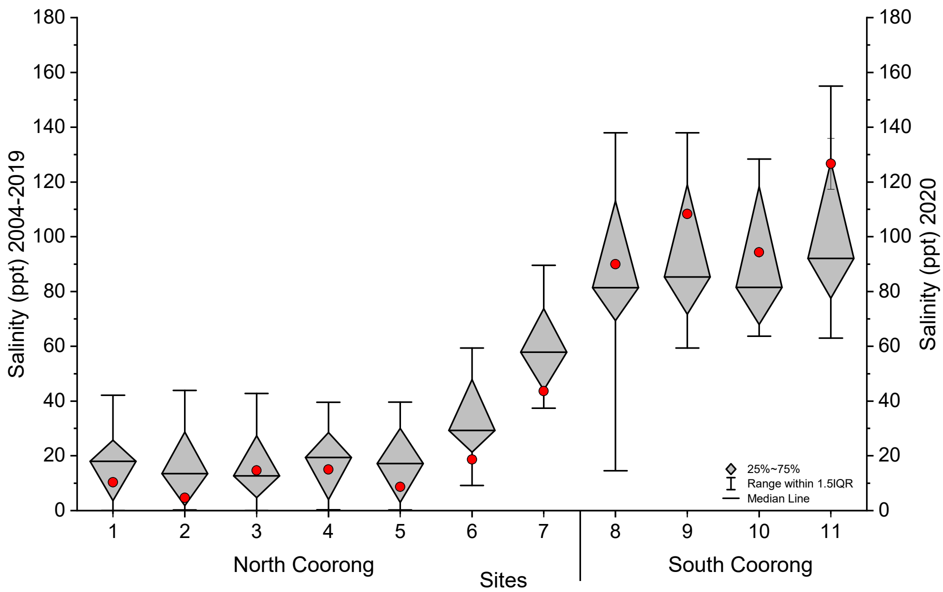


Figure 4: Salinity gradient in the North and South Coorong in December 2020 (red symbols, average ± SE) in relation to the long term salinity gradient in the Coorong (diamond box plots for salinities at the study sites from 2004 to 2019).

4.1.1.2 Dissolved oxygen and temperature

The water overlying the mudflats was oversaturated with dissolved oxygen (DO) at most sites, but close to the trigger value of the ANZECC (2000) Guidelines at Mulbin Yerrok (site 6) and Loop Road (site 11) (Figure 5a). At Mundoo Channel (site 3) and Ewe Island (site 4), where a dense cover of filamentous algae occurred on the mudflats, DO saturation levels were high. The DO concentration was on average 10 mg/L across all sites, and highest at sites 3 and 4 (Figure 5b). Both the DO saturation level and the DO concentration varied significantly between sites within regions, but not between the regions (SM-Table 2).

In long-term comparison, values for DO saturation and DO concentration were higher than, or in the upper range of values recorded in previous monitoring years (Figure 6). Values were more comparable to the range of long-term values at Mulbin Yerrok (site 6). Dissolved oxygen saturation and concentration differed significantly over the monitoring years (SM-Table 2). The concentration of DO also varied significantly between the regions (higher in North than South Coorong), while regional differences in DO saturation were subject to the monitoring year (SM-Table 2).

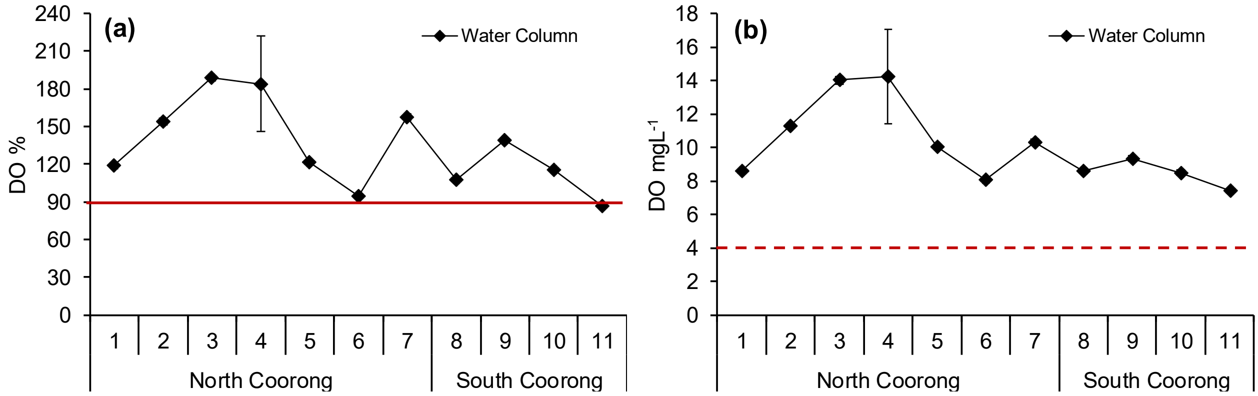


Figure 5: Dissolved oxygen (DO) (average ± SE, n=3) in the water above the mudflats at the study sites during the survey in December 2020. (a) DO saturation, red line indicates the 90% trigger value of the ANZECC Guidelines; (b) DO concentration, the dotted red line indicates low DO (<4 mg/L).

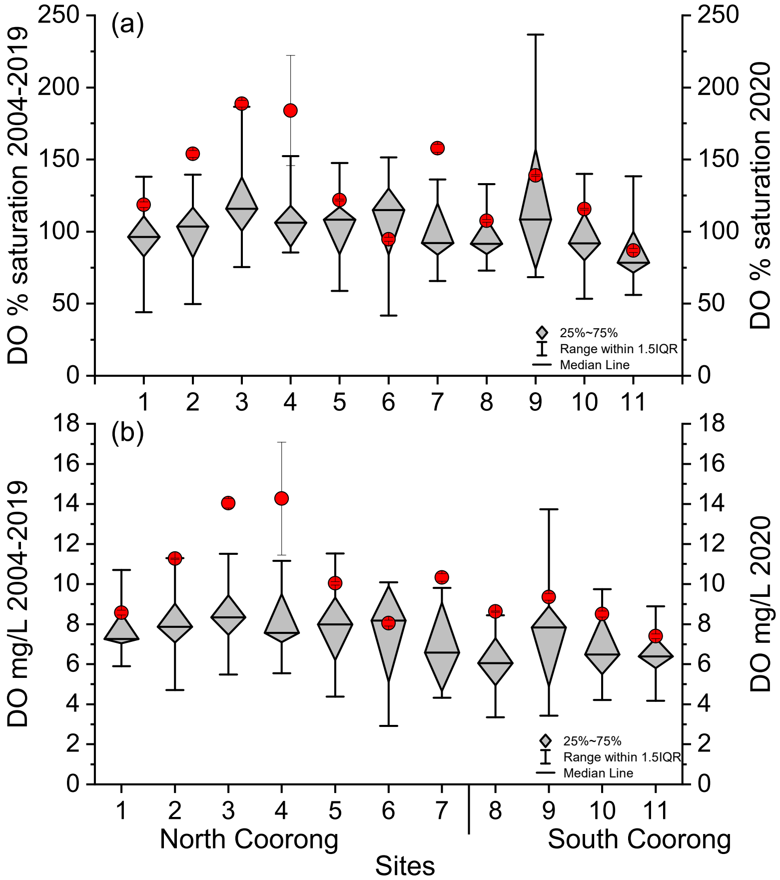


Figure 6: Dissolved oxygen (DO) in the North and South Coorong in December 2020 (red symbols, average ±SE) in relation to long-term values (diamond box plots) based of DO measurements from 2004 to 2019). a) DO saturation; b) DO concentration.

The average water temperature at the mudflats at the time of sampling in December 2020 was 25 °C (range 17 – 32 °C), warm for spring/early summer. The range in water temperature also reflects the time of day of sampling, as sediment heats up during the day.

4.1.1.3 pH in water and sediment

The pH of water above the mudflats and in the sediment was similar and on average pH 6.1 (Figure 7a), and each differed significantly between sites within regions (SM-Tables 2 and 3). Compared to previous monitoring years, the pH in the water was more acidic, but fell within a range of previous values at site 3 (Mundoo Channel), site 4 (Ewe Island) and Noonameena (site 7) (Figure 7b). Over the longer term monitoring, pH in the water was significantly different between years, but not regions (SM-Table 2).

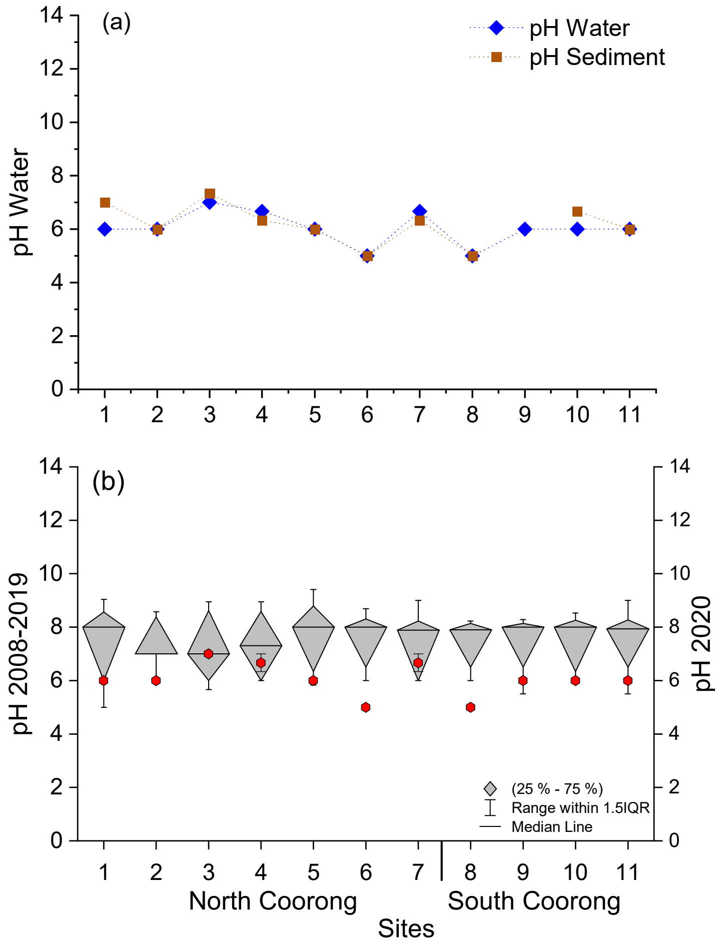


Figure 7: a) pH in sediment and water above the mudflats at the study sites in December 2020. b) Diamond box plots of long-term comparison of pH in water overlying the mudflats, measured since 2008, and values from the survey in December 2020 shown in red symbols (average ± SE).

4.1.2 Sediment characteristics

The targets for sediment grain size and organic matter were only partially met, as sediments were coarser and less well sorted at several sites, and organic matter content higher in the mudflat sediments of the South Lagoon. The target for provision of microphytobenthic food was also only partially met, as chlorophyll-a values in sediments were lower than in previous years at several sites. The sediment characteristics were still habitable for macroinvertebrates at most sites, but effects of eutrophication continued and the extent of filamentous algae intensified on mudflat sediments in the North Coorong.

4.1.2.1 Grain size composition

Sediment characteristics changed from the North Coorong into the South Coorong. At sites between the Goolwa barrage (site 1, Monument Road) and the Ewe Island barrage (site 4), the median grain size was fine sand with moderately sorted grain size composition (Table 2, Figure 8). At the sites from Pelican Point (site 5) southward, sediments were medium sand and moderately or poorly sorted. While the sediments were mostly fine to medium sands, they included coarser particles at several sites and were only moderately sorted or poorly sorted and the target that '*Sediments are maintained as fine to medium sands and are mostly moderately well sorted*' has thus only been partially met.

The median grain size differed significantly between sites within regions for the monitoring in December 2020, but not between regions (SM-Table 3). At Jack Point (site 10), sediments were coarse sand, similar to conditions from previous years (SM-Figure 2). At Mulbin Yerrok (site 6) and at Noonameena (site 7), sediments were slightly coarser than in previous monitoring years, however, similar grain size compositions have been found in earlier monitoring years at these sites (SM-Figure 2). Over the longer term, sediment grain size composition did not change between years, but was significantly different between regions, irrespective of the monitoring year (SM-Table 3).

The median grain size for the Murray Mouth region was within the reference range from long-term monitoring, but deviated beyond the confidence intervals for the North and South Lagoon (Figure 9a). The sorting coefficient was below the lower bounds of the reference range for all regions (Figure 9c). The Index of Relative Change showed significant differences for the recent survey in December 2020 to the long-term reference, for both the median grain size and the sorting coefficient (Figure 9 b, d; SM-Table 5).

Table 2: Sediment characteristics (organic matter content in percent dry weight, median grain size and sorting coefficient) from the survey in December 2020 at the eleven sites in the North and South Coorong. Values are averages from three replicate samples per site.

Region	Site	Organic Matter (% DW)	Grain size (µm)	Grain size description	Sorting σ_G	Sorting description
North Coorong	1	1.61	188	Fine sand	1.67	Moderately Sorted
	2	2.22	185	Fine sand	1.92	Moderately Sorted
	3	1.37	247	Fine sand	1.99	Moderately Sorted
	4	1.17	174	Fine sand	1.72	Moderately Sorted
	5	1.18	323	Medium sand	1.93	Moderately Sorted
	6	1.61	382	Medium sand	2.42	Poorly Sorted
	7	1.30	376	Medium sand	2.24	Poorly Sorted
South Coorong	8	2.02	303	Medium sand	1.71	Moderately Sorted
	9	10.53	256	Medium sand	2.33	Poorly Sorted
	10	2.12	502	Coarse sand	2.04	Poorly Sorted
	11	3.23	316	Medium sand	1.92	Moderately Sorted

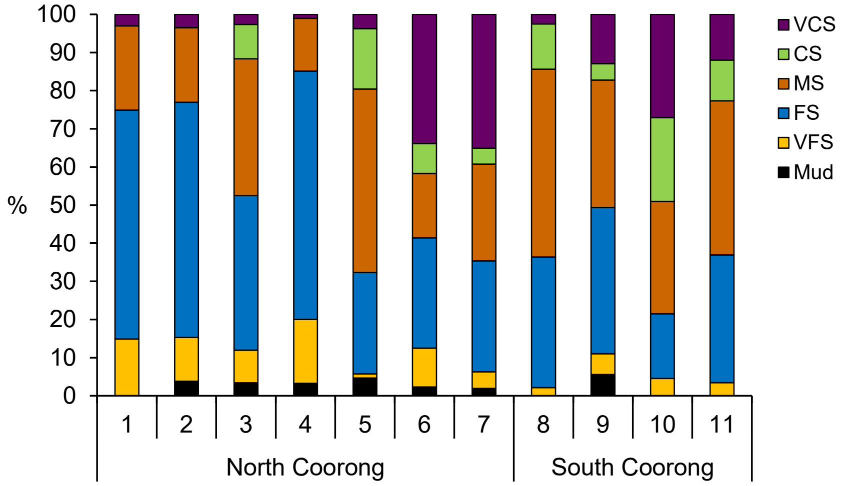


Figure 8: Grain size composition (%) of sediments at the study sites in the North and South Coorong in December 2020. The grain size fractions are: Mud <63 µm, VFS (very fine sand) 63-125 µm, FS (fine sand) 125-250 µm, MS (medium sand) 250-500 µm, CS (coarse sand) 500-1000 µm, VCS (very coarse sand) >1000 µm.

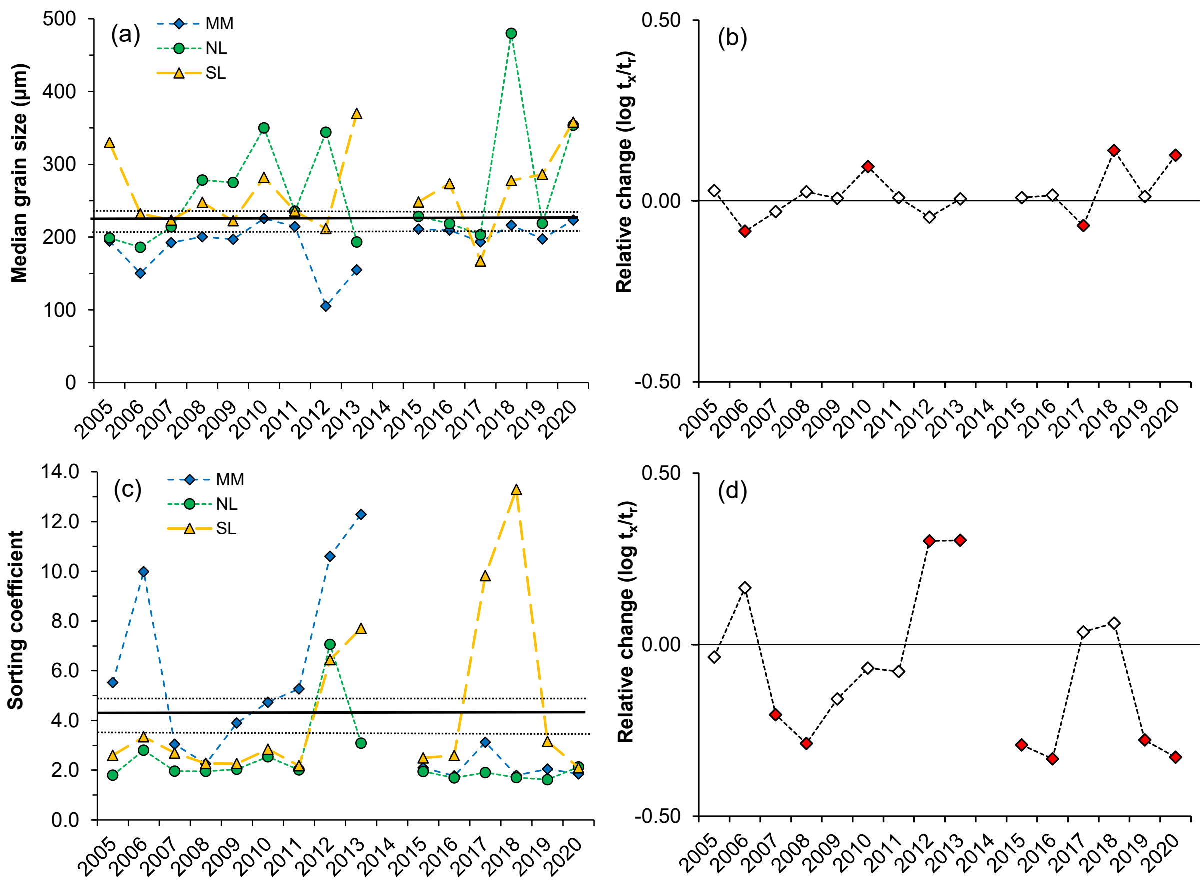


Figure 9: Long term changes in median grain size (a, b) and sorting coefficient (c, d) over the mudflat monitoring from 2005 to 2020 (no TLM monitoring in 2014). Average of a) median grain sizes and c) sorting coefficient for the Murray Mouth (MM), North (NL) and South Lagoon (SL) of the Coorong. The horizontal black line indicates a reference, based on the monitoring years from 2005-2017, with 90% confidence intervals (dotted lines) (see SM-Table 4). The Index of Relative Change is plotted for the b) median grain size and d) sorting coefficient for all monitoring sites (irrespective of region) as a ratio of observed values (averages per year t_x) to reference data (t_r) from the monitoring period from 2005-2017. The black line at 0 marks where observed values equal the reference values. The effect sizes (rho) in 2020 were 0.06 for median grain size and 0.20 for the sorting coefficient. Red symbols indicate significant changes of values > or < the respective effect size.

4.1.2.2 Sediment organic matter

The organic matter content of the mudflat sediments differed significantly between sites within the regions, and between regions, with higher values recorded in the South Coorong (average organic matter content 4.4 - 7%) than the North Coorong (average content 1.49 %) (Figure 10a; SM-Table 3). The highest sediment organic matter (10.53 ± 2.38, average ± standard error) was again recorded at Villa de Yumpa (site 9), similar to previous years, and exceeded the long-term range (Figure 10). Sediment organic matter was also above the long-term range at Hunters Creek (site 2). At all other sites, the values from December 2020 fell within the range of previous records. Over the long-term, sediment organic matter was significantly different across monitoring years and regions (SM-Table 3).

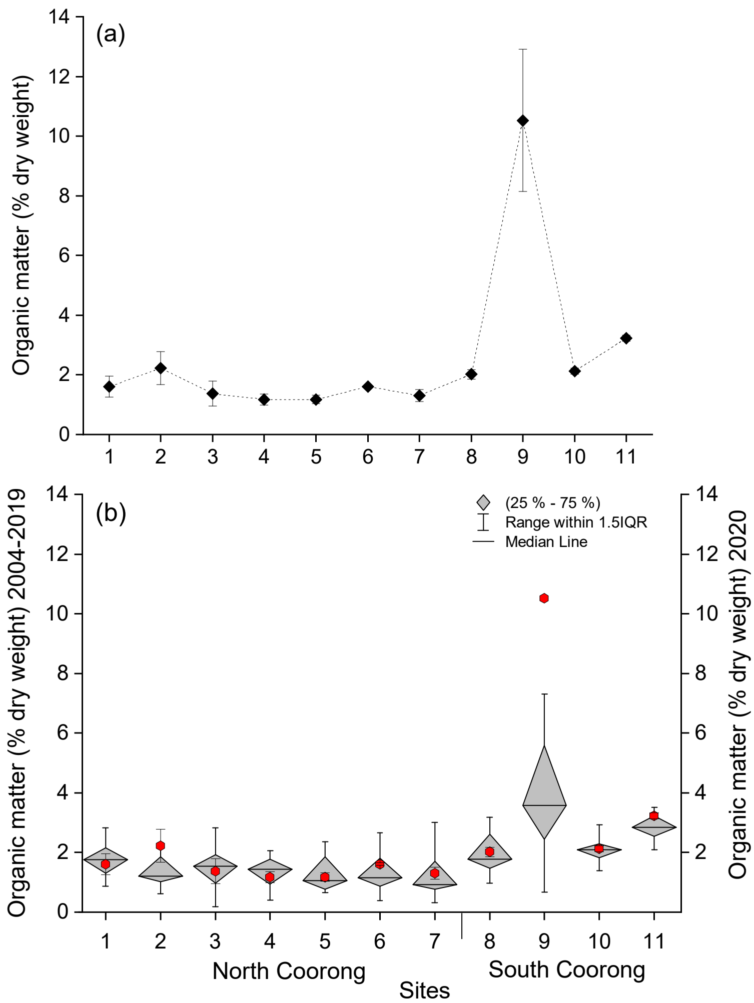


Figure 10: Sediment organic matter content (a) at the study sites in the North and South Coorong in December 2020 (average ± SE, n=3), and (b) for December 2020 (red symbols, average ± SE) in relation to long term monitoring from 2004 to 2019 (diamond box plots).

The Index of Relative Change revealed a significant deviation from the reference level, which was due to a significant deviation in the South Lagoon (Figure 11, SM-Figure 3, SM-Table 5). While sediment organic matter fell within the range of previous records at most of the sites in the Murray Mouth and North Lagoon, the significant deviation in the South Lagoon leads to the target that '*Sediment organic matter is maintained*' being only partially met for 2020.

