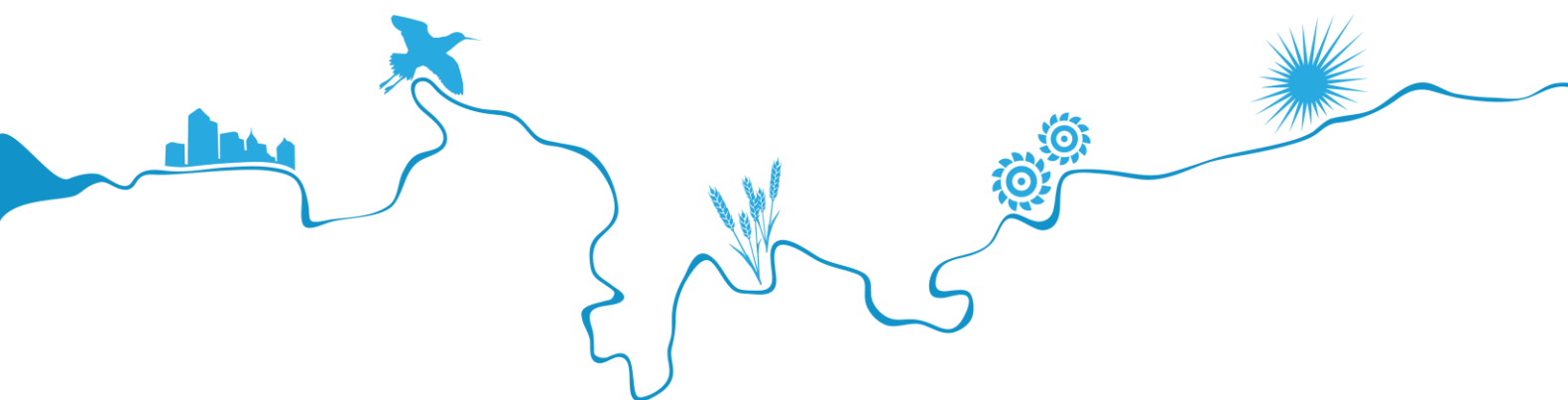


Distribution and seasonality of the *Ruppia* dominated aquatic macrophyte community and filamentous algae in the southern Coorong

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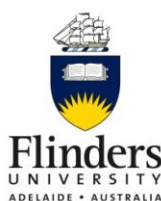


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Respect and reconciliation

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.

Executive summary

The Coorong and Lakes Alexandrina and Albert wetland in South Australia is recognised under the Ramsar Convention as a wetland of international importance. The Coorong provides ecological services to a range of biota, the foundation of which is the aquatic macrophyte community dominated by *Ruppia tuberosa* (the *Ruppia* Community). The Millennium Drought (in particular during the period 2001 to 2010) significantly impacted the southern Coorong including an almost complete decline of the *Ruppia* Community including their seed banks, which has decreased the resilience of the system. The recovery of the *Ruppia* Community has been gradual with the number of sites occupied by plants increasing over the past decade. Recovery has been impeded most strongly in the Coorong South Lagoon because of the hyper-eutrophic state and algal blooms, including mat forming filamentous algae that physically disrupt the aquatic macrophytes' ability to flower and set seed.

The South Australian Government's Healthy Coorong, Healthy Basin (HCHB) Program Trials and Investigations (T&I) project includes an aquatic plants and algae component (*Component 2*) that was undertaken between July 2020 and April 2022. This component aims to support management by investigating factors that limit filamentous algal growth and restore *Ruppia* distribution and abundance. This may require additional management interventions to facilitate direct recovery of ecological functions in the Coorong South Lagoon. To achieve this, the goal is to shift the southern Coorong ecosystem from algal dominated to a *Ruppia* dominated habitat, improve water quality, and restore the ecological function associated with a *Ruppia* dominated state.

The aim of 'T&I Component 2 – Activity 2.2: Undertake baseline surveys and map the extent of *Ruppia* and filamentous algae communities' was to establish the current extent and condition of the *Ruppia* and filamentous algae communities in the southern Coorong at times of the year critical to the lifecycle of *Ruppia*. This study, undertaken between October 2020 and December 2021, undertook four baseline surveys to document the extent of *Ruppia* and filamentous algae communities using direct field-based observations. These observations were compared to project-specific and externally monitored environmental conditions to establish if there were measurable changes in condition of the *Ruppia* Community concurrent with water delivery and accepted ecological drivers of change (i.e. water levels and salinity) at times of the year critical to the lifecycle.

A different methodology to the The Living Murray (TLM) condition monitoring program was applied to survey the aquatic macrophyte community in the Coorong. The new methods have improved understanding of the baseline status of these important aquatic plants. The methods applied here could be further modified into a sampling protocol streamlined for future application as a routine monitoring program to provide evidence of the condition and trend in the Coorong ecosystem.

The aquatic macrophyte community of the Coorong South Lagoon is an assemblage of several species. This assemblage has a suite of characteristics that are different from a monoculture of the well documented *Ruppia tuberosa*. In the Coorong South Lagoon, *Ruppia tuberosa* is much more common than the two other co-occurring species - *Althenia cylindrocarpa* and an unresolved species of *Ruppia*. In addition, two haplotypes of *Ruppia tuberosa* were detected, the less common haplotype only found in samples from the South Lagoon in the 2020 reproductive sample. A DNA toolkit to easily identify these species using eDNA techniques has now been developed. We recognise that a multi-species aquatic macrophyte community existed historically. Evidence from reports, collections lodged in the herbarium of the Botanic Gardens of South Australia, Adelaide, and published field photographs, support a mixed *Ruppia/Althenia* community prior to the Millennium Drought (1996-2010). The terminology '*Ruppia* Community' is proposed for this assemblage.

By the end of the Millennium Drought in 2010, the *Ruppia* Community had dwindled to highly isolated remnants throughout the sampling area of this study, and the seed bank was largely exhausted. The *Ruppia* Community is now found the along the extent of the surveyed area, with a range from north of Long Point to south of Salt Creek, and, albeit uncommonly, at Tea Tree Crossing, (approximately 112 km from the Murray Mouth), where the absence of plants is likely due to rocky substrate or elevation (shallowness). The current spatial extent of the *Ruppia* Community appears to have followed a dispersal limited recruitment trajectory.

Comparing the current seed bank data with historical TLM monitoring data, it appears that the current seed bank is at ~30% of pre-drought levels. The results and observations from these surveys support the ongoing concern that while *Ruppia* plants are increasing in extent, they are not performing well, having poor vigour and limited resilience due to the production of seed in many areas remaining low. The observation that low plant abundance within the *Ruppia* Community was associated with poor sediment quality has emphasised the need for developing options to enhance sediment quality through management strategies.

The La Niña summer weather cycles in 2020-21 and 2021-22 have led to longer-term availability of water entering the Coorong system. The water is predominantly from unregulated flows from the River Murray but also water released through Salt Creek from Morella Basin. This appears to have encouraged the formation of more mature populations of *Ruppia tuberosa*, and an increase in turion formation. However, given the cyclic nature of El Niño–Southern Oscillation (ENSO) conditions, interpretation of long-term trends should be made with caution.

During warmer months, large areas of high biomass filamentous algae can form across the southern Coorong and have multiple effects: shading of the *Ruppia* Community reducing productivity; increasing the high turnover organic production cycle in and over aquatic macrophyte habitat; physical interference with flowering, fruiting and completion of the *Ruppia* Community members' lifecycles; and generation of organic sludge that creates anoxic and sulfidic environments, poor sediment quality and impacts on aquatic macrophyte growth and productivity. Based on the results of this study, a management target of reducing algal mat formation to less than 100 g DW m⁻² was identified to maintain and improve the condition and resilience of the *Ruppia* Community.

The lack of algae in the very southern sections of the Coorong South Lagoon may provide an opportunity to understand strategies to manage the presence of algae in the entire Coorong system. This region of the Coorong has similar salinity to other areas that contain significant algal mats; hence, other processes limit the formation of filamentous algae. For example, sufficient water movement may act as an impediment to dispersal of algae further south, or more likely, that even modest levels of flow over aquatic macrophyte meadows may act as an impediment to physical attachment to plants.

The return of high *Ruppia* Community biomass and shoot counts at many sites is a positive indication for the current state of the Coorong system. Implementing options to enhance resilience to future changing environmental conditions through increasing seed banks, removing algal loads and encouraging higher biomass populations are proposed as options to achieve the best outcomes for the long-term viability of the Coorong.

The previously abundant charophyte, *Lamprothamnium papulosum* was absent from the southern Coorong ecosystem in this study. The loss of this species could be indicative of the decline in water quality although responses of other, non-macrophyte species were not specifically studied in this project. *Lamprothamnium* presence may have value as an additional indicator of improved ecological health of the South Lagoon.

Based on the results of this research, the following key points are highlighted:

- The mixed community of aquatic macrophytes found in the *Ruppia* Community should be managed to maintain the mixed assemblage rather than one dominant species (i.e. *Ruppia tuberosa*), and allow for potential inclusion of other species as the ecological health of the Coorong improves.
- The presence of *Lamprothamnium* in the Coorong could act as an indicator in the future for changing ecological health, particularly when this charophyte can form larger populations and act as food for birds.
- An improved evidence base derived from the principles established by this study could be utilised to analytically inform a revised TLM condition monitoring program for the *Ruppia* Community.
- Environmental conditions in 2020–2021, in particular La Niña weather patterns during the summer periods, coincided with the extended growth of many populations and a dominance of turions being formed instead of seed banks. Overall resilience based on seed banks remains low. Interpretation of

the results of this research must take into account the environmental conditions present during the period of this study and should not be generalised without further work.

- Reducing nutrient loads to minimise the relative advantage the filamentous algae have over the *Ruppia* Community will be critical.
- When algal biomass exceeds 184 g DW m⁻² there is a significant decline in aquatic macrophyte biomass. Consequently, we suggest that waterway management needs to achieve algal biomass of <100 g DW m⁻² as an urgent outcome.
- The relationship between low *Ruppia* Community biomass and poor sediment quality emphasises the need for developing options to enhance sediment quality through management strategies.

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1 Introduction

1.1 Background

The Coorong is culturally, environmentally and economically important at local, national and international scales but has experienced a long-term decline in its ecological condition due to reductions in fluvial inflows. Whilst there has been recovery of some elements of the Coorong ecosystem associated with increased inflows since the Millennium Drought ended in 2010, the South Lagoon has not recovered to the levels expected. There has been a switch of the ecosystem from being dominated by aquatic macrophytes to algae associated with eutrophication (nutrient enrichment), with subsequent impacts on invertebrates, fish and waterbirds. These changes in the ecosystem and the lack of recovery are likely caused by a number of complex, interacting factors, which are not well understood. This is limiting the capacity to forecast the ecological response to future management scenarios and therefore the capacity of water managers to identify management interventions required to improve the health of the Coorong.

The Phase One Trials and Investigations (T&I) project of the Healthy Coorong, Healthy Basin (HCHB) program consists of a series of integrated components that will collectively provide knowledge to inform the future management of the Coorong. Component 2 of the T&I project is titled: *Knowledge to inform the restoration of the Ecological Character of the South Lagoon of the Coorong: drivers and controls of filamentous algae and aquatic plants*. The component includes seven main activities outlined in Figure 1 addressing research knowledge gaps at a range of spatial and temporal scales.

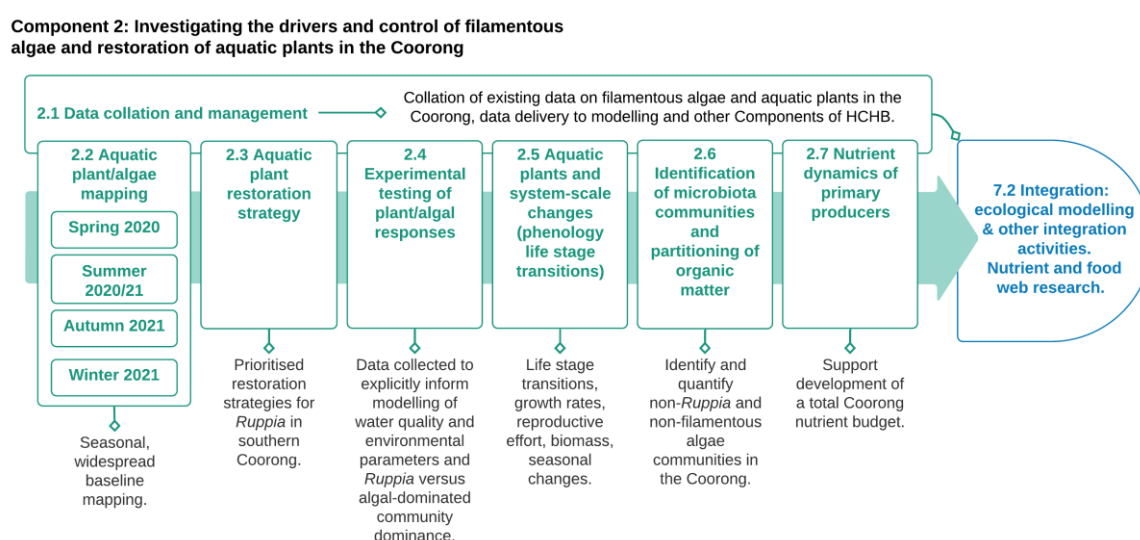


Figure 1. Summary of the main research activities in T&I Component 2 Investigating the drivers and controls of filamentous algae and restoration of aquatic plants in the Coorong and the primary outputs for each activity and a direct linkage to the modelling activities in T&I Component 7 Integration.

*Activity 2.2: Undertake baseline surveys and map the extent of *Ruppia* sp. and filamentous algae communities* forms part of Component 2 of the T&I project. Its broad objectives were to undertake surveys and map the extent of the *Ruppia* and filamentous algae communities at critical times of the year, to track the extent of the problem and monitor changes in the extent in response to water delivery.

The submergent *Ruppia tuberosa* community, (hereafter referred to as the *Ruppia* Community) is a keystone component for the southern Coorong – contributing to maintaining water quality and trapping sediment while providing habitat for invertebrates and fish, and food for waterbirds (Asanopolous and Waycott 2020, Collier et al. 2017, Department for Environment and Water 2021, Paton 2010, Paton et al. 2021, Paton 1986). Excessive filamentous algal growth in the Coorong, starting in late spring and continuing over summer,

negatively impacts *Ruppia* growth and seed production throughout the system, although these impacts are more detectable across the southern Coorong, with flow-on effects for the Coorong ecosystem (Asanopolous and Waycott 2020, Collier et al. 2017, Department for Environment and Water 2021, Paton et al. 2021). These impacts are hampering the long-term recovery of the Coorong ecosystem following the large-scale losses of the *Ruppia* Community in the Coorong during the Millennium Drought (in particular 2001–2010) (Collier et al. 2017, Rogers and Paton 2009) despite translocation efforts that led to recruitment of *Ruppia* across areas where seeds were deployed in sediments (Asanopolous and Waycott 2020, Collier et al. 2017, Paton et al. 2018a, Paton et al. 2018b). The current state of the previously robust *Ruppia* Community, was described in the findings of an expert panel review (Brookes et al. 2018) as being in a vulnerable condition requiring significant effort to restore resilience and associated ecological functions.

The ecological character of the Coorong exhibited during the period 2015–2019 did not support critical ecological functions, in particular provision of food and habitat for water birds and other highly valued components of the system (Figure 2). Field observations over the last 4–5 years suggest that the South Lagoon of the Coorong has remained in the Hypersalinity–High Nutrient state (Figure 2 – lower left panel).

The Coorong South Lagoon and the southern region of the North Lagoon was classified as hyper-eutrophic based on water quality parameters including chlorophyll *a* and total nutrient concentrations (Mosley and Hipsey 2019). Ongoing field surveys (Asanopolous and Waycott 2020, Paton et al. 2020, 2021, Waycott et al. 2021a, Waycott et al. 2020) indicate this combined Hypersalinity–High Nutrient State of the southern Coorong is characterised by:

- Filamentous algal mat-forming species gaining an advantage over the *Ruppia* Community through the combined, complex interactions of nutrient availability, salinity, water temperature and water level (Collier et al. 2017, Hipsey et al. 2020).
- Increased prevalence of filamentous algae, compared to the *Ruppia* Community, altering nutrient cycling away from relatively stable fixed carbon (i.e. submergent vegetation) persisting throughout the season to more rapid dynamic trophic cycling typical of eutrophic systems (filamentous algae and micro-biota) (Mosley et al. 2020b).
- Algae accumulating on shorelines, forming mats that prevent shorebirds from accessing food resources from within mudflats (Paton et al. 2021).
- Filamentous green algae forming mats at the water surface shading plants and entangling *Ruppia tuberosa* flowers resulting in them breaking away from stems, leading to failure to set seed (Paton et al. 2021, Waycott et al. 2020).
- Failed reproduction of *Ruppia tuberosa* contributing to a depleted seed bank and lowered resilience of the population, which affects the invertebrates, fish and waterbird for which it provides food and/or habitat (Paton et al. 2021).
- The breakdown of algae that accumulates on shorelines reducing oxygen levels within the sediment, affecting the invertebrate communities, and thus food for key fish and waterbird populations (Collier et al. 2017, Dittmann 2017, Paton et al. 2017a).
- Filamentous green algae creating a barrier at the water's surface impeding foraging by birds (Paton et al. 2018b).

Ruppia community in the Coorong recent time changes

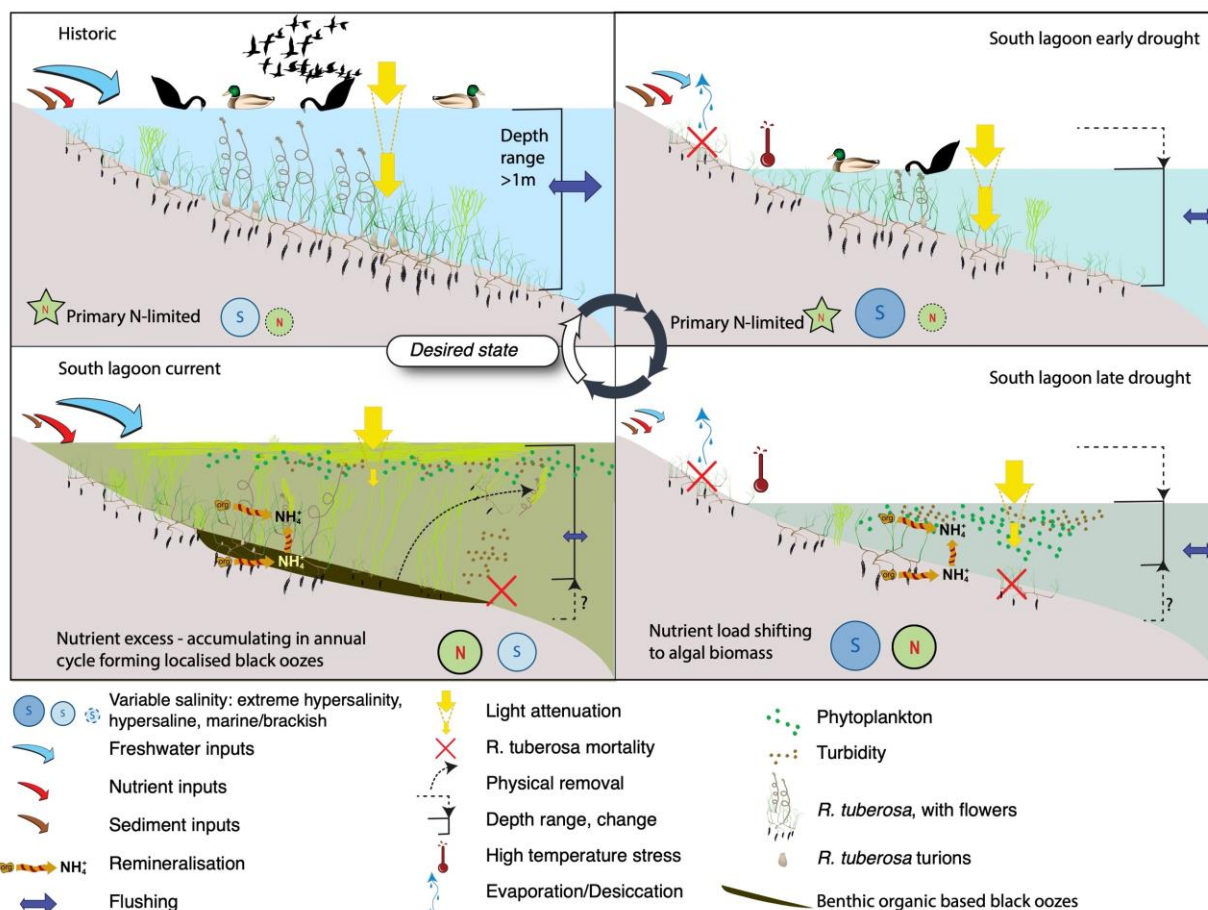


Figure 2. Conceptual diagram summarising alternative states for the southern Coorong, emphasising primary producers, based on observed ecological conditions (revised from Collier et al. 2017).

Substantial information dating back to the early 1970s exists regarding the distribution and abundance of submergent vegetation in the Coorong (Lucas and Womersley 1972, Womersley 1975). Womersley (1975) recorded *Ruppia*, *Lamprothamnium*, *Lepilaena* (now *Althenia*) and filamentous algae (identified then as *Cladophora* and *Enteromorpha*) and suggested that even then the Coorong was in a degraded state. Sporadic investigations and monitoring occurred until 1999 when regular monitoring of *Ruppia* in the Coorong commenced (Paton 1999). In 2007 The Living Murray Program (TLM) commenced funding *Ruppia* monitoring in the Coorong (Rogers and Paton 2009) and this has continued through regular monitoring in winter (July), spring (October to assess flowering) and summer (January) ever since.

Regular monitoring under TLM documented the decline in the *Ruppia* Community during the Millennium Drought, in particular, during the period 2001–2010 and partial recovery with increased inflows post 2010 (Nicol et al. 2018, Paton et al. 2018b). However, additional information required for the restoration of the *Ruppia* Community (not collected as part of TLM monitoring) was identified and justified further investigation of the distribution and abundance of the *Ruppia* and filamentous algal communities under HCHB, leading to the development of Activity 2.2. Extra information identified as important for the restoration of the *Ruppia* Community that was not collected as part of TLM included:

- Greater spatial coverage of the southern Coorong aquatic macrophyte community; TLM condition monitoring currently has 26 sites. In this study, we have expanded the spatial coverage of sites to approximately 100 (total number dependent on conditions at the time of sampling), georeferenced to the sample level locations and adopting a detailed sampling technique extending previous observations, but ensuring TLM data are comparable for some features (e.g. utilising same sized cores).

- Expanded and realigned sampling times to cover known life history stages of the *Ruppia* Community (Collier et al. 2017). The timing of TLM monitoring (July, October and January) may not be optimal. Sampling in winter (July) when water levels are high prevents sampling at lower elevations using the TLM method (PVC corer). Plants are also not flowering in winter (Asanopolous and Waycott 2020), making it not possible to differentiate between *Ruppia tuberosa* and *Althenia cylindrocarpa*. Additional sampling in spring/early summer at the end of the growing season allows sampling at lower elevations when plants are flowering.
- Laboratory-based counts have been undertaken as part of this study (HCHB). Field-based shoot, turion and seed counts are generally less accurate than those conducted in the lab.
- Investigation of alternative sampling methods in deep water, such as towed submersible videography, waterproof still camera and video sampling at regular intervals and grab samples.
- Identification all aquatic macrophyte components of the *Ruppia* Community to characterise a more diverse (and potentially resilient) primary producer community.
- Development of DNA markers to determine which species are present in the *Ruppia* Community at a site (i.e. whether only *Ruppia tuberosa*, or other taxa are present).

The overall focus of the HCHB T&I project is to support management of the Coorong to shift the system from algal dominated to a *Ruppia* dominated habitat, improve water quality and restore the ecological function associated with an aquatic plant dominated state (Healthy Coorong, Healthy Basin 2021). Reviews of previous research and monitoring activities provided the framework for undertaking the evaluation of the *Ruppia* dominated aquatic macrophyte and filamentous algal community in the southern Coorong over the course of this study (Asanopolous and Waycott 2020, Brookes et al. 2009, Nicol et al. 2018, Paton et al. 2021 Waycott et al. 2019, Waycott et al. 2020). The results of this study will help to address the overarching management questions of how to optimise the use of existing infrastructure to limit filamentous algal growth and restore *Ruppia* distribution and abundance; and what additional management interventions are required to assist with the recovery of ecological functions.

1.2 Aim

The Aim of 'T&I Component 2 – Activity 2.2: Undertake baseline surveys and map the extent of *Ruppia* and filamentous algae communities' was to use direct field-based observations to establish the current extent and condition of the *Ruppia* and filamentous algae communities in the southern Coorong at times of the year critical to the lifecycle of *Ruppia*. In addition, Activity 2.2 utilised project specific and externally monitored environmental conditions to establish if there were measurable changes in ecological condition resulting from water delivery and accepted ecological drivers of change.

This final technical report presents the results of work completed during the period from July 2020 to June 2022.

2 Methods

To evaluate the *Ruppia* dominated aquatic macrophyte community and filamentous algal distribution across the Coorong, field-based surveys were undertaken four times over a 15-month period. These document the presence and abundance of the aquatic macrophyte community, along with the extent and variability of substantial filamentous algal blooms. Observations of community composition, specifically which species of aquatic macrophyte were present, were made in the field. If no reproductive structures were present, it was not possible to determine the presence of different species at each site. To account for this, environmental DNA testing (eDNA) methods were developed to screen for macrophyte species in the field samples of plant material. These applied a next generation sequencing toolkit developed for coastal submerged and emergent aquatic flowering plants (Foster et al. 2022, Waycott et al. 2021b). Previous exploration to apply remote sensing techniques concluded that the current poor water quality (Mosley et al. 2020b) impeded the use of aerial surveys and/or satellite imagery to detect submerged aquatic vegetation (Waycott et al. 2020). Further application of remote sensing methods was not explored during this project. In addition, remote sensing applications for detecting surface algal mats during peak growth using satellite imagery and spatial analysis tools may have application in the Coorong as previously reported by Auricht et al. (2019) and this will be discussed in Section 4.2.

2.1 Field based surveys

The primary survey area covered the length of the main body of the Coorong from Long Point (-35.695404°, 139.161932°) to the area just south of Salt Creek where the channel starts to narrow (-36.140916°, 139.628937°) (Figure 3). Baseline surveys to document the aquatic macrophyte community distribution were done four times. Each sample period aligned with a discrete stage in the lifecycle of the dominant macrophyte in the system, *Ruppia tuberosa*, (i.e. vegetative, reproductive (x2) and aestivation) providing additional knowledge of the abundance and distribution of critical reproductive outputs in addition to vegetative standing stock.

Sampling was *post-hoc* stratified into three regions within the primary survey area. The three regions were defined in our analyses based on latitude (decimal degrees) as follows; 'north' less than -35.838217°, 'central' between -35.905129° and -35.838217° 'south' greater than -35.905129°.

Collection locations for the initial surveys were between +0.5 and -1.2 m Australian Height Datum (AHD) in the main body of the Coorong within the primary survey area. The locations where samples were taken on each survey are referred to as 'Sites' throughout our reporting (Figure 4). The locations for Sites in the initial survey were chosen haphazardly to cover the extent of the primary survey region, to represent a range of depths, positions relative to east or west shorelines and subject to accessibility. The Site collections covered both embayments and island sectors to provide good coverage of the length and breadth of the region.

The periods of sampling and type/number of samples taken (Table 1) were aligned with the current season's conditions. Hence, surveys were not undertaken at the same time each year. *Reproductive* sampling was conducted when plants were flowering or fruiting, *Aestivation* sampling was conducted when the sites experienced the lowest water levels and many sites were dry as water levels had dropped, *Vegetative* samples were taken over a longer period when plants had initiated growth following return of higher water levels (autumn) until mature plants initiated flowering when water levels were at their highest.

