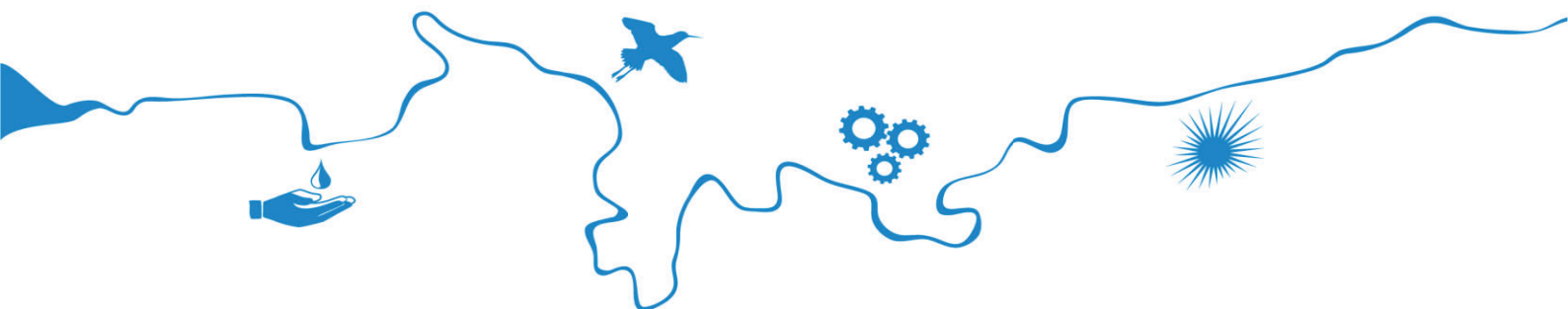# South Australian Murray-Darling Basin 2022-2023 Flood Environmental Response in the Coorong

Luke Mosley, Sabine Dittmann, Qifeng Ye, Emily Leyden, Anthony Newbery, Luciana Bucater, Travis Howson, George Giatas, Dhafeena Ahmed, Sunira Reardon, Jade Teigeler, Georgia Tiller, Josh Nitschke, Laura Schroder, Chris Sepe, Abbie Hay, Noah Morris, Cassandra Urgl, Jason Nicol, Michelle Waycott

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Disclaimer

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Respect and reconciliation

Aboriginal people are the First Peoples and Nations of South Australia. The Coorong, connected waters and surrounding lands have sustained unique First Nations cultures since time immemorial.

The Goyder Institute for Water Research acknowledges the range of First Nations' rights, interests and obligations for the Coorong and connected waterways and the cultural connections that exist between Ngarrindjeri Nations and First Nations of the South East peoples across the region and seeks to support their equitable engagement.

Aboriginal peoples' spiritual, social, cultural and economic practices come from their lands and waters, and they continue to maintain their cultural heritage, economies, languages and laws which are of ongoing importance.

Acknowledgments

Executive Summary

Background

This report presents the findings of a study conducted on the environmental response of the Coorong to the 2022-2023 River Murray floods. This flood was larger than any that have occurred since 1956, with barrage discharges peaking at 120,000 ML/day on 30 January 2023.

The study, part of the South Australian Government's *Healthy Coorong, Healthy Basin* (HCHB) Program, aimed to understand the hydrological, water and sediment quality, and ecological (aquatic plants, fish, invertebrates) impacts of significant flood events on the Coorong ecosystem.

Methods

The research involved field and laboratory assessments of samples collected from various sites within the Coorong from 2023-2024. Key components included hydrological assessment, water and sediment quality analysis (e.g. salinity, nutrient concentrations), and ecology (e.g. aquatic plant distribution and diversity, macroinvertebrate abundance and diversity, and fish distribution, diversity and dietary analysis). Where possible data were compared across different temporal phases: pre-flood, flood, and post-flood periods, allowing for an analysis of changes induced by the flood event.

Key Findings

Environmental conditions

- The 2022-2023 floods resulted in significant changes in water levels and salinity in the Coorong, affecting both the North and South Lagoons.
- The system is still eutrophic and there is a need for ongoing flushing and export of nutrients from the Coorong, which high flows could facilitate. It remains to be determined if the system returns to a hyper-eutrophic state in subsequent seasons.
- Nutrient levels reduced during the high flow periods. Water quality improved, but is transitioning back to pre-high flow levels, particularly in the Coorong South Lagoon indicating there has not been a permanent change in the system.
- Sediments were better oxygenated during the high flow period and an overall improved sediment quality was observed.
- There were improvements to water clarity once the sediment resuspension and flood plume associated with the high flow had abated.
- As the Coorong water levels returned to normal, there was a return to anoxic sediments with increased concentrations of porewater sulfide and ammonia, at high levels potentially toxic to the biota.

Aquatic plant community

- The typical hypersalinity tolerant *Ruppia* Community (including *Ruppia tuberosa*) was resistant to the flood effects until salinity levels went below 35 g/L throughout the southern Coorong and plants died off fully when salinities reduced to 10 g/L.
- With lower salinities persisting, other aquatic macrophytes recruited, the more freshwater tolerant aquatic plant *Ruppia megacarpa* and the Charophyte *Lamprothamnium papulosum*.
- Tissue nutrient concentrations (%C and %N) were higher in 2023 than previous samples (2016-17 and 2021-22) for both the *Ruppia* Community and algal plants analysed.
- Seed banks of the *Ruppia* Community persisted although at a moderate or low level following the flood event.
- The seeds condition was vulnerable as the outer seed coat was soft, not hard as usual, however seedlings were observed to be germinating and populations recruiting in the most recent (June 2024) growing season.

- Water levels dropped rapidly and salinity rose in late 2023 which led to a *Ruppia* Community die-off. as is expected to happen with the more regular annual cycle of warmer weather associated water level, high salinity change.
- The consequences of the floods will not be evident for several more growing seasons given the timing and life cycle of the *Ruppia* Community species.
- The *Ruppia* Community is behaving as a dynamic multispecies community and further regular monitoring regularly will provide greater insight into status rather than one off sampling.

Macroinvertebrate community

- Despite the initial impact of the flood event on macroinvertebrates communities in the area adjacent to the Murray Mouth, they started to recover quickly.
- The diversity and abundance of macroinvertebrates were highest in the North Lagoon and these animals provided a source for colonisation of the South Lagoon.
- The availability of macroinvertebrate prey for fish and shorebirds changed as range and diversity changed with recolonisation into a wide range of locations.
- The rapid change in distribution and abundance of macroinvertebrates is evidence of resilience in the system for this component of the Coorong biota.

Fish community

- The post flood fish community was characterised by increased number of freshwater species, enhanced recruitment of diadromous species (congolli and galaxias), and greater abundance of sandy sprat.
- There was a substantial increase in fish species richness in the North and South Lagoons. With salinity reducing to below 60 g/L, South Lagoon had species from all four functional groups (freshwater, estuarine, marine and diadromous).
- Fish biomass increased evidenced by the increase in catch-per-unit-effort in 2022-23 of key fishery species, including black bream and greenback flounder.
- Salinity reduction, increased estuarine habitat and food resources, particularly in the North and South Lagoons, have shown to benefit fish populations in the Coorong.

Conclusions and Recommendations

The study concludes that the 2022-2023 River Murray floods had profound impacts on the Coorong's hydrological regime, water quality, and ecology. Based on the observed responses to the flood event and comparing observations from the outcomes of previous high flow events, further improvements to the Coorong ecosystem can be expected if the system experiences moderate to good flows in the next couple of years. Already, the water level drop going into summer of 2023 had a negative effect on habitat quality, with localised anoxia, sulfide and ammonia production. The draw down of nutrients during higher flow periods was observed, however return to low flows led to a halt to this. In addition, the *Ruppia* Community died off quickly as water levels dropped reducing seed bank production and higher salinities impacted fish communities and macroinvertebrates.

Key recommendations include the need for ongoing monitoring of the flood response over a longer time period coupled with adaptive management and initiatives to support the resilience of the Coorong in the face of future climate variability. Understanding the responses of the Coorong ecosystem to flood events is essential for developing sustainable Murray-Darling Basin water management practices that balance ecological health with human demands. Building on outcomes of the *Healthy Coorong, Healthy Basin* major project this study improves the foundation for further research, policy development, and strategies aimed at preserving the health of the Coorong.

1 Introduction

1.1 Background

Estuaries are dynamic and productive environments where rivers meet the sea. Floods can significantly impact estuaries by transporting large amounts of freshwater, sediment and nutrients into them (Voynova et al. 2017). High flows can rapidly flush material (e.g. salt, sediment, nutrients, organic matter) out to sea, bypassing normal cycling processing in the estuary (Eyre and Twigg 1997). Floods often bring nutrients that can stimulate primary production, but they can also lead to eutrophication and hypoxic conditions once they recede (Yoynova et al. 2017). The salinity reductions induced by floods can disrupt the delicate balance of estuarine ecosystems, affecting the distribution and abundance of plant and animal species (Dittmann et al. 2015, James et al. 2020). The physical structure of estuaries can be reshaped by the deposition of sediments during floods, changing habitats such as mudflats and seagrass meadows, and their associated faunal populations, and can take some time to return to conditions prior to the event (Thrush et al. 2007). On the other hand, flood/high flows have been reported to benefit fish populations and increase fishery productions in estuaries (Gilson 2011, Halliday et al. 2012). Understanding the effects of floods on estuaries is crucial for the management and conservation of these environments, especially in the context of climate change, which is expected to increase the frequency and intensity of flooding events (Kennish 2002, Gillanders et al. 2011).

Australia's Murray-Darling Basin is one of the most heavily regulated river systems in the world. Hydrological drought due to water extraction in the Murray-Darling Basin has been shown to impact the quantity and quality of water reaching the Murray Mouth and Coorong estuary, with cascading impacts of eutrophication and salination on the estuarine and lagoon ecosystem (Brookes et al. 2022, Dittmann et al. 2015, Mosley et al. 2023). However, there is little information on the responses of the Coorong to large River Murray floods as floods in the Murray-Darling Basin have been intermittent since water resource development (e.g. 1917, 1956 and 1976) (Bloss et al. 2015). Higher rainfall in the Murray-Darling Basin during 2022, in combination with full storages in the basin, led to large increases in River Murray flows and flooding in many parts of the Basin during late 2022 and early 2023. This flood was larger than any that have occurred since 1956, with barrage discharges peaking at 120,000 ML/day on 30 January 2023. The flood event provided an ideal opportunity to evaluate water and sediment quality and ecological outcomes for the Coorong in South Australia.

1.2 Aims of project and management questions

The overarching aim of the project is to evaluate the response of the Coorong to the 2022-2023 River Murray flood. A conceptual diagram underpinning the project is shown in Figure 1.
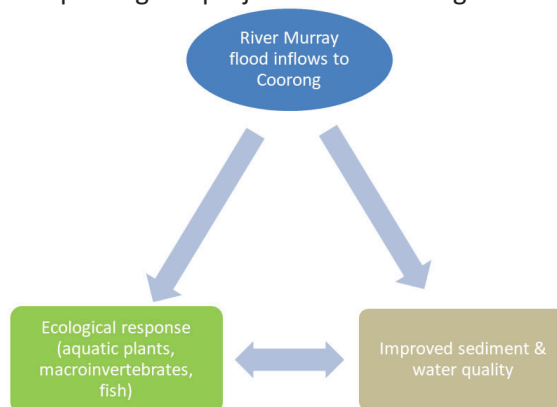
Figure 1 Conceptual diagram of hypothesized flood response and interactions between ecological components, sediment and water quality

The specific outcome sought by the project is to fill critical knowledge gaps on the influence of the 2022–23 flood event on the water quality, nutrient dynamics, abundance and diversity of key biota and food web structure in the Coorong as well as ecological restoration options through experimental treatments.

The key management questions provided by the South Australian Government to be addressed in the project were:

Impacts on the Coorong ecosystem – Response of Coorong nutrient store and dynamics

- How have nutrient and related conditions in the sediment changed in response to the 2022-2023 flood? Have surface sediment nutrient concentrations reduced, compared to previous years, due to increased flushing and bioturbation during and following the flood?
- How did the floods affect macroinvertebrate distribution, diversity, abundance and biomass in the Murray Mouth Area and Coorong lagoons, and are their functional processes (e.g. bioturbation) affecting sediment nutrient loads and bioremediation?

Impacts on the Coorong ecosystem – Response of Coorong food web

- How did the flood impact on the Coorong food web and key biota including aquatic plants, macroinvertebrates and fish? Specific questions include:
    - What effect have the floods had on macroinvertebrates (distribution, diversity, abundance, biomass and functions) in the Murray Mouth Area, North and South Lagoons of the Coorong?
    - What effect have the floods had on fish (distribution, diversity, abundance, biomass) and the dietary composition of key fish species in the Murray Mouth Area, North and South Lagoon of the Coorong.
    - What is the status of the *Ruppia* Community, the filamentous algal community and the environmental condition of the littoral zone following the River Murray Floods of 2022-2023

On-ground ecological restoration actions – Macroinvertebrate and aquatic plant translocations

- Does ecological restoration enabled by the lower salinity conditions in the flood help promote sediment remediation by macroinvertebrate bioturbation and aquatic plants in the South Lagoon?
- Can the addition of burrowing macroinvertebrates function as natural remediation of sediment conditions in the southern Coorong lagoon?

2 Water and sediment quality

2.1 Introduction

Significant changes to the environmental and ecological conditions in the Coorong occurred during the flood and subsequent high flow period, including water quality and sediment nutrient conditions. The aim was to investigate the changes to sediment and water quality in response to the 2022-2023 flood and infer the extent of the flow impacts to the environment. In addition, the changes to surface sediment nutrient concentrations during and after the flow event were examined by comparing previous years data to data collected over the flood and high flow period, to investigate whether the increased flushing and bioturbation had a net impact on the nutrient status of the system over and above levels inputted during the flood event.

2.2 Methods

Long term hydrological flow and salinity data was collected from the continuous Department for Environment and Water (DEW) monitoring stations on the Coorong and from monthly grab samples data (Water Data SA) historically collected by State Government departments. These locations are shown on Figure 2 in green.

Sediment and water quality at specific locations were conducted in August 2023, November 2023 and March 2024 at 10 locations along the North and South Lagoon of the Coorong (Figure 2 (shown in red), Figure 3). The August sampling event coincided with the river still at high flow (>40 000 ML/day over the barrages), but flows were beginning to recede by the November 2023 (~30 000 ML/day over the barrages) sampling event and were lower still at the March 2024 sampling event (~10 000 ML/day – Figure 6). As the water levels receded over the sampling period, the locations sampled in August were dry in November and March. This meant cores were taken in November and March at closer toward the water's edge at each location.
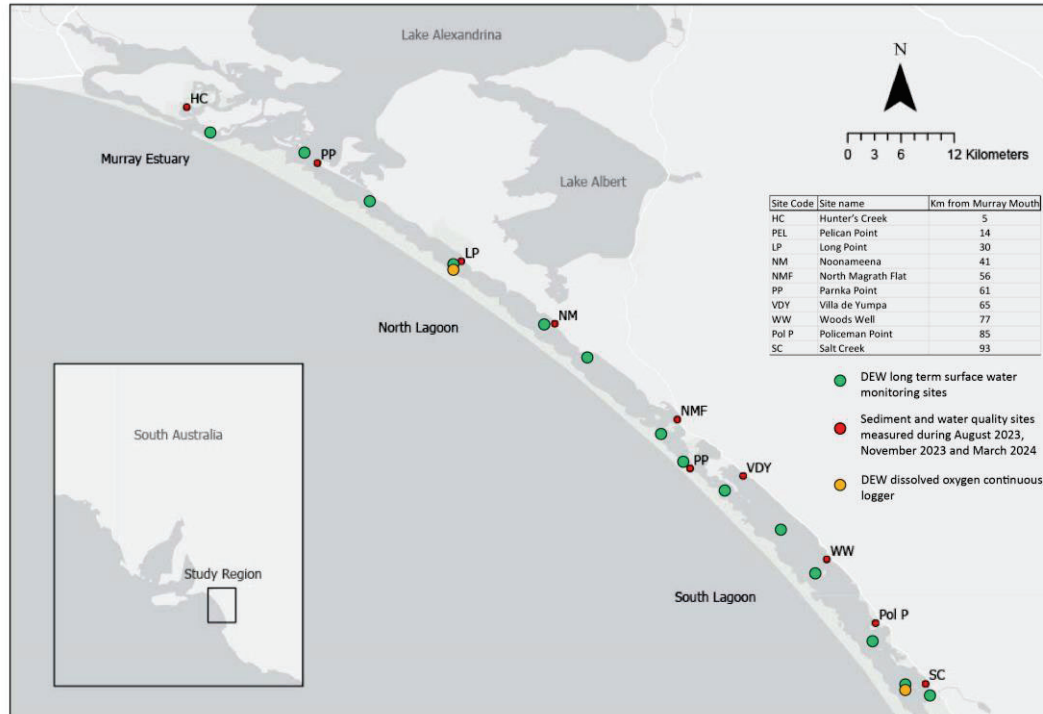
Figure 2 Map of DEW long term surface water monitoring locations (green), sediment and surface water site locations (red) and DEW dissolved oxygen continuous logger locations (orange)

2.3 Field sampling protocol

At each location (*n* = 10), surface water quality field parameters were measured, including pH, dissolved oxygen (DO), electrical conductivity (EC), turbidity (NTU) and temperature (degrees C) using a calibrated YSI 556 multi-meter. A surface water sample was also filtered through a pre-washed 0.45 µm filter for analysis of dissolved nutrients. Samples were immediately stored in an ice box and then frozen within 8 hours of sampling.

A 20 cm deep sediment core was then taken using a 60 cm long clear acrylic tube. The tube was pushed into the sediment and then surface sediment was dug out around the base of the acrylic tube using a shovel, allowing the intact core to be retrieved by hand (Figure 3). Once retrieved, a rapid assessment protocol (RAP) score was recorded for the core as per Hallett et al (2019). Briefly, RAP assigns scores from 1 to 5 based on sediment colour, texture and odour criteria. Low scores indicate poorer sediment quality for each criterion. The scores for individual criteria were summed to produce a total RAP score. Presence of aquatic plants and macroinvertebrates in the sediment core are also recorded.

Following RAP assessment, the core was photographed and sectioned into 6 x 2.5 cm increments (0-2.5 cm, 2.5-5 cm, 5-7.5 cm, 7.5-10 cm, 10-12.5 cm, 12.5-15 cm), and placed in a plastic vial with no air gap for analysis of total nitrogen, total organic carbon, phosphorus, electrical conductivity (EC) and pH and carbon and nitrogen isotopes. A composite sample from 0-10 cm was also taken. Samples were immediately stored in an ice box, and frozen within 8 hours of sampling.

An additional intact core was taken at each site for porewater measurements, using an acrylic tube with predrilled holes at increments of 1.5 cm, 4 cm, 6.5cm, 8 cm, 9.5 cm and 11 cm (for measurement of porewater between the 0-2.5 cm, 2.5-5 cm, 5-7.5 cm, 7.5-10 cm, 10-12.5 cm, 12.5-15 cm increments sampled for sediment analysis). Holes were taped over with PVC tape prior to collection. A water head above the sediment surface in the tube was maintained after collection and rubber bungs were inserted at the top and bottom and secured with tape. Cores were stored upright for transport and immediately returned for processing on land in Meningie (<1hr transit time).

Figure 3 Core collection procedure and intact core after collection.

2.4 Laboratory methods

The cores were processed within approximately 3 hours of sampling. The cores were set vertically using a retort stand and clamps to keep in position (Figure 4).
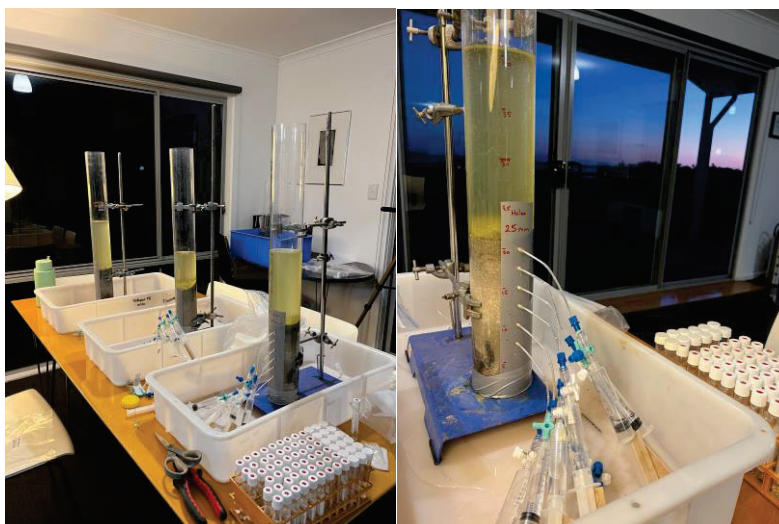
Figure 4 Porewater collection using Rhizon samplers and syringes under vacuum pressure.

The tape covering the holes on the acrylic tube was punctured, and a Rhizon Flex sampler (Rhizosphere Research Products) was inserted into each increment in the sediment core. The Rhizon extended across the internal 60 mm diameter of the acrylic tube at each depth. Rhizon Flex samplers consist of a hydrophilic microfiltration membrane with a pore diameter filter of 0.15 µm. The Rhizon sampler formed a watertight seal with the hole in the acrylic tube, with tubing and a sampling port extending outside the PVC pipe. A 10 ml luer lock syringe and 3-way stopcock were connected to the sampling port at the end of the Rhizon sampler. The syringe was kept under suction pressure in order to extract the sample. Once the syringe was full, the stopcock was turned to the off position for the syringe and the syringe was removed from the Rhizon sampling port. This prevented oxygen ingress into the syringe during sampling. For measurement of hydrogen sulfide, a needle was connected to the stopcock and was then inserted through the double septum exetainer cap of a 12 ml soda glass vial (Labco limited). The glass vial was preloaded with 0.1 ml of zinc acetate for sulfide preservation and was under vacuum pressure (i.e. all oxygen extracted prior to sample insertion). Samples were stored below 4 degrees C until analysed. An additional 10 ml sample was extracted for dissolved nutrients by reconnecting the syringe to the Rhizon sampler under suction pressure. This sample was extracted into in a plastic vial and frozen immediately after collection.

Once back at the laboratory, colourimetric analysis of sulfide was measured via Cline (1969) using a double beam spectrophotometer (GBC UV/VIS 916). Calibration standards for quantification were prepared using sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$). Procedural and instrument blanks were used, and clean handling techniques were applied throughout.

The sediment samples and the surface/porewater samples (except the porewater samples in glass for sulfide analysis), remained frozen and were then sent for analysis at the Environmental Analysis Laboratory, Lismore, Australia, a NATA accredited laboratory.

For the sediments, total nitrogen and total carbon were measured via high temperature combustion and infra-red detection using a LECO CNS TruMAC Analyser. Total organic carbon (%TOC) was measured using the same analytical method following pre-treatment of the sample with dilute hydrochloric (HCl) acid added to remove inorganic carbon. Phosphorus was measured with a 1:3 Nitric/HCl digest via method APHA 3125 and measured via ICP MS. Electrical conductivity (EC) and pH were measured on a 1:5 soil:water extract using methods from Rayment and Lyons (2011) (4A1 and 4B1 methods).

Carbon and nitrogen isotopes were measured using a continuous flow isotope ratio mass spectrometer (CF-IRMS. The C/N molar ratio was estimated based on beam area using internal standards with known amounts of carbon and nitrogen along with the samples for each analysis.

Surface and porewater dissolved nutrients, nitrate, nitrite, phosphate and ammonia, were measured via APHA 4500 NO_3, APHA 4500 NO_2^-, APHA 4500 P-G, APHA 4500 NH_3^- H respectively.

2.5 Results

2.5.1 Hydrology

The official duration of the flood (i.e. defined as >100,000 ML/day flow at the SA Border) was from 15 November 2022 to 26 January 2023, although high flows persisted for longer (Figure 5). Flows peaked at 186,000 ML/day at the SA Border on 22 December 2022. Discharges over the barrages were increased at a similar time to the start of the River Murray flood, but the flood peak (~120,000 ML/day) at the barrages did not arrive until 30 January 2023 (Figure 6).

Average water levels increased substantially in the Coorong during the flood period and remained high throughout most of 2023 (Figure 7). Average salinities, measured at continuous DEW monitoring stations on the Coorong, decreased in response to higher flows in mid-2022, and remained relatively low through the flood peak and most of 2023 (Figure 7). Salinities were <60g/L in both lagoons for a substantial period of time, which will be discussed and analysed in more detail below.
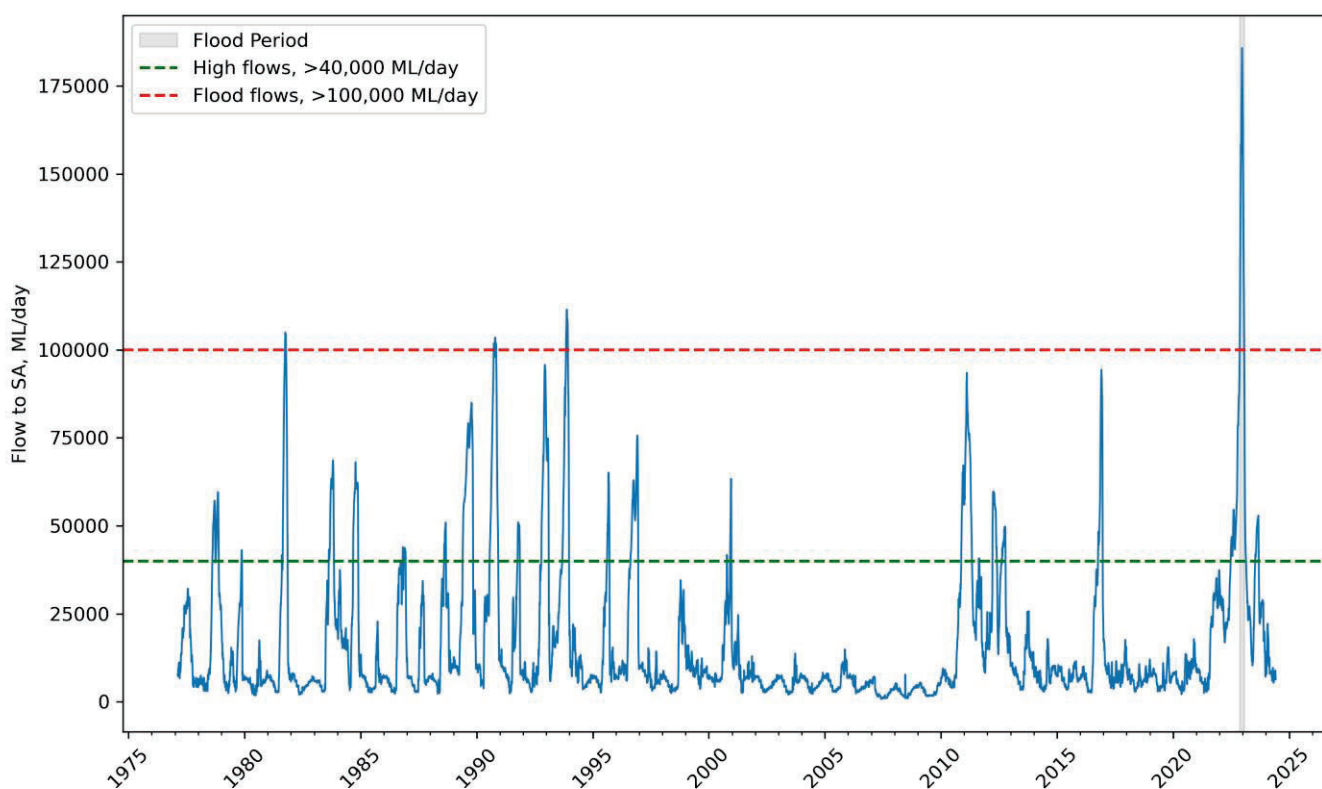
Figure 5 Flow on the River Murray from 1977-2024. The 2022-2023 River Murray flood period is highlighted in grey, with high and flood flow thresholds shown as the horizontal dashed lines.
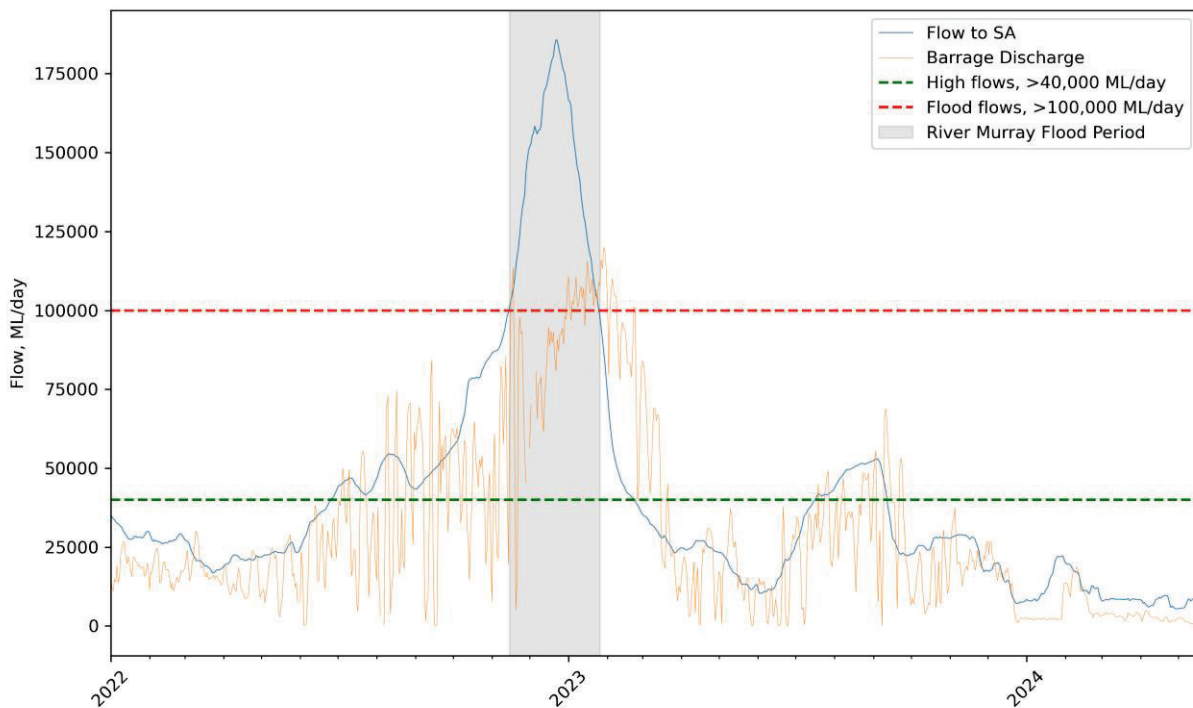
Figure 6 Flow on the River Murray and total discharge from the barrages from 2022-2024. The 15 November 2022 to 26 January 2023 River Murray flood period is highlighted in grey, with high and flood flow thresholds shown as horizontal dashed lines.
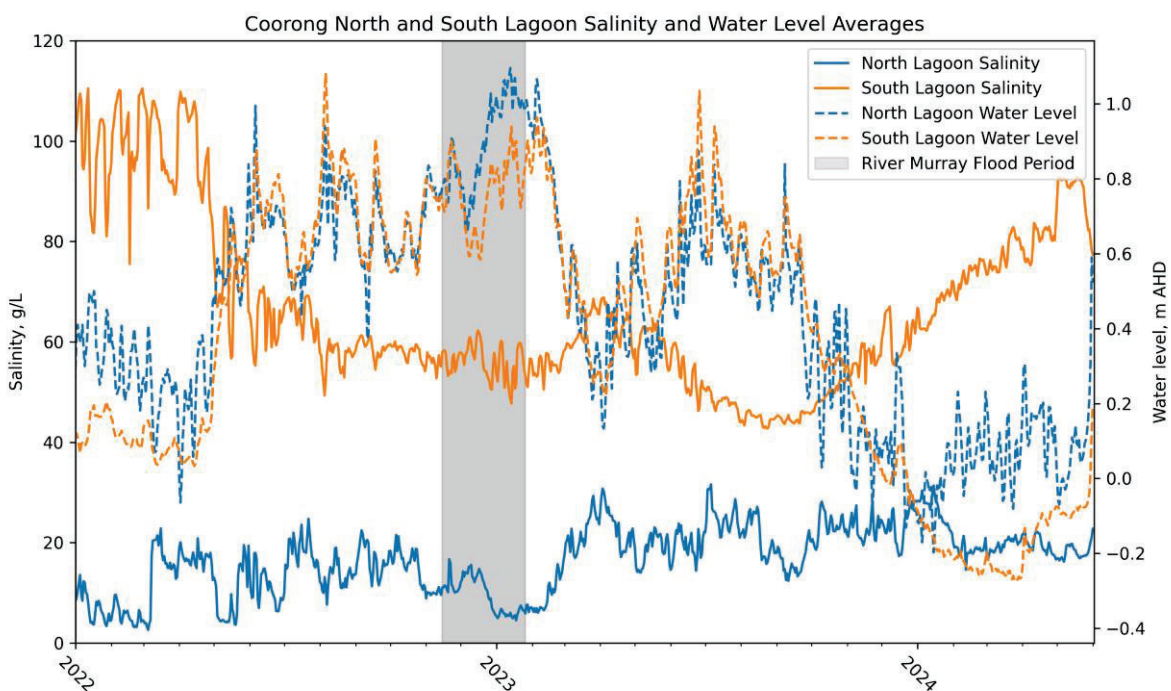
Figure 7 Average water level and salinity in the North Lagoon and South Lagoon from continuous monitoring stations (source: Water Data SA).

2.5.2 Water and Sediment Quality

Pore and surface water results

Surface water quality results

Salinity, total nitrogen and phosphorus (TN and TP), and chlorophyll *a* are shown in for the long-term DEW monitoring sites (Figure 8). As outlined above the salinity decreased throughout both lagoons in 2022-2024. Total nitrogen decreased during the flood, particularly in the South Lagoon. Total phosphorus remained low in the flood and post flood period (July 2022-December 2023; orange line in (Figure 8) from the locations closest to the receiving flood waters (<20km from the Murray Mouth) which recorded higher TP during the flood. This is likely a consequence of the increased concentrations of phosphorus in flood waters from the Murray Darling Basin catchments and floodplains. Chlorophyll *a* levels lowered in the south of the North Lagoon but increased substantially in the South Lagoon during the flood period. In general, levels of salinity, TN and TP were much lower than in the Millennium Drought.
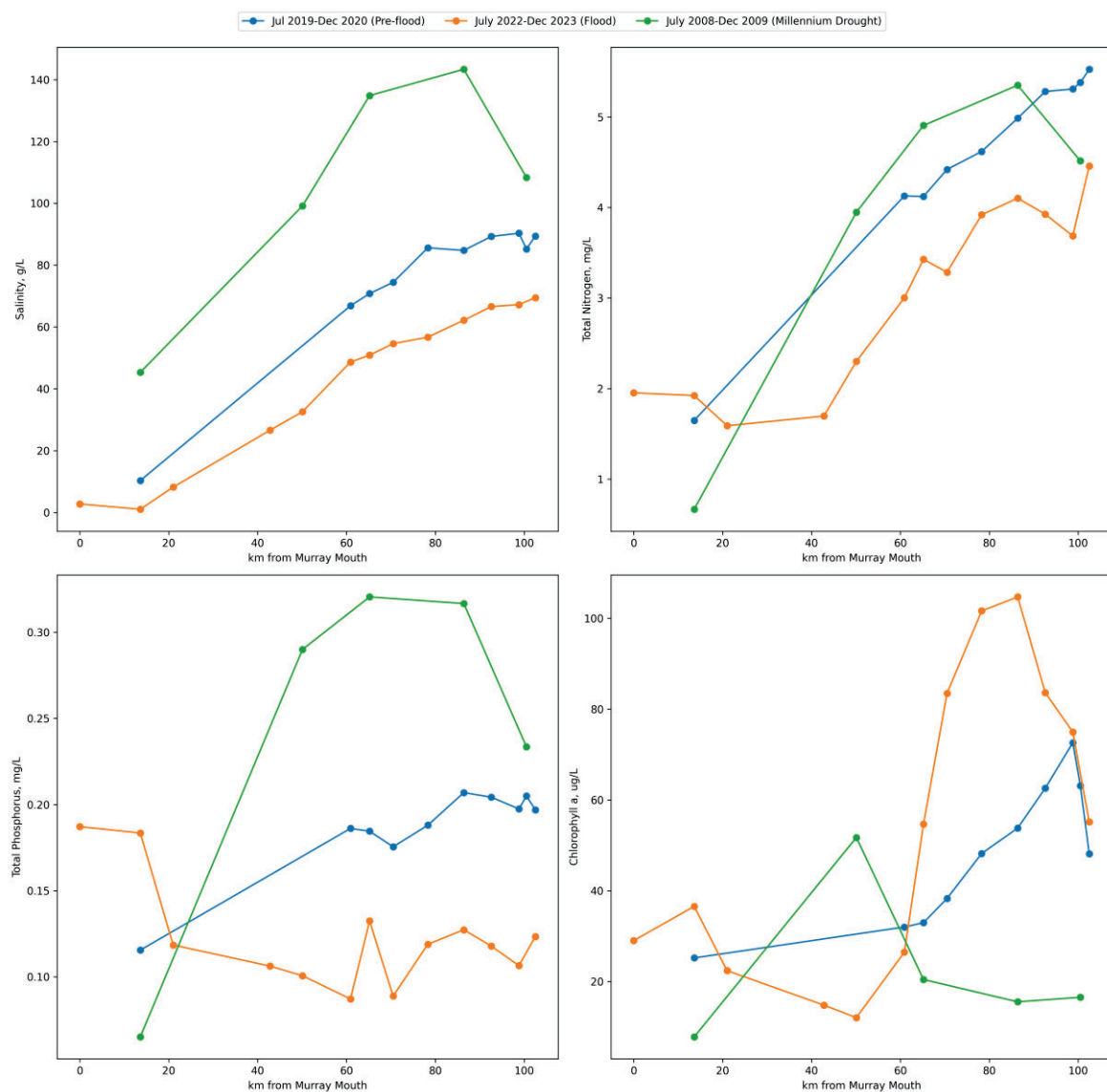
Figure 8 Salinity, total nutrients (TN, TP) and chlorophyll *a* for locations from the North to South Lagoon, as shown with distance from the Murray Mouth and grouped into the periods July 2019 to December 2020 ('pre-flood'), July 2022 to December 2023 ('flood'), and July 2008 to December 2009 ('Millennium Drought').

Filterable reactive phosphorus (FRP) and turbidity were generally higher and more variable in floodwater entering the North Lagoon (<20km from the Murray Mouth, Figure 9). The South Lagoon showed lower FRP in the flood period, and much lower levels than in the Millennium Drought. Turbidity levels were slightly lower in the South Lagoon during the flood period.
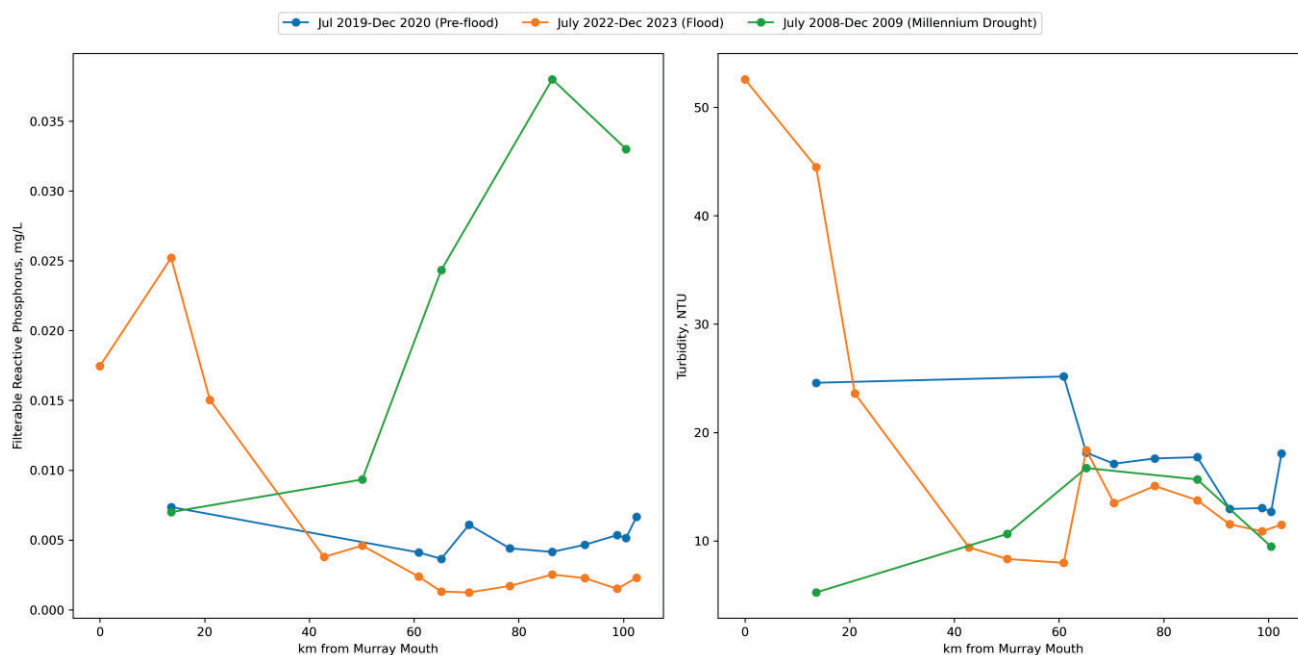
Figure 9 Average filterable reactive phosphorus and turbidity for locations from the north to South Lagoon, as shown with distance from the Murray Mouth and grouped into the periods July 2019 to December 2020 ('pre-flood'), July 2022 to December 2023 ('flood'), and July 2008 to December 2009 ('Millennium Drought').