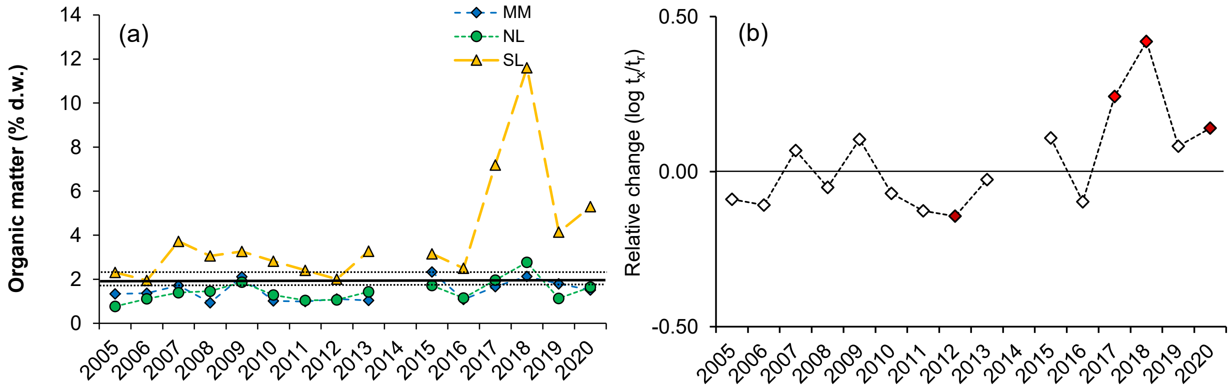


Figure 11: Long term changes in sediment organic matter since 2005 (no TLM monitoring in 2014). (a) Average of organic matter across the study sites in each of the regions Murray Mouth (MM), North (NL) and South Lagoon (SL) of the Coorong. The horizontal black line indicates a reference, based on the monitoring years from 2005-2017, with 90% confidence intervals (dotted lines). (b) Index of Relative Change for sediment organic matter for all monitoring sites (irrespective of region). Index values are plotted as a ratio of observed values (averages per year t_x) to reference data (t_r) obtained from the monitoring 2005-2017. The black line at 0 marks where observed values equal the reference values. The effect size (rho) for sediment organic matter in 2020 is 0.14, and red symbols indicate significant change of values > rho.

4.1.2.3 Sediment chlorophyll-a

Concentrations of sediment chlorophyll-a were on average 1.22 mg m^{-2} in the North Coorong compared to 0.24 mg m^{-2} on average in the South Coorong (Figure 12a). As sediment chlorophyll-a was highly variable and also low at some sites in the North Coorong (Figure 12a), there were no significant differences between regions, nor between sites within regions (SM-Table 2). In comparison to values from long-term monitoring of chlorophyll-a, the concentrations from December 2020 were at the lower range at sites 1 (Monument Road), 2 (Hunters Creek) and 6 (Mulbin Yerrok) in the North Coorong, and at all sites in the South Coorong (Figure 12b). At sites 3 (Mundoo Channel), 4 (Ewe Island), 5 (Pelican Point) and 7 (Noonameena), the chlorophyll-a concentrations were in the upper ranges of long-term values. The chlorophyll-a concentrations were significantly different across the years and regions (SM-Table 3). Chlorophyll-a is measured as a proxy of microphytobenthos, but could also be derived from filamentous algae covering the mudflats at some sites.

Compared to previous monitoring years, the sediment chlorophyll-a values in December 2020 were lower than a long-term reference for the North and South Lagoon, and at the lower confidence interval for the Murray Mouth (Figure 13a). The Index of Relative Change was also below the reference value, but not a significant deviation (Figure 13b). For each of the three regions, the Index of Relative Change was below the reference value as well, with a significant deviation for the South Lagoon (SM-Figure 4). As chlorophyll-a was lower than in previous years at several sites in the North and South Coorong, the target that '*Sediments provide microphytobenthic food for the benthic food web*' has been partially met.

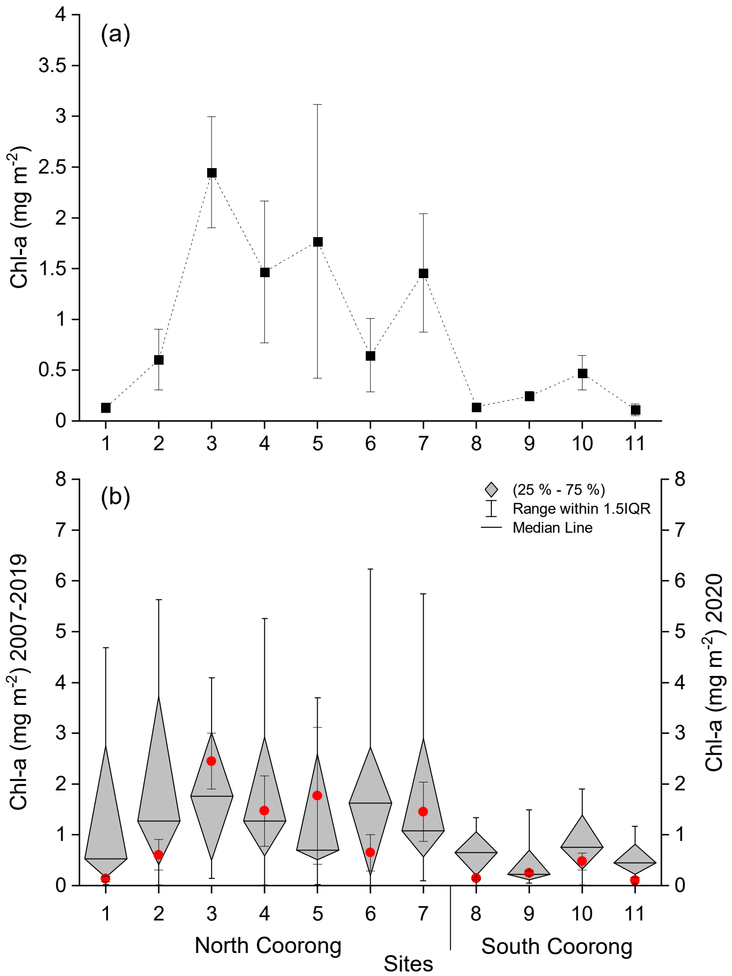


Figure 12: Sediment chlorophyll-a content a) for the survey in December 2020 at the study sites in the North and South Coorong (average ± SE, n=3); and b) from December 2020 (red symbols, average ± SE) in relation to long term monitoring values (2007-2019, shown as diamond box plots).

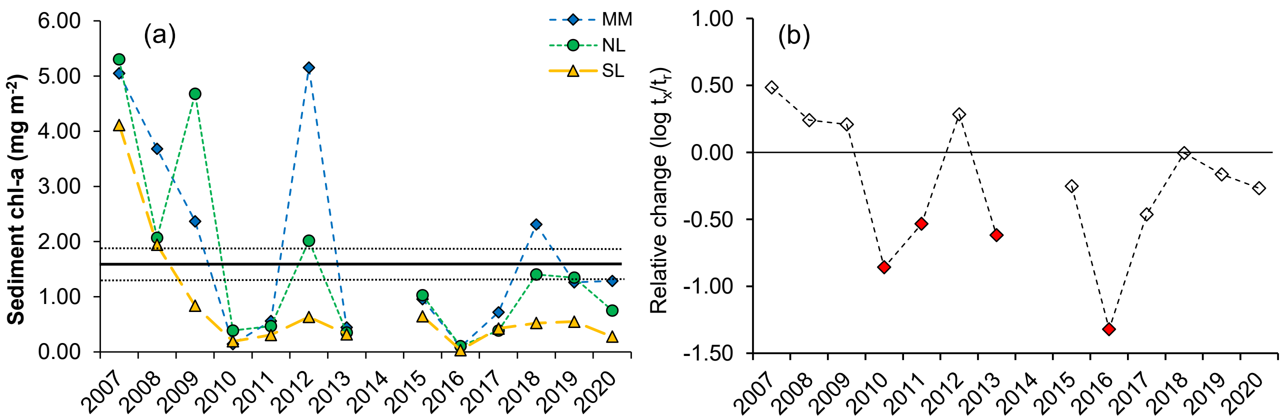


Figure 13: Long term changes in sediment chlorophyll-a (chl-a) since 2007 (no TLM monitoring in 2014). (a) Average of chl-a across the study sites in each of the regions Murray Mouth (MM), North (NL) and South Lagoon (SL) of the Coorong. The horizontal black line indicates a reference, based on the monitoring years from 2007-2017, with 90% confidence intervals (dotted lines). (b) Index of Relative Change for chl-a for all monitoring sites (irrespective of region). Index values are plotted as a ratio of observed values (averages per year t_x) to reference data (t_r) obtained from the monitoring 2005-2017. The black line at 0 marks where observed values equal the reference values. The effect size (rho) for sediment chl-a in 2020 is 0.50, and red symbols indicate significant change of values < rho.

4.2 Macroinvertebrate populations

In the 2020 monitoring, macroinvertebrates occurred with similar diversity, abundance and community composition, but lower biomass than in previous years. The occurrence of several species was contracted compared to 2019, but species rarely seen before were also recorded. The North Coorong macroinvertebrate community was very similar to the one found in recent years with continuous flow. The macroinvertebrate community in the South Coorong continued to be dominated by chironomid larvae, and had very low diversity, abundance and biomass. Salinity continued to explain the differentiation of macroinvertebrate communities, with a main threshold between the regions of 64 ppt.

4.2.1 Macroinvertebrate species richness, diversity and distribution

Through sampling and observations in the field, 22 macroinvertebrate taxa were recorded in the December 2020 monitoring (Table 3). Almost all of the taxa occurred in the North Coorong (20 taxa), and two further taxa (Hexapoda, insect larvae) were found only in the South Coorong. The total number of species recorded from the South Coorong was seven, including four different taxa of insect larvae (Table 3). Annelida accounted for most of the macroinvertebrate species (9 species), followed by Mollusca (6 species), Hexapoda (4 taxa) and Crustacea (3 taxa) (Table 3). Species of Mollusca and Annelida were only recorded from the North Coorong, apart from one Annelida species (*Capitella*) found at Jack Point (site 10) in the South Coorong (Table 3). The average number of species found in the North Coorong was 12 ± 2, compared to 4 ± 2 in the South Coorong. The species richness, density and composition of major taxa in the two regions was very similar to the previous monitoring. The target that '*Macroinvertebrate species richness increases throughout the Murray Mouth and Coorong'* was partially met.

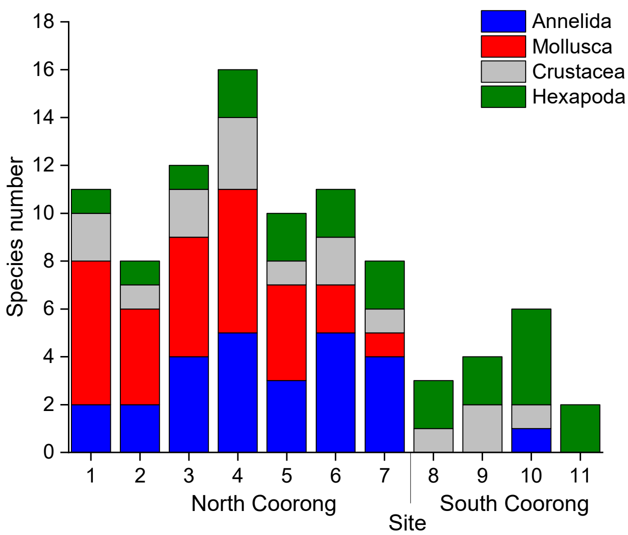


Figure 14: Species numbers (total and species for major taxonomic groups) at the eleven monitoring sites sampled in December 2020. The figure does not include species from field observation only (see Table 3).

Table 3: Species list of macroinvertebrates recorded at the study sites through sampling (√) and field observations (#) during the 2020 monitoring. The total number of taxa per site is given based on samples

and observations, as well as the number of taxa for the two main regions. 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 de Yumpa; Site 10 = Jack Point and Site 11 = Loop Road. See Figure 2 for site locations.

			North Coorong							South Coorong			
Phyla/Class/Order		**Family/Genus/Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Annelida	Oligochaeta					✓	✓	✓	✓				
	Polychaeta	*Capitella spp.*		#	✓	✓	✓	✓	✓			✓	
		Simplisetia aequisetis	✓	✓	✓	✓	✓	✓					
		Australonereis ehlersi							✓				
		Nephtys australiensis				✓							
		Boccardiella limnicola	✓	✓	✓			✓					
		Euchone variabilis						✓	✓				
		Ficopomatus enigmaticus	#	#	✓			#					
		Phyllodoce novaehollandiae		#		✓							
Crustacea	Amphipoda		✓	✓	✓	✓	✓	✓	✓		✓	✓	
	Ostracoda					✓				✓	✓		
	Malacostraca	*Mysidacea*	✓		✓	✓		✓					
Mollusca	Bivalvia	*Arthritica semen*	✓	✓	✓	✓	✓	✓	✓				
		Hiatula (Soletellina) alba	✓	#		✓							
	Gastropoda	Hydrobiidae sp. 4	✓	✓	✓	✓	✓	✓					
		Hydrobiidae sp. 5	✓		✓	✓							
		Hydrobiidae sp. 6	✓	✓	✓	✓	✓						
		Salinator fragilis	✓	✓	✓	✓	✓						
Hexapoda	Diptera	Chironomidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Dolichopodidae				✓	✓	✓	✓	✓	✓	✓	
		Ceratopogonidae										✓	✓
		Stratiomyidae										✓	
Total species number per site			12	12	12	16	10	12	8	3	4	6	2
Species number per region			20							7			

Compared to previous years, the number of species recorded in the South Coorong was unchanged. In the North Coorong, the number of species in 2020 was higher than in 2019 and within the range of species numbers over the years since flows were restored (Figure 15). The polychaete *Euchone variabilis* (Sabellidae), which was rarely seen in earlier surveys, was recorded in the North Lagoon at Mulbin Yerrok (site 6) and Noonameena (site 7). Mysid shrimps were also found in samples throughout the North Coorong, possibly reflecting the higher water level.

The diversity of macroinvertebrates was higher throughout most of the North Coorong compared to the South Coorong, based on the number of species per site and the Shannon-Wiener diversity index H' as well as Margalef's dominance index d (Figure 16). At Jack Point (site 10) in the South Coorong, the diversity values were similar to those at Noonameena (site 7) in the North Coorong. Apart from Loop Road (site 11), evenness was similar across all sites (Figure 16). All of the diversity measures were lowest at Loop Road, which was also the case in previous years.

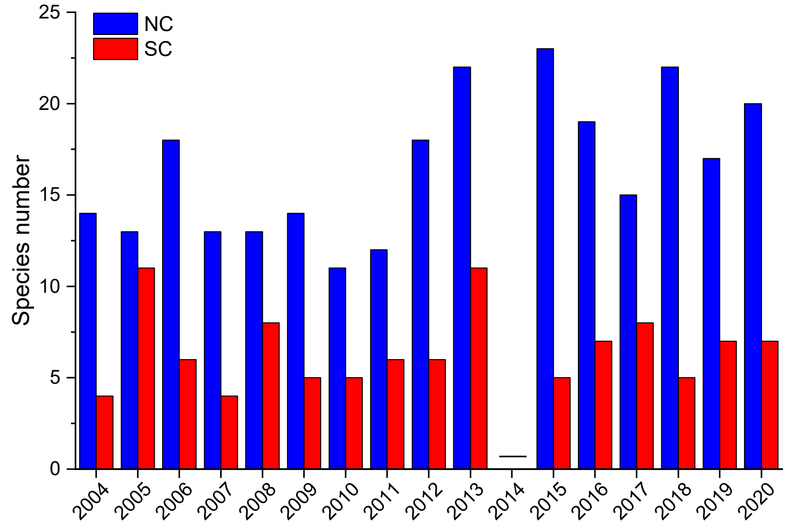


Figure 15: Total number of macroinvertebrate species in the North Coorong (NC) and South Coorong (SC) regions for each monitoring years since 2004, based on annual surveys in spring/early summer. No TLM survey for macroinvertebrates was carried out in 2014.

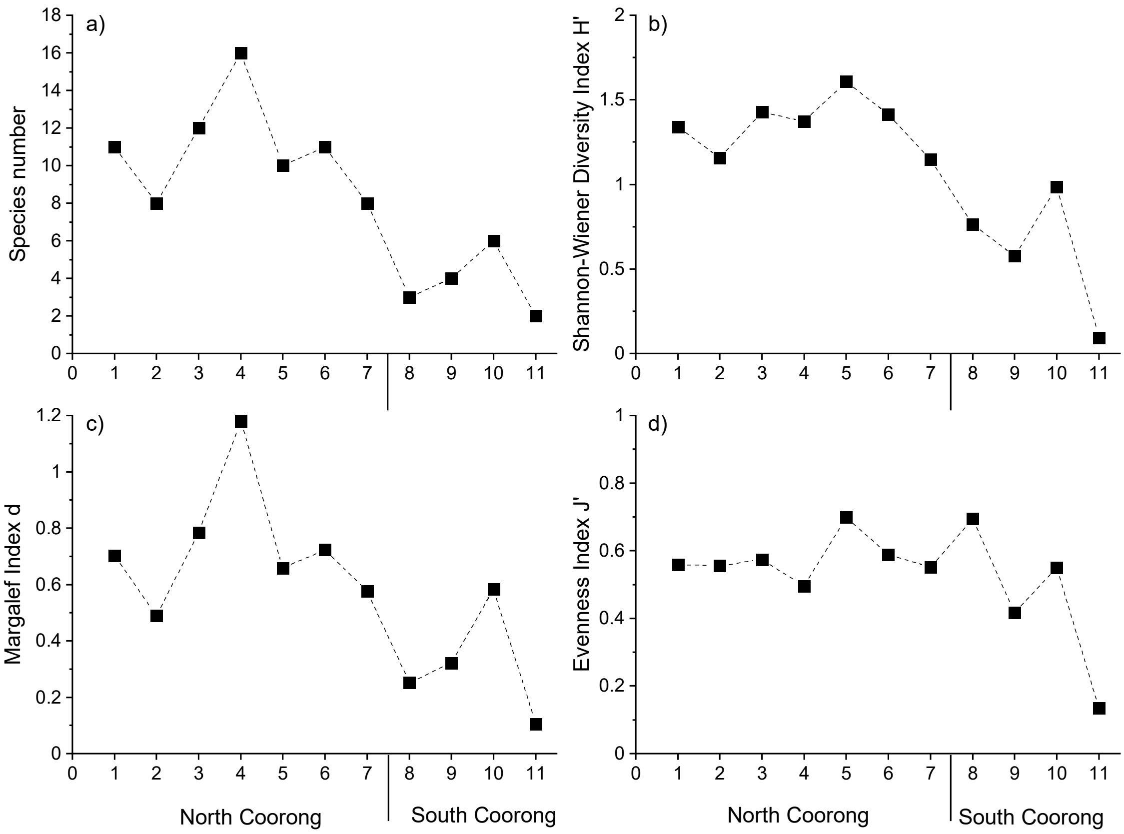


Figure 16: Diversity measure for macroinvertebrates from mudflats in the Murray Mouth, North and South Lagoon of the Coorong in December 2020, showing a) the total number of species, b) Shannon-Wiener diversity H', c) Margalef's index for dominance, and d) evenness J'. Sites 1 (Monument Road) – 7 (Noonameena) are located in the North Coorong, and sites 8 (Parnka Point) – 11 (Loop Road) in the South Coorong. See Figure 2 for site locations and Table 1 for site names.

Species numbers in the North Coorong continued to be above the long-term reference range, indicating improvements in macroinvertebrate diversity in mudflats of this region (Figure 17). The further diversity indices (Margalef's dominance and the Shannon-Wiener Index) were also higher. For the South Coorong, species numbers fell within the confidence intervals of a long-term reference, and the diversity indices were also comparable to earlier years (Figure 17). Macroinvertebrate diversity has thus not improved in the South Coorong.

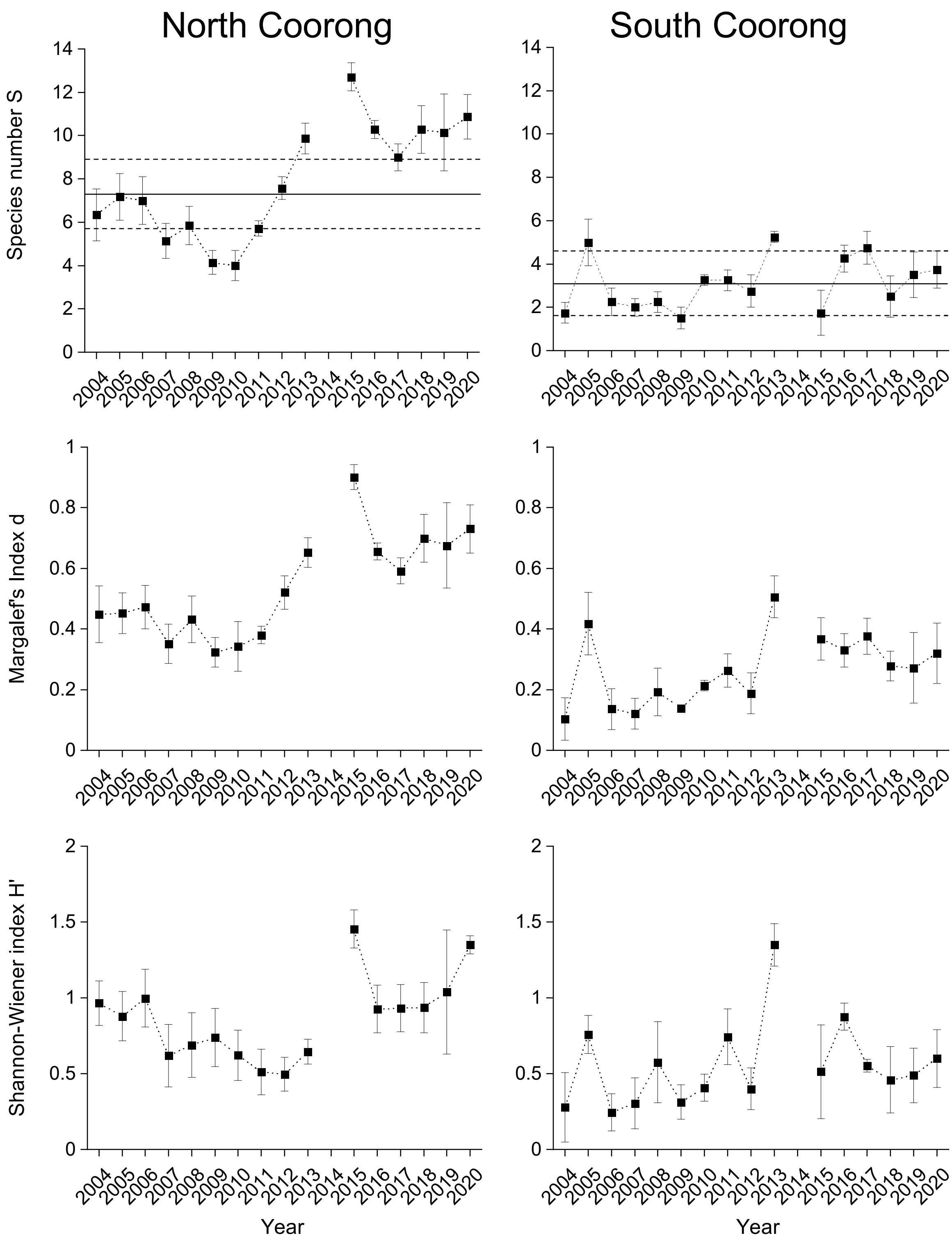


Figure 17: Changes in diversity measures over the monitoring years since 2004 for the North and South Coorong (average ± SE, n=7 for the North Coorong and n=4 for the South Coorong). No TLM survey was carried out in 2014. For species numbers, average (black line) and upper and lower tolerance ranges (dotted lines) are indicated based on the TLM refinement using data 2004-2017 (see SM-Table 7).