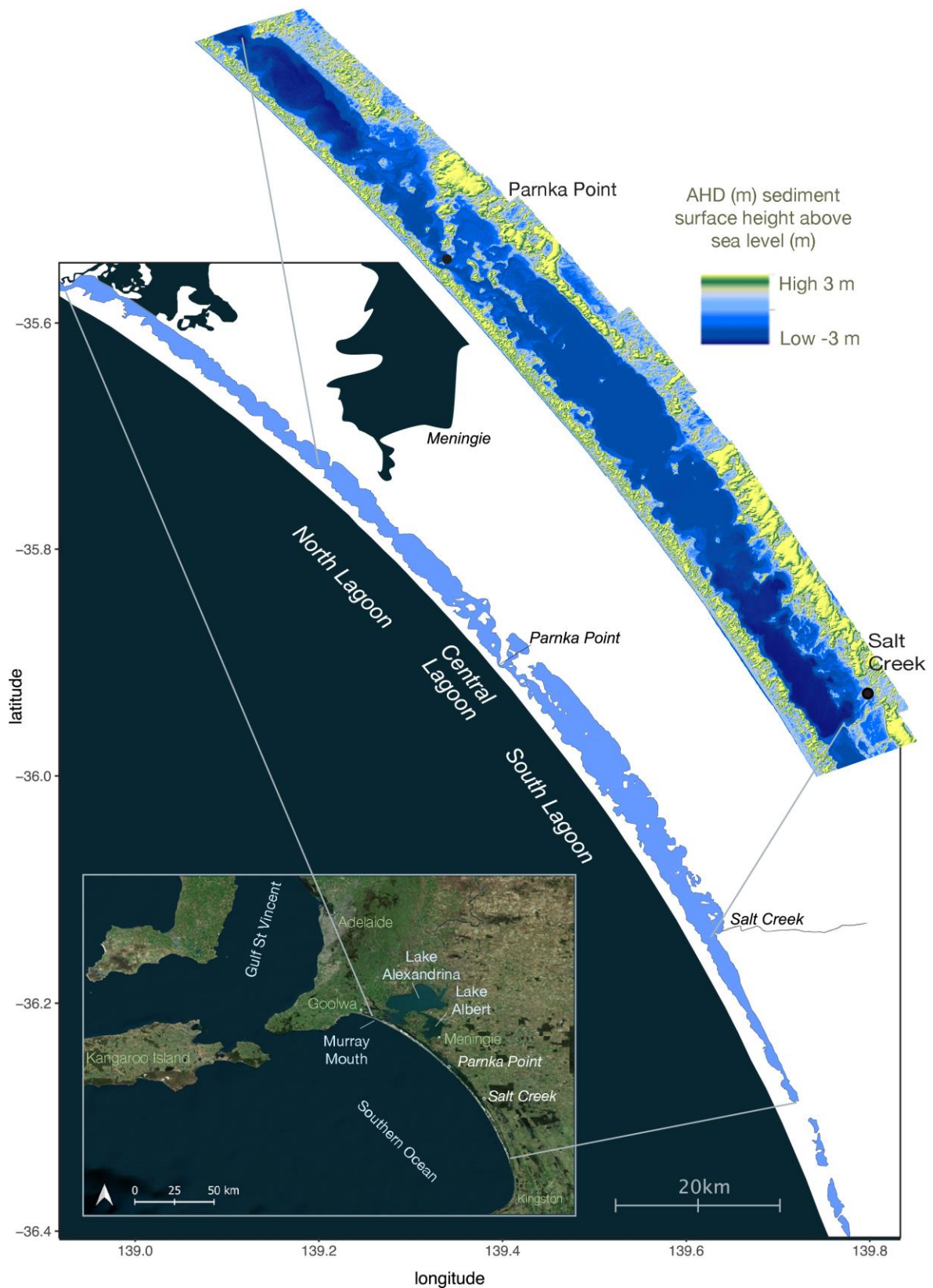


Figure 3. Maps of the location of the study region in the Coorong (lower inset) with key landmarks indicated for cross referencing purposes. An illustration of the Coorong main waterbody highlighted (blue) and the Coorong Lagoon sections referred to in this report. The outset section depicts the sediment surface digital elevation model (DEM; relative to the Australian Height Datum, m AHD) on a colour gradient based on the 2018 update to the Coorong DEM (Hobbs et al. 2019).

The data collected during the single *Vegetative* survey period was focused on establishing greater knowledge of the depth range of the *Ruppia* Community (Table 1) during the rapid colonisation growth phase of the plants. Sampling for the *Vegetative* survey period included transects across sections of the primary survey area at varying spatial scales (Figure 4, Table 1). Data recorded were georeferenced observations of the presence and absence of plants at sampling points and salinity and water depth measurements. In addition, six reference sites were monitored more frequently (every two to three weeks) throughout the period from shortly after germination until senescence to investigate transition times and plant phenology and productivity, these data are reported separately (Waycott et al. in preparation).

Table 1. Sampling periods and numbers of sites and points sampled included in all four surveys.

SAMPLING PERIOD	MONTHS SAMPLES TAKEN	NUMBER OF POINTS SAMPLED (I.E. CORES) OR OBSERVED (I.E. ± PLANTS)	NUMBER OF SITES SURVEYED
2020 Reproductive	30 Sep 2020 – 17 Dec 2020	1535	103
2021 Aestivation	2 Mar 2021 – 12 Apr 2021	1344	92
2021 Vegetative*	3 Aug 2021 – 17 Nov 2021	610	96
2021 Reproductive	1 Dec 2021 – 16 Dec 2021	1358	90

*Only presence/absence data collected.

Figure 4 shows the locations of each aquatic macrophyte survey site (quantitative and presence absence) over the study period. Surveys conducted during 2020 Reproductive, 2021 Aestivation and 2021 Reproductive periods had some variation in the number of sites accessed occurred due to local conditions and changes to site access. Sites were sampled by three teams and accessed using either a vehicle (then to site on foot), boat or hovercraft. In the 2020 Reproductive survey, 59 sites were sampled by vehicle access, 39 sites by boat and 5 sites by hovercraft. For the 2021 Aestivation survey, 60 sites were accessed by vehicle, 16 sites by boat and 16 sites by hovercraft. In the 2021 Reproductive survey, 59 sites were accessed by vehicle, 21 sites by boat and 10 sites by hovercraft.

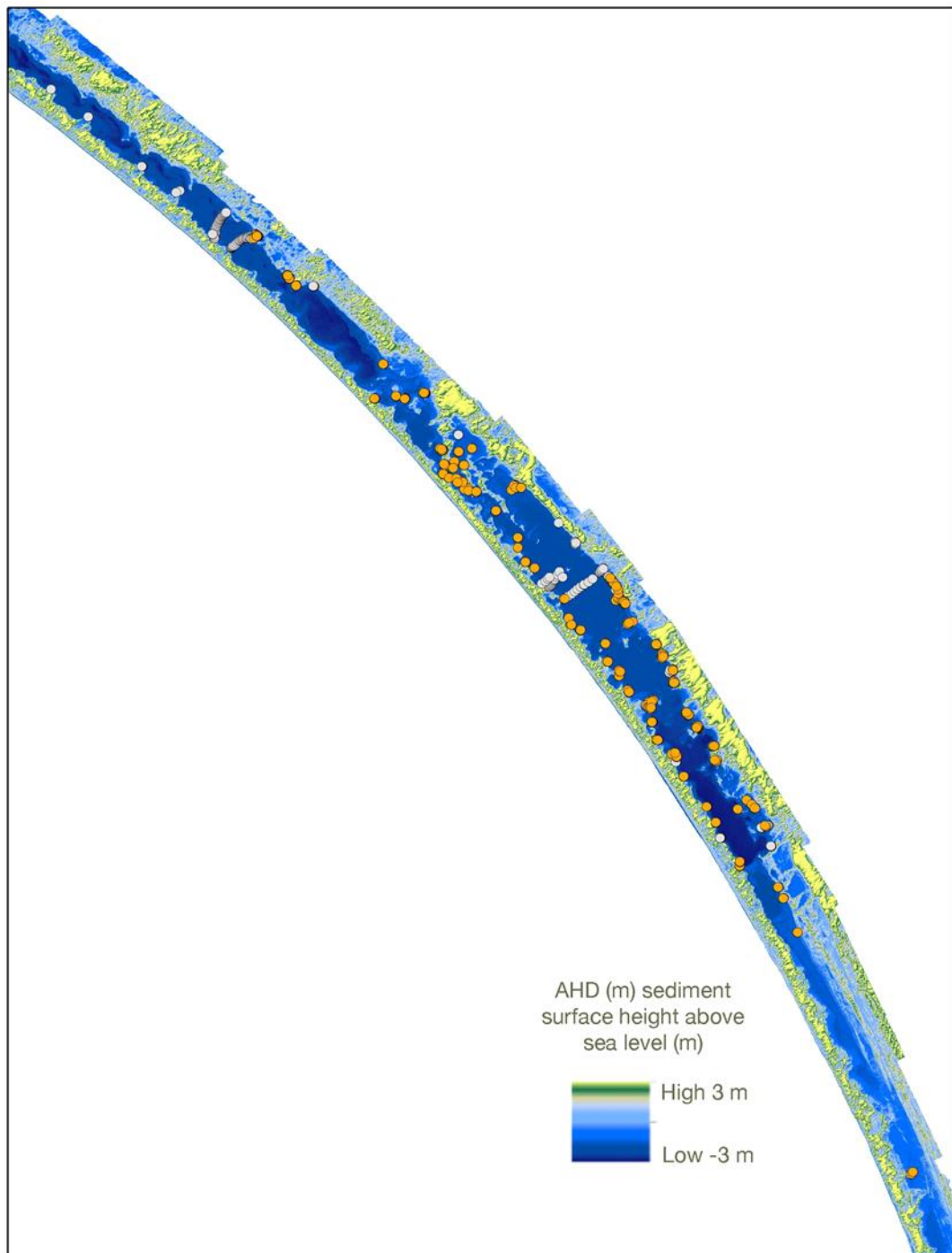


Figure 4. Survey site locations from Reproductive 2020, Aestivation 2021 and Reproductive 2021 quantitative baseline surveys (orange dots) and the 2021 Vegetative presence/absence survey (grey dots) across the Coorong. The relative position and geographical orientation are depicted in Figure 3. The shading represents digital elevation model (Hobbs et al. 2019) - yellow-green > 0.9 m AHD, Pale-mid blue 0.9–0 m AHD and dark blue <0 m AHD.

For the quantitative baseline surveys, at each individual site, 15 replicates of 75 mm diameter sediment cores were taken, to a depth of up to 8 cm. This is consistent with TLM monitoring (Paton et al. 2017a) and was adopted so that results were consistent with existing *Ruppia* condition monitoring.

At each site, the first core was taken at a central sampled point (i.e. central core) and the remaining 14 in a haphazard circle with an approximate radius of 5 m from the central core (Figure 5). Georeferencing of sites and samples was undertaken, the position of each core taken was recorded by GPS. In addition to the replicate cores, physico-chemical measurements of salinity and water depth were recorded at the central core, and plant material was collected for DNA analysis to determine species presence (*Ruppia* spp. or *Althenia cylindrocarpa*). If flowering plants were present, flowers of *Ruppia tuberosa* and *Althenia*

cylindrocarpa were collected for DNA analysis to determine genetic markers for each species to be used to differentiate individual genus or species.

We adopted a haphazard sampling design revisiting approximate locations (i.e. intended to resample the same meadow but not the exact same points) but sites may have been in slightly different sampling areas (Figure 5). While every effort was made to be consistent in our sampling protocol, changes in access, water depth and GPS accuracy were all factors which lead to slightly different sites in some surveys. These site differences were evaluated analytically and database records were linked where sites were within 100 metres from each other and treated as if they were from the same 'Location' (Figure 5).

Sampled area = *Location*

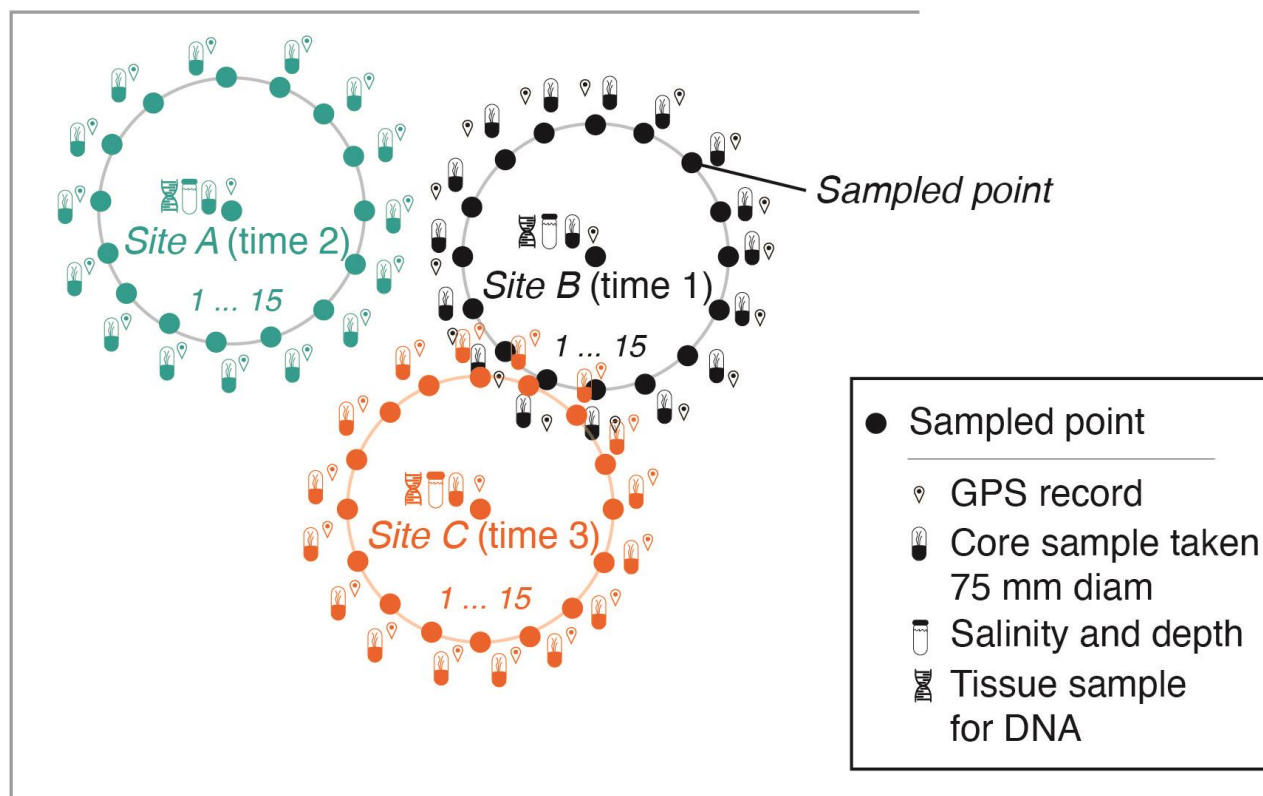


Figure 5. Conceptual diagram of sampling strategy, sample nomenclature for the surveys conducted to include measures of abundance; Reproductive 2020, Aestivation 2021 and Reproductive 2021. The central sampled point also acts as a reference to the samples taken on each survey. Symbols represent sample type taken at each sampled point. Note that Site samples from different surveys were considered to be in the same Location as long as they were within 100 m of each other. All calculations on relative positions were made on the georeferenced central sampled point unless specifically utilising georeferenced individual sampled points.

A Rapid Assessment Protocol (RAP) developed by Hallett et al. (2019) was used to categorise sediment condition at the central core of each site. The RAP assigns scores from 1 to 5 based on colour, texture and odour of sediments (Table 2). The sum of these scores forms the overall Sediment Condition Score. This was first adopted in the 2021 Aestivation survey assessing one core per site; however, in the 2021 Reproductive survey this was extended to be assessed at every third core. We were not able to complete Sediment Condition RAP on the 2021 Vegetative survey as intact core samples in the deeper water were unable to be collected.

Each core sample was placed into individual, numbered calico bags. All cores were frozen (-20°C) upon return to laboratory to preserve their contents. All DNA samples were collected in individually numbered plastic bags and upon return, placed in labelled tea bags and put on silica to dry. The silica was changed frequently to ensure all samples were able to fully dry and stay dry until analysed.

Table 2. Sediment Condition assessment criteria as defined by Hallett (2019). Sediment condition score is based on an assessment of sediment colour, texture and odour, scores are added up, higher scores indicate good sediment condition.

CRITERIA	SCORE: 5	SCORE: 4	SCORE: 3	SCORE: 2	SCORE: 1
Colour	Yellow/brown to depth of core	Yellow/brown overlying grey	Yellow/brown overlying black	Grey to depth of core	Black to depth of core
Texture	Coarse grainy/coarse sand	Fine grainy/fine sand		Smooth and silky	Oozy and slick/sticky
Odour	No odour of H ₂ S	Mild odour of H ₂ S		Moderate odour of H ₂ S	Strong odour of H ₂ S

At each site the algae % cover and level of interference with plants was recorded. An Algal Severity Score was developed based on research outcomes from earlier HCHB Phase 0 activities (Waycott et al. 2019, Waycott et al. 2020) where filamentous algal biomass and attachment to substrates was surveyed (Table 4). We later adapted the data collected through the Algal Severity Score observations into estimated algal biomass, using calculations based on the mean dry weight biomass of 100% surface cover. Categories for visual estimates of filamentous algal severity and estimated dry weight biomass are found in Table 4.

Table 3. Filamentous algal scoring matrix based on observations that there is a seasonal lifecycle for the formation of surface mats of filamentous green algae in the Coorong (Auricht et al. 2019, Collier et al. 2017, Waycott et al. 2020).

ALGAL SEVERITY SCORE*	DESCRIPTION OF ALGAL FORM AND QUANTITY
1	No filamentous algae observed in area sampled; category <i>none</i>
2	Only as drift (i.e. floating on surface, unattached); category <i>drift</i>
3	Attached to sediment only (not attached to plants), category <i>sediment</i>
4	Attached to plants at sediment level (basal), category <i>basal</i>
5	Attached to plants in water column (not just basal), category <i>column</i>
6	Attached to plants in water column, obscuring plants, category <i>obscuring</i>
7	Attached to plants as a surface mat, category <i>surface</i>

*See Table 4 for estimated algal biomass calculations per severity score.

The 2021 Vegetative survey was conducted as transect surveys across 20 sampling areas, 6 transects in the North Lagoon, 2 in the central section and 12 in the South Lagoon (Figure 4) either accessed by vehicle or boat. Observations were taken haphazardly along a transect using two approaches: from shore (vehicle), each transect started shallow and continued to sample areas as deep as practicable; alternatively using a boat, transects were run from shore to shore or as close as practically possible. Observations taken along each transect included presence/absence of aquatic plant material (including life stage), water depth and sediment characteristic score (where possible) as per Hallett et al. (2019). At least one water sample was taken per transect to measure salinity. Transects completed on foot (vehicle) involved up to 10 observations per GPS reference site: once aquatic macrophytes were determined to be present along those transects, no further observations were undertaken; if 10 observations were made and no plant material was observed, the site was recorded as absent of aquatic macrophytes.

2.2 Environmental data sets

Water depth in metres (m) and salinity in Parts Per Thousand (PPT) were measured *in-situ* during field surveys. Salinity samples were collected in 25 mL Falcon Tubes and analysed in the laboratory using a Milwaukee digital refractometer to determine salinity (in Parts Per Thousand, PPT).

There is a high degree of variability in water depth in the Coorong influenced by tide, external water inputs (including barrage flows and flows from Salt Creek), wind (seiching) and evaporation. As a result, utilising *in-situ* water depth data must be done with caution as it represents water present over a local site at that point in time. New tools for modelling the combined effect of these influences are underway (Hipsey et al. 2020). Therefore, we utilised the Digital Elevation Model (DEM) indicating the sediment surface height above sea-level supplied by the Department of Environment and Water (DEW) (Hobbs et al. 2019) as a more consistent indicator of site elevation in relation to Australian Height Datum (AHD). Throughout this report 'elevation' refers to the elevation of the site (m AHD), where we refer to 'depth' it corresponds to our field measurements of water level above the sediment at the time of survey.

To investigate the relationship between abiotic environmental factors and aquatic macrophytes and filamentous algae, we obtained Electrical Conductivity (EC, mS/m^{-1}), Total Dissolved Solids (TDS g L^{-1}), temperature ($^{\circ}\text{C}$) and depth (m) data from the DEW Water Data SA website (accessed at www.water.data.sa.gov.au, on 3 April 2022). Electrical Conductivity data were exported as a monthly aggregate in mS m^{-1} , our field data were measured in PPT but a conversion between the two units was not reliably accurate. The Coorong water quality monitoring stations used were: The Coorong NW Snipe Island (A4261165), Coorong near Woods Well (A4261209), Coorong at Parnka Point (A4260633) and The Coorong at Robs Point (A4260572) (water.data.sa.gov.au).

2.3 Laboratory processing of field samples

Individual core samples were thawed, and sample numbers cross-referenced with site data for quality control. The contents of each calico sample bag containing individual cores were placed into a 500 μm sieve, and the sample bag rinsed inside-out into the sieve to ensure all sample material was included in processing. The samples were then sieved, to retain seeds and all plant material, using manual, mechanical action and running water. All sediment was collected for return to the Coorong to fulfil permit requirements.

Once thoroughly sieved, the material was rinsed and emptied into a plastic tray for further sorting. The tray was elevated on one side, and its contents were spread across and up the tray such that seeds and plant biomass are separated from sediment, the contents of the tray were then inspected thoroughly. The first sample from each site was photographed pre- and post-sieving with its corresponding sample number for future reference.

Only plant material where the overall structure of the plant i.e. rhizome, roots and shoots were attached and present prior to collection was included in counts and biomass estimates. Loose, unstructured and otherwise dead biomass was not included in counts and biomass estimates.

Small aluminium foil containers were used for weighing biomass. These were weighed empty to the nearest 0.01 mg. Living aquatic macrophyte biomass was collected from each sample and placed into the corresponding foil container and dried in the oven at 60°C for 48 hours. Once dry, the sample was weighed to the nearest 0.01 mg. The original weight of the container was subtracted to determine the total dry weight of the sample i.e. biomass (g DW) and this weight recorded. The aluminium foil container was then tightly folded shut with the label clearly visible and stored in a vacuum-sealed bag with other biomass samples from the location for future analysis. Any seeds found were enclosed together in empty unused nylon teabags which were labelled with their corresponding sample number and sealed in a container with silica gel-based desiccant.

Once finalised, data sheets were digitised and their contents manually recorded in the project database (created in FilemakerPro™). As data were entered, images of the lab data sheet were included in the database for quality control cross checking purposes. Data quality assurance included a second data entry reviewer checking records.

2.4 Algal biomass sampling

Algae biomass was measured on 3 and 17 November 2021 at Parnka Point, where 100 percent algal surface cover was present. These data supplemented previous data on algal biomass collected on 29 November 2019 when 10–90% algal surface cover was present.

Samples for filamentous algal biomass estimation were taken using a plastic tube with a diameter of 400 mm (0.125 m²). The tube was pushed down into the water column, cutting the algae floating in the water on the way through, and then pushed into the sediment. All algae were then removed from the water inside the tube, while removing as much macrophyte material as possible and put into a labelled bag for return to The University of Adelaide where the algae was placed into paper bags and dried at 60°C for 48 hrs and then weighed (g DW).

This sampling in 2021 focussed on areas of 100% surface algae cover. We experimented with sampling across a variety of algal densities; however, due to the complex morphology and structure of the filamentous algae it was difficult to quantify biomass for percentage cover less than 100%. Due to these complexities, we relied on sampling 100% algal cover, then used our other data to compile the Algal Severity Index (Table 4). This index relies on field estimations of algae cover to deduce an assumed cover and its proportion of 100% biomass. We found that below an algal severity score of two, algal biomass was negligible, and consequently we then used the estimates of proportion of biomass to deduce an estimated dry weight (g DW m⁻²). Although this method did not give an exact biomass measurement, it enabled us to use existing algal cover data from surveys to estimate algal biomass. When referenced to samples which were less than 100% cover, the estimated values were within 10% of the actual value.

Table 4. Algal Severity Index and calculated biomass for filamentous algae in the Coorong 2020/2021.

ALGAL SEVERITY	ASSUMED % COVER [^]	PROPORTION OF 100% BIOMASS	ESTIMATED G/DW PER M ² *
0	0	0	0.0
1	0	0	0.0
2	0	0	0.0
3	5	0.5	3.7
4	20	5	36.8
5	50	25	184.1
6	80	50	368.2
7	100	100	736.5

[^]Based on field estimates of algae cover proportion, *based on proportion of 100% cover from sampling.

2.5 Statistical analyses

Figures were created using Data Graph V. 5.0 unless otherwise specified. Q-Q Plots were used to check data for normality, and data were transformed where necessary.

Differences between seasonal biomass and shoot, seed and turion counts, were compared using Multivariate General Linear Models (GLM) including the variables; biomass, shoots, seeds and turions, with season as the fixed factor and a Bonferroni Correction post-hoc test to identify seasonal differences. Data were log transformed to improve normality. Pearson's Correlation Coefficient was used to assess the association between shoot counts and biomass. This analysis was also used to show the relationship between biomass and distance from Murray Mouth for the 2020 and 2021 Reproductive seasons.

Estimated algal biomass data were checked for normality then log transformed. A univariate GLM was conducted using Survey Period as the fixed factor and a Bonferroni Correction post-hoc test to identify

seasonal differences. A One-way ANOVA was conducted on log transformed data to analyse aquatic macrophyte biomass at different levels of estimated algal biomass, and a Tukey's HSD post-hoc test was used to assess difference in aquatic macrophyte biomass between levels of estimated algal biomass.

To assess the effect of season on salinity and depth we used a One-way ANOVA and Tukey's HSD post-hoc test. Salinity data were natural log transformed to improve normality.

The effect of environmental factors on biological variables (macrophyte biomass, shoot count, seed count and turion count) were tested using PERMANOVA Distance-based Linear Model (DistLM). Data were fourth root transformed to correct for skewness. DistLM produced a graphical representation in the way of a distance-based Redundancy Analysis (dbDRA) to assess relationships visually. We assessed each biotic variable individually except for biomass and shoot counts, which we combined. We used a step-wise routine to select variables for model building, then excluded environmental factors based on the results and ran the model again, forcing exclusion of the insignificant variables. Akaike's Information Criterion (AIC) was used to select and evaluate models.

All statistical analyses, were undertaken using the software package IBM SPSS Statistics V. 11 except DistLM and dbDRA, which were undertaken using Primer-e V.7 + PERMANOVA.

2.6 DNA based assessment of aquatic macrophyte community composition

Identification of aquatic macrophytes present at each sampling site using next generation sequencing techniques was undertaken for samples collected at the central core georeferenced point during the 2020 Reproductive survey (i.e. October 2020). A lack of consistent vegetative characters for identification of species and the patchiness in space and time of reproductive structures led to this requirement to confirm taxonomy. As a result, to estimate the overall distribution of aquatic macrophytes an environmental DNA assessment method based on a newly developed target capture toolkit (Foster et al. 2022, Waycott et al. 2021b) based on multiple chloroplast gene regions and mixed DNA sources was used. The main sample set for the analysis was the central core taken at each site during the 2020 Reproductive survey. These samples were central at each site collection providing a representative sample of material present.

2.6.1 DNA extraction and sequencing

Samples collected during the 2020 Reproductive survey from those Sites where it was possible for cores to be taken (i.e. the benthic substrate was not rocky) were included in DNA extraction and library preparation, a total of 96 samples. Additionally, samples of known previously collected *Ruppia* and *Althenia* species were also processed to serve as reference material. Because the HCHB samples were collected in 75 mm diameter cores there is a very high probability that multiple plants, and even species, were collected in one core. To perform a representative DNA extraction from the core, all organic material was separated, dried and ground up by mortar and pestle. A subsample of ~30 µg was taken for DNA extraction and placed in a 2 mL screwcap tube.

DNA extractions were preformed using the DNeasy® Plant Mini Kit (Qiagen). Initial analysis steps were done according to the manual, and after ice precipitation step extractions were transferred to an S-plate (Qiagen) and finalised on a QIAcube HT (Qiagen) following the manufacturer's Qiacube instructions. Final DNA elutions were done in 100 µL EB buffer. DNA extractions were stored in a 96-well DNA storage plate at -20°C.