Dissolved oxygen data from the continuous DEW loggers at Long Pt (North Lagoon) and Snipe Island (South Lagoon and data from the loggers installed in the shallow margins at Villa de Yumpa in this study, are shown in Figure 10. All loggers show large diurnal fluctuations in dissolved oxygen saturation with ubiquitous periods of overnight hypoxia (<50% saturation) and no clear changes due to the floodwaters.
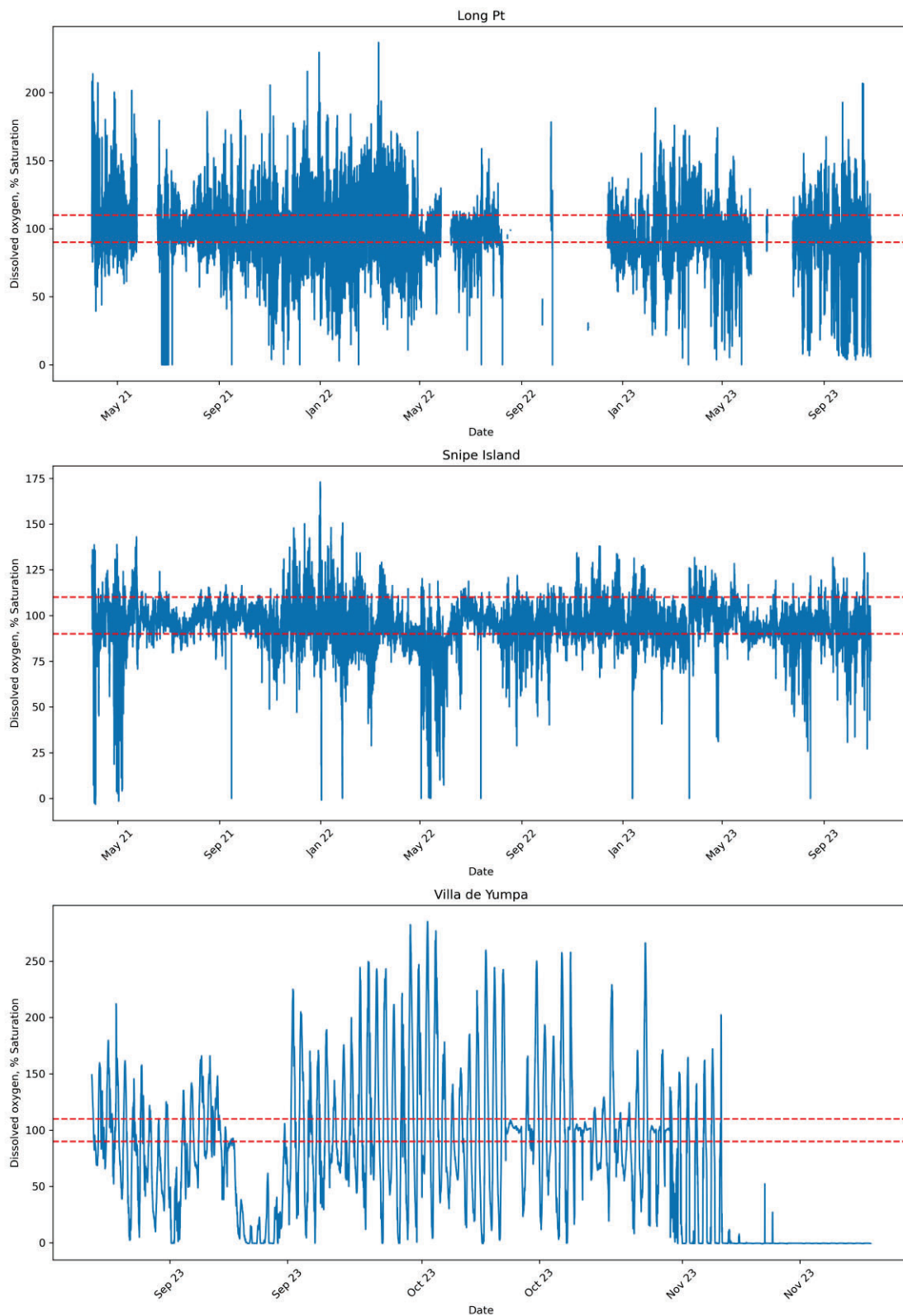
Figure 10 DEW logger data from Long Pt (North Lagoon), Snipe Island (South Lagoon) and study logger data from Villa de Yumpa (shallow water on margin of northern South Lagoon) – ANZECC guidelines for estuaries are shown as red dashed lines.

Surface water

Surface water quality at increasing distances from the Murray Mouth are shown Figure 11 for, salinity (g/L), turbidity, nitrate and phosphorus is shown in Figure 11. Site specific field data for EC, NTU and pH at each sampling period is shown in Table 1 (Appendix A). Locations were measured at the time of shallow margin sediment and porewater sampling (August 2023, November 2023 and March 2024). This sampling period was conducted in the post flood period, with August sampling conducted during the high flow period. Water levels had begun to drop in both the North and South Lagoon by November 2023 and March 2024, with salinity also beginning to rise from January 2024 in the continuous monitoring station in the North Lagoon and from March 2024 in the South Lagoon (Figure 7).

Surface water sampling in the marginal areas in March 2024 shows salinity increasing, particularly in the middle of the Coorong and South Lagoon. Increases were also seen in turbidity in November and March sampling, compared to August, when the flows were the highest. Surface water phosphate was higher in the surface water in August and November in the marginal locations closer to Murray Mouth, again likely due to higher concentrations of TP being brought in with the floodwaters. Nitrate is generally similar along the Coorong except for the mid Coorong locations where it was higher. Surface water pH (shown in Table 1; Appendix A) slightly decreases at most locations between August 2023 and March 2024.
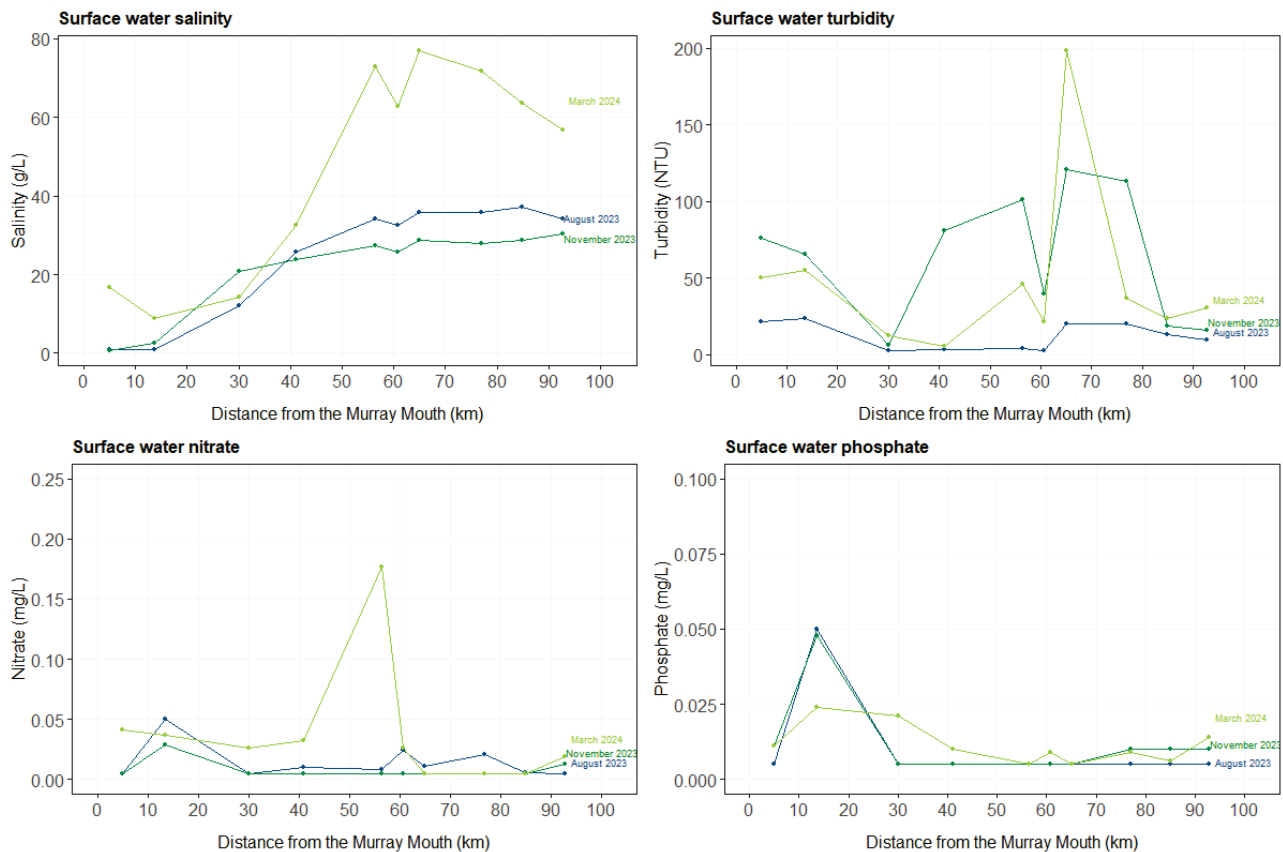
Figure 11 Surface water salinity, turbidity, nitrate and phosphate in the Coorong with distance from the Murray Mouth at three time periods; August 2023, November 2023 and March 2024.

Porewater

Porewater sulfide and ammonia at each site with depth is shown in Figure 12. As the high flows recede, sulfide and ammonia in sediment porewaters increases. Locations further away from the Murray Mouth (i.e. locations in the South Lagoon) have higher concentrations of amonia and sulfide in the porewaters, and generally ammonia and sulfide are higher in the lower 15 cm portion of the profile compared to the top few cm. Increasing concentrations of ammonia and sulfide in the porewaters indicate that anoxic conditions are

increasing in sediments by November 2023, and dominating at several locations in March 2024. Low dissolved conditions overnight (Figure 10) further supports these observations of anoxic conditions in the sediments.

The mean and maximum concentrations of sulfide (Table 2–Appendix A) are below toxicity thresholds for common seagrass species where experimental data is available. Specifically, Pedersen and Kristensen (2015) found sulfide toxicity occurred for *Ruppia maritima* at 1.8 mM (~60 mg/L) and Zostera marina 0.27 mM (~9 mg/L) which is higher than the mean and maximum sulfide concentrations in the profiles in the Coorong during the study period (Figure 12, Table 2–Appendix A).

Increases in ammonium occurred at North Lagoon locations Hunter's Creek, Pelican Point and North Magrath Flat and at South Lagoon locations Villa de Yumpa, Woods Well, Policeman's Point and Salt Creek (Figure 12). Overall, increases in ammonium were larger in the South Lagoon, than the North Lagoon and were typically in higher concentrations in the lower 15 cm portion of the profile, similar to sulfide. The unionised form of ammonia (NH_3) is potentially toxic. The Australian Water Quality Guidelines for ammonia[1] have trigger values at pH 8.5 and 9.0 of 0.35 and 0.14 mg/L for total ammonia as N respectively. The ammonium values in the Coorong sediment (Figure 12) often exceeded these values, particularly in the South Lagoon as water levels receded. This suggests the possibility of free ammonia toxicity to aquatic organisms and this likely promoted by low dissolved conditions overnight (Figure 10), and limited burrowing invertebrates in the South Lagoon when salinity exceed 60 g/L (Figure 7)

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[1] https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants/ammonia-2000
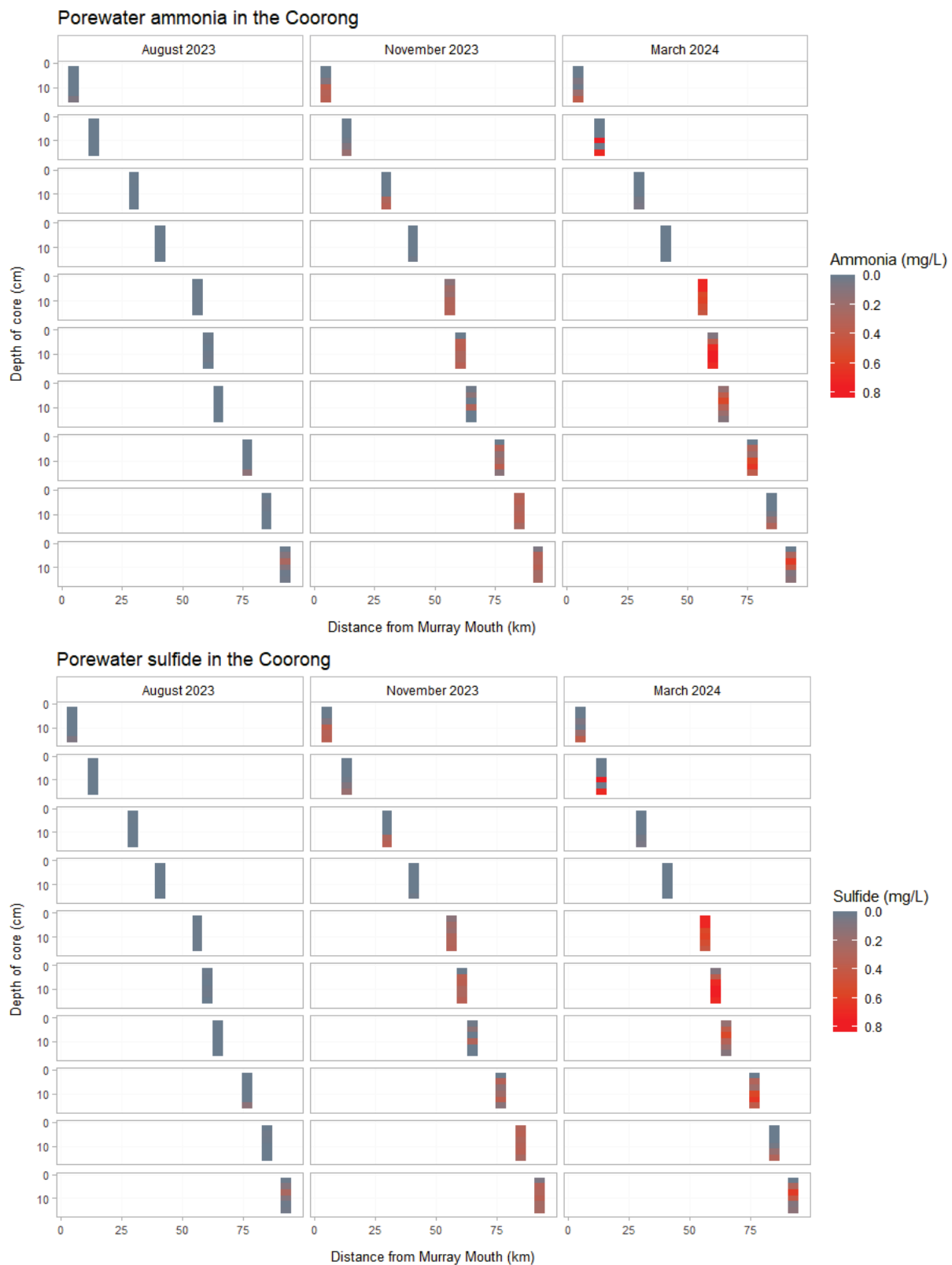
Figure 12 Ammonia (top) and sulfide (bottom) concentrations at each location at each time period with distance from the Murray Mouth, illustrating the concentration gradient at depth.

Sediment quality and isotopic composition

Decreasing RAP scores (Figure 13) from August 2023, November 2023 and March 2024 generally corresponded to the higher sulfide concentrations in the porewater as shown in Figure 12. Some sites, such as Salt Creek, Policeman's Point and North Magrath Flat, corresponded very accurately with the strongly decreasing RAP score, whereas locations such as Villa De Yumpa and Woods Well did not record a significant decrease in RAP score but did increase in porewater sulfide from August to March.
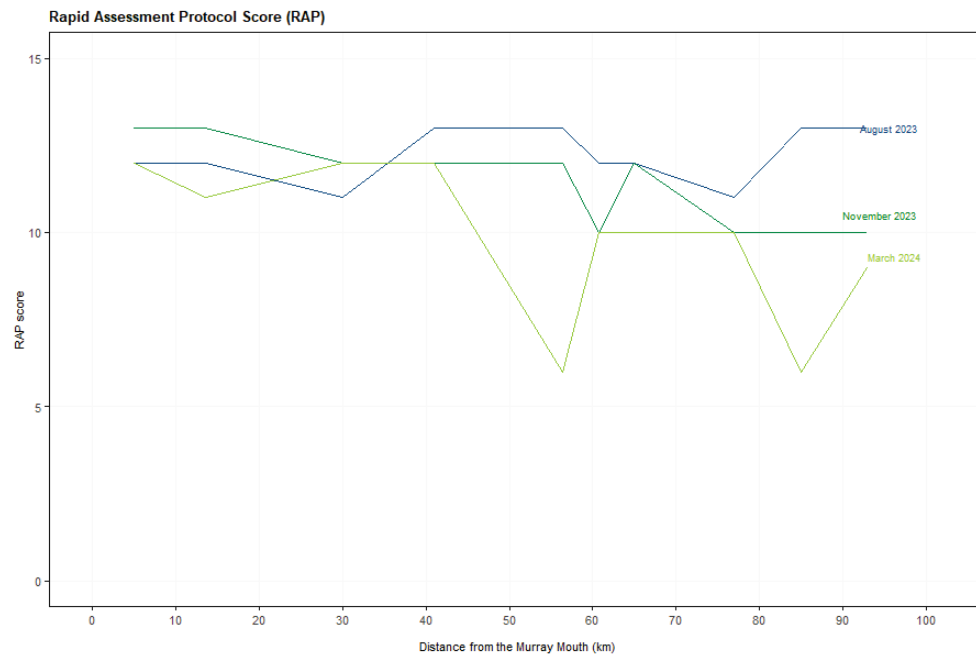
Figure 13 Rapid Assessment Protocol (RAP) scores in the Coorong with distance from the Murray Mouth at three time periods; August 2023, November 2023 and March 2024.
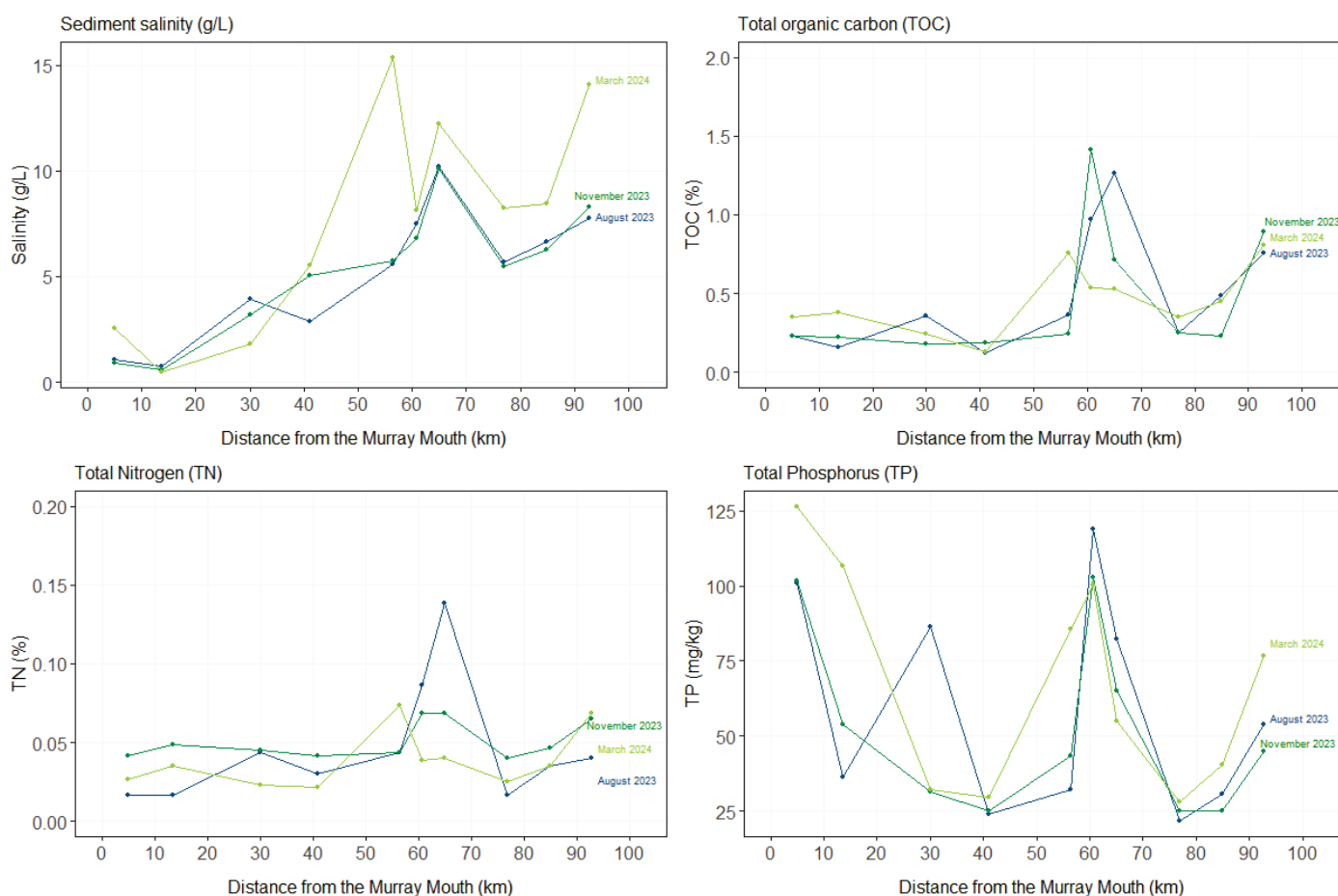
Figure 14 Sediment data (0-15 cm) for locations in the Coorong, as distance from the Murray Mouth for Salinity (dS/m), TOC (%), TN (%) and TP (%) at three time periods August 2023, November 2023 and March 2024.

Sediment salinity (Figure 14 – top left) generally increased with distance from the Murray Mouth. However, salinity was generally lower in March 2024 in North Lagoon, but had increased in the South Lagoon by March 2024. TOC is highest in the middle of the Coorong (Figure 14 – top right). TN and TP (Figure 14 – bottom left and right) also have higher sediment concentrations around this point, indicating this constriction point accumulates organic nutrients which settle into the sediments. Total sediment phosphorus (Figure 14 – bottom right) was also elevated at locations closest to the Murray Mouth, and was highest in the sediments in March 2024, again illustrating that TP in surface flood waters was likely accumulating in the sediments at this time.

2.6 Discussion

Water and sediment quality

Average water levels increased substantially in the Coorong during the flood period, and remained high throughout most of 2023 (Figure 7). Salinity at all locations measured continuously in the Coorong decreased in response to higher flows in mid-2022, and remained relatively low throughout 2023, with salinities < 60 g/L in both lagoons for a substantial period of time in response to the flood. The estuarine area (<35 g/L) expanded greatly in the Coorong during the flood, extending almost

completely through the North Lagoon at its peak. This is a similar finding to that reported in the 1976 flood by Geddes and Butler (1984).

Overall total nutrient levels decreased in the Coorong, particularly in the South Lagoon. This appears due to increased flushing (Mosley et al. 2023), and this is consistent with recent hydrological and mass balance modelling that suggested large floods are needed to flush the South Lagoon of nutrients and salt (Priestley et al. 2022). Despite these overall positive effects on much of the Coorong, floodwaters brought higher concentrations of phosphorus into the North Lagoon, as evident in phosphorus patterns in surface water and sediments. Nutrients in the Coorong also still tended to accumulate in the middle of the estuary around the constriction points (Narrows-Parnka Pt region). Chlorophyll *a* increased substantially in the South Lagoon on the flood recession which may indicate increased nutrient availability or mineralisation of organic matter promoted by the flood and recolonisation by invertebrates or indicate resuspension of benthic microalgae.

As the high flows from the River Murray began to recede, surface sediments also became more anoxic, leading to porewater concentrations of sulfide and ammonia. While sulfide concentration measured in porewaters were below toxic concentrations, porewater ammonia concentrations were possibly toxicity to aquatic organisms (i.e. in excess of Australian Water Quality guidelines for marine systems).

The flood oxygenated sediments and decreased salinity in both surface waters and sediments. This occurred rapidly and persisted through the subsequent high flow period, allowing for conditions favourable for aquatic plant, macroinvertebrates and fish as outlined further below.

3 Aquatic plants and algae

3.1 Introduction

The status of the *Ruppia* Community and the persistent filamentous algal community has been of concern due to the fundamental role that submerged aquatic macrophytes have in the maintenance of healthy coastal lagoon ecosystems (Waycott and Lewis 2022). This *Ruppia* Community includes *Ruppia tuberosa* and *Althenia cylindrocarpa* during periods when hypersalinity is extreme (>60 g/L over the year) and other species during periods of lower salinity (Asanopoulos and Waycott 2020). The condition of the macrophyte community in the southern Coorong following the River Murray Floods of 2022-2023 was of particular interest as it had only recovered to a reasonable level following the Millennium Drought (Waycott et al. 2022). In addition, locally relevant, site-specific observations of the environmental condition of the littoral zone where plants were proposed to be sampled including sediment condition, water level and local salinity. Results were compared to previous data sets available using equivalent methodologies specifically those reported in Lewis et al. (2022) and Waycott et al. (2022).

The contemporary model of the growing season for the *Ruppia* community, summarised in detail in Asanopoulos and Waycott (2020) and Waycott et al. (2022), is linked to water levels, salinity and temperature. In summary the warmer conditions at the end of spring (October–November) lead to increasing salinities and falling water levels in the littoral zone. During this period, as water levels drop and salinity increases, the regular cycle is that the submerged aquatic plants senesce (die-back) and reproduce via set seed and/or forming turions. The success of reproduction is dependent on a number of factors which have been modelled in detail (e.g. Collier et al. 2017, Hipsey et al. 2020, Hipsey et al. 2022) with considerable field validation in the Coorong from these recent studies. Widespread surveys during the period when flowering and/or turion formation are indicative of the status of the system before, or as, the *Ruppia* Community dies off as water levels drop and salinity increases leading to reduction in biomass and the annual cycle of local die-back. When combined with regular surveys to capture the inter-annual variation in the status of the system, monthly sampling for example, the condition estimates can be better interpreted given the hyper-variability evident in the system (Waycott et al. 2022).

3.2 Methods

3.2.1 Aquatic plant community field sampling and sample processing

The condition of the *Ruppia* Community was evaluated to assess its status at two spatial and temporal scales following the River Murray Floods of 2022-2023. Additional data was obtained from sampling undertaken prior to the 2023 River Murray flood conditions using similar protocols the year before to monitor the *Ruppia* Community following unregulated flows in Spring of 2021.

Widespread surveys of the *Ruppia* Community and filamentous algae were conducted late in the growing season (early-mid Summer) were conducted following protocols established in Lewis et al. (2022). In the 2022/23 growing season 108 locations were visited and in the 2023/24 growing season 132 locations were visited (Figure 15; location data available on request).

Monthly sampling was undertaken at seven reference sites (Table 1, see Figure 2 for locations) using the methodology of Waycott et al. (2022) to assess biomass, reproductive status (flowering, seed bank, turion abundance), local site environmental conditions including sediment condition (Rapid Assessment Protocol, RAP Hallett et al. 2019 see section 2.3 for description). Sample processing of

turion counts and seed banks was changed to an estimation method (Table 2) due to time constraints and where data is combined it is noted in the results.

Note that sediments were captured where possible during sample processing and any remaining were stored and returned to the Coorong on a later trip in to support the concerns of First Nations communities regarding removal of sediments from the region.

Plant tissue nutrients (see Waycott et al. 2022) for the *Ruppia* Community were analysed from samples collected using standard core samplers (75 mm diameter core) and collecting replicate cores where seagrass was present. It was necessary to collection up to five replicates to obtain enough material for analysis (i.e. final amount per sample required was 1-3 g DW).

Filamentous algae were collected using a scoop of 0.8-1 mm mesh size, macrophyte material was removed manually and the algae placed into a labelled bag for return to the laboratory where the algae was placed into paper bags and dried, as were the *Ruppia* Community plant material, at 60°C for 48 hrs and then weighed on a 5 decimal point balance (g DW). These samples were submitted to the laboratory for nutrient analysis.

Where needed, samples were kept on ice in transit to the laboratory where they were frozen before transportation on ice by courier to the laboratory for analysis. Laboratory analysis for tissue nutrients was conducted by Environment Analysis Laboratory (EAL), Southern Cross University, New South Wales.
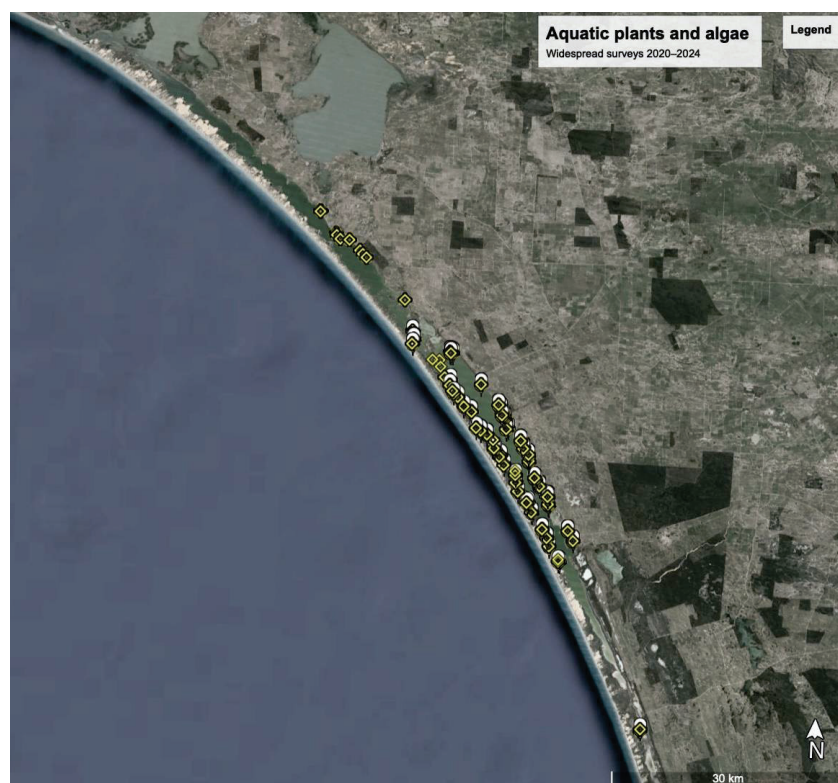
Figure 15 Map of aquatic plant widespread survey sampling sites included in this study.

Table 1 Sites where monthly sampling of aquatic plants and algae occurred (location code in Figure 2).

REFERENCE SITE NAME	LOCATION CODE	SECTION	LATITUDE	LONGITUDE
Noonameena	NM	north	-35.756382	139.261225
North Magrath Flat	NMF	central	-35.853038	139.38466
Parnka Point	PP	central	-35.902472	139.398427
Villa de Yumpa	VDY	south	-35.909831	139.451147
Woods Well	WW	south	-35.993921	139.538071
Policeman Point	Pol P	south	-36.058815	139.585598
North of Salt Creek	SC	south	-36.119907	139.636311

Table 2 Seed and turion scoring categories adopted in 2024 (per core, 7.5 cm diameter and 10 cm deep) based on typical numbers seen in previous results (e.g. Lewis et al. 2022, Waycott et al. 2022).

CATEGORY NAME	ESTIMATED NUMBER (AS RANGE) OF SEEDS OR TURIONS	MID-POINT OF RANGE FOR COMPARATIVE PURPOSES
None	0	0
Very few	1–10	5
Few	11–50	30
Many	50–100	75
Lots	101+	150

3.3 Results

3.3.1 Widespread survey of the *Ruppia* Community in the southern Coorong

Using Figure 7 as a reference point to summarise comparisons of the timing (date of year) of an equivalent water level drop in the southern Coorong system, the month of year that a drop to 0.2 AHD varied between February (2022), March (2023) and November 2023 (staying low into 2024) and comparison to data available on Water Data SA the month of year the water level dropped in 2020/21 would have been November 2020. The trends in biomass across these four years (Figure 16) indicate widespread and significant biomass across each year, the most recent year having lowest results. The timing of the series of widespread sampling events was significant as for each year the sites were experiencing different conditions:

- 2020/21 was sampled from September–October 2020 when water levels began dropping (Lewis et al. 2022).
- 2021/22 was sampled in December 2021 when water levels were similar to the previous year (Lewis et al. 2022).
- 2022/23 was sampled in January 2023 as water levels remained high due to unregulated flows occurring in late Spring 2022 (Figure 17).
- 2023/24 was sampled November 2023 with high water levels making some sampling difficult (Figure 17).

However, the surveys have captured the high biomass of plants at sites after a period of high water levels and lower salinity (2021/22–2022/23) (Figure 16) and the subsequent decline of biomass in 2023/24 when the water levels dropped rapidly in late 2023 leaving the *Ruppia* Community exposed. Interestingly water levels are slow to return to more typical early winter levels this year (June 2024). At the time of sampling, the water levels above plants were similar in the first three sampling periods (Figure 17), however in 2023/24 water levels had dropped by the time sampling was undertaken. Visualising the overall trend in more detail (Figure 18) there is a trend in plant performance despite highly variable results for individual samples. The poor performance of the 2023/24 survey is discussed further in the context of monthly sampling below.

The reproductive status of the *Ruppia* Community also varied with year sampled (Figure 19), the most notable being a shift from seed production in 2020/21 to turion production in 2021/22. The highest production of seeds and turions occurred in 2022/23 following the year of higher water levels and lower salinities for longer periods, however, the flood levels dropping rapidly led to a short term reduction in the biomass do not appear to have impacted the seed bank in 2023/24 (Figure 19 A.) when viewed in these categories.

The presence of significant filamentous algal mats continues, few higher coverage areas were observed in the southern end of the Coorong South Lagoon, and few outside the sheltered constricted areas of the central region of the Coorong (Figure 20). The majority of sites had only drift algae or no algae, the highest coverage occurring in 2021/22 and 2023/24, when water levels had already dropped.
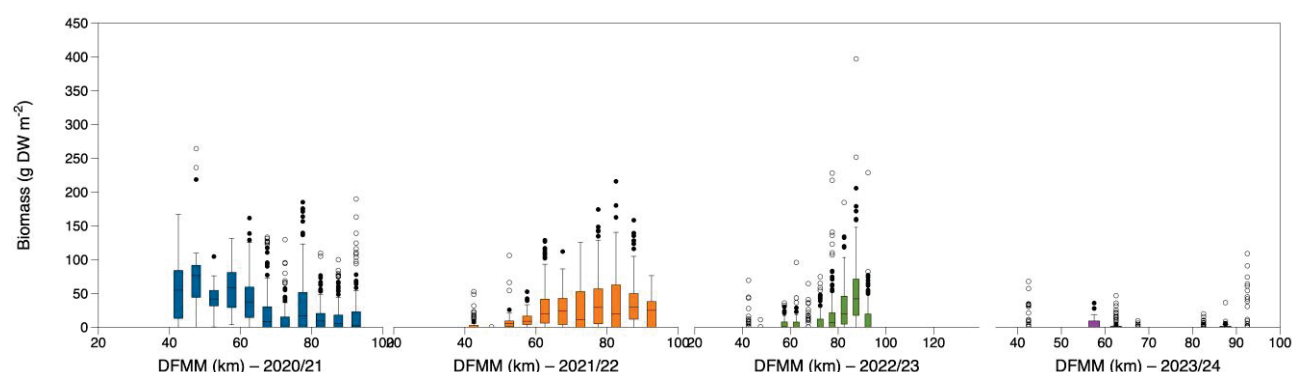
Figure 16 Box plots (inter quartile range, IQR, plus outliers) for Biomass (g DW m^{-2}) plotted along the distance from Murray Mouth (DFMM; linear) for the different growing seasons with available data. More than 100 site samples (Figure 15) are included in the survey each year and are from comparable locations.
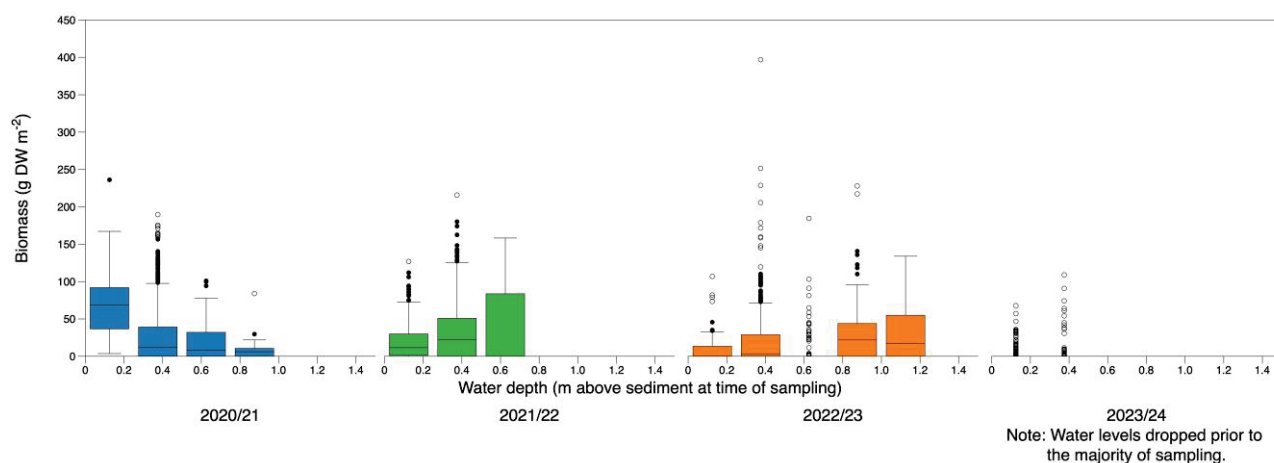
Figure 17 Box plots (inter quartile range, IQR, plus outliers) for Biomass (g DW m^{-2}) plotted against water depth at time of sampling for the four different growing seasons.
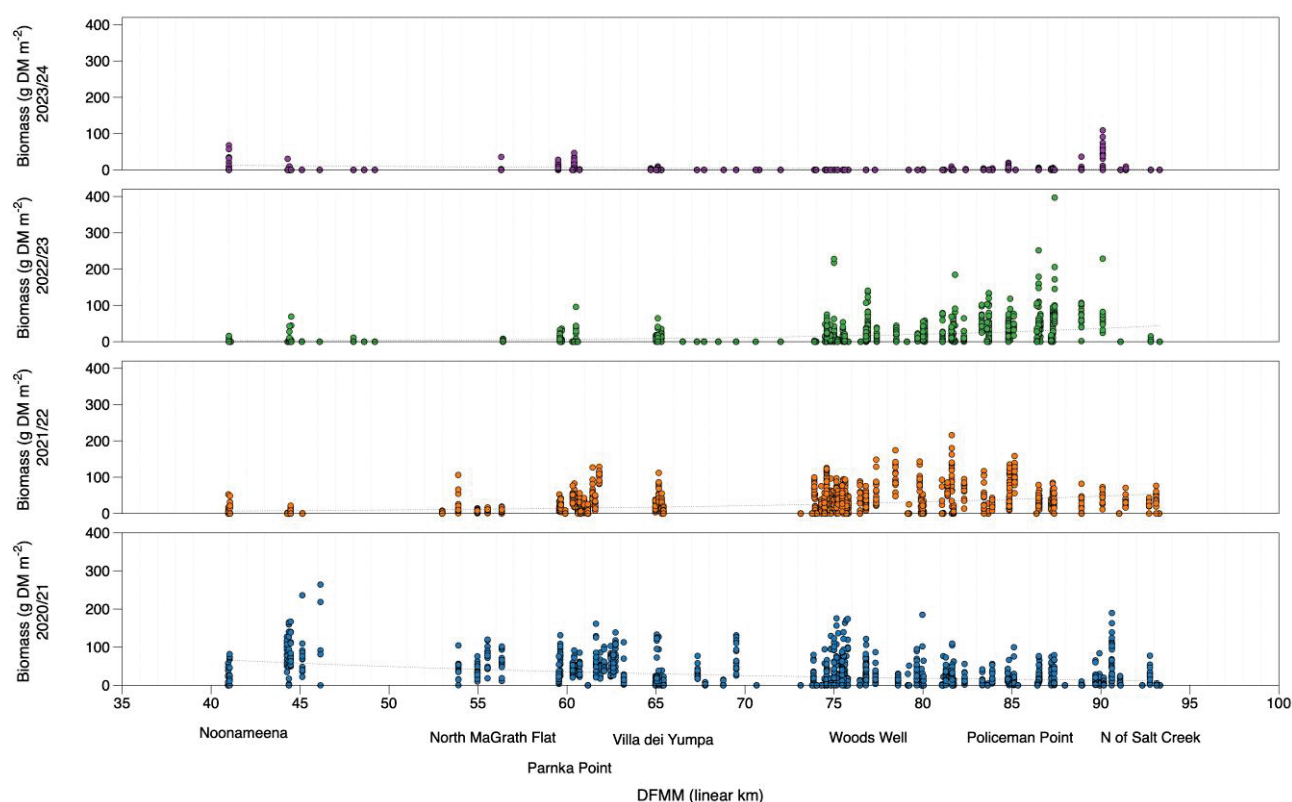
Figure 18 Individual sample biomass estimates (g DW m^{-2}) plotted along the distance from Murray Mouth (DFMM; linear) for the different growing seasons with available data. For reference key sites are indicated below the axis.

A.

B.