4.2.2 Macroinvertebrate occurrence and abundance

4.2.2.1 Macroinvertebrate occurrence and distribution ranges

The occurrence of typical estuarine benthic macroinvertebrates like annelids, crustaceans and molluscs was confined to the North Coorong, with only single individuals of three species detected at some sites in the South Coorong (Table 3). The macroinvertebrates recorded in the South Coorong were mainly insect larvae. The target that '*Macroinvertebrate occurrence extends along the Coorong into the South Lagoon*' has not been met.

The occurrence of macroinvertebrate species within the Coorong can be measured in relation to the number of sites at which they are found. Expressed as a Constancy Index, chironomid larvae and amphipods continue to be the most widespread and were classified as 'constant', similar to previous years (Figure 18). Further key species of polychaetes and molluscs also remained 'very common' or 'common' in their occurrence. Of the polychaetes and bivalves which had emerged as 'not very common' or 'common' in the 2019 monitoring, only the bivalve *Hiatula alba* remained as 'not very common' in the 2020 monitoring, while the other species were again rarely found (Figure 18).

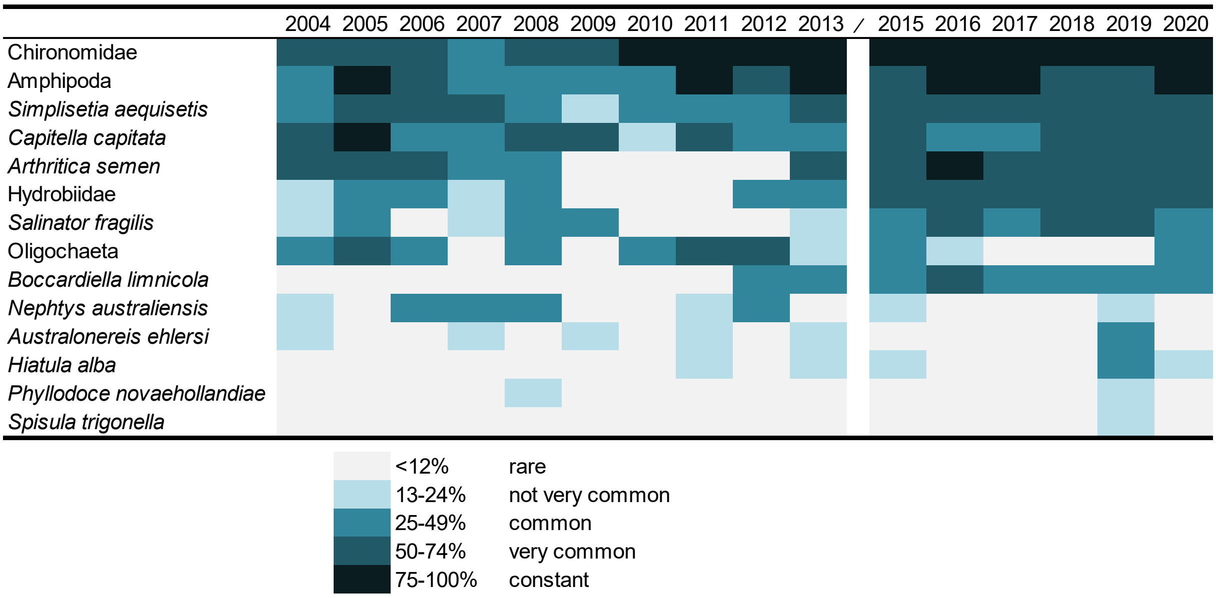


Figure 18: Constancy Index for the presence of the more common macroinvertebrate species across the study sites in the Murray Mouth and Coorong over the monitoring years since 2004. The darker the colour, the more widespread a species is in occurrence. No TLM monitoring occurred in 2014.

The Index of Occurrence revealed that chironomid larvae were the only taxon found at all sites in the 2020 monitoring, and have been close to their reference since flows resumed in 2010 (Figure 19). For several other macroinvertebrate species, the Index of Occurrence increased in the 2020 monitoring to be closer to the reference. This was the case for amphipods, oligochaetes, larvae of Dolichopodidae, and mysid shrimp (Figure 19). A contracted distribution range was indicated by a lower Index of Occurrence for the polychaetes *Capitella*, *Simplisetia*, *Salinator*, *Boccardiella*, *Australonereis*, *Nephtys*, *Phyllodoce* and the bivalves *Hiatula* and *Spisula*. There was no change in the Index of Occurrence for *Arthritica* and hydrobiid snails. The distribution range for many key macroinvertebrate species in the Coorong thus remained below their potential occurrence as indicated by the reference (Figure 19). The target that '*The area of occupation for typical estuarine and marine macroinvertebrate species exceeds 60% of the sites sampled'* was partially met.

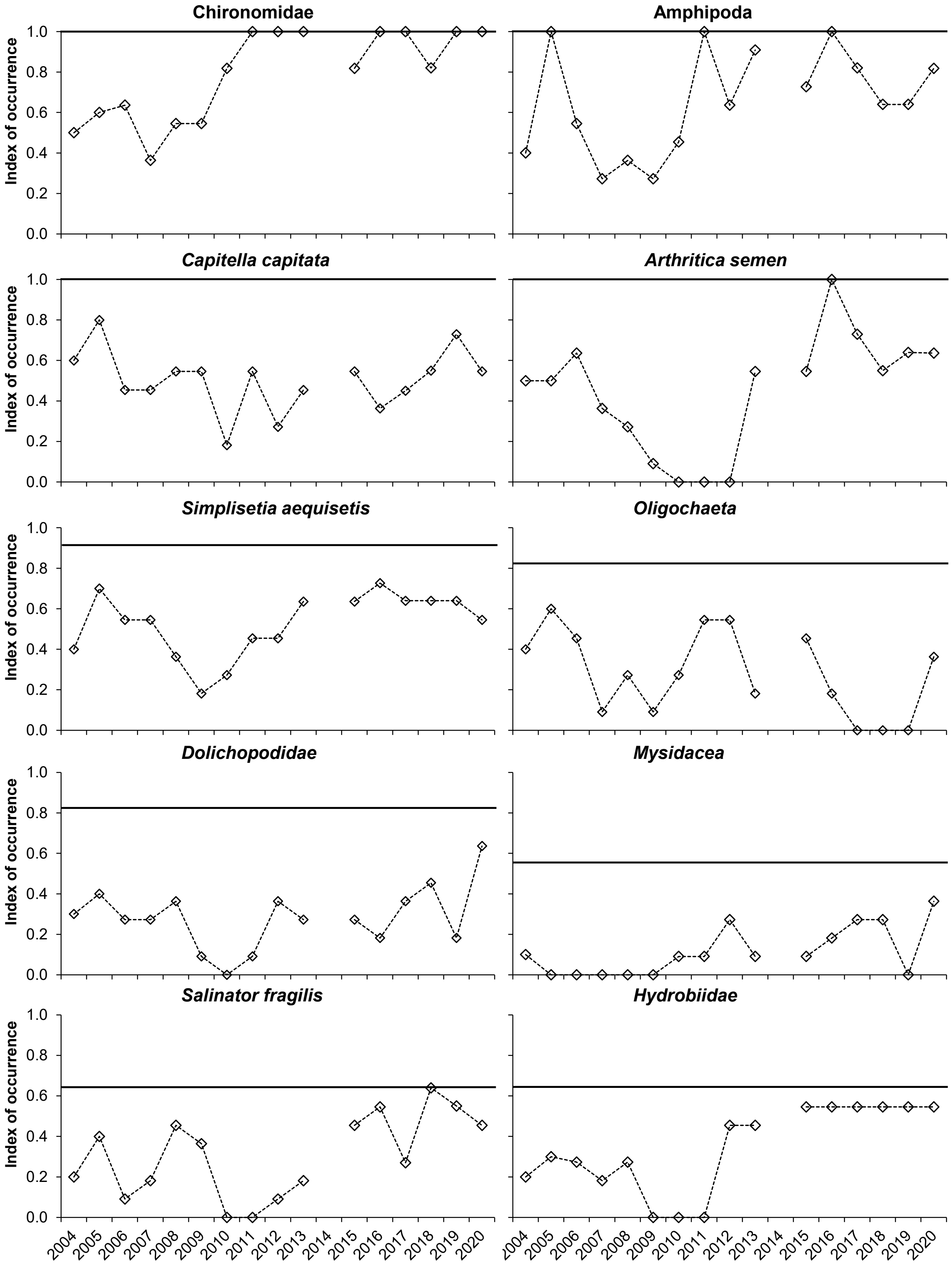


Figure 19: Index of Occurrence (continued next page)

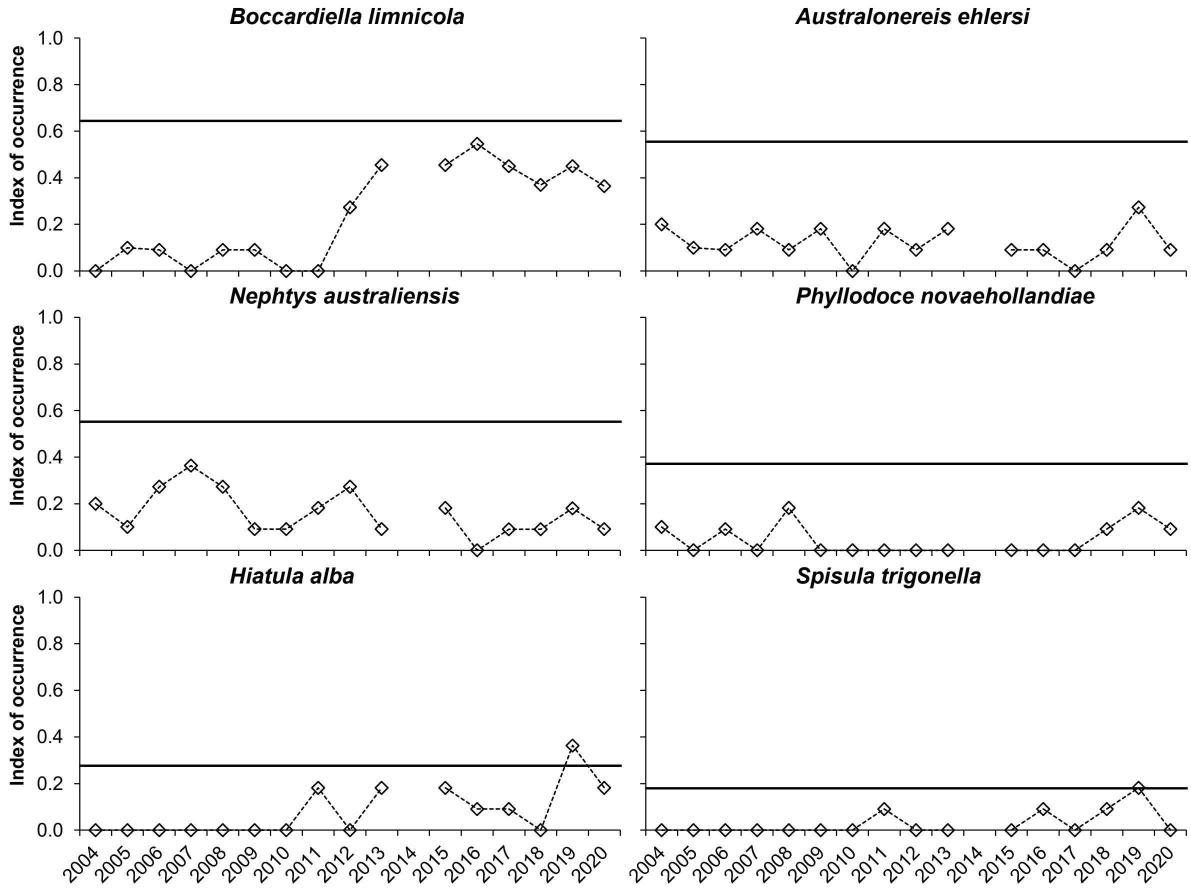


Figure 19 (cont.): Index of Occurrence for several macroinvertebrate species over the TLM monitoring years 2004 to 2020. No TLM sampling occurred in 2014. The Index shows the number of sites at which a species was found, out of all the sites sampled in that survey (e.g. the index is 1 if a species occurred at all 11 sites in a year, and 0.45 if found at 5 out of 11 sites), shown by the symbols and dotted lines. The solid black line indicates a reference for this index, based on the number of sites the species was found over the TLM surveys from 2004-2017 (see SM-Table 6). The plots of the single species are arranged in a sequence from the highest to the lowest reference value for the Index.

4.2.2.2 Macroinvertebrate abundances

The individual densities of macroinvertebrates were significantly higher in the North Coorong (average 96,910 individuals m^{-2} ± 68,914) than in the South Coorong (average 810 individuals m^{-2} ± 989) (Figure 20; SM-Table 8). The total abundance of macroinvertebrates also varied significantly between sites within the North Coorong region. Abundances were highest at sites 1, 2, 3 and 6 (Monument Road, Hunters Creek, Mundoo Channel and Mulbin Yerrok), and lowest within the North Coorong at sites 4, 5 and 7 (Ewe Island, Pelican Point and Noonameena) (Figure 20). In the South Coorong, the low abundances varied little between sites, and were only significantly higher at Loop Road (site 11) compared to Parnka Point (site 8) (pairwise t-test, P=0.03).

For the major taxa, abundances of annelids, crustaceans and molluscs were also significantly different between regions, and at sites within regions (Figure 21; SM-Table 8). These major taxa occurred almost exclusively in the North Coorong, where they accounted for almost all of the abundance of macroinvertebrates (Figures 20, 21). Abundances of Hexapoda were not significantly different between regions, but between sites within regions (Figure 21).

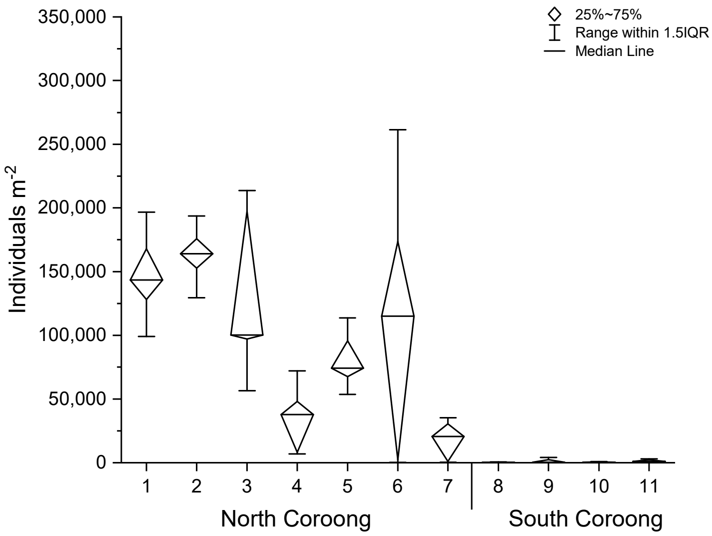


Figure 20: Box plots of individual densities for total macroinvertebrates at the study sites from the North and South Coorong in the survey from December 2020.

For single species, individual densities varied significantly between sites within regions, and in most cases also between regions (SM-Table 9). Amphipoda were the most abundant taxon with significantly higher individual densities in the North than South Coorong (Figure 22a). Within the North Coorong, abundances of amphipods differed significantly between sites, which was also reflected in the site differences of the total macroinvertebrate abundance count which was dominated by these small crustaceans (Figures 20, 21 b, 22a). The individual density of *Arthritica* was high at several sites in the North Coorong, but low at Ewe Island (site 4), and only 2 individuals were found at Noonameena (site 7). This small bivalve dominated the abundance pattern of molluscs (Figures 21, 22b).

Chironomid larvae were the third most abundant taxon and present at all sites, with significantly higher abundances at Monument Road (site 1), Mundoo Channel (site 3) and Pelican Point (site 5) in the North Coorong (Figure 22c). Other taxa of insect larvae occurred in lower individual densities and chironomid larvae accounted for the abundance pattern of Hexapods (Figures 21, 22c).

The polychaete *Simplisetia* was the fourth most abundant macroinvertebrate overall and found in high individual densities in the North Coorong, apart from the Ewe Island mudflat (site 4) and Noonameena (site 7) (Figure 22d). Together with *Capitella*, these two species accounted for the abundance pattern of polychaetes (Figures 21, 22d, e). The pollution indicator species *Capitella* was most abundant again at sites 6 and 7 (Mulbin Yerrok and Noonamena) (Figure 22e), similar to previous years.

Other macroinvertebrates found throughout the North Coorong occurred with highest abundances at single sites, the hydrobiid snails at Pelican Point (site 5, Figure 22f), the sabelllid polychaete *Euchone* at Noonameena (site 7, Figure 22g), and the spionid polychaete *Boccardiella* near the Murray Mouth between sites 1 to 3 (Figure 22h).

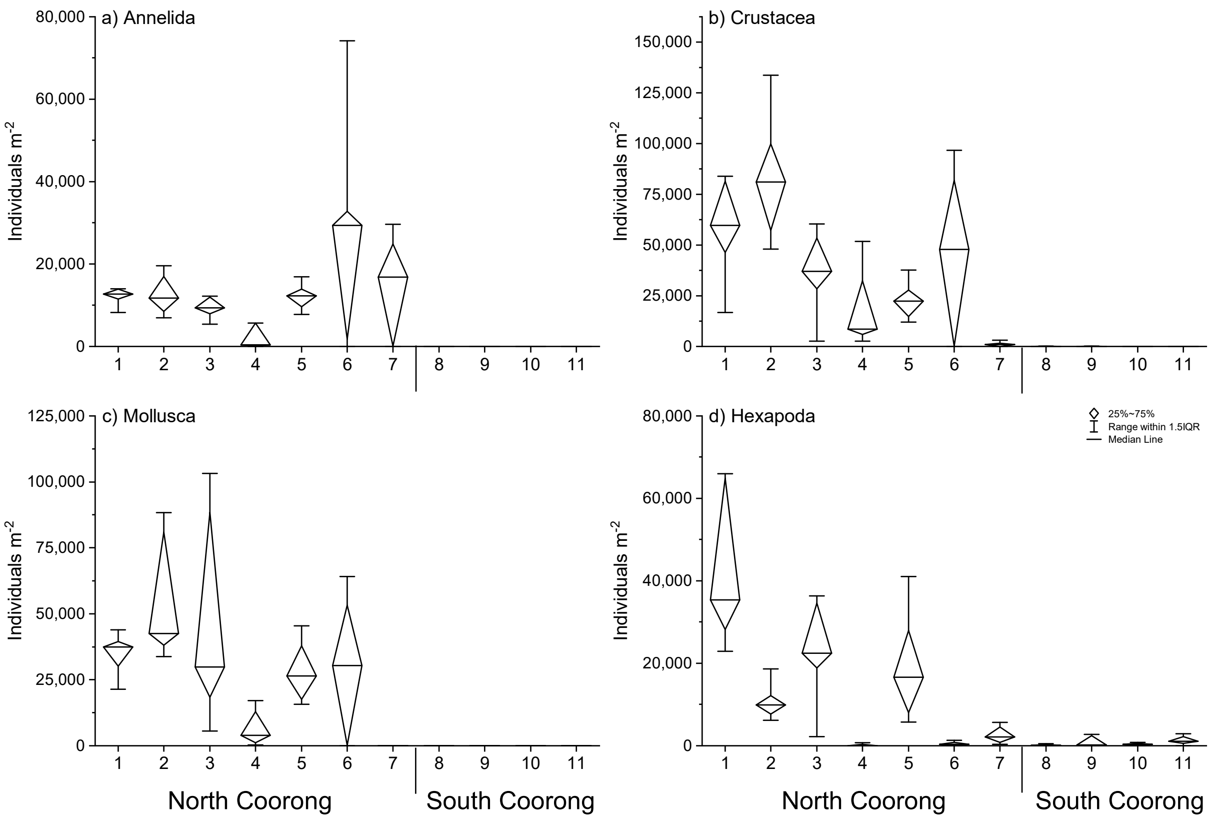


Figure 21: Box plots of individual densities for the four major taxa represented in the macroinvertebrate samples at the study sites in the North and SouthCoorong in December 2020. Note the different y-axes scales.

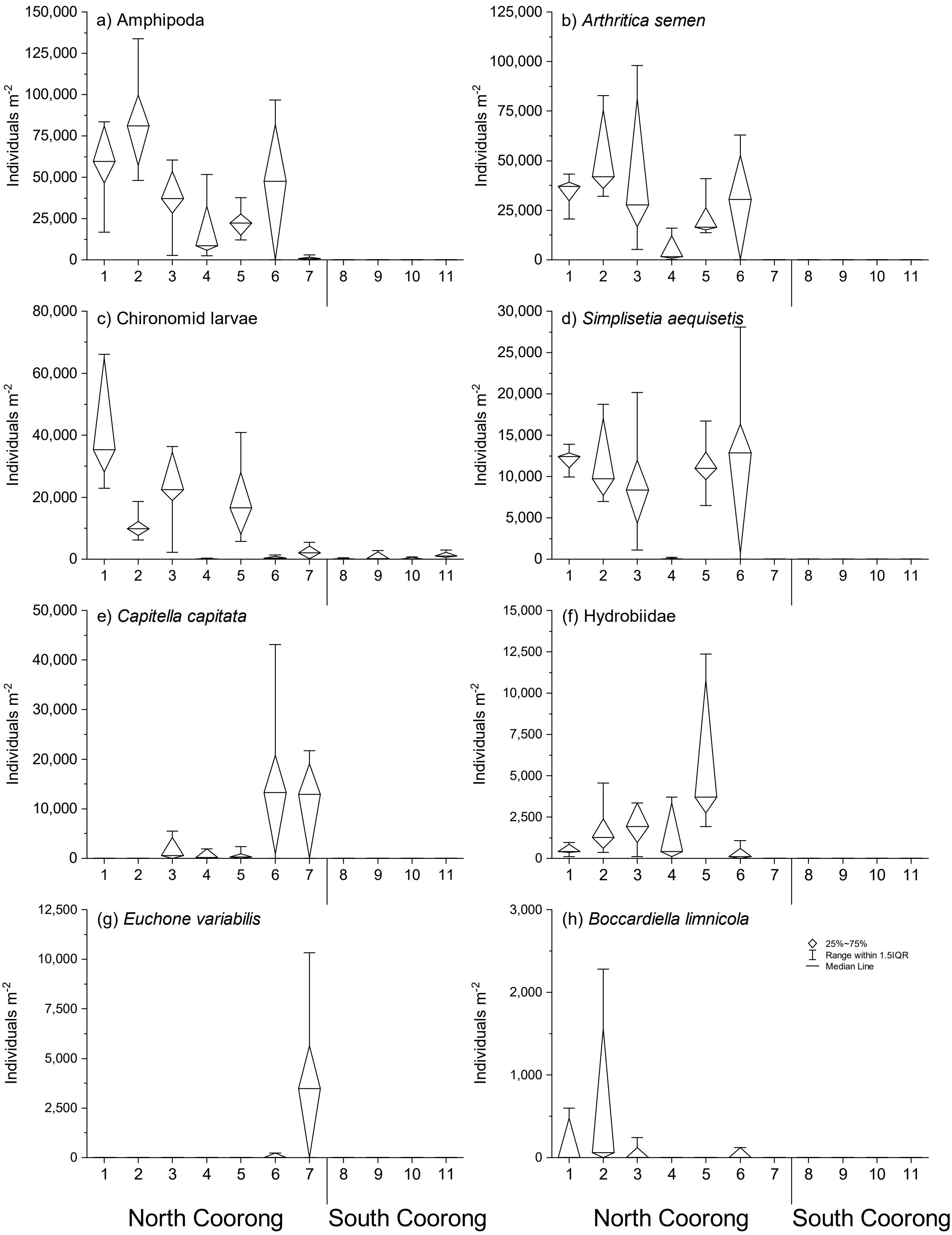


Figure 22: Box plots of individual densities for the most abundant species/taxa in the survey from December 2020 at sites in the North (sites 1 to 7) and South Coorong (sites 8 to 11). The species are listed in a sequence of decreasing rank order of abundance over samples from all sites. Note the different y-axes scales.