Illumina sequencing library generation and RNA bait capture were performed using the method described in (Waycott et al. 2021b). For species identification we used the myBait target enrichment system (Arbor Science) with the single copy plastid loci bait set developed by the Waycott research group (Waycott et al. 2021b). For this project, the target capture utilised an updated version of the bait set consisting of ~50 chloroplast loci instead of 19 loci. Library preparation was done with the NEBNext® Ultra™ II DNA Library Prep kit with fragmentase (FS) and Sample Purification Beads (New England Biolabs). Neat DNA extracts of both HCHB and reference samples were used as the starting material and reactions were done in 1/3

(Waycott et al. 2021b). To enable bioinformatics processing following myBaits capture, Y-adaptors (compatible to Illumina TruSeq primers) with synthetic 'barcodes' were annealed to the ends of the DNA fragments. These barcodes allow for samples to be identified by a unique synthetic 8 nucleotide (nt) DNA barcode situated on the 3' end of the Read 1 and 2 primer binding sites. 96 unique adapters (a full Polymerase Chain Reaction (PCR) plate worth) were used for this study. Hybrid capture was performed following the manufacturer's manual (v4.0), hybridization was performed at 63°C and incubated for 24 h. Post capture PCR was performed on the half build libraries to fuse the remaining sequencing adapters with 8nt P5 and P7 indexes to the ends of the DNA fragments (Waycott et al. 2021b). Libraries were pooled in equimolar concentrations and size selected to 350-600 base pairs (bp) with a Pippin Prep 2% agarose cassette (Sage Science). Subsequently they were quantified and sent for Illumina paired-end sequencing (2 × 150) on a Novaseq S1 lane to the Australian Genome Research Facility (AGRF) in Melbourne.

2.6.2 Bioinformatic processing

Raw Illumina reads were imported into the NGS bioinformatics platform CLC Genomics Workbench v12 (Qiagen). Reads were demultiplexed and named based on the 8nt barcodes, this was followed by a trimming step to remove residual adapter sequences and low-quality sequence calls.

Initial species diversity assessment was done by mapping the reads of all 96 HCHB samples and reference samples to the publicly available chloroplast genome of *Ruppia sinensis* (NCBI accession number [MN233650](#)). This was done under relaxed conditions (in CLC; 80% of reads matching at least 80% of the sequence) to isolate any chloroplast sequences from each sample. Mapped reads were extracted into separate files and *de novo* assembled under strict conditions (in CLC; 95% of reads matching at least 99% of the sequence) in order to identify divergent sequences for each gene region that might represent different taxa within a single sample. Consensus sequences for each sample were generated with a minimum read depth of 12 reads and a minimum length of 100 bp. Consensus sequences for each HCHB sample were exported as Genbank files and imported into Geneious Prime v2022 and renamed. All sample sequences were mapped back to the *Ruppia sinensis* genome, and mappings to *matK* and *rbcl* were extracted, trimmed and realigned. Unique sequences were blasted online (BLASTN, NCBI) to The National Center for Biotechnology Information (NCBI) database to determine which species (or genera) were present in the mixed samples. *Althenia* and *Ruppia* were the only angiosperm genera found in the mixes. Reference samples were also blasted to confirm their respective genera. It is worth noting that as species level taxonomy remains unresolved within these genera, our classification the samples to species is tentative (e.g. Waycott et al. 2014).

An all-purpose mapping reference was generated to represent the captured loci only for each genus (reducing the full 150Kb Cp genome to ~20-30Kb gene regions). This was done by mapping a known sample (collected in the Coorong) of either *Ruppia* or *Althenia* to a close reference chloroplast genome (in CLC; *Ruppia sinensis* [MN233650](#) and for *Althenia* the close relative *Potamogeton perfoliatus* [NC_029814](#) was used). Consensus sequences were extracted using a coverage cut-off of 50X (i.e. each base in the mapped region is represented by at least 50 reads) and a contig length of > 50 bp, and gene annotations were added from the reference genome. Consensus sequence extractions resulted in ~40 loci for each genus (100–5400 bp in length).

To establish which loci to use in the final data set all samples (raw trimmed reads of both HCHB and reference) were mapped to both references under strict conditions (85% of length of each read had to map with 98% similarity). These conditions were selected in order to separate the *Ruppia* reads from the *Althenia* reads in the potentially mixed HCHB samples. Again, consensus sequences were extracted from mappings for each sample but with a lower coverage threshold (12X read depth) and calling ambiguous bases (Ns) below this cut-off (this resulted in each extracted locus sequence being the same length). All sequences were then exported to Geneious, renamed and mapped back to the two new mapping references (*Ruppia* and *Althenia*) to generate a per sample alignment for each targeted gene region. All mappings were visually assessed and loci with good recovery of samples and high alignment quality were selected for further processing. This resulted in a set of 13 Loci including 21 coding and 17 Loci including 22 coding regions for *Ruppia* and *Althenia*, respectively.

The selected mapping alignments were cleaned-up further by trimming ambiguous regions (i.e. a high number of Ns) from 3' and 5' ends and by removing poorly aligned regions (sections that did not assemble well like poly T and A areas). Samples that were poorly recovered (i.e. low coverage, low locus recovery) were also removed. The cleaned-up mappings were then concatenated separately for the *Althenia* and the *Ruppia* species identification. Finally, both concatenations were masked for ambiguities resulting in alignments lengths of 22,627 bases for *Ruppia* and 14,912 bases for *Althenia*.

To determine the number of operational taxonomic units (OTUs) reflecting the number of species, maximum likelihood phylogenies were constructed using the PhyML (Guindon et al. 2010) plugin for Geneious using the masked alignments. PhyML analysis were based on utilising the SPRs topology search, initial tree defined by BioNJ protocol, substitution model K80 for the data set that included 106 taxa, gamma shape parameter of 0.032m Ti/Tv ratio of 3.147 and equal nucleotide frequencies and 100 bootstrap replicates. The final consensus tree was visualised using the interactive tree of life online tool (Letunic and Bork 2021) enabling classification of OTUs with a high degree of confidence.

3 Results

3.1 Distribution and abundance of aquatic macrophytes

Overall, 4,847 observations were made during surveys undertaken over four different time-periods (2020 Reproductive; 30 Sep 2020–17 Dec 2020. 2021 Aestivation; 2 Mar 2021–12 Apr 2021; 2021 Reproductive; 1 Dec 2021–16 Dec 2021; 2021 Vegetative; 3 Aug 2021–17 Nov 2021). Aquatic macrophytes were present in 67% (3,011) of samples across all surveys (Table 5). It is worth noting that *Lamprothamnium* sp. or other charophytes were rarely observed during sample processing, or in the field forming large patches.

Table 5. Sampling effort outcomes for surveys of the distribution of the *Ruppia* Community across the southern Coorong region from Long Point to south of Salt Creek, 2020–2021, refer to Table 1 for sampling dates.

SAMPLING PERIOD	NO. CORES WITH PLANTS	NO. CORES WITHOUT PLANTS	% CORES WITH PLANTS	NO. SITES WITH PLANTS	NO. SITES WITHOUT PLANTS	% SITES WITH PLANTS
Surveys including abundance						
2020 Reproductive	1072	463	69.8	88	15	85.4
2021 Aestivation	741	603	55.1	75	17	81.5
2021 Reproductive	1003	355	73.9	80	10	88.9
Survey taking observations only (presence absence)						
2021 Vegetative	195	415	32	40	56	41.7
Overall	3011	1836	67	283	98	74

Aquatic macrophyte biomass varied across both season and site. The 2020 and 2021 Reproductive seasons had significantly higher biomass than the 2021 Aestivation period ($p < 0.001$); however, no significant difference was observed between the biomass of the 2020 and 2021 Reproductive seasons ($p > 0.05$) (Figure 6, Figure 7A, Figure 8).

Shoot density was slightly higher in the 2021 Reproductive season when compared with 2020; however, this difference was not significant ($p > 0.05$) (Figure 7B, Figure 9). This higher mean shoot density in 2021 might

relate to a trend in biomass; however, 2020 and 2021 Reproductive seasons both had a mean biomass of 28 g of dry weight m⁻² (Figure 7). There was a strong positive relationship between aquatic macrophyte biomass and shoot density ($R^2=0.844$, $p<0.001$) (Figure 10), suggesting that either variable may be used to predict the other depending on what is viable in the field sampling process.

Although biomass and shoot density were not significantly different between the 2020 and 2021 Reproductive seasons, the trends across the system, measured as distance from Murray Mouth, were different. In the 2020 Reproductive period, biomass generally decreased with increasing distance from the Murray Mouth. Contrastingly for the 2021 Reproductive survey, biomass increased with increasing distance from the Murray mouth peaking between 70 and 90 km from the Murray Mouth (Figure 8, Figure 11).

Aquatic macrophyte distribution across elevation was modelled using Digital Elevation data provided by the Department for Environment and Water (Hobbs et al. 2019). The elevation range of our samples was +0.89 – -0.84 m AHD for 2020 Reproductive, 2021 Aestivation and 2021 Reproductive surveys where core sampling was possible. The 2021 Vegetative survey was conducted with a different method, as the water depth at the time did not allow for core sampling, therefore presence/absence was used, with sampling undertaken between +0.65 and -1.3 m AHD. Plants were present in cores between +0.403 and -0.82 m AHD but were present but not quantified.

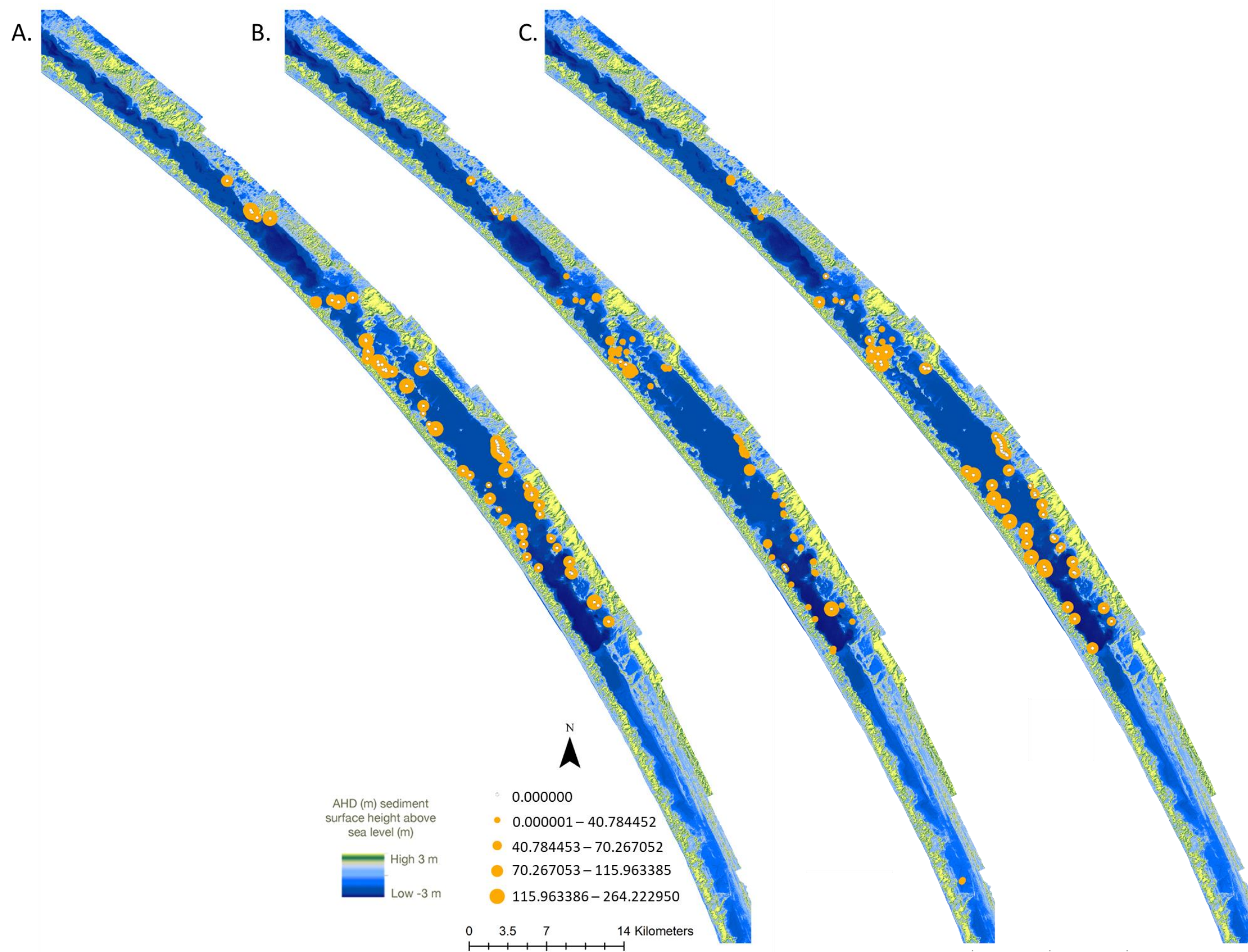


Figure 6. Macrophyte biomass ($\text{g dry weight m}^{-2}$) based on core samples taken during baseline surveys across the Coorong (A) Reproductive 2020, (B) Aestivation 2021 and (C) Reproductive 2021 (for sampling dates see Table 1). The relative position and geographical orientation are depicted in Figure 3. Map shading represents digital elevation model (Hobbs et al. 2019).

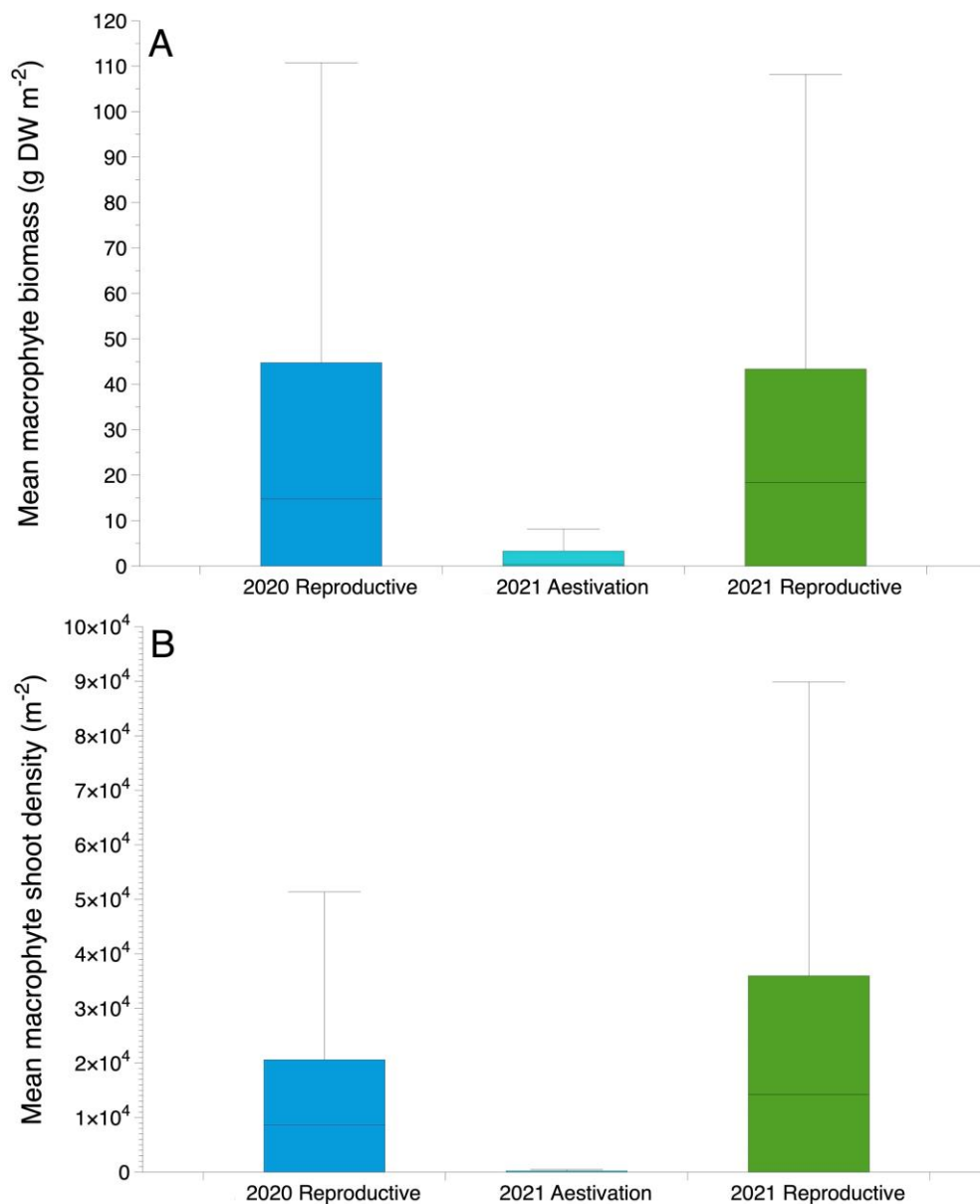


Figure 7. Box and Whisker plots representing the Inner-Quartile-Range (IQR; lower value 25%, upper value 75%), mean, and 95% confidence interval (error bars) for aquatic macrophyte A. biomass m⁻² and B. shoot density across three survey seasons (Table 1).

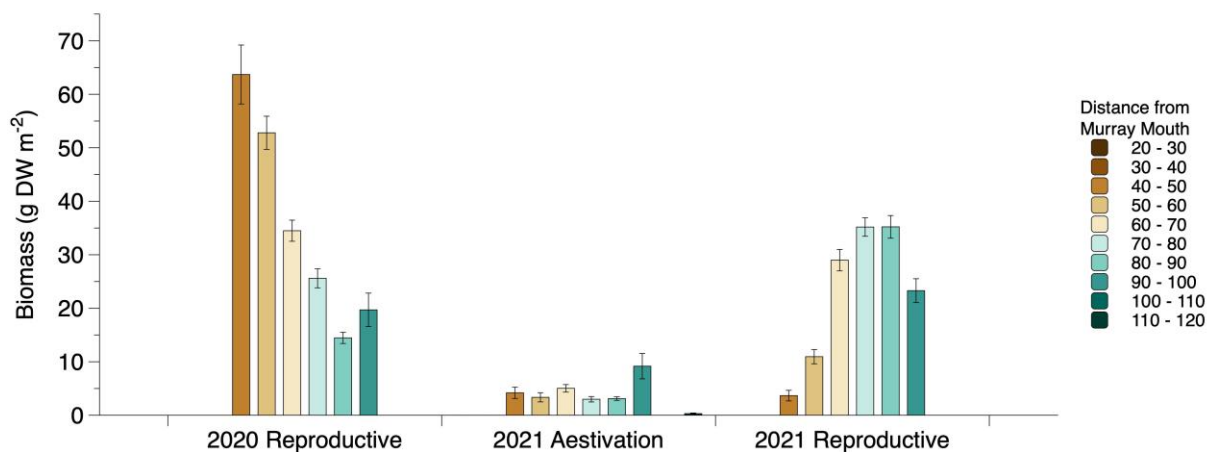


Figure 8. Mean biomass (g DW.m²) ± s.e. of aquatic macrophytes present in the *Ruppia* Community as a function of their distance from Murray Mouth (distances in kilometers from Murray Mouth calculated as linear distance to georeferenced collection point of analysed cores and grouped into 10 km bins).

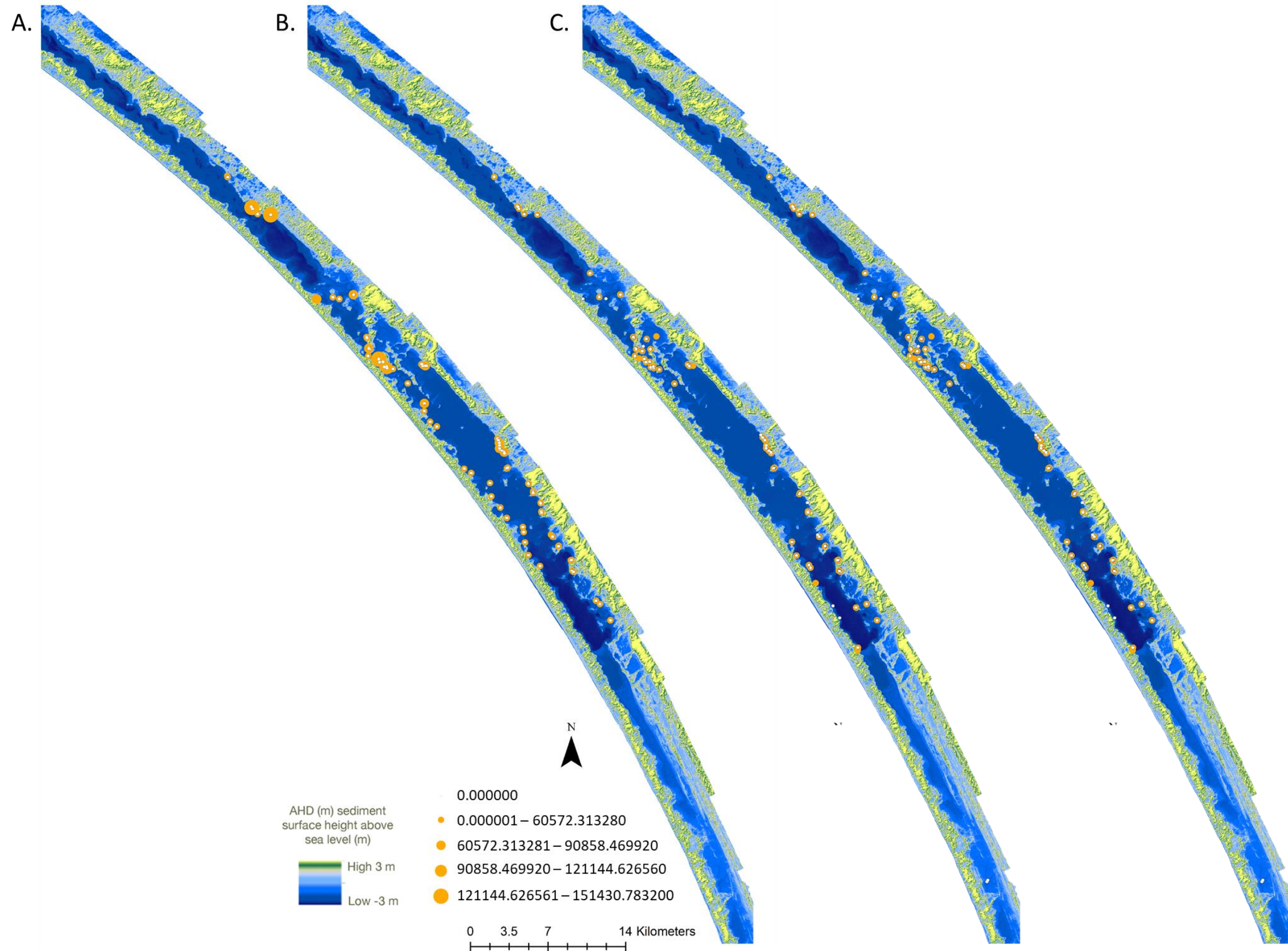


Figure 9. Shoot density (shoots m^{-2}) based on core samples taken during baseline surveys across the Coorong (A) Reproductive 2020, (B) Aestivation 2021 and (C) Reproductive 2021 (for sampling dates see Table 1). The relative position and geographical orientation are depicted in Figure 3. Map shading represents digital elevation model (Hobbs et al. 2019).

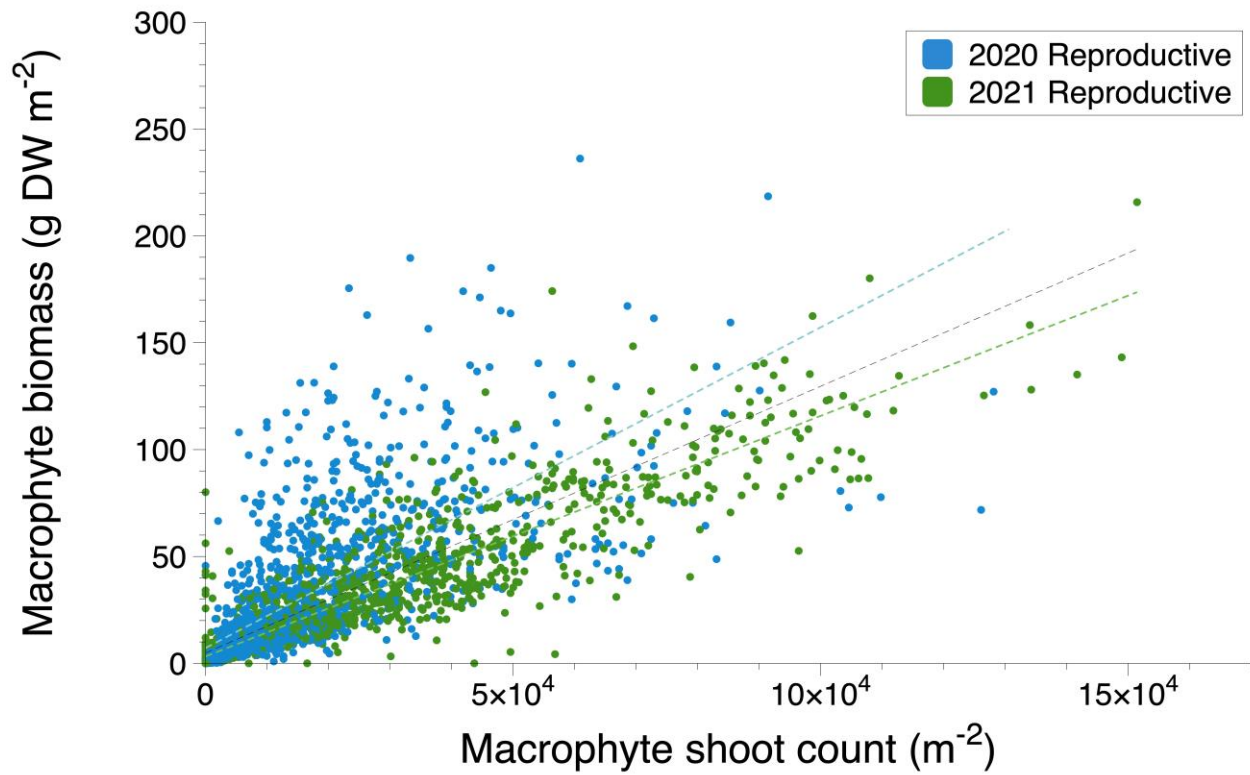


Figure 10. Aquatic macrophyte biomass and shoot counts m⁻² (cores) across the 2020 and 2021 Reproductive seasons with linear trendlines. 2020 Reproductive $R^2=0.5516$ (blue dashed line), 2021 Reproductive $R^2=0.82331$ (green dashed line), 2020 and 2021 combined $R^2=0.844$ (grey dashed line).

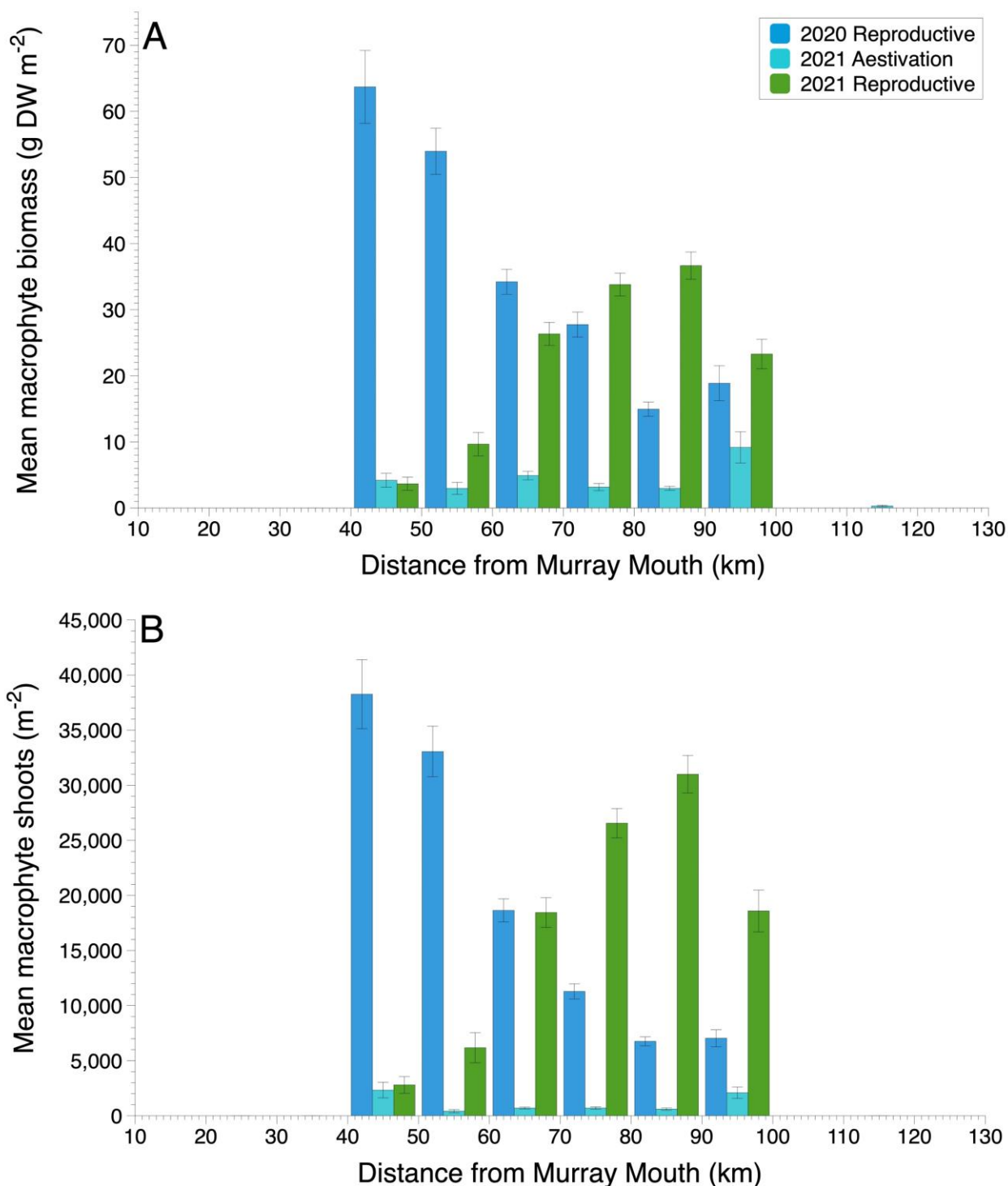


Figure 11. Mean aquatic macrophyte (A) biomass \pm s.e and (B) shoot density \pm s.e measured against distance from the Murray Mouth (MM) for the three surveys (Table 1). No biomass or shoot counts were measured for the 2021 Vegetative period due to high water levels. For reference: Nooneameena – 40 km from MM, Parnka Point – 60 km from MM, Woods Well – 77 km from MM, Salt Creek – 93 km from MM.