C.
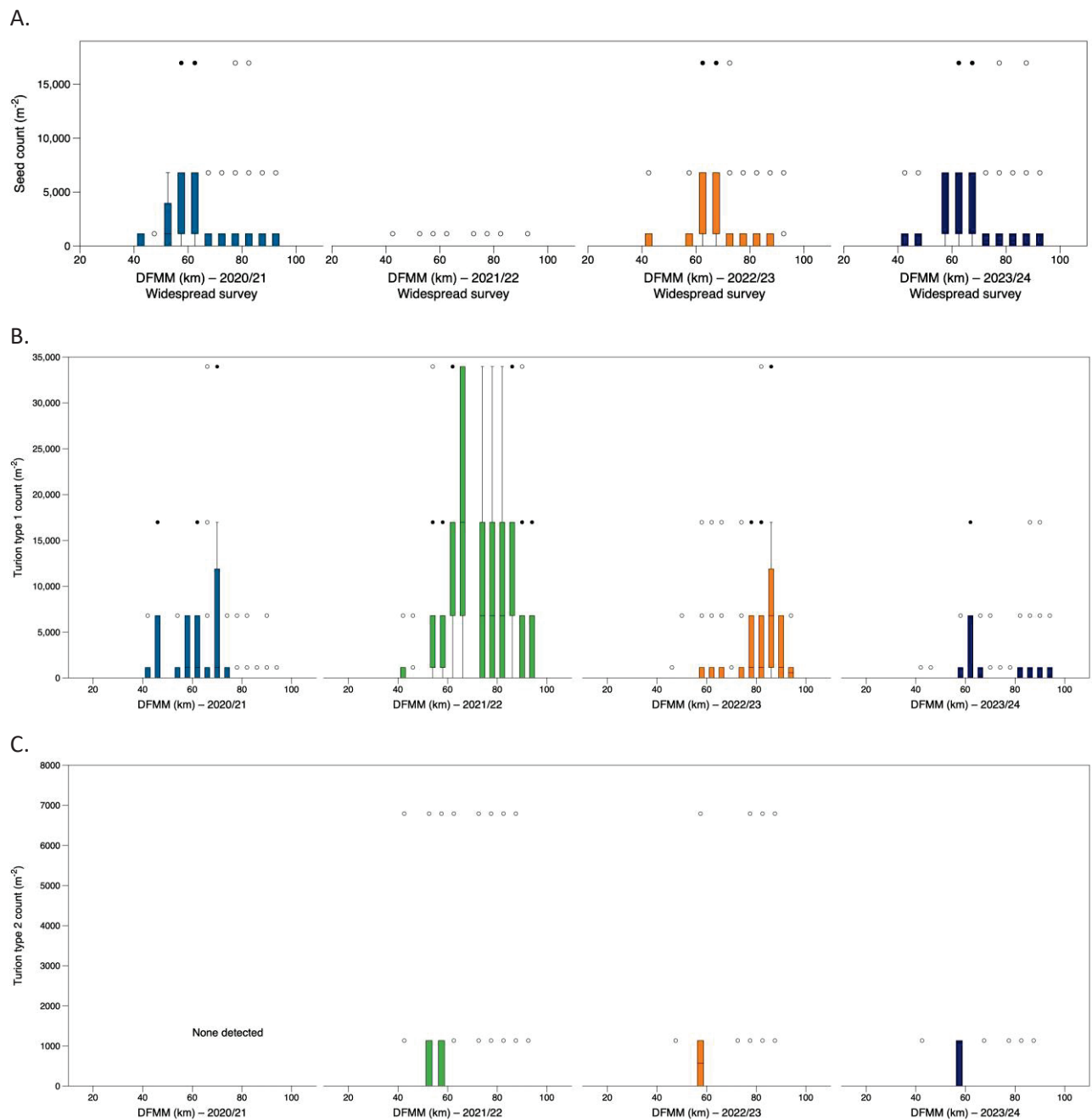
Figure 19 Box plots (inter quartile range, IQR, plus outliers) for A) seed count (number of seeds m^{-2}), B) turion (type 1) count (number type 1 turions m^{-2}) C) turion (type 2) (number type 2 turions m^{-2}) plotted against distance from Murray Mouth (DFMM; linear) for the four different growing seasons. Note for comparative purposes the scoring has been summarised to the estimate clusters used in the most recent sampling period (Table 2).
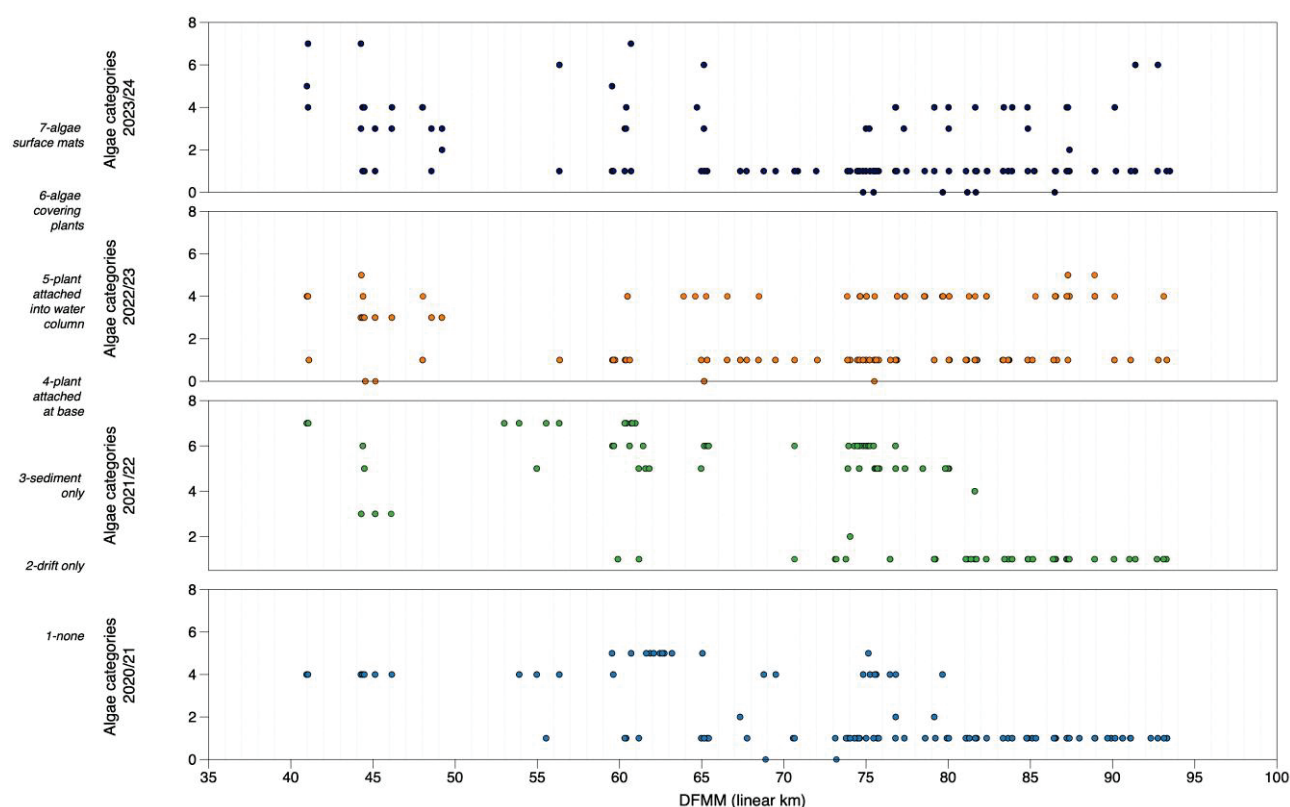
Figure 20 Category scoring based algal community status at each site sampled plotted along the distance from Murray Mouth (DFMM; linear km). Years plotted separately, the most recent at the top.. For reference the algal growth form categories from Waycott et al. (2022) are included on the y-axis.

3.3.2 Observations of a diversifying *Ruppia* Community

The ongoing presence of a multispecies *Ruppia* Community which normally includes *Ruppia tuberosa, Althenia cylindrocarpa* along with another to be named *Ruppia* species, was reinforced by the detection of both *Ruppia megacarpa* and the Charophyte *Lamprothamnium papulosum* during field surveys in this study and others (Faith Coleman personal communication and samples provided 2023). The lower salinities and improved water quality (both water clarity and change in chemistry; see Chapter 2.5) will have enabled the recruitment of these species from seed banks and persistent cysts. Waycott et al. (2022) observed the growth of *Lamprothamnium* in experimental treatments from sediment cores containing *Ruppia* Community plants and the recent recovery of numerous sites of this previously widespread species suggests their reproductive structures remain in sediments for a long time. The seeds of *Ruppia megacarpa* have been observed in seed samples throughout the four sampling seasons to date, although previously at low frequencies. It is likely these seeds enter the Coorong from adjacent lower salinity water bodies e.g. the Salt Creek regulator outflow and the Coorong North Lagoon Goolwa Channel and the lower lakes, where this species is common.

3.3.3 Monthly surveys of the *Ruppia* Community and associated filamentous algae

To evaluate the changing cycles of growth each year monthly sampling was undertaken across seven reference sites (see Table 1 and refer to Figure 2 for locations). Plant performance parameters measured included biomass (g DW m^{-2}), seed counts (m^{-2}), turion counts (both type 1 and type 2, m^{-2}), site sediment condition (RAP scores), water levels and salinity at the time of sampling. Except for a period between January 2022 and November 2022 monthly sampling has been underway since December 2020 (Figure 21). Sites showed a decline in biomass with the onset of lower water levels, most recently leading into the summer of 2024 (e.g. Figure 21 A. 'Woods Well'). Overall, despite the floods plants remain present in the system and anecdotally seedlings germinating in a June 2024 survey indicate ongoing recovery (C. Urgl field observation 7 June 2024). In southern locations turions remain present, however these are dominantly type I turions, so relatively unformed and not ready to act as a perennating organ.

Since the establishment of monthly sampling the seasonal signal of biomass is well established (Figure 22 A.). The very high biomass in the summer of 2022/23 followed a season with extended periods of high water levels and moderate salinities. Seed counts remained consistent throughout the period on average across all reference sites (Figure 22 B.) although as would be expected, a high degree of spatial heterogeneity in seed counts exists (Figure 21 B.). Turion formation also continues, although at a reduced numbers (Figure 22 C.).

Maximum individual samples for seed counts regularly exceeds the current threshold for resilience (>2000 seeds m^{-2}) and even exceeds the 2029 long term resilience targets (Paton et al. 2017) of >10,000 seeds m^{-2} for some samples.

Filamentous algal mat formation continued to occur where the *Ruppia* Community remained protected and well established (e.g. Figure 24 North Magrath Flat). Southern sites, particularly Policeman Point and North of Salt Creek, remained virtually clear of filamentous algae.
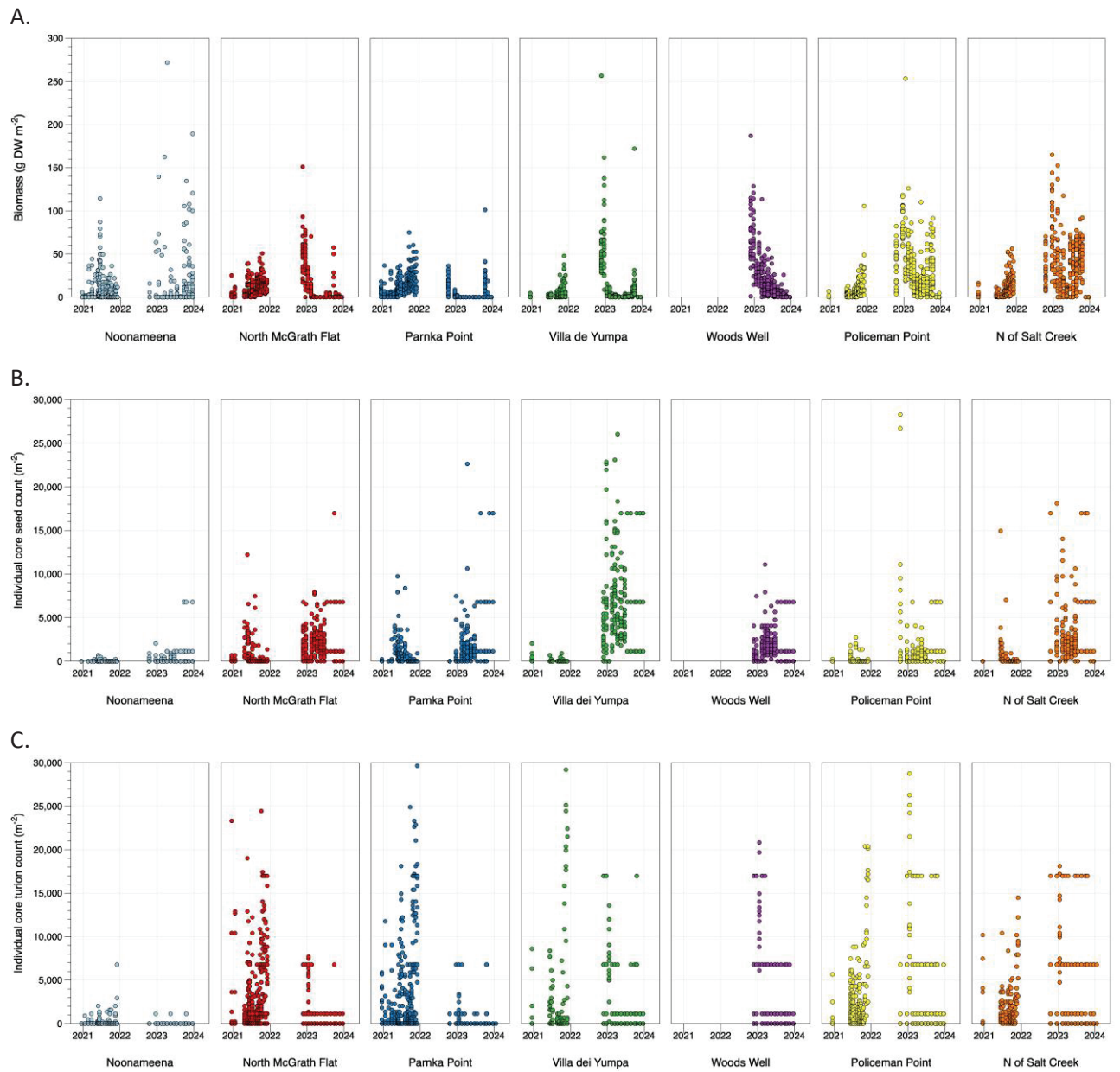
Figure 21 Individual plots for samples collected at monthly samples (plotted separately by site) for A) biomass (g DW m⁻²) B) seed count (number of seeds m⁻²), C) turion count (type 1 and 2) (number turions m⁻²) plotted by date.
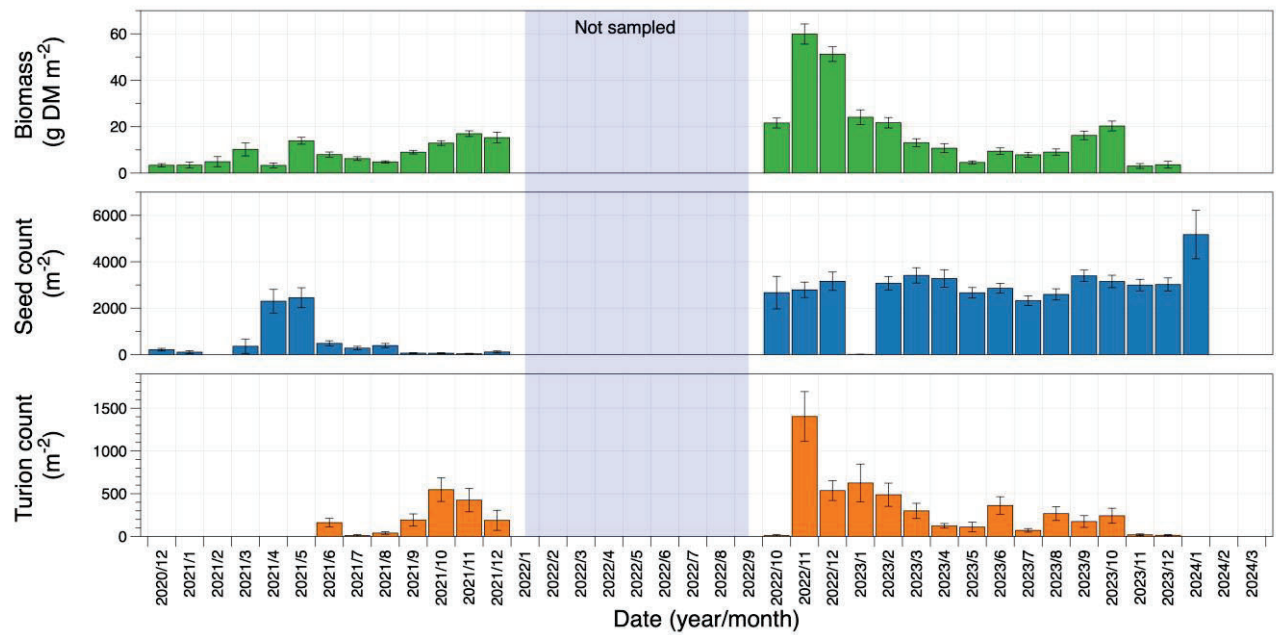
Figure 22 Mean of three measures of *Ruppia* Community status for samples collected (plotted combining all seven sites; see Table 1) for A) biomass (g DW m⁻²) B) seed count (number of seeds m⁻²), C) turion count (type 1 and 2) (number turions m⁻²) plotted by date of month sampled.
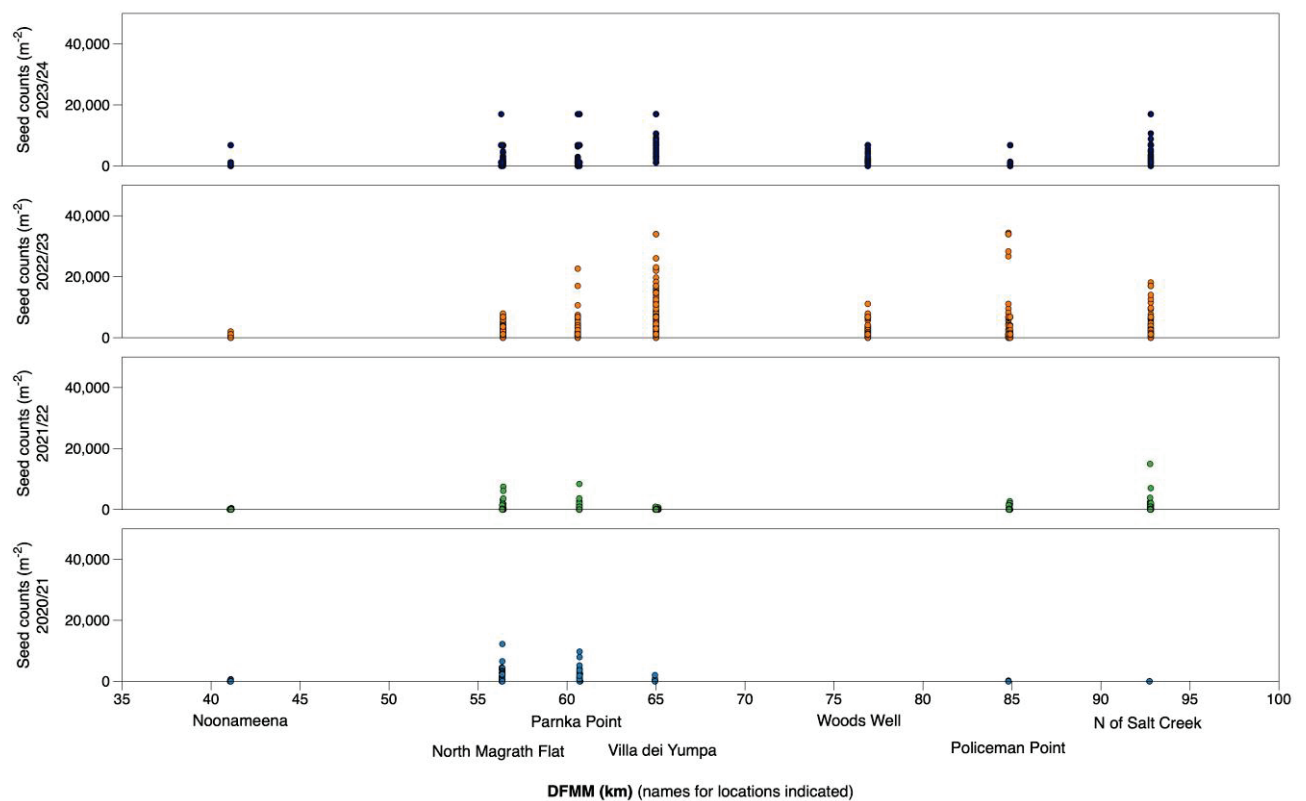
Figure 23 Individual seed core values (count per m⁻²) for *Ruppia* Community samples collected at all seven sites for all sampling times in each growing season; see Table 1) for A) biomass (g DW m⁻²) B) seed count (number of seeds m⁻²), C) turion count (type 1 and 2) (number turions m⁻²) plotted by date of month sampled.
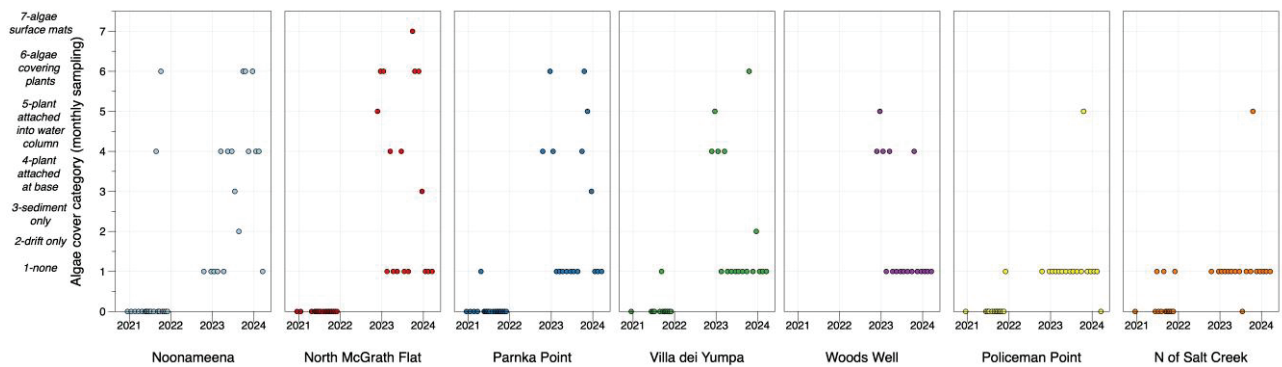
Figure 24 Category scoring for individual site algal community status from observations collected when monthly sampling of the *Ruppia* Community were undertaken (separately by site) plotted by date. For reference the algal growth form categories from Waycott et al. (2022) are included adjacent to the y-axis.

3.3.4 Sediment condition, salinity changes and the *Ruppia* Community

The critical nature of the relationship between the performance of the *Ruppia* Community as measured by its biomass and seed production and the condition of sediment remains of interest given the dynamics of the Coorong system. Comparing all sites surveyed in the widespread survey and across the four years of samples, the highest biomass occurs where the plants are growing in a high score Rapid Assessment Protocol, (RAP; Hallett et al. 2019; section 2.3) (Figure 25). Comparing the three different years the RAP surveys have been undertaken in, despite the proximate location of sampling sites (estimated to be ± 100 m between years), different sediment conditions prevailed on average (Figure 26). In a few cases sediments rated more poorly were observed in sampled from the most recent season (Figure 27), perhaps as a result of the sediment mobilisation during the high flow periods experienced during the recent flooding event although the overall trend was improvement across the southern Coorong region sampled.
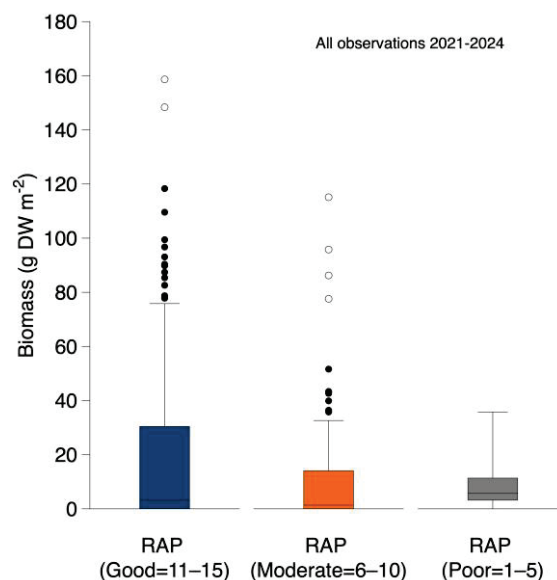
Figure 25 Box plot of plant performance (biomass g DW m^{-2}) for three classes of sediment condition evaluated with the Rapid Assessment Protocol (RAP; Hallett et al. 2019; section 2.3) as described in Waycott et al. (2022).
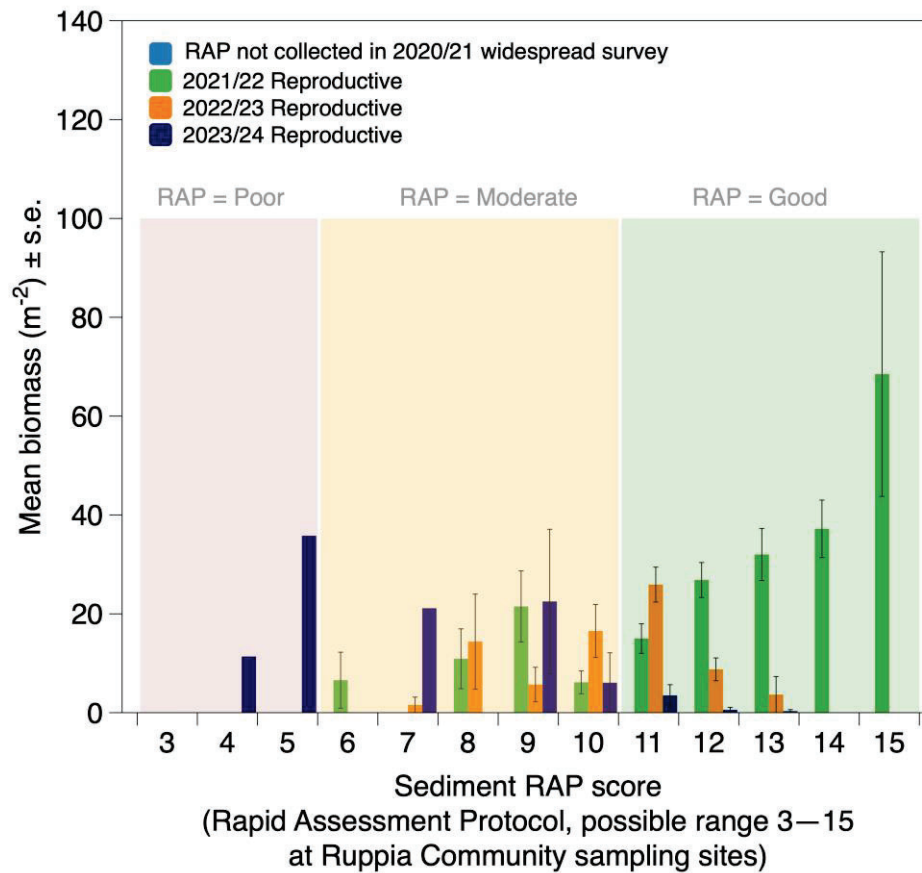
Figure 26 Mean plant performance (biomass g DW m^-2 ± s.e.) associated with sediment condition categories evaluated with the Rapid Assessment Protocol (RAP; Hallett et al. 2019; section 2.3) as described in Waycott et al. (2022). Data were collected during three separate widespread surveys and relative performance ratings identified in the following figure are overlayed in the body of the figure.
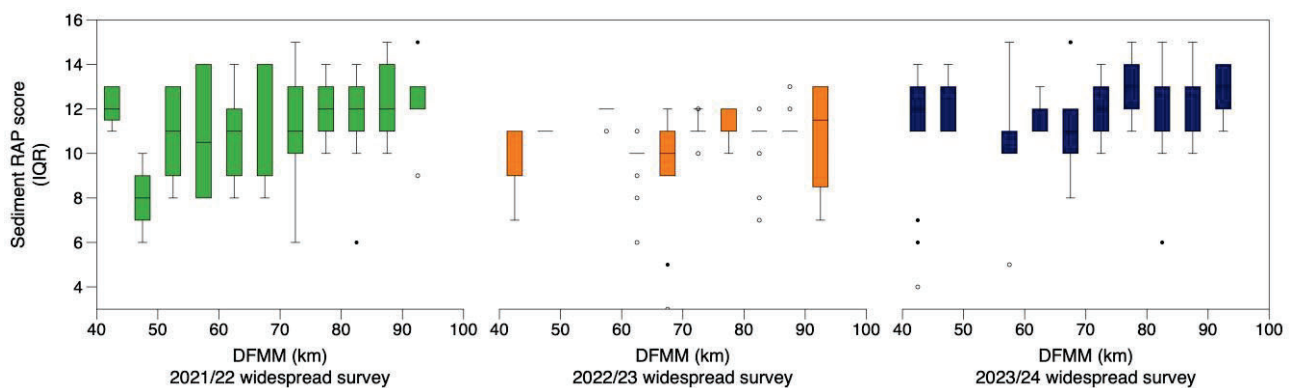
Figure 27 Box plots of sediment condition categories in each of three growing years (2021/22, 2022/23, 2023/24) evaluated using the Rapid Assessment Protocol (RAP; Hallett et al. 2019; section 2.3) as described in Waycott et al. (2022) plotted along the distance from Murray Mouth (DFMM; linear km).

The profile of salinities during the different widespread survey periods was variable (Figure 28) the highest values recorded during the 2021/22 sampling period where some sites had salinities over 100 g/L. Lower salinities were observed during the high flow periods of both 2022 and 2023 (Figure 28).

The Coorong North Lagoon site, Noonameena, lost its *Ruppia* Community when salinities were persistently below 15 g/L (Figure 29) and fewer sites had plants were seen when salinities were lower than 35 g/L.
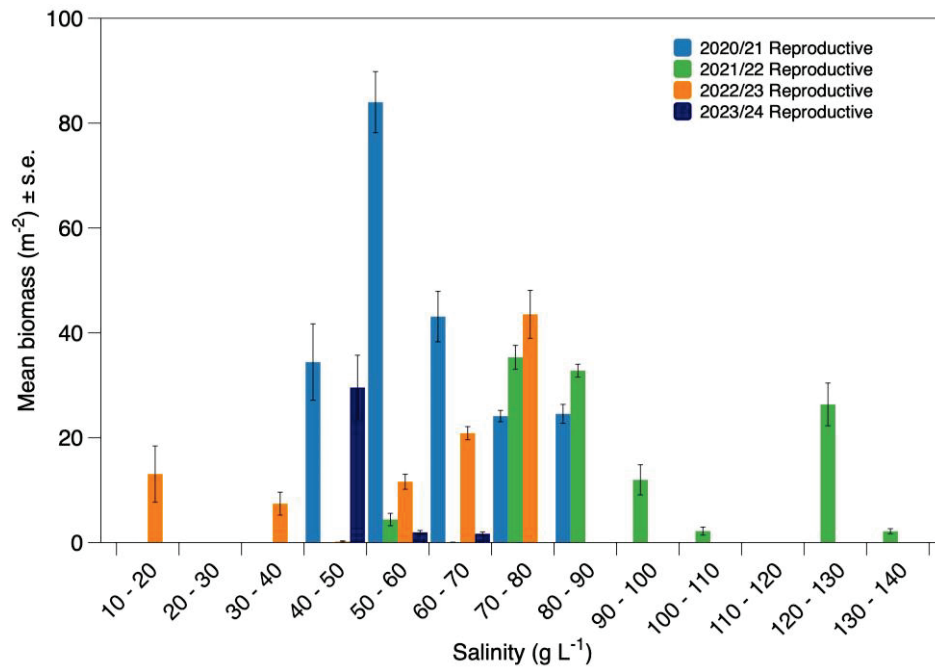
Figure 28 Mean plant performance (biomass g DW m^{-2} ± s.e.) associated with salinity categories (based on salinity measured on site when plants were sampled) at four separate widespread surveys.
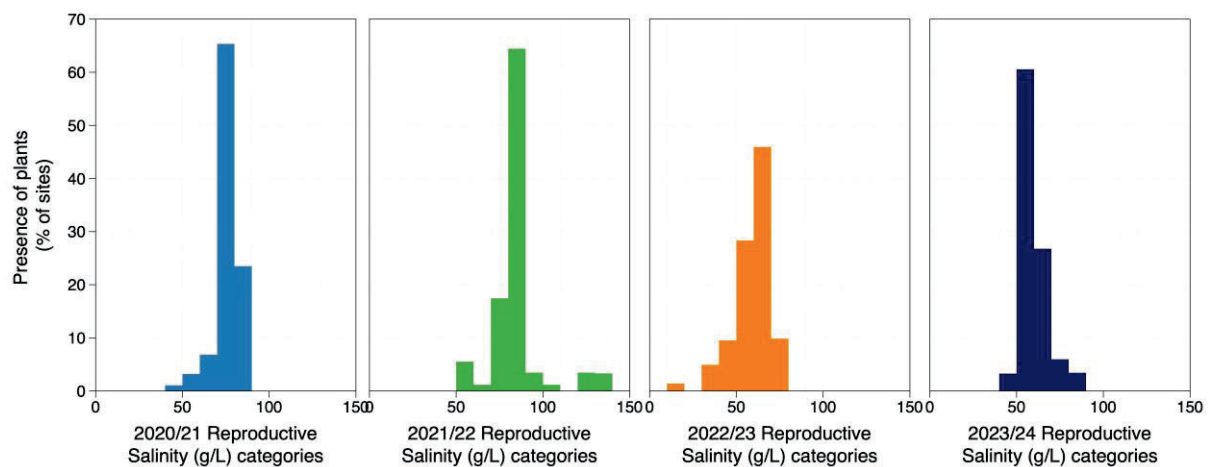
Figure 29 Presence of plants (% of sites with any *Ruppia* Community present) with salinity categories (based on salinity measured on site when plants were sampled) during the four separate widespread surveys.

3.3.5 Plant tissue nutrient status

The tissue nutrient concentrations estimated from three different classes of plants, *Ruppia* Community, filamentous algae and two samples of the charophyte (*Lamprothamnium*) in 2023 were compared to those collected previously from the same locations (Figure 30) overall indications that both the *Ruppia* Community and filamentous algae had higher %C and %N in the most recent samples. There were only a limited number of samples available for conducting charophyte tissue nutrient analysis however measured concentrations were similar to the *Ruppia* Community samples. The stable isotope results (Figure 31) indicate that the 2023 samples are grouped with those from 2016/17 than the 2021/22 sampling. Note that the isotope analyses conducted in 2021/22 were to provide insights into the implications of unregulated water release from Salt Creek, and samples were taken over a longer time period.
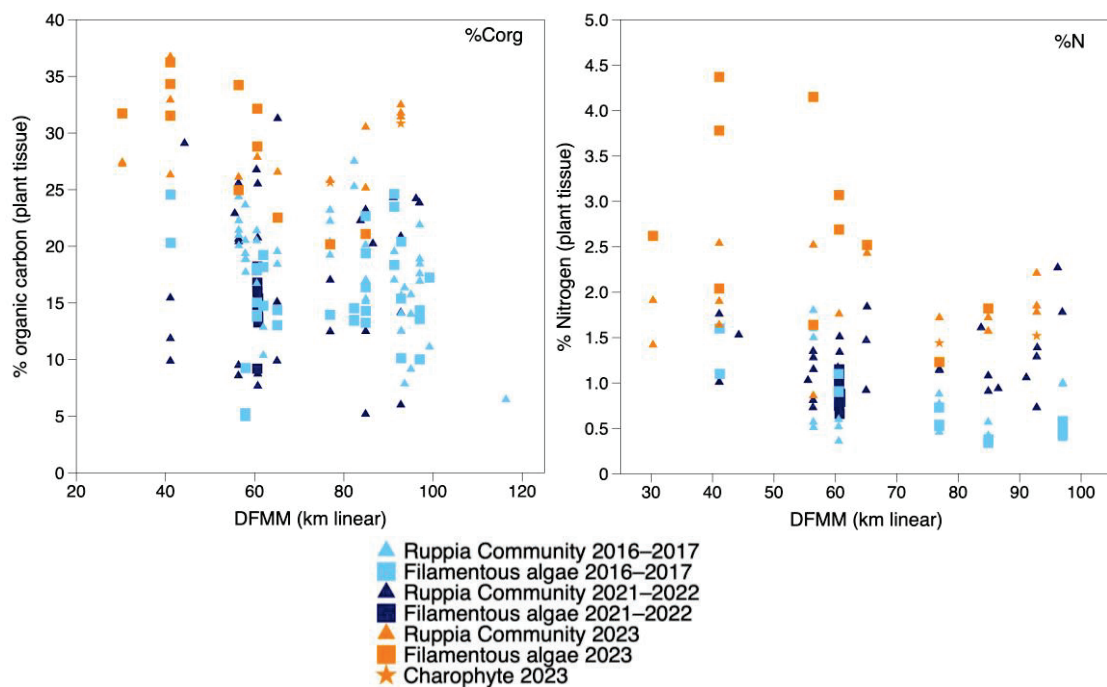
Figure 30 Plant tissue nutrients, total organic Carbon (% DW, left) and total Nitrogen (% DW, right) from samples taken at three times (2021/22, 2022/23, 2023) (previous data from Waycott et al. 2022), plotted against distance from Murray Mouth (DFMM; linear km).
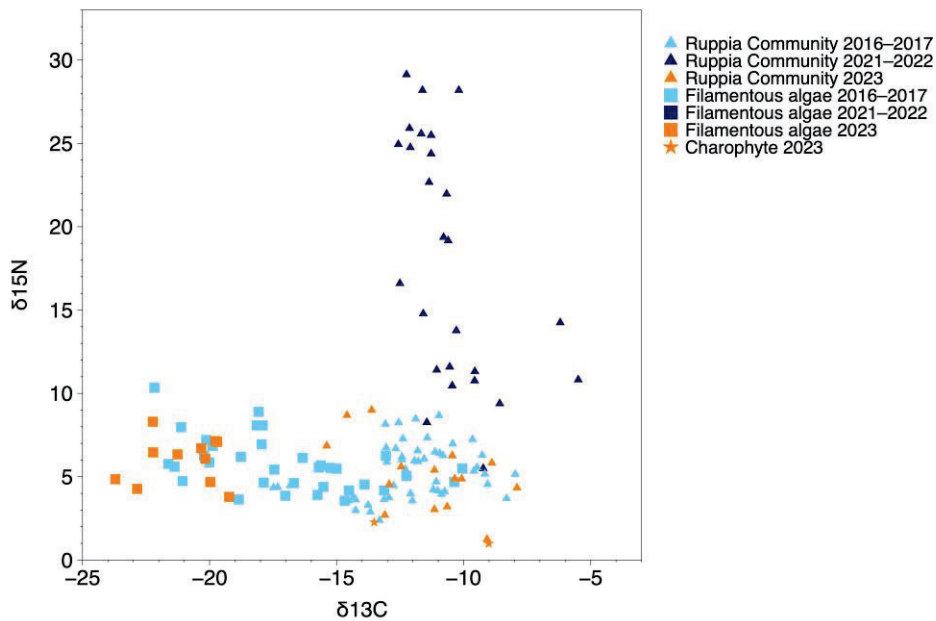
Figure 31 Plant tissue nutrients stable isotope biplot for δ^{13}C and δ^{15}N, different plant types (*Ruppia* Community, filamentous algae and one sample of Charophyte) and at three times (2021/22, 2022/23, 2023) (previous data from Waycott et al. 2022).

3.4 Discussion—Aquatic plants and algae

Prior to the flood event the *Ruppia* Community had become widespread and was increasing in biomass and was present to an extent as described for the system before the Millennium drought (see Waycott et al. 2022). Previously determined experimental evidence (Waycott et al. 2022) indicated that in the presence of persistent water levels, lowering salinities from the extreme hypersaline conditions, where the Coorong South Lagoon is continuously > 60 g/L, would yield an increase in biomass and encourage plants to grow in a more perennial manner. Prior to the 2022/23 floods there had been higher water levels in the Coorong due to unregulated flows from upstream water that had been released through Salt Creek and the barrages. This had provided the *Ruppia* Community with good growing conditions and there were observed to be plants forming perennial populations in some locations.

The condition of the *Ruppia* Community, based on biomass measurements (Lewis et al. 2022), was found to improve during and immediately after the highest flow period associated with the River Murray Floods 2022/23. This observation supports experimental evidence that the *Ruppia* Community has an ability to resist the effects of reduced salinities observed in previous studies (Collier et al. 2017, Waycott et al. 2022). As a result we document that the higher water levels that resulted in reduced salinity across the southern Coorong sites surveyed (Noonameena to Salt Creek) had a positive effect on *Ruppia* Community growth, i.e. an increase in biomass, as long as salinities remained at or above standard marine levels (<35 g/L). However, when salinities went below these levels for prolonged periods (e.g. based on our sampling periods, more than a month), plants rapidly declined and died off. In addition, as salinities persisted for longer periods, below ~40 g/l and above ~10-15 g/L additional aquatic macrophyte species, *Ruppia megacarpa* and the charophyte *Lamprothamnium papulosum*, were detected and future observations of these aquatic plants in the southern Coorong would be indicative of a more benign salinity environment.

These results contrast with the expectations of Paton et al. (2017) who infer that if there is freshening of the southern Coorong to levels below 60 g/L there would be a negative effect on the ability of *Ruppia tuberosa* (and indeed the *Ruppia* Community) to survive. The expected poor performance at lower salinities for *Ruppia tuberosa* relate to the prevailing higher salinities experienced in the southern Coorong during the annual cycle of lower water levels and high salinity experienced in the mud flats and shallow water. This annual cycle represents the most critical period of growth for the *Ruppia* Community during spring and early summer where plants must either set viable seed or form turions before water levels drop and plants die off. Following periods of extended drought (Paton et al. 2015) salinities remain high to extreme all year round and as a result many aquatic macrophyte species are lost from the Coorong ecosystem. However, when water levels are high and/or salinities decline the community composition changes as identified here. Resilient aquatic macrophyte communities in the southern Coorong must survive this annual cycle (i.e. Collier et al. 2017, Paton et al. 2015, Asanopoulos and Waycott 2020, Waycott et al. 2022) as well and the long term variability as climate variability changes.

The timing of the high flow was such that the plants of the *Ruppia* community had already begun producing seeds or/or to establish turions. As a result the areas where the *Ruppia* Community were lost were associated with the Coorong North Lagoon locations that experienced greatest change in salinities, on site measurements as low as 10 ppt. The major change following the flood was the early water level drop in the Coorong South Lagoon (refer to Figure 7), such that the plants were rapidly exposed to more than a 0.5 m AHD drop in a few weeks, inadequate to complete seed set in locations where plants had colonised over the previous 12+ months. Under more regular conditions (i.e. non-flood and high water levels of the 2022/period) the plants would be habituated to a narrower depth range for their growth established by Lewis et al. (2022 and Waycott et al. (2022) to be ±0.4 m AHD.

The overall response of the *Ruppia* Community is one where the community has adapted to its annual cycle, the plants rely heavily on seasonal environmental cues including water temperature, water depth, water clarity, and salinity. In fact, they experience regular seasonal cycles that although may vary interannually still occur and are typified by flowering in spring/early summer and then senescing as water levels drop and salinity increases.

The impact of changed hydroclimatic conditions on reproduction was observed, lowered seed counts throughout the system, and seed coats were found to soften during the period of high flow, likely a chemical affect. These softened seeds are now returning to normal hard coat state in 2024 sampling. The challenge of monitoring this environment is that survey timing is critical. Our monitoring of the system monthly provided some of the most useful observations enabling us to take the variance in the hydro-climatic conditions into account. Abundance and distribution measured within the active growth period and seedbank measured post-seed/turion production are critical parameters to measure for interpreting the broader scale responses as demonstrated by the changing abundance and distribution between years. In addition, it is worth noting that throughout the period where salinities and water quality was affected by the flood waters the seeds being sampled appeared to be soft and not hardened. Initially it was expected that this may result in the seeds being non-viable, however, field germination and the recent detection of hardened seeds suggest it was a temporary phenomenon. There is a need for future work to establish if this would occur at other times when salinities are lowered.

The ongoing presence of excessive filamentous algal growth and also the presence of phytoplankton (e.g. chlorophyll *a*, see Figure 8 lower right panel) continue to impact the reproductive capacity of the *Ruppia* Community in the Coorong, particularly *R. tuberosa*. Although during the high flow period, water movement kept the production of surface mats down, the high degree of water column obscuring algae was high (e.g. Figure 20, algal categories 5 and 6 in 2022 years). The elevated nutrient levels in the Coorong remain an important factor in the ecosystem status for aquatic plants.

4 Macroinvertebrates

4.1 Introduction

Macroinvertebrates in estuaries are adapted to a dynamic environment that varies on temporal and spatial scales subject to riverine flow and mixing with oceanic waters (Jones 1990; Verissimo et al. 2013). Flood events can, however, be a major disturbance affecting the salinity profiles, inundation frequencies, and water quality through low dissolved oxygen (Nishijima et al. 2013; Mayjor et al. 2023). Floods can be accompanied by sedimentation or erosion, which varies between river- and tide-dominated estuaries (Cooper 2002). Both erosion and sedimentation by flood waters can effect macroinvertebrates in estuarine mudflats (Woodruff et al. 2001; Norkko et al. 2002; Thibault de Chanvalon et al. 2016). Floods have been reported to cause declines in macroinvertebrate populations in estuaries around the world, which are followed by differential and often life-history specific recolonisation after flood peaks recede (Conde et al. 2013; Nishijima et al. 2013; Lowe et al. 2022).

In the Murray Estuary and Coorong, the only floods which have been studied for effects on biota occurred in 1983/84 (Geddes 1987) and in 2010/11 after the Millenium Drought (Dittmann et al 2015). In the decade since the 2010/11 flood, some further high flow events occurred and flow over the barrages has been continuous. The flood in early 2023 occurred after a period of high flows and was the largest flood event in the estuary and lagoon since the 1950s (Chapter 2). For macroinvertebrates, impacts arising from the flood could affect the ecosystem functions performed by these benthic organisms, i.e. for biogeochemical processes in the sediments (Lam-Gordillo et al. 2022a, b) as well as the provision of prey for higher trophic levels such as shorebirds and fish (Ye et al. 2020). Yet, the freshening effect of the flood could also be beneficial by lowering salinities in the South Lagoon of the Coorong below the salinity threshold for macroinvertebrates and enabling recolonisation, which was not possible during the previous persistently high (>60 g/L) hypersaline conditions.