4.2.2.3 Long term changes in macroinvertebrate abundances

The total macroinvertebrate abundances from the monitoring in December 2020 were not significantly different to the previous year, while abundances did vary significantly across all monitoring years, regions and sites within regions (Figure 23; SM-Table 8 and further pairwise tests). For the North Coorong, differences across years occurred between the drought years (2004, 2007-2009), the drought breaking flood in 2010 and the years with flow since 2011 (Figure 23a). The recent survey from 2020 had similar total abundance to recent years with continuous flow. In the South Coorong, the recent survey was not significantly different to any earlier monitoring year, apart from 2004 (Figure 23b). The target that '*Macroinvertebrate abundance is maintained at, or increases above, reference levels'* has been met.

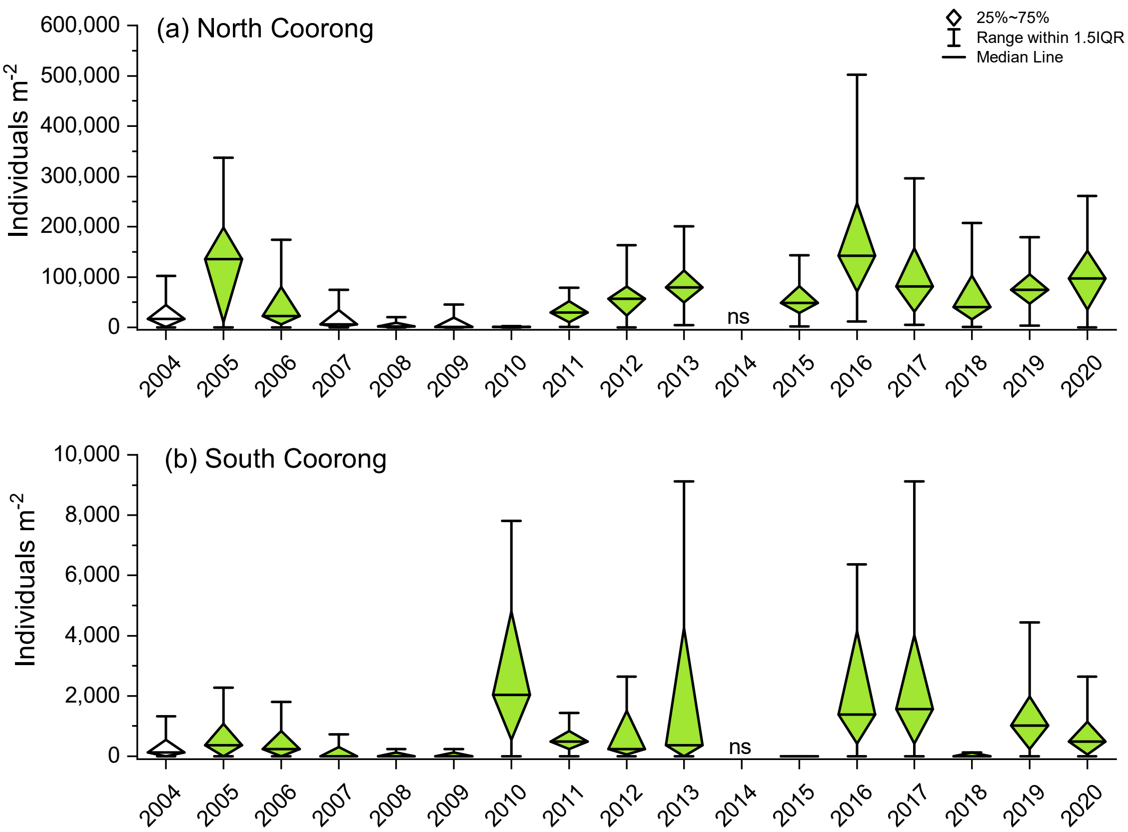


Figure 23: Box plots of individual densities for total macroinvertebrates (all species) for a) the North Coorong (sites 1 – 7) and b) the South Coorong (sites 8-11) for each of the monitoring years. ns = not surveyed, as no TLM monitoring occurred in 2014. Note the different y-axes scales for a) and b). Box plots highlighted in green are not significantly different to 2020 based on pairwise tests.

The long-term changes of total macroinvertebrate abundance in the North Coorong can be attributed to annelids (with high abundance in 2005-2006, decreasing during prolonged drought and increasing in recent years), crustaceans (abundant after higher flow), molluscs (abundances increasing since 2015) and, to a smaller degree, hexapods since flows resumed a decade ago (SM-Figure 6). In the South Coorong, annelids, crustaceans and molluscs contributed episodically in small numbers to the abundances, which are mainly reflecting the pattern of hexapod abundances over the years (SM-Figure 6). Hexapods were not significantly different between regions, but other major taxa were (SM-

Table 8). With the changes in abundance with drought and flow years, abundances differed significantly between years and subject to sites within the regions (SM-Table 8).

For total macroinvertebrate abundances, the Index of Relative Change did not detect any significant deviation from the reference values. For the North Coorong, the 2020 Index value remained above the long-term reference values similar to 2019 (Figure 24). For the South Coorong, the 2020 value was a bit beneath the reference value.

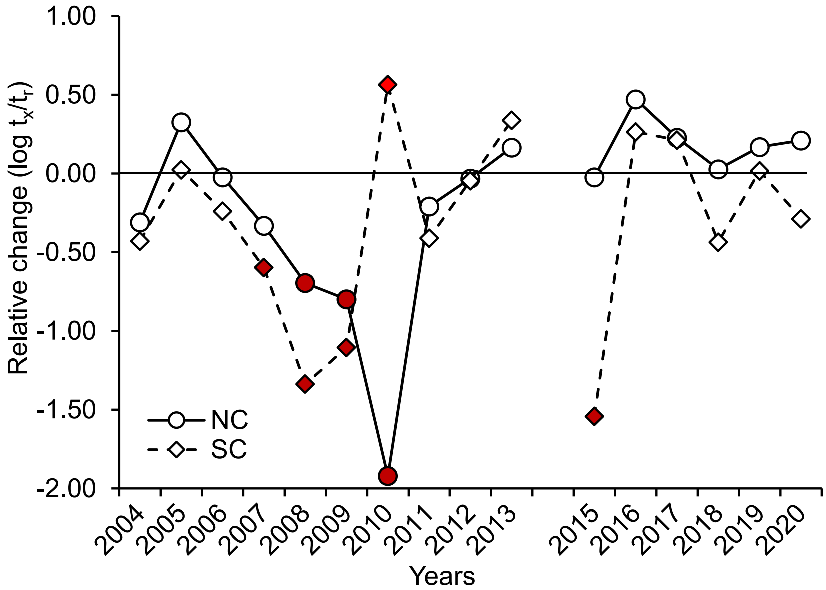


Figure 24: Index of Relative Change showing long-term changes in the abundance of macroinvertebrates (all species) for the North Coorong (NC, 7 sites) and South Coorong (SC, 4 sites). Index values plotted are a ratio of observed (t_x, any particular year) to reference data (t_r based on monitoring from 2004-2017, SM-Table 7), based on average values per region and year. No TLM monitoring occurred in 2014. The black line at 0 marks where observed values equal reference values. The effect sizes (rho) in 2020 were 0.50 for NC and 0.52 for SC (SM-Table 10). Red symbols indicate significant change of values > or < the effect size.

For single taxa, the Index of Relative Change detected several significant deviations from the long-term reference value (Figure 25; SM-Table 11). In the North Coorong, *Arthritica* continued to be significantly more abundant with an index value above the reference. For two other species in the North Coorong, *Nepthys* and oligochaetes, the Index of Relative Change was significantly below the reference. In the South Coorong, the index value was significantly lower than the reference for amphipods and *Capitella*.

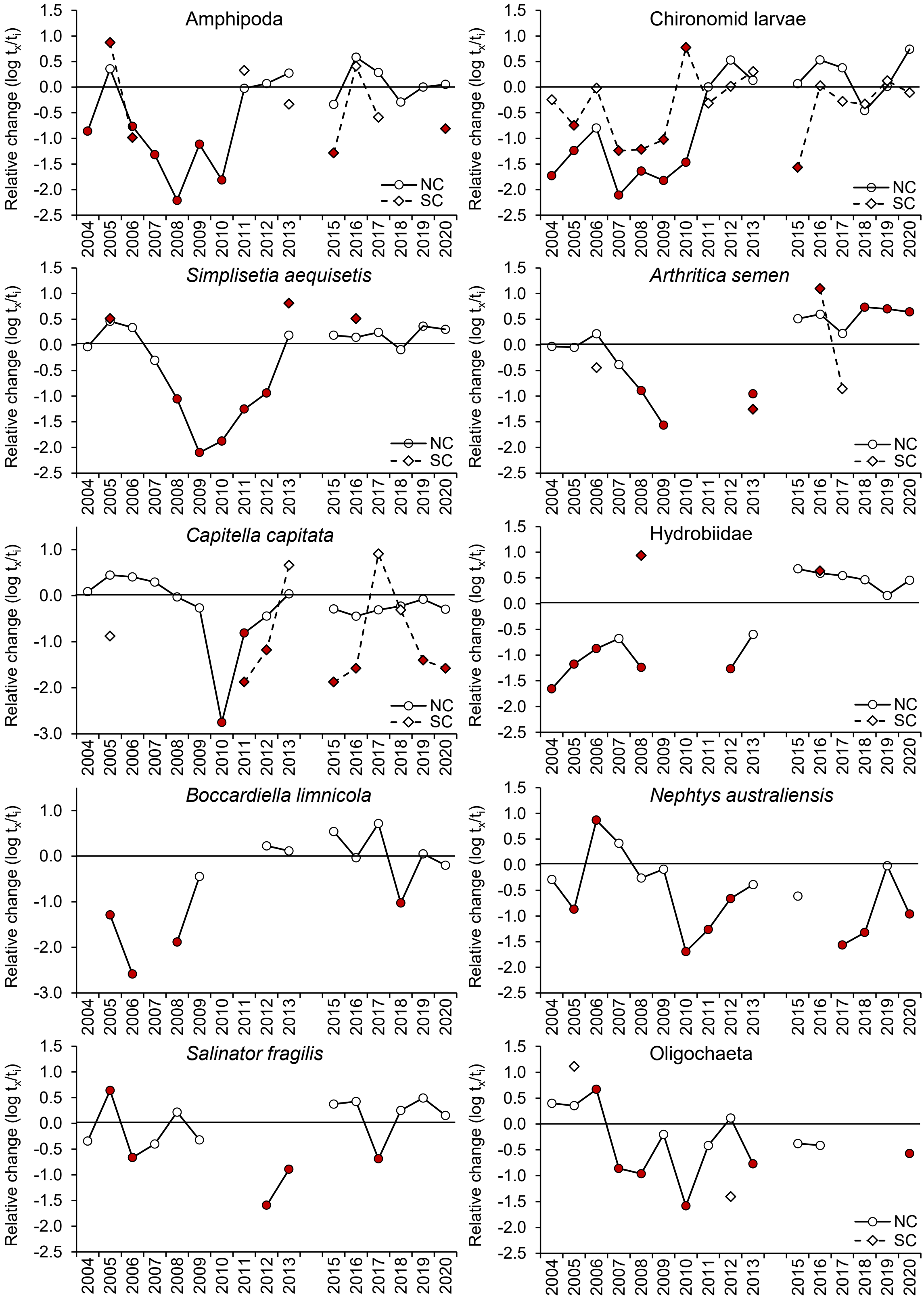


Figure 25: Index of Relative Change for long-term changes in the abundance of several key macroinvertebrate species for the North Coorong (NC, 7 sites) and South Coorong (SC, 4 sites). Index values are a ratio of observed (t_x, any particular year) to reference data (t_r based on monitoring from 2004-2017), for average values per region and year. No TLM monitoring occurred in 2014. *Boccardiella*, *Nepthys* and *Salinator* occurred only in the North Coorong. The black lines at 0 mark where observed values equal reference values. Red symbols indicate significant change of values > or < the effect size (see SM-Table 11 for effect sizes for single taxa in 2020).

As the Ewe Island mudflat had some of the lowest macroinvertebrate abundances in the 2019 and 2020 monitoring (Figure 20), the long-term change in abundance was explored further, and compared to abundances at the nearby mudflat at Pelican Point. Both mudflats are located in front of the barrages (Ewe Island and Tauwitchere, respectively). Macroalgal mats have been accumulating on the Ewe Island mudflat in recent years (SM-Figure 1, and see Dittmann et al. 2020). At both sites, total abundances in 2020 were similar to the 2019 monitoring, but had significantly decreased over recent years at Ewe Island (Figure 26a) compared to increased abundances recorded at Pelican Point (Figure 26b).

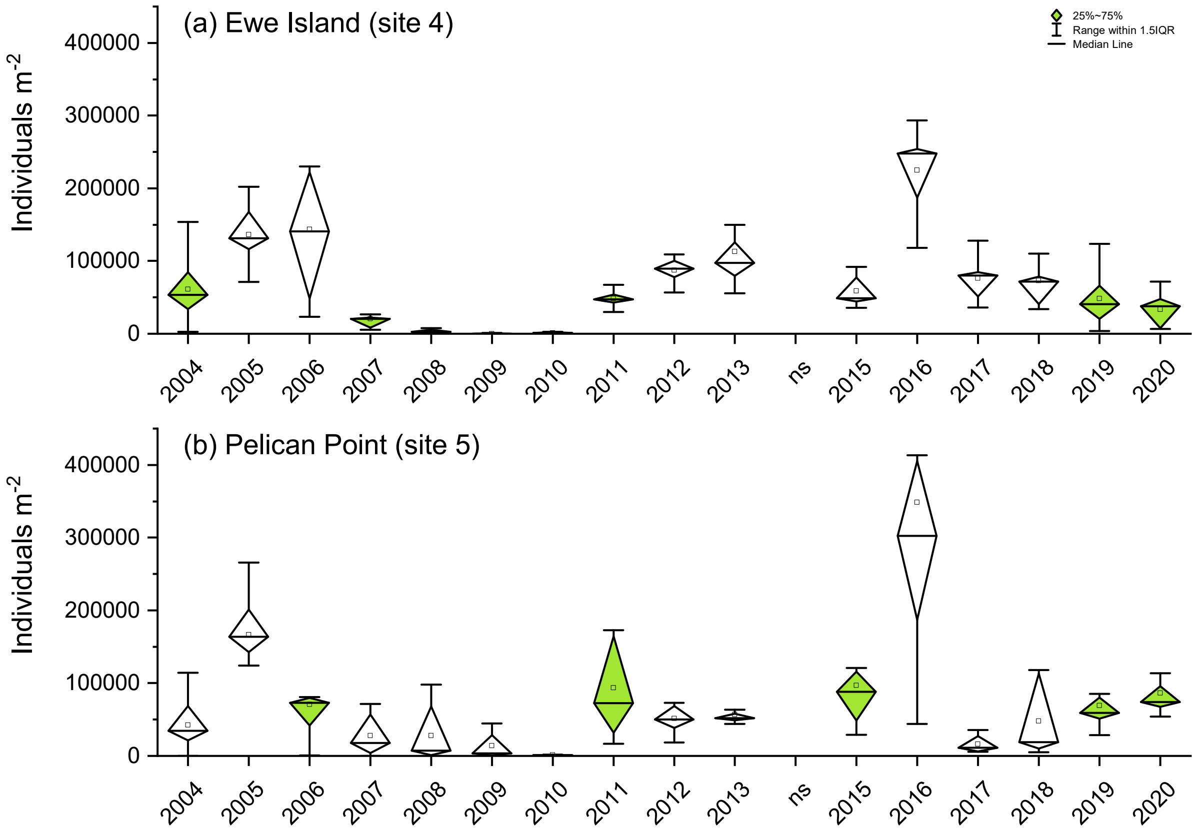


Figure 26: Box plots of individual densities for total macroinvertebrates (all species) for mudflats at a) Ewe Island and b) Pelican Point, for each of the monitoring years. ns = not surveyed, as no TLM monitoring occurred in 2014. Box plots highlighted in green are not significantly different to 2020 based on pairwise tests.

4.2.3 Macroinvertebrate biomass

4.2.3.1 Biomass 2020

The biomass of macroinvertebrates was significantly higher in the North (21.73 ± 17.09 g AFDM m^{-2} average ± SD) than the South Coorong (0.15 ± 0.32 g AFDM m^{-2}) (Figure 27, SM-Table 12). Within the North Coorong, pairwise tests showed that biomass was significantly lower at Ewe Island (site 4) and Noonameena (site 7) than other sites in this region. In the North Coorong, species contributing most to the biomass were the snail *Salinator*, the bivalves *Arthritica* and *Hiatula*, the polychaete

Simplisetia, and amphipods, and differences in the abundance of these species contribute to the differences in biomass across sites. Yet, biomass at Mulbin Yerrok (site 6) was not significantly different to other sites due to the high variability (Figure 27). There was no significant difference in biomass between sites in the South Coorong, where chironomid larvae accounted for most of the macroinvertebrate biomass.

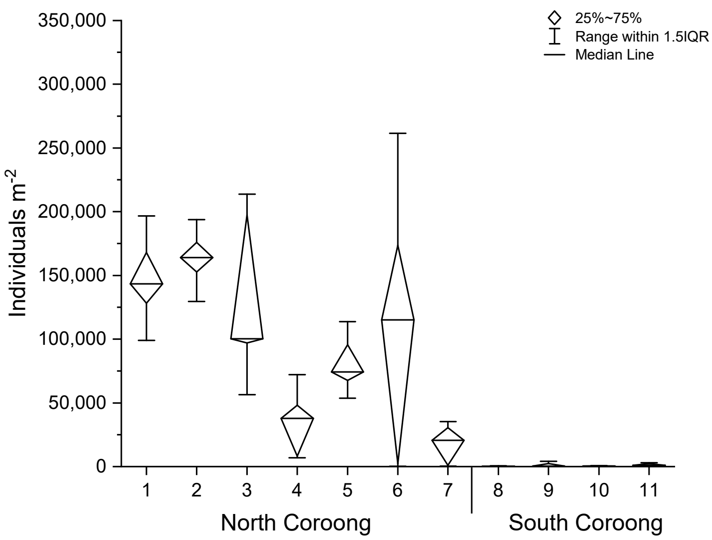


Figure 27: Box plots of total macroinvertebrate biomass at the study sites in the North and South Coorong from the December 2020 monitoring.

4.2.3.2 Long term changes in macroinvertebrate biomass

The increase in biomass recorded in 2019 for the North Coorong did not continue, with a significant decrease in the 2020 monitoring (Figure 28a). Yet, biomass from December 2020 was comparable to other monitoring years since flows returned, and the small flow year 2005. As the total abundance of macroinvertebrates was not significantly different between the 2019 and 2020 monitoring for the North Coorong, the reduced biomass can be attributed to a change in the abundance of species with high biomass, such as the bivalve *Hiatula.* In the South Coorong, biomass was lower than in the previous year, but not significantly different to almost all of the monitoring years, apart from the 2010 and 2017 monitoring years (Figure 28b). Biomass varied significantly across all years, regions and sites within regions over the long-term monitoring (SM-Table 12).

The values for the Index of Relative Change decreased from the monitoring in 2019, but showed no significant deviation from the reference for either the North or South Coorong (Figure 29, SM-Table 13). While the Index value was still above the reference for the North Coorong, it did not continue the increasing trend seen in previous years. For the South Coorong, the Index value deviated further below the reference value. The target that '*Macroinvertebrate biomass is maintained at, or increases above, reference levels'* was thus only partially met.

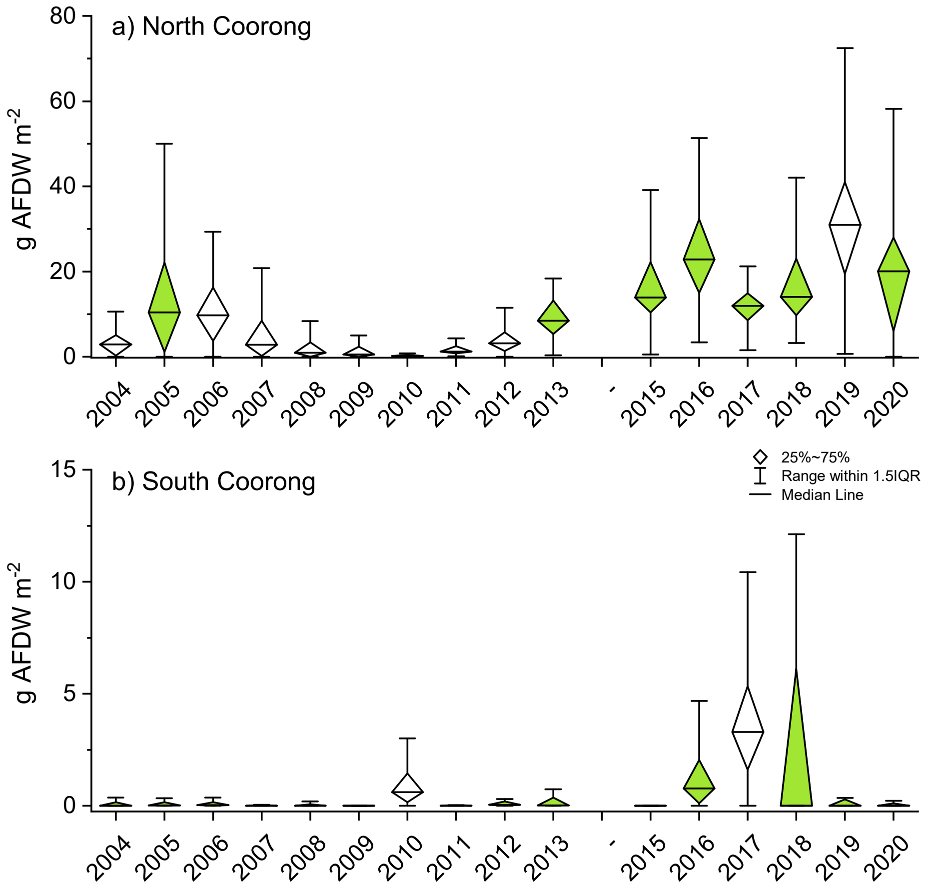


Figure 28: Box plots of macroinvertebrate biomass for the a) North Coorong (sites 1 – 7) and b) the South Coorong (sites 8-11) for each of the monitoring years. No TLM monitoring occurred in 2014, indicated by a /. Note the different y-axes scales for a) and b). Box plots highlighted in green are not significantly different to 2020 based on pairwise tests.