The highest mean aquatic macrophyte biomass was recorded in the 2021 Reproductive survey between -0.8 and -0.6 m AHD (101.5 g DW m⁻²) and -1.0 and -0.8 m AHD (99.4 g DW m⁻²), which were both significantly higher ($p < 0.001$) than biomass at all other elevation ranges surveyed. The elevation range with the highest biomass across all sampling periods was -0.6 – -0.8 m (Figure 12). Shoot density generally reflected the trends of biomass across elevation; however, at -1.0 – -0.8 m AHD, although average biomass was similar to -0.8 –

0.6 m AHD, shoot density was significantly higher, 99,940 and 86,780 shoots m^{-2} respectively ($p=0.042$). This may suggest that at below -0.8 m AHD productivity starts to become limited.

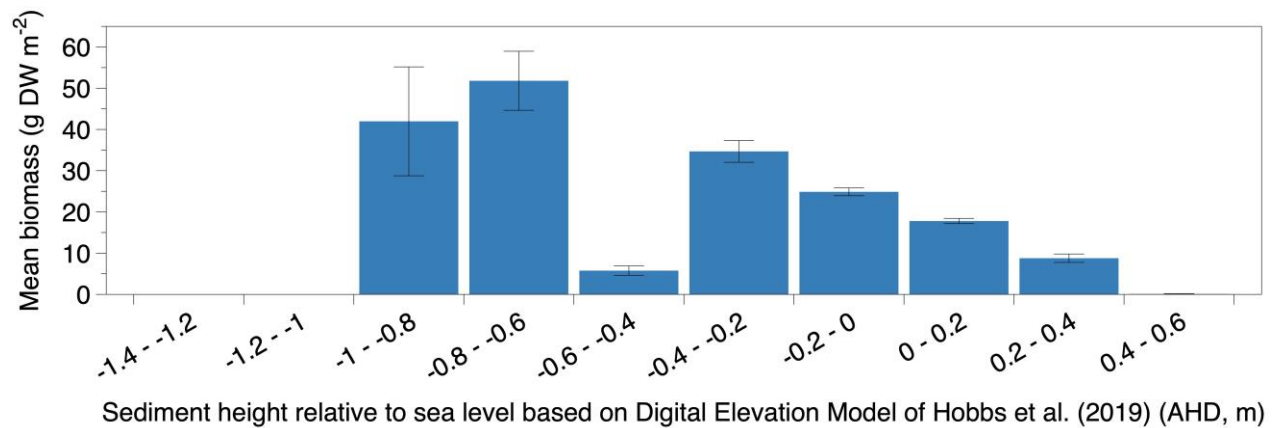


Figure 12. Mean biomass \pm s.e of the *Ruppia* Community classified by 0.2 m ranges of the sediment surface digital elevation model (DEM; relative to the Australian Height Datum, m AHD based on the 2018 update to the Coorong DEM (Hobbs et al. 2019).

We compared our results to data from TLM condition monitoring program (Paton et al. 2021), the only long-term data set collected within the Coorong system (Figure 13). These data show that there has been an increase in aquatic macrophyte shoot density since the end of the Millennium Drought (2010). This slowly increasing trend in aquatic macrophyte abundance contrasts with the significant increase in abundance observed in this study (Figure 13). However, it should be noted that there are differences in sampling methods at each location, site distribution and scoring of shoot counts.

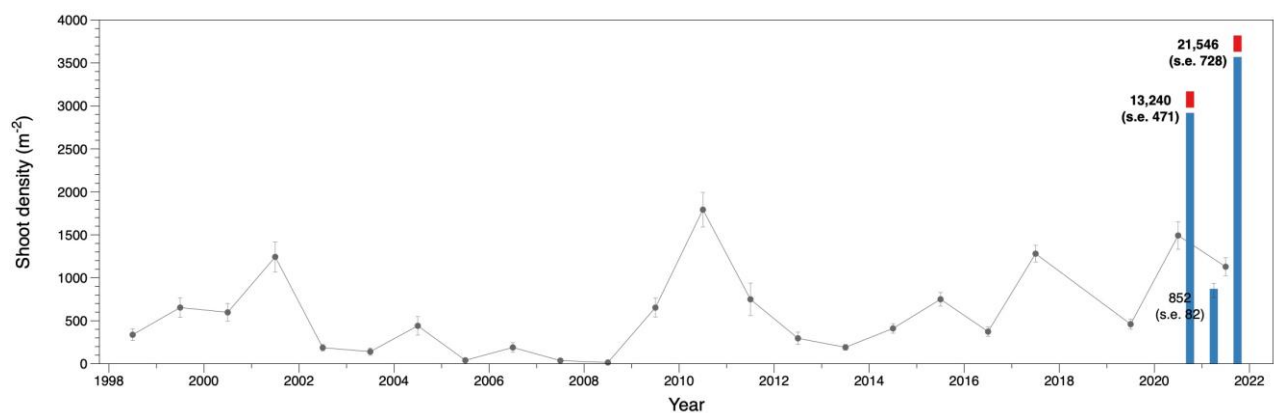


Figure 13. Aquatic macrophyte shoot counts m^{-2} for The Living Murray (TLM; Paton et al. (2021)) monitoring program as an mean of all sites sampled in that year (\pm s.e.) (grey line) from 2008-2021 during the vegetative growing period–winter sampling, July each year and Healthy Coorong, Healthy Basin Trials and Investigations Component 2 mean of all sites sampled (\pm s.e.) (columns) for the 2020 Reproductive, 2021 Aestivation and 2021 Reproductive surveys. Note that Summer TLM sampling commenced in 2011 and HCHB sampling only includes Spring 2020 and Autumn, Summer 2021 (Table 1).

3.2 Resilience of the aquatic macrophyte community

Seed and turion density were variable across the 2020 and 2021 Reproductive seasons and in the 2021 Aestivation period. No seed or turion counts were possible in 2021 Vegetative survey due to it being conducted as presence/absence for aquatic macrophytes without assessing abundance or presence of reproductive structures. There was no significant difference between seed density in the 2020 Reproductive and 2021 Aestivation surveys ($p>0.05$); however, there was a significantly lower seed density in the 2021 Reproductive season compared to the 2020 Reproductive and 2021 Aestivation surveys ($p<0.001$) (Figure 14,

Figure 15A). A mean of 1,559 seeds m^{-2} were found in the 2021 Aestivation period, while 1,009 m^{-2} were found in the 2020 Reproductive period, in contrast only 8.5 m^{-2} were found in the 2021 Reproductive period (Figure 15A). The difference between the two reproductive periods may suggest that seed set had not yet occurred at the time of the 2021 Reproductive survey.

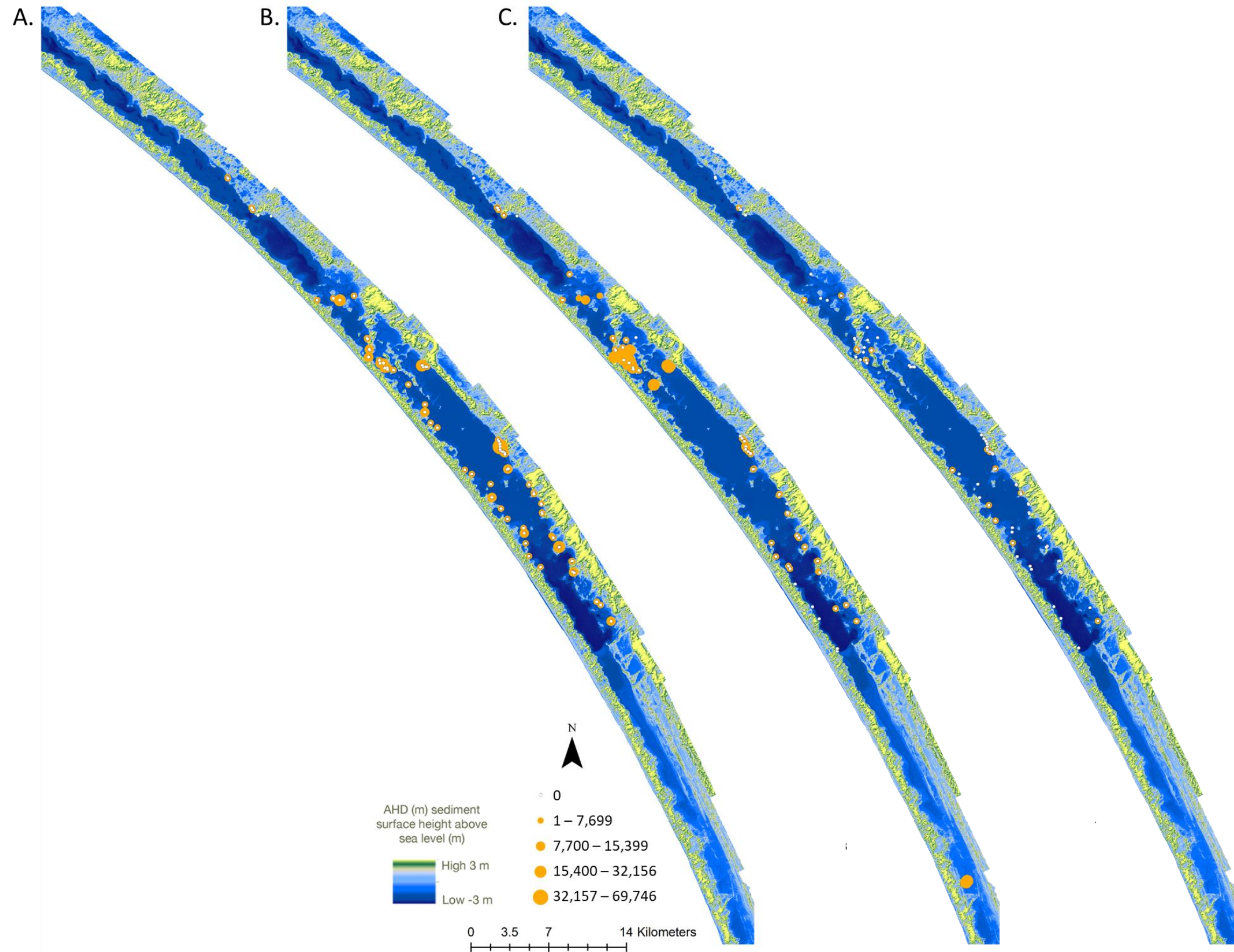


Figure 14. *Ruppia* spp. seed density (seeds m^{-2}) based on core samples taken during baseline surveys across the Coorong (A) Reproductive 2020, (B) Aestivation 2021 and (C) Reproductive 2021 (for sampling dates see Table 1). The relative position and geographical orientation are depicted in Figure 3. Map shading represents digital elevation model (Hobbs et al. 2019).

Turion counts varied significantly across all surveys ($p < 0.001$) (no counts were conducted for 2021 Vegetative survey) (Figure 16). We observed the highest average turion density in the 2021 Reproductive season, (9,286 turions m^{-2}) compared to the 2020 Reproductive and 2021 Aestivation seasons where an average of 226 and 1,992 turions m^{-2} were observed respectively (Figure 15). Interestingly, seed and turion production across the 2020 and 2021 Reproductive periods appeared to be different, with high seed and low turion numbers in 2020, and low seed and high turion numbers in 2021 (Figure 15, Figure 16).

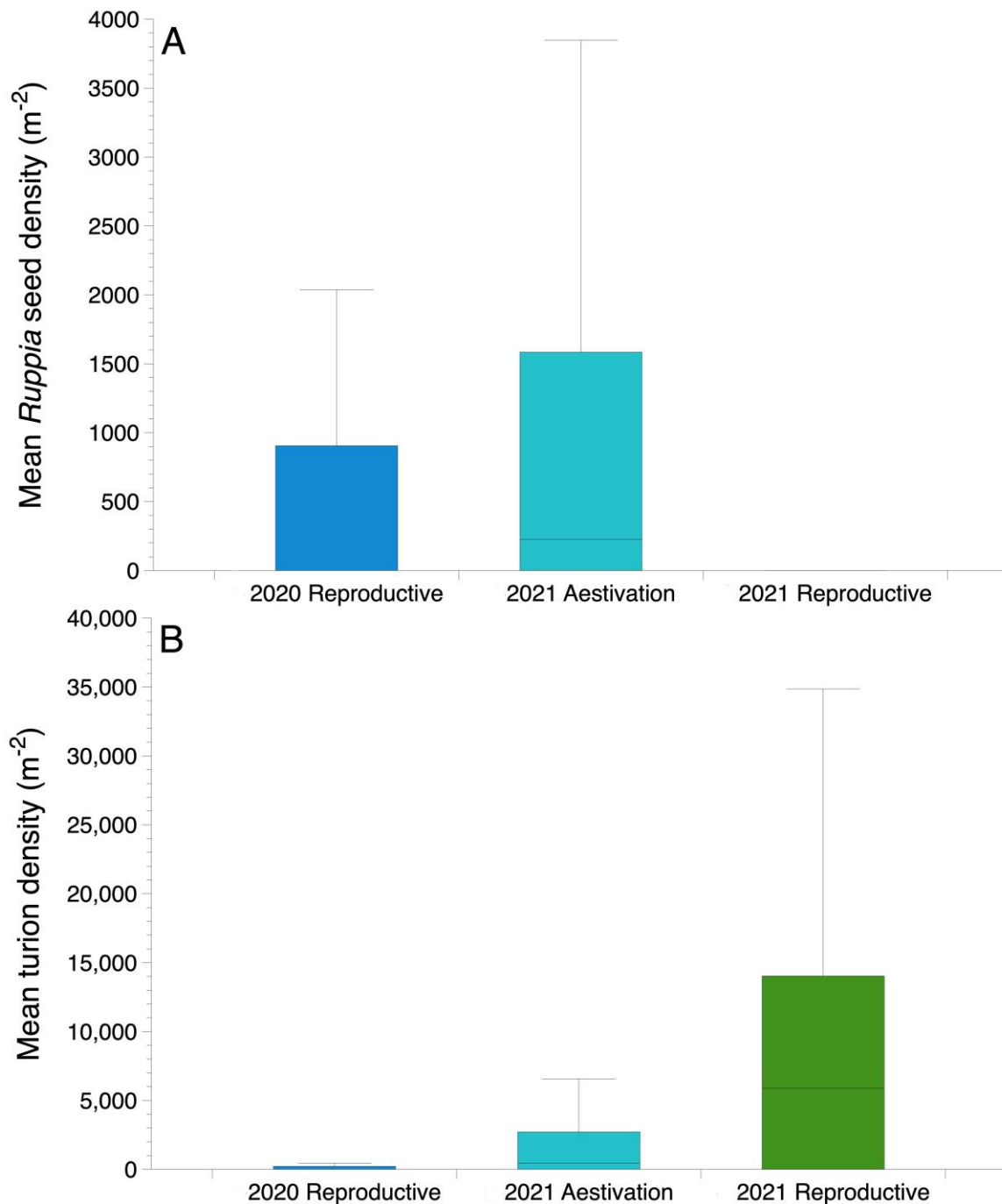


Figure 15. Box and Whisker plots representing the Inner-Quartile-Range (IQR; lower value 25%, upper value 75%), mean, and 95% confidence interval (error bars) for (A) *Ruppia* spp. seed density and (B) *R. tuberosa* turion density across three survey seasons (Table 1).

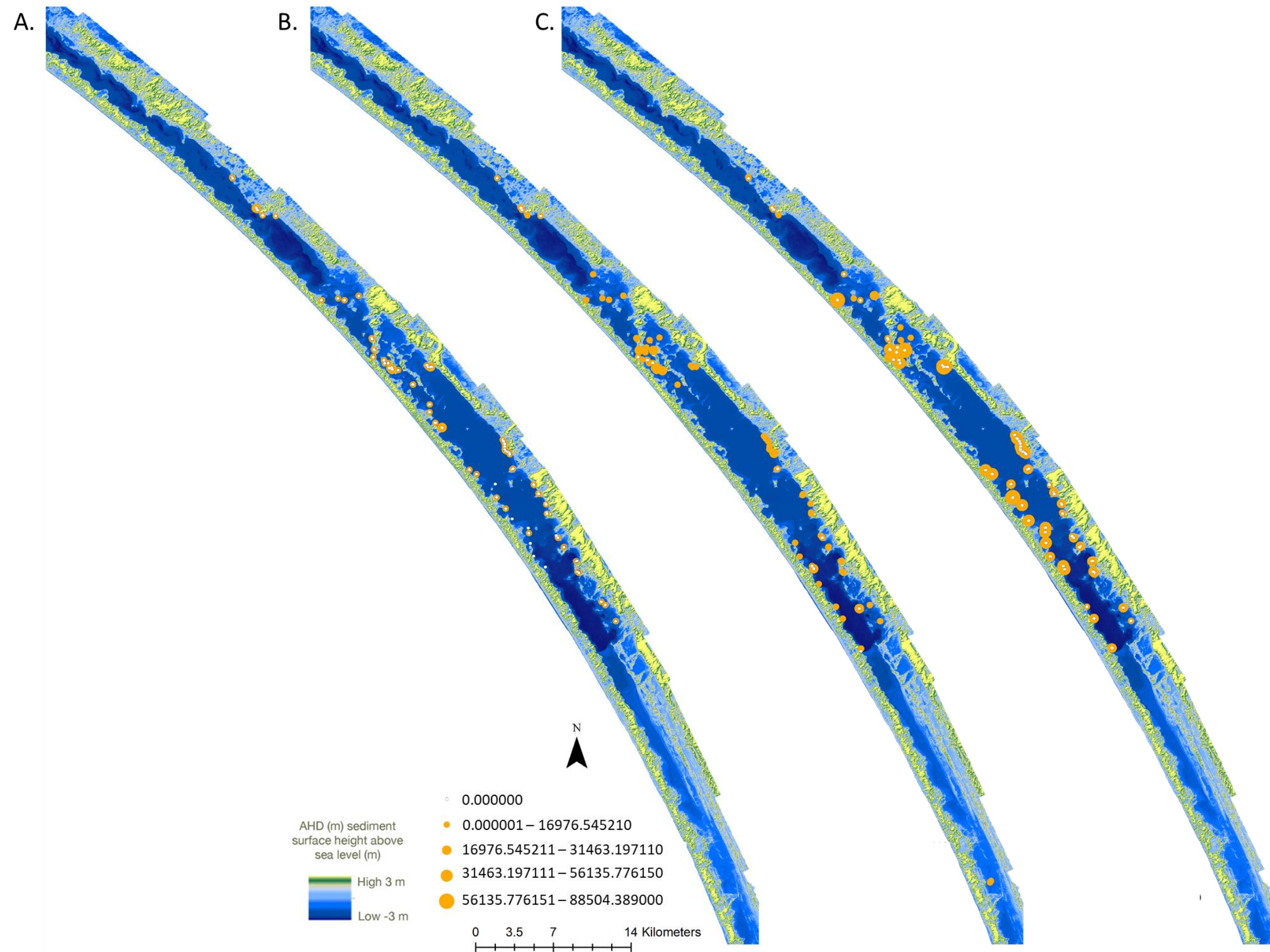


Figure 16. *Ruppia tuberosa* turion density (turions m^{-2}) based on core samples taken during baseline surveys across the Coorong (A) Reproductive 2020, (B) Aestivation 2021 and (C) Reproductive 2021 (for sampling dates see Table 1). The relative position and geographical orientation are depicted in Figure 3. Map shading represents digital elevation model (Hobbs et al. 2019).

It is also worth noting that there were living plants present even during the aestivation season which indicates the presence of a perennial *Ruppia* Community.

There were two types of *Ruppia* seeds observed: those that were classified as typical *Ruppia tuberosa* seeds; and those that were *Ruppia megacarpa* seeds. The seeds of *Ruppia megacarpa* are distinctive in being larger and beaked, we counted 4,062 *Ruppia megacarpa* seeds among the total of 38,182 *Ruppia* seeds (10.6%). The small slender seeds of *Althenia* were not able to be reliably separated from sediment due to size, shape and colour. As a result, the *Althenia* seed bank was not able to be scored directly and so the presence of flowering shoots were counted as an alternative. Flowering shoots were found in 114 of the 2,729 cores (4.2%), noting each *Althenia* flowering shoot contains numerous flowers potentially resulting in many seeds per flowering shoot.

There was a notable absence of seeds (Figure 17A) yet high turion density (Figure 17B) from the 2021 Reproductive survey. The highest production of both reproductive structures (seeds and turions) occurred around 70 km from the Murray Mouth, an area which is south of Parnka Point near Woods Well, which is consistent with field observations (Figure 14, Figure 16). These counts only consider areas which were able to be surveyed; therefore, where counts are low or absent it is also possible that no survey was undertaken, although see Figure 4 for survey extent. The highest seed density was observed at Lake Cantara (-36.332339°, 139.744002°), an isolated coastal hypersaline lagoon that does not experience algal blooms and completely dries most years thus must be obligately annual and growth is not impacted by algal blooms.

When categorised into 0.2 m elevation intervals, *Ruppia* spp. seed density was significantly higher above +0.6 m AHD in the 2021 Aestivation survey when compared to all other elevations across surveys, with +0.8 to +1.0 m AHD having an average of 23,731 seeds m⁻² and +0.6 to +0.8, 16,169 seeds m⁻² ($p < 0.001$) (Figure 16, Figure 18A). Seeds were present between -1.0 and +1.0 m AHD across all seasons, which is consistent with the overall depth range at which we found plant biomass (Figure 18). Turion density appeared to be in contrast to seed density in relation to elevation with the majority of turions found below +0.4 m AHD and in the 2021 Reproductive survey (Figure 16, Figure 18B). Significantly higher turion numbers were present at -1.0 – -0.8 m AHD (23,731 turions m⁻²) and declined with increasing elevation (Figure 18B) ($p < 0.001$). This may suggest that turion production is preferential where water coverage is less variable, and/or at depths where flowers are not able to reach surface for pollination.

Comparison of the long-term trends in seed and turion density from both TLM and HCHB monitoring programs (Figure 19; Figure 20) indicate that the timing of the 2021 Aestivation survey in autumn may be more effective at detecting seeds. The 2020 and 2021 HCHB Reproductive surveys, which were based on plant lifecycle rather than a set time of year, appeared to observe higher numbers of both seeds and turions overall. The HCHB surveys were conducted over a larger spatial scale, across a wider range of depths and sections of the system, which would potentially detect pockets high plant growth and seed deposition undetected by TLM monitoring.

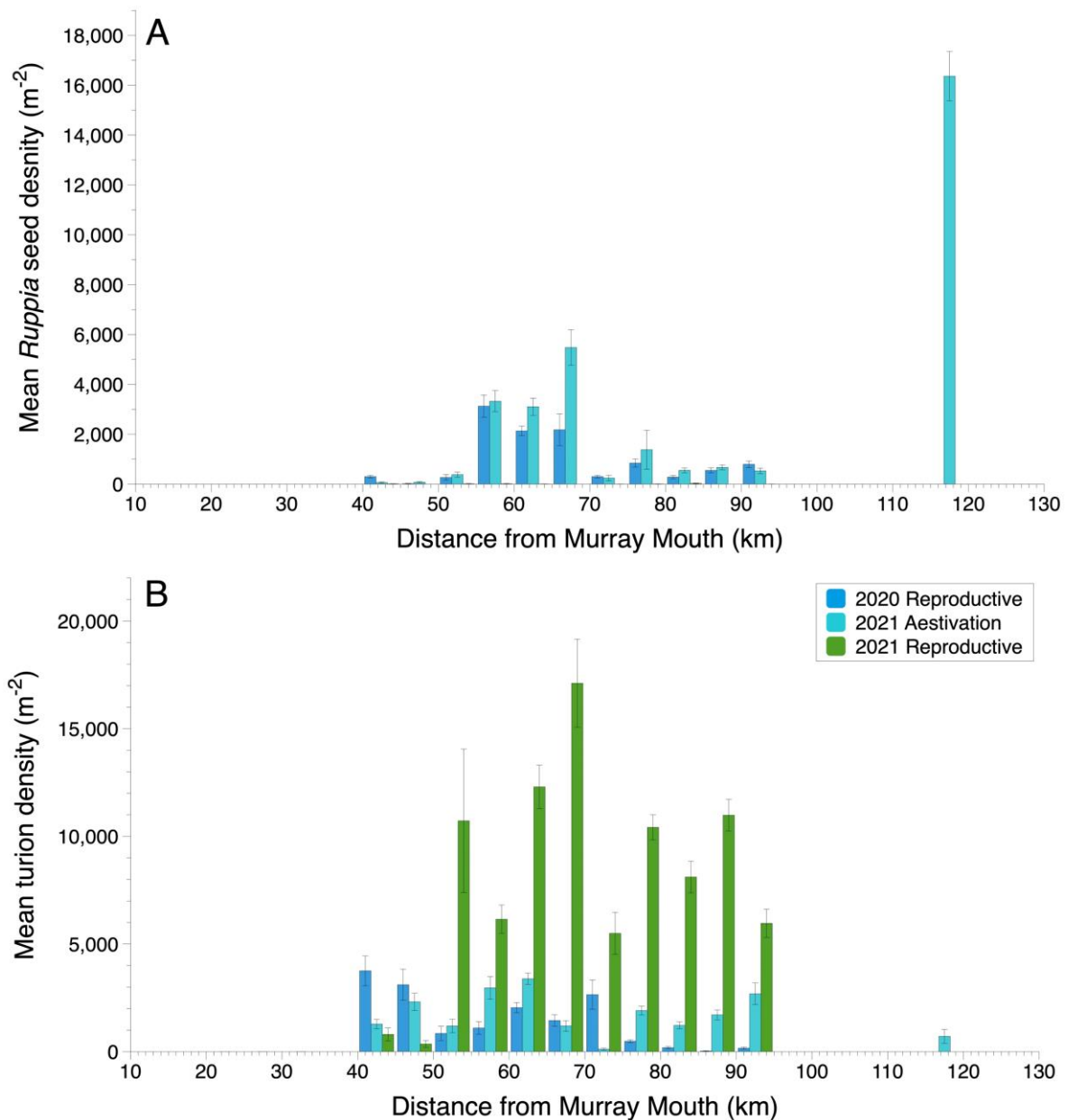


Figure 17. Mean \pm s.e of (A) *Ruppia* spp. seed density and (B) *Ruppia tuberosa* turion density measured against location distance from the Murray Mouth (MM) across three survey seasons (Table 1). For reference: Nooneameena – 40 km from MM, Parnka Point – 60 km from MM, Woods Well – 77 km from MM, Salt Creek – 93 km from MM.

