Growth, phenology, nutrient responses and ecological limits of the Ruppia Community and associated filamentous algal blooms of the southern Coorong

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is less than 24 then they are N-limited (Duarte 1990). The primary limiting nutrient is independent of N and P concentrations. Median tissue N concentration of seagrasses globally is 1.8% and values above this indicate high N availability; high N can occur when either N or P are limiting. Equivalent thresholds are different in plankton and macroalgae. 50

Figure 83. Conceptual diagram summarising the seasonal development of the filamentous algal community and indicative conditions observed or measured during the 2019–2021 field surveys reported here and by Lewis et al. (2022). The different stages of filamentous algal mat formation are summarised in Table 5 and Figure 11 and associated text. 92

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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 the Ngarrindjeri Nation 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 foundation of the Coorong aquatic ecosystem is the macrophyte community dominated by *Ruppia tuberosa* but which also includes *Althenia cylindrocarpa* and another, potentially new, species of *Ruppia* (which together form the 'Ruppia Community'). The Ruppia Community provides a range of critical ecosystem functions to the Coorong ecosystem, ranging from sediment stabilisation and maintaining water quality, through to a food source for water birds and shelter from predation for fish. The Millennium Drought (in particular the period from 2001 to 2010) significantly impacted the southern section of the Coorong (from Long Point to south of Salt Creek) 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 area occupied by plants increasing over the past decade. Recovery of the Ruppia Community in the Coorong South Lagoon has been limited by widespread, algal blooms; symptomatic of the lagoon's hyper-eutrophic state. Algae, including mat-forming filamentous algae, physically disrupt the ability of the Ruppia Community to flower and set seed and have led to the widespread formation of black oozes in upper sediment layers.

The South Australian Government's *Healthy Coorong, Healthy Basin* (HCHB) program's Trials and Investigations 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 actions to limit filamentous algal growth and restore *Ruppia* distribution and abundance. This may require additional management interventions to facilitating direct recovery of ecological functions in the Coorong South Lagoon. Restoration of ecological function would require a shift in the southern Coorong ecosystem from algal dominated to a Ruppia Community dominated habitat, improvement of water and sediment quality.

This report presents results of experimental evidence, field observations and analyses that inform an improved understanding of the Ruppia Community and the formation of filamentous algal blooms across the southern Coorong. Mesocosm experiments are used to assess survival thresholds of filamentous algae to a range of salinity, temperature and nutrient conditions. The growth (and factors which influence growth) of filamentous algae in the field are documented and a new model for growth of filamentous algal biomass is proposed and applied. The phenology of the Ruppia Community based on repeated, field observations over the growing season and seed banks are documented in detail. Nutrient content in the sediment, water column and plants were surveyed across a period that included high volume water release from the Salt Creek regulator in the southern end of the Coorong South Lagoon. An overall understanding of changes to the southern Coorong Ruppia Community is summarised in key findings at the end of this report.

Experimental evidence of the range of environmental tolerances for the Ruppia Community and filamentous algae of the southern Coorong indicates that the aquatic macrophyte species that comprise the Ruppia Community are capable of actively growing, i.e. increasing biomass, at or below marine salinities (i.e. 35 g L^{-1}) to 90 g L^{-1}. Plants persist at higher salinities, however they appear to become physiologically stressed, allocating resources to turion production or seeds rather than new shoots. Plants became demonstrably stressed morphologically (leaf length and shoot number), physiologically (growth rate) and in their overall productivity (biomass) when light was reduced below 16% of surface light over a 4 week period. These conditions were observed in the Coorong South Lagoon using in situ data loggers at two locations with heavy surface algal mats forming in the same period.

Phenology of the Ruppia Community indicates that plants develop strong root structures prior to allocating resources to other structures. Such a high root:shoot ratio suggests colonising behaviour which aligns with the annual cycle of the community. The investment in below ground biomass provides advantages including increased sediment oxygenation reducing anoxia, sediment stabilisation, increased nutrient and water availability in the event of variable water levels and secure attachment to substrate. However, the relative low proportion of photosynthetic tissue results in a vulnerability if light availability changes significantly shifting the balance between photosynthesis and respiration.

The Ruppia Community throughout the Coorong had no charophytes present as described in our seasonal and other ad hoc surveys (i.e. between October 2019–March 2022). Historically charophytes were prevalent

in sections of the main Coorong water body and an additional source of food for waterbirds although likely to be dynamic in there extent and biomass. However, in mesocosms, *Lamprothamnium* sp. emerged from translocated cores when placed in clean, lower salinity water. In these mesocosm experiments, at marine salinity under a range of different shading conditions, charophytes presented significantly reduced biomass when shading was reduced to 16% surface light. The emergence of charophytes is a positive sign that their propagules remain viable in sediments for a long period so if water quality is improved they may become an overt component of the system again in the future as they have been historically.