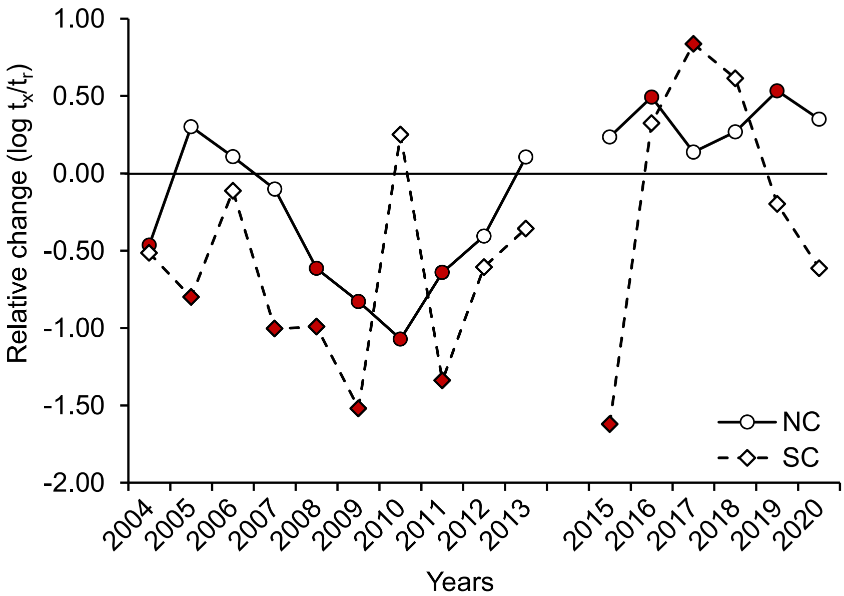


Figure 29: Index of Relative Change showing long-term changes in the biomass of macroinvertebrates for the North Coorong (NC, 7 sites) and South Coorong (SC, 4 sites). Index values plotted are a ratio of observed (t_x, any particular year) to reference data (t_r based on monitoring from 2004-2017, SM-Table7), based on average values per region and year. No TLM monitoring occurred in 2014. The black line at 0 marks where observed values equal reference values. The effect sizes (rho) in 2020 were 0.43 for NC and 0.64 for SC (SM-Table 13). Red symbols indicate significant change of values > or < the effect size.

4.2.4 Macroinvertebrate communities

4.2.4.1 Community compositions

Community compositions in 2020

The macroinvertebrate community was significantly different between the North and South Coorong, and between sites within the North Coorong (Figure 30a, SM-Table 14). The mudflats at sites 1, 2, 3 and 5 (Monument Road, Hunters Creek, Mundoo Channel and Pelican Point) were inhabited by a very similar macroinvertebrate community. The macroinvertebrate community at site 4 (Ewe Island) in the Murray Mouth was more similar to the one at site 6 (Mulbin Yerrok) in the North Lagoon (Figure 30). The southernmost site in the North Coorong, Noonamena (site 7) had a distinct macroinvertebrate community more similar to the South Coorong. The community in the South Coorong was not well defined, given the more ephemeral nature of insect larvae who were the main macroinvertebrates present here. This pattern of macroinvertebrate communities across the North and South Coorong resembled the pattern seen in the previous 2019 monitoring.

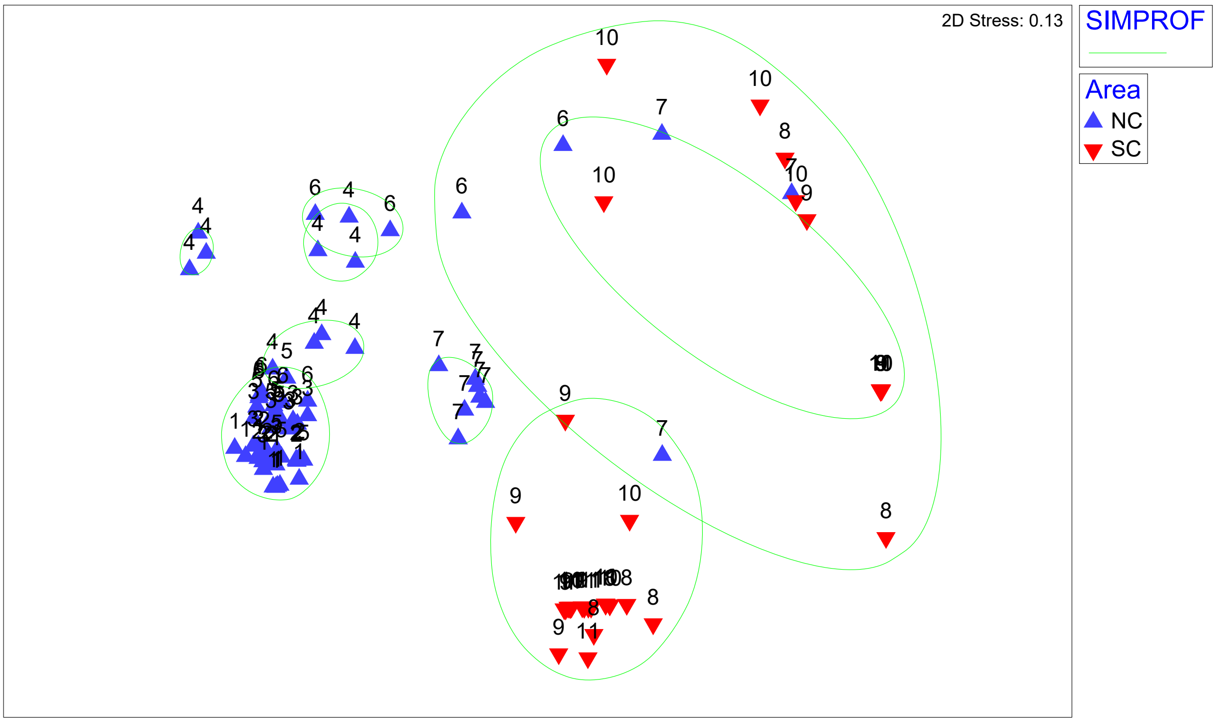
The macroinvertebrate community at sites 1, 2, 3 and 5 near the Murray Mouth were characterised by high abundances of several macroinvertebrate species: amphipods, *Arthritica*, *Simplisetia* and *Boccardiella*, as well as chironomid larvae (Figure 30b, SM-Figure 7). At sites 4 and 6, the macroinvertebrate communities were not well defined, and differentiated from the community at the other sites in the Murray Mouth by relatively high diversity but lower abundance, and several species not recorded at other sites (SM-Figure 7). Both sites had accumulations of filamentous algae. The pollution indicator species *Capitella* was a main part of the communities here, and became dominant in the community at Noonameena (site 7) (Figure 30b). The macroinvertebrate community at the South Coorong sites was characterised by chironomid larvae, and other macroinvertebrates found made little contribution to the community with the low densities of organisms recorded at these sites (Figure 30b, SM-Figure 7).

Long term changes in macroinvertebrate communities

Macroinvertebrate communities were significantly different across the monitoring years, regions, and sites within regions (SM-Table 14). Patterns of change were more pronounced in the North Coorong than in the South Coorong (Figure 31).

In the North Coorong, long-term changes differentiated macroinvertebrate communities before the drought, after several years of drought (2009), the 2010 flood event, early recolonisation (2011-2012) and the recent years of continuous flow since 2013 (Figure 31a). While the macroinvertebrate community in the North Coorong stabilised in recent years, it remained significantly different to the community present before the drought. A trajectory indicated that the community in 2020 was more similar to the one observed in 2015 and 2016 (Figure 31a). The community occurring in mudflats of the North Coorong since 2013 to 2020 had higher abundances of several typical benthic macroinvertebrates than the community found from 2004 to 2008 (SM-Figure 8a). This shift in community composition could be attributed to an increase of *Boccardiella*, chironomids, hydrobiid snails, amphipods, mysid shrimp, *Hiatula* and *Arthritica*, whereas oligochaetes, *Nepthys* and *Australonereis* had decreased (SM-Figure 8a).

a)



b)

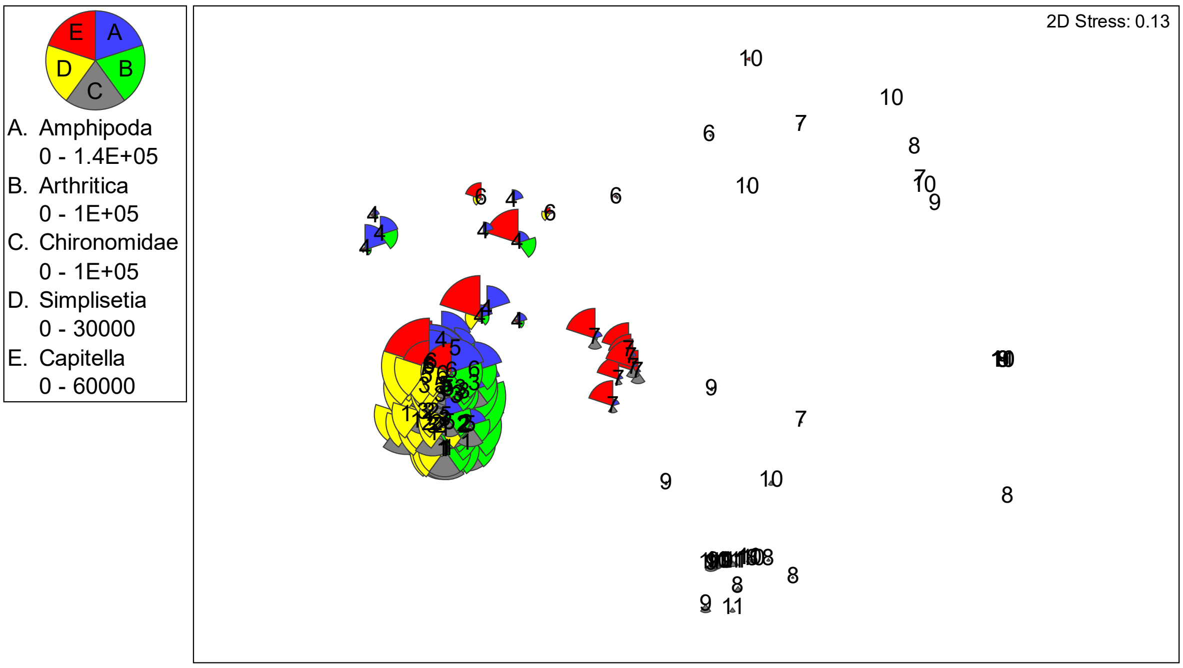


Figure 30: Macroinvertebrate community analysis using non-metric multidimensional scaling (nMDS) a) nMDS plot based on all replicate samples for the eleven TLM monitoring sites in 2020. The green circles show significantly different groupings based on SIMPROF tests. Regions are differentiated in a) as NC = North Coorong and SC = South Coorong. b) the same nMDS plot but with bubble overlay for the five most abundant macroinvertebrate species differentiating the communities. The scale of the bubble sectors is reflecting their abundance.

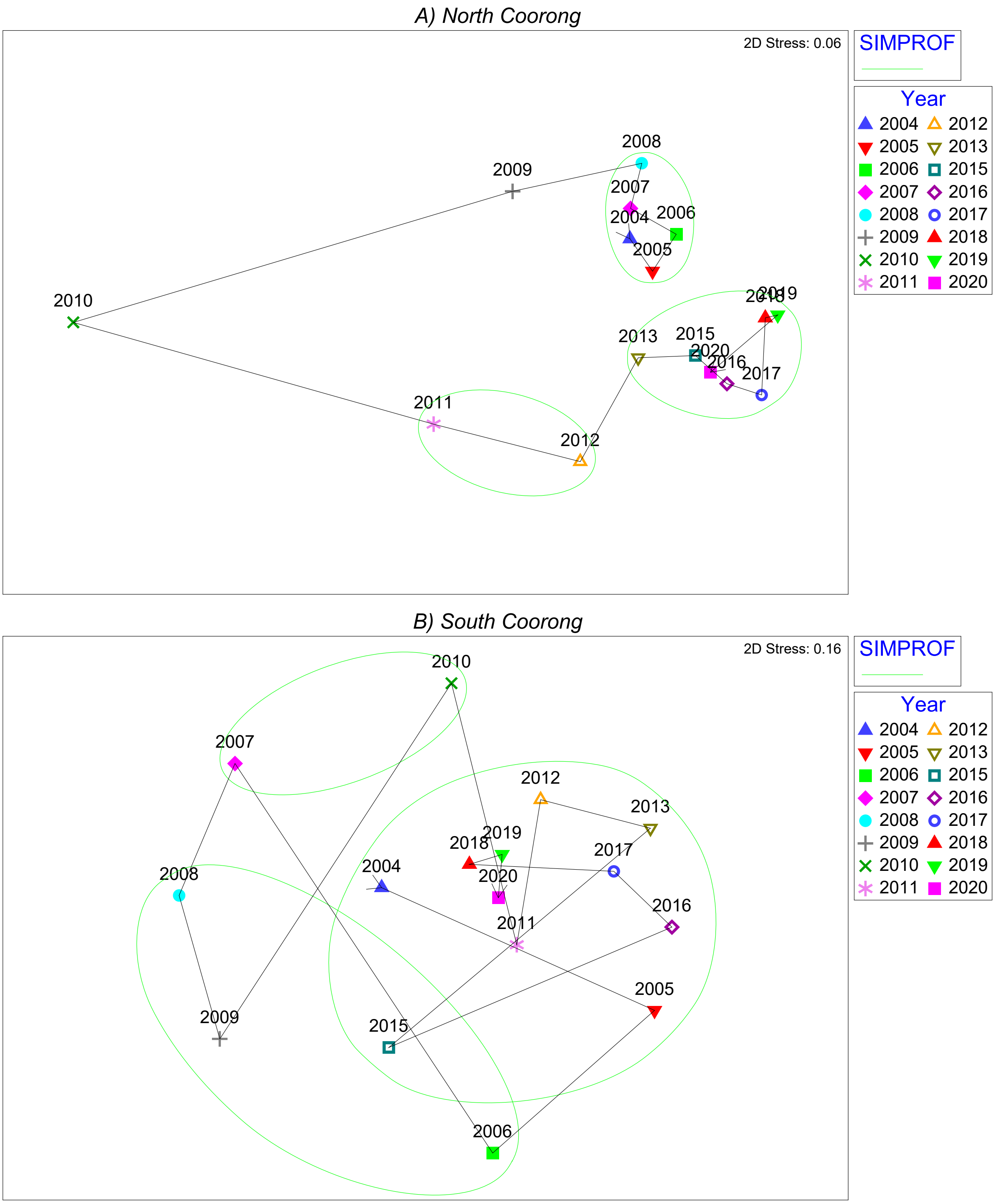


Figure 31: Trajectories of changes in the macroinvertebrate communities based on non-metric multidimensional scaling (nMDS) on average values per region and monitoring year (no TLM monitoring in 2014). A) Macroinvertebrate communities in the North Coorong. B) Macroinvertebrate communities in the South Coorong. The green circles show significantly different groupings based on SIMPROF tests. The direction of the connecting black lines shows a trajectory over consecutive monitoring years.

In the South Coorong, the trajectory did not indicate any clear direction of change in relation to years with distinct environmental flows, as the macroinvertebrate communities in years with small (2006) and large flows (2010) were similar to communities in drought years (2007, 2008 and 2009) (Figure 31b). The macroinvertebrate community found in the South Coorong in all other years fell into one cluster and within this cluster, 2020 was most similar to 2018 and 2019 (Figure 31b). Chironomid larvae characterised the community in all clusters, with Ostracods, *Parartemia* and *Capitella* contributing to the communities in some years and the differentiation between the cluster groups (SM-Figure 8b).

As a strong salinity gradient characterises the system (Figure 4), variability in macroinvertebrate communities within each region can be a further measure of change over the monitoring years, subject to environmental flows. Using the Bray-Curtis Index as a similarity measure for macroinvertebrate communities, a more homogenous community emerges for the North Coorong, and greater distinction between sites for the South Coorong (Figure 32). Within the seven sites of the North Coorong region, the community present had relatively similar species composition and abundances in the December 2020 monitoring. Such greater similarity was also found in previous years since flows resumed, whereas a greater distinction of communities occurred during the drought (e.g. 2007-2009) (Figure 32). In the South Coorong, the deviation from the reference indicated a significant change in December 2020 (Figure 32, SM-Table 15). Variability in the macroinvertebrate community between the four sites in the South Coorong has occurred in several previous monitoring years (Figure 32).

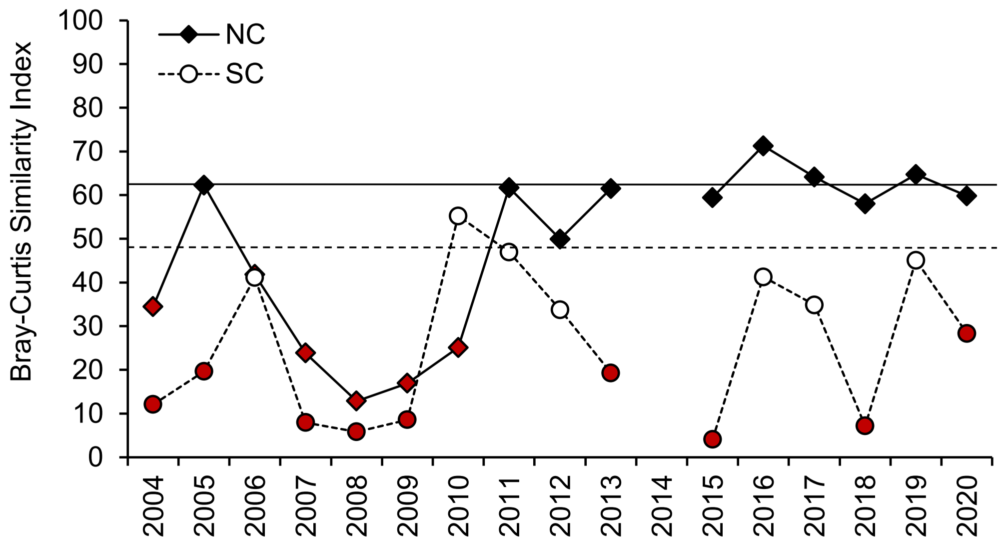


Figure 32: Average Bray-Curtis similarity index for similarity of macroinvertebrate communities within the North (NC) and South (SC) Coorong respectively for each of the monitoring years. No TLM monitoring occurred in 2014. The horizontal lines indicate reference similarities for each region, taking selected years as reference (2005 and 2013 for the NC, 2006 and 2010 for SC). A higher index shows greater similarity between the sites in each region for the respective year. Red colour indicates significant change (see SM-Table 15 for effect size).

The relatedness of macroinvertebrate communities across years was measured with the Spearman rank correlation coefficient (rho). The relatedness of the macroinvertebrate community in 2020 was highest with the previous two years (2018 and 2019), and also high in comparisons with every other year since 2013, and with 2006 (Figure 33). The lowest relatedness for 2020 was with the monitoring years during the drought (2007-2009) and the flood year 2010. The target that '*Macroinvertebrate communities are similar to those occurring under intermediate continuous flows'* was met.

The pattern of relatedness was driven by the macroinvertebrate community in the North Coorong, with highest relatedness of 2020 to 2013, 2019, and other years since flows resumed. For the South Coorong, relatedness of macroinvertebrate communities across years was lower than respective annual comparisons for communities in the North Coorong. The 2020 community from the South Coorong was more similar to the previous two years, but not significantly different to all other years (Figure 33, SM-Table 16).

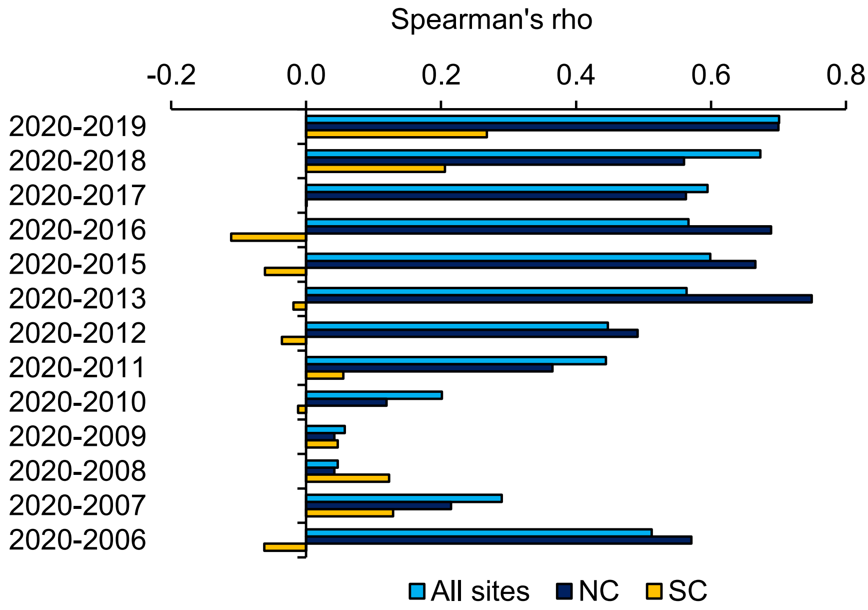


Figure 33: Spearman's rank correlation coefficient rho (δ) comparing the similarity matrices of macroinvertebrate communities from 2020 to previous years. Comparisons are not made to 2004 and 2005 as one sampling site was missing in each of these years. The coefficient δ was calculated using data from all 11 sites (light blue), the North Coorong (NC), and the South Coorong (SC). Low values for δ indicate that communities in the two years are unrelated.

4.2.4.2 Environmental conditions as predictor variables for macroinvertebrate communities

Environmental conditions as predictor for communities in 2020

The macroinvertebrate community differentiation between the North and South Coorong could be attributed almost exclusively to salinity, which explained over 63% of the total variation (Figure 34, SM-Table 17). The other environmental variables included in the analyses (DO saturation, sediment organic matter, chlorophyll-a, grain size and sorting) each explained approximately 15 to 23% of the variation in macroinvertebrate communities, whereby sediment organic matter made the only slightly significant contribution (SM-Table 17). Coarser sediment was found at sites 6 (Mulbin Yerrok), 7 (Noonameena) and 10 (Jack Point) and accounted for the differentiation along the second axis (Figure 34, dbRDA2). Salinity has been the main environmental predictor variable in previous monitoring years as well.

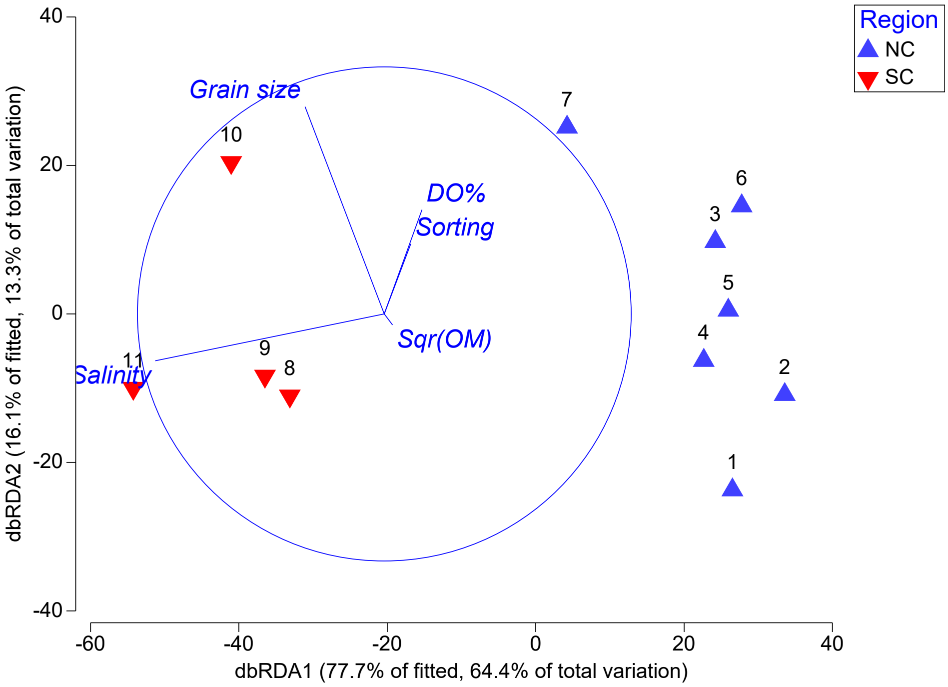


Figure 34: Plot from distance-based redundancy analysis (dbRDA) for the survey in 2020. The plot shows a constrained ordination of macroinvertebrate communities subject to the environmental predictor variables displayed in the vector overlay.