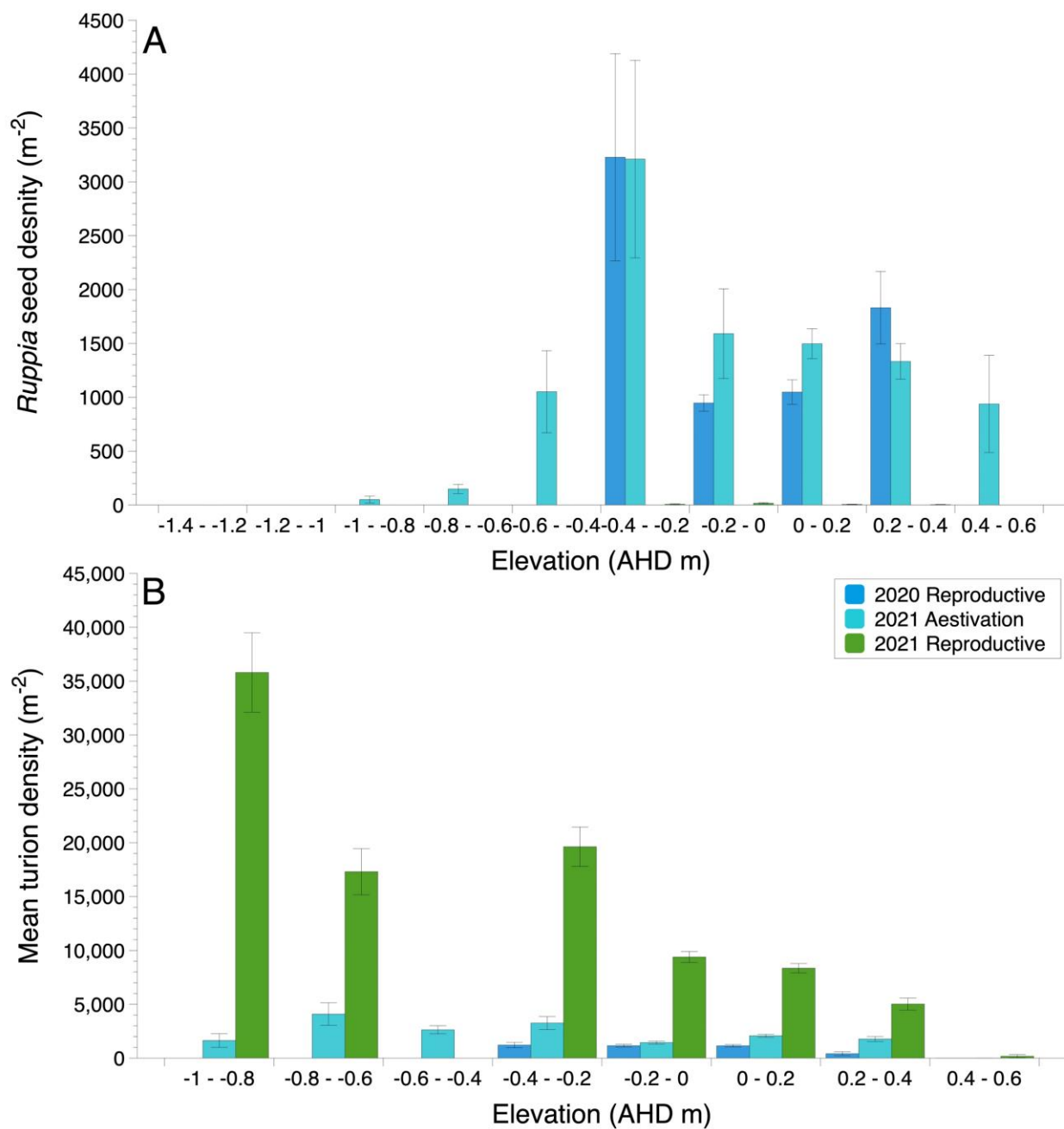


Figure 18. Mean \pm s.e of (A) *Ruppia* spp. seed density and (B) turion density measured against digital elevation modelling in AHD m, across three survey seasons (Table 1).

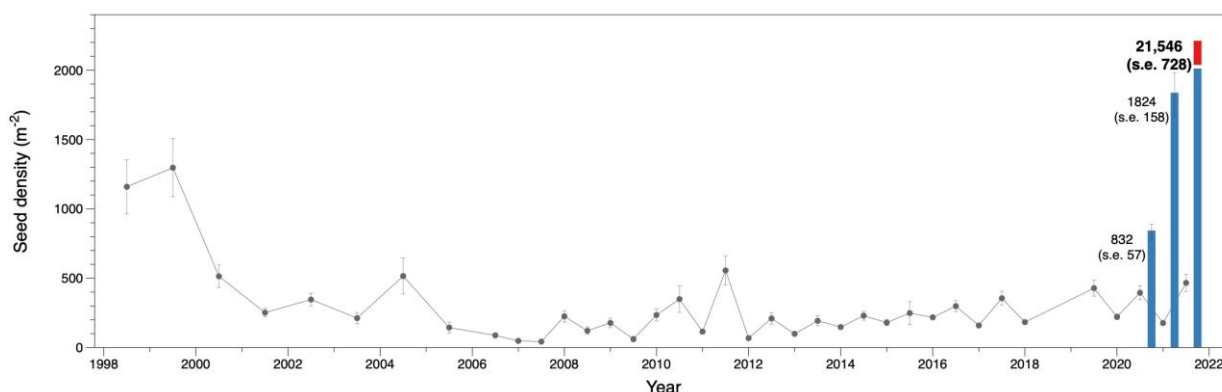


Figure 19. Aquatic macrophyte seed density measured by The Living Murray (TLM; Paton et al. (2021)) monitoring program as an average of all sites sampled in that year (\pm s.e.) (grey line) from 2008-2021 for summer and winter sampling, January and July each year, and Healthy Coorong, Healthy Basin Trials and Investigations Component 2 (columns) average of all sites sampled in that year (\pm s.e.) 2020 Reproductive, 2021 Aestivation and 2021 Reproductive. Note that Summer TLM sampling commenced in 2011 (note that the sampling methodology differs between summer and winter for the TLM surveys Paton et al. (2017b) and HCHB sampling only includes Spring 2020 and Autumn, Summer 2021 (Table 1).

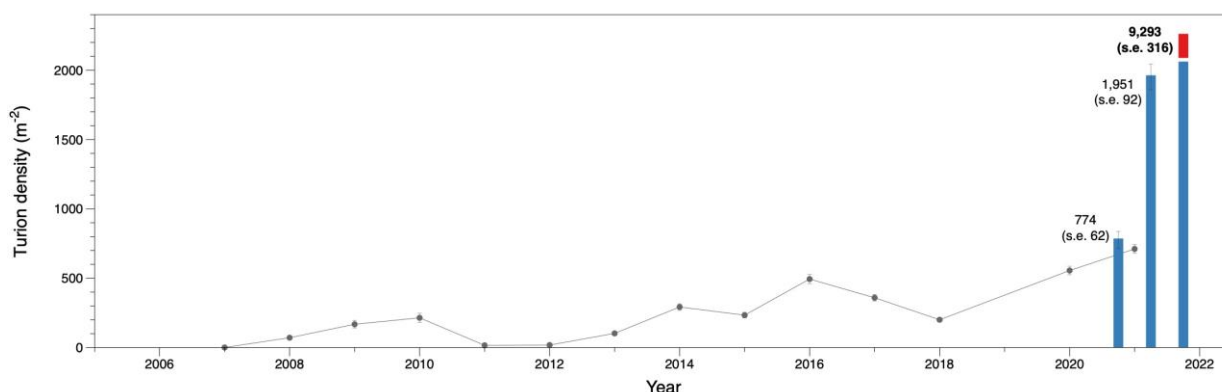


Figure 20. Aquatic macrophyte turion density measured by: The Living Murray (TLM; Paton et al. (2021)) monitoring program as an average of all sites sampled in that year (\pm s.e.) (grey line) from 2008-2021 during the turion aestivation period—summer sampling, January each year, and Healthy Coorong, Healthy Basin Trials and Investigations Component 2 (columns) average of all sites sampled in that year (\pm s.e.) 2020 Reproductive, 2021 Aestivation and 2021 Reproductive. Note that Summer TLM sampling commenced in 2011 and HCHB sampling only includes Spring 2020 and Autumn, Summer 2021 (Table 1).

3.3 Distribution and biomass of filamentous algae

The majority of algal biomass coincided with the 2020 and 2021 Reproductive surveys, although there was significantly higher algal biomass present in the 2021 Reproductive survey with mean algal biomass of 218.3 g dry weight m⁻² (Figure 21A, Figure 22). The 2020 Reproductive survey had a mean of 28.5 g dry weight m⁻², with 4.9 g and 46.3 g dry weight m⁻² in 2021 Aestivation and 2021 Vegetative periods respectively. All survey seasons varied showing significant variation in algal biomass ($p < 0.001$), increasing towards summer (i.e. lowest biomass in 2021 Aestivation, highest in 2020 and 2021 Reproductive surveys) (Figure 21A). Whilst not directly comparable to the quantitative surveys, algal biomass was low during the 2021 Vegetative survey (Figure 22).

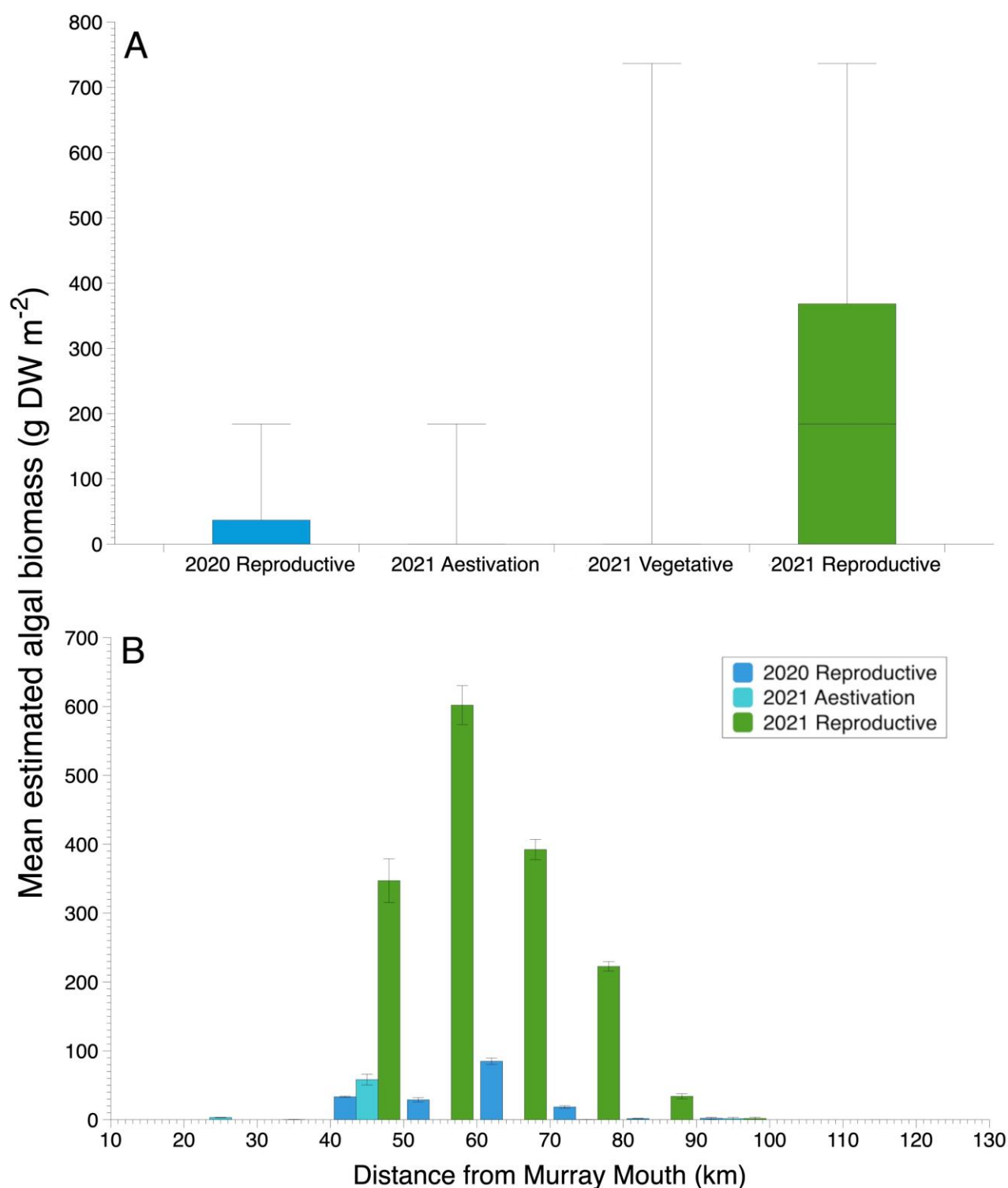


Figure 21. (A) Mean estimated filamentous algae biomass (g DW m⁻²) across survey seasons (\pm s.e.) and (B) mean estimated algal biomass (\pm s.e.) against distance from the Murray Mouth (MM) measured against location distance from the Murray Mouth across all survey seasons. For reference: Noonameena – 40 km from MM, Parnka Point – 60 km from MM, Woods Well – 77 km from MM, Salt Creek – 93 km from MM.

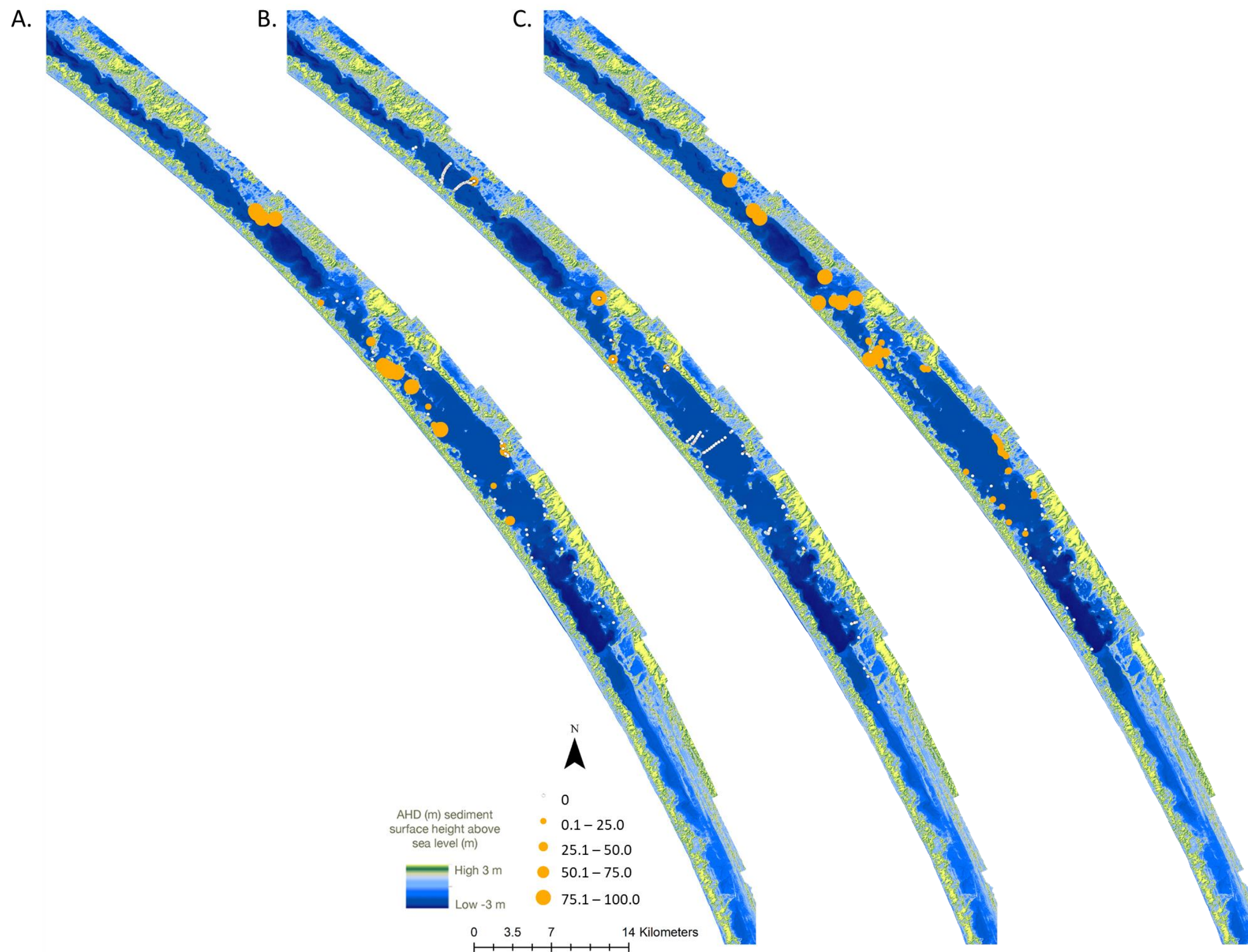


Figure 22. Filamentous algae cover (%) based on samples taken during baseline surveys across the Coorong (A) Reproductive 2020, (B) Vegetative 2021 and (C) Reproductive 2021 (for sampling dates see Table 1). The relative position and geographical orientation are depicted in Figure 3. Map shading represents digital elevation model (Hobbs et al. 2019).

Estimated algal biomass was highest and most widespread in the 2021 Reproductive survey, ranging from Noonameena (40km from MM) to Salt Creek (90 km to MM) (Figure 22). The highest estimated mean algal biomass was around Parnka Point (60 km to MM) at 600.6 g dry weight m^{-2} (Figure 21B, Figure 22). The highest mean biomass in the 2020 Reproductive period was also at Parnka Point, but considerably lower at 84.7 g dry weight m^{-2} (Figure 21B). Algal biomass was most consistently observed across all surveys at Noonameena, although biomass was not as high as that observed at Parnka Point, potentially due to differences in environmental factors such as salinity or water flow (Figure 22). Whilst algal biomass was low or absent at most sites for the 2021 Vegetative survey, >50% cover was observed at Magrath Flat and 21–40% cover at Noonameena and Parnka Point (Figure 22).

Figure 23 compares aquatic macrophyte biomass across the 2020 and 2021 Reproductive surveys when most algal biomass was present. In the 2020 Reproductive survey, aquatic macrophyte biomass was highest (92.6 g DW m^{-2}) at 3.7 g estimated algal dry weight m^{-2} , while in 2021 macrophyte biomass was highest at 36.8 g estimated algal dry weight m^{-2} . The full results of the Tukey's HSD post-hoc test of aquatic macrophyte biomass across the gradient of estimated algal biomass can be found in Table 6. The differences between levels of estimated algal biomass were variable but indicated the trends shown in Figure 23, with significant differences between mid-range algal biomass (36.8–368.2 g DW m^{-2}) and absent/low (0–3.2 g DW m^{-2}) and high (736.5 g DW m^{-2}) (Table 6).

The results indicate that where high algal biomass is present there was a reduction in aquatic macrophyte biomass, although the results also suggest that at low algal biomass there is decreased macrophyte biomass, i.e. below 36.8 g dry weight algal biomass m^{-2} . This anomaly might suggest that macrophyte biomass needs to be present for filamentous algal attachment; therefore, limiting the ability for filamentous algae to stay anchored in position. The decrease of aquatic macrophyte biomass at greater than 36.8 g m^{-2} estimated dry weight biomass could, in turn be the algae limiting macrophyte growth, although this would require further studies to investigate.

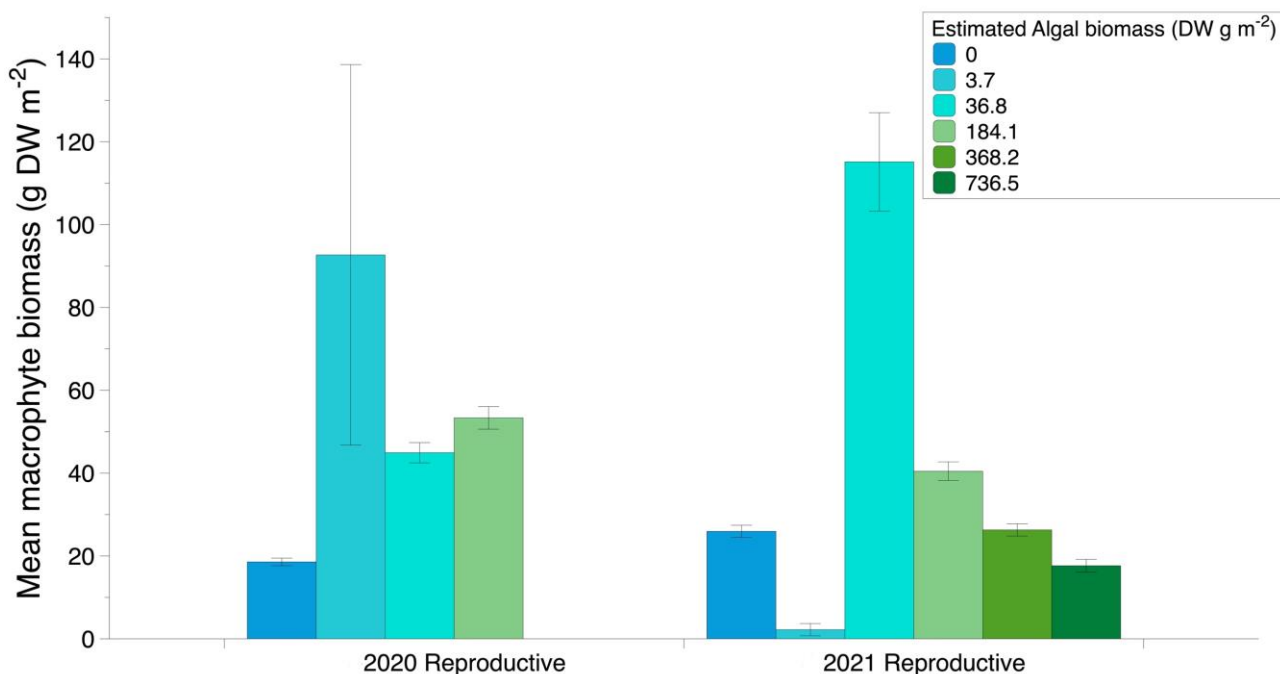


Figure 23. Mean aquatic macrophyte biomass (\pm s.e.) across the two reproductive seasons measured against the estimated quantity of algal biomass, both measured in grams of dry weight biomass m^{-2} .

Table 6. Tukey's Honest Significant Difference post-hoc test results for aquatic macrophyte biomass m⁻² across the gradient of estimated algal biomass m⁻². Values in bold are significant.

ESTIMATED MEAN ALGAL BIOMASS (G DW M ⁻²)		MEAN DIFFERENCE IN AQUATIC MACROPHYTE BIOMASS (G DW M ⁻²)	STD. ERROR	P VALUE
0	3.7	-0.02190	0.51122	1.000
	36.8	-1.57637*	0.10223	<0.001
	184.1	-1.17878*	0.08221	<0.001
	368.2	-0.93939*	0.10737	<0.001
	736.5	-0.37006	0.13670	0.074
3.7	0	0.02190	0.51122	1.000
	36.8	-1.55447*	0.51848	0.033
	184.1	-1.15688	0.51490	0.217
	368.2	-0.91749	0.51952	0.488
	736.5	-0.34816	0.52636	0.986
36.8	0	1.57637*	0.10223	<0.001
	3.7	1.55447*	0.51848	0.033
	184.1	0.39759*	0.11928	0.011
	368.2	0.63698*	0.13783	<0.001
	736.5	1.20631*	0.16173	<0.001
184.1	0	1.17878*	0.08221	<0.001
	3.7	1.15688	0.51490	0.217
	36.8	-0.39759*	0.11928	0.011
	368.2	0.23939	0.12371	0.381
	736.5	0.80871*	0.14988	<0.001
368.2	0	0.93939*	0.10737	<0.001
	3.7	0.91749	0.51952	0.488
	36.8	-0.63698*	0.13783	<0.001
	184.1	-0.23939	0.12371	0.381
	736.5	0.56932*	0.16503	0.008
736.5	0	0.37006	0.13670	0.074
	3.7	0.34816	0.52636	0.986
	36.8	-1.20631*	0.16173	<0.001
	184.1	-0.80871*	0.14988	<0.001
	368.2	-0.56932*	0.16503	0.008

* The mean difference is significant at the 0.05 level.

3.4 Abiotic environmental factors and their influence on aquatic macrophytes and filamentous algae

3.4.1 Trends in environmental data

Reviewing the 5-year period prior to the end of sampling in December 2021 the overall trends in major environmental parameters included an increase in total dissolved solids (TDS, as an indicator of salinity) in the Coorong South Lagoon ($R^2=0.34$, Woods Well) and no change for Coorong North Lagoon station at Robs Point (Figure 24). The mean maximum temperatures were slightly lower in 2020 and 2021 (Figure 25) and a small trend to lower water depths (Figure 26) although neither was significant.

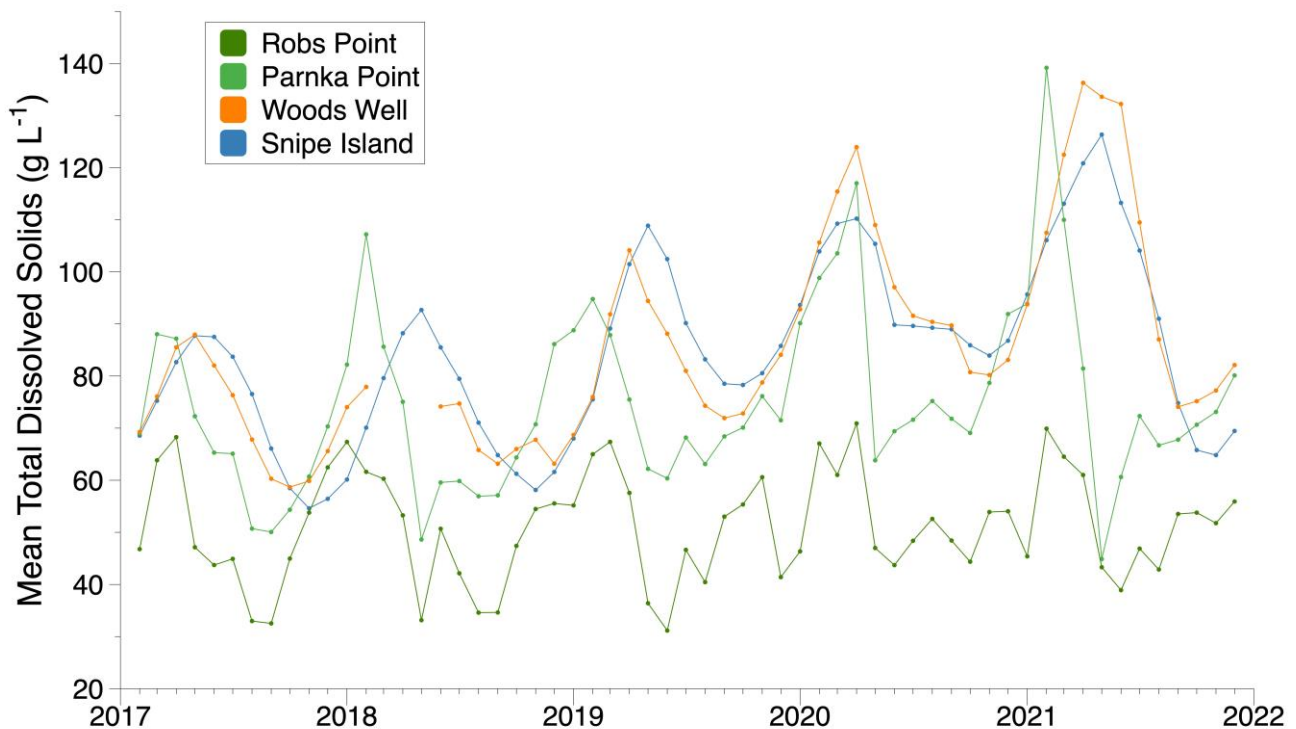


Figure 24. Mean monthly total dissolved solids measured in (g L⁻¹) for four sites across the Coorong, Robs Point, Parnka Point, Woods Well and Snipe Island from 1 January 2017 to 4 December 2021. Data obtained from www.water.data.sa.gov.au on 3 April 2022.