The macroinvertebrate component of the flood response investigations was split into two parts, in relation to the nutrient dynamics and the food web. The objectives were to assess flood effects on macroinvertebrate communities in the Murray Estuary and Coorong lagoons, and whether changes to macroinvertebrates would impact sediment nutrient loads and food supply for higher trophic levels. The investigations carried out for both tasks are presented here in one chapter. Comparisons with pre-flood conditions were possible through long-term monitoring by The Living Murray Program (TLM).

4.2 Methods

Two major field surveys were carried out as part of this flood response investigation, in August and November/December 2023 (abbreviated as Nov/Dec hereafter). The August survey occurred about seven months after the flood peak and during a second high flow event (see Figure 6)The survey in Nov/Dec occurred when flood waters had receded. A further survey was carried out in March 2024 at a subset of the locations, but processing of samples from this third survey has not started and the data are not provided here.

4.2.1 Study sites

The sampling locations for the flood response investigations covered the entire length of the Murray Estuary and Coorong lagoons, with 15 locations and transect zones at 12 of these sites (Figure 32; Appendix Table B.1). Mudflats on the mainland shore were referred to as 'intertidal' although water levels in the Coorong lagoons are not tidal but affected by wind seiching and overall water level in the ecosystem. At four of the locations (Mundoo Channel, Mulbin Yerrok, Sandpiper and Loop Road), only

mudflats were sampled. The transect zones in the channel were referred to as 'subtidal', and mudflats on the peninsula as 'peninsula'. The subtidal and peninsula zones were reached by boat, but when low water levels prevented launching the vessel, subtidal samples were collected by wading out as far as safely possible and taking samples with the Ekman grab, but peninsula zones could not be accessed (Figure 33). Not all locations and zones were thus sampled in each survey.

The sampling locations covered the entire length of the Coorong and included locations for which earlier data are available from the TLM condition monitoring as well as the HCHB T&I food web component investigations. Appendix Table B.1 has details of the locations and survey dates as well as the distances from the Murray Mouth. North Magrath Flat was not previously sampled for macroinvertebrates, and Sandpiper was added in the South Lagoon to cover the distance gap between survey locations in the northern end of this lagoon. A further criterion for selection of locations was also overlap with locations sampled by other teams for nutrients, fish and macrophytes.
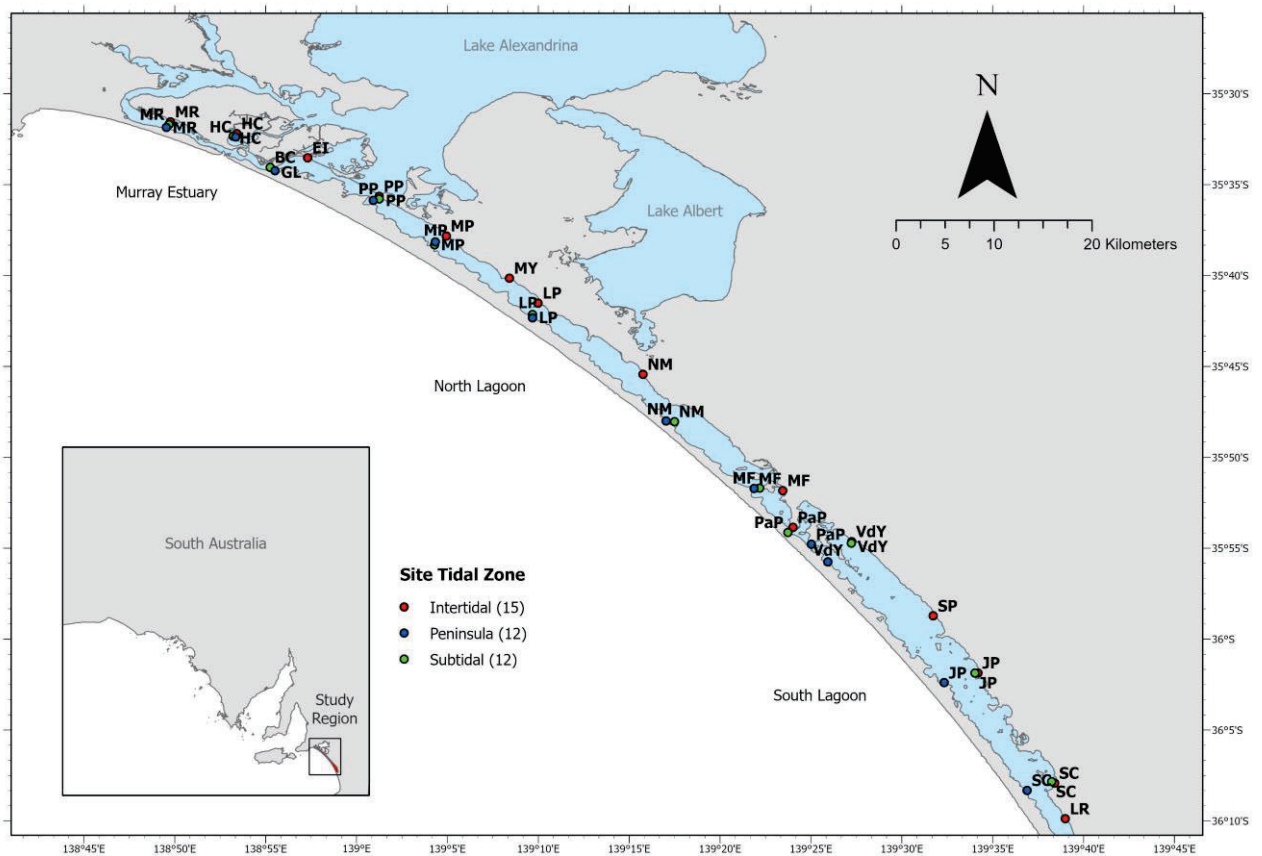
Figure 32 Macroinvertebrate sampling locations in the Murray Estuary and North and South Lagoon of the Coorong, surveyed in August 2023 and Nov/Dec 2023. The colour represents the transect zone of the sampling area; red (intertidal), blue (peninsula) and green (subtidal). Note that not all locations on the peninsula in the Coorong could be reached in Nov/Dec due to low water levels. See Appendix Table B.1 for codes and full names of locations.

4.2.2   Field methods

To collect macroinvertebrates, sediment samples were taken using either a handheld corer (83.32 cm^2 surface area) or an Ekman grab (225 cm^2 surface area). The sampling depth for the corer was ~15 cm on average, but with the Ekman grab, sampling depth was often less, especially with black ooze from subtidal sediments. All sediment samples were sieved in the field through a 0.5 mm mesh and stored

in 70% ethanol until further processing. At each of the locations, five replicate samples were taken per zone, and where only the intertidal mudflat could be reached (i.e. Mulbin Yerrok, Sandpiper and Loop Road), ten replicates. For the Coorong flood response, the total number of macroinvertebrate samples for August 2023 was 210 and for Nov/Dec 2023, i195, as peninsula zones could not always be reached. Thus, for all surveys, 10 or 15 replicate samples were taken per locations (see Appendix Table B.1). At the optional additional survey in March 2024, 110 samples were taken at the long-term TLM monitoring sites (n=10 per site). These are still to be processed.

For sediment solid nutrients, separate samples were taken from three additional corer or Ekman grab samples and pooled into composite samples for each sediment depth per location and zone. The samples were taken with a syringe (140 mL) cut-off at the tip, which allowed separating the sediment samples by depth into sample jars. The 0 – 5 cm depth could be taken at all locations, and at the intertidal and peninsula mudflats the depths 5 – 10 cm and 10 – 15 cm could be separated as well. For subtidal sediments, these deeper layers could not be obtained at all locations as the sediment was often black ooze. A total of 92 sediment samples were collected in August 2023 and 85 sediment samples in the Nov/Dec 2023 survey. The samples for nutrients were put into a freezer and kept at -20°C until further processing.

In the field, the salinity of the water was measured at all sites and locations using an optical refractometer Hanna HI96822 and an optical refractometer IWAKI (Japan) if salinities exceeded the detection limit or for sites accessed by boat.

Figure 33 Field work for macroinvertebrate sampling by boat and on foot, in August 2023 and Nov/Dec 2023 respectively.

4.2.3   Laboratory methods

Macroinvertebrate samples were preserved in 70% ethanol and rinsed through a 0.5 mm mesh prior to processing and sorted from sorting trays and under dissecting microscopes. 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, but the terminology 'species' is used regardless of the specific taxonomic level. In the text and figures of the results, we mostly refer to the genus name only. To avoid overinflation of abundances, polychaete specimens were only included in the count if they had a complete anterior region (prostomium). All macroinvertebrates were further preserved in 70% ethanol.

The sediment solid nutrient samples were kept frozen (-20 °C) and sent to the NATA accredited Environmental Analysis Laboratory at Southern Cross University in Lismore, Australia, for analyses.

4.2.4 Data analyses

Data were analysed across the fixed factors location and survey. Some data are also plotted differentiating transect zones. Diversity was calculated as species number and Shannon-Wiener diversity (H') based on average values per location and survey. Tests for significant differences in abundance for all taxa and for each main taxonomic group were carried out with PERMANOVA based on fourth-root transformed data and Euclidean distance, with 9999 permutations. For multivariate analyses and a test for difference in communities, a dummy value of 1 was added because of many zero values (absence of a lot of species from many samples as well as entire samples with no fauna) prior to calculating Bray-Curtis similarity. A Similarity Profile test (SIMPROF) was used to detect significantly different groups of macroinvertebrate communities. Ordination plots were created based on averaged data over locations and surveys, with Principal Coordinate analysis (PCO) and vector overlays to show species correlated with the ordination axes. Data analyses were carried out in PRIMER v7 with PERMANOVA add-on, or Origin –Pro 2021b. Figures were prepared in Origin-Pro or Arc GIS Pro v3.3.0. For the creation of heatmaps, data were converted from point values to a surface raster layer in ArcGIS Pro using the inverse distance weighting (IDW) spatial analysis tool. The values were displayed low to high throughout the study area. At each location, the colour of the point indicates the true abundance. Where heatmaps are shown for consecutive surveys, the heatmap scale range was kept the same.

4.3 Results

4.3.1 Macroinvertebrate diversity patterns after the flood

Following the flood, the macroinvertebrate diversity patterns resembled a unimodal curve with highest species numbers in the North Lagoon and low numbers at locations near the Murray Mouth and at the southern end of the Coorong lagoons (Figure 34a). The number of species at single locations ranged from 3 (at Loop Road) to 18 (at Noonameena). The pattern of higher species numbers in the North Lagoon was also reflected for species numbers per transect zones at the sites (Appendix Figure B.1) While the number of species per location showed a similar pattern across the August and Nov/Dec surveys, the Shannon-Wiener diversity index H' revealed some greater differences in diversity at single locations, and mostly lower values in Nov/Dec than August (Figure 34b). The higher diversity in Nov/Dec at Monument Road, Pelican Point and Noonameena aligned with higher numbers of species than in August, whereas the higher diversity index at Salt Creek occurred with similar species numbers across both surveys.
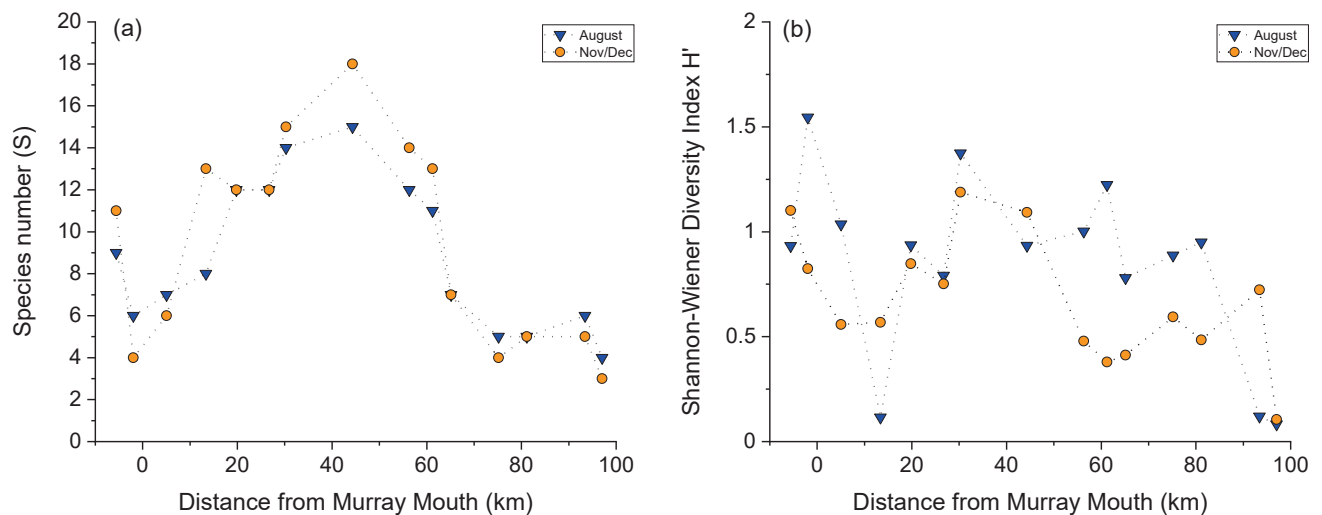
Figure 34 Species number (a) and diversity (b) of macroinvertebrates in the surveys from August 2023 and Nov/Dec 2023.

Over both surveys in August and Nov/Dec, 31 macroinvertebrate species were found, with Annelida and Mollusca each contributing ten species, Crustacea five and Hexapoda (insect larvae) six (Appendix Table B.2). Annelida included nine species of polychaetes and oligochaetes, and Mollusca the bivalves *Spisula trigonella*, *Hiatula alba* and *Arthritica semen*, as well as seven species of gastropods. Crustacea comprised amphipods, ostracods, mysids, isopods and the crab *Amarinus laevis*. Hexapods included insect larvae of several families of Diptera, such as Chironomidae. Annelids, crustaceans and insect larvae contributed to the macroinvertebrate communities at almost all locations, whereas molluscs were mostly found in the North Lagoon locations (Figure 35). By Nov/Dec, molluscs, crustaceans and annelids had colonised further into the South Lagoon and were found with one or two species almost to the southern end (Figure 35b). At locations where species numbers were lower in the subtidal sediments than adjacent intertidal and peninsula mudflats, the decrease was mostly due to lower numbers of annelids and molluscs. Where subtidal sediments contained more species than the other transect zones, it was mostly due to greater numbers of annelid species (Figure 35).
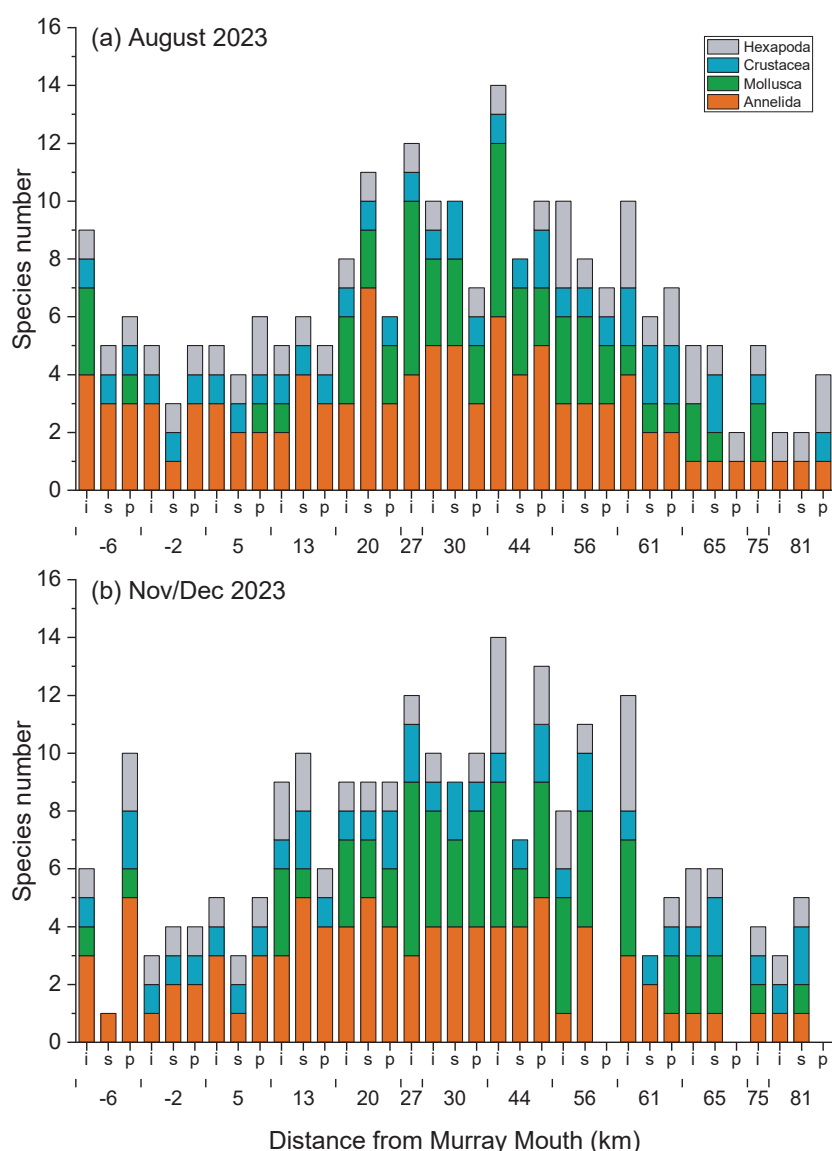
Figure 35 Stacked bar graphs of macroinvertebrate species numbers by main taxonomic group and for each of the transect zones I = intertidal, s = subtidal, p = peninsula per locations along the distance of the Coorong from the Murray Mouth. (a) Data for August 2023 (b) Data for Nov/Dec 2023. Not all peninsula transect zones could be reached in the Nov/Dec survey.

4.3.2 Macroinvertebrate abundance patterns after the flood

Following the flood, macroinvertebrate abundances were very low in the vicinity of the Murray Mouth and high throughout the North Lagoon (Figure 36). Amphipods accounted for the very high abundances of Crustacea at Pelican Point (km 13) in the August and Nov/Dec surveys. In August 2023, the high abundances in the North Lagoon were attributable to individual numbers of annelids, crustaceans and molluscs, while insect larvae accounted for most of the individuals found in the South Lagoon (Figure 36, Figure 37). By Nov/Dec 2023, annelids and molluscs occurred in higher abundances in the South Lagoon, which was mostly attributable to capitellid polychaetes, the bivalve *Spisula trigonella* and the snail *Salinator fragilis* (Figure 36, Figure 38). Abundances of total macroinvertebrate fauna, and the main taxonomic groups (Annelida, Mollusca, Crustacea and Hexapoda) were significantly different across locations and surveys, but Crustacea and Hexapods did not differ significantly between the two surveys (Table 3). Chironomid larvae were abundant throughout the South Lagoon and also in the Murray Mouth region. The recolonisation of the South Lagoon did not

persist over summer, based on field observations of dead gastropods and bivalves in the South Lagoon during March 2024.
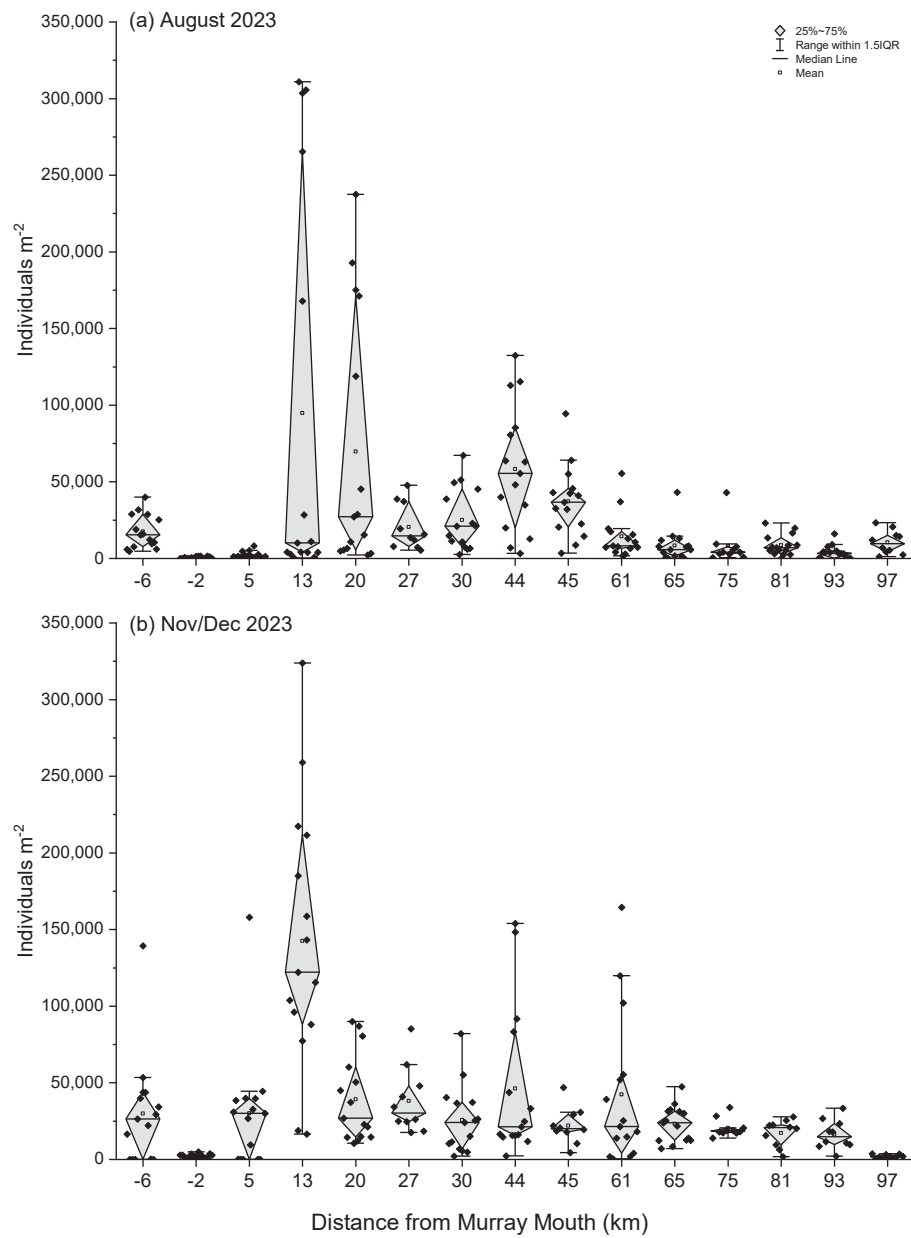
Figure 36 Boxplots with data overlay of total macroinvertebrate abundance (individuals m^{-2}) at the survey locations from (a) August and (b) Nov/Dec 2023.

Table 3 Test outcomes from PERMANOVA for macroinvertebrate abundances across locations and the surveys (August and Nov/Dec 2023). Not significant test results are indicated with ns. See Table B.3 for further detail on test outcomes.

Site	df	Total benthos $P_{(perm)}$	Annelida $P_{(perm)}$	Mollusca $P_{(perm)}$	Crustacea $P_{(perm)}$	Hexapoda $P_{(perm)}$
Location (Loc)	14	0.0001	0.0001	0.0001	0.0001	0.0001
Survey (Su)	1	0.0001	0.0001	0.0003	ns	ns
Loc x Su	14	0.0001	0.0001	0.0001	0.0001	0.0001
Residual	375					
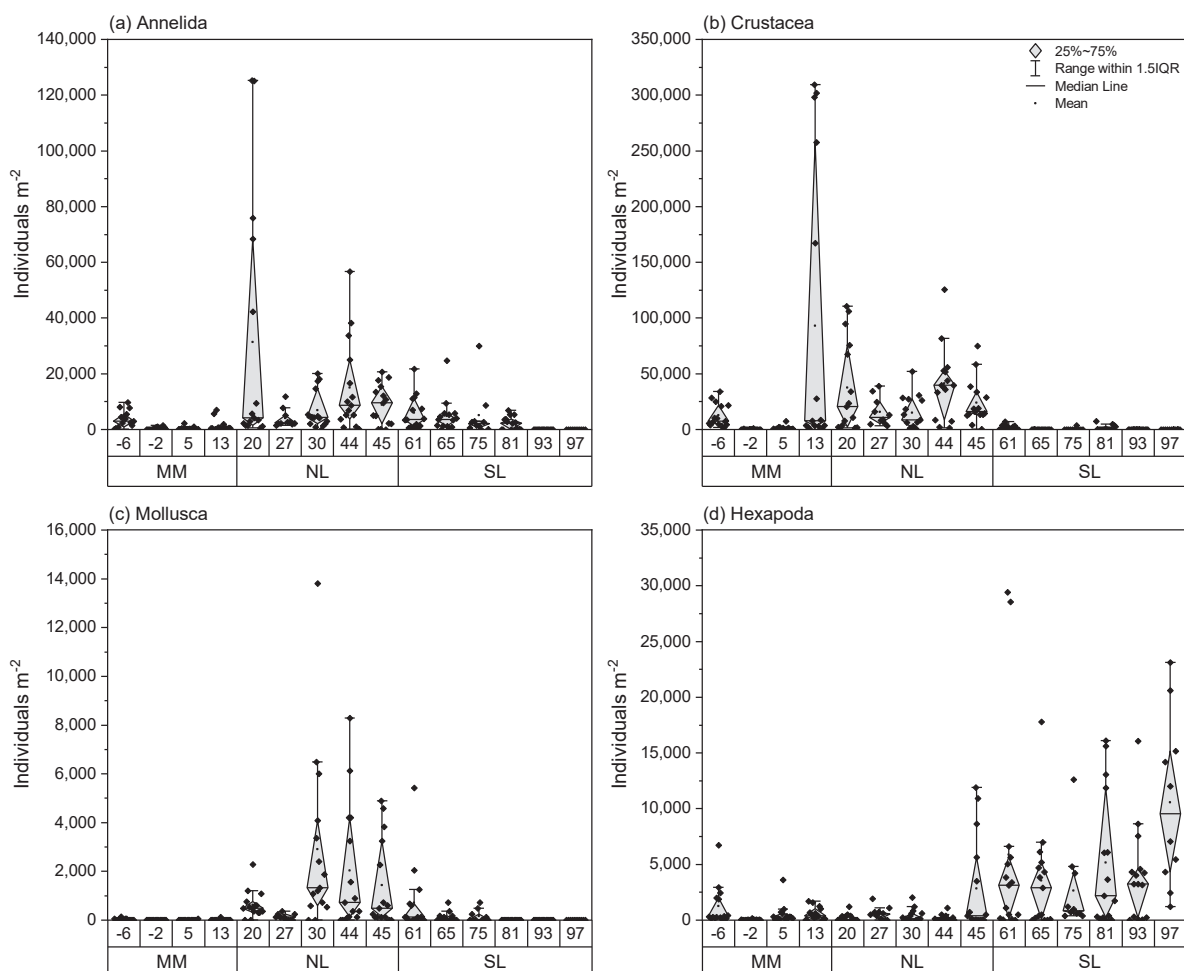
Figure 37 Boxplots with data overlay of macroinvertebrate abundance (individuals m^{-2}) at the survey locations from August 2023, for the four main taxonomic groups (a) Annelida, (b) Crustacea (c) Mollusca, (d) Hexapods. Note the different y-axis scales. The x-axis shows the distance from the Murray Mouth for the surveyed locations in the Murray Mouth (MM) region, the North Lagoon (NL) and South Lagoon (SL).
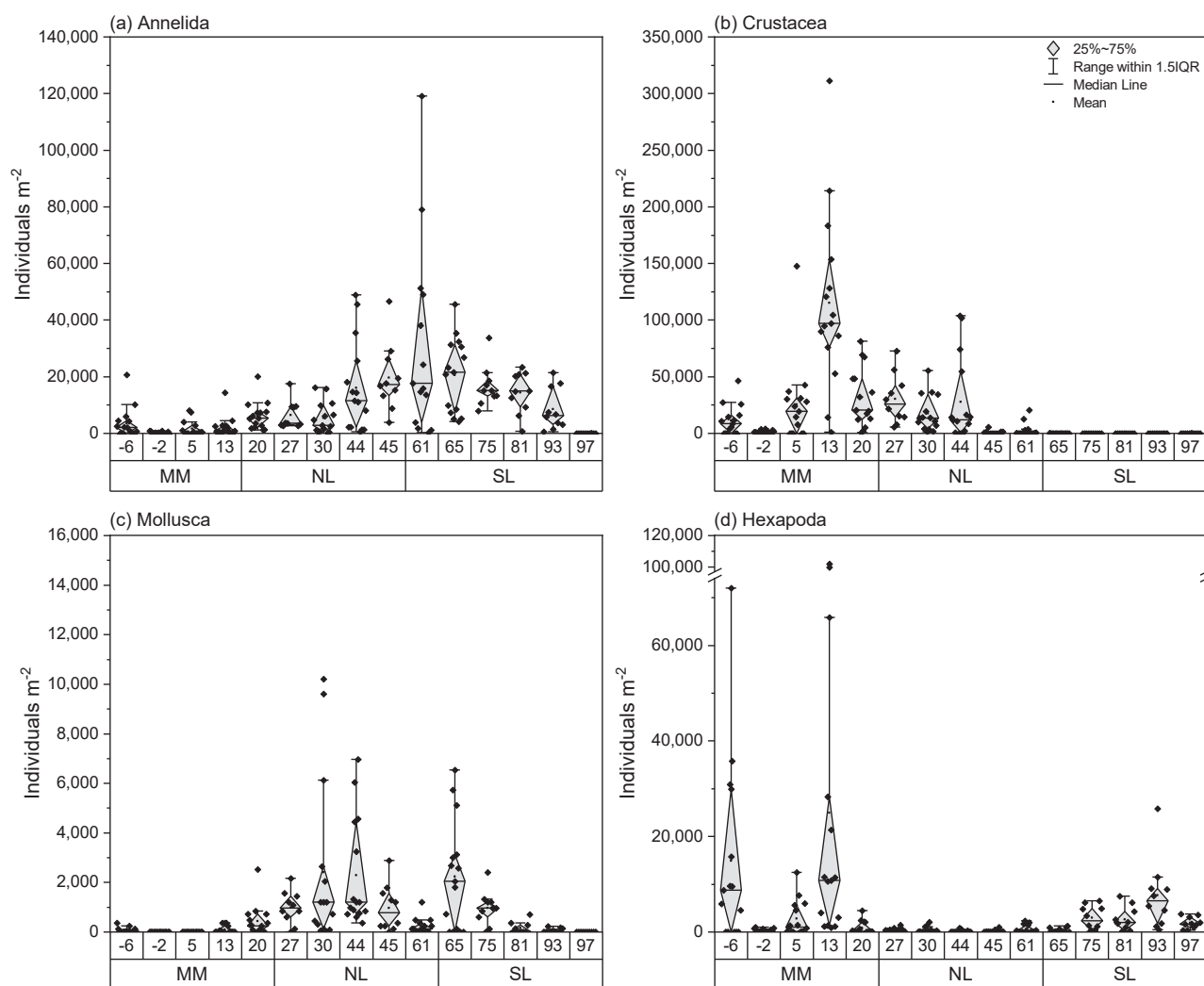
Figure 38 Boxplots with data overlay of macroinvertebrate abundance (individuals m^{-2}) at the survey locations from Nov/Dec 2023, for the four main taxonomic groups (a) Annelida, (b) Crustacea, (c) Mollusca, (d) Hexapods. Note the different y-axis scales. The x-axis shows the distance from the Murray Mouth for the surveyed locations. See Figure 37 for more detail.

Using data from the transect zones at the survey locations, heatmaps of abundance illustrate the dynamic of shifts in the spatial distribution pattern of macroinvertebrates across the Coorong after the flood (Figure 39). These maps reflect the particularly rapid re-colonisation of the South Lagoon by molluscs and annelids between August and Nov/Dec surveys, while their numbers remained low in the Murray Mouth. Chironomid larvae had a bimodal distribution in Nov/Dec 2023 with high numbers in the Murray Mouth and South Lagoon, but were less common in the North Lagoon. The high abundances of amphipods in subtidal sediments near Pelican Point and Mark Point resulted in high abundances for Crustacea, particularly in August (Appendix Figure B.2). By Nov/Dec 2023, recolonisation of macroinvertebrates in the Murray Mouth was detectable at intertidal and peninsula mudflat locations, but abundances remained low in subtidal sediments (Appendix Figure B.2).

(a) August 2023
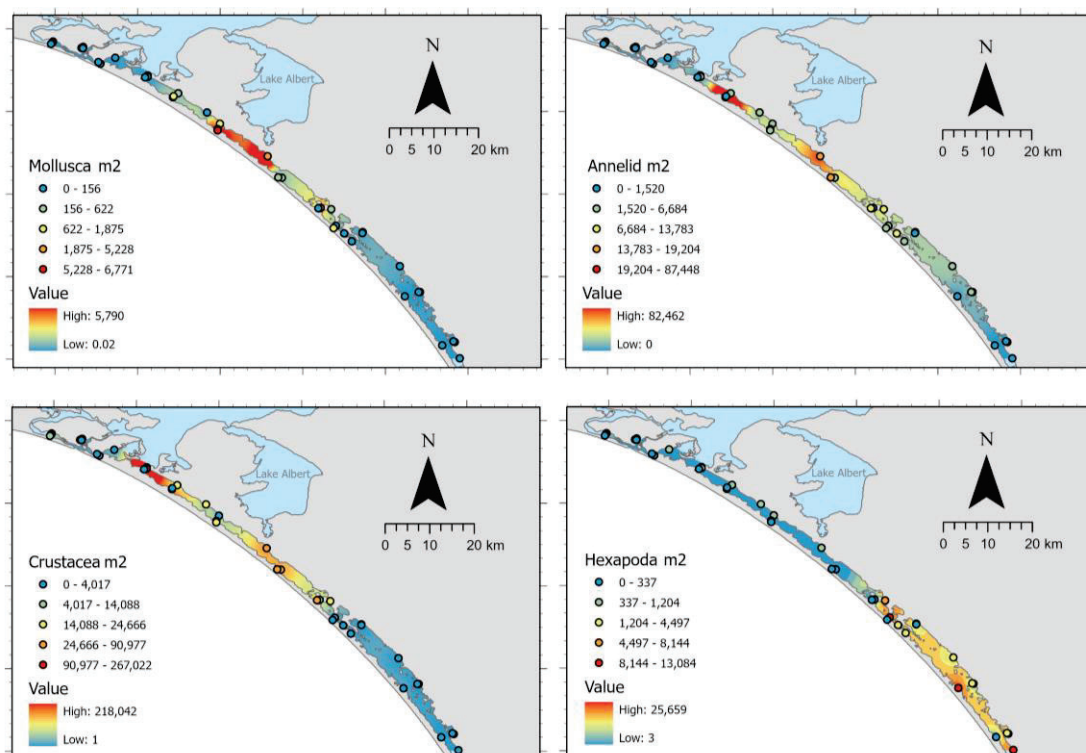
b) Nov/Dec 2023
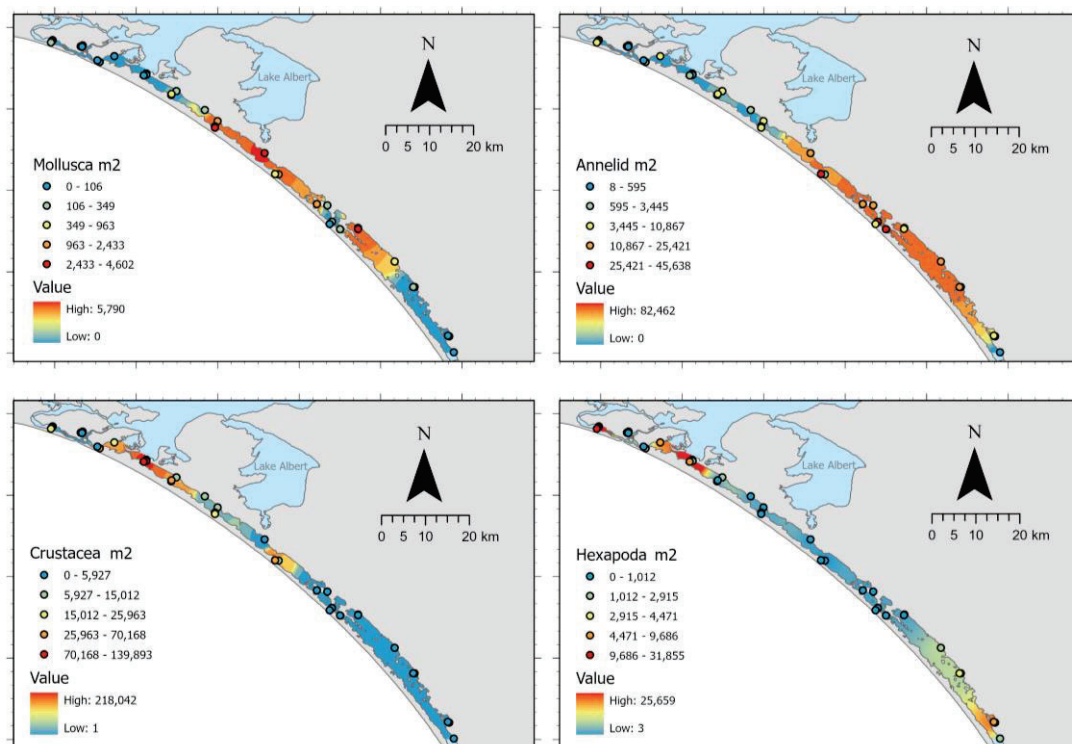
Figure 39 Heatmaps of abundances for main taxonomic groups (Mollusca, Annelida, Crustacea and Hexapods) across the sampling locations and transect zones for (a) August 2023 and (b) Nov/Dec 2023. The colour within the point of each sampling location indicates the true value range, while the heatmap colour scale was set to the same range over both surveys.

4.3.3 Macroinvertebrate community patterns after the flood

Macroinvertebrates in the Coorong could be differentiated into two main groups, one group comprising mostly South Lagoon sites and the second group the North Lagoon and Murray Mouth, which were further split as North Lagoon sites formed a distinct cluster (Figure 40; Table B.4). The pattern of community differentiation was more shaped by region than survey, and was also similar across the transect zones of locations. Some differences are noteworthy the subtidal sediments at two locations from the Murray Mouth (Ewe Island and Hunters Creek), which contained very few macroinvertebrates, grouped with the South Lagoon locations, as did the macroinvertebrates from intertidal sediments at North Magrath Flat; while the subtidal sediment from Parnka Point shared similarity with the North Lagoon in August (Figure 40). The macroinvertebrates from the intertidal sediments at Mark Point grouped with the Murray Mouth community.