Environmental conditions as predictor for long term changes in communities

Based on the long-term monitoring, the macroinvertebrate communities in the North and South Coorong could be mainly explained by salinity (Figure 35). Several environmental variables contributed significantly to the variation in macroinvertebrate communities in the two regions over time. Salinity explained about 24% of the total variation, while other environmental variables (organic matter, dissolved oxygen and grain size) explained only 2 to 6% of the variation (SM-Table 18).

The pattern emerging from the long-term analysis of macroinvertebrate communities and environmental conditions up to December 2020 was very similar to the pattern derived from analyses in the previous monitoring year. The LINKTREE analyses corroborated the main environmental drivers (Figure 36). Salinity split the macroinvertebrate communities between the two regions, with communities in the North Coorong occurring at salinities <50 ppt, and the communities in the South Coorong when salinity exceeded 64.3 ppt. This main salinity threshold has been persistent over all monitoring years. Within the North Coorong, the difference in macroinvertebrate communities between years with continuous flow (2012-2020) and drought (2006-2009) was explained by a salinity threshold of <25.5 ppt and >33.7 ppt respectively (Figure 36, split D). Low dissolved oxygen during the flood in 2010 explained the distinct community present that year, and sediment organic matter for the community in 2011. Within the South Coorong, salinities >120 ppt separated the community from the drought years to all other years (Figure 36, split L).

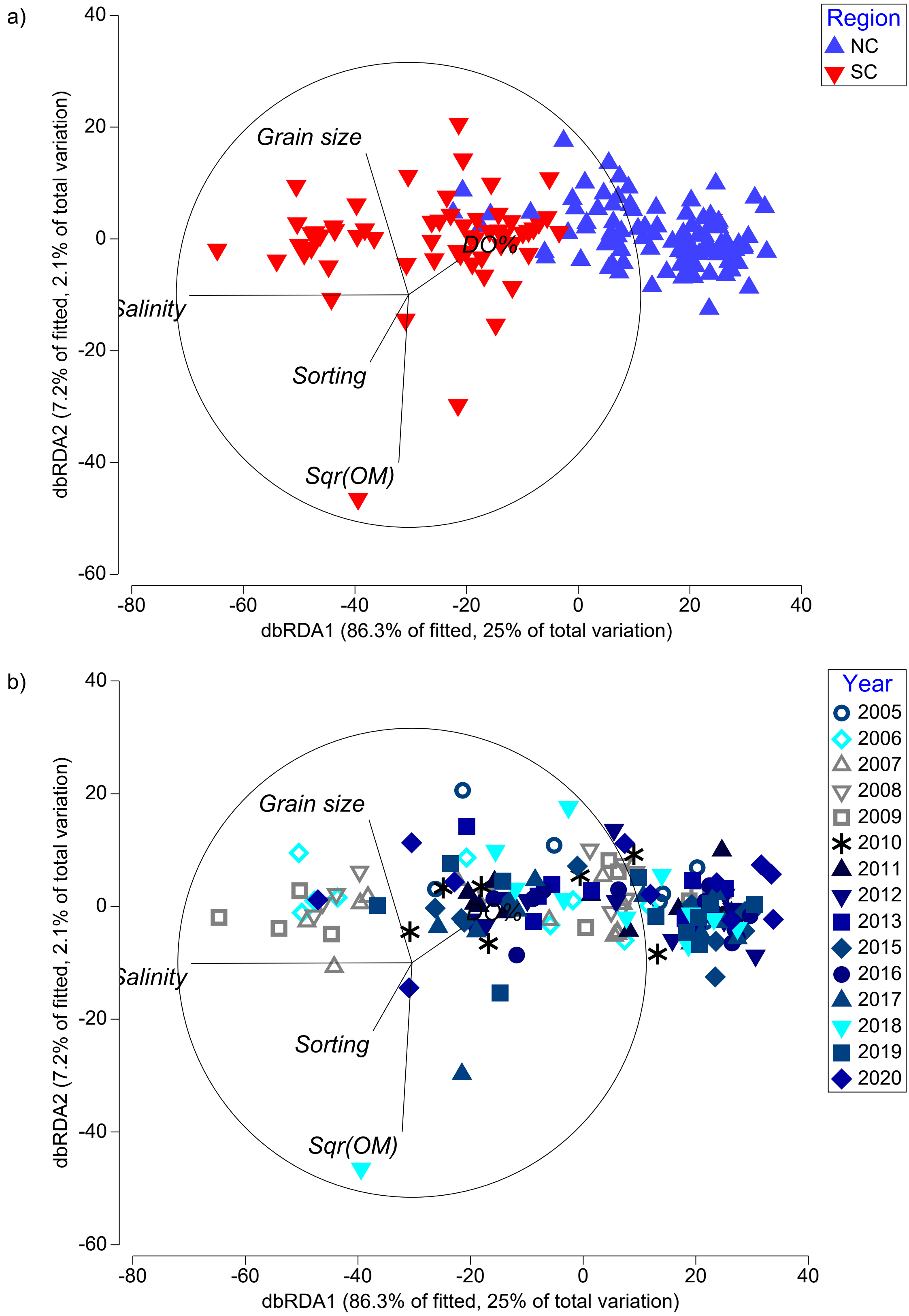


Figure 35: Plots from distance-based redundancy analysis (dbRDA) for all of the monitoring years since 2005 (with consistent grain size analysis using laser diffraction). Environmental variables recorded in each monitoring were included here (except for chlorophyll-a which was only measured since 2007). The same plot is shown with a) regional differentiation into North (NC) and South (SC) Coorong, and b) year differentiation. The plots show a constrained ordination of macroinvertebrate communities subject to the environmental predictor variables displayed in the vector overlays.

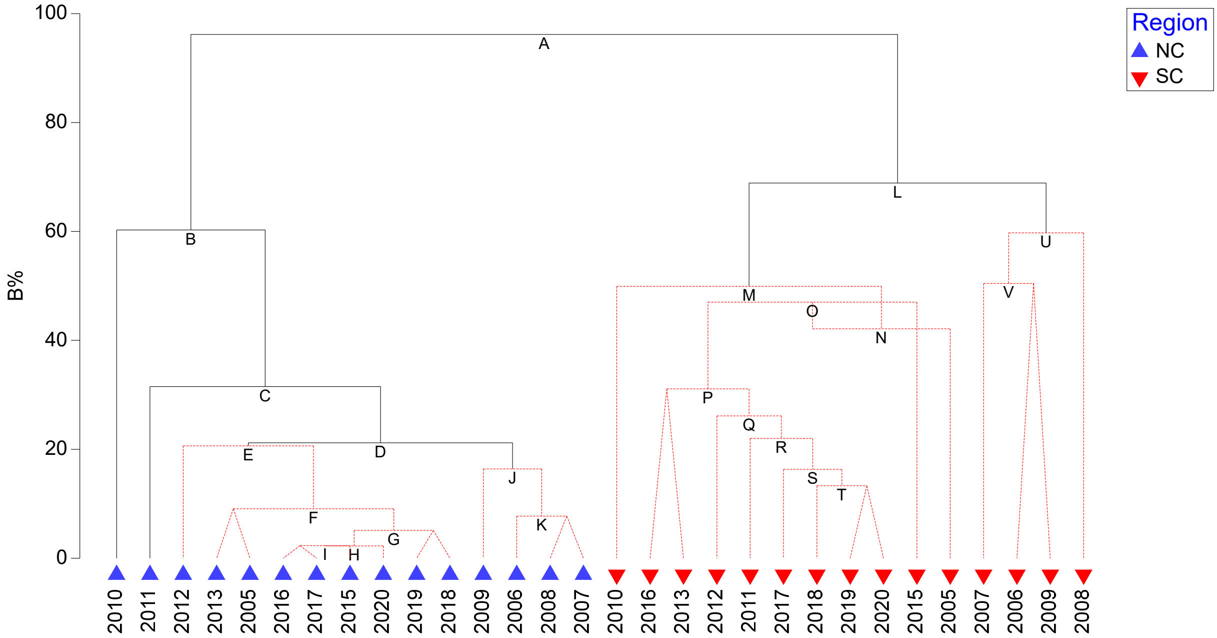


Figure 36: LINKTREE of macroinvertebrate community data from monitoring since 2005 (as different method for grain size analysis in 2004), based on average values per region (North Coorong (NC) and South Coorong (SC)) and year. Black lines are separating significantly different groups based on SIMPROF tests. The splits are explained by environmental variables, with significant splits at; A: Salinity<49.9(>64.3) *P*=0.001, B: DO%<80.4(>91) *P*=0.001, C: Sqr(OM)<0.971(>1.01) *P*=0.004, D: Salinity<25.5(>33.7) *P*=0.007, L: Salinity<105(>120) *P*=0.004. Red lines indicate groups that are not significantly different. The B% scale splits groups using original rank dissimilarities.

5. Discussion

Results from the 2020 macroinvertebrate and mudflat monitoring are assessed below against the ecological objectives and targets from the TLM LLCMM Icon Site *Condition Monitoring Plan (Revised)* (DEWNR 2017).

5.1 Macroinvertebrate populations

The condition of mudflat invertebrate communities has been maintained.

Macroinvertebrate species richness increases throughout the Murray Mouth and Coorong

The pattern of species richness, being high in the North Coorong and decreasing into the South Coorong, remained unchanged. Compared to previous years, species richness in the North Coorong increased above the reference range in 2020, reflecting a diversification with continuing flows. The beneficial effects of environmental flows for species diversity seen in the North Coorong have not reached the South Coorong, where salinity remains above the threshold for macroinvertebrate species. Yet in 2020, species richness was slightly above the reference value for the South Coorong, similar to 2019, and diversity indices were also similar to 2019 and remained at a low level. This target has thus been partially met.

Macroinvertebrate occurrence extends along the Coorong into the South Lagoon

The macroinvertebrates occurring in the South Coorong remain predominantly chironomid larvae, with larvae of further insect families (esp. Dolichopodidae) found as well. Salt tolerant ostracods were recorded at Parnka Point and Villa de Yumpa. The only other macroinvertebrates found in samples from the South Coorong were two individuals each of the polychaete *Capitella* and amphipod crustaceans. These taxa occur with hundreds of individuals in samples from the North Coorong. Macroinvertebrate occurrence has thus not extended into the South Lagoon, as the high salinity is inhibiting their colonisation. This target has thus not been met.

The area of occupation for typical estuarine and marine macroinvertebrate species exceeds 60% of the sites sampled

The area of occupation exceeded 60% of the sites sampled for chironomid larvae, amphipods, *Arthritica* and dolichopodid larvae. Further key macroinvertebrate species (e.g., the polychaetes *Simplisetia* and *Capitella*, and hydrobiid snails) were present at >50% of the sites sampled. The Index of Occurrence increased for mysid shrimp and oligochaetes in 2020. However, the area of occupation was slightly contracted for many macroinvertebrate species compared to 2019. Several macroinvertebrate species, which were found with an increased area of occupation in the 2019 monitoring, had a rare occurrence again in 2020. The occurrence of macroinvertebrates species in estuaries can not only be determined by flow related variability in salinity, but also suspended particulate matter loads and eutrophication (Blanchet et al. 2014; Coelho et al. 2015). The target was thus partially met.

Macroinvertebrate abundance is maintained at, or increases above, reference levels

This target has been met, as abundances were maintained at reference levels. The Index of Relative Change did not indicate any significant deviation from the reference for total abundances in the North and South Coorong. The total macroinvertebrate abundance for the North Coorong was similar to years with continuous flows, as polychaete, amphipod and mollusc abundances have been higher since flows resumed. Species-specific patterns of abundance trends emerged, with *Arthritica* significantly above, and *Nephthys* and oligochaetes below the reference, while abundances of other species remained unchanged. For the South Coorong, total abundances were at the reference level, due to chironomid larvae, and abundances of amphipods and *Capitella* were significantly below the reference.

Macroinvertebrate biomass is maintained at, or increases above, reference levels

Biomass was lower than in the previous monitoring year, but the Index of Relative Change did not detect a significant decrease or deviation from the reference. A reduction in biomass compared to the previous year could arise from fewer large bivalves in the samples in 2020. Biomass of macroinvertebrates in the North Coorong was still comparable to other years with flow, whereas biomass in the South Coorong was one of the lowest values observed across the long-term sampling. This target has been partially met.

Macroinvertebrate communities are similar to those occurring under intermediate continuous flows

The macroinvertebrate communities in the North and South Coorong in December 2020 were very similar to those in 2019 (Dittmann et al. 2020), and other years with continuous flow. This target has thus been met.

The community in the North Coorong has stabilised since 2013 and shifted into a different state, characterised by higher diversity, abundance and biomass than the community found before the prolonged drought in the previous decade. Long-term community shifts in estuarine benthic communities in other parts of the world have been attributed to pollution, eutrophication and macroalgal mats, as well as restoration (Derolez et al. 2020; Taupp & Wetzel 2019; Elliott et al. 2016; Viaroli et al. 2008). Salinity and sediment organic matter have been found to be most influential for estuarine macrobenthic communities after changes in water management of catchment basins (Vinagre et al. 2015). Salinity is the main driver for the macroinvertebrate community pattern in the Coorong and Murray Mouth as well, and the community present in the North Coorong in years with continuous flow was differentiated by salinities <25.5 ppt (Figure 36). Maintaining estuarine conditions in the North Coorong could maintain the current community. However, the effects of high nutrient load and algal mats discussed below can deteriorate mudflat habitat conditions.

5.2 Mudflat habitat conditions

The 2020 monitoring occurred after winter and spring flows, which reduced salinities in the North Coorong. Based on water quality meters in the Coorong (data supplied by MDBA) for the months preceding the survey (July – early December 2020), salinities were on average 33 ppt in the North Coorong and 80 ppt in the South Coorong. Salinities recorded at the time of our monitoring reflected this pattern, with brackish conditions recorded at sites in the Murray Mouth, while salinities at Villa de Yumpa and Loop Road in the South Coorong exceeded 100 ppt. Throughout the North Coorong, salinities were lower than the threshold (64 ppt) for macroinvertebrates based on long-term records (Dittmann et al. 2015) (Figure 36).

All four of the targets for mudflat habitat conditions were partially met during the monitoring in spring/summer 2020-21.

Habitable sediments are occurring along the Coorong into the South Lagoon

This target was partially met. In the South Coorong, habitability of sediments was affected by a combination of hypersalinity and high organic matter loads. The salinities recorded exceeded the threshold of most macroinvertebrates, apart from a few salt-tolerant species. High organic matter load in sediments is often accompanied by anoxic sediments and reduced benthic habitat quality (Sutula et al. 2014).

While salinities and sediment conditions were habitable for macroinvertebrates at many sites in the North Coorong, macroalgal mats had a noticeable impact on the sedimentary environment and benthic communities. Extensive filamentous algae with anoxic sediment beneath the mats were noted during the December 2020 survey at several sites in the North Coorong. An increase in filamentous algae in the Murray Mouth area has been already apparent on remote sensing maps of the Coorong from 2017 and 2018 (Auricht et al. 2019). Filamentous algae have become an increasing environmental issue on mudflats in the North Coorong, and their growing extent was noted in 2019 at the Ewe Island mudflat (site 4) (Dittmann et al. 2020). This site has one of the largest mudflats in the estuary of the Murray Mouth region, but the continued accumulation of macroalgal mats affected macroinvertebrate abundances and biomass, which have now decreased over several consecutive years, a trend not seen at another nearby mudflat (Pelican Point) which has little filamentous algal cover.

Filamentous algae mats are often a consequence of eutrophication and can themselves further impact on biogeochemical processes and benthic fauna in sediments (Corzo et al 2009; Le Moal et al. 2019). The contribution of filamentous algae to the high chlorophyll-a concentrations and high dissolved oxygen saturation recorded in sediment and water near mudflats with algal mats remains unknown. The impacts of algal mats on microphytobenthos are not easily generalised (Besterman & Pace 2018) and require further targeted investigations. Filamentous algae have wide effects on the food web, as seen in the reduced macroinvertebrate abundances at the Ewe Island mudflat. Algal mats are not only known to impact benthic macroinvertebrates, but also flatfish and shorebirds (Engelsen et al. 2010; Green et al. 2014; Quillien et al. 2018; Harris et al. 2020; Thornton et al. 2020). Unregulated flow events could help to remove algal mats from the mudflats and reduce nutrient loads in the system to lower the risk of their reestablishment (De Wit et al. 2020; Derolez et al. 2020).

Sediments are maintained as fine to medium sands and are mostly moderately well sorted

This target was partially met. While sediments were mostly fine to medium sands, they were not ‘moderately well’ sorted. Sediment composition was more heterogenous and moderately or poorly sorted. Sites in the southern North Lagoon (Mulbin Yerrok and Noonameena) and Jack Point also contained coarser sediment. The Index of Relative Change showed that both median grain size and sorting deviated significantly from the long-term reference.

Causes for changes in sediment grain size composition could be an influx of fine particles with riverine flow, trapping of finer mud particles by floating algal mats, and aeolian deposition of sand blown across from the nearby dunes. Slight variation between years can also arise from sampling distance from shore as accessibility is subject to the water level, especially at sites like Mulbin Yerrok with a steeper shore profile and rocky outcrops.

Sediment organic matter is maintained

This target was partially met, as sediments in the South Lagoon had higher organic matter content than in previous years. At Villa de Yumpa (site 9), the organic matter content has been high in several monitoring years, but exceeded the range of long-term values in December 2020. The Index of Relative Change revealed a significant deviation from the reference level.

The high organic matter in mudflats of the South Coorong reflect the eutrophic conditions in this ecosystem (Mosley et al. 2020). Eutrophication can also lead to hypoxia which affects benthic communities (Rosenberg et al. 1992; Norkko et al. 2015; Hale et al. 2016). As examples from Europe have shown, habitat conditions in eutrophic lagoons can be restored through reduction of nutrient loads, but this re-oligotrophication can take several decades (Derolez et al. 2020) and has not commenced in the Coorong.

Sediments provide microphytobenthic food for the benthic food web

This target was partially met, as chlorophyll-a concentrations were lower than in previous years at several sites. The Index of Relative Change showed a significant difference for the South Coorong, while the deviation was not significantly different for the entire system. A reduction in microphytobenthic biomass can affect macroinvertebrates such as amphipods, snails and surface-deposit-feeding infauna which are grazing on the microalgae in the sediment (Hagerthey et al. 2002; Daggers et al. 2020). Yet, there was no pronounced effect on abundances of grazers and surface-deposit-feeders in the recent survey, and chlorophyll-a did not emerge as a predictor variable for macroinvertebrate communities in 2020.

5.3 Conclusion

Monitoring in December 2020 found that the conditions of macroinvertebrate communities in the North Coorong, which improved as a result of the flood events and continuous flows since late 2010, have stabilised, although not all of the mudflat habitat targets were met (Table 4). Deteriorating conditions for macroinvertebrates could develop if filamentous algal mats persist. The monitoring in 2020 found no improvement in the degraded conditions of the South Coorong. The beneficial effects of continuous flows were detectable in the North Coorong, but did not reach the South Coorong, where hypersalinity prevented the establishment of more diverse and abundant macroinvertebrate communities.

Table 4: Summary table of how monitoring targets have been met over time in recent monitoring years.

Monitoring target	2016	2017	2018	2019	2020
Flow year	Wet	Dry-moderate	Dry	Dry-moderate	Wet-dry
Macroinvertebrates					
1. Species richness increases throughout the Murray Mouth and Coorong.	Partially met	Partially met	Partially met	Partially met	Partially met
2. Occurrence extends along the Coorong into the South Lagoon.	Partially met	Not met	Not met	Not met	Not met
3. Area of occupation exceeds 60% of the sites sampled.	Met	Partially met	Partially met	Partially met	Partially met
4. Abundance is maintained at, or increases above, reference levels.	Met	Partially met	Partially met	Met	Met
5. Biomass is maintained at, or increases above, reference levels.	Met	Met	Partially met	Partially met	Partially met
6. Communities are similar to those occurring under intermediate continuous flows.	Met	Met	Met	Met	Met
Mudflat habitat condition					
1. Habitable sediments are occurring along the Coorong into the South Lagoon.	Partially met	Partially met	Partially met	Partially met	Partially met
2. Sediments are maintained as fine to medium sands and are mostly moderately well sorted.	Met	Partially met	Partially met	Met	Partially met
3. Sediment organic matter is maintained.	Met	Partially met	Not met	Met	Partially met
4. Sediments provide microphytobenthic food for the benthic food web.	Not met	Met	Met	Met	Partially met

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8. Supplementary Material 1: Data analysis and additional figures for mudflat and macroinvertebrate monitoring 2020

SM-Table 1: Sampling sites and dates of the TLM macroinvertebrate and mudflat monitoring in December 2020. GPS coordinates, sampling dates and times of sampling are given for the survey in late spring/early summer. For region, both old and new groupings are given, and the site numbers are given as per TLM condition monitoring plan (revised).

Region		Site		2019			
Old	**New**	**New**	**Name**	**Southing**	**Easting**	**Sampling dates**	**Sampling time**
Murray Mouth	**North Coorong**	**1**	Monument Road	35°31.524'	138°49.750'	04/12/20	16:00 pm
		2	Hunters Creek	35°32.192'	138°53.376'	04/12/20	14:30 pm
		3	Mundoo Channel	35°32.245'	138°54.086'	04/12/20	13:00 pm
		4	Ewe Island	35°33.462'	138°57.381'	04/12/20	12:00 pm
		5	Pelican Point	35°35.572'	139°01.297'	04/12/20	11:00 am
North Lagoon		**6**	Mulbin Yerrok	35°40.144'	139°08.337'	04/12/20	10:00 am
		7	Noonameena	35°45.470'	139°15.729'	03/12/20	15:30 pm
	South Coorong	**8**	Parnka Point	35°53.815'	139°24.060'	03/12/20	18:20 pm
South Lagoon		**9**	Villa de Yumpa	35°54.661'	139°27.243'	03/12/20	14:00 pm
		10	Jack Point	36°01.894'	139°34.186'	03/12/20	12:00 pm
		11	Loop Road	36°10.082'	139°39.081'	03/12/20	9:00 am

Site 1: Monument Road

Mudflat at Monument Road

Site 2: Hunters Creek

Mudflat at Hunters Creek

Sediment at Hunters Creek with burrows from polychaetes (*Simplisetia aequisetis*) and snails (*Salinator fragilis*) at the surface

Site 3: Mundoo Channel

Mudflat at Mundoo Channel

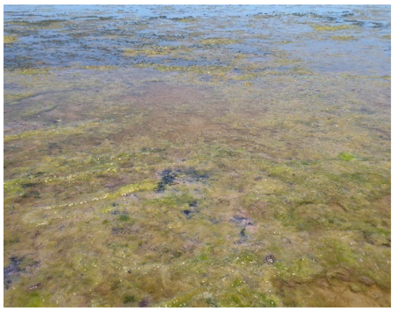
Sediment at Mundoo Channel with burrows from polychaetes (*Simplisetia aequisetis*)

Sediment surface with filamentous algae, decomposing accumulating algae and black ooze. Snails (*Salinator fragilis*) can also be seen at the surface

Site 4: Ewe Island

Ewe Island mudflat covered in algae, decomposing algae, and sediment beneath anoxic

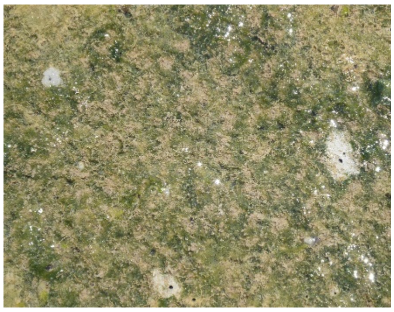
Decaying filamentous algae accumulating near the shore at Ewe Island



Mudflat surface smothered by filamentous algae

Filamentous algae on the mudflat

Sediment beneath algae



Burrow openings of some macroinvertebrates through the algal mats

Site 5: Pelican Point


Sampling at Pelican Point


Sampling at Pelican Point


Pelicans and Red-necked avocets

Site 6: Mulbin Yerrok


Sharp-tail sandpiper on algal mats accumulating on shore at Mulbin Yerrok

Site 7: Noonameena


Mudflat at Noonameena

Site 10: Jack Point


Mudflat at Jack POint

Site 9: Villa de Yumpa


Sampling on mudflat at Villa de Yumpa


Sediment cut showing thin surface layer above black anoxic layer, no signs of bioturbation


Red-capped plover at Villa de Yumpa

Site 11: Loop Road


Sampling on mudflat at Loop Road


Salt crust at sediment surface


Sediment cut showing surface layer above band of anoxic black sediment, no signs of bioturbation

SM-Figure 1: Photos illustrating the sampling activities and mudflat habitats at the study sites during the monitoring in December 2020. No photos were taken at Site 8 (Parnka Point). High water levels prevented taking photos of sediment cuts at several sites. All photos by Sabine Dittmann.