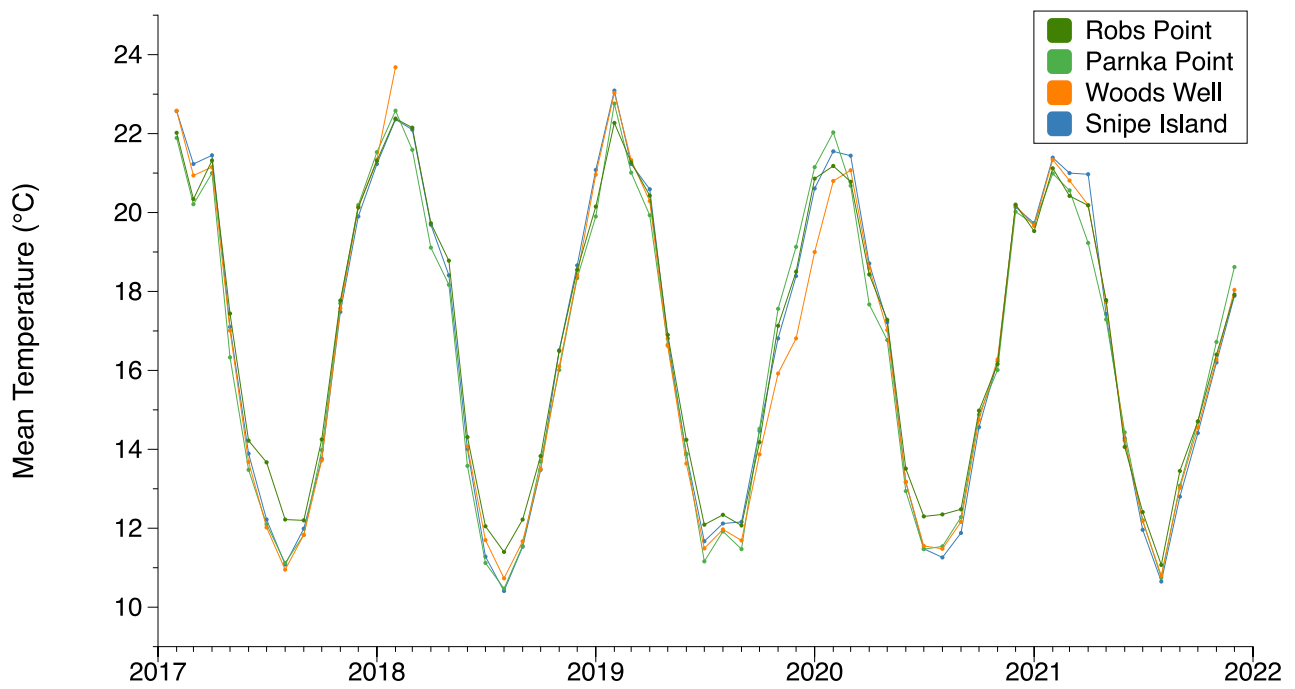


Figure 25. Mean monthly temperature (°C) for four sites across the Coorong, Robs Point, Parnka Point, Woods Well and Snipe Island from 1 January 2017 to 4 December 2021. Data obtained from www.water.data.sa.gov.au on 3 April 2022.

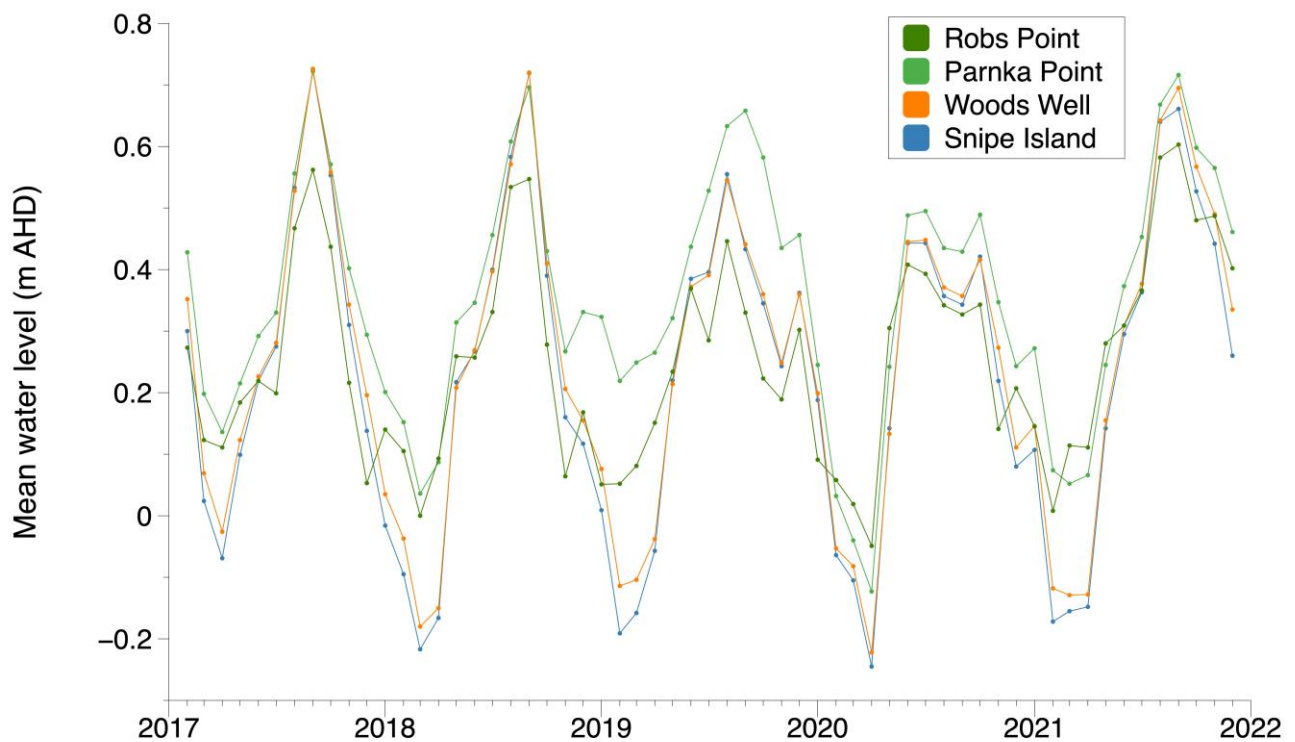


Figure 26. Mean monthly water level (m AHD) for four sites across the Coorong, Robs Point, Parnka Point, Woods Well and Snipe Island from 1 January 2017 to 4 December 2021. Data obtained from www.water.data.sa.gov.au on 3 April 2022.

3.4.2 Water salinity and depth (field measured)

Overall, the salinity recorded across all field sites and season ranged from 9 – 137 parts per thousand (PPT), and salinity varied significantly across all seasons ($p < 0.001$). The highest mean salinity was recorded in the 2021 Aestivation survey at 98.3 PPT with a range of 24 – 131 PPT (Figure 27A) as would be expected given at this time of year water levels are low and evaporation rates high. The lowest salinity was recorded in the 2021 Vegetative survey with a mean of 64.4 PPT and across all sites salinities ranged between 9 – 84 PPT. Salinities also varied significantly across regions ($p < 0.001$) with the highest mean salinity being found in the central region (88.9 PPT), followed by the south at 82.1 PPT and was lowest in the north with a mean salinity of 56.2 PPT (Figure 27B).

Mean water depth (i.e. as measured in the field) also varied significantly between seasons ($p < 0.001$), and was lowest in the 2021 Aestivation survey with an average of 0.06 m, and highest in the 2021 Vegetative survey which was conducted as presence/absence due to high water levels at an average of 0.42 m (Figure 28). The depth range across all surveys ranged from dry (no water at site) through to 1.7 m deep (at which depth the survey was conducted as presence/absence from a vessel). Depths in the 2020 and 2021 Reproductive periods were not significantly different ($p > 0.05$) with mean water depths of 0.366 m and 0.313 m respectively (Figure 28).

Aquatic macrophyte biomass m^{-2} varied with salinity; however, it appeared to be able to survive within a range of 40 – 140 PPT, in the 2020 and 2021 Reproductive seasons. The majority of biomass was found between 40 – 80 PPT and 50 – 140 for the 2020 and 2021 Reproductive seasons respectively, although biomass was reduced at salinities above 90 ppt. The highest mean biomass m^{-2} was observed during the 2020 Reproductive survey between 50 – 60 PPT at 83.8 g dry weight m^{-2} (Figure 29A).

Algal biomass was present across a salinity range of 20 – 140 PPT but was more common between 40 and 90 PPT for most sampling periods. In the 2021 Reproductive survey large amounts of filamentous algal biomass were observed at numerous sites where salinity exceeded 90 PPT, specifically around the Parnka Point region (Figure 29B).

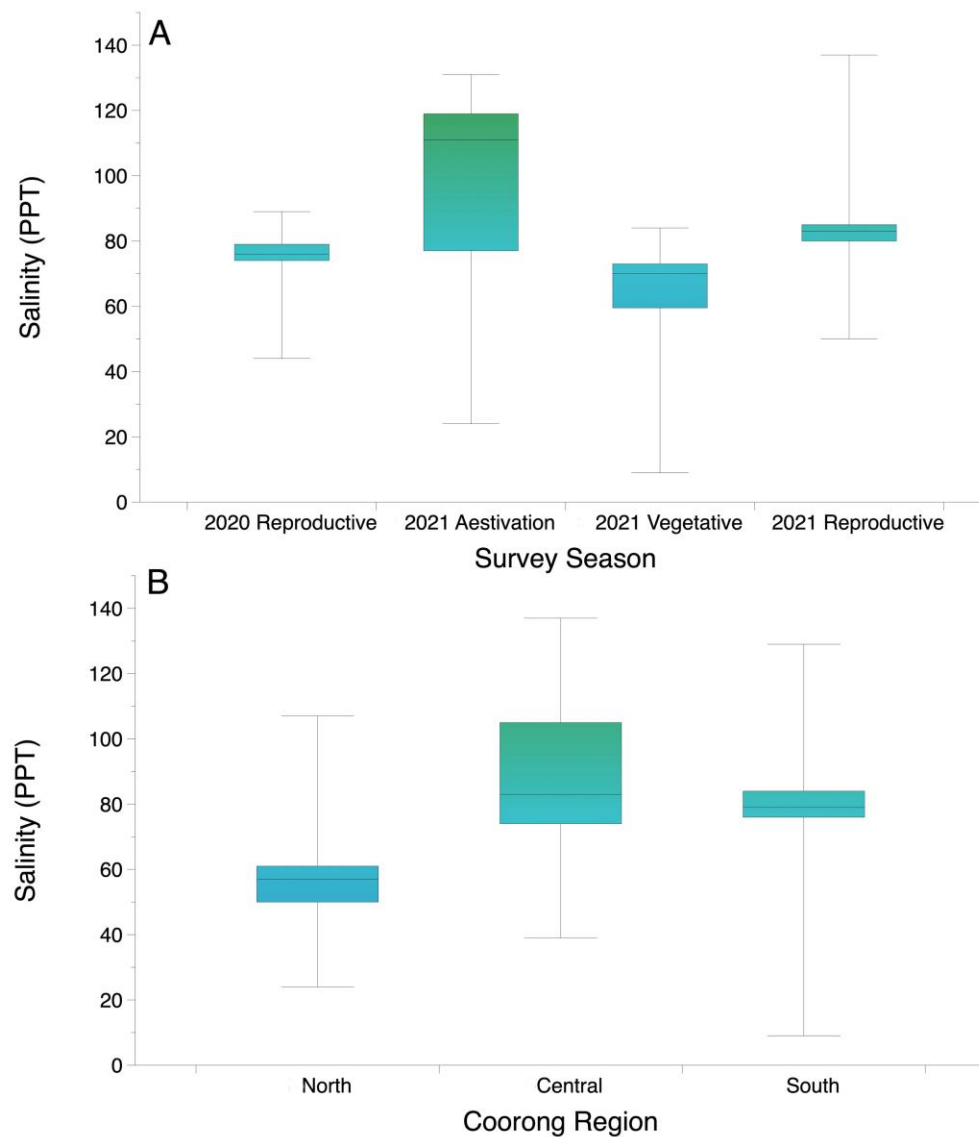


Figure 27. Box and Whisker plots representing the Inner-Quartile-Range (IQR; lower value 25%, upper value 75%), mean, and 95% confidence interval (error bars) for mean salinity gradients across (A) all survey seasons and (B) three regions, north (north of McGrath Flat), central (Parnka Point) and south (south of Parnka Point).

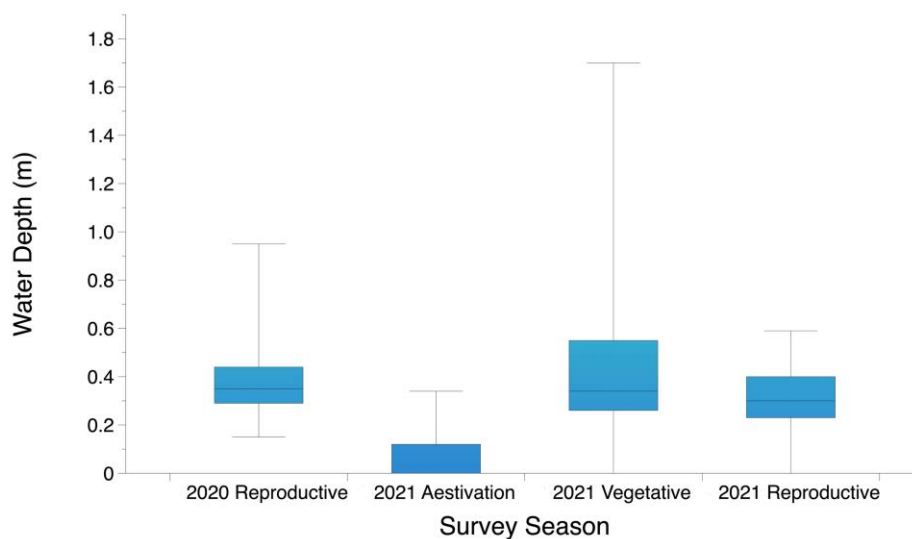


Figure 28. Box and Whisker plots representing the Inner-Quartile-Range (IQR; lower value 25%, upper value 75%), mean, and 95% confidence interval (error bars) for mean field measured water depths across all survey seasons.

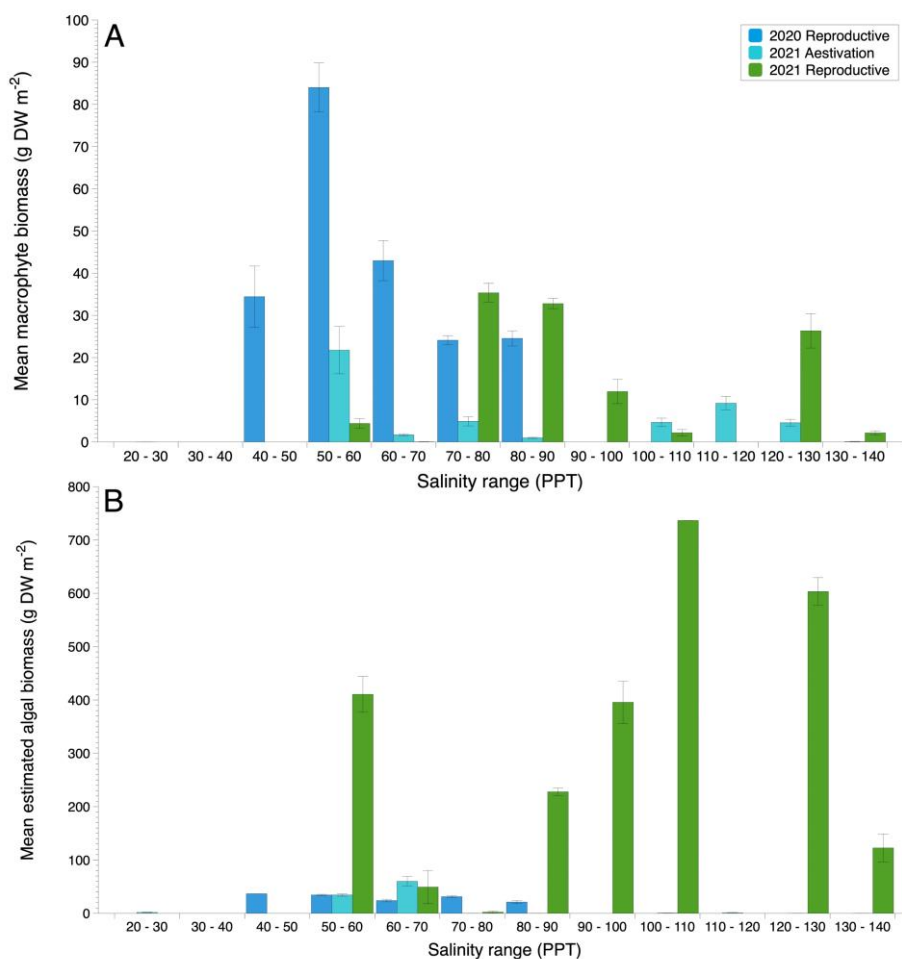


Figure 29. Mean salinity measured across all survey seasons in relation to (A) average aquatic macrophyte biomass in grams of dry weight m⁻² (\pm s.e.), and (B) estimated mean algal biomass in grams of dry weight m⁻² (\pm s.e.).

3.4.3 Sediment condition

Sediment condition varied across the system as assessed using the Rapid Assessment Protocol (RAP) (Hallett et al. 2019). We considered overall Sediment Condition score (Figure 30A) and each component data set contributing to the RAP, which considers sediment texture (Figure 30B), sediment colour (Figure 30C) and sediment odour (Figure 30D). Although the differences between Sediment Scores were not significant ($p>0.05$), there appears to be a trend that aquatic macrophyte biomass increases with Sediment Condition. There was no significant correlation between sediment texture and aquatic macrophyte biomass in this study ($p>0.05$); however, Figure 30B shows a general trend of increasing biomass as sediment texture becomes coarser, i.e. an increase in biomass from a score of 1 to 5. This trend also appeared to be present in the results for sediment colour (Figure 30C) and odour (Figure 30D). These trends were supported by field observations where aquatic macrophytes appeared less likely to be present in cores where sediment did not appear to be as consolidated or where very fine sediment was present.

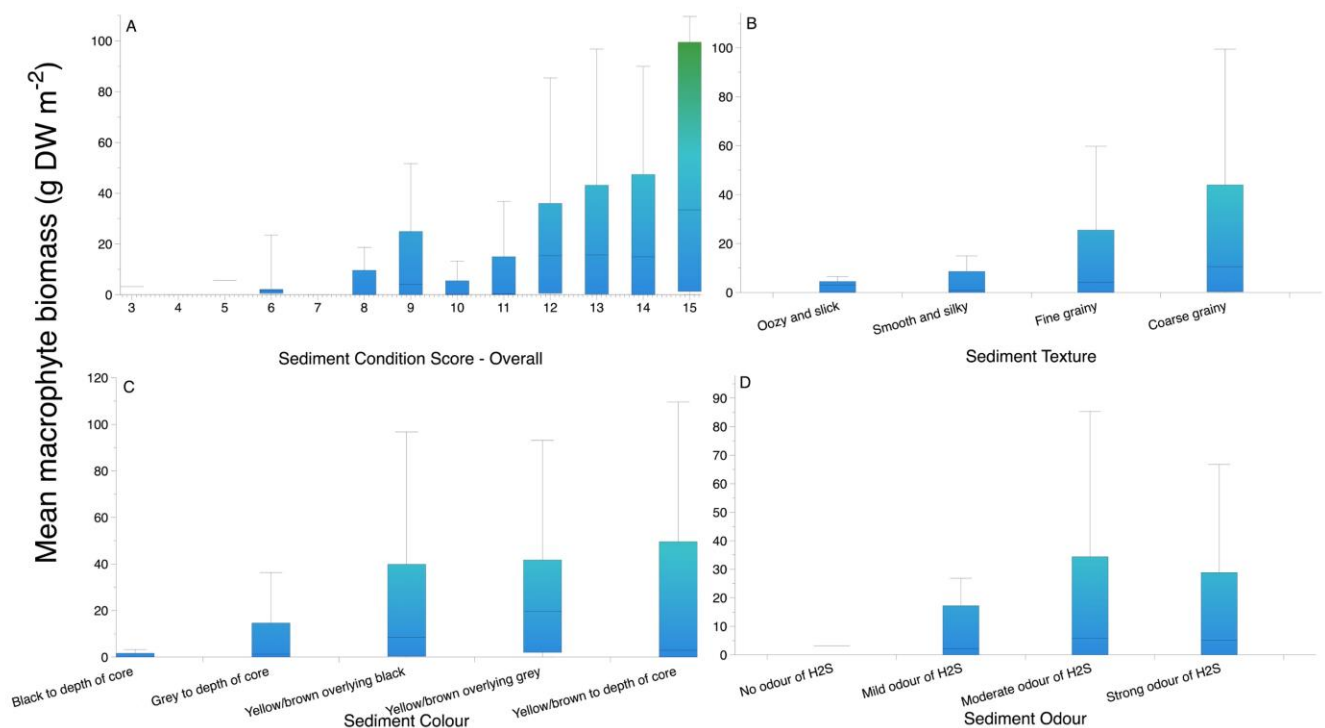


Figure 30. Box and Whisker plots representing the Inner-Quartile-Range (IQR; lower value 25%, upper value 75%), mean, and 95% confidence interval (error bars) for aquatic macrophyte biomass m⁻² and: (A) Sediment Condition Score as defined in Hallett (2019), consists of the sum of categories in panels B, C and D and provides a rapid assessment of overall sediment condition. (B) Sediment texture categories/descriptions as defined by Hallett et al. (2019). (C) Sediment colour descriptions as defined by Hallett et al. (2019). (D) Sediment textures as defined by Hallett et al. (2019).

The DistLM analysis of aquatic macrophyte biomass indicated that all variables apart from depth ($p>0.05$) influence the distribution of aquatic macrophytes across all sample locations ($p<0.0001$). Distance from the Murray Mouth and salinity explained 17.46% of the variance in biomass or 9.06% and 8.40% respectively (Table 7, Figure 31). Algal biomass accounted for 3.52% of variation in macrophyte biomass, while elevation accounted for 2.5%. Overall, the variables measured and which were significant in the model accounted for 23.48 % of variation in the data leaving 76.52% of variance unexplained. This is to be expected given the limited environmental list of variables which were measured in the field, the limited temporal scale and the high variability of the aquatic macrophyte data (Table 7).

Table 7. DistLM sequential test for relationship between aquatic macrophyte biomass and environmental factors. DFMM = linear calculated Distance from Murray Mouth; salinity = field survey measurements at time of sampling; depth = height of water above sediment at time of sampling; elevation = AHD based on digital elevation model compared to sea-level.

VARIABLE	ADJUSTED R ²	PSEUDO-F	P	PROP. OF VARIANCE EXPLAINED (%)
DFMM	0.08978	29.305	0.0001	9.06
Salinity	0.083693	111.72	0.0001	8.4
Depth	0.090148	2.7112	0.0567	0.005
Algal Biomass per m ²	0.034943	154.34	0.0001	3.52
Elevation	0.059725	112.59	0.0001	2.50

Figure 31 shows the dbDRA plot of data across the three surveys where biomass measurements were taken. Variability within the two reproductive seasons were grouped and variation was mostly influenced by distance from Murray Mouth (DFMM), while the separation between the 2021 Aestivation survey and the 2020 and 2021 Reproductive surveys appears to be driven by DFMM, algal biomass, elevation and salinity.

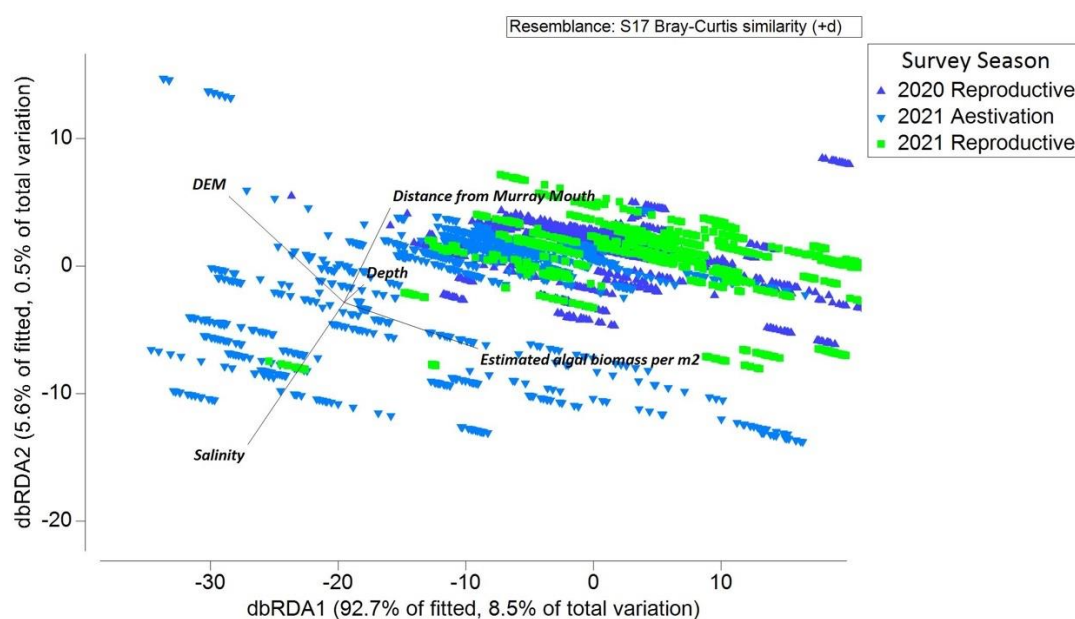


Figure 31. dbRDA ordination as predicted by DistLM model of aquatic macrophyte biomass and shoot counts m⁻² and relationships with the environmental factors across 2020 Reproductive, 2021 Aestivation and 2021 Reproductive seasons. DFMM = linear calculated Distance from Murray Mouth; salinity = field survey measurements at time of sampling; depth = height of water above sediment at time of sampling; elevation = AHD based on digital elevation model compared to sea-level.

The environmental variables included in the model, which looked at *Ruppia* spp. seed counts, only accounted for 6.8% of total variation in the data, leaving 93.2% unexplained (Table 8). Algal biomass accounted for the most variation in the model; however, was only explained 4.4% of the variance. Distance from the Murray Mouth explained 1.8% of variation in the model (Table 8). Again, in the model for seed counts, depth was not significant ($p > 0.05$) and only explained 0.004% of the variation in the data (Table 8).

Table 8. DistLM sequential test for relationship between aquatic macrophyte seed counts and environmental factors. DFMM = linear calculated Distance from Murray Mouth; salinity = field survey measurements at time of sampling; depth = height of water above sediment at time of sampling; elevation = AHD based on digital elevation model compared to sea-level.

VARIABLE	ADJUSTED R ²	PSEUDO-F	P	PROP. OF VARIANCE EXPLAINED (%)
DFMM	0.061199	81.121	0.0001	1.8
Salinity	0.066146	23.423	0.0001	0.05
Depth	0.071667	1.9702	0.1456	0.004
Algal Biomass per m ²	0.043434	193.29	0.0001	4.4
Elevation	0.071454	25.193	0.0001	0.5

The dbRDA plot of seed counts shows that some overlap occurred between seasons; however, 2021 Reproductive was influenced by estimated algal biomass and Distance from the Murray Mouth (DFMM), while the vertical patterns in the data which appear random across seasons appears to be driven by depth, elevation (DEM) and salinity, although the variability in the data makes it difficult to interpret strong causes of variation in the model (Figure 32).

Algal biomass and elevation explained 13.11% of the variation in turions counts (5.91 and 7.2% respectively), which is also supported by the data from Figure 18. Distance from Murray Mouth and salinity were also significant ($p < 0.0001$), yet depth was not significant ($p > 0.05$) (Figure 30, Table 9). There did not appear to be much separation between survey seasons in the dbRDA plot, apart from the 2021 Reproductive season potentially being more influenced by DFMM and algal biomass (Figure 33).