The macroinvertebrate communities at locations level over both surveys also split by region in a PCO plot (Figure 41a). Both ordination axes explained over 76% of the total variation. The species contributing to the differentiation of the communities include the polychaete *Boccardiella limnicola* and oligochaetes for the Murray Mouth, amphipods and the polychaete *Simplisetia aequisetis* for the North Lagoon, and the bivalve *Spisula trigonella* and the polychaete *Capitella* for the South Lagoon (Figure 41b). SIMPER analyses also showed that chironomid larvae were typical for the similarity within the South Lagoon locations. The sabellid polychaete *Euchone variabilis* which occurred in some higher abundances in the North Lagoon also contributed to differentiate these locations from those in the Murray Mouth.
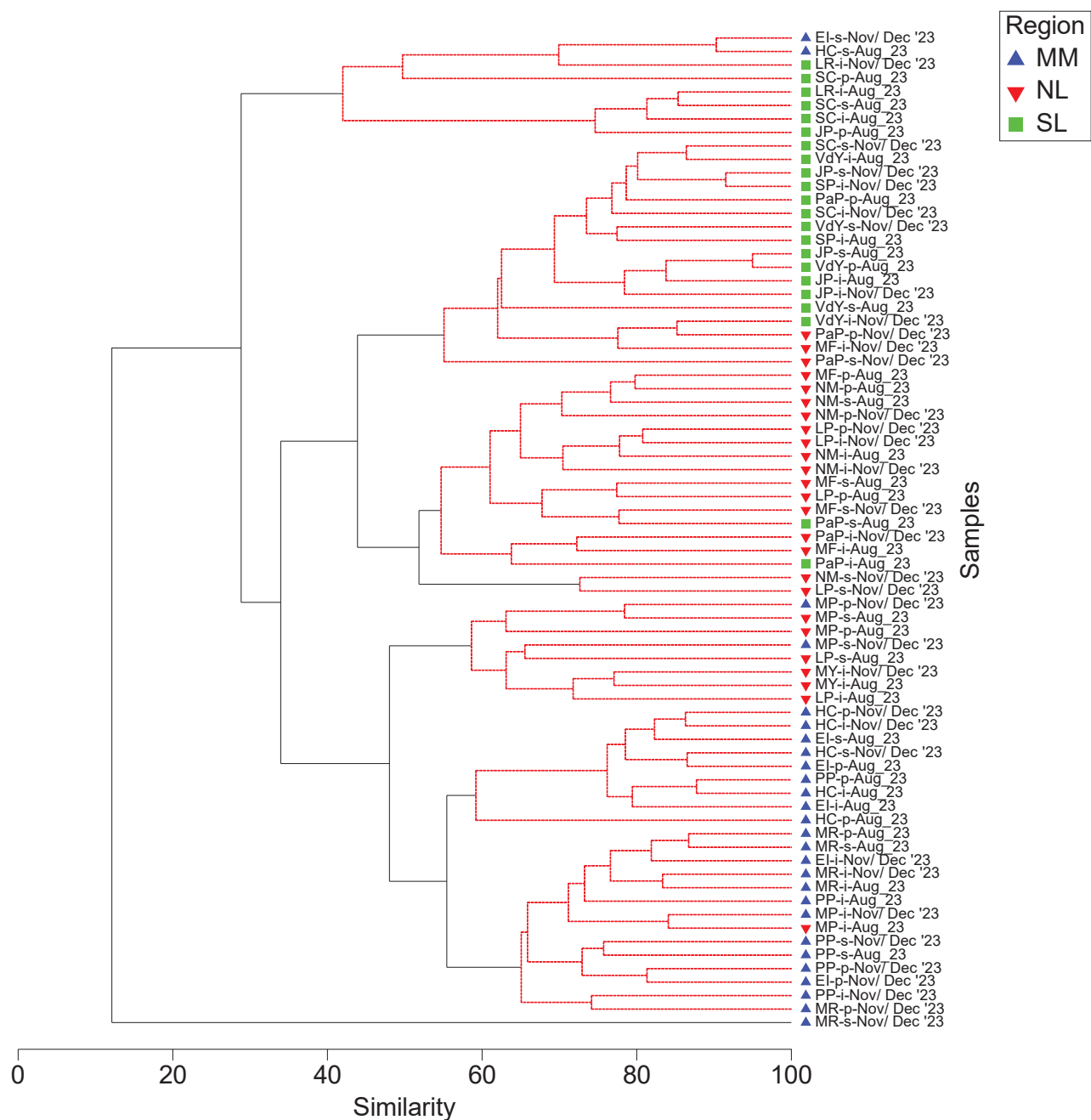
Figure 40 Dendrogram of macroinvertebrate communities for each locations and transect zone sampled at the two surveys in August and Nov/Dec 2023. The black lines delineate significantly different groups based on SIMPROF tests. The transect zones are indicated with the letters i = intertidal, s = subtidal and p = peninsula. For location codes and distance in km from the Murray Mouth see Appendix Table B.1
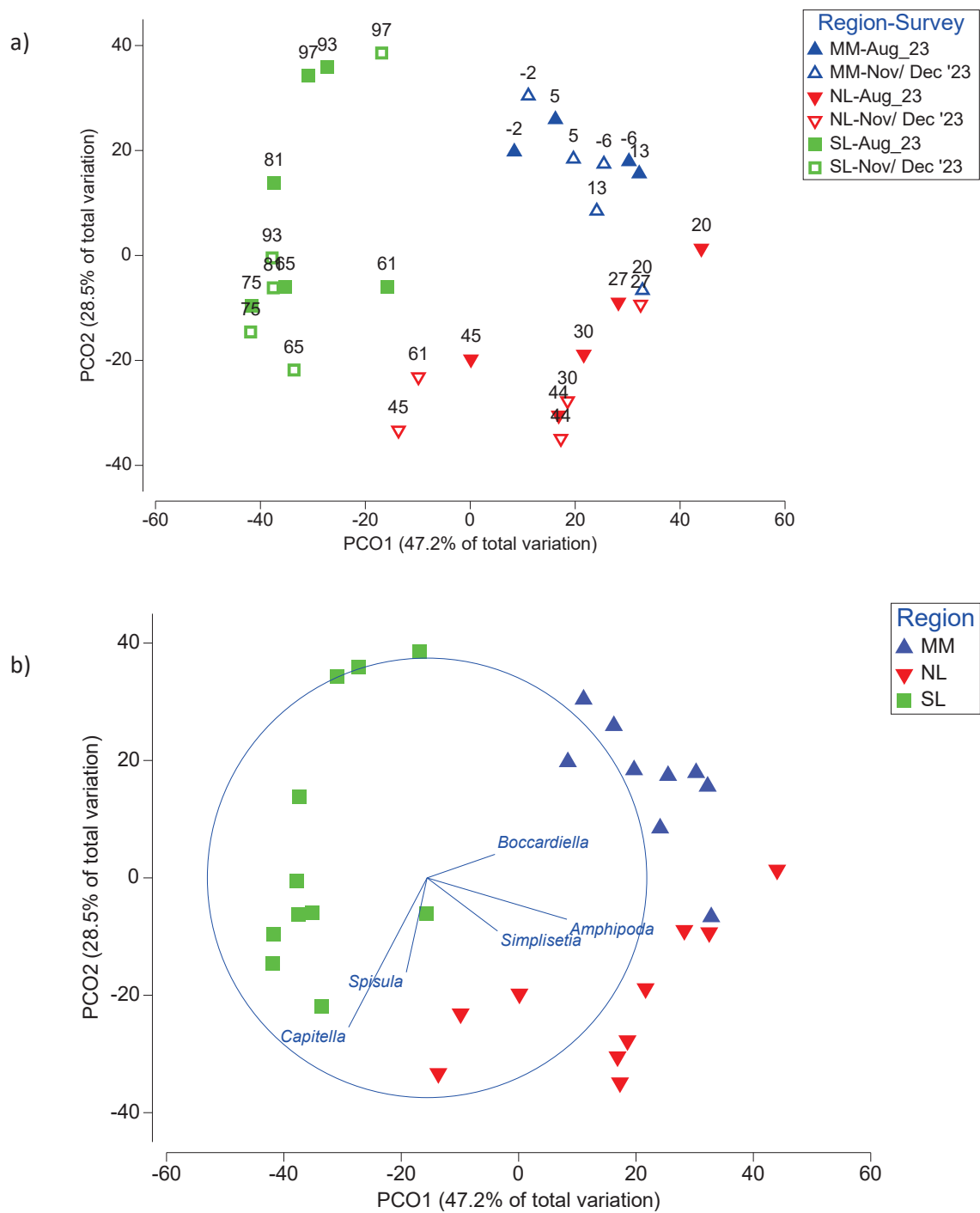
Figure 41 Principal Coordinates analysis (PCO) plots for macroinvertebrate communities for a) each location (in km distance from the Murray Mouth) and region sampled for the two surveys in August and Nov/Dec 2023 and b) vector overlay of correlations for key species in the communities sampled. For location codes and distance in km from the Murray Mouth see Appendix Table B.1

4.3.4 Longer-term response of macroinvertebrates before and after the flood

As the flood response monitoring commenced late, the focus of the surveys from August 2023 was on the effects and recovery as seen several months post flood peak, as presented in the result chapters above. Here, we present a longer-term comparison with surveys just before (November 2022) and after the flood peak (April 2023) for locations which are also regular TLM condition monitoring locations (intertidal mudflats) (Dittmann et al. 2023). The TLM locations in Mundoo Channel was not included in all figures as it was not surveyed as part of the flood response.

The annual TLM monitoring occurred before the flood peak in November 2022 and showed a pattern for macroinvertebrates that had been persistent for several years, i.e. with highest abundances in the Murray Mouth and North Lagoon and very low numbers in the South Lagoon (Figure 42). Right after the flood peak, macroinvertebrates in the Murray Mouth and most of the North Lagoon were decimated, but macroinvertebrates (mostly insect larvae) still occurred in the South Lagoon. Several months after the flood, recovery in the Murray Mouth and re-colonisation of the South Lagoon started to occur, which further increased abundances there. The comparison over these four surveys during and after the flood year thus illustrates impact and commencing recovery.

The recovery is also apparent at the community level. The macroinvertebrate communities which were statistically distinguished (SIMPROF test overlay) grouped mainly by region or location (Figure 43). The community at Parnka Point and Villa de Yumpa (sites 8 and 9 on Figure 43) were on a trajectory to shift into a new state during and after the flood, as macroinvertebrates started to recolonise the sediments, and more species occurred and became abundant (Figure 44). At Jack Point (location 10, at 81 km distance from the Murray Mouth), the community did not change over time. At Loop Road, the community in the last survey was similar to those in the Murray Mouth immediately after the flood peak, where a decrease in diversity and abundance had occurred (see the shadeplot in Figure 44). The trajectories indicate a large shift for macroinvertebrate communities at location in the Murray Mouth and North Lagoon immediately before and after the flood peak, but a return towards pre-flood communities in the August and Nov/Dec surveys 2023. This pattern is indicative of resilience present in the macroinvertebrate communities in response to this flood event. The diverse and abundant communities which had established at the North Lagoon location (see shadeplot Figure 44, location 6 and 7) following a longer period of high flows have enabled such response.
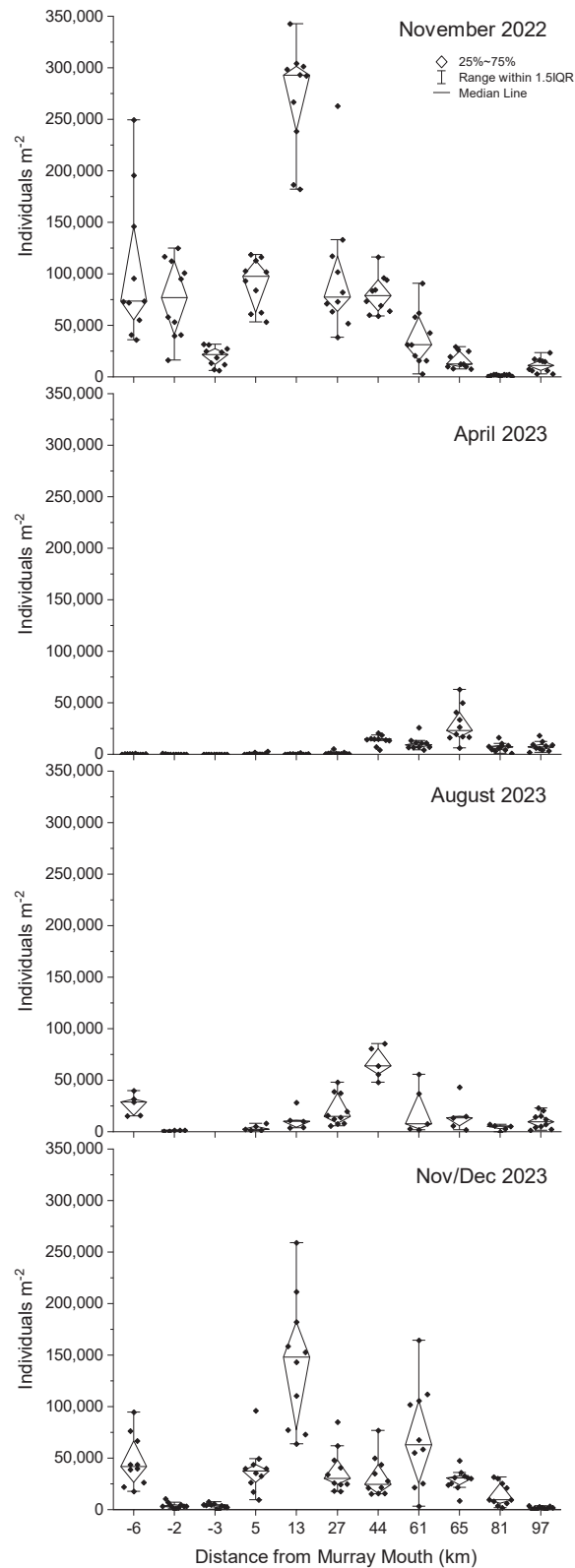
Figure 42 Boxplots with data overlay of macroinvertebrate abundance for monitoring locations of The Living Murray (TLM) condition monitoring surveyed before the flood peak in November 2022, shortly after the flood peak in April 2023, and in August and Nov/Dec2023 as per flood response investigations also reported above. Note that the site at distance-3 was not part of the flood response surveys.
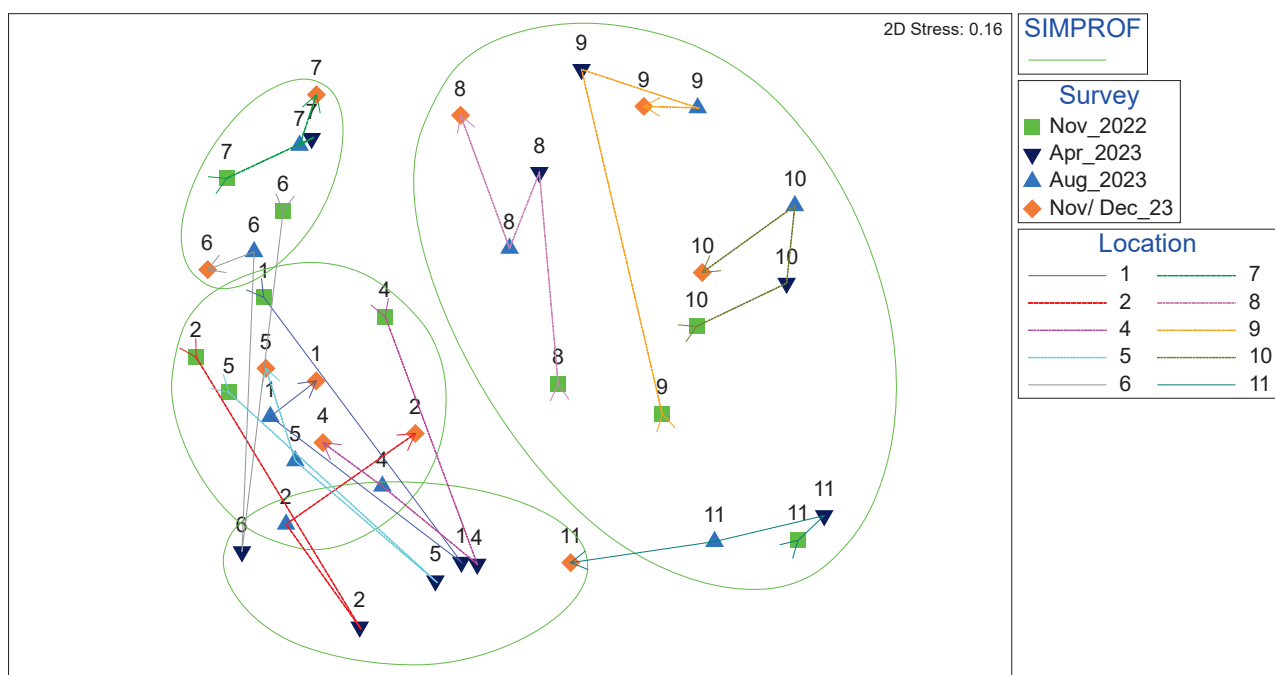
Figure 43 nMDS plot of macroinvertebrate communities at monitoring locations of The Living Murray (TLM) surveyed before the flood peak in November 2022, shortly after the flood peak in April 2023, and in August and Nov/Dec 2023. The location numbers relate to the distances seen on the x-axis of Figure 42, and see Table B.1. Note that the -3 km distance site was not included here as not sampled in August 2023. Locations 1 to 5 are in the Murray Mouth, locations 6 to 7 in the North Lagoon and locations 8 to 11 in the South Lagoon. The green circles separate significantly different communities as tested by a SIMPROF test. The trajectories link consecutive surveys at each location from before and after the flood peak.
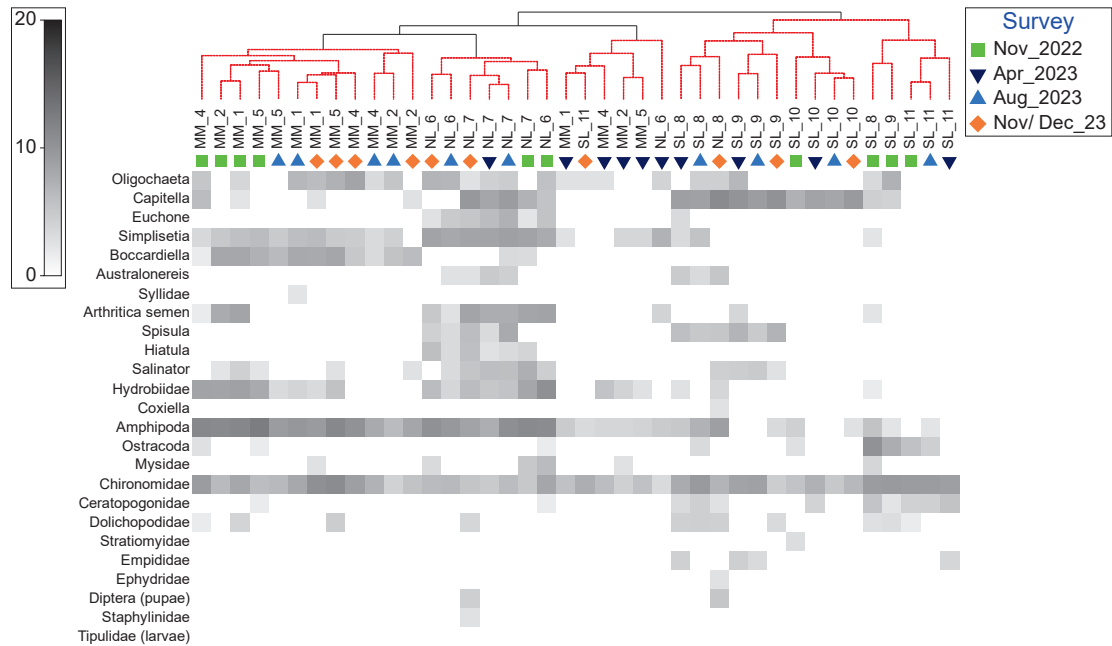
Figure 44 Shadeplot of macroinvertebrate communities at monitoring locations of The Living Murray (TLM) surveyed before the flood peak in November 2022, shortly after the flood peak in April 2023, and in August and Nov/Dec 2023. The depth of the colour for the macroinvertebrate species corresponds to their abundances. The location numbers relate to the distances seen on the x-axis of Figure 42, and see Table B.1. The shadeplot is constrained by the significantly different community groups also encircled in Figure 43.

4.3.5 Sediment nutrients and macroinvertebrate patterns

Macroinvertebrates can play functional roles, including improving sediment biogeochemistry but are themselves also affected by sediment conditions. We investigated the sediment nutrient loads at the August and Nov/Dec 2023 survey locations and transect zones which indicated large scale shifts. In August, concentrations of total organic carbon (TOC), nitrogen (TN) and phosphorous (TP) were highest between Noonameena and Villa de Yumpa (Figure 45a). These concentrations decreased in the northern end of the South Lagoon by Nov/Dec 2023, but increased throughout the North Lagoon and Murray Mouth (Figure 45).

A more detailed look at the sediment nutrient loads over the sediment depths and transect locations gives indications for potential drivers of these changes, which could include further influx, internal processes with or without interaction by macroinvertebrates. For TOC, the higher values emerging throughout the Murray Mouth and the North Lagoon were mainly driven by very high contents in subtidal sediments, which often exceeded 4% (Figure 46). Differences between the survey months were less pronounced for TOC at the intertidal or peninsula mudflat locations. The decrease at the northern end of the South Lagoon occurred as these sediments started to become recolonised by macroinvertebrates. The southernmost locations in the South Lagoon, which still had very few macroinvertebrates, had higher contents of TOC.

The subtidal sediments had exceedingly high contents of nitrogen and phosphorous as well, which showed the same pattern of increase in the Murray Mouth and North Lagoon by Nov/Dec 2023 (Figure 47 and Figure 48). This indicates some accumulation of organic matter in the deeper sections of the Coorong Channel. For TOC and TN, little variation between surveys was seen at the intertidal and peninsula mudflats, apart from the peninsula at Hunters Creek and Parnka Point. TP concentrations appeared to be higher at the peninsula mudflat shores compared to the mainland intertidal mudflats.

(a) August 2023
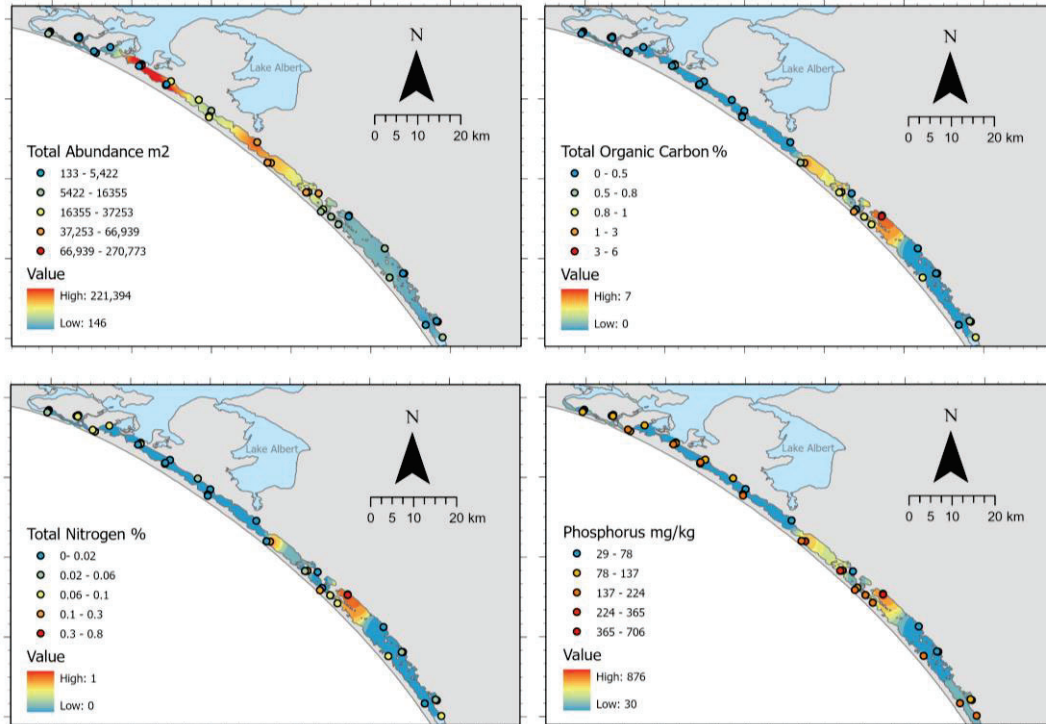
b) Nov/Dec 2023
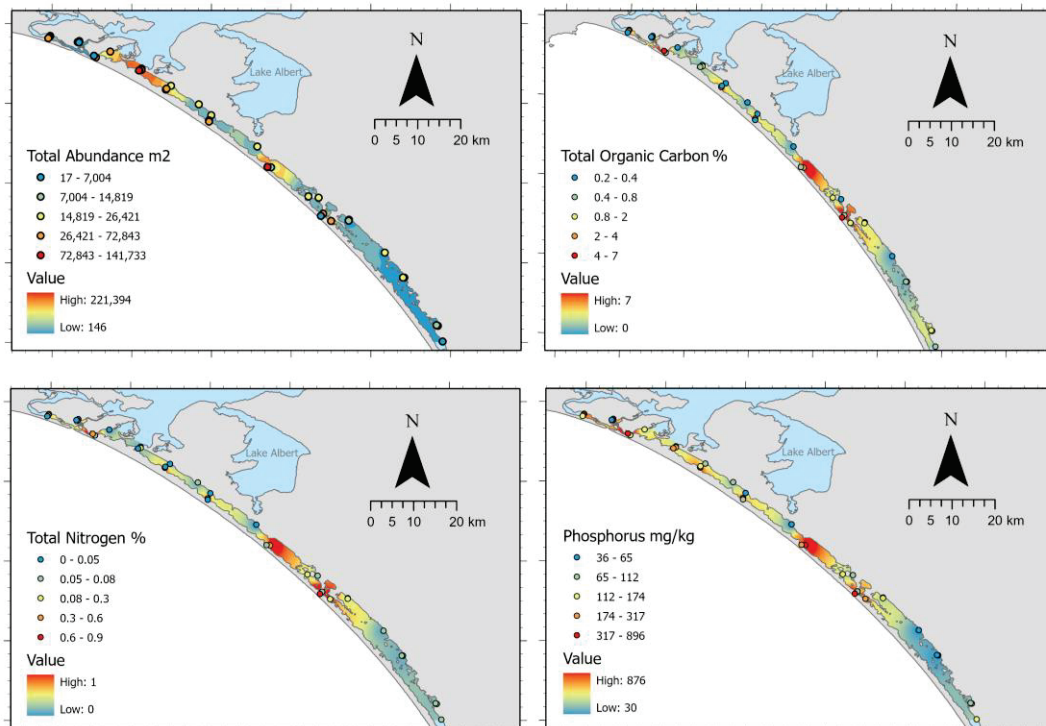
Figure 45 Heatmaps of total macroinvertebrate abundance and sediment nutrient loads as indicated by total organic carbon, total nitrogen and total phosphorous in the 0-5 cm sediment depth layer across the sampling locations and transect zones for (a) August 2023 and (b) Nov/Dec 2023. The colour within the point at each sampling site indicates the true value range, while the heatmap colour scale was set to the same range over both surveys.
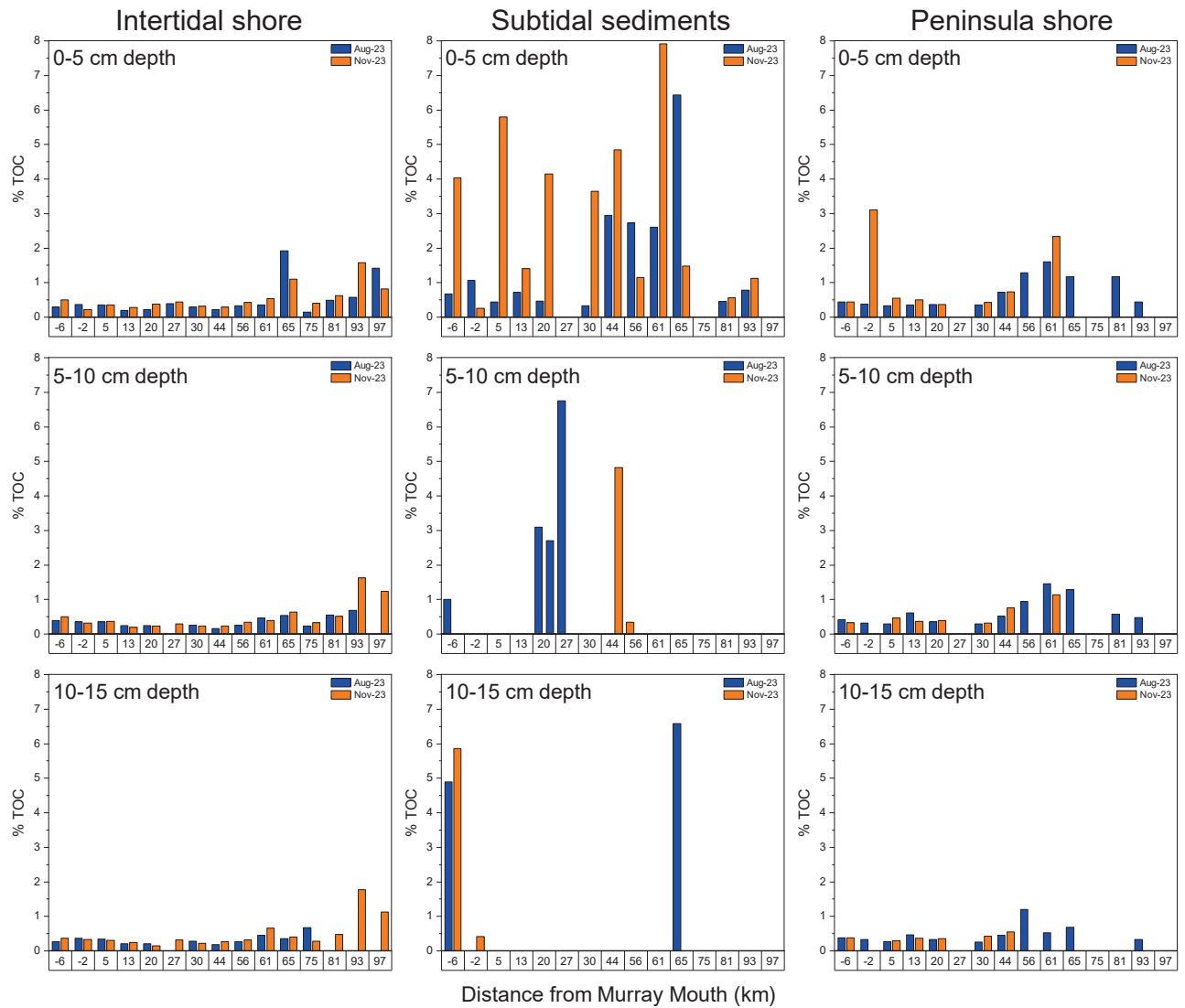
Figure 46 Total organic carbon (TOC) content of sediments at the macroinvertebrate sampling locations and transect zones, separated by sediment depths. The bar graphs show the contents for the surveys in August and Nov/Dec 2023, based on composite samples at each location and zone. Note that not all peninsula zones could be reached in each survey, and deeper sediment layers could often not be obtained from subtidal sediments. The gaps in the graphs are thus missing values.
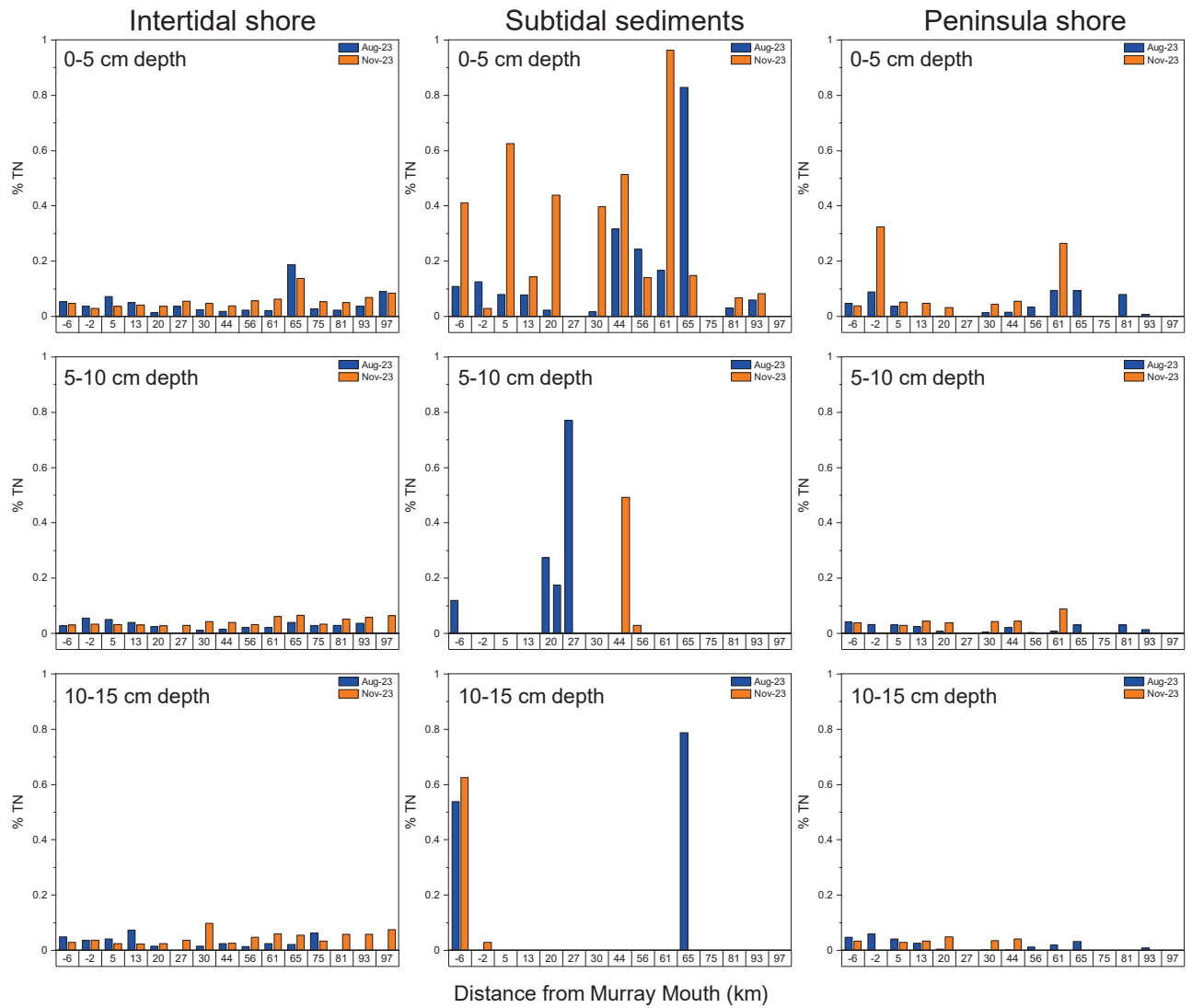
Figure 47 Total nitrogen (TN) content of sediments at the macroinvertebrate sampling locations and transect zones, separated by sediment depths. The bar graphs show the contents for the surveys in August and Nov/Dec 2023, based on composite samples at each location and zone. Note that not all peninsula zones could be reached in each survey, and deeper sediment layers could often not be obtained from subtidal sediments. The gaps in the graphs are thus missing values.
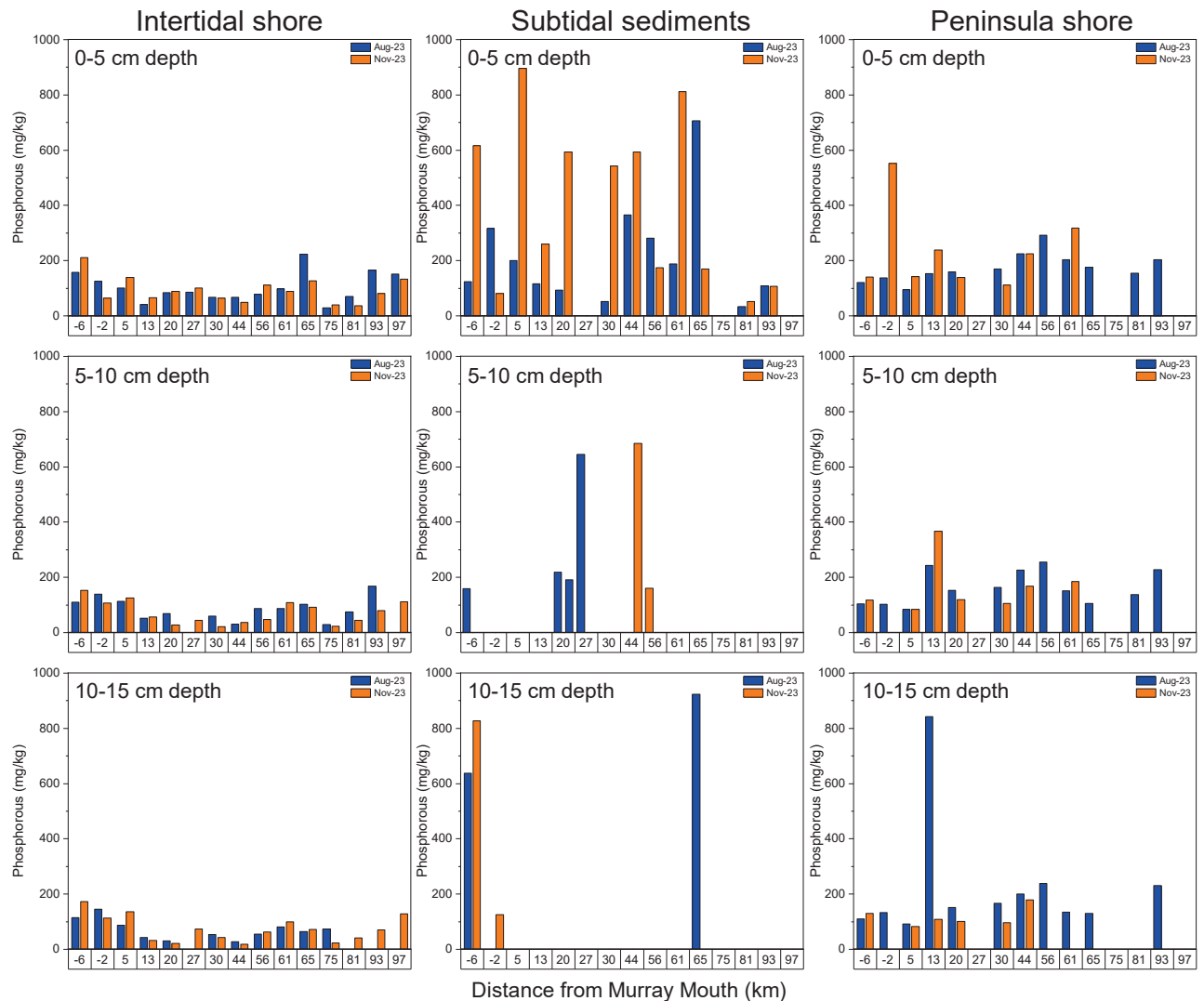
Figure 48 Total phosphorous (TP) concentration of sediments at the macroinvertebrate sampling locations and transect zones, separated by sediment depths. The bar graphs show the contents for the surveys in August and Nov/Dec 2023, based on composite samples at each location and zone. Note that not all peninsula zones could be reached in each survey, and deeper sediment layers could often not be obtained from subtidal sediments. The gaps in the graphs are thus missing values.

4.4 Discussion

The macroinvertebrate investigations of the flood response detected major changes in the Coorong, including the recolonisation of the South Lagoon and the beginning of a recovery in the Murray Mouth, where an impact from the flood had occurred. These shifts had respective effects on prey availability for zoobenthivorous fish, with an initial decrease in prey followed by an increase in the North and South Lagoon, and was reflected in the distribution, condition and diet of fish (see chapter 5) and nutrient dynamics (see chapter 2). For the nutrient conditions in the sediments, effects were not easily linked with macroinvertebrate abundances, which would require further experimentation. In the period after the flow, many environmental conditions changed and both flushing of nutrients out of the system as well as deposition of organic matter could have occurred. Sediment loads as well as accumulation and decomposition of matter in the deeper channels, which could have arisen from

smothering by sediment deposition, similar to the deposition of 5-10 cm of sediment onto mudflats reported after floods in other estuaries around the world (Norkko et al. 2002; Thibault de Chanvalon et al. 2016). This can affect sediment chemistry by itself and reduces the beneficial effects of estuarine bioirrigation of sediments (Thibault de Chanvalon et al. 2016). Recolonisation will be important to reestablish these functions.

The increase in macroinvertebrate abundance into the South Lagoon in Nov/Dec compared to August was possible due to high flow occurring in mid-2023 which further reduced salinities in the South Lagoon (Figure 7). This freshening effect was, however, short-lived as water levels rapidly fell and salinity increased sharply in the South Lagoon from spring 2023 (Figure 11) and exceeded the tolerance limit for most macroinvertebrate species. Yet, for the first time in the last two decades of quantitative data, a recolonisation of the South Lagoon by annelids and molluscs occurred.

Effects of floods on macroinvertebrates have been reported to be severe in estuaries in other parts of the world (Conde et al. 2013), where a decline in diversity, abundance and biomass was detected in certain macroinvertebrates, similar to our findings. One of the macroinvertebrate key species in the Coorong is the burrowing polychaete *Simplisetia aequisetis*, which decreased in abundance at sites in the Murray Mouth affected by the flood. This was similar to the disappearance of a related nereid polychaete (*Ceratonereis* sp.) in mudflats in Japan in years with intense flooding and sediment scouring (Nishijima et al. 2013). Scouring also effected macroinvertebrate communities in shallower sediments of the Swan Estuary after high discharge (Kanandjembo et al. 2001). Nishijima et al. (2013) found an increase in capitellid polychaetes after high flows, whereas we found a decrease in areas of the Murray Mouth and North Lagoon where sediment conditions had improved by the bioturbating activities of other macroinvertebrates over the last few years. We did, however, detect an increase in *Capitella* in the South Lagoon, where it recolonised in high abundances. The *Capitella* species complex is a known pollution indicator that can sustain populations in anoxic and eutrophic sediments, which can explain the decrease in the North Lagoon and increase in the South Lagoon.

Our surveys revealed a decline in mollusc populations in the Murray Mouth. Jones (1987) found a decrease in *Spisula trigonella* after floods in the Hawkesbury River, New South Wales. A complete loss of bivalves has been found in tropical estuaries after floods (Lowe et al. 2022). Yet, our surveys also detected increases of molluscs, with recruitment events of the larger bivalves *Hiatula alba* and *Spisula trigonella* in the North Lagoon and into the South Lagoon, and increases in the populations of the pulmonate snail *Salinator fragilis* in the southern North Lagoon and northern South Lagoon. These increases were possible with lower salinities in the Coorong, however, several other environmental factors have to be considered which can contribute to the changing macroinvertebrate patterns seen in such a complex system as the Coorong after a flood. This includes, for example, changes in the amount of filamentous algal cover which previously occurred on many mudflats. In the Mondego estuary in Portugal, Cardoso et al. (2005) detected a positive effect of floods on hydrobiid snails, which showed a resilient response facilitated by a release from effects of eutrophication after the flood flushed excess nutrients from their estuarine system.

As the effects of floods on macroinvertebrate communities are manyfold, the response and recovery is also species specific subject to adaptations, tolerance ranges and life history strategies (Lowe et al. 2022). Furthermore, past events can be important and influence a hysteresis in estuaries. The macroinvertebrate response seen after this flood also reflects the decade of continuous and higher flow since the drought-breaking flood in 2010/11, where an impact on macroinvertebrates in the Murray Mouth lasted several years (Dittmann et al. 2015). The investigations presented here for the flood response, in combination with the use of data from TLM monitoring just before and after the flood, are indicating the resilience which has been gained in the system through the improved flow and water management of the last decade, in combination with the three years of La Niña rainfall which led to the flood. This had enabled the establishment of an abundant and diverse

macroinvertebrate community in the North Lagoon, which functioned as a source population in two directions, to recolonise the Murray Mouth and the South Lagoon. The evaluation of the flood effects also highlights the relevance of long-term data, without which patterns of change and understanding of effects and resilience would be impossible. A continuation of long-term monitoring is recommended to provide the data for effective water management in the Coorong and Murray Mouth estuary.

5 Fish

5.1 Introduction

As a result of the River Murray Floods 2022-23 we would expect there to be a change in fish assemblage structure and that the North and South Lagoons of the Coorong may differ in the timing of that response. Assessing the impact of the floods on fish populations, including their distribution, diversity, abundance, biomass, growth rate, body condition, and diet composition will provide insights into the status of the fish community and contributing to the understanding of the overall food webs. Specifically, the focus of these surveys was to establish the effect of the floods on fish diversity, abundance, and distribution and the how the diet composition of a key species, yelloweye mullet, may have changed in the Murray Mouth Area, North and South Lagoons of the Coorong.

5.2 Methods

5.2.1 Fish sampling

Sampling was conducted at 12 sites in the Coorong, with four sites in each region (Murray Mouth Area, North Lagoon and South Lagoon), during both March and December from 2020 to 2023 and during March 2024. Table 4 shows the sampling sites in each region and their approximate distance from the Murray Mouth. Sampling in March 2023, December 2023 and March 2024 was conducted in conjunction with TLM Coorong fish condition monitoring while fish data from TLM/HCHB T&I between March 2020 and December 2022 were included for before and after 2022-23 flood comparison.

Table 4 Fish sampling sites across three regions in the Coorong.

REGION	SITES	DISTANCE TO MURRAY MOUTH (KM)
Murray Mouth Area	Beacon 19	-4.9
Murray Mouth Area	Boundary Creek	4.0
Murray Mouth Area	Godfrey's Landing	5.0
Murray Mouth Area	Pelican Point	12.7
North Lagoon	Mark Point	19.7
North Lagoon	Long Point	32.0
North Lagoon	Mount Anderson	39.0
North Lagoon	Noonameena	49.5
South Lagoon	Hells Gate	60.6
South Lagoon	Villa de Yumpa	66.0
South Lagoon	Jack Point	82.9
South Lagoon	Salt Creek	93.8

At each site, sampling was conducted during the day using a standard seine net (61 m net length, 29 m wing length, 22 mm mesh, 3 m bunt length (eight mm mesh); *n* = three hauls). The seine net was

deployed mainly by boat but also from shore at shallower sites in a semi-circle, which sampled to a maximum depth of 2 m and swept an area of ~592 m^2. All fish collected in each haul were identified to species, and the total number of individuals of each species recorded. A random subsample of yelloweye mullet were kept for further laboratory processing to determine changes in their diet composition in the Coorong post flood (March and December 2023). Up to 10–16 individuals for both small (<150 mm Total Length (TL)) and large (>150 mm TL) size classes per region per sampling period were selected for stomach/gut content and stable isotope analyses. However, number in some regions/months was lower due to lower number of individuals collected.

5.2.2 Fish statistical analysis

Fish diversity is reported as species richness (number of species identified within the samples) and relative abundance is reported as the number of individuals per seine net shot (number of fish/seine net shot). Species richness and relative abundance of fish and multivariate assemblage patterns were analysed using permutational Analysis of Variance (PERMANOVA). Analyses were carried out using PRIMER Version 7 with PERMANOVA+ add-on (Clarke and Gorley 2006; Anderson et al. 2008). The design for analyses presented in this report included two fixed factors region and survey month/year. This statistical analysis design was used to test for differences in species richness, relative abundance and assemblage structure.