SM-Table 2: Test results from permutational ANOVA on differences in water quality parameters measured; a) during the December 2020 survey and, b) over the monitoring years since 2004 (for pH since 2008). ns = non-significant.

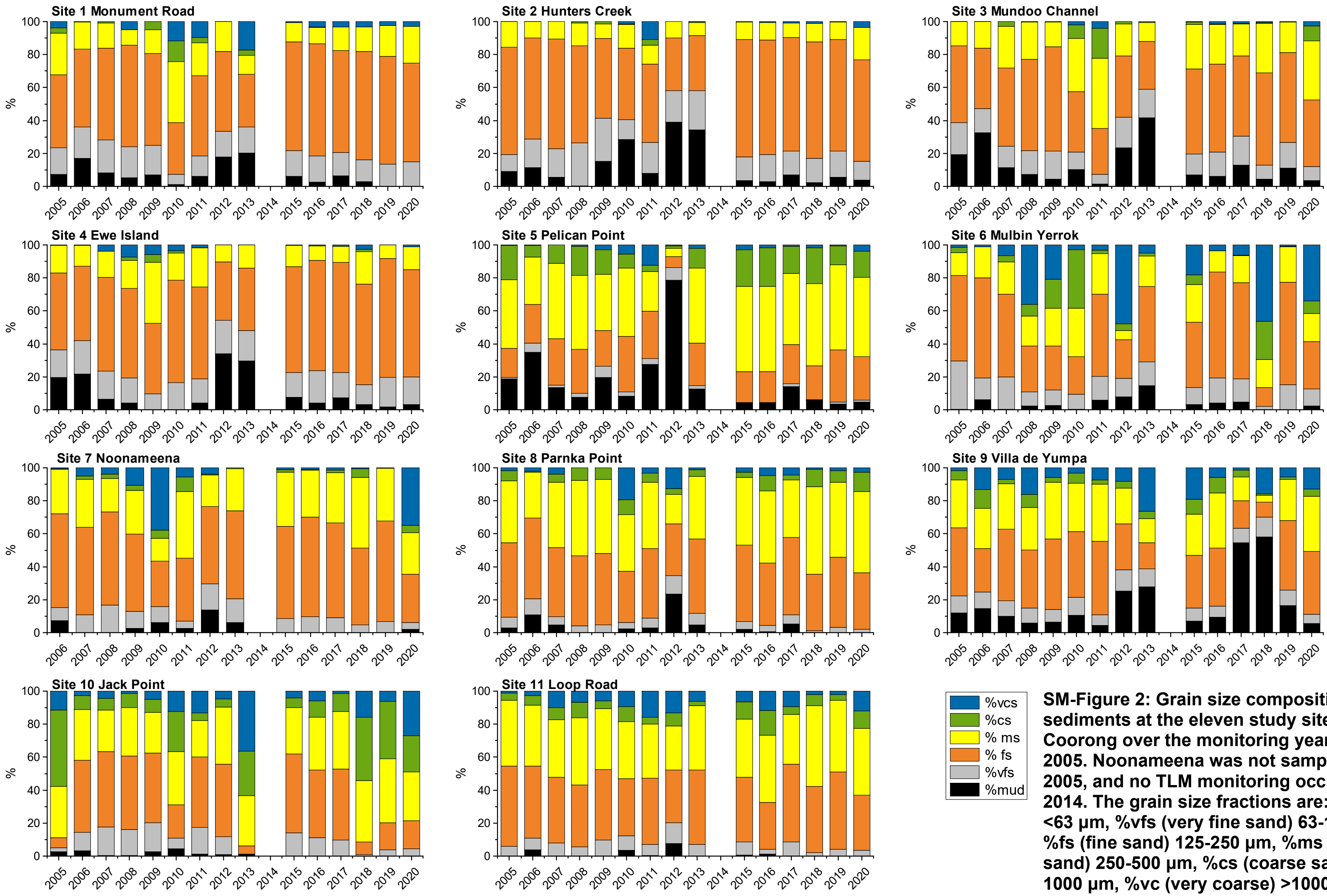
	Salinity		DO %		DO mg/L		pH water	
	df	$P_{(perm)}$	df	$P_{(perm)}$	df	$P_{(perm)}$	df	$P_{(perm)}$
a) 2020 survey								
Region	1	0.0034		ns		ns		ns
Site(Region)	9	0.0001		0.0001		0.0002		0.0001
Residual	22							
b) All years								
Year	15	0.0001	15	0.0001	15	0.0001	11	0.0001
Region	1	0.0001	1	ns	1	0.0003	1	ns
Year*Region	15	ns	15	0.001	11	ns	11	ns
Residual	142		141		119		105	

SM-Table 3: Test results from permutational ANOVA on differences in sediment parameters measured; a) during the survey in the December 2020 and, b) over the monitoring years since 2005 (2004 not included as different method used for grain size analysis). Chlorophyll-a in sediments was measured since 2007. ns = non-significant.

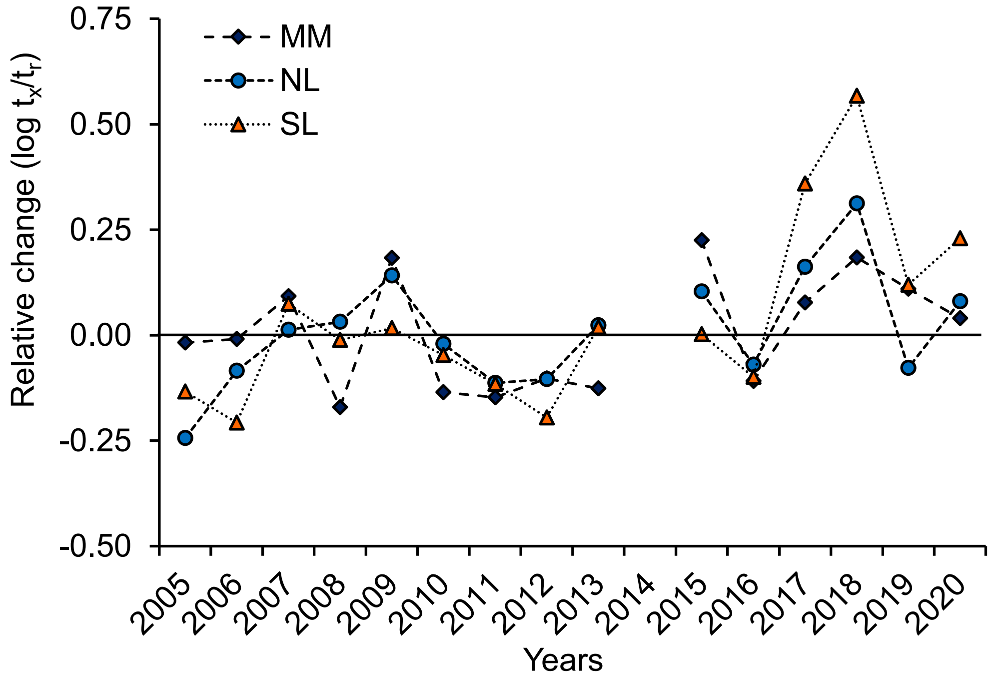
	Median grain size		Organic matter		Chl-a		pH sediment
	df	$P_{(perm)}$	df	$P_{(perm)}$	df	$P_{(perm)}$	$P_{(perm)}$
a) 2020 survey							
Region	1	ns		0.0096		ns	ns
Site(Region)	9	0.0002		0.0001		ns	0.0001
Residual	22						
b) All years							
Year	15	ns		0.0006	12	0.0001	
Region	1	0.0043		0.0001	1	0.0001	
Year*Region	15	ns		ns	12	0.0004	
Residual	142				117		

SM-Table 4: Reference values and 90% confidence intervals (lower and upper bound) for sediment characteristics based on the TLM refinement for all sites of the mudflat monitoring years and updated to include years to 2017 for the reference, see Dittmann et al. 2019.

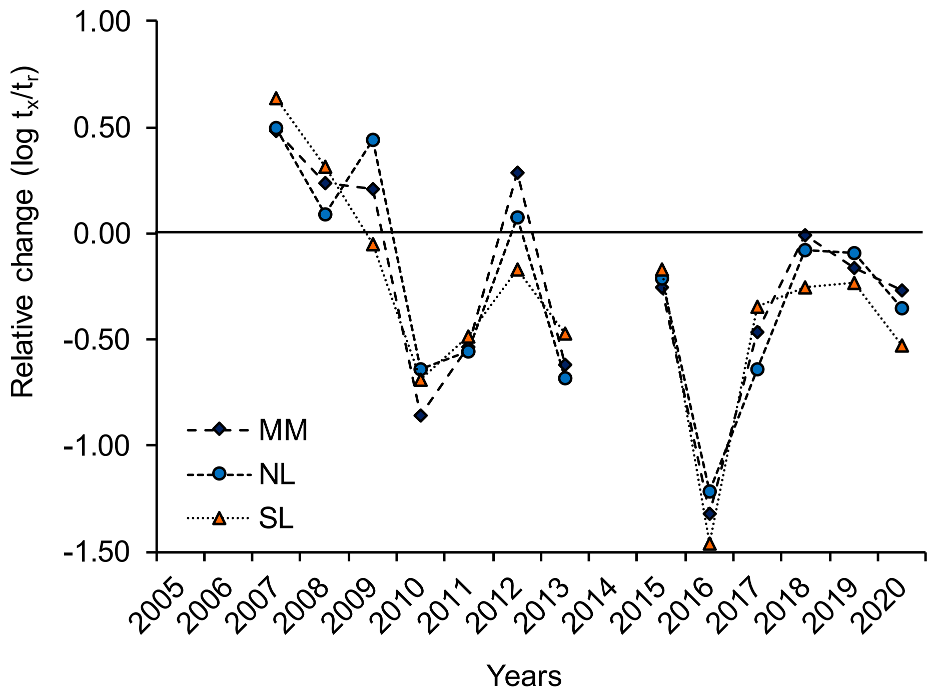
		Median grain size	Sorting coefficient	Organic matter	Chlorophyll-a
Mean		221.00	4.24	1.94	1.59
90% Confidence Interval for Mean	Lower Bound	208.00	3.59	1.66	1.28
	Upper Bound	234.00	4.89	2.22	1.89



SM-Figure 2: Grain size composition of sediments at the eleven study sites in the Coorong over the monitoring years since 2005. Noonameena was not sampled in 2005, and no TLM monitoring occurred in 2014. The grain size fractions are: %mud <63 µm, %vfs (very fine sand) 63-125 µm, %fs (fine sand) 125-250 µm, %ms (medium sand) 250-500 µm, %cs (coarse sand) 500-1000 µm, %vc (very coarse) >1000 µm.



SM-Figure 3: Index of relative change for sediment organic matter for the monitoring sites in each region: MM=Murray Mouth, NL=North Lagoon, SL=South Lagoon. Index values are plotted as a ratio of observed values (averages per year t_x) to reference data (t_r) obtained from the monitoring 2005-2017. The black line at 0 marks where observed values equal the reference values. No TLM monitoring occurred in 2014. In 2020, the Index value for the South Lagoon was a significant deviation.



SM-Figure 4: Index of relative change for sediment Chlorophyll-a for the monitoring sites in each region: MM=Murray Mouth, NL=North Lagoon, SL=South Lagoon. Index values are plotted as a ratio of observed values (averages per year t_x) to reference data (t_r) obtained from the monitoring 2007-2017. The black line at 0 marks where observed values equal the reference values. Chlorophyll-a in sediments has been recorded since 2007. No TLM monitoring occurred in 2014. In 2020, the Index value for the South Lagoon was a significant deviation.

SM-Table 5: Annual sediment characteristics for mudflats from all sites in the monitoring from 2005-2020 (chlorophyll-a measured since 2007, and no TLM monitoring in 2014). Average values per year are given (t_x) and for each year in relation to the long-term average (t_r), calculated to 2017. The t_r value is used as a reference for calculating the index of relative change (log (t_x/t_r). The effect size (rho δ) for the index of relative change was calculated over the monitoring years considered here (15 years, but 13 years for chlorophyll-a). The shaded fields indicate changes detected as significant change (> or < rho).

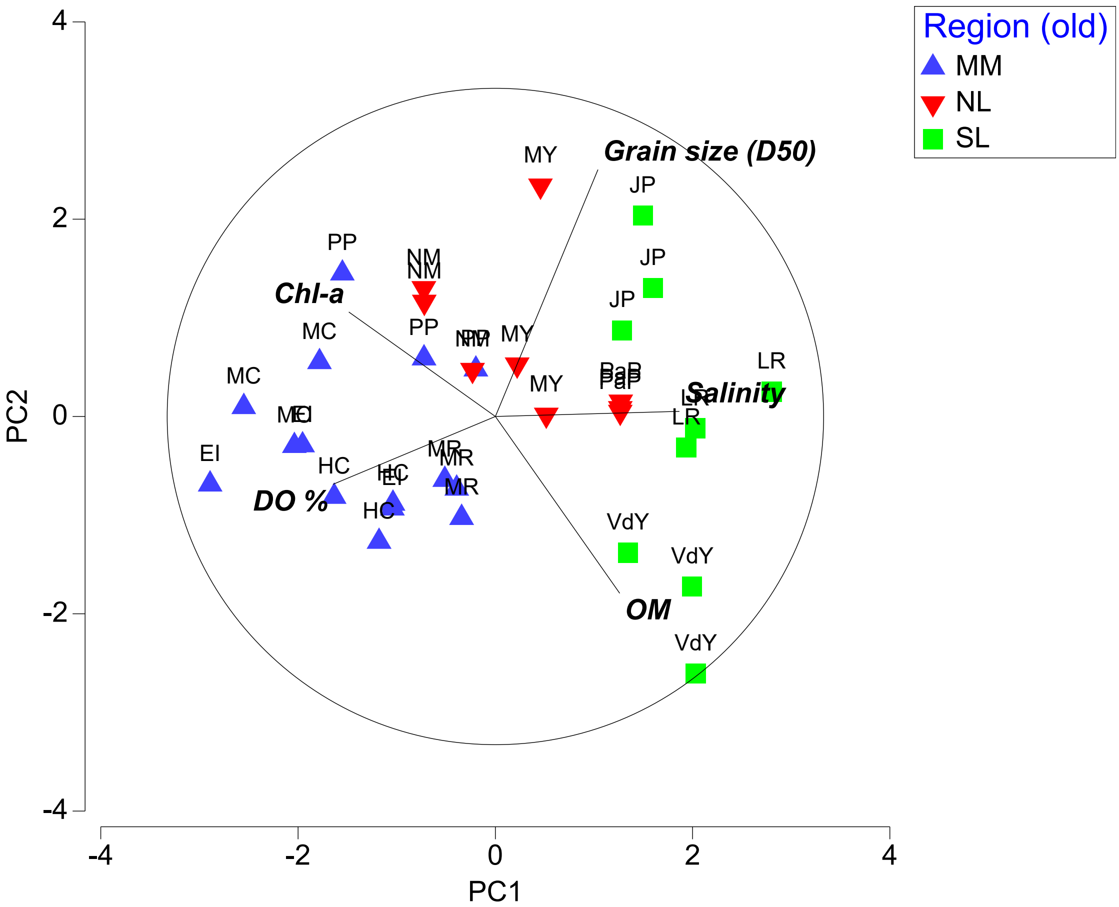
	Median grain size			Sorting coefficient			Sediment organic matter (% d.w.)			Sediment Chlorophyll-a (mg m^{-2})		
Year	(t_x)	t_x/t_r	log (t_x/t_r)	(t_x)	t_x/t_r	log (t_x/t_r)	(t_x)	t_x/t_r	log (t_x/t_r)	(t_x)	t_x/t_r	log (t_x/t_r)
2005	235.96	1.07	0.03	3.90	0.92	-0.04	1.51	0.81	-0.09			
2006	182.25	0.82	-0.08	6.21	1.47	0.17	1.45	0.78	-0.11			
2007	206.68	0.93	-0.03	2.65	0.63	-0.20	2.18	1.17	0.07	4.86	3.06	0.49
2008	234.57	1.06	0.03	2.18	0.51	-0.29	1.66	0.89	-0.05	2.77	1.74	0.24
2009	224.99	1.02	0.01	2.94	0.69	-0.16	2.36	1.27	0.10	2.58	1.62	0.21
2010	274.89	1.24	0.09	3.62	0.85	-0.07	1.58	0.85	-0.07	0.22	0.14	-0.86
2011	225.86	1.02	0.01	3.54	0.84	-0.08	1.39	0.75	-0.13	0.47	0.29	-0.53
2012	199.32	0.90	-0.04	8.50	2.01	0.30	1.34	0.72	-0.14	3.06	1.93	0.28
2013	224.06	1.01	0.01	8.53	2.01	0.30	1.75	0.94	-0.03	0.38	0.24	-0.62
2014												
2015	225.77	1.02	0.01	2.16	0.51	-0.29	2.39	1.28	0.11	0.89	0.56	-0.25
2016	229.37	1.04	0.02	1.97	0.46	-0.33	1.49	0.80	-0.10	0.08	0.05	-1.32
2017	188.91	0.85	-0.07	4.62	1.09	0.04	3.25	1.75	0.24	0.55	0.34	-0.46
2018	304.84	1.38	0.14	4.90	1.16	0.06	4.89	2.63	0.42	1.57	0.99	0.00
2019	227.36	1.03	0.01	2.23	0.53	-0.28	2.25	1.21	0.08	1.09	0.69	-0.16
2020	295.55	1.34	0.13	1.99	0.47	-0.33	2.58	1.39	0.14	0.86	0.54	-0.27
Mean (to 2017) (t_r)	221.05		0.02	4.24		-0.08	1.86		0.03	1.59		-0.25
StDev			0.06			0.22			0.16			0.51
rho			**0.06**			**0.20**			**0.14**			**0.50**

$t_{0.975,14} = 2.145$

$t_{0.2,14} = 1.345$

$t_{0.975,12} = 2.179$

$t_{0.2,12} = 1.356$



SM-Figure 5: Principal Component Analysis (PCA) plot of environmental variables measured at the study sites in December 2020. The ordination shows a separation of sites from the North and South Coorong along the PC1 axis, which explained 46.4% of the variation. PC2 explained a further 23.2 % of variation and was largely driven by the higher organic matter at Villa de Yumpa (VdY) and the coarse sediment at Jack Point (JP). In the North Coorong, the high sediment chlorophyll-a and high dissolved oxygen saturation (DO%) separates some of the sites with filamentous algae. Further site codes are Monument Road (MR), Hunters Creek (HC), Mundoo Channel (MC), Ewe Island (EI), Pelican Point (PP), Mulbin Yerrok (MY) and Noonameena (NM), Loop Road (LR).

SM-Table 6: Reference values for Index of Occurrence for macroinvertebrate species and taxa, based on data from 2004 to 2017. This table shows the reference values for further species and taxa than displayed in Figure 19 of the report.

Species/taxa	Reference value for Index of Occurrence
Capitella sp.	1.00
Amphipoda	1.00
Arthritica helmsi	1.00
Chironomidae	1.00
Simplisetia aequisetis	0.91
Oligochaeta	0.82
Ostracoda	0.82
Dolichopodidae	0.82
Salinator fragilis	0.64
Hydrobiidae	0.64
Boccardiella limnicola	0.64
Australonereis ehlersi	0.55
Nephtys australiensis	0.55
Mysid sp.	0.55
Phyllodoce novaehollandiae	0.36
Haloniscus searlei	0.36
Paragrapsus gaimardii	0.27
Hiatula alba	0.27
Spisula trigonella	0.18

SM-Table 7: Reference values for macroinvertebrate characteristics as updated in Dittmann et al. (2019) to include years to 2017. Diversity: 90% confidence intervals (lower and upper bound) for species numbers. For abundance and biomass: reference values for Index of Relative Change. NC=North Coorong, SC=South Coorong.

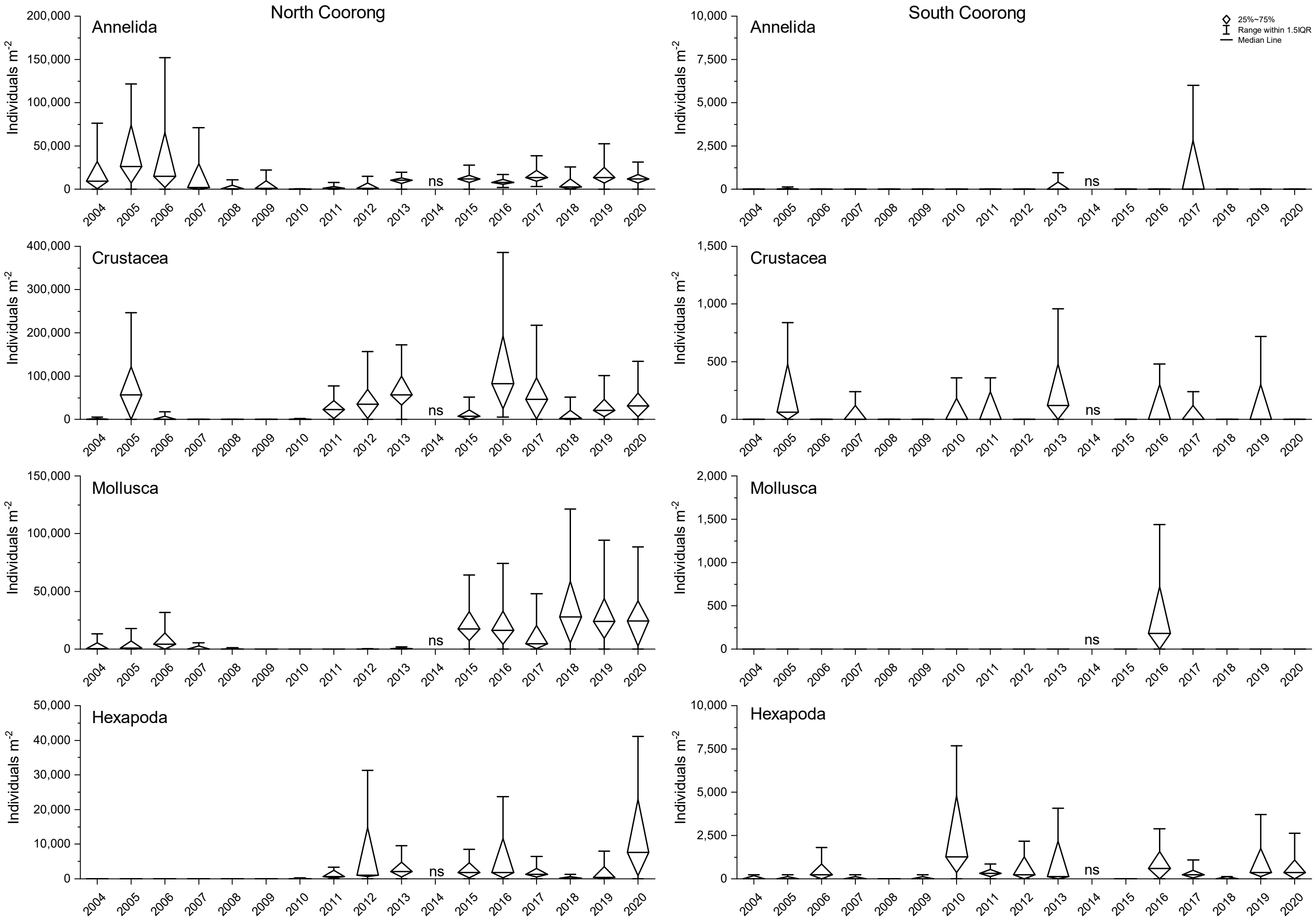
		Species numbers		Total abundance Individuals m^{-2}		Biomass g AFDW m^{-2}	
		NC	SC	NC	SC	NC	SC
Mean		7.3	3.1				
90% Confidence Interval for Mean	Lower Bound	5.7	1.6				
	Upper Bound	8.9	4.6				
Index of Reative Change reference value				59,787	1,569	9.70	0.62

SM-Table 8: Test results from permutational ANOVA on differences in individual densities for total macroinvertebrates and major phyla, a) during the 2020 monitoring, and b) over the monitoring years since 2004. $P_{(MC)}$ = Monte Carlo test *P* values for sparse data. ns = non-significant.