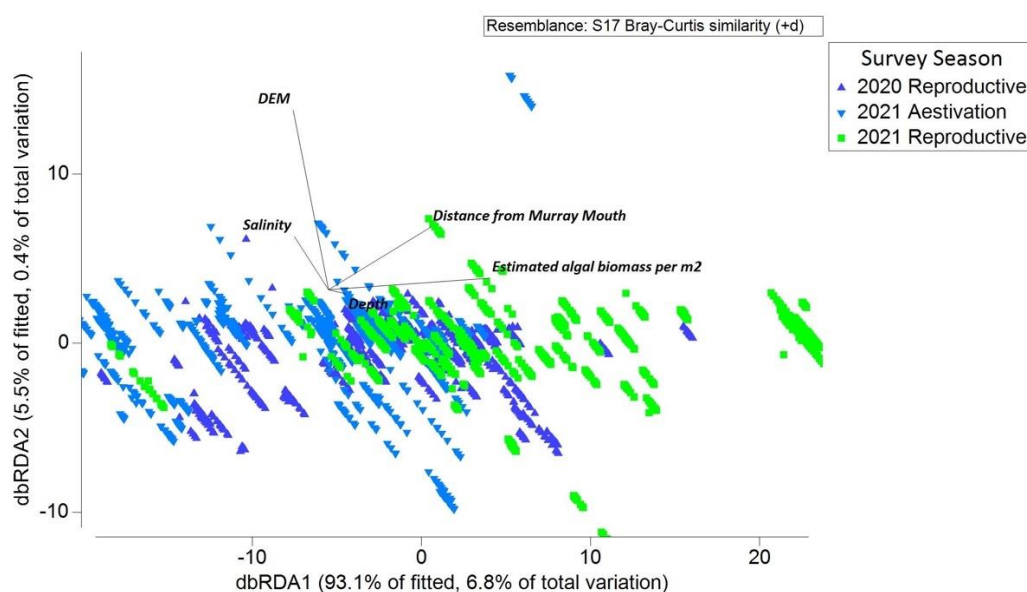


Figure 32. dbRDA ordination as predicted by DistLM model of seed counts m⁻² and relationships with the environmental factors: depth, elevation (DEM), distance from Murray Mouth, estimated algal biomass and salinity across 2020 Reproductive, 2021 Aestivation and 2021 Reproductive seasons.

Distance from the Murray Mouth accounted for 7.5% of variation in the model in which we considered variation in estimated algal biomass, salinity, depth and elevation also all were significant in explaining variation in the model; however, only explained 2.4% of variation overall (Table 10). In Figure 34 there appeared to be some separation between the higher (>184.1) and lower levels (<184.1) of estimated algal biomass which was driven by salinity and DFMM; however, salinity only accounted for 0.48% of variation in the model (Figure 34).

Table 9. DistLM sequential test for relationship between aquatic macrophyte turion counts and environmental factors. DFMM = linear calculated Distance from Murray Mouth; salinity = field survey measurements at time of sampling; depth = height of water above sediment at time of sampling; elevation = AHD based on digital elevation model compared to sea-level.

VARIABLE	ADJUSTED R ²	PSEUDO-F	P	PROP. OF VARIANCE EXPLAINED (%)
DFMM	0.080245	42.452	0.0001	0.92
Salinity	0.08916	42.42	0.0001	0.91
Depth	0.089492	2.5414	0.0791	0
Algal Biomass per m ²	0.058889	266	0.0001	5.91
Elevation	0.71239	57.297	0.0001	7.2

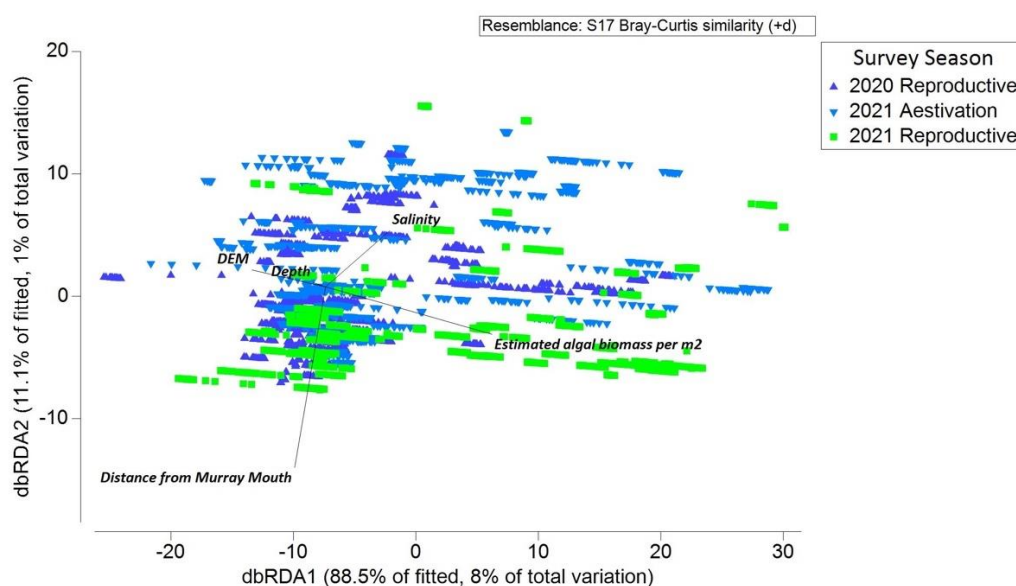


Figure 33. dbRDA ordination as predicted by DistLM model of turion counts m⁻² and relationships with the environmental factors: depth, elevation (DEM), distance from Murray Mouth, estimated algal biomass and salinity across 2020 Reproductive, 2021 Aestivation and 2021 Reproductive seasons.

Table 10. DistLM sequential test for relationship between estimated algal biomass and environmental factors. DFMM = linear calculated Distance from Murray Mouth; salinity = field survey measurements at time of sampling; depth = height of water above sediment at time of sampling; elevation = AHD based on digital elevation model compared to sea-level.

VARIABLE	ADJUSTED R ²	PSEUDO-F	P	PROP. OF VARIANCE EXPLAINED (%)
DFMM	0.075446	346.58	0.0001	7.5
Salinity	0.13317	23.192	0.0001	0.48
Depth	0.12862	73.367	0.0001	1.51
Elevation	0.13704	20.001	0.0001	0.41

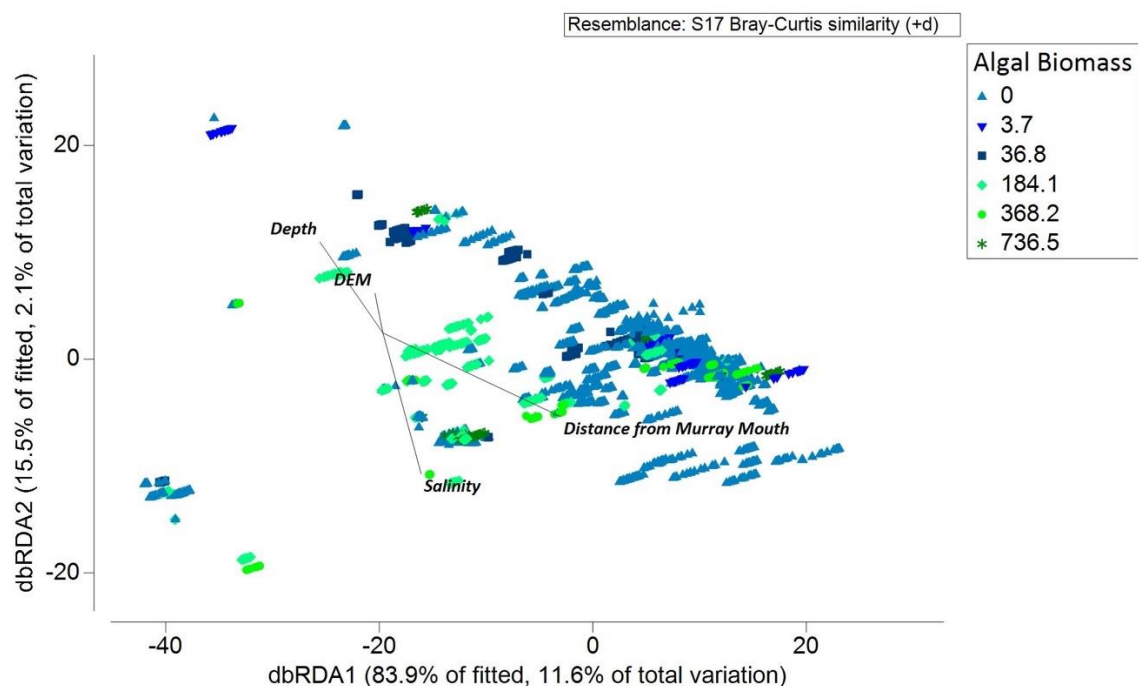


Figure 34. dbRDA ordination as predicted by DistLM model of estimated algal biomass m^{-2} (g dry weight/ m^2) and relationships with the environmental factors: depth, elevation (DEM), distance from Murray Mouth and salinity across 2020 Reproductive, 2021 Aestivation and 2021 Reproductive seasons.

The depth range for the aquatic macrophyte community where the majority of plants were found, was determined to be ± 0.2 m AHD (Figure 35). When compared to the months where water levels are required to persist through this depth range to complete the reproductive life cycle (i.e. months of September to December), highlighted as the shaded bars in Figure 35, the two years of this study were observed to have relatively high water levels during the reproductive period.

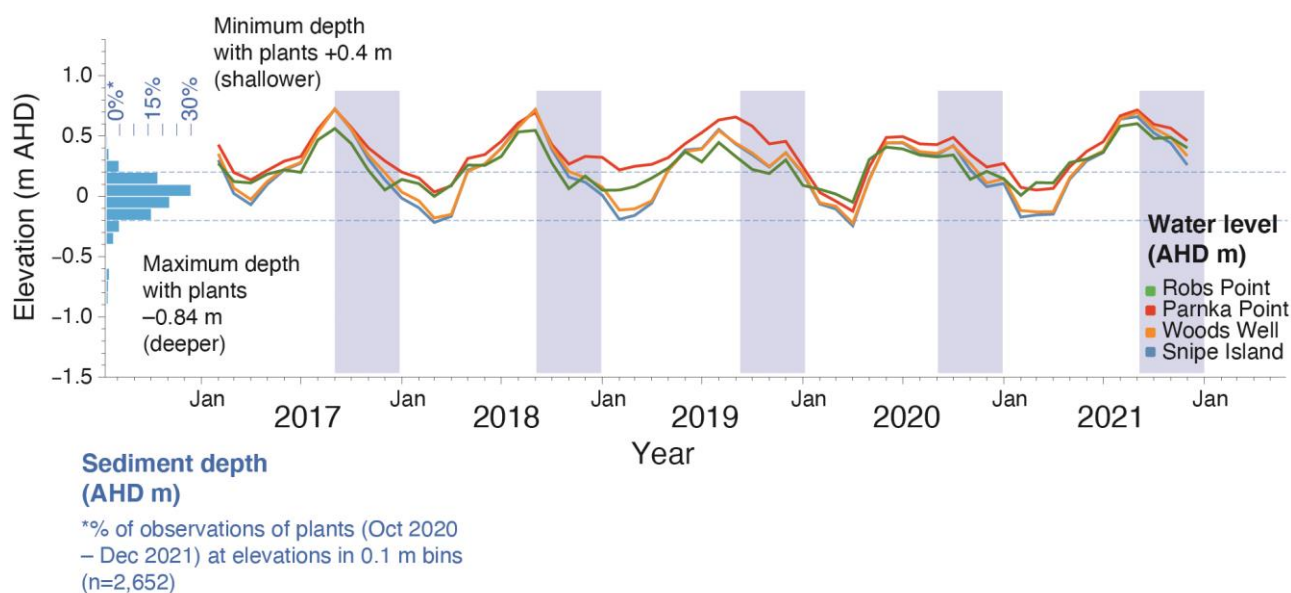


Figure 35. Mean monthly water level (m AHD) for 4 sites across the Coorong, Robs Point, Parnka Point, Woods Well and Snipe Island from 1 January 2017 to 4 December 2021. Shaded areas the months that the *Ruppia* Community are completing their lifecycle to produce viable seeds and turions, horizontal grey lines the depth range proposed as 'optimal' for enabling seed and turion set during the grey period. The bar graph plotted on the left hand side is the proportion of all Coorong collected samples with living plants found at sediment depths classified into 0.1 m AHD depths during the 2020 and 2021 Reproductive surveys. Water level data obtained from www.water.data.sa.gov.au on 03 April 2022.

3.5 DNA based assessment of aquatic macrophyte community composition

The analysis successfully identified aquatic macrophyte to species level from 92 of the core samples, four samples were removed from analyses, as the number of reads and locus recovery were low. There was a clear dominance by one genetic OTU, *Ruppia tuberosa* haplotype 2 (Table 11). From the more than 14,000 bp alignment determined for sequences matching to *Althenia* only one OTU was detected. This was identical to previously collected *Althenia cylindrocarpa* samples based on the 'strict' *Althenia* mappings and concatenated alignment.

Table 11. DNA based identification of aquatic macrophyte presence across 2020 Reproductive sampling sites.

SPECIES	<i>Althenia cylindrocarpa</i>	<i>Ruppia tuberosa</i> h1	<i>Ruppia tuberosa</i> h2	<i>Ruppia</i> sp.	Two OTU's present (any)	OTU's with both <i>Ruppia tuberosa</i> haplotypes (i.e. multiple genotypes)
Number of cores with OTU* present	10	11	81	4	14	7
Percentage of cores containing each OTU*	11%	12%	88%	4%	15%	8%

*Operational Taxonomic Unit

In the *Ruppia* concatenated alignment and tree, three OTUs can be observed within the HCHB core samples (Figure 36). We are confident that at least two *Ruppia* species are found among the HCHB samples from the Coorong. There were two major chloroplast haplotypes for *Ruppia tuberosa*; however, at this stage we did not establish the unknown *Ruppia* species detected in the Coorong, as associated voucher material did not match.

There was an initial expectation that some of the core samples had multiple plants, and potentially species, in them and in fact, bioinformatically we detected 7 cores with *Althenia* and *Ruppia* in them. We also detected 7 cores that had both *Ruppia tuberosa* haplotypes. These observations were inferred from the hybrid nature of the consensus sequence detected in the cores.

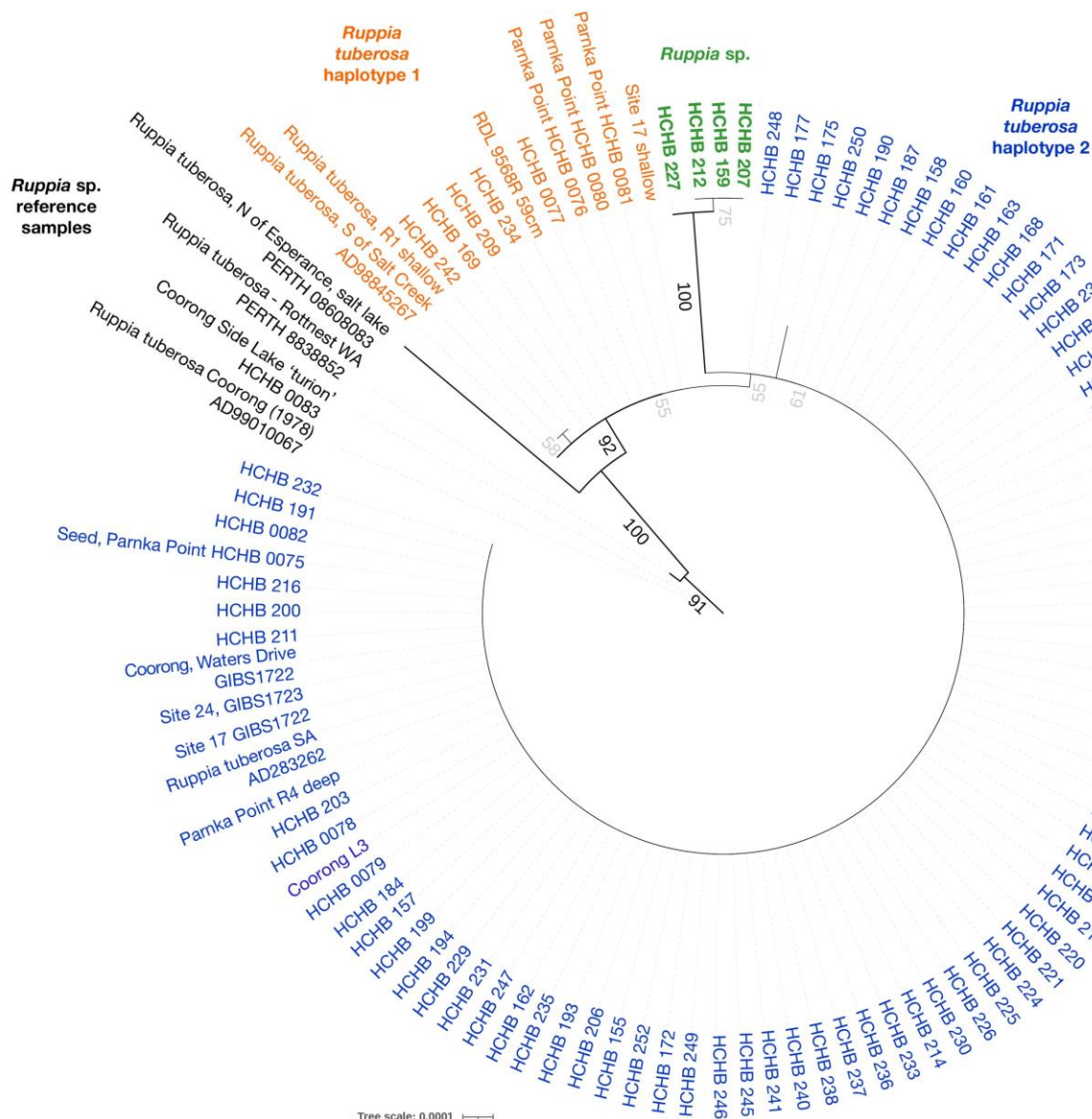


Figure 36. PhyML Maximum Likelihood tree based on 22,627 base pairs of cpDNA sequences generated using target capture Illumina sequencing obtained from mixed samples in field collected cores. Sample codes refer to site codes, colours associate bootstrap supported haplotypes and bootstrap support values are indicated on >90% supported nodes (black text). Tree visualisation was using iTOL v5 online tool (Letunic and Bork 2021).

The distribution of genetically identified species and haplotypes along the sampled distribution was compared by plotting the presence of genotypes detected against the sampled distance from the Murray mouth (Figure 37). *Ruppia tuberosa* haplotype 2 was the dominant OTU detected. *Althenia cylindrocarpa* was found across a wide geographic range including North and South Lagoon sites. The additional haplotypes, *Ruppia tuberosa* haplotype 1 and *Ruppia* sp. were only found among South Lagoon locations. Overall, we were able to detect a higher species diversity in the South Lagoon, contrasting with much of the literature of the last 15 years (Paton et al. 2017a, Paton et al. 2021) although contrasting results have also been presented (Asanopolous and Waycott 2020, Collier et al. 2017).

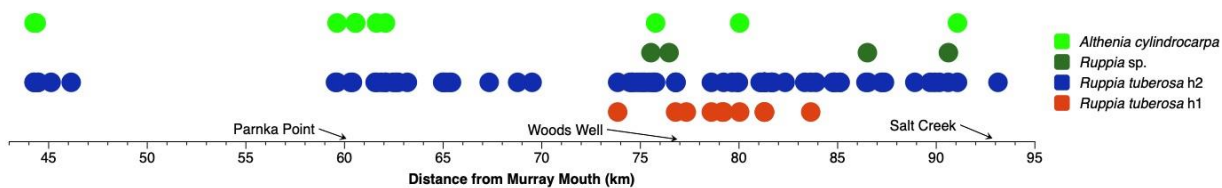


Figure 37. Presence of OTUs as distance from Murray Mouth (colours represent OTU and distance from Murray Mouth calculated as linear distance to georeferenced collection point of analysed core).

4 Discussion

This study was able to generate a more detailed understanding of the southern Coorong aquatic macrophyte community, its distribution, community composition and the trends associated with environmental factors at a larger scale than has been reported previously. Through a sampling approach that surveyed a wider range of locations, encompassing different sediment types, east and west shorelines and in between, and a wider range of water depths, we were able to address significant knowledge gaps identified in previous studies (Asanopolous and Waycott 2020, Brookes et al. 2009, Collier et al. 2017). The results and conclusions presented here are related to the hydrological conditions present during the period of this study (July 2020 to March 2022) as well as the preceding conditions which have influenced the distribution of aquatic macrophytes and filamentous algae throughout the Coorong system. During the period of sampling for these surveys, back to back La Niña conditions occurred across southern Australia; the Bureau of Meteorology declared La Niña conditions confirmed for Southern Australia in September 2020 continuing until March 2021 and again November 2021 continuing into April 2022 (<http://www.bom.gov.au/climate/enso/>). La Niña typically results in cooler and wetter conditions in southern Australia, and these conditions have been coincident with the collection of our reproductive and peak biomass samples. It should also be noted that these conditions will continue to influence the status of the Coorong system as a whole in the upcoming growing season. The results we present need to be interpreted considering these seasonal conditions and the new sampling design. The implications of our results will reflect the confluence of these factors as well as the limitations of previous sampling methodologies.

4.1 The distribution of *Ruppia* and other aquatic macrophytes in the southern Coorong: the ‘*Ruppia* Community’

During the peak biomass period in 2020 and 2021, a mixed community of aquatic macrophytes was widely distributed across the study area. Indeed, the aquatic macrophyte community now exceeds the measured extent of previous monitoring although the area of extent is consistent with descriptions of the extent prior to the Millennium Drought (Geddes 1987, Nicol et al. 2018, Womersley 1975). Our surveys documented plant populations distributed across a diversity of sites and local conditions, confirmed by the range of environmental conditions such as salinity, sediment type and water depth. The focus of this study was the southern Coorong, and thus the majority of our survey sites, other than in a few sites included for reference further north, were concentrated in the area southeast of Noonameena and northwest of Salt Creek (40 and 93 km from Murray Mouth respectively). The distribution of the *Ruppia* Community along the extent of the southern Coorong sampled demonstrated different trends for the two peak biomass/reproductive seasons sampled (Figure 8). The more northern sites had higher biomass in 2020 Reproduction and southern sites in 2021 Reproduction.

Across sampling seasons, the presence of plants corresponded to the seasonality of the Coorong ecosystem, dry summer with low water levels and cool winter with higher water levels. At the sample taken level (i.e. each core taken) overall 67% of samples contained plants (Table 5). At the site level (i.e. all samples taken at the same location at the same time) 74% of sites contained plants. These overall results included the depth

profiles of the 2021 Vegetative survey, if removed, the site level proportion of plant presence increases to 84% and sample level to 66%. Achieving more than 80% presence across the southern Coorong for plant presence, given the diversity of locations covered is indicative that the *Ruppia* Community has recovered its extent of occurrence to pre-drought levels.

Outside the main study area, we received anecdotal observations from other researchers and the local community that aquatic macrophytes were distributed further to the north and south of our survey area, many of which we were able to confirm. In addition, *Ruppia megacarpa* was observed during the study period forming dense populations in water courses adjacent to the Coorong. Among these were the channel to the Salt Creek fishway and in the channels around Goolwa. Finally, collections of *Zostera muelleri* have been made close to the Murray Mouth in the North Lagoon. In addition to pre-Millennium Drought observations, the long term paleo-ecological record for the region (Dick et al. 2011) includes these species as components of the Coorong ecosystem. The observations of other species were not quantified in this series of surveys except for *Ruppia megacarpa* seed counts, where 10.64% were counted as *Ruppia megacarpa* among the total number of seeds counted in sediments.

The southern Coorong has a mixed-species aquatic macrophyte community, consistent with much of the pre-Millennium Drought literature (Dick et al. 2011, Geddes 1987, Nicol 2005, Nicol et al. 2018, Paton 1986, Womersley 1975). This community composition was established using two independent methodologies, first through direct observations of plants in the field when reproductive, and second using DNA based markers generated from samples collected at each site. The DNA evidence found the surveyed region dominated by *Ruppia tuberosa*. *Althenia cylindrocarpa*, a common species found in salt lakes and estuaries of southern Australia, was also prevalent in many locations. In addition, a potentially novel species of *Ruppia* was detected that was unable to be matched with reference samples of other common *Ruppia* species of the region (Figure 37). We will refer to the community as the '*Ruppia* Community' and assume that it contains the documented species and genetic variants described above.

The monitoring conducted under TLM program is unlikely to have observed the less common or unusual components of this mixed community as flowering and fruiting of *Althenia cylindrocarpa* occurs earlier in the season than *Ruppia tuberosa*. As a result, it is possible that *Althenia* is likely to have completed its life cycle before TLM summer sampling; and during winter TLM sampling, vegetative features alone would be insufficient to discriminate between *Ruppia* and *Althenia* with any confidence. However, with sampling now occurring in October *Althenia* should be detected by TLM monitoring. This emphasises the need to sample at times of the year that capture key aquatic macrophyte community members to inform our understanding of the state of the community.

4.2 Filamentous algae distribution and association with aquatic macrophytes

Filamentous algae that form large areas of dense surface mats were observed to be almost exclusively associated with the presence of aquatic macrophyte habitat. The filamentous algal community was confirmed to be comprised of *Ulva paradoxa*, *Cladophora* sp. and *Rhizoclonium* sp. as found previously (Collier et al. 2017). Filamentous algae such as these have been observed in coastal estuaries of southern Australia since early collections were made (Womersley 1975, Womersley 1984). Observations of *Cladophora* being associated with the end of season 'dry off' pools in shallow areas were made but these were not extensive (Lucas and Womersley 1972). These early authors infer that typically these filamentous green algae exhibit variable abundance following local conditions leading to extensive rapid growth when high nutrients and warmer water occur at modest salinity. Little other macroalgae has been associated with the southern Coorong with the exception of *Lamprothamnium papulosum* being frequently recorded and collected prior to the Millennium Drought (Lucas and Womersley 1972, Nicol 2005, Nicol et al. 2018).

Observations of *Lamprothamnium* sp. or other charophytes in the field or during sample processing was very rare, nor were they observed with any prevalence in the field during previous field collections in late 2016 (Collier et al. 2017). This contrasts with earlier records where *Lamprothamnium* (common name: Stonewort) was often observed in high frequencies before the Millennium Drought, and where it was also found in earlier

studies to be a significant part of the diet of some species of bird (Delroy 1974, Dick et al. 2011, Geddes 1987, Nicol 2005, Nicol et al. 2018, Paton 1986). The loss of the charophyte *Lamprothamnium papulosum* from the Coorong ecosystem since the Millennium Drought is potentially due to the significant change in water quality. Charophytes are typically found in fresher water and are often referred to as indicators of habitat quality due to their sensitivity to high nitrogen and high pH (Barbosa et al. 2021, García and Chivas 2004). The species of *Lamprothamnium* from the Coorong is one of only a few species found in Australia that are salt tolerant (Casanova 2013, Womersley 1984). Given the description of *Lamprothamnium* being eaten by waterbirds (Delroy 1974) the reduction in its prevalence is of concern.

Large areas (km²) of filamentous algal mats formed as Coorong ecosystems recovered from the loss of a well-established *Ruppia* Community at the end of the Millennium Drought (Collier et al. 2017, Paton et al. 2021, Waycott et al. 2020). These large areas contain high biomass of algae. For example, in 2019 within the central section of the southern Coorong (between -35.839317° 139.363192° and -35.927042° 139.426823°), an area of 26.8 km² was estimated using remote sensing from an high resolution aerial survey to have a total algal biomass between 1,700 tonnes and 6,400 tonnes, equivalent to 34–703 tonnes km⁻² (Waycott et al. 2020). The formation of the algal mats was observed to continue until a combination of higher water temperatures and salinity lead to the decline in algal productivity (Waycott et al. in preparation).

To better understand the formation and dynamics of these filamentous algal mats and how they reach large, densely occupied areas (Waycott et al. 2020), we developed a scoring system to track the presence and status of algal mat formation during all surveys. This algal mat formation makes them difficult to measure. While surface percentage cover is a rapid and potentially useful method, yet it does not provide insight into the biomass that varies dependent on the thickness of the mat, its composition under the surface and water depth. The method we developed was based on developing a scoring system, which described the composition of the filamentous algae and then derived an estimated biomass. The formation of these mats follows a clear life cycle pathway summarised in the following stages (drift only filamentous algae was classified separately):

1. As water levels rise early in the growing season, filamentous algae may start growing attached to benthic substrate/s. Early stages are only visible as strands or small clumps at sediment surface.
2. Filamentous algae form conspicuous clumps and begins to shroud the substrate above the sediment.
3. Filamentous algal clumps begin filling the water column, and if attached to plants they inhibit light getting to the plant. At this stage the clumps are not yet reaching the water's surface.
4. Filamentous algae now reach the water's surface, attached to substrate but filling the water column, spreading over the surface forming surface mats which fully obscure the macrophytes below.
5. The algal mat spreads beyond the immediate area of benthic substrate it was initially attached to. The mat becomes detached with minimal energy required, and can cover 100% of the water surface.