For univariate analyses, Euclidean distance was used as the resemblance matrix. For tests of differences in abundance and multivariate fish assemblage analyses, data were fourth-root transformed before calculating the Bray-Curtis similarity with a dummy value of 1 added because of the large number of 0 values. All PERMANOVA tests were run with 9,999 permutations. Pairwise tests were carried out when interaction terms were significant. Significant (P<0.05) values are set in bold in tables throughout the report.

The fish assemblage pattern was visualised using averaged data in multi-dimensional scaling (MDS) plots with trajectory overlay (survey date and split by region). Principal coordinates analysis for the ordination (PCO) of fish samples in multivariate space was performed with vector overlays to indicate fish species that were correlated (Spearman rank correlation, r > 0.8) with the ordination axes.

5.2.3 Stomach content analysis

A total of 65 yelloweye mullet was analysed for dietary items over March and December 2023. In March, 38 guts were examined with 10 being empty (26.3%), whereas in December, one out of 27 guts was empty (3.7%) (Table 5). Empty guts were excluded from further analyses. Historical stomach content data from a previous diet study of yelloweye mullet in the Coorong during March 2012 were also included for comparison (Giatas 2012) (Table 5).

Table 5 Number of gut samples examined with dietary items found and number empty for both small (<150 mm) and large (>150 mm) size classes in the Coorong for yelloweye mullet. * Note provided March 2012 diet data did not include the number of empty stomachs. Murray Mouth Area = MMA, North Lagoon = NL, South Lagoon = SL.

		MMA		NL		SL	
		with content (n)	empty (n)	with content (n)	empty (n)	with content (n)	with content (n)
2012 - Mar		**52**	-	**47**	-	**2**	-
	<150	44	-	18	-	1	-
	>150	8	-	29	-	1	-
2023 - Mar		**13**	**7**	**12**	**2**	**3**	**1**
	<150	7	3	5	2	2	0
	>150	6	4	7	0	1	1
2023 - Dec		-	-	**20**	**0**	**6**	**1**
	<150	-	-	10	0	6	1
	>150	-	-	10	0	-	-

To assess the diet of yelloweye mullet, stomach and alimentary tracts (hereby referred to as guts) were examined with food items identified to the lowest taxonomic level possible under a dissecting microscope (x 8–56 magnification) and measured to 1mm3 to determine volume. Parasites (acanthocephalans and nematodes) and sand found in guts were not considered to be a part of the diet and disregarded from any analysis. Food items were calculated quantitatively by the percentage of volume contribution (V%) and frequency of occurrence (F%), following the equations below (Hyslop 1980).

$$V\% = \frac{total\ volume\ of\ a\ food\ item\ for\ all\ guts}{total\ volume\ all\ food\ items\ for\ all\ guts} \text{ x } 100\%$$

$$F\% = \frac{number\ of\ guts\ containing\ food\ item}{total\ number\ of\ guts} \text{ x } 100\%$$

Diet statistical analysis

Prior to analyses, filamentous algae, nereidid polychaetes and chironomid of different life stages (i.e. larvae, pupae, adult) were grouped to their respective families. Additionally, unidentified and identified fish were grouped to the infraclass Teleostei. All other prey items were analysed as identified. Unidentified crustacea (i.e. fragments of exoskeleton from various small crustaceans) were common among samples in March 2023. To reduce the influence of unknown dietary items, this item was excluded from analysis. Gut capacity of yelloweye mullet increases with fish length (Giatas 2012). To mitigate such effect, diet composition data based on percentage of volume contribution was standardised by total percentage of volume contribution. Due to low sample numbers or nil fish captured, South Lagoon samples were excluded from statistical analysis (Table 5).

To investigate the potential change in diet composition of yelloweye mullet after the 2022-23 flood, North Lagoon samples from March 2023 and December 2023 were compared across two mullet size classes (n=2) using a 2-factor fixed design (size, month). To compare the diet of yelloweye mullet with historical data, March 2023 samples were compared to March 2012 data (Giatas 2012) across two size classes (n=2) using a 3-factor fixed design (size, region, year). Statistical tests were conducted using the software program PRIMER version 7 with PERMANOVA+ (Clarke and Gorley 2006; Anderson et al. 2008). A Bray–Curtis similarity matrix was constructed from fourth root transformed data. Outliers were removed to achieve equal variance across data sets. The threshold for significance was set at *p* = 0.05 and *p* – values were obtained using 9,999 permutations under an unrestricted permutation of raw data. To visually present dissimilarities in diet composition by chosen factors, a two – dimensional multi-dimensional scaling (MDS) plot was used. When a significant difference occurred, PERMANOVA

pair-wise comparison tests were undertaken. To determine the contribution of different prey items to the dissimilarity in diet composition, a similarity percentage (SIMPER) analysis was used (Clarke 1993). A 60% cumulative contribution cut-off was applied.

5.2.4 Stable isotope analysis of δ ^{13}C and δ ^{15}N

Collected samples of yelloweye mullet were stored frozen until sample processing. Upon processing, fish were thawed, measured for length and weight, dissected. The gut was removed and muscle samples (approximately 1-3 grams) were carefully extracted without skin and bone from fillets of the flanks using a scalpel. All tools used in dissections were washed between each fish using Milli-Q water (Millipore). Tissue samples upon removal from fish were stored in 5 mL vials, stored in foam trays and frozen at -18 ^{O}C.

Sample preparation and mass spectrometry

For isotopic analysis, tissue samples were initially freeze-dried for a period of 24 hours and then subsequently grounded using a mortar and pestle or in a ball mill grinder (Mawson Analytical Spectrometry Services, University of Adelaide) until a fine powder consistency was reached.

Stable isotope analysis was conducted at Mawson Analytical Spectrometry Services, University of Adelaide. Approximately 1 mg of each sample was accurately weighed into tin capsules and sealed ready for analysis. The samples were then analysed for δ^{13}C and δ^{15}N using a continuous flow isotope ratio mass spectrometer (Nu Horizon, Wrexham, UK) equipped with an elemental analyser (EA3000, EuroVector, Pavia, Italy).

Stable isotope ratios were expressed in δ notation as deviations from a standard in parts per mil (‰)

$$\delta^{13}C = [(R_{sample}/R_{standard})-1] \times 1000.$$
$$\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 1000.$$

Where R_{sample} is the ratio of abundance of ^{13}C /^{12}C or ^{15}N /^{14}N in the sample, and $R_{standard}$ is this ratio in the standard. δ^{13}C was reported relative to the standard Vienna Pee Dee Belemnite (VPDB) and δ^{15}N was reported relative to the atmospheric abundance. All samples were corrected for instrument drift and normalized according to reference values using in-house standards (n=20); δ^{13}C = glycine -31.2‰, glutamic acid -16.72‰ & triphenylamine (TPA) -29.2‰ and δ^{15}N = glycine 1.32‰ , glutamic acid -6.18‰ and triphenylamine (TPA) -0.54‰ calibrated against USGS and IAEA certified reference materials (USGS40, USGS 41, IAEA-2). USGS40 glutamic acid (n=4) δ^{13}C = -26.39‰ and δ^{15}N = -4.52 was used as a check standard CRM with the analyses.

Stable isotope statistical analysis

PERMANOVA (a single multivariate test) was used to test for group-level d^{13}C and d^{15}N differences in fish samples belonging to separate Region (Fixed, 3 levels), Time-period (Fixed, 3 levels) and fish sizes (Fixed, 2 levels). Euclidean distance was selected to measure similarity *P-values* were generated using Monte-Carlo permutations. Upon finding significant interaction terms, pairwise comparisons were undertaken to determine which combination of factors differed.

5.2.5 Fisheries catch and CPUE

Lakes and Coorong commercial fishery catch and effort data from 1984-85 to 2022-23 were used to calculate catch-per-unit-effort (CPUE) (Sarakinis and Earl 2024), which provides an indication of

biomass variation for four key species in the Coorong. For greenback flounder, yelloweye mullet and mulloway, annual CPUE was calculated based on targeted catch and effort from large-mesh gill nets (LMGN). For mulloway, targeted CPUE from swinger net catch along the ocean beaches adjacent to the Murray Mouth was also calculated. For black bream, there were low or no targeted catch in most years, thus smoothed incidental CPUE (three years rolling average, Salakinis and Earl 2024) was presented, along with total annual catch.

5.3 Results

5.3.1 Fish species richness

Fish species richness showed a general decline from the Murray Mouth Area toward the South Lagoon of the Coorong (Figure 49). With increased barrage flows in 2021-22, there was a substantial increase in species numbers, particularly at sites in the Murray Mouth Area in December 2021, and across the sites in both the Murray Mouth Area and northern North Lagoon in March 2022. During the flood in December 2022, species richness decreased in the North Lagoon but increased slightly in the northern sites of the South Lagoon. Species richness continued to increase in the South Lagoon in March 2023, which was at a similar level to the North Lagoon and Murray Mouth Area by December 2023, however, the species number in the Murray Mouth Area were lower than that in December 2022. The spatial pattern of species richness in March 2024 was similar to that in December 2023 although the species number started to reduce at several sites in the southern Coorong. Overall, species numbers varied spatially (between regions) and temporarily (between years) with a significant interaction term (P=0.001) between year and region (Table 6), suggesting the spatial pattern differed across years.

5.3.2 Total abundance

The relative total abundance of all fish species combined varied across the sites in the Coorong from March 2020 to March 2024 (Figure 50). The pattern was mainly driven by the abundance (by number) of sandy sprat and smallmouth hardyhead, which dominated the northern and southern Coorong, respectively (Appendix table 1). For example, in the South Lagoon, fish abundance was generally higher in March compared to December, due to the annual recruitment of smallmouth hardyhead following their reproductive season in spring/early summer. In December 2022 and 2023, relative abundance of fish was lower across the Coorong compared to December 2020 and 2021. Fish abundance in March 2024 was lower than in March 2020–2023. Statistical analysis indicated different spatial patterns in fish abundance across years with a significant interaction term (P=0.001) (Table 6).
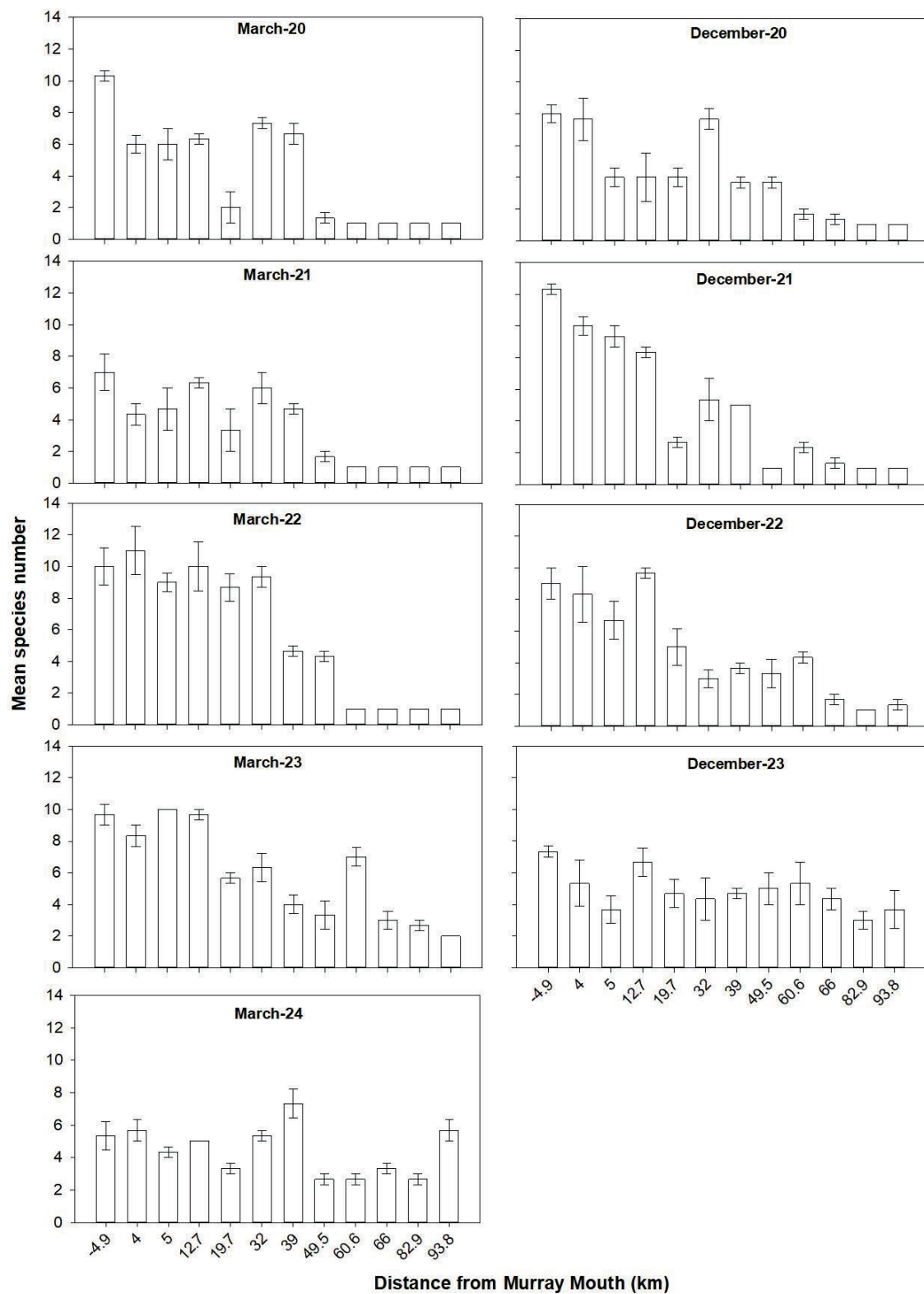
Figure 49 Fish species richness (mean species number with standard error) at each site (distance from Murray Mouth) along the Coorong between March 2020 and March 2024.
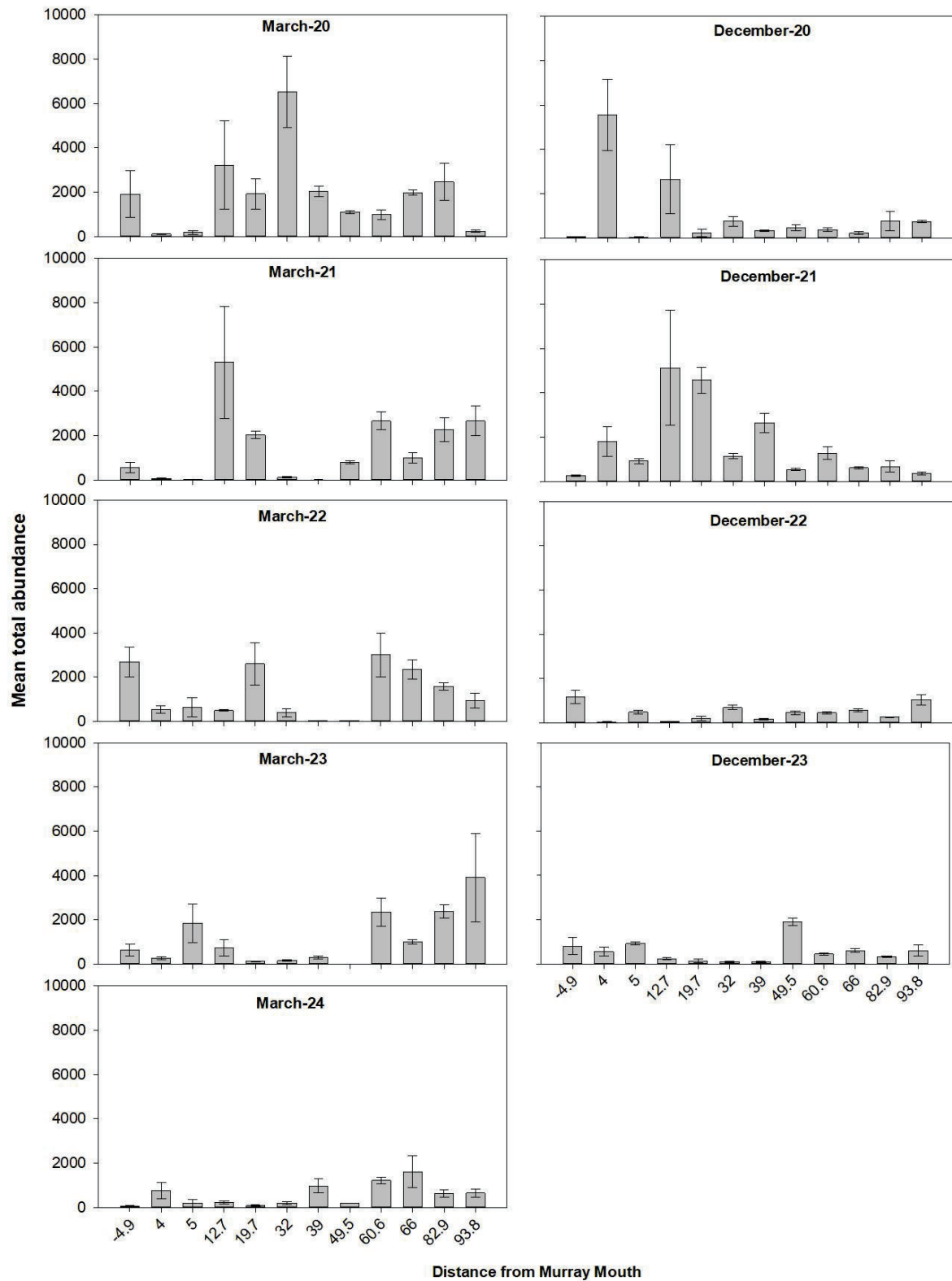
Figure 50 Relative total abundance (number of fish/seine net shot with standard error) at each site (distance from Murray Mouth) along the Coorong between March 2020 and March 2024.

Table 6 Test results from permutational ANOVA (PERMANOVA) on differences in fish species richness, relative total abundance of all species combined and assemblage structure, during the different years and across the regions. Significant *P*-values are shown in bold.

MAIN TEST	df	SPECIES RICHNESS $P_{(PERM)}$	TOTAL ABUNDANCE $P_{(PERM)}$	FISH ASSEMBLAGE $P_{(PERM)}$
Year	4	**0.001**	**0.001**	**0.001**
Region	2	**0.001**	**0.001**	**0.001**
Year x Region	8	**0.001**	**0.001**	**0.001**
Residual	309			

5.3.3 Fish community

Fish assemblage structure showed significant variation between the three regions of the Coorong and from March 2020 to 2024 (Table 6; Figure 51). For each region, fish assemblage showed a clear shift post high flows/flood (i.e. in the Murray Mouth Area in December 2021, March 2022, December 2022, March 2023 and December 2024, although there was a time lag in response in the North Lagoon and South Lagoon). Assemblages in the three regions were distinct, although there were some interspersed points in the data clouds between adjacent regions (Figure 51). This interspersion indicates that following high flow/flood, fish assemblages in the North Lagoon (i.e. in December 2023) became similar to those in the Murray Mouth Area, whereas assemblages in the South Lagoon (i.e. December 2023 and March 2024) were similar to those in the North Lagoon.

The spatio-temporal differences in fish assemblages were influenced mainly by highly abundant small-bodied species (i.e. smallmouth hardyhead and sandy sprat), the nonindigenous freshwater redfin perch, and the diadromous congolli (Figure 52). The Principal Coordinate Analysis (PCO) graph of fish assemblage data accounted for 74.4% of the total variation in the first two axes (Figure 52). Post high flow/flood (December 2021, March 2022, December 2022, March 2023 and December 2023) fish responses were mainly driven by increased abundance of sandy sprat, congolli and redfin perch while fish assemblages were strongly characterised by smallmouth hardyhead in the North and South Lagoons (Figure 53).
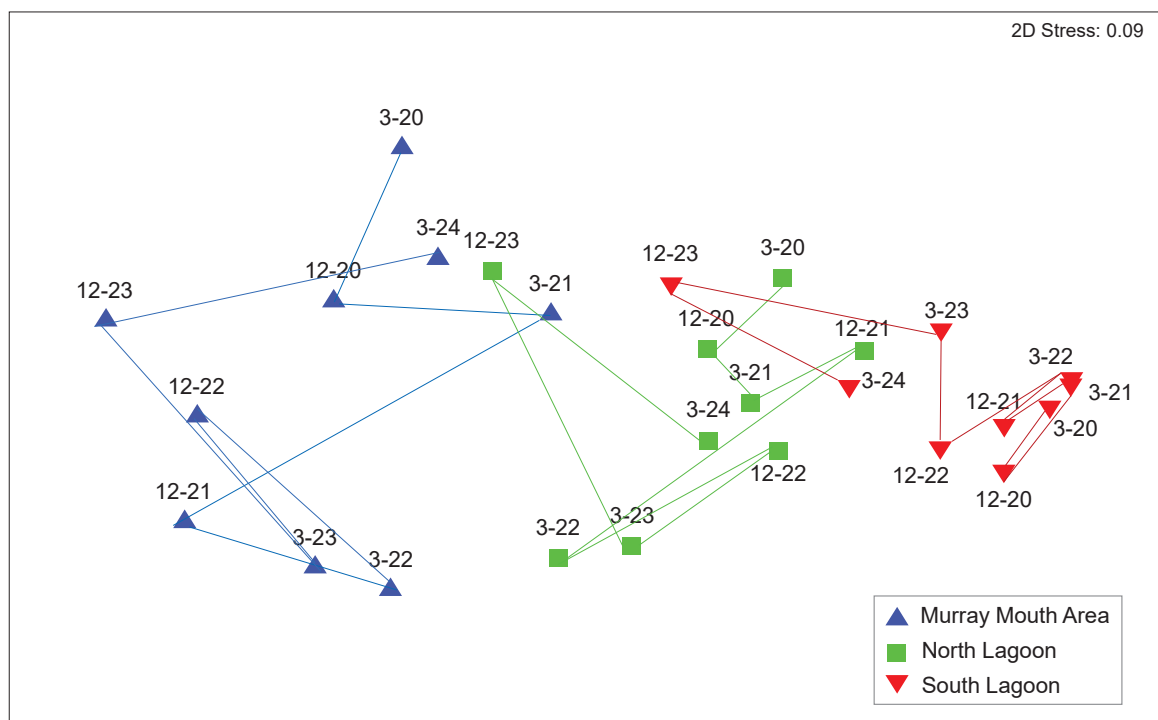
Figure 51 MDS plot for Coorong fish assemblages from the Murray Mouth Area, North Lagoon and South Lagoon, based on averaged data of large seine net samples between March 2020 and March 2024.
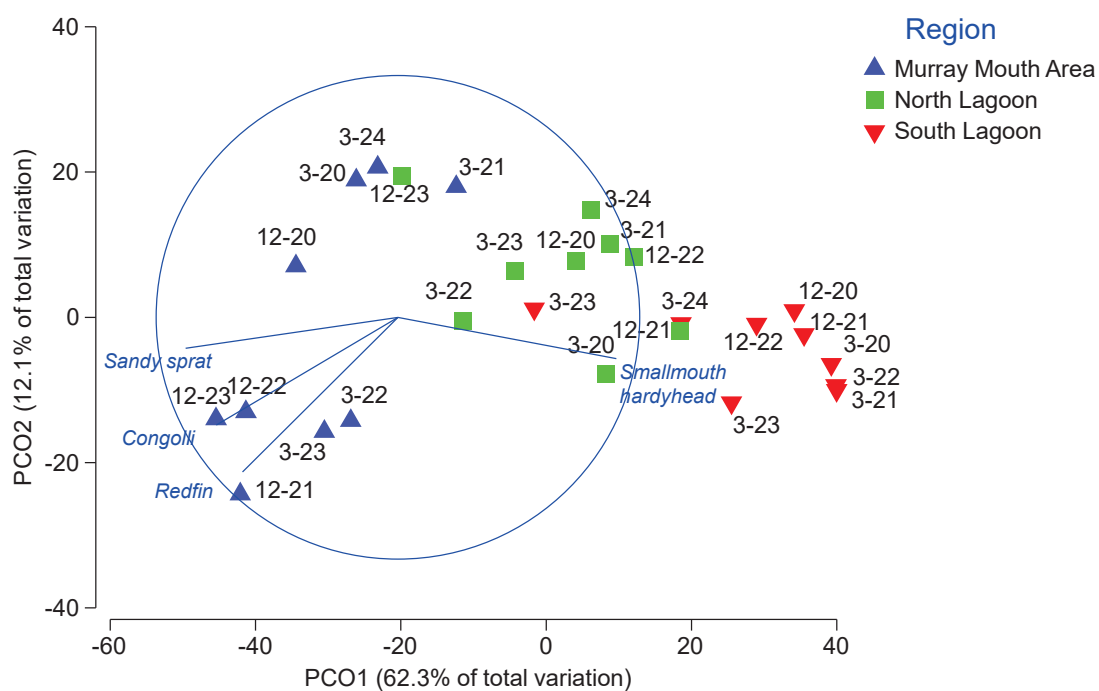
Figure 52 PCO of abundance samples of fish species collected by seine net in Murray Mouth Area, North Lagoon and South Lagoon regions in the Coorong from March 2020 to March 2024. The vector overlay indicates Pearson rank correlations between species and PCO axes 1 and 2 (Pearson correlation R >0.85). Labels indicate month-year.
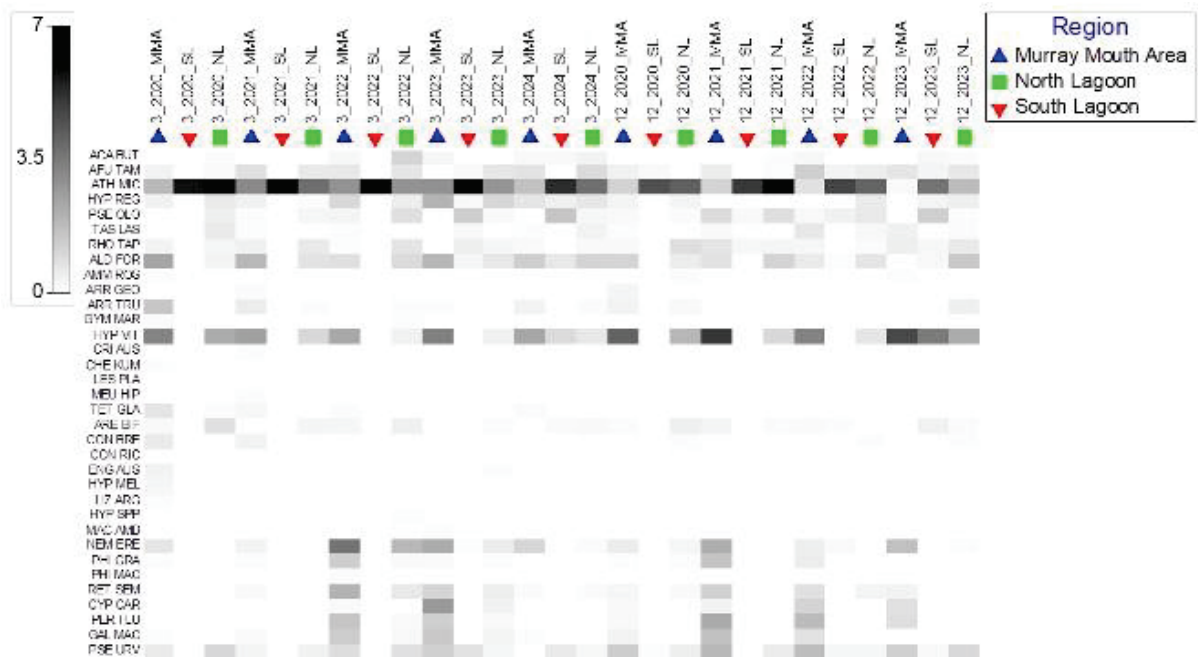
Figure 53 Shadeplot of fish communities during fish surveys in the Murray Mouth Area (MMA), North Lagoon (NL) and South Lagoon (SL) of the Coorong using seine netting between March 2020 and March 2024. The depth of the colour for the fish species corresponds to their abundances. See Appendix table 11 for species codes.

5.3.4 Fishery catches and CPUE

Long-term fisheries data indicated the variation of fish biomass of four key commercial species in the Coorong (Figure 54). The CPUE (smoothed incidental 3 year rolling average) of black bream suggested a considerable increase in biomass over the last two high flow years. The CPUE in 2022-23 flood year has been the highest since 1988-89. For greenback flounder, the CPUE in 2022-23 reached the highest since 1984-85, and was greater than two folds compared to majority of previous years. For yelloweye mullet, although the CPUE in 2022-23 was among the high levels and showed an increase from 2021-22, it was less than in 2019-20 and 2020-21. Similarly for mulloway, the 2022-23 CPUE in the Coorong was among historical highs, it was lower than 2016–2019. The CPUE of mulloway from swinger net catch along the ocean beaches adjacent to the Murray Mouth in 2021-22 and 2022-23 were the highest and second highest, respectively, compared to all previous records.
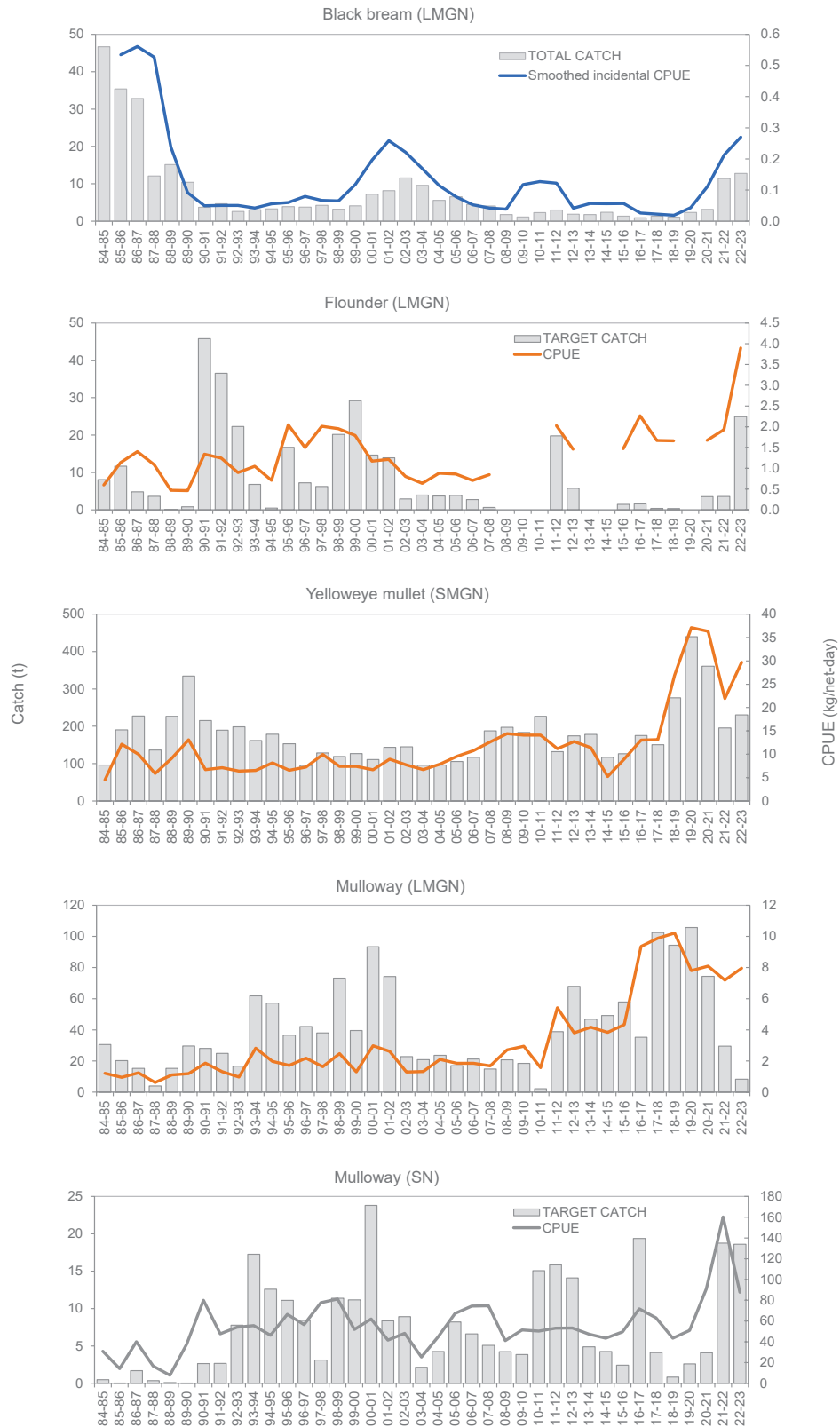
Figure 54 Annual fisheries catch and catch-per-unit-effort (CPUE) for black bream, flounder, yelloweye mullet and mulloway presented by financial year from 1984-85 to 2022-23. Note annual total catches were presented for black bream while targeted catches for other species. Large-mesh gillnets (LMGN), small-mesh gillnets (SMGN), swinger nets (SN). Graphs adapted from Salakinis and Earl 2024.

5.3.5 Yelloweye mullet diet

In March 2023, detritus was the most important dietary item for both small (<150 mm TL) and large (>150 mm TL) size groups of yelloweye mullet in the Murray Mouth Area and North Lagoon regions (Figure 55a; Appendix figure 1), followed by small crustaceans for small mullet, and filamentous green algae for large mullet (Appendix table 12). Within the Murray Mouth Area, the range of items consumed by both size groups was low, particularly for large mullet where detritus comprised almost the entirety of the diet; while small mullet primarily fed on detritus, small crustaceans, and fish (Appendix table 12). In contrast, yelloweye mullet in the North Lagoon showed a wider consumption of various dietary items for both size groups, yet still detritus remained the most important food item (Figure 555a; Appendix table 12). In the South Lagoon, diet composition of mullet was unique compared to the northern regions, with diatoms (*Bacillariophyceae*) and capitellid polychaetes being the main dietary items for small mullet and small crustaceans comprising 65.2% (by volume) of the diet for large mullet (Figure 555a). Nevertheless, the sample size was low for both small (*n* = 2) and large (*n* = 1) mullet in the South Lagoon.

In December 2023, macroinvertebrates accounted for a substantial proportion of dietary biomass for yelloweye mullet with polychaetes being the most significant food item for both small and larger mullet (Figure 55b). Most of the polychaetes in the gut content were the large 'ragworms' from the family Nereididae (Appendix table 13). In the North Lagoon, amphipods were also commonly preyed upon by small mullet, while larger mullet fed on a variety of bivalves, such as the microbivalve *Athritica semen* and large bivalve *Hiatula alba* (Appendix table 13). Diets of small mullet in the South Lagoon were comparable to that of the North Lagoon, although with a greater consumption of small crustaceans (Figure 55b, Appendix table 13).

Yelloweye mullet in March 2012 showed a difference in diet composition between size classes, which was more pronounced in the Murray Mouth Area (Figure 55c, Appendix table 14). Large mullet (>150 mm) focused predominantly on the consumption of detritus and algae/plant matter in both the Murray Mouth Area and North Lagoon. However, smaller mullet (<150 mm) shifted their diet across regions, favouring the consumption of invertebrates and fish in the Murray Mouth Area while primarily feeding on organic material (i.e. detritus and algae/plants) in the North Lagoon (Appendix table 14). Small (*n=1)* and large (*n*=1) mullet in the South Lagoon largely fed on chironomids and diatoms (Bacillariophyceae), respectively (Appendix table 14, Figure 55c).
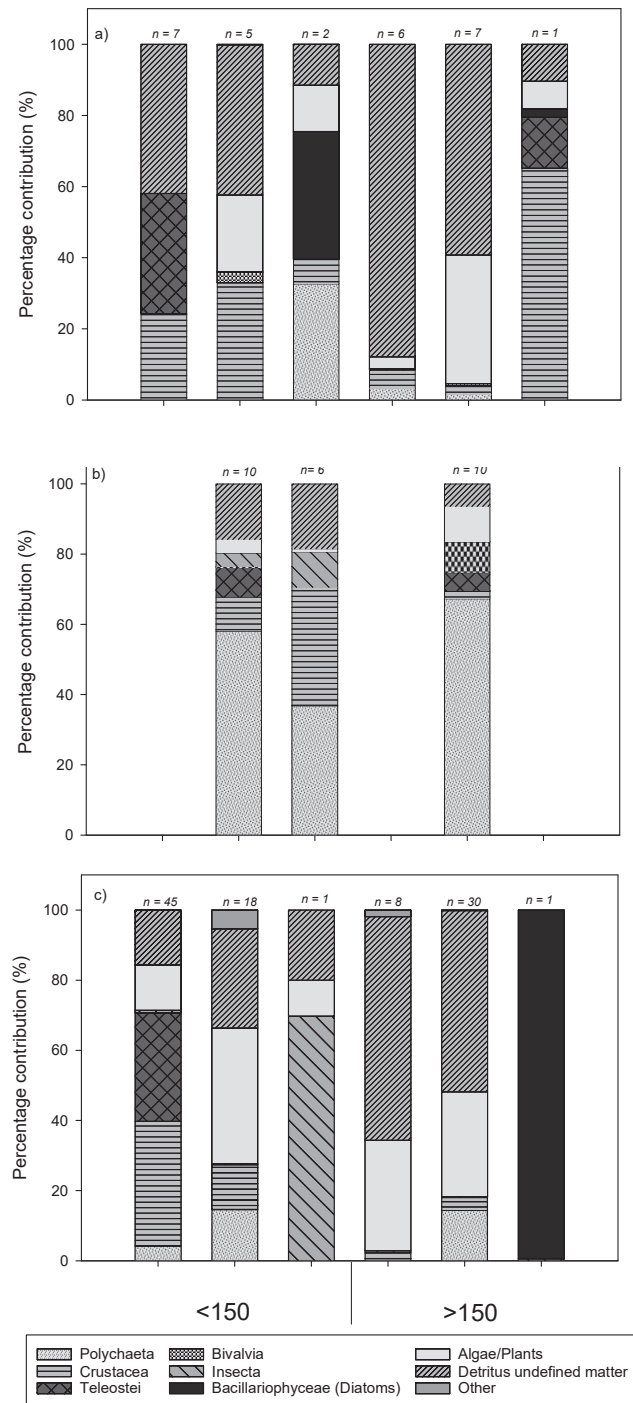
Figure 55 Percentage contribution of diet groups by volume (V%) to diet composition of small (< 150 mm) and large (>150 mm) yelloweye mullet in the Coorong. a) March 2023, b) December 2023, c) March 2012. Diet group 'other' = Foraminifera and unidentified invertebrate tissue. MMA = Murray Mouth Area, NL = North Lagoon, SL = South Lagoon.

Effect of fish size and time on diet composition in the Coorong post flood 2023

There was a significant difference in yelloweye mullet diet composition in the Coorong between March and December 2023 (*p=0.0001*, Table 7), but no significant dietary difference between mullet sizes. The MDS ordination plot revealed a clear segregation between March and December data clouds, with no distinct grouping when size was considered (Figure 56). Similarity Percentage Routine (SIMPER) revealed that differences in diet composition between March and December were predominantly attributed to the greater volumes of the nereid polychaetes in December 2023, and higher volumes of detritus in March 2023 (Table 8).

Table 7. PERMANOVA test results for size and month effects on diet composition of two different size classes (<150 mm, > 150 mm) of yelloweye mullet in the North Lagoon of the Coorong in March and December 2023. *p* values in bold are significant differences.

Factor	df	Pseudo-F	p(perm)
Size	1	1.1575	>0.05
Month	1	11.021	**0.0001**
Size x Month	1	1.0419	>0.05
Residual	28		
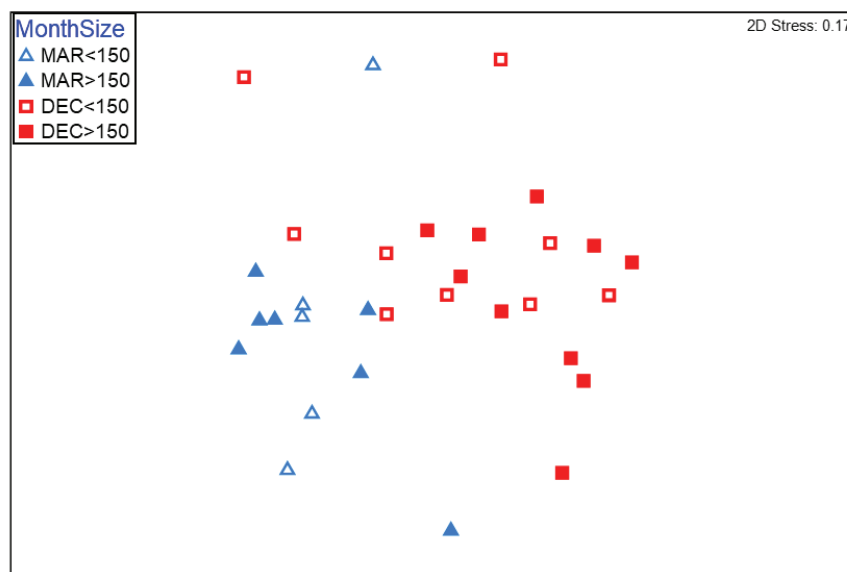
Figure 56 Two dimensional MDS ordination of the diet composition (standardised) of two size classes (<150 mm, >150 mm) of yelloweye mullet for March and December 2023 in the North Lagoon, Coorong.

Table 8 Dietary items/groups selected by SIMPER signifying the major contributors to dissimilarities of diet composition for yelloweye mullet in the Coorong between March and December 2023. A 60% cumulative contribution cut off was applied.

Av. diss= 79.31	Mar Av. volume	Dec Av. volume	Av. Diss	Diss/SD	Contrib%	Cum.%
Nereididae	0.41	2.30	16.19	1.59	20.14	20.41
Detritus	2.42	0.90	15.73	1.33	19.84	40.25
Amphipoda	0.27	0.81	6.51	0.78	8.21	48.46
Ulvaceae	0.85	0.00	6.48	0.66	8.17	56.63
Copepoda	0.78	0.04	5.68	0.94	7.16	63.79

Effects of fish size, region, and year on diet composition in the Coorong between 2012 and 2023

PERMANOVA indicated that there was a significant interaction in the diet composition of yelloweye mullet for size and year, and size and region, in the Coorong in March between 2012 and 2023 ($p <$ 0.05, Table 9a). This suggested that the pattern of diet composition difference between mullet size groups varied between years and regions. Pairwise comparisons revealed significant diet differences between years for small and large mullet, with a greater difference for the small size group ($p <0.05$, Table 9b; Appendix figure 2). SIMPER revealed that temporal dissimilarities in small mullet was attributed mostly to higher volumes of detritus and amphipods in 2012, and greater amounts of copepods in 2023 (Table 10a). For larger mullet, detritus was the significant contributor to dissimilarity between years, which was in greater volume in 2023; while algae, plant matter, and amphipods were higher in 2012 (Table 10b).