		Total benthos	Annelida	Mollusca	Crustacea	Hexapoda
	df	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$
a) 2020 survey				$P_{(MC)}$		
Region	1	0.003	0.0033	0.0015	0.0025	ns
Site(Region)	9	0.0035	0.0128	0.0001	0.0001	0.0001
Residual	99					
b) All years						
Year	15	0.0001	0.0001	0.0001	0.0001	0.0001
Region	1	0.0003	0.0002	0.0001	0.002	ns
Site(Region)	9	0.0001	0.0001	0.0001	0.0001	0.0001
Year*Region	15	0.0001	0.0077	0.0001	0.0001	0.0001
Year*Site(Region)	133	0.0001	0.0001	0.0001	0.0001	0.0001
Residual	1565					

SM-Table 9: Test results from permutational ANOVA on differences in individual densities for some of the more abundant macroinvertebrate species throughout the Coorong in the 2020 monitoring, $P_{(MC)}$ = Monte Carlo test *P* values for sparse data. ns = non-significant.

		Amphipoda	*Arthritica helmsi*	Chironomid larvae	*Simplisetia aequisetis*	*Capitella* sp.	*Boccardiella limnicola*	Hydrobiidae	*Salinator fragilis*
	df	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$	$P_{(perm)}$
2020 survey			$P_{(MC)}$		$P_{(MC)}$	$P_{(MC)}$	$P_{(MC)}$	$P_{(MC)}$	$P_{(MC)}$
Region	1	0.0028	0.0016	ns	0.0089	0.0318	ns	0.006	ns
Site(Region)	9	0.0001	0.0001	0.0001	0.0001	0.0001	0.002	0.001	0.002
Residual	99								



SM-Figure 6: Box plots of individual densities for the higher taxa of macroinvertebrates in the North Coorong (left column) and South Coorong (right column) for each of the monitoring years. No TLM monitoring occurred in 2014 (ns = not surveyed). Note the different y-axes scales for each plot.

SM-Table 10: Index of Relative Change and effect size (rho δ) for macroinvertebrates from all sites in the monitoring from 2004-2020. Average values per year are given (t_x) and set in relation to the long-term average (t_r), calculated here to 2017. The t_r value is used as a reference for calculating the index of relative change (log (t_x/t_r). The shaded fields indicate changes detected as significant change (> or < rho).

Year	North Coorong (tx)	t_x/t_r	log (t_x/t_r)	South Coorong (t_x)	t_x/t_r	log (t_x/t_r)
2004	29381	0.49	-0.31	585	0.37	-0.43
2005	126796	2.12	0.33	1656	1.06	0.02
2006	56570	0.95	-0.02	906	0.58	-0.24
2007	27931	0.47	-0.33	396	0.25	-0.60
2008	12051	0.20	-0.70	72	0.05	-1.34
2009	9497	0.16	-0.80	123	0.08	-1.11
2010	716	0.01	-1.92	5737	3.66	0.56
2011	37028	0.62	-0.21	609	0.39	-0.41
2012	55595	0.93	-0.03	1413	0.90	-0.05
2013	87367	1.46	0.16	3418	2.18	0.34
		0.00			0.00	
2015	56697	0.95	-0.02	45	0.03	-1.54
2016	176768	2.96	0.47	2877	1.83	0.26
2017	100833	1.69	0.23	2559	1.63	0.21
2018	63634	1.06	0.03	576	0.37	-0.44
2019	87948	1.47	0.17	1632	1.04	0.02
2020	96910	1.62	0.21	810	0.52	-0.29
Mean (t_r)	59787		-0.17	1569		-0.31
StDev			0.58			0.60
rho			**0.50**			**0.52**

$t_{0.975,15}$ =2.131

$t_{0.2,15}$ = 1.341

SM-Table 11: Index of Relative Change and effect size (rho δ) for several key macroinvertebrate species from all sites in the monitoring from 2004-2020. The long-term reference for calculating the index of relative change (log (t_x/t_r) is based on the average (t_r), calculated here to 2017. The t_r value is used as a reference. The shaded fields indicate changes detected as significant change (> or < rho).

	Simplisetia	Capitella	Nepthys	Boccardiella	Oligochaeta	Amphipoda	Arthritica	Chironomid	Salinator	Hydrobiidae
North Coorong										
2004	-0.04	0.09	-0.29		0.40	-0.86	-0.03	-1.73	-0.34	-1.66
2005	0.46	0.45	-0.87	-1.29	0.35	0.36	-0.05	-1.24	0.64	-1.17
2006	0.34	0.41	0.87	-2.58	0.67	-0.76	0.22	-0.80	-0.66	-0.87
2007	-0.30	0.30	0.42		-0.86	-1.32	-0.38	-2.11	-0.40	-0.68
2008	-1.05	-0.03	-0.26	-1.88	-0.96	-2.21	-0.89	-1.64	0.22	-1.24
2009	-2.10	-0.27	-0.09	-0.45	-0.20	-1.12	-1.57	-1.82	-0.32	
2010	-1.88	-2.76	-1.70		-1.58	-1.81		-1.47		
2011	-1.25	-0.81	-1.26		-0.42	-0.02		0.01		
2012	-0.94	-0.44	-0.66	0.23	0.12	0.07		0.53	-1.59	-1.27
2013	0.19	0.04	-0.39	0.12	-0.77	0.27	-0.96	0.13	-0.89	-0.60
2014										
2015	0.19	-0.29	-0.61	0.54	-0.38	-0.34	0.51	0.07	0.38	0.68
2016	0.15	-0.45		-0.03	-0.41	0.58	0.60	0.53	0.43	0.59
2017	0.24	-0.31	-1.56	0.72		0.29	0.22	0.38	-0.69	0.55
2018	-0.09	-0.23	-1.32	-1.03		-0.29	0.73	-0.46	0.25	0.47
2019	0.37	-0.08	-0.02	0.05		0.00	0.70	0.01	0.49	0.16
2020	0.30	-0.30	-0.96	-0.20	-0.57	0.05	0.64	0.74	0.16	0.46
Mean	-0.34	-0.29	-0.58	-0.48	-0.36	0.44	-0.02	-0.55	-0.17	-0.35
StDev	0.83	0.74	0.72	1.01	0.63	0.82	0.73	0.98	0.64	0.86
rho	**0.72**	**0.64**	**0.63**	**0.88**	**0.54**	**0.71**	**0.64**	**0.85**	**0.56**	**0.74**
South Coorong										
2004								-0.25		
2005	0.51	-0.88			1.11	0.87		-0.75		
2006						-0.99	-0.44	-0.02		
2007								-1.24		
2008								-1.22		0.94
2009								-1.02		
2010								0.77		
2011		-1.88				0.33		-0.31		
2012		-1.18			-1.40			0.02		
2013	0.81	0.66				-0.33	-1.26	0.30		
2014										
2015		-1.88				-1.29		-1.57		
2016	0.51	-1.58				0.41	1.10	0.03		0.64
2017		0.91				-0.59	-0.86	-0.28		
2018		-0.31						-0.33		
2019		-1.40						0.13		
2020		-1.58				-0.81		0.11		
Mean	0.61	-0.91			-0.14	0.30	-0.37	-0.37		0.79
StDev	0.17	1.01			1.78	0.76	1.03	0.63		0.21
rho	**0.15**	**0.88**			**1.54**	**0.66**	**0.89**	**0.55**		**0.18**

SM-Table 12: Test results from permutational ANOVA on differences in macroinvertebrate biomass a) during the 2020 monitoring, and b) over the monitoring years since 2004.

	df	Biomass $P_{(perm)}$
a) 2019 survey		
Region	1	0.0029
Site(Region)	9	0.0001
Residual	99	
b) All years		
Year	15	0.0001
Region	1	0.0001
Site(Region)	9	0.0001
Year*Region	15	0.0001
Year*Site(Region)	133	0.0001
Residual	1565	

SM-Table 13: Index of Relative Change and effect size (rho δ) for macroinvertebrate biomass from all sites in the monitoring from 2004-2020. Average values per year are given (t_x) and related to the long-term average (t_r), calculated here to 2017. The t_r value is used as a reference for calculating the index of relative change (log (t_x/t_r). The shaded fields indicate changes detected as significant change (> or < rho).

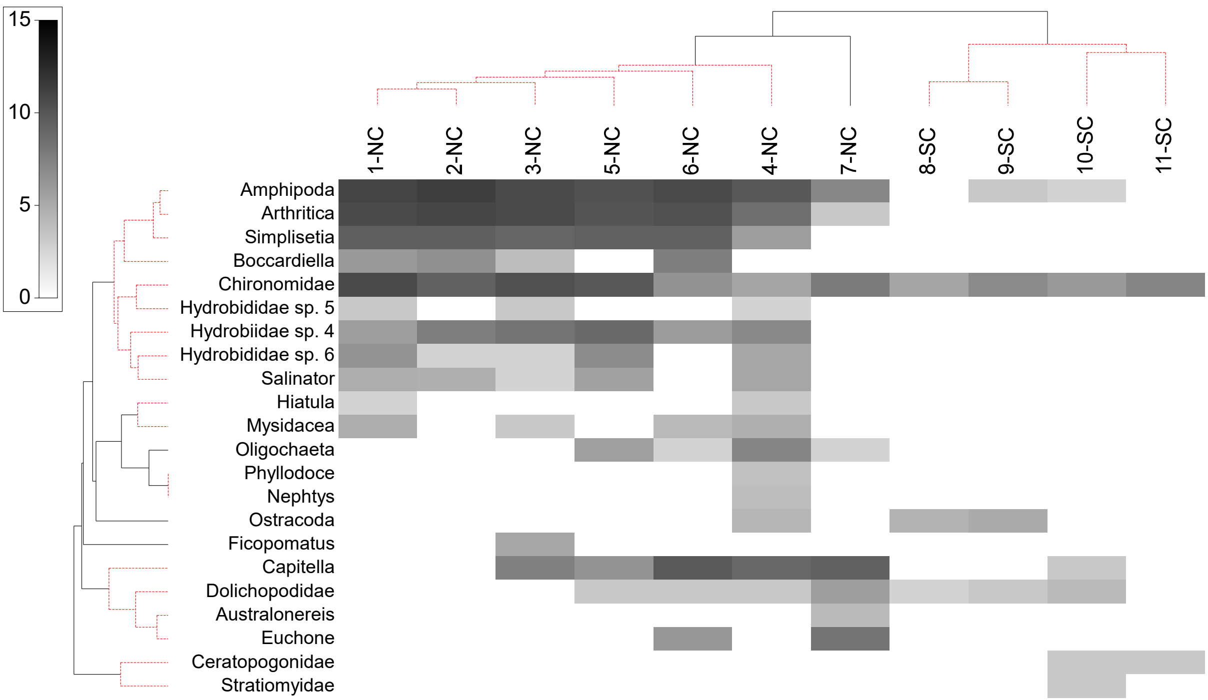
Year	North Coorong (tx)	t_x/t_r	log (t_x/t_r)	South Coorong (t_x)	t_x/t_r	log (t_x/t_r)
2004	3.33	0.34	-0.46	0.19	0.31	-0.51
2005	19.43	2.00	0.30	0.10	0.16	-0.80
2006	12.43	1.28	0.11	0.48	0.77	-0.11
2007	7.68	0.79	-0.10	0.06	0.10	-1.00
2008	2.36	0.24	-0.61	0.06	0.10	-0.99
2009	1.44	0.15	-0.83	0.02	0.03	-1.52
2010	0.82	0.08	-1.07	1.10	1.78	0.25
2011	2.22	0.23	-0.64	0.03	0.05	-1.34
2012	3.82	0.39	-0.41	0.15	0.25	-0.61
2013	12.38	1.28	0.11	0.27	0.44	-0.36
2015	16.68	1.72	0.24	0.01	0.02	-1.62
2016	30.19	3.11	0.49	1.30	2.11	0.32
2017	13.30	1.37	0.14	4.23	6.88	0.84
2018	17.98	1.85	0.27	2.53	4.11	0.61
2019	33.13	3.42	0.53	0.39	0.63	-0.20
2020	21.73	2.24	0.35	0.15	0.24	-0.61
Mean (t_r)	9.70		-0.10	0.62		-0.48
StDev			0.50			0.74
rho			**0.43**			**0.64**

$t_{0.975,15} = 2.131$

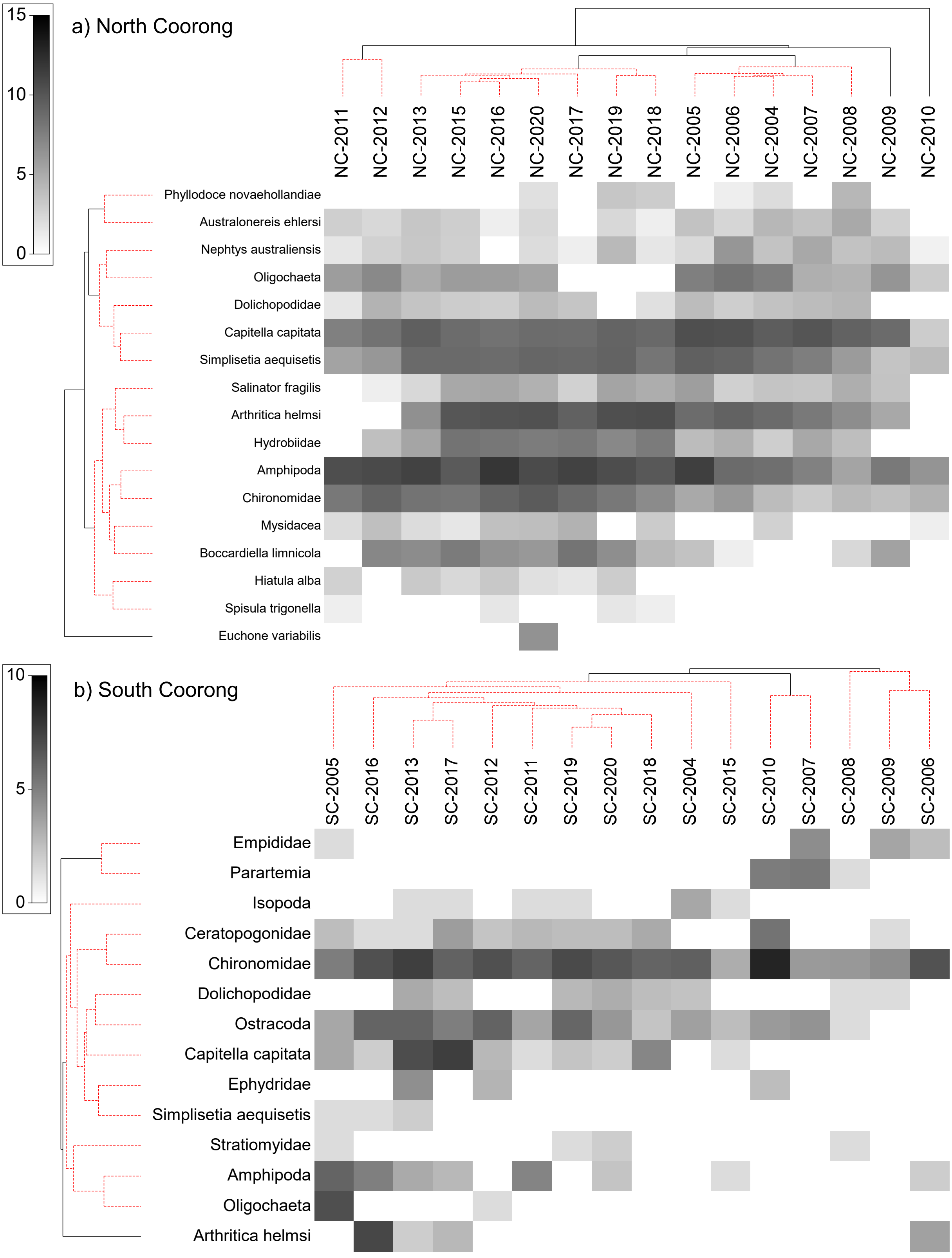
$t_{0.2,15} = 1.341$

SM-Table 14: Test results from permutational ANOVA on differences in macroinvertebrate communities a) during the survey in the 2020 monitoring, and b) over the monitoring years since 2004.

		Community
	df	$P_{(perm)}$
a) 2020 survey		
Region	1	0.0027
Site(Region)	9	0.0001
Residual	99	
b) All years		
Year	15	0.0001
Region	1	0.0001
Site(Region)	9	0.0001
Year*Region	15	0.0001
Year*Site(Region)	133	0.0001
Residual	1565	



SM-Figure 7: Shade plot for macroinvertebrate species based on average values (log-transformed) per site. The depth of the shading corresponds to abundances. The species are ordered through a cluster analysis on species, and the sampling sites are ordered through a cluster analysis, whereby black lines indicate significantly different clusters (SIMPROF tests) and red lines indicate not significantly different groups.



SM-Figure 8: Shade plots for macroinvertebrate species in a) North Coorong, and b) South Coorong, based on average values per year for each region. The depth of the shading corresponds to abundances. The species are ordered through a cluster analysis on species, and the sampling sites are ordered through a cluster analysis. Black lines indicate significantly different clusters (SIMPROF tests) and red lines indicate not significantly different groups.

SM-Table 15: Average Bray-Curtis similarity Index S_{jk} for macroinvertebrate communities for each of the monitoring years from 2004 to 2020. The reference for each region was obtained as average of the index from two years with flow and good condition (see TLM refinement and revised condition monitoring plan). The difference between the index in each year and the reference is included as change in condition. The effect size (rho δ) for was calculated over a 16-year monitoring period. Shaded fields are changes detected as significant change.

	North Coorong		South Coorong	
Year	**S_{jk}**	**S_{jk} - Mean**	**S_{jk}**	**S_{jk} - Mean**
2004	34.47	-27.43	12.08	-36.11
2005	62.27	0.38	19.64	-28.55
2006	41.80	-20.10	41.13	-7.06
2007	23.85	-38.05	7.94	-40.25
2008	12.86	-49.04	5.82	-41.89
2009	16.92	-44.98	8.57	-39.62
2010	25.10	-36.80	55.24	7.06
2011	61.68	-0.22	46.98	-1.21
2012	49.92	-11.98	33.76	-14.43
2013	61.52	-0.38	19.29	-28.63
2014				
2015	59.42	-2.48	4.05	-44.14
2016	71.27	9.37	41.30	-6.89
2017	64.19	2.29	34.86	-13.33
2018	58.02	-3.88	7.15	-41.04
2019	64.72	2.83	45.09	-2.64
2020	59.84	-2.06	28.34	-19.85
Mean	61.95#	-13.90	48.19*	-22.48
StDev		19.26		17.16
rho		**16.71**		**14.90**

$t_{0.975,15} = 2.131$

$t_{0.2,15} = 1.341$

Mean from 2005 and 2013 as reference

* Mean from 2006 and 2010 as reference

SM-Table 16: Spearman's rank correlation coefficient rho for comparisons between similarity matrices of macroinvertebrate communities from the latest (here: 2019) with each previous monitoring year for all sampling sites and the North (sites 1-7) and South Coorong (sites 8-11). *P* is the significance level from permutation tests.

	All sites		North Coorong		South Coorong	
Yearly comparison	Spearman's rho	*P*	Spearman's rho	*P*	Spearman's rho	*P*
2020-2019	0.7010	0.0001	0.7000	0.0001	0.2680	0.0007
2020-2018	0.6730	0.0001	0.5600	0.0001	0.2060	0.0101
2020-2017	0.5950	0.0001	0.5630	0.0001	0.0010	ns
2020-2016	0.5670	0.0001	0.6890	0.0001	-0.1110	ns
2020-2015	0.5990	0.0001	0.6660	0.0001	-0.0610	ns
2020-2013	0.5640	0.0001	0.7490	0.0001	-0.0190	ns
2020-2012	0.4470	0.0001	0.4910	0.0001	-0.0360	ns
2020-2011	0.4440	0.0001	0.3650	0.0001	0.0550	ns
2020-2010	0.2010	0.0001	0.1190	0.0152	-0.0120	ns
2020-2009	0.0570	ns	0.0420	ns	0.0470	ns
2020-2008	0.0470	ns	0.0420	ns	0.1230	ns
2020-2007	0.2900	0.0001	0.2150	0.0001	0.1290	ns
2020-2006	0.5120	0.0001	0.5710	0.0001	-0.0620	ns

SM-Table 17: Test results from distance-based redundancy analysis (DISTLM) using environmental variables as predictor for macroinvertebrate community data in 2020. Marginal tests show how much each variable explains when taken alone, while sequential tests are conditional on variables added sequentially based on how much they add to the explanation of variability. Only variables adding with significant *P*-values are shown for sequential tests. The percent values give the proportion of the variability in macroinvertebrate community data that is explained by the respective environmental variable. ns = non significant.

	2020	
Variable	*P*	%
Marginal tests		
Salinity	0.0004	63.43
Dissolved oxygen (%)	ns	15.43
Organic matter	0.0426	22.40
Chl-a	ns	23.89
Grain size	ns	21.12
Sorting	ns	6.48
Sequential tests		
Salinity	0.0002	63.43

SM-Table 18: Test results from distance-based redundancy analysis (DISTLM) using environmental variables as predictor for macroinvertebrate community data from long-term monitoring for all years and sites (2005-2020, but no TLM monitoring in 2014, and site 2 in 2009 was left out due to a missing value for DO saturation). Marginal tests show how much each variable explains when taken alone, while sequential tests are conditional on variables added sequentially based on how much they add to the explanation of variability. Only variables adding with significant *P*-values are shown for sequential tests. The percent values give the proportion of the variability in macroinvertebrate community data that is explained by the respective environmental variable. ns = non significant.

	Long-term monitoring	
Variable	*P*	%
Marginal tests		
Salinity	0.0001	23.81
Dissolved Oxygen (%)	0.0007	2.91
Organic matter	0.0001	5.96
Grain size	0.0022	2.33
Sorting	ns	1.08
Sequential tests		*Cumulative %*
Salinity	0.0001	23.81
Organic matter	0.0023	25.38
Dissolved Oxygen (%)	0.0145	26.58
Sorting	0.0237	27.64
Grain size	0.0061	29.01