Undertaking observations of algal mat formation using the life cycle stages, across all surveys confirmed an association between the presence of filamentous algal mat formation and aquatic macrophytes (Figure 38). In fact, only 2 sites sampled of the 114 where filamentous algae was present had no obvious aquatic plant substrate.

Filamentous algal mats were most common in the southern North Lagoon and central region around Parnka Point. There is a clear cut-off where no filamentous algae appear in the lower section of the Coorong South Lagoon just north of Policeman Point (Figure 38). There was significantly more algae observed in the 2021 Reproductive survey than in 2020; however, this did not appear to impact aquatic macrophyte biomass overall. This difference between seasons could be due to the differences in water levels; however, salinity did not differ significantly between the two seasons. We also observed filamentous algal mats persisting where salinity was in excess of 100 PPT; however, the 'point-in-time' nature of these surveys does not consider whether these algal mats were healthy or in decay due to the high salinity present. The difference

in algal biomass in the northern and central regions, may be due to topographic complexity, where islands and bays protect algal mats from movement via wind or water; however, this is based on field observations and not quantified.

This very strong association of filamentous algal mat formation with the presence of a *Ruppia* Community makes managing the reduction in filamentous algae even more difficult. Principally, any algal extraction process will likely disturb the *Ruppia* Community leading to further damage of the system (Waycott et al. in preparation, Waycott et al. 2020). Experimental testing of survival thresholds of filamentous algal mats in culture indicate that at higher temperatures and salinity algae may be able to survive longer if there are high levels of nitrate in the water column (Waycott et al. in preparation).

Interestingly, where there was low aquatic macrophyte biomass, there were also low levels of filamentous algae. It is possible that this is a feedback of algae requiring macrophyte presence to provide substrate for attachment. Conversely, we also observed that as filamentous algae biomass increased, macrophyte biomass declined. There appears to be thresholds where if aquatic macrophyte biomass is below $\sim 4 \text{ g DW m}^{-2}$, algal growth (or at least its ability to stay in place) is inhibited. While if algal biomass is above $\sim 100 \text{ g DW m}^{-2}$, it begins to inhibit aquatic macrophyte growth. We suggest the quantity of filamentous algae present has the greatest negative influence in the *Ruppia* Community. Filamentous algae may also be providing habitat complexity (i.e. protection from predation), or an additional food source for detritivores, invertebrates, fish and bird species, although this is speculative. Our improved understanding of the lifecycle and influence of dense algal mat formation to plant growth through shading and contribution to a hyper-eutrophic nutrient cycling pathway may lead to a more diverse array of options to find mechanisms to reduce their impact on ecological function in the southern Coorong.

4.3 Condition of the aquatic macrophyte community in the southern Coorong

Despite different seasonal conditions in the 2020 and 2021 reproductive surveys, biomass did not differ significantly, suggesting it is possible the aquatic macrophyte community is reaching a threshold. Our study showed that biomass was widely, albeit patchily, dispersed across the southern Coorong. The majority of sites, where plant growth was not limited by rocky substrate, had plants present, specifically the *Ruppia* Community. Visualisation of the distribution of the *Ruppia* Community is underway such that the results of our surveys can inform an overall system distribution based on probability modelling of the available data (Waycott et al. in preparation). The interaction between the *Ruppia* Community based on the surveys and some of the major system scale drivers can then be compared using the Coorong Dynamics Model. If current major drivers persist, in particular, if water levels are maintained during late spring-early summer so plants can complete their lifecycle forming reproductive structures, the aquatic macrophyte community may slow in biomass production, but the system will be more likely to continue to build resilience (i.e. production of seeds and turions). This is already recognised in condition monitoring for the region through TLM, and to inform water management (Department for Environment and Water 2017).



Figure 38. Presence of filamentous algae during field surveys 2020–2021. Filamentous algae associated with 1. benthic aquatic plants in the *Ruppia* Community (blue circles) and 2. Bare sediments (red circles). Landmark locations are annotated on the map.

In a system with the high biotic and abiotic variability (i.e. patchy plant populations, changing water depths, salinity and overall water availability) such as the Coorong, careful consideration of the life history of the subject is important to interpret annual trends. For example, life history stages may occur at different times of the year, the 2020 and 2021 Reproductive surveys were conducted at approximately the same point of the macrophyte life cycle (similar biomass and water levels) but were conducted in October 2020 and December 2021. Detailed knowledge of plant phenology and environmental conditions will assist in developing strategies to interpret incoming information, and for use in developing monitoring and evaluation options.

The survey design aimed to conduct surveys at all life history stages, aestivation (dormant), vegetative, and reproductive. Given the importance of reproductive success on the condition and resilience of the aquatic macrophyte community, we sought to replicate surveys of this season; hence, the 2020 and 2021 Reproductive surveys. The aestivation survey was important to provide seed and turion data as a measure of resilience and the vegetative survey aimed to provide a measure of condition (vigour, health, abundance).

During the 2021 Vegetative survey, high water levels throughout the system made it difficult to follow the method used in previous surveys with most sites inaccessible. Although the water levels made our sampling

method unviable, we still required data from this season. Whilst it was not possible to collect core samples and measure biomass at our seasonal sites, the conditions provided an opportunity to focus on some previously unsurveyed sites which were now able to be accessed by vessel. We ran several longer transects and collected presence/absence data as well as environmental variables including salinity, water depth, sediment condition and the presence of filamentous algae. This study, through assessing the presence of aquatic macrophyte communities across the deeper regions of the southern Coorong has shown a wider range of depths where plants are growing, at least under current conditions, than previously recorded post-Millennium Drought.

Overall, sites where macrophytes were present varied across the depth range of -0.84 m to +0.40 m AHD (Figure 35). A clear depth range where the majority of plants are found, ± 0.2 m AHD, is shown in Figure 35. This conforms with our general observations of preference for shallow water, but that the water levels persist to complete the reproductive life cycle during the months of September to December, highlighted as the shaded bars in Figure 35. Over the past five years (i.e. 2017-2022), the water levels have been sufficient during the period of September to December to achieve inundation of the aquatic macrophyte habitat across the southern Coorong for reproduction to occur. It is worth noting that the 2021 reproductive period had excellent water levels for submerged aquatic macrophytes, and this is a likely factor in the improved shoot counts seen in December 2021 and the formation of turions.

The range of depths that can be successfully occupied by aquatic macrophytes is highly dependent on annual environmental variables: in addition to water levels, water clarity and light penetration are important factors (Olsen et al. 2015). The 2021 reproductive season, where water persisted in the system significantly longer than the previous 4 years (Figure 35), resulted in increased aquatic macrophyte biomass across a wider range of elevations and changed distribution in relation to distance from the Murray Mouth. In the 2020 Reproductive survey, there was a significantly higher proportion of biomass observed in the southern sites of the North Lagoon and central section around Parnka Point. In contrast for 2021 we observed a shift where high levels of biomass still occurred in the north and central sections but there was increased biomass in the South Lagoon.

Surveys elsewhere have relied on shoot counts as a measure of density and productivity (Paton et al. 2017a, Paton et al. 2021); however, counting shoots can be time consuming and may be less accurate when time is limited, or field conditions are difficult. This study counted shoots under laboratory conditions and measured total biomass of dry weight. We found that these two measures were strongly correlated, meaning that depending on the scope and method of future studies, either metric is a suitable measure of aquatic macrophyte populations. However, we believe that biomass is a more integrative measure of plant condition which does not rely on the form and/or structure, and it also provides information in a metric which can be compared to other aquatic macrophyte communities (Ishikawa et al. 2019, Krumholz 2019).

The persistence of seed and turion populations, despite highly variable environmental conditions (including high shallow water temperatures, desiccation, high salinity) supports the aquatic macrophyte community maintaining a high level of resilience and adaptability. Interestingly, it appears that between the 2020 and 2021 Reproductive surveys there was a change in reproductive strategy, from favouring sexual reproduction (seed production) in 2020 to vegetative reproduction (turions) in 2021. The change to this alternative reproductive strategies from seed to turion production in *Ruppia tuberosa* is likely the response to seasonal environmental conditions (Porter 2007), specifically water levels and salinity (Brock 1983), although further studies would be required to determine this.

4.4 Relationship between environmental drivers and the condition of the *Ruppia* Community

There is often a strong association between the morphological traits of plants and environmental conditions (Mabry et al. 2000). *Ruppia tuberosa* and its associated macrophyte community are well known for their ability to persist in extreme environments with high salinity and temperatures and fluctuating water levels (Porter 2007, Rogers and Paton 2009). *Ruppia tuberosa* and its associated community responds to a several co-dependent environmental and physical factors including water depth, light availability, water quality

(nutrients, salinity, turbidity, sediment biogeochemistry), disturbance through grazing and competition with filamentous algae (Asanopolous and Waycott 2020). The Coorong is well known for its hyper-salinity, shallow water leading to high water temperatures and evaporation and frequently fluctuating water levels (Frahm and Gehrig 2015).

Following surveys across 13 months, including two aquatic macrophyte reproductive seasons, we observed how variable conditions were in the system. *In-situ* measurements of depth were highly variable due to the influence of environmental variables such as wind, rainfall and inflows. Australian Height Datum (elevation) measurements provided a more accurate way to compare the relative depth distribution of aquatic macrophytes across a depth gradient although recognising the level of water in the exact location above the plants has had value for interpreting patterns of growth and local conditions.

The higher and longer persisting water levels in 2021 may have supported higher turion growth by limiting the depths at which flowers could reach the surface and effectively pollinate. Turion counts were highest in deeper water (-0.8 m AHD; 2021 Reproductive survey), whilst the majority of seeds were counted above -0.4 m AHD and were abundant up to 0.6 m AHD, possibly due to water movement and prevailing winds moving seeds towards shore. In the 2021 Reproductive season, asexual (turion) reproduction was more common. The production of turions likely requires longer availability of water levels later in the season as has been proposed by Brock (1983).

Salinity was highly variable spatially and temporally as has been well documented elsewhere (Collier et al. 2017, Phillips and Muller 2006, Rogers and Paton 2009), with a range of 40 – 140 PPT recorded across seasons. Aquatic macrophytes were able to persist at all levels of salinity recorded; however, there was a clear reduction in biomass above 90 PPT which supports observations following the Millennium Drought where a decline in macrophyte biomass was attributed to rising salinity (Asanopolous and Waycott 2020, Whipp 2010). While the aquatic macrophyte community is highly resilient and tolerant of high salinity, it does appear to be a limiting factor and result in lower biomass and productivity.

Poor sediment condition and quality is known to lead to a decline in macrophyte growth (Barko et al. 1991, Barko and Smart 1986, Idestam-Almqvist and Kautsky 1995), and sediment enrichment and eutrophication are known to impact sediment condition and regular processes in the Coorong (Hallett et al. 2019, Mosley et al. 2020a).

We adopted sediment condition assessment by a Rapid Assessment Protocol (RAP) (Hallett et al. 2019) after our first survey in October 2020, aiming to quantify the effects of different sediment qualities on aquatic macrophyte growth. Due to the number of samples being collected per survey and the number of cores per site, it was not viable to conduct the assessment for each sample. It was also not possible to incorporate such assessment into the laboratory processing, as it required cores to be intact, which was not logistically possible.

Our results did not indicate a significant difference in aquatic macrophyte biomass between sediment conditions; however, it was evident that there was a pattern of increasing macrophyte biomass as sediment condition increased. This was evident in the overall sediment condition score and also specifically the sediment texture rating. Although neither were statistically significant, we believe this was due to variability and low sample size rather than actual effect. It is also likely that the presence of aquatic macrophytes influence sediment condition, and therefore increases in macrophyte biomass increase sediment condition, although further research is required to quantify this. We believe that this study has highlighted the relationship between sediment condition and aquatic macrophyte productivity and that the RAP is an effective tool for future condition monitoring programs in eutrophic lagoons.

How environmental variables interact with biotic variables is important for understanding the drivers behind stressors within natural systems such as the Coorong (Nicol 2005). We used multivariate analyses to explore the relationships between aquatic macrophytes and environmental variables. All variables incorporated into the model, apart from depth (field visit measured), had a significant effect on biomass, shoots, seeds and turions. In relation to biomass, distance from the Murray Mouth and salinity accounted for the most variation (9.06 and 8.4% respectively); however, algal biomass and elevation also accounted for some variation (3.52 and 2.5% respectively). Cumulatively, the environmental variables included in the model only explained 23.5% of the variation in the model, which suggests that other factors (not investigated in this study) were

at play. Biotic factors such as macrophyte patch dynamics or differences between biomass caused by different species composition could be important (Braun et al. 2012, Fu et al. 2013).

Similar patterns were observed in seed and turion counts, with only small amounts of variation being able to be explained by the environmental variables we measured/considered (7.7 and 14.94% respectively). Interestingly, Elevation accounted for 7.2% of the modelled variation, which is consistent with our observations that water levels influenced the reproductive strategies chosen by aquatic macrophytes, with consistent water levels appearing to favour turion production. Although the variation of seed production explained by algal biomass was quite low (4.4%), it would make sense when considering that the aquatic macrophytes conduct sexual reproduction at the air-water interface, a process of which success could potentially be reduced by algal interference (Paton et al. 2017b, Waycott et al. 2019).

The majority of variation in algal biomass can be attributed to distance from the Murray Mouth (7.5%), although the other variables of salinity, depth and elevation were also significant in the model but only accounted for less than 2.5% of variation in the model combined. This is consistent with field observations, analysis and previous discussion, which indicated that the majority of algal biomass was located in the southern North Lagoon and central area around Parnka Point. In this model it was also consistent with aquatic macrophytes, that only a small amount of variation could be explained in the modelling, this is likely a product of the high levels of variation in the system overall and potential that other factors which we have not measured or examined are at play.

4.5 Management implications

The aquatic macrophyte community of the Coorong South Lagoon should be considered a community of several co-occurring species some of which have characteristics that differ to the well-documented *Ruppia tuberosa*. In the Coorong South Lagoon, *Ruppia tuberosa* is more common than the other species present: *Althenia cylindrocarpa* along with an unresolved species of *Ruppia*. In addition, two haplotypes of *Ruppia tuberosa* were detected, the less common haplotype only found in the South Lagoon samples in the 2020 Reproductive sample. We now have a DNA toolkit to identify these species relatively easily and with confidence using eDNA samples.

There are management implications associated with the presence of a mixed community of aquatic macrophytes. First due to the future potential need to undertake intervention actions to facilitate recovery of the *Ruppia* Community following extreme conditions such as those experienced during the Millennium Drought. It would be desirable that any interventions would be advised to maintain a mixed species in the community, or find mechanisms to encourage recruitment in the future. Secondly, as the community is mixed and difficult to discriminate among species unless reproductive, many of the experiments and results from surveys conducted in this study are reflective of this community as a whole, termed here as the *Ruppia* Community, rather than *Ruppia tuberosa* alone. The historical presence of a multi species aquatic macrophyte community historically in the southern Coorong ecosystem is a reminder that this is the typical state for this environment. Detailed evidence from reports, collections lodged in the herbarium, and photographs, support the observation that there was a mixed *Ruppia* / *Althenia* community prior to the Millennium Drought (Delroy 1974, Geddes 1987, Lucas and Womersley 1972, Paton 1986, Womersley 1975).

The surveys conducted in this study indicate there has been a loss of the charophyte, *Lamprothamnium papulosum* from the southern Coorong ecosystem. This charophytic algae species was almost completely absent in our survey samples, and where present, specimens were very small and exhibiting poor growth. The loss of this species could be indicative of the decline in water quality although species responses were not specifically studied in this project. The presence of *Lamprothamnium* could act as an indicator in the future for improved ecological health if conditions are able to return to be suitable for their survival.

The different methodology developed to survey the aquatic macrophyte community in the Coorong has improved the outcomes for understanding the baseline status of these important aquatic plants in the system, critical aspects of the population ecology including an accurate estimate of plant growth relative to depth, increased range of population locations including the finding that there are numerous populations in deeper water (-0.8 m AHD) that produce high biomass populations. The survey method we adopted detects

higher numbers for characteristics such as shoot, seed and turion counts listed as critical inputs to Condition Monitoring. There is considerable additional value of the application of our methodology to monitoring, evaluation and reporting of change in the Coorong system. Through the generation of an improved evidence base that can be utilised analytically, we suggest developing revised condition monitoring program with principles established by this study that builds on the long-term data set. A modified sampling protocol that would not require excessive laboratory analysis could be developed that provide improved data sets for future application. Shoot counts and biomass are both good estimates of abundance, and laboratory analysis of total biomass would be an easy and quick processing task to achieve the outcome of a robust measure that has the added benefit of informing Food Web development, modelling and plant energetics estimates. Furthermore, processing samples in the laboratory would free up time in the field to allow more sites to be sampled giving greater spatial coverage of the Coorong. Counts of seed banks (and turions) using cores and sifting through to find seeds is an irreplaceable tool but is more efficient when done in the laboratory compared to the field.

The *Ruppia* Community is now present along the extent of the surveyed area, from just to the north of Long Point to south of Salt Creek (albeit uncommonly) at Tea Tree Crossing, approximately 112 km from the Murray Mouth. There are several areas where plants were found not to occur due to a rocky substrate. The recovery of this extent of occurrence following the end of the Millennium Drought in 2010 appears to have followed a dispersal recruitment trajectory. This is a reasonable concept as at the end of the Millennium Drought the *Ruppia* Community had dwindled to highly isolated remnants at best with an exhausted seed bank (Kim et al. 2013, Nicol et al. 2018, Rogers and Paton 2009). Given this, rapid recovery with the return of more positive water levels since 2010 would be unlikely. In fact, the trajectory of aquatic macrophyte population recovery following a catastrophic loss, such as happened during the Millennium Drought in the Coorong, would be determined by the rate at which local populations could establish, generate seeds or turions, followed by dispersal to other locations. In addition, short distance dispersal among sites facilitated by birds may be possible; however, the lower seed banks and variable bird numbers make this a relative low frequency event and not to be relied on. A review of the seed bank information from the TLM monitoring program (Figure 19) suggests that the current seed bank observed in this study is only ~30% of pre-drought levels. These results and observations during our surveys support the ongoing concern that aquatic macrophytes are not performing well, having poor vigour and resilience with the production of seeds in many areas remaining low. The return of numerous turions (Figure 18) as observed in the 2021 Reproductive season is promising for the year to year ability of populations to be healthy but does not improve long term resilience.

Since *Ruppia* translocation activities commenced in 2012/13 (Department for Environment and Water 2014) there have been slow increases in the numbers of sites where the *Ruppia* Community is present. The most recent increases over the last three years (2019-2022) reflect a period of relative positive environmental conditions supporting an improved growth period for the *Ruppia* Community: cooler maximum temperatures in warmer months; higher water levels for longer periods into summer; and the resultant improved habitat availability. The anomaly appears to be a trend of increased salinity despite the higher water levels. Speculatively, this higher salinity and longer-term availability of water and the formation of more mature populations of *Ruppia tuberosa* has led to the dramatic increase in turion formation observed in our study. However, given the unusual conditions of the past two La Niña summer weather cycles in 2020-21 and 2021-22, interpretation of the long-term trends should be cautious at best.

The ongoing presence of large areas, generating a high biomass, of filamentous algae across the southern Coorong remains a significant concern. We identify a target of reducing algal mat formation to less than 100 g DW m⁻². Unfortunately, the direct association of the filamentous algae forming mats with the presence of the *Ruppia* Community leads to the need to consider trade-offs for managing the impact of the algae. We suggest, most importantly, reducing nutrient loads to minimise the relative advantage the filamentous algae have over the *Ruppia* Community will be critical. Physical interventions to remove algae are not practical due to the large area occupied by filamentous algae. Even if practical, it will lead to damage to the *Ruppia* Community present in those locations which often have a fragile rhizome-root interface due to the very high organic biomass in the surface layers of the sediments.

The association of poor plant abundance of the *Ruppia* Community present with poor sediment quality has emphasised the need for developing options to enhance sediment quality through management strategies. The lack of algae in the very southern sections of the Coorong South Lagoon could relate to higher flow rates experienced with water coming from the South East Flows through the Salt Creek regulator. This may provide sufficient water movement to act as an impediment to dispersal of algae further south, or more likely, that even low levels of flow over meadows may act as an impediment to physical attachment to *Ruppia* plants minimising the impact of algae. This could be studied *in situ* relatively easily and would increase the value of infrastructure options that enhance these outcomes.

The implications of our surveys are that a *Ruppia* Community may occupy a range of deeper locations than has been accepted historically. We trialled several methods for surveying deeper water without needing to wait for low water levels or diving; including camera/video drops and underwater vehicles. Results were poor from these methods due to the poor water quality in particular high turbidity making visualisation difficult with standard equipment. A purpose-built video drop may work if able to work under low light conditions with sufficiently high resolution. There would be rapid outcomes possible from a survey method applying such a tool. Further evaluation of this as a potential evaluation tool for deeper water (>1 m) is important. Another consideration is that we were able to utilise the availability of a hovercraft to access some areas without significant disturbance. The access a hovercraft can give to areas with fine, unconsolidated sediments containing an extensive *Ruppia* Community was invaluable. In the future, to access sites such as this, out of range of small boat access and with walking to sites being highly disruptive to sediments and plants, a method that can minimise the disturbance footprint will be essential.

The return of many sites to high biomass and shoot counts is a positive indication for the current state of the system. Implementing options to enhance resilience through increasing seed banks, reduce algal loads through reduced nutrient loading and encourage higher biomass populations will achieve the best outcomes for the long-term viability of the Coorong.

List of shortened forms and glossary

AHD	Australian height datum. Note: the mean sea level for 1966-1968 was assigned a value of 0.000 m on the Australian Height Datum (AHD) at 30 tide gauges around the coast of the Australian continent (Geosciences Australia).
Algal Severity Index	A scale of algae cover and level of interference with <i>Ruppia</i> scored from 1 - 7.
Aquatic Macrophyte	A submerged aquatic flowering plant, large enough to be seen by the naked eye
Biomass	The total mass of sampled organisms, measured as wet or dry and for a particular sampled area such as m ² .
Central Coorong	In this report, the central part of the Coorong stretching from the Needles to Hack Point connecting the North Lagoon and the South Lagoon.
Charophyte	The charophytes (Streptophyta, Viridiplantae) are the extant group of green algae that are most closely related to modern land plants.
Chlorophyll <i>a</i> (chl <i>a</i>)	One of the photosynthetic pigments of plants that is used as an indicator of the eutrophic state of water bodies with degraded water quality showing an increase of chlorophyll <i>a</i> .
DW	Dry weight (of a sample).
Eutrophication	The increase in the supply of organic matter to an ecosystem. Eutrophic systems are typically characterised by excessive plant and algal growth due to the increased availability of one or more limiting plant growth factors needed for photosynthesis including light, carbon dioxide, and nutrients.
Filamentous algae	The green filamentous algal community which occurs in the Coorong, consisting of <i>Ulva paradoxa</i> , <i>Rhizoclonium</i> sp. and <i>Cladophora</i> sp. defined in Collier et al. 2017.
Haplotype	A specific combination of jointly inherited nucleotides or DNA markers from polymorphic sites in the same chromosomal segment such as the chloroplast.
HCHB	Healthy Coorong, Healthy Basin
Hypersalinity	Water having higher salinity than seawater
IQR	InterQuartile Range, a measure of statistical dispersion in descriptive statistics.
Life history stages	The different growth stages of an organism that reflect investments in growth, reproduction and survivorship. For <i>Ruppia tuberosa</i> , an organism goes through during its lifetime, i.e. seed, seedling, vegetative growth of mature plant, flowering, fruiting, turion formation, senescing plant.
Macrophyte	An aquatic plant large enough to be seen by the naked eye, in this report specifically a flowering plant (angiosperm).
Millennium Drought	The Millennium Drought occurred from 1996-2010 (van Dijk et al. 2013), the impacts to the Coorong occurring over the period that El Niño was experienced across South Australia 2001-2010.
North Lagoon	As a region in this report we refer to the North Lagoon as the part of the Coorong from the Murray Mouth to the Needles. Literature elsewhere refer to the North Lagoon as area from the Murray Mouth to Parnka Point.
OTUs	Operational Taxonomic Unit, an operational definition used to classify groups of closely related individuals.
Primary productivity	The conversion of light energy to organic compounds through photosynthesis, can be referred to as carbon fixation. The primary producer is therefore an organism that fixes carbon, otherwise known as photosynthesising.
Plant morphology	Physical form, shape and external structure of the aquatic plants

Rapid Assessment Protocol

(RAP)	Characterisation of sediment characteristics based solely on qualitative assessments of sediment colour, texture and odour, provides an informative and robust proxy for the degree and effects of sediment enrichment (Hallett et al. 2019).
<i>Ruppia</i> Community	The multi species assemblage identified in this study that has become established across the southern Coorong and includes <i>Ruppia tuberosa</i> , <i>Althenia cylindrocarpa</i> along with an as yet unresolved species of <i>Ruppia</i> .
Salinity	<p>Measure of the concentration of salt in the water.</p> <p>Commonly reported at PPT (parts per thousand) equivalent to g/kg Historically salinity was calculated as</p> $\text{salinity} = 1.80655 * \text{chlorinity (ppt or g/kg)}$
Sampling core 'Cores'	A standard core referred to will be a sampled taken that is 7.5 cm diameter (0.0044 m ²) and 8 cm deep, including all above and below ground plant community as well as sediment. The device used to take a core is typically a cylinder (such as plumping pipe) with a mark made at 8 cm to indicate depth that can be pushed into the sediment to isolate the area to be sampled.
South Lagoon	In this report, the part of the Coorong south of Hack Point.
Spatial	Refers to the dimension of space or area
Temporal	Refers to the dimension of time
TDS	The Total concentration of Dissolved Solids in water
TLM	The Living Murray
Trophic	Feeding and nutrition of plants and animals and where they fit into niches and levels of the food web.
T&I	Trials and Investigations (a project associated with the Healthy Coorong, Healthy Basin program)
Turion	Reproductive structure that <i>Ruppia polycarpa</i> (Type I) and <i>Ruppia tuberosa</i> (Type I and Type II) produce underground that is capable of forming into a new plant.

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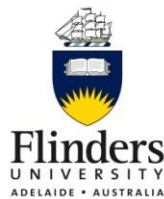
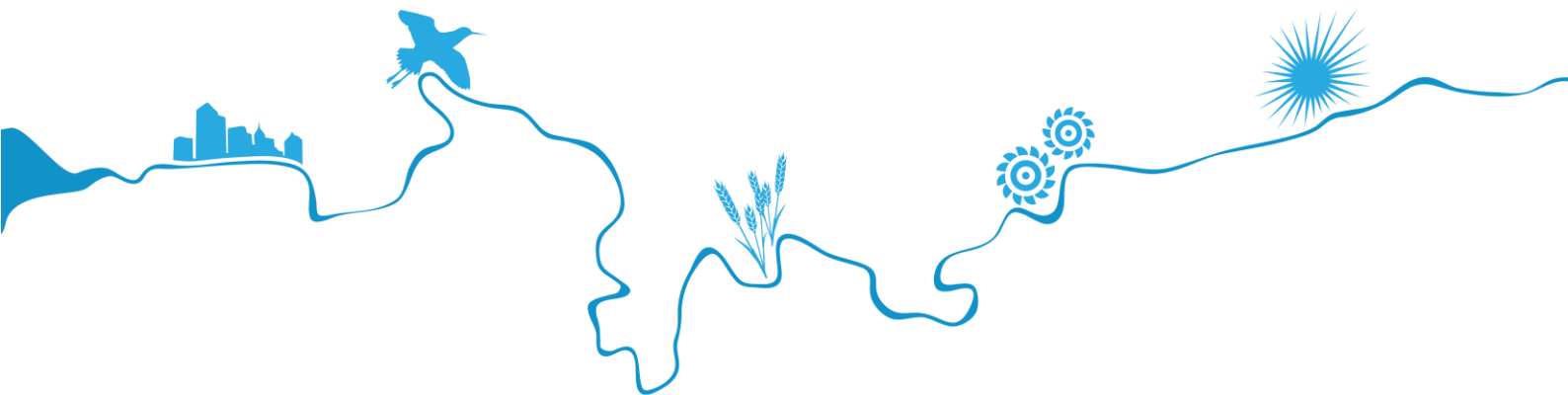
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