Table 9 a) PERMANOVA test results with subsequent b) pair – wise comparisons for region and year effects on diet composition of two different size classes (<150 mm, > 150 mm) of yelloweye mullet in the Coorong in March 2012 and 2023. p values in bold are significant differences. MMA = Murray Mouth Area, NL = North Lagoon.

a)

Factor	df	Pseudo-F	p(perm)
Size	1	6.9611	**0.0002**
Region	1	3.7814	**0.0062**
Year	1	8.1502	**0.0001**
Si x Re	1	2.5239	**0.0357**
Si x Ye	1	4.8278	**0.0014**
Re x Ye	1	1.4938	>0.05
Si x Re x Ye	1	0.67933	>0.05
Residual	111		

b)

Pairwise comparisons	t	p(perm)
Size between year		
<150		
2012, 2023	3.2856	**0.0001**
>150		
2012, 2023	1.7665	**0.016**
Size between region		
<150		
MMA,NL	2.4276	**0.0005**
>150		
MMA, NL	1.027	>0.05
Size within region		
MMA		
<150,>150	2.7853	**0.0001**
NL		
<150,>150	1.0801	>0.05

Table 10 Dietary items/groups selected by SIMPER signifying the major contributors to dissimilarities of diet composition for yellow – eyed mullet in size class (a) <150 mm and (b) >150 mm in the Coorong between March 2012 & 2023. Indicated by pair-wise comparisons to be statistically different. A 60% cumulative contribution cut off was applied.

a)

Av diss = 70.92 <150 mm	2012 Av. volume	2023 Av. volume	Av. Diss	Diss/SD	Contrib%	Cum.%
Copepoda	0.89	**1.89**	14.59	1.14	20.57	20.57
Detritus	**2.19**	1.48	12.94	1.12	18.25	38.82
Amphipoda	**1.52**	0.13	12.85	1.06	18.12	56.94
Ulvaceae	0.02	**0.52**	4.14	0.46	5.84	62.78

b)

Av. diss = 59.64 >150 mm	2012 Av. volume	2023 Av. volume	Av. Diss	Diss/SD	Contrib%	Cum.%
Detritus	2.21	**2.75**	10.18	0.74	17.07	17.07
Unident. Algae/plant	**1.00**	0.45	9.64	0.88	16.16	33.23
Amphipoda	**0.90**	0.15	8.50	0.72	14.25	47.48
Cladophoraceae	**0.36**	0.24	4.53	0.47	7.60	55.08
Copepoda	0.29	**0.31**	4.17	0.69	7.00	62.08

Comparing between regions, there was a significant difference in diet composition of small mullet (<150 mm) (p=0.0005, Table 9b; Appendix figure 3) but not large mullet. The diet dissimilarities for small mullet were mainly attributed to greater volumes of copepods and unidentified algae/plant material in the Murray Mouth Area, whereas there were more amounts of amphipods and detritus in the North Lagoon (Table 11a). Furthermore, diet composition differed significantly between small and large mullet in the Murray Mouth Area (p =0.0001, Table 9). Indicated by SIMPER, small mullet consumed a greater volume of invertebrates (i.e. copepods and amphipods), while large mullet diet comprised higher amounts of organic material (i.e. detritus and unidentified algae/plant material) (Table 11b).

Table 11 Dietary items/groups indicated by SIMPER signifying the major contributors to dissimilarities of diet composition between regions for one size class of yelloweye mullet (a), and two size classes within MMA region (b), in the Coorong with March 2012 and 2023 combined. Indicated by pair-wise comparisons to be statistically different. MMA = Murray Mouth Area, NL = North Lagoon. A 60% cumulative contribution cut off was applied.

a)

Av.diss = 60.75 <150 mm	MMA Av. volume	NL Av. volume	Av. Diss	Diss/SD	Contrib%	Cum.%
Amphipoda	1.25	**1.42**	11.75	1.11	19.34	19.34
Copepoda	**1.39**	0.35	11.12	1.03	18.3	37.64
Detritus	1.95	**2.34**	9.16	0.92	15.08	52.73
Mysidacea	0.04	**0.58**	4.38	0.63	7.21	59.94
Unident. Algae/plant	**0.26**	0.21	3.26	0.47	5.37	65.31

b)

Av.diss = 67.06	<150 mm MMA Av. volume	>150 mm Av. volume	Av. Diss	Diss/SD	Contrib%	Cum.%
Copepoda	**1.39**	0.17	12.99	1.00	19.37	19.37
Detritus	1.95	**2.26**	12.98	1.00	19.35	38.73
Amphipoda	**1.25**	0.38	12.41	0.90	18.5	57.23
Unident. Algae/plant	0.26	**1.06**	10.59	0.79	15.8	73.03

5.3.6 Stable isotope analysis

Eighty yelloweye mullet were collected across three regions in 2023, with 45 collected during March (autumn) and 36 collected during December (early summer) 2023 (Table 12). Data from an additional 35 fish collected in March 2012 were incorporated into the analyses for comparisons (Table 12). Upon initial data screening, one individual returned an extreme value for δ^{13}C of -80.7665, and subsequently this outlier was removed from further analysis.

Table 12 Number of tissue samples collected from Yellow-eye mullet (< 150 and >150mm FL) across the three study time periods and three regions. Note * denotes one fish sample removed from further analysis, - denote no available samples for those time-period and regions. MMA = Murray Mouth Area, NL = North Lagoon, SL = South Lagoon.

Region	March 2012		March 2023		December 2023	
	<150 mm	>150 mm	<150 mm	>150 mm	<150mm	>150mm
MMA	11	11	15	13	-	-
NL	6	7	7	7*	15	14
SL	-	-	2	1	7	0
Total	17	18	24	21	22	14

PERMANOVA indicated that there were several complex statistical differences in δ^{13}C and δ^{15}N across Regions, Time-periods and Size group according to detecting significant Region x Time-period (*Psuedo-F$_{1,107}$* = 4.7318, *P* = 0.0184), Region x Size group (*Psuedo-F$_{2,107}$* = 5.679, *P* = 0.0017) and Time-period x Size group (*Psuedo-F$_{2,107}$* = 11.541, *P* = 0.0001) interactions. These differences can be observed in Figure 57 and Appendix figure 4.

Pairwise test examining the Region x Time-period interaction revealed that δ^{13}C and δ^{15}N values for fish samples, were:

- lower in Autumn 2023 than March 2012 ($t_{1,49}$ = 5.076, *p* = 0.0001) for Murray Mouth
- lower in Autumn 2023 than December 2023 ($t_{1,51}$ = 1.9449,   *p* = 0.0334) for North Lagoon
- higher in Autumn 2023 than December 2023 ($t_{1,7}$ = 5.812,   *p* = 0.02) for South Lagoon

Pairwise test examining the Region x Size group interaction revealed that δ^{13}C and δ^{15}N values for fish samples, were:

- lower for <150 mm Size group ($t_{1,49}$ = 5.3684, *p* = 0.0001) for the Murray Mouth
- lower for <150 mm Size group ($t_{1,49}$ = 4.0482, *p* = 0.0002) for the North Lagoon
- not different between Size groups ($t_{1,49}$ = 1.1394, *p* = 0.3053) in the South Lagoon

Pairwise test examining the Time-period x Size group interaction revealed that δ^{13}C and δ^{15}N values for fish samples, were:

- lower for <150 mm ($t_{1,47}$ = 5.4043, *p*= 0.0045) in March 2023 than March 2012
- lower for <150 mm ($t_{1,37}$ = 2.823, *p* = 0.0001) in March 2023 than December 2023
- lower for >150 mm ($t_{1,40}$ = 2.1851, *p* = 0.0154) in December 2023 than March 2023
- not different for >150 mm ($t_{1,49}$ = 0.76901, *p* = 0.5394) in March 2012 and March 2023
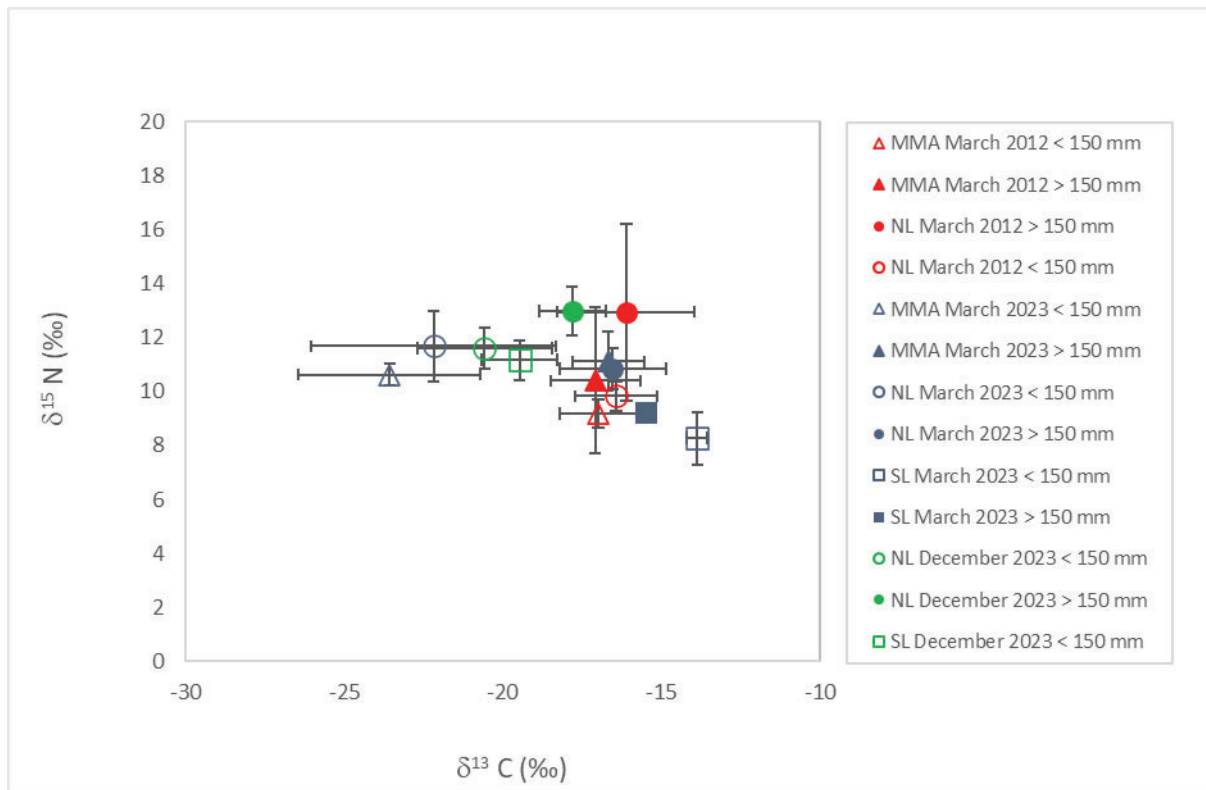
Figure 57 Stable isotope biplot of δ^{13}C and δ^{15}N across Regions, Time-periods and Size groups. Markers represent means with error bars indicating ± 1 standard deviation. MA = Murray Mouth Area, NL = North Lagoon, SL = South Lagoon.

5.4 Discussion

5.4.1 Fish species richness, abundance/biomass and assemblage structure

Freshwater inflows (particularly from the River Murray) affect fishes in the Coorong by influencing the following critical factors: (1) salinity; (2) connectivity within, and between, marine, estuarine and lake environments; and (3) productivity, by transporting carbon, nutrients and microbiota from upstream (Ye et al. 2016, Bice et al. 2018). The effects have been demonstrated through fish ecology research and monitoring, particularly over the last two decade in the Coorong (Ye et al. 2020, Dittmann et al. 2022). This study investigated Coorong fish responses to recent high flows, particularly to the 2022-23 flood using data collected from March 2020 to March 2024. Compared to low flow periods (2019-20 and 2020-21 with barrage discharge generally <10,000 ML/day), barrage flows increased substantially to 20,000–30,000 ML/day throughout most of 2021-22. A remarkable increase in fish species richness was observed in the Murray Mouth Area in December 2021 with a reduction in mean salinities to 1–18 g/L (e.g. from 12–32 g/L in December 2020), as many freshwater fish species entered the estuary. In the North Lagoon, the number of species increased substantially in March 2022 corresponding to reduced mean salinities to 15–23 g/L compared to 37–40 g/L in March 2021. As the salinity in the South Lagoon remained high, being 92–126 g/L in March 2020, 2021 and 2022, and 80–96 g/L in December 2020 and 2021, mean species number remained low (1–2) in this region; for instance, only the most salt tolerant fish species, smallmouth hardyhead, (50% lethal concentration, LC_{50} = 108 mg/L, Lui 1969) was present in this region in March.

During the peak flood flows in December 2022, there was an extensive reduction in salinity throughout the entire Coorong, with the mean salinities being 0.5–6 g/L in the Murray Mouth Area, 12–16 g/L in the North Lagoon and 57–62 g/L in the South Lagoon. This led to a considerable increase in species richness in the South Lagoon. Previous research in the Coorong also indicated a substantial reduction in species numbers at >70 g/L in the Coorong (Ye et al. 2020), and in fact, most fish species are unable to tolerate salinities >60 g/L (Dittmann et al. 2022, SARDI unpublished data). During the flood peak, a slight reduction in species richness was also observed in the Murray Mouth Area and North Lagoon, likely due to flood disturbance and a reduction in marine species due to much 'fresher' conditions in these regions in the Coorong. In March 2023, about 2–3 months after the flood peak, salinity in the Coorong generally remained similar to that during December 2022 with a further increase in species richness observed in the South Lagoon although species numbers in the North Lagoon remaining less compared to March 2022, possibly reflecting continued flood/high flow disturbance. It is interesting to see by December 2023, about 11–12 months after the flood peak, species number across the sites in the Coorong became homogenised despite that salinity gradient remained ranging 0.6–67 g/L. There was a general reduction in species richness in the Murray Mouth Area, as less freshwater species were caught, whereas an increase in species richness in the North and South Lagoons, potentially due to the improvement in estuarine habitat including macroinvertebrate food resources after the flood in these regions (see Figure 39 in section 4.3.2). In March 2024, species richness reduced at most sites in the southern Coorong associated with reduced barrage flows to <4,000 ML/day and increased salinities in the South Lagoon to 75–83 g/L.

Fish sampling by seine net did not show an increase in relative abundance post 2022-23 flood. This may be due to flood disturbance, but more likely reflects an artefact of decreased sampling efficiency. During post-flood fish sampling in 2023, there were greater flow discharge volumes to the Coorong with much high water levels relative to preceding years. This could have a 'dilution' effect on fish density in the Coorong. On the contrary, fishery CPUE indicated a substantial increase in fish biomass for black bream and greenback flounder in 2022-23, likely due to the expansion of estuarine habitat through salinity reduction and increased food resources in the Coorong. Despite no distinct post-flood increase in CPUE for mulloway and yelloweye mullet, the 2022-23 CPUE for these species were among historically high levels in the Coorong. Additionally, there was a substantial increase in mulloway abundance/biomass along the ocean beaches adjacent to the Murray Mouth post-high flows/flood, indicated by the highest swinger net CPUE in 2021-22 and 2022-23. This species forms spawning aggregations during spring/summer in the marine waters near the Murray Mouth with young juveniles then entering the Coorong when environmental conditions are favourable and utilising estuarine habitat as nursery and feeding ground, before returning to the marine environment as 4–6-year-olds (Hall 1986, Ferguson et al. 2008, 2014). The importance of freshwater inflows in estuarine fishery production has been reported in many studies (e.g. Gillson 2011, Halliday et al. 2012, Ferguson et al. 2018).

In the current study, fish assemblage structure showed a distinct shift after high flow (2021-22) and flood (2022-23), with a lag response in the North Lagoon and further lag in the South Lagoon. Post high flow/flood fish responses were mainly driven by increased abundance in freshwater species, particularly in the Murray Mouth Area; increased recruitment success of diadromous species (congolli and common galaxias), likely due to improved connectivity between marine, estuarine and freshwater environments; and increased abundance of sandy sprat, which may have benefited by the consumption of increased input of freshwater zooplankton into the Coorong (Bice et al. 2016) associated with the flood. In contrast, fish assemblages were strongly characterised by smallmouth hardyhead in the southern Coorong during years with lower flows. Smallmouth hardyhead and other small atherinids are important fish species in many temperate Australian estuaries, where they are often the dominant species (>50% of total number of fish), particularly where salinities are near or above that of seawater (e.g. Potter and Hyndes 1994, Griffiths and West 1999, Young and Potter 2002, Hoeksema and Potter 2006). Sandy sprat is a marine-estuarine opportunist species, which spawns in

inshore waters of southern Australia, and frequently enters and uses estuaries as a feeding and nursery ground (Roger and Ward 2007). High densities of sandy sprat were observed in the Murray Estuary, typically following high barrage flows (e.g. in 2011–2013), and their distribution also extended into the North Lagoon, with peak numbers generally associated with marine salinity (35 g/L) (Ye et al. 2020). Being primarily planktivorous feeders, the increased discharge of freshwater flows to the Murray Mouth Area and Coorong offers greater food availability from increases in freshwater zooplankton abundance as found in a previous study in the Coorong (Bice et al. 2016) and could promote sandy sprat recruitment. Smallmouth hardyhead and sandy sprat are the two most important prey for piscivorous fish and waterbirds in the Coorong (Giatas et al. 2018, 2022).

5.4.2 Yelloweye mullet diet

The current study, with comparison to historical data supports that mugilid species are generalist feeders which consume fish, invertebrates, and organic matter with a clear ontogenetic shift in diet (Cotta-Ribeiro and Molina-Urena, 2009, Salvarina et al. 2018). Post flood, diet composition was shown to be variable for both years, and that variability was more pronounced in smaller individuals. Small mullet may be restricted to intertidal zones due to predation pressure (Munsch et al. 2016), and therefore prey availability may be limited. Additionally, as they do not share the same capacity to digest organic material like larger individuals (Platell et al.2006, Giatas 2012), their diet is predominantly carnivorous (e.g. invertebrates). Unlike organic material, the availability of which is generally consistent temporally and spatially, invertebrate communities can change drastically through time and space. Therefore, a greater susceptibility to changes in diet is expected for smaller yelloweye mullet, as opposed to larger individuals that favour the consumption of widely available organic matter.

Freshwater input to estuarine environments can alter invertebrate assemblages due to changes in salinity, and modification of macrohabitats by sedimentation and nutrient influxes (Thrush et al. 2004, Ahmadi et al. 2011, Dittmann et al. 2022). The Murray Mouth Area is influenced by large volumes of freshwater input from the Murray River.Further distanced from this source, regions of the Coorong would be less impacted by direct freshwater input. This may allow for general food items to be more commonly widespread and available, resulting in less differences in diet between small and larger mullet, as seen in the North Lagoon in March across years. In contrast, the ever – changing environmental conditions experienced in the Murray Mouth Area, especially in periods of high flows may explain the greater variability in yelloweye mullet diet within that region. It was found that copepod consumption greatly attributed to the differences in diet between small and large individuals in the Murray Mouth Area. This may largely correspond to freshwater zooplankton (i.e. copepods) being introduced in high abundance during periods of high flows (Bice et al. 2016).

Post 2022-23 flood, there was a substantial change in the diet composition of yelloweye mullet over time. Detritus, likely readily available and encountered in high abundance during foraging shortly after flood, comprised the largest volume contribution to the diet composition of yelloweye mullet (both small and large size classes) in March 2023. Transportation of abundant organic material into the Coorong during periods of high flow has been reported previously (Mosley and Leyden 2023) and in this study (see Section 2.5.2). In December 2023, a distinct shift in diet from organic material to polychaetes exhibited by both size groups, could display opportunistic feeding particularly in large mullet, consistent with other studies on this species (Crinall and Hindell 2004, Platell et al. 2006). Polychaetes may have been more widely available in December, consumed in higher amounts. This seemed to correspond with post flood response in the availability of macroinvertebrate prey, showing increased abundance of benthic annelids (including polychaetes) during periods of high flows in the Coorong (See Section 3.3.4, Dittmann et al. 2022). Nereididae polychaete, also known as 'ragworms',

have been reported as a rich food source, particularly the large *Australonereis ehlersi* (Dittmann et al. 2022). High diet composition of the nereid polychaete for both size groups of yelloweye mullet may also suggest an element of selective feeding, where they prioritise the consumption of quality food items with higher energy content.

Further research should be conducted to confirm the impact that flood events have on the diet composition of yelloweye mullet. This may include the identification of zooplankton of freshwater origin in the diet, particularly for small mullet, as zooplankton consumption was shown to be high in the Murray Mouth Area for both high flow years in March 2012 and 2023. Such research will improve understanding of the flood impacts on estuarine fish diets.

5.4.3 Stable isotope analysis

Carbon (δ^{13}C) and Nitrogen (δ^{15}N) isotope values were observed to differ in yelloweye mullet tissues, spatially, temporally and specifically for sizes of fish. These changes likely reflect the influence of flood on the diet of yelloweye mullet in the Coorong estuary.

During March 2023, a short time after the peak of the flood arriving at the Coorong, it was noticed that δ^{13}C average values (-22 to -23) were among the lowest for <150 mm fish in both the Murray Mouth Area and North Lagoon regions and these values were substantially lower than what was observed in March 2012 (-16 to -17). Despite that 2011-12 was also a high flow year, barrage flow releases to the Coorong were much lower compared the 2022-23, which was the highest flood year since 1956. These autumn 2023 signatures for smaller fish are more consistent with basal carbon signatures for the lower River Murray (McInerney et al. 2019), suggesting greater freshwater subsidy in fish diets during March 2023. Furthermore, gut examination of these <150 mm class of fish during March, indicated that the diet increasingly consisted of copepods (see Section 5.3.5), which taken in line with the δ^{13}C results may indicate that flood waters either brought down volumes of freshwater zooplankton, which were opportunistically consumed by smaller fish, or alternatively reflect zooplankton (including estuarine species) ingesting primary producers from freshwater sources. Freshwater subsidy in fish diets, through the consumption of freshwater zooplankton species, has been shown for sandy sprat (*Hyperlophus vittatus*) in the Coorong during moderate barrage inflows (Bice et al. 2016). Depletion of δ^{13}C in the muscle tissue owing to the flood was not detected in larger fish, as integration of carbon in fish muscle tissue (i.e. turnover) is expected to be longer for large compared to small fish (Weidal et al. 2011), especially the flood effects were short term in nature. Furthermore, the enrichment of nitrogen (δ^{15}N) in large mullet in December 2023 compared to March 2023 seemed to align with diet shifts from primarily detritus to polychaete worms. Further analyses with other element isotopes, such as sulphur can also assist with better discrimination of potential carbon and nitrogen from basal resources including primary producers (Conolly et al. 2004).

6 The response of the Coorong to the 2022/23 River Murray Flood

During the high flow period of the River Murray Flood 2022/23, which was larger than any that have occurred since 1956, with barrage discharges peaking at 120,000 ML/day on 30 January 2023, there were significant changes to the environmental and ecological conditions in the Coorong. This provided an opportunity to understand the environmental response of the Coorong from the physical, chemical and ecological perspective. The nature of these changes may have positive outcomes such as the increased ecological diversity and biomass production expected from the reduction in salinity, greater connectivity and associated flushing of nutrients. Alternatively, the changes may have negative outcomes, where physical scouring, high water levels and changing water chemistry lead to the loss of ecosystem functions and reduced resilience.

This study observed changes in the Coorong physical and biological components of the environment as a result of the flood event. The changes were spatially and temporally variable as further described below. Water levels were significantly higher than normal and covered the normally exposed mudflats throughout the summer months across most of the southern Coorong in early 2023.

Sediment and water quality

During the period of the flood event salinities were reduced from a long-term average of around 55 ppt (1998-2020) to around 10 ppt in the North Lagoon, and from around 100 ppt (pre-2022) to around 60 ppt in the South Lagoon (Chapter 2). There were areas of physical scouring where the high energy of the flow from the floods occur (i.e. around the Murray Mouth). Sediments were better oxygenated and an overall improved sediment quality was observed. There were improvements to water quality in the Coorong South Lagoon, including reductions in total nitrogen and total phosphorus, and improvements in water clarity once the high flow had abated.

Following the flood, as the Coorong water levels returned to normal, there was a return to anoxic sediments and sediments were seen to have increased concentrations of porewater sulfide and ammonia. Both the North and South Coorong lagoons were still classed as eutrophic (i.e. contain excessive nutrients), reflecting the need for ongoing flushing and export of nutrients from the Coorong, which high flows could facilitate.

Ruppia community

During the period of high flow the *Ruppia* Community was resistant to changes until salinity levels went below 35 ppt where the hypersalinity adapted plants (e.g. *Ruppia tuberosa*) began to decline and which appeared to die off when salinities reduced to 10 ppt. With lower salinities persisting, other aquatic macrophytes recruited, in particular the more freshwater-tolerant aquatic plant *Ruppia megacarpa* and the charophyte *Lamprothamnium papulosum*. This is consistent with paleolimnology findings of Dick et al. (2012) that suggest the Coorong had much lower salinity in the past. Propagules of these two aquatic plants must be present in the system for their rapid growth as conditions changed. The rapid change in water levels in late 2023 led to *Ruppia* Community die-off as is expected to happen with the annual cycle of water level and salinity changes. The full consequences of the floods will likely not be evident for several more growing seasons given the timing and life cycle of the *Ruppia* Community species.

Seed banks of the *Ruppia* Community persisted although at a moderate or low level following the flood event. The condition of seeds appeared vulnerable, seeds were soft coated, not hard as usual, however they are recruiting in the most recent (June 2024) growing season. The *Ruppia* Community is behaving as a resilient multispecies community and further monitoring regularly will provide greater insight into status rather than one-off sampling.

Invertebrate community

There was a recolonisation of macroinvertebrates into the South Lagoon as salinities reduced to levels that were able to be tolerated by a wide range of macroinvertebrates. The diversity and abundance of macroinvertebrates were highest in the North Lagoon. These Coorong North Lagoon animals provided a source for colonisation of the South Lagoon observed.

There was an initial impact of the flood event to the macroinvertebrates communities in the area adjacent to the Murray Mouth, but they started to recover quickly once the immediate impact was over. There were shifts in distribution and abundance of macroinvertebrates after the flood which affected the food availability for fish and shorebirds. The rapid change in distribution and abundance of macroinvertebrates is evidence of resilience in the system.

Macroinvertebrates resilience has been enabled by management strategies that have led to improved flow and connectivity associated with and water management over the last decade. This has led to the increased populations of macroinvertebrates in the North Lagoon which further support recovery when salinity conditions prevail in the Coorong South Lagoon to enable this.

Fish community

The biota responded positively, fish diversity increased, and there was an increase in the southwards distribution in many species including congolli, black bream, greenback flounder and yelloweye mullet. In fact, the post flood fish community was characterised by increased number of freshwater species, enhanced recruitment of diadromous species (congolli and galaxias), and greater abundance of sandy sprat, whereas the community was more dominated by smallmouth hardyhead in low flow years in the southern Coorong. Sandy sprat and smallmouth hardyhead were the two most abundant prey species for fish-eating waterbirds and larger fish. Fish biomass increased evidenced by the increase in CPUE in 2022-23 of key fishery species, including black bream and greenback flounder. Carbon isotope analysis suggested freshwater food subsidy from the lower River Murray post flood for small yelloweye mullet in the Coorong. Diet shifts were observed in both small and larger mullet with increased consumption of polychaete worms corresponding to macroinvertebrate recovery in the North Lagoon. Salinity reduction, increased estuarine habitat and food resources, particularly in the North and South Lagoons, have shown to benefit fish populations in the Coorong.

Ecological change in the Coorong following the flood event

Based on the observed responses to the flood event, and comparing observations from the outcomes from previous high flow events, further improvements to the Coorong ecosystem can be expected if the system experiences moderate to good flows in the next couple of years. Already, the water level drop going into summer of 2023 had a negative effect on habitat quality, with local anoxia, sulfide and ammonia production. The draw down of nutrients during higher flow periods was observed, however return to low flows led to a halt to this. In addition the *Ruppia* Community died off reducing seed bank production and higher salinities impacted fish communities and macroinvertebrates.

7 List of shortened forms and glossary

Laboratory detection limits	The smallest amount or concentration of an analyte that can be reliably distinguished from the baseline.
T&I	Trials and Investigations project

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Appendices

Appendix A – Water Quality and Sediment

Table 13 A1 Surface Water EC, pH and Turbidity (NTU) in each sampling period

	AUGUST			NOVEMBER			MARCH		
	EC (dS/m)	PH	NTU	EC (dS/m)	PH	NTU	EC (dS/m)	PH	NTU
Hunters Creek	1.4	9.22	21.5	1.0	9.08	76.2	26.2	8.52	49.9
Pelican Point	1.4	9.89	23.5	4.1	8.8	65.8	13.7	9.16	54.7
LP	18.7	8.18	2.3	32.4	7.71	5.7	22.5	8.56	12.3
NOO	40.1	8.23	3.4	37.2	8.1	80.6	50.6	8.42	5.0
NMF	53.4	8.23	4.0	42.8	8.55	101.5	11.4	8.2	46.2
Parnka Point	50.6	8.32	2.7	40.2	8.43	39.5	98.0	7.88	21.25
VDY	56.0	8.43	19.9	44.7	8.22	120.6	120.2	7.88	198.8
Woods well	55.0	8.48	19.5	43.5	8.39	113.2	112.0	8.04	36.8
Policeman's Point	58.0	8.47	12.8	45.1	8.48	18.7	99.6	7.69	23.3
Salt Creek	53.4	8.4	9.6	47.3	7.96	16.1	88.7	8.04	30.1

Table 14 A2 Porewater sulfide mean across all depths and maximum sulfide in profile (mM) in each sampling period at each site. Sulfide does not exceed toxicity concentration in literature of 0.25 mM.

	AUGUST	NOVEMBER	MARCH	AUGUST	NOVEMBER	MARCH
	mM (mean)	mM (mean)	mM (mean)	mM (max)	mM(max)	mM(max)
Hunters Creek	0.0	0.005	0.003	0.00000	0.011	0.011
Pelican Point	0.0003035	0.001	0.007	0.00182	0.005	0.023
LP	0.0000098	0.003	0.000	0.00006	0.010	0.001
NOO	0.0000055	0.0	0.0	0.00003	0.0	0.0
NMF	0.0001722	0.007	0.017	0.00019	0.009	0.021
Parnka Point	0.0002512	0.008	0.017	0.00063	0.010	0.024
VDY	0.0000051	0.002	0.008	0.00003	0.009	0.016
Woods well	0.0005943	0.006	0.010	0.00357	0.011	0.019
Policeman's Point	0.0001707	0.009	0.002	0.00064	0.010	0.009
Salt Creek	0.0024025	0.007	0.007	0.00771	0.010	0.018

Table 15 A3 Sediment rapid assessment protocol (RAP) based on visual assessment of retrieved sediment core at each site

	AUGUST 2023			NOVEMBER 2023			MARCH 2024		
	RAP	AQUATIC PLANTS	INVERTABRATES PRESENT	RAP	AQUATIC PLANTS	INVERTABRATES PRESENT	RAP	AQUATIC PLANTS	INVERTABRATES PRESENT
Hunters Creek	12	NO	YES	13	NO	NO	12	NO	NO
Pelican Point	12	NO	YES	13	NO	NO	11	NO	NO
LP	11	YES	YES	12	NO	NO	12	NO	NO
NOO	13	NO	YES	12	YES	YES	12	NO	NO
NMF	13	YES	YES	12	YES	NO	6	NO	NO
Parnka Point	12	YES	YES	10	NO	NO	10	NO	NO
VDY	12	YES	YES	12	NO	NO	10	NO	NO
Woods well	11	NO	NO	10	NO	NO	10	NO	NO
Policeman's Point	13	YES	NO	10	NO	NO	6	NO	NO
Salt Creek	13	YES	NO	10	NO	NO	9	NO	NO

Appendix B – Macroinvertebrates

LOCATION	LOCATION CODE	ZONE	TLM SITE NUMBER	DISTANCE FROM MURRAY MOUTH (KM)	AUGUST 2023	N	NOVEMBER 2023	N	MARCH 2024
Monument Road	MR	Intertidal	1	5.6	7/8/2023	5	27/11/2023	5	14/3/2023
Monument Road	MR	Subtidal		5.57	7/8/2023	5	4/12/2023	5	ns
Monument Road	MR	Peninsula		5.59	7/8/2023	5	4/12/2023	5	ns
Hunters Creek	HC	Intertidal	2	2.21	7/8/2023	5	27/11/2023	5	14/3/2023
Hunters Creek	HC	Subtidal		1.85	7/8/2023	5	13/12/2023	5	ns
Hunters Creek	HC	Peninsula		1.86	7/8/2023	5	13/12/2023	5	ns
Ewe Island	EI	Intertidal	4	6.81	9/8/2023	5	27/11/2023	5	18/3/2023
Godfrey's Landing	GL	Peninsula		4.44	7/8/2023	5	4/12/2023	5	ns
Boundary Creek	BC	Subtidal		3.95	7/8/2023	5	4/12/2023	5	ns
Pelican Point	PP	Intertidal	5	13.46	9/8/2023	5	27/11/2023	5	18/3/2023
Pelican Point	PP	Subtidal		13.56	10/8/2023	5	5/12/2023	5	ns
Pelican Point	PP	Peninsula		13.15	10/8/2023	5	5/12/2023	5	ns
Mark Point	MP	Intertidal		20.12	10/8/2023	5	27/11/2023	5	ns
Mark Point	MP	Peninsula		19.67	10/8/2023	5	6/12/2023	5	ns
Mark Point	MP	Subtidal		19.6	10/8/2023	5	6/12/2023	5	ns
Mulbin Yerrok	MY	Intertidal	6	26.74	10/8/2023	10	1/12/2023	10	18/3/2023
Long Point	LP	Intertidal		30.06	10/8/2023	5	1/12/2023	5	ns
Long Point	LP	Subtidal		30.27	10/8/2023	5	4/12/2023	5	ns
Long Point	LP	Peninsula		30.45	10/8/2023	5	4/12/2023	5	ns
Noonameena	NM	Intertidal	7	41.26	11/8/2023	5	29/11/2023	5	19/3/2023
Noonameena	NM	Subtidal		46.14	10/8/2023	5	6/12/2023	5	ns
Noonameena	NM	Peninsula		45.53	10/8/2023	5	6/12/2023	5	ns
North Magrath Flat	MF	Intertidal		57.53	11/8/2023	5	30/11/2023	5	ns

Site	Code	Zone	Ref	Value	Date	n	Date	n	Date
North Magrath Flat	MF	Subtidal		55.83	9/8/2023	5	6/12/2023	5	ns
North Magrath Flat	MF	Peninsula		55.51	9/8/2023	5	ns	ns	ns
Parnka Point	PaP	Intertidal	8	60.5	8/8/2023	5	30/11/2023	5	19/3/2023
Parnka Point	PaP	Subtidal		60.48	9/8/2023	5	4/12/2023	5	ns
Parnka Point	PaP	Peninsula		62.75	9/8/2023	5	4/12/2023	5	ns
Villa de Yumpa	VdY	Intertidal	9	65.19	8/8/2023	5	29/11/2023	10	19/3/2023
Villa de Yumpa	VdY	Subtidal		65.26	9/8/2023	5	15/12/2023	5	ns
Villa de Yumpa	VdY	Peninsula		64.96	9/8/2023	5	ns	ns	ns
Sandpiper	S	Intertidal		75.17	8/8/2023	10	30/11/2023	10	ns
Jack Point	JP	Intertidal	10	81.7	8/8/2023	5	30/11/2023	5	19/3/2023
Jack Point	JP	Subtidal		81.51	8/8/2023	5	15/12/2023	5	ns
Jack Point	JP	Peninsula		80.27	8/8/2023	5	ns	ns	ns
Salt Creek	SC	Intertidal		93.89	8/8/2023	5	28/11/2023	5	ns
Salt Creek	SC	Subtidal		93.6	8/8/2023	5	5/12/2023	5	ns
Salt Creek	SC	Peninsula		92.76	8/8/2023	5	ns	ns	ns
Loop Road	LR	Intertidal	11	97.04	8/8/2023	10	28/11/2023	10	19/3/2023

Table B.2 Species list for macroinvertebrates recorded as present (√) at the sampling locations in the surveys in August 2023 (A) and November/December 2023 (N/D). For location codes see Table B.1. Post-larvae of nereid polychaetes and pupae of unidentified dipteran insects were not included in the table.

Site code, distance from the Murray Mouth (km) and survey

Phyla/Class/Order	Family/Genus/Species	MR -6 A	MR -6 N/D	HC -2 A	HC -2 N/D	EI 5 A	EI 5 N/D	PP 13 A	PP 13 N/D	MP 20 A	MP 20 N/D	MY 27 A	MY 27 N/D	LP 30 A	LP 30 N/D	NM 44 A	NM 44 N/D	MF 45 A	MF 45 N/D	PaP 61 A	PaP 61 N/D	VdY 65 A	VdY 65 N/D	SP 75 A	SP 75 N/D	JP 81 A	JP 81 N/D	SC 93 A	SC 93 N/D	LR 97 A	LR 97 N/D
Annelida Oligochaeta		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√
Polychaeta	Capitella spp.	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		
	Simplisetia aequisetis	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			√	√	√				
	Australonereis ehlersi	√	√	√		√	√	√		√	√	√	√	√	√	√	√	√	√	√	√										
	Boccardiella limnicola	√	√	√	√	√		√		√	√	√		√	√	√	√	√	√	√	√										
	Spionidae indet.									√					√	√			√												
	Aglaophamus australiensis	√						√		√			√																		
	Syllidae indet.							√	√	√		√	√	√	√	√	√	√	√		√										
	Euchone variabilis							√		√				√																	
	Ficopomatus enigmaticus							√		√				√																	
Crustacea Amphipoda		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√		√	√	√	√	√
Ostracodae														√	√							√	√			√	√	√	√	√	√
Isopoda	Haloniscus searlei		√										√		√		√										√				
Mysidae		√	√						√		√						√														
Decapoda	Amarinus laevis								√																						
Mollusca Bivalvia	Arthritica semen									√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√					
	Hiatula alba	√	√							√		√	√	√	√	√	√	√	√	√		√	√	√	√	√					
	Spisula trigonella	√	√								√	√	√	√	√					√	√	√	√					√			
Gastropoda	Salinator fragilis																			√	√	√	√		√	√					
	Hydrobiidae sp. 1						√	√	√																						
	Hydrobiidae sp. 3	√	√						√			√	√																		
	Hydrobiidae sp. 4	√	√						√	√		√	√	√		√	√	√	√												
	Hydrobiidae sp. 5	√	√		√				√		√																				
	Hydrobiidae sp. 6																	√	√												
	Coxiella striata								√																						
Hexapoda Diptera	Chironomidae	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	Ceratopogonidae	√	√	√		√	√	√	√								√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	Dolichopodidae							√	√											√	√	√	√								
	Empididae																	√	√				√								
	Ephydridae																		√		√										
	Staphylinidae																	√													
Total species number per site and survey		9	10	6	4	7	6	8	13	12	12	12	12	14	15	15	16	12	14	11	12	7	7	5	4	5	5	6	5	4	3

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Table B.3 PERMANOVA test results for univariate tests of macroinvertebrate abundances for total counts and major taxa, across a two factor design with survey and location as fixed factors. Ns = not significantly different. All tests had >9000 permutations.

	df	SS	MS	Pseudo-F	P(perm)
Total					
Location (Loc)	14	2928	209	17.04	0.0001
Survey (Su)	1	201	201	16.37	0.0001
Loc x Su	14	594	42	3.46	0.0001
Residual	375	4603	12		
Annelida					
Location (Loc)	14	3359	240	31.38	0.0001
Survey (Su)	1	382	382	50.01	0.0001
Loc x Su	14	795	57	7.43	0.0001
Residual	375	2868	8		
Mollusca					
Location (Loc)	14	1680	120	31.47	0.0001
Survey (Su)	1	70	70	18.26	0.0003
Loc x Su	14	187	13	3.50	0.0001
Residual	375	1430	4		
Crustacea					
Location (Loc)	14	8631	616	49.69	0.0001
Survey (Su)	1	6	6	0.46	0.5
Loc x Su	14	878	63	5.05	0.0001
Residual	375	4653	12		
Hexapoda					
Location (Loc)	14	1478	106	12.46	0.0001
Survey (Su)	1	4	4	0.48	0.4871
Loc x Su	14	749	54	6.31	0.0001
Residual	375	3179	8		

Table B.4 PERMANOVA test results for multivariate macroinvertebrate community composition for total counts and major taxa, across a two factor design with survey and location as fixed factors. Ns = not significantly different. The test had >9000 permutations.

	df	SS	MS	Pseudo-F	P(perm)
Location (Loc)	14	491490	35106	39.80	0.0001
Survey (Su)	1	13907	13907	15.77	0.0001
Loc x Su	14	64842	4632	5.25	0.0001
Residual	375	330760	882		
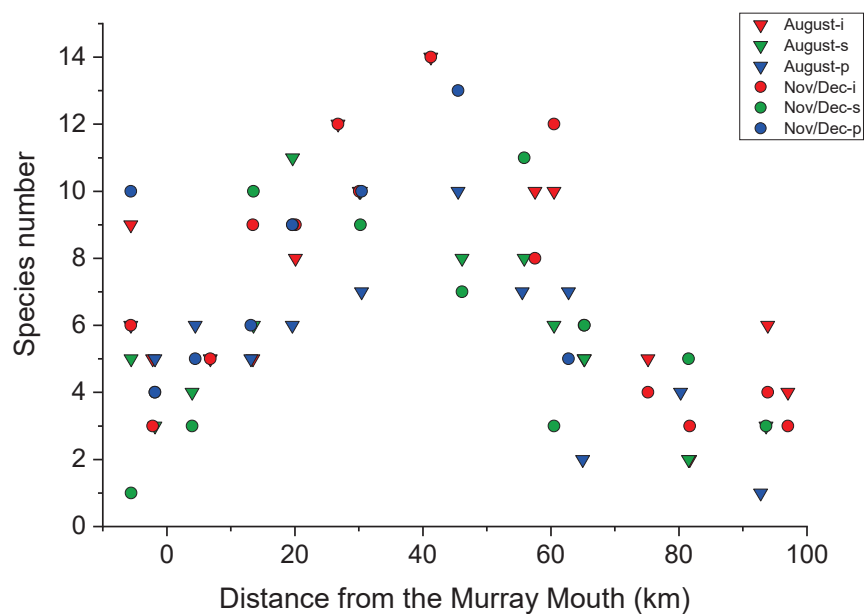
Figure B.1. Number of macroinvertebrate species found at the locations along the Coorong in August and Nov/Dec 2023, differentiated by the sampling zones across transects with i = intertidal mudflat, s = subtidal sediment in channel, p = peninsula mudflat.
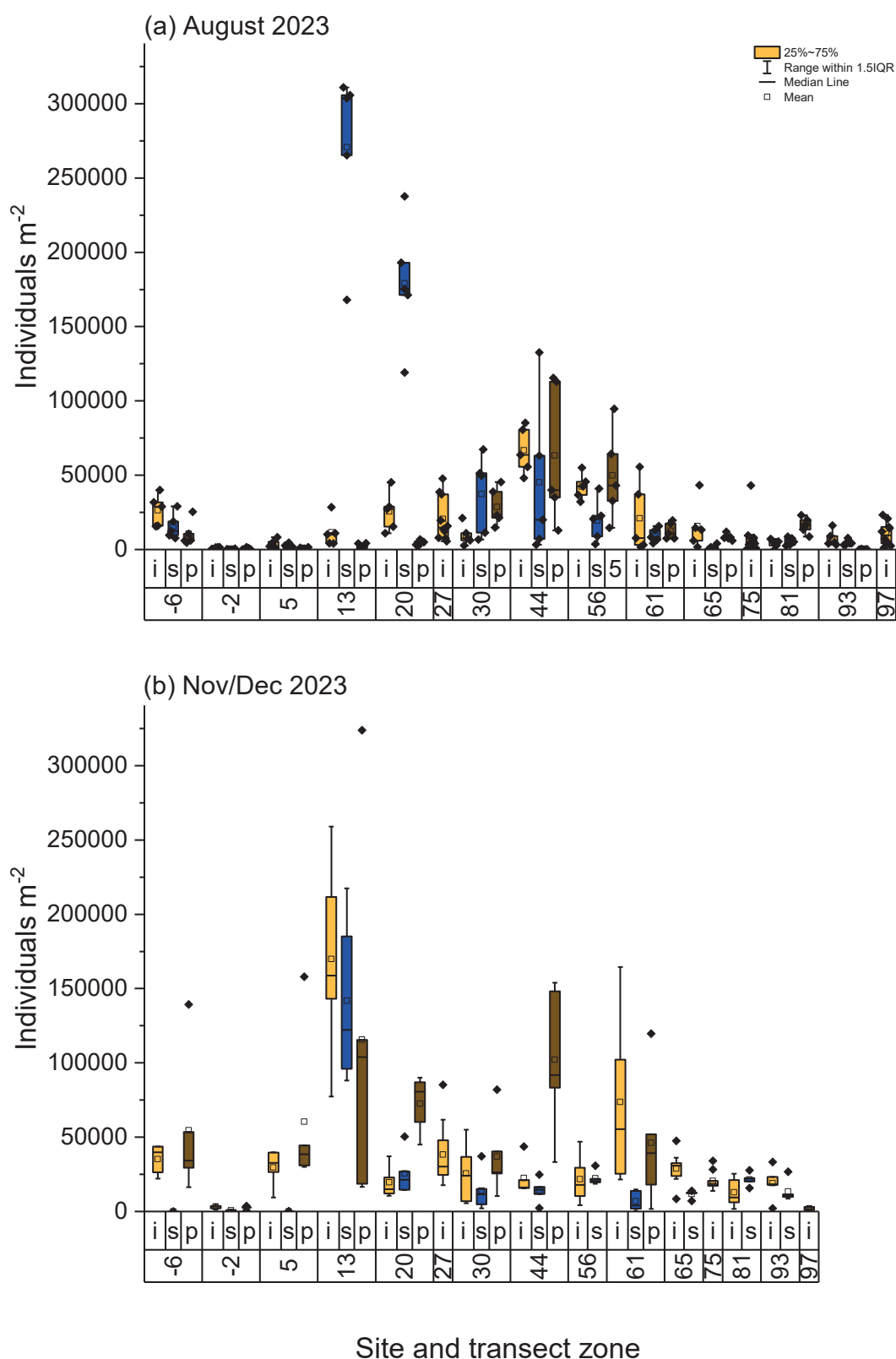
Figure B.2. Boxplots of macroinvertebrate abundance from the surveys in (a) August and (b) Nov/Dec 2023, showing the total macroinvertebrate numbers per locations and transect zones, with i = intertidal mudflat, s = subtidal sediment in channel, p = peninsula mudflat. Note that not all peninsula zones could be sampled in Nov/Dec 2023. See Table B.1 for location codes and distances from Murray Mouth.

Appendix C – Fish

Appendix table 1 – Catch summary from fish surveys in the Coorong across three regions (Murray Estuary, North Lagoon and South Lagoon) using seine netting between March 2020 and March 2024.

Murray Estuary Area	Mar-20	Dec-20	Mar-21	Dec-21	Mar-22	Dec-22	Mar-23	Dec-23	Mar-24	Total
Australian anchovy	18									18
Australian herring		7	3							10
Australian smelt		4	2	68	308	29	59	6	2	478
Black bream					3	3	4		5	15
Bluespot goby		2	1	27	7	4				41
Bony herring	187	89	8	375	3,560	16	394	290	91	5,010
Bridled goby	3	2		6	5	5				21
Carp		3		6	2	21	1,911	19		1,962
Common galaxias	1	5	2	212	106	26	56		1	409
Congolli	21	79	12	232	21	157	124	59		705
Dwarf flat-headed gudgeon					3					3
Flat-headed gudgeon	1	1	1	141	58	62	2			266
Golden perch							1			1
Goldspot mullet	1									1
Greenback flounder	7	3	9	16	1	3		17	2	58
Horseshoe leatherjacket			1							1
Longsnout flounder	1							1		2
Prickly toadfish	12		6							18
Red gurnard	1									1
Redfin perch		3		1,882	96	467	76	24		2,548
River garfish	8	10	20		41		361		14	454
Sandy sprat	14,712	24,430	11,994	20,763	1,121	4,210	4,731	7,086	2,820	91,867
Scary's Tasman goby		1	1	3	1	43		11	1	61
Smallmouth hardyhead	364	98	5,384	384	7,518	20	2,030	2	586	16,386
Smooth toadfish	33		11		1				2	47

	Mar-20	Dec-20	Mar-21	Dec-21	Mar-22	Dec-22	Mar-23	Dec-23	Mar-24	Total
Soldier	2									2
Southern crested weedfish			1							1
Southern garfish	5									5
Tamar goby	6	9	53	15	24	90		64	32	293
Western Australian salmon	132	5	22				1		6	166
Yelloweye mullet	797	93	378	129	177	55	710		146	2,485
Sub-total	**16,312**	**24,844**	**17,909**	**24,259**	**13,053**	**5,211**	**10,460**	**7,579**	**3,708**	**123,335**

North Lagoon	Mar-20	Dec-20	Mar-21	Dec-21	Mar-22	Dec-22	Mar-23	Dec-23	Mar-24	Total
Australian anchovy							1			1
Australian smelt		2			63	19	43		4	131
Black bream				1	252		2	4	5	264
Bluespot goby	36	2	5	48	14	16	2	1	3	127
Bony herring		5			1,764		77	1	2	1,849
Bridled goby	51	49	7	2	9		2	3	4	127
Carp							15			15
Carp gudgeon spp.					1					1
Common galaxias					11		11			22
Congolli	127	16	8	10	33	4	4	83	10	295
Flat-headed gudgeon		1			3		1			5
Greenback flounder	5	36	9	8	20	2	4	32	5	121
Longsnout flounder		2								2
Prickly toadfish		1				1		1		3
Redfin perch					11		4			15
River garfish	21	8	1		19	24	76	9	28	186
Sandy sprat	7,597	526	240	936	122	205	90	5,914	238	15,868
Scary's Tasman goby	154					7		3	6	170
Smallmouth hardyhead	26,710	4,568	8,582	25,500	6,695	4,141	1,418	447	3,688	81,749
Smooth toadfish	1									1
Soldier					1					1

	Mar-20	Dec-20	Mar-21	Dec-21	Mar-22	Dec-22	Mar-23	Dec-23	Mar-24	Total
Tamar goby	2		8	1	53	11	38	21	147	281
Western Australian salmon		5	1	1	1			6	4	17
Yelloweye mullet	15	14	35	128	55	70	18	173	130	638
Sub-total	**34,721**	**5,235**	**8,896**	**26,634**	**9,127**	**4,500**	**1,804**	**6,698**	**4,274**	**101,889**

South Lagoon	Mar-20	Dec-20	Mar-21	Dec-21	Mar-22	Dec-22	Mar-23	Dec-23	Mar-24	Total
Black bream								3	3	6
Bluespot goby				3		19	50	656	186	914
Bony herring						2	1			3
Bridled goby						1		7	9	17
Congolli		1				5	47	3	14	70
Greenback flounder				3			8	3		14
River garfish							3	7	31	41
Sandy sprat								3,056	680	3,736
Scary's Tasman goby							3			3
Smallmouth hardyhead	17,060	6,229	25,800	8,761	23,580	6,923	28,797	2,253	11,348	130,751
Tamar goby		4				10	4	7		25
Yelloweye mullet						1	4	6	18	29
Sub-total	**17,060**	**6,234**	**25,800**	**8,767**	**23,580**	**6,961**	**28,917**	**6,001**	**12,289**	**135,609**

Appendix table 2 – PERMANOVA results for fish species richness, main test.

	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Year	4	1.1366	0.28415	12.628	**0.001**	999
Region	2	12.416	6.2078	275.88	**0.001**	996
Year x region	8	2.7237	0.34046	15.131	**0.001**	997
Residual	309	6.953	0.022502			
Total	323	23.226				

Appendix table 3 – PERMANOVA results for fish species richness, pairwise test between water years for each region.

Region	t	P(perm)
Murray Mouth Area		
2019/20, 2020/21	1.8785	0.071
2019/20, 2021/22	4.5057	**0.001**
2019/20, 2022/23	2.7328	**0.018**
2019/20, 2023/24	2.6566	**0.008**
2020/21, 2021/22	6.6803	**0.001**
2020/21, 2022/23	5.1365	**0.001**
2020/21, 2023/24	0.30125	0.765
2021/22, 2022/23	2.0888	**0.043**
2021/22, 2023/24	8.8303	**0.001**
2022/23, 2023/24	6.7636	**0.001**
North Lagoon		
2019/20, 2020/21	0.63071	0.536
2019/20, 2021/22	1.0164	0.324
2019/20, 2022/23	0.8059	0.411
2019/20, 2023/24	1.2414	0.208
2020/21, 2021/22	0.70908	0.499
2020/21, 2022/23	0.17992	0.87
2020/21, 2023/24	0.86361	0.413
2021/22, 2022/23	0.61862	0.53
2021/22, 2023/24	0.059599	0.963
2022/23, 2023/24	0.78956	0.431
South Lagoon		
2019/20, 2020/21	1.2724	0.524
2019/20, 2021/22	1.4526	0.246
2019/20, 2022/23	4.2029	**0.001**
2019/20, 2023/24	9.5901	**0.001**
2020/21, 2021/22	0.59688	0.772
2020/21, 2022/23	5.1862	**0.001**
2020/21, 2023/24	11.597	**0.001**
2021/22, 2022/23	4.7153	**0.001**
2021/22, 2023/24	10.39	**0.001**
2022/23, 2023/24	2.5039	**0.023**

Appendix table 4 – PERMANOVA results for fish species richness, pairwise test between regions for each year

Year	t	P(perm)
2019/20		
Murray Mouth Area, South Lagoon	18.532	**0.001**
Murray Mouth Area, North Lagoon	2.8268	**0.011**
South Lagoon, North Lagoon	4.1993	**0.002**
2020/21		
Murray Mouth Area, South Lagoon	13.964	**0.001**
Murray Mouth Area, North Lagoon	2.2806	**0.032**
South Lagoon, North Lagoon	10.117	**0.001**
2021/22		
Murray Mouth Area, South Lagoon	30.543	**0.001**
Murray Mouth Area, North Lagoon	6.2772	**0.001**
South Lagoon, North Lagoon	8.1724	**0.001**

Appendix table 5 – PERMANOVA results for fish total abundance, main test.

	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Year	4	457.38	114.35	8.3277	**0.001**	998
Region	2	1739.6	869.78	63.346	**0.001**	999
Year x region	8	532.02	66.502	4.8433	**0.001**	996
Residual	309	4242.8	13.731			
Total	323	6971.8				

Appendix table 6 – PERMANOVA results for fish total abundance, pairwise test between water years for each region.

Region	t	P(perm)
Murray Mouth Area		
2019/20, 2020/21	0.94077	0.382
2019/20, 2021/22	2.7607	**0.001**
2019/20, 2022/23	2.3711	**0.001**
2019/20, 2023/24	2.0141	**0.01**
2020/21, 2021/22	2.7878	**0.001**
2020/21, 2022/23	2.0925	**0.01**
2020/21, 2023/24	1.4852	0.081
2021/22, 2022/23	2.3317	**0.001**
2021/22, 2023/24	3.1933	**0.001**
2022/23, 2023/24	2.4651	**0.001**
North Lagoon		
2019/20, 2020/21	3.2073	**0.001**
2019/20, 2021/22	2.1872	**0.009**
2019/20, 2022/23	4.2922	**0.001**
2019/20, 2023/24	3.7521	**0.001**
2020/21, 2021/22	1.6221	0.058

2020/21, 2022/23	1.8223	**0.018**
2020/21, 2023/24	1.6179	**0.037**
2021/22, 2022/23	2.0975	**0.008**
2021/22, 2023/24	2.206	**0.004**
2022/23, 2023/24	1.5566	**0.038**
South Lagoon		
2019/20, 2020/21	0.35561	0.726
2019/20, 2021/22	0.25886	0.893
2019/20, 2022/23	1.3775	0.132
2019/20, 2023/24	3.1362	**0.001**
2020/21, 2021/22	0.58324	0.565
2020/21, 2022/23	1.6772	0.067
2020/21, 2023/24	3.9045	**0.001**
2021/22, 2022/23	1.6938	0.053
2021/22, 2023/24	4.2518	**0.001**
2022/23, 2023/24	3.4287	**0.001**

Appendix table 7 – PERMANOVA results for fish total abundance, pairwise test between regions for each year

Year	t	P(perm)
2019/20		
Murray Mouth Area, South Lagoon	3.9576	**0.001**
Murray Mouth Area, North Lagoon	3.2671	**0.001**
South Lagoon, North Lagoon	2.4142	**0.004**
2020/21		
Murray Mouth Area, South Lagoon	4.3965	**0.001**
Murray Mouth Area, North Lagoon	2.2521	**0.006**
South Lagoon, North Lagoon	3.5658	**0.001**
2021/22		
Murray Mouth Area, South Lagoon	6.8819	**0.001**
Murray Mouth Area, North Lagoon	3.9656	**0.001**
South Lagoon, North Lagoon	2.4234	**0.004**

Appendix table 8 – PERMANOVA results for fish assemblage, main test.

	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Year	4	40851	10213	10.525	**0.001**	998
Region	2	1.8464E+05	92321	95.144	**0.001**	998
Year x region	8	53931	6741.4	6.9475	**0.001**	999
Residual	309	2.9983E+05	970.33			
Total	323	5.7926E+05				

Appendix table 9 – PERMANOVA results for fish assemblage, pairwise test between water years for each region.

Region	t	P(perm)
Murray Mouth		
Area	1.334	**0.118**
2019/20, 2020/21		
2019/20, 2021/22	4.7785	**0.001**
2019/20, 2022/23	3.959	**0.001**
2019/20, 2023/24	2.9862	**0.001**
2020/21, 2021/22	4.0349	**0.001**
2020/21, 2022/23	3.1966	**0.001**
2020/21, 2023/24	2.3608	**0.001**
2021/22, 2022/23	2.6531	**0.001**
2021/22, 2023/24	3.7784	**0.001**
2022/23, 2023/24	2.8651	**0.001**
North Lagoon		
2019/20, 2020/21	2.1843	**0.002**
2019/20, 2021/22	2.0332	**0.004**
2019/20, 2022/23	2.7265	**0.001**
2019/20, 2023/24	2.7749	**0.001**
2020/21, 2021/22	1.9881	**0.005**
2020/21, 2022/23	2.0673	**0.004**
2020/21, 2023/24	1.8522	**0.006**
2021/22, 2022/23	2.1488	**0.001**
2021/22, 2023/24	2.1547	**0.001**
2022/23, 2023/24	1.784	**0.007**
South Lagoon		
2019/20, 2020/21	0.39141	0.843
2019/20, 2021/22	0.76867	0.521
2019/20, 2022/23	2.2379	**0.011**
2019/20, 2023/24	3.6403	**0.001**
2020/21, 2021/22	1.1065	0.286
2020/21, 2022/23	2.7773	**0.001**
2020/21, 2023/24	4.8011	**0.001**
2021/22, 2022/23	2.6594	**0.001**
2021/22, 2023/24	4.9413	**0.001**
2022/23, 2023/24	3.4511	**0.001**

Appendix table 10 - PERMANOVA results for fish assemblage, pairwise test between regions for each year

Year	t	P(perm)
2019/20		
Murray Mouth Area, South Lagoon	5.6729	0.001
Murray Mouth Area, North Lagoon	3.9005	0.001
South Lagoon, North Lagoon	3.0982	0.002
2020/21		
Murray Mouth Area, South Lagoon	5.8006	0.001
Murray Mouth Area, North Lagoon	2.5813	0.001
South Lagoon, North Lagoon	4.3312	0.001
2021/22		
Murray Mouth Area, South Lagoon	12.208	0.001
Murray Mouth Area, North Lagoon	4.9808	0.001
South Lagoon, North Lagoon	4.236	0.001

Appendix table 11 - Species abbreviation for fish scientific name and common name.

Species abbreviation	Genus	Species	Common name
ACA BUT	Acanthopagrus	butcheri	Black bream
AFU TAM	Afurcagobius	tamarensis	Tamar goby
ALD FOR	Aldrichetta	forsteri	Yelloweye mullet
AMM ROS	Ammotretis	rostratus	Longsnout flounder
ARE BIF	Arenigobius	bifrenatus	Bridled goby
ARR GEO	Arripis	georgianus	Australian herring
ARR TRU	Arripis	truttaceus	Western Australian salmon
ATH MIC	Atherinosoma	microstoma	Smallmouth hardyhead
CHE KUM	Chelidonichthys	kumu	Red gurnard
CON BRE	Contusus	brevicaudus	Prickly toadfish
CON RIC	Contusus	richei	Barred toadfish
CRI AUS	Cristiceps	australis	Southern crested weedfish
CYP CAR	Cyprinus	carpio	Carp
ENG AUS	Engraulis	australis	Australian anchovy
GAL MAC	Galaxias	maculatus	Common galaxias
GYM MAR	Gymnapistes	marmoratus	Soldier
HYP MEL	Hyporhamphus	melanochir	Southern garfish
HYP REG	Hyporhamphus	regularis	River garfish
HYP SPP	Hypseleotris	spp.	Carp gudgeon spp.
HYP VIT	Hyperlophus	vittatus	Sandy sprat
LES PLA	Lesueurina	platycephala	Flathead sandfish
LIZ ARG	Liza	argentea	Goldspot mullet
MAC AMB	Macquaria	ambigua	Golden perch
MEU HIP	Meuschenia	hippoocrepis	Horseshoe leatherjacket
NEM ERE	Nematolosa	erebi	Bony herring
PER FLU	Perca	fluviatilis	Redfin perch
PHI GRA	Philypnodon	grandiceps	Flat-headed gudgeon
PHI MAC	Philypnodon	macrostomus	Dwarf flat-headed gudgeon
PSE OLO	Pseudogobius	olorum	Bluespot goby
PSE URV	Pseudaphritis	urvillii	Congolli
RET SP1	Retropinna	sp.1	Australian smelt
RHO TAP	Rhombosolea	tapirina	Greenback flounder
TAS LAS	Tasmanogobius	lasti	Scary's Tasman goby
TET GLA	Tetractenos	glaber	Smooth toadfish

Appendix table 12 - Percentage contribution of diet items by volume (V%) and frequency of occurrence (F%) in the stomach and alimentary tract of two different size classes (<150 mm, >150 mm) of yelloweye mullet in the Coorong, for March 2023. Major diet groups are in bold. MMA = Murray Mouth Area, NL = North Lagoon, SL = South Lagoon. Individual dietary items or groups included in statistical analysis are indicated by *

Size class (mm): <150 | >150. n= (MMA 7, NL 5, SL 2, Total 14) | (MMA 6, NL 7, SL 1, Total 14) | All sizes 28.

Diet items	<150 MMA V%	<150 MMA F%	<150 NL V%	<150 NL F%	<150 SL V%	<150 SL F%	<150 Total V%	<150 Total F%	>150 MMA V%	>150 MMA F%	>150 NL V%	>150 NL F%	>150 SL V%	>150 SL F%	>150 Total V%	>150 Total F%	All V%	All F%
Polychaeta			0.1	60	32.7	100	3.7	36	3.4	17	1.7	29			1.5	21	2.7	29
Capitellidae*					32.6	50	3.7	7	3.4	17					0.2	7	2.1	7
Nephtyidae*																		
Nereididae*			0.1	60	0.1	50	0.1	29			1.7	29			1.3	29	0.6	21
Australonereis ehlersi																		
Simplisetia aequisetis																		
Unidentified Nereididae			0.1	60	0.1	50	0.1	29			1.7	29			1.3	14	0.6	21
Spionida*																		
Unidentified polychaeta*																		
Sipuncula*																		
Crustacea	24.3	86	32.9	80	6.9	100	27.7	86	5.1	33	2.2	57	65.2	100	12.7	50	20.7	68
Amphipoda*			0.6	20			0.4	7			0.4	29			0.3	14	0.4	11
Cladocera*	1.5	29					0.4	14	4.8	17			0.1	100	0.3	14	0.4	14
Copepoda*	6.7	71	6.4	80			5.8	64	0.3	17	0.5	29	2.3	100	0.8	29	3.5	46
Mysidacea*					6.5	50	0.7	7									0.4	4
Ostracoda*			0.6	40	0.3	100	0.4	29			<0.1	14	0.1	100	0.0	14	0.2	21
Zoea Larvae*	0.5	14	<0.1	20			0.2	14									0.1	7
Unidentified Crustacea	15.5	86	25.2	80			19.9	71			1.3	29	62.6	100	11.2	21	15.8	46
Teleostei*	33.8	43					8.9	21					14.3	100	2.3	7	5.8	14
Atherinosoma microstoma																		
Pseudaphritis urvillii	31.4	14					8.2	7									4.4	4
Unidentified fish	2.4	29					0.6	14					14.3	100	2.3	7	1.4	11
Bivalvia			3.0	60			1.9	21			0.7	29			0.5	14	1.2	18
*Arthritica helmsi**			3.0	60			1.9	21			0.7	29			0.5	14	1.2	18
*Hiatula alba**																		
*Spisula trigonella**																		
Unidentified bivalve*																		
Insecta			<0.1	40	<0.1	50	<0.1	21	0.3	17			<0.1	100	0.0	14	0.0	18
Chironomidae*																		
Coleoptera larvae*									0.3	17					0.0	7	0.0	4
Collembola*																		
Corixidae*																		
Empididae larvae*																		
Ephydridae larvae*																		
Formicidae*																		
Pterygota*													<0.1	100	0.0	7	0.0	4
Thysanoptera*																		
Unidentified insecta*			<0.1	40	<0.1	50	<0.1	21									<0.1	11
Unidentified invertebrate tissue*																		
Foraminifera*			0.2	40	<0.1	50	0.1	21									0.1	11
Bacillariophyceae (Diatoms)*					35.9	100	4.0	14					2.3	100	0.4	7	2.3	11
Algae/Plants			21.7	60	13.1	50	15.0	29	3.4	17	36.2	57	7.8	100	29.5	43	21.7	36
Cladophoraceae*											21.9	14			17.0	7	7.9	4
Cladophora spp.											21.9	14			17.0	7	7.9	4
Rhizoclonium spp.																		
Ulvacacae / *Ulva* spp.*			21.7	40			13.6	14			13.7	29			10.6	14	12.2	14
Unidentified Red Algae*																		
Unidentified Algae/Plant material*			<0.1	20	13.1	50	1.5	14	3.4	17	0.6	43	7.8	100	1.9	36	1.7	25
Detritus/unidentifiable matter*	41.9	14	42.2	100	11.4	100	38.7	57	87.8	83	59.2	71	10.4	100	53.0	79	45.3	68

Appendix table 13 - Percentage contribution of diet items by volume (V%) and frequency of occurrence (F%) in the stomach and alimentary tract of two different size classes (<150 mm, >150 mm) of yelloweye mullet in the Coorong, for December 2023. Major diet groups are in bold. MMA = Murray Mouth Area, NL = North Lagoon, SL = South Lagoon. Individual dietary items or groups included in statistical analysis are indicated by *

Size class (mm)									>150								All sizes	
n=	0		10		6		16		0		10		0		10		26	
Region	MMA		NL		SL		Total		MMA		NL		SL		Total		All sizes	
Diet items	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%
Polychaeta			**58.1**	**80**	**38.9**	**50**	**54.5**	**69**			**67.4**	**100**			**67.4**	**100**	**63.5**	**81**
Capitellidae*			1.5	10	3.2	17	1.8	13			4.9	30			4.9	30	4.0	19
Nephtyidae*																		
Nereididae*			**56.6**	**80**	**35.7**	**33**	**52.6**	**63**			**62.4**	**100**			**62.4**	**100**	**59.5**	**77**
Australonereis ehlersi			28.8	30			23.3	19			5.2	10			5.2	10	10.7	15
Simplisetia aequisetis			24.3	30	31.8	17	25.7	25			36.5	40			36.5	40	33.3	31
Unidentified Nereididae			3.5	30	4.0	17	3.6	25			20.7	60			20.7	60	15.5	38
Spionida*																		
Unidentified polychaeta*																		
Sipuncula*																		
Crustacea			**9.6**	**70**	**32.6**	**50**	**14.0**	**63**			**2.1**	**60**			**2.1**	**60**	**5.7**	**62**
Amphipoda*			9.4	50			7.6	31			2.0	40			2.0	40	3.7	35
Cladocera*			<0.1	10	0.7	17	0.1	13									<0.1	8
Copepoda*			<0.1	10	0.2	33	0.1	19									<0.1	12
Mysidacea*					19.1	17	3.6	6									1.1	4
Ostracoda*			0.1	30	12.7	50	2.5	38			0.1	30			0.1	30	0.8	35
Zoea Larvae*																		
Unidentified Crustacea			0.1	10	<0.1	17	0.1	13									0.0	8
Teleostei*			**8.5**	**10**			**6.9**	**6**			**5.2**	**20**			**5.2**	**20**	**5.7**	**12**
Atherinosoma microstoma											5.2	10			5.2	10	3.6	4
Pseudaphritis urvillii																		
Unidentified fish			8.5	10			6.9	6			<0.1	10			<0.1	10	2.1	8
Bivalvia			**0.2**	**20**			**0.2**	**13**			**8.7**	**40**			**8.7**	**40**	**6.2**	**23**
*Arthritica helmsi**			0.2	20			0.2	13			2.6	30			2.6	30	1.9	19
*Hiatula alba**											0.7	10			0.7	10	0.5	4
*Spisula trigonella**											5.5	20			5.5	20	3.8	8
Unidentified bivalve*																		
Insecta			**3.8**	**10**	**9.8**	**33**	**4.9**	**19**			**0.1**	**10**			**0.1**	**10**	**1.5**	**15**
Chironomidae*					9.7	33	1.8	13									0.6	8
Coleoptera larvae*																		
Collembola*											0.1	10			0.1	10	0.1	4
Corixidae*																		
Empididae larvae*			3.0	10			2.4	6									0.7	4
Ephydridae larvae*																		
Formicidae*			0.8	10			0.7	6			<0.1	10			<0.1	10	0.2	8
Pterygota*																		
Thysanoptera*																		
Unidentified insecta*					0.1	17	<0.1	6									<0.1	4
Unidentified invertebrate tissue*																		
Foraminifera*			<0.1	10			<0.1	6			<0.1	20			0.0	20	<0.1	12
Bacillariophyceae (Diatoms)*																		
Algae/Plants			**3.9**	**50**	**0.9**	**33**	**3.3**	**44**			**10.1**	**70**			**10.1**	**70**	**8.0**	**54**
Cladophoraceae*			2.2	10	0.8	17	2.0	6			9.4	30			9.4	30	7.2	15
Cladophora spp.			2.2	10			1.8	6									0.5	4
Rhizoclonium spp.					0.8	17	0.2	6			9.4	30			9.4	30	6.6	15
Ulvacacae / *Ulva* spp.*																		
Unidentified Red Algae*																		
Unidentified Algae/Plant material*			1.7	50	0.1	33	1.4	44			0.6	50			0.6	50	0.8	46
Detritus/unidentifiable matter*			15.8	50	17.8	83	16.2	63			6.4	40			6.4	40	9.3	54

Appendix table 14 - Percentage contribution of diet items by volume (V%) and frequency of occurrence (F%) in the stomach of two different size classes (<150 mm, >150 mm) of yelloweye mullet in the Coorong, for March 2012. Major diet groups are in bold. MMA = Murray Mouth Area, NL = North Lagoon, SL = South Lagoon. Individual dietary items or groups included in statistical analysis are indicated by *

| Size class (mm)	<150								>150								All sizes	
n=	44		18		1		63		8		29		1		38		101	
Region	MMA		NL		SL		Total		MMA		NL		SL		Total		All sizes	
Diet items	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%	V%	F%
Polychaeta	**4.3**	**15.9**	**14.6**	**39**			**7.2**	**22**			**14.5**	**45**			**14.3**	**34**	**12.0**	**27**
Capitellidae*			14.2	28			4.2	8			10.5	21			10.4	16	8.4	11
Nephtyidae*	1.8	4.5					1.2	3									0.4	2
Nereididae*	2.5	13.6	0.3	11			1.8	13			0.1	14			0.1	11	0.6	12
Australonereis ehlersi	0.2	2.3					0.1	2			<0.1	3			<0.1	3	0.1	2
Simplisetia aequisetis	0.9	6.8	<0.1	6			0.6	6									0.2	4
Unidentified Nereididae	1.4	9.1	0.3	6			1.1	8			<0.1	10			<0.1	8	0.4	8
Spionida*											1.5	17			1.5	13	1.0	5
Unidentified polychaeta*			0.1	11			0.0	3			2.4	21			2.4	16	1.6	8
Sipuncula*			5.3	11			1.6	3			0.1	3			0.1	3	0.6	3
Crustacea	**35.8**	**82**	**12.8**	**78**			**28.1**	**79**	**2.5**	**38**	**3.6**	**69**			**3.6**	**61**	**11.5**	**72**
Amphipoda*	19.7	57	8.9	78			16.0	62	1.4	25	2.8	59			2.7	50	7.0	57
Cladocera*	<0.1	5					<0.1	3									<0.1	2
Copepoda*	16.0	48	<0.1	11			10.9	37	0.2	13	0.4	17			0.4	16	3.8	29
Mysidacea*	0.1	5	3.8	44			1.2	16			0.4	10			0.4	8	0.7	13
Ostracoda*	<0.1	5					<0.1	3	0.9	13	<0.1	7			<0.1	8	<0.1	5
Zoea Larvae*	<0.1	2					<0.1	2									<0.1	1
Unidentified Crustacea											<0.1	3			<0.1	3	<0.1	1
Teleostei*	**31.2**	**20**					**21.2**	**14**									**6.9**	**9**
Atherinosoma microstoma																		
Pseudaphritis urvillii																		
Unidentified fish	31.2	20					21.2	14									6.9	9
Bivalvia			**<0.1**	**6**			**<0.1**	**2**			**<0.1**	**10**			**<0.1**	**8**	**<0.1**	**4**
Arthritica helmsi*			<0.1	6			<0.1	2			<0.1	10			<0.1	8	<0.1	4
Hiatula alba*																		
Spisula trigonella*																		
Unidentified bivalve*											<0.1	3			0.0	3	<0.1	1
Insecta	**0.8**	**27.3**	**0.2**	**11**	**69.8**	**100**	**2.3**	**24**	**0.4**	**25**	**0.2**	**21**	**0.6**	**100**	**0.2**	**24**	**0.9**	**24**
Chironomidae*	0.8	25.0	0.1	6	69.8	100	2.3	21	0.4	25	0.2	14	0.6	100	0.2	18	0.9	20
Coleoptera larvae*																		
Collembola*																		
Corixidae*	<0.1	2.3					<0.1	2									<0.1	1
Empididae larvae*																		
Ephydridae larvae*			0.1	6			<0.1	2									<0.1	1
Formicidae*																		
Pterygota*																		
Thysanoptera*											<0.1	3			<0.1	3	<0.1	1
Unidentified insecta*											<0.1	3			<0.1	3	<0.1	1
Unidentified invertebrate tissue*											<0.1	7			<0.1	5	<0.1	2
Foraminifera*	0.1	15.9	<0.1	6			<0.1	13	1.9	13	<0.1	7			<0.1	8	<0.1	11
Bacillariophyceae (Diatoms)*													99.4	100	1.0	3	0.7	1
Algae/Plants	**12.8**	**18.2**	**38.8**	**22**	**10.2**	**100**	**20.4**	**21**	**31.5**	**75**	**29.8**	**69**			**29.6**	**68**	**26.6**	**39**
Cladophoraceae*			31.6	6			9.4	2			26.4	17			26.1	13	20.6	6
Cladophora spp.			31.6	6			9.4	2			26.4	17			26.1	13	20.6	6
Rhizoclonium spp.																		
Ulvacacae / Ulva spp.*			0.7	6			0.2	2			0.3	7			0.3	5	0.2	3
Unidentified Red Algae*	10.9	4.5	4.5	6			8.7	5	1.4	13					<0.1	5	2.8	5
Unidentified Algae/Plant material*	1.9	13.6	2.0	17	10.2	100	2.1	16	30.1	63	3.2	45			3.2	47	2.9	28
Detritus/unidentifiable matter*	15.1	84	28.3	94	20.0	100	19.1	87	63.8	63	51.7	86			51.2	79	40.8	84
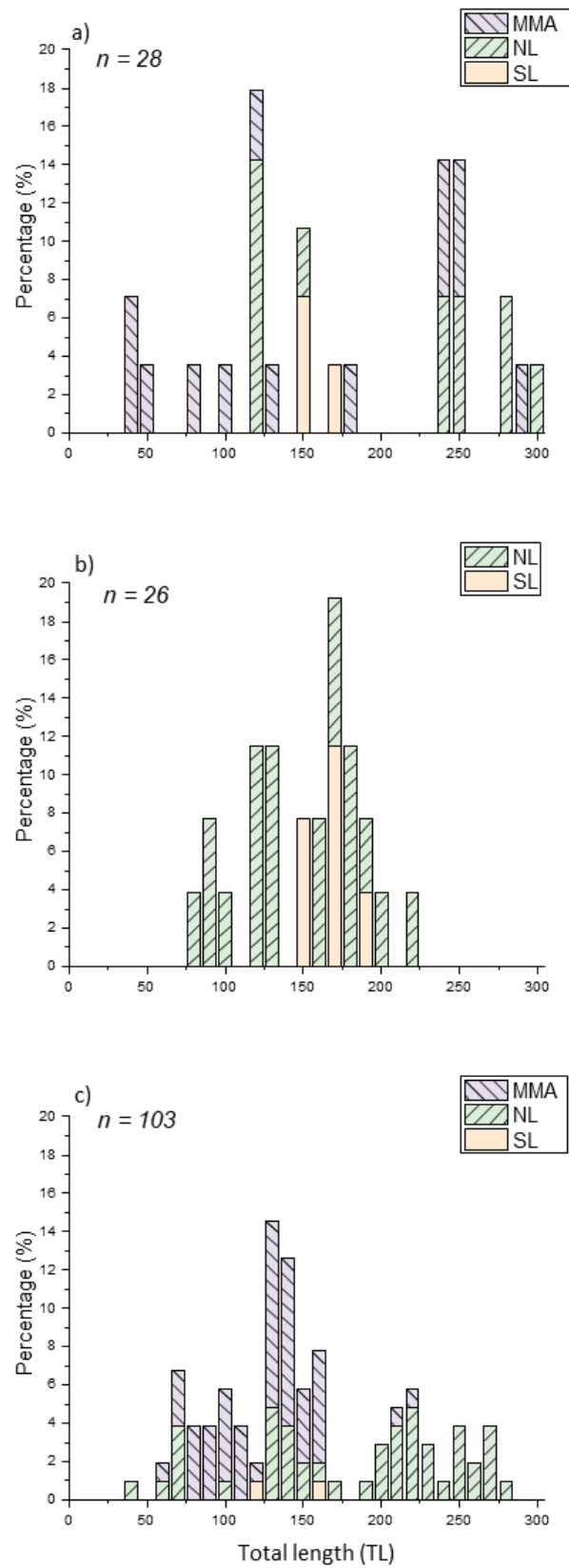
Appendix figure 1. Size frequency distribution of yelloweye mullet total length (TL) in a) March 2023, b) December 2023, c) March 2012. MMA = Murray Mouth Area, NL = North Lagoon, SL = South Lagoon.
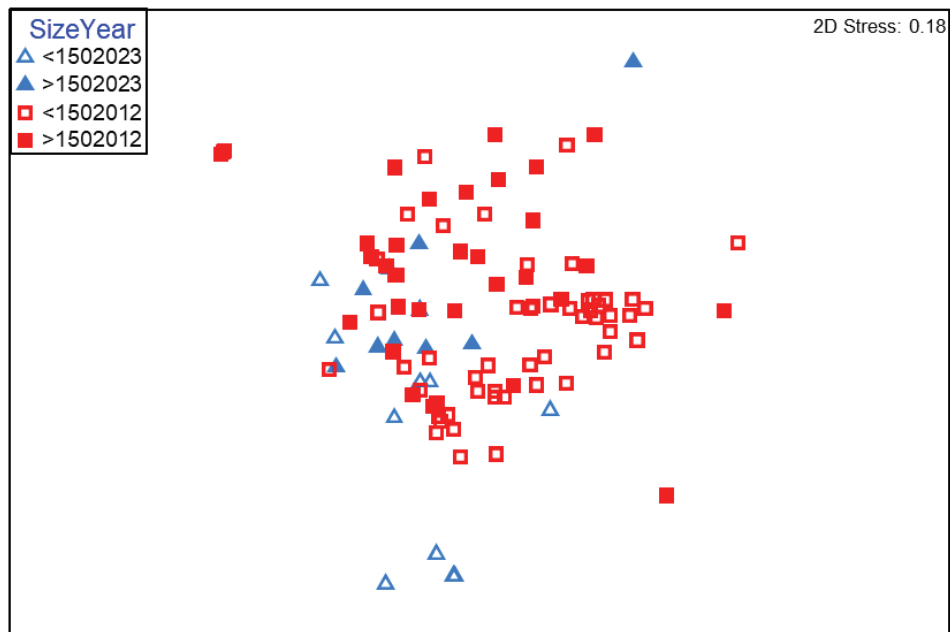
Appendix figure 2. Two dimensional MDS ordination of the diet composition (standardised) of two size classes (< 150 mm, > 150 mm) of yelloweye mullet with regions combined, in March 2012 and 2023 in the Coorong.
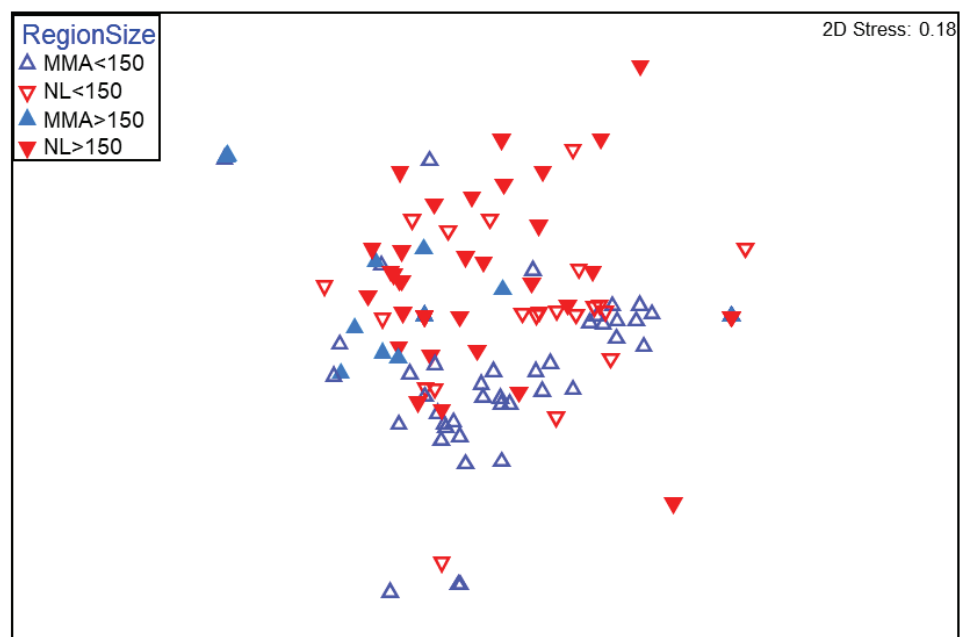
Appendix figure 3. Two dimensional MDS ordination of the diet composition (standardised) of two size classes (< 150 mm, > 150 mm) of yelloweye mullet in Murray Mouth Area (MMA) and North Lagoon (NL), with years 2012 and 2023 combined, in the Coorong.
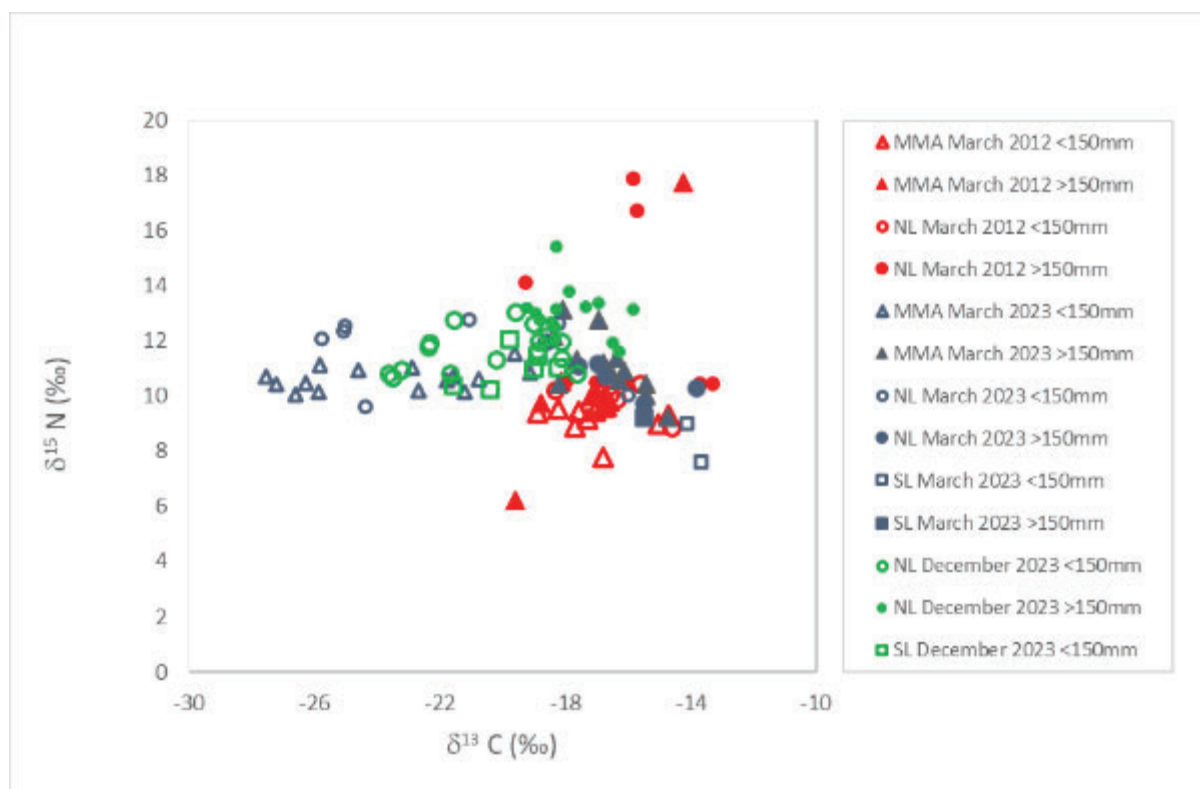
Appendix figure 4. Stable isotope biplot of δ^{13}C and δ^{15}N values across Regions, Time-periods and Size groups. Markers represent values for individual fish samples. MMA = Murray Mouth Area, NL = North Lagoon, SL = South Lagoon.