Salinity and temperature are seasonally driven processes *in situ* as both salinity and temperature increase throughout the growing season. Previous experimental testing in mesocosms of the filamentous algal community under a range of salinity and temperature conditions supports the upper threshold of 90 g L^{-1} for filamentous algal growth to stop, however death was not observed until higher salinities. Experimental maximum daily growth in biomass was observed at lower salinities (< 40 g L^{-1}) and negative growth (i.e. loss of biomass) occurred at salinities ≥ 90 g L^{-1}.

The ongoing generation of high biomass of algal blooms has led to the deposition of organic carbon onto the sediment, which promotes sulfate reducing bacteria activity, formation of Acid Volatile Sulfide and the development of monosulfidic black ooze (MBO) sediment. We observed this in the Coorong South Lagoon at all field sites with high filamentous algal cover. These sites also experienced periods of shading during the Ruppia Community growth season and while flowering was occurring. The link of these biochemical and biological processes continues to provide the feedback for the hyper-eutrophic cycle through the action of the filamentous algae.

Based on extensive field observations we propose the filamentous algal community that develops in the southern region of the Coorong, from Long Point to south of Salt Creek, is directly associated with the presence of a high biomass Ruppia Community. Prior to this study, the close association between filamentous algal mat formation and the presence of the Ruppia Community was not understood. Based on these observations and development of the new model, the filamentous algal mat formation is being included as a new component of the Coorong Dynamics Model (developed by the Department of Environment and Water) to improve accuracy of predictions of habitat suitability for the Ruppia Community and other biota.

The inter-connectivity between filamentous algae mat formation and the Ruppia Community means that manual, physical removal of filamentous algae from the Coorong waterbody would unavoidably also damage the Ruppia Community leading to losses. Following the trial algal removal activities undertaken in this study, we propose that intervention activities would disrupt the Ruppia Community and inhibit reproductive success making it a poor strategy for reducing algal biomass. However, there is potential for mechanical removal of aggregations of filamentous algae that end up decaying on shorelines. The removal of these decaying filamentous algae mats would improve the habitat quality of these mudflats through sediment oxygenation and improved overall sediment accessibility, enable colonisation by benthic macroinvertebrates, and support un-impeded shorebird foraging.

The ongoing presence of the filamentous algae does cause impacts, particularly at higher abundances, including the physical prevention of successful seed set due to removal of flowering stalks and the inhibition of pollination. Although there may be interannual differences in timing, it is likely that rapid algal growth and flowering of the species in the Ruppia Community will consistently be coincident with the increasingly warmer conditions during spring and early summer. The long-term solution would be to prevent algae growing in the first place or to prevent the algal-*Ruppia* physical connection from becoming established.

The Ruppia Community in the southern Coorong in 2021 has an excess of nitrogen compared to phosphorus. Plant tissue samples taken from the Ruppia Community in 2017 also showed an excess of nitrogen, however there has been a substantive increase in the 2021 samples. In addition, higher phosphorus concentrations in the plant tissue were detected in the 2021 samples, in particular towards the salt creek end of the southern Coorong. Filamentous algae in the southern Coorong appears to have established a feedback mechanism, whereby the decay of dense organic matter forms organic-rich black ooze sediments over areas of the Ruppia Community and other sites where algae detritus settles. The increased nutrient loads detected in the Coorong ecosystem measured since 2017 support the need for intervention to reduce this continuing as an upward cycle.

Options for intervention, as outlined in the Ruppia Restoration Strategy (technical report produced by HCHB T&I Component 2), include the potential for seed translocation activities. Currently, the primary donor site of seeds is Lake Cantara, located approximately 25 km south of Salt Creek in the Coorong South Lagoon. Lake Cantara is a modest sized lake that has very high densities of Ruppia Community seed. As long-term impacts of seed removal were observed at Lake Cantara as a result of sediment removal for translocation, this study aimed to identify other potential donor sites. A survey of more than 50 field locations across South Australia from 2019-2022 found potential populations of submerged aquatic macrophytes that were external to the Coorong and adjacent areas. Some of the sites with potential donor populations had submerged aquatic macrophyte species that were likely to survive in the Coorong. However, to our current knowledge, most of these species would require significantly lower salinities in the Coorong South Lagoon than present to survive.

This report presents experimental evidence, field observations and analyses, which confirm that the Ruppia Community continues to survive the extreme range of salinity and light conditions experienced in the southern Coorong. Our new knowledge has been directly applied to parameterise Ruppia Habitat Suitability and filamentous algae components of the Coorong Dynamics Model, which informs decision-making by forecasting changes in these ecosystem components under different management scenarios. This Ruppia Community has adapted to the extreme southern Coorong environment using multiple complementary plant traits leading to a high level of resilience, likely the reason the Ruppia Community have been able to recover from the severe impacts observed during the Millennium Drought. However, without addressing the impacts and negative feedbacks caused by the current nutrient, hydrological flow and connectivity conditions, it is likely that minor additional changes may exacerbate stressors impacting the aquatic macrophytes in this ecological community.

Acknowledgments

1 Background

The Coorong, is a long, narrow lagoon at the end of the River Murray which runs more than 100 kilometres parallel to the coast (Figure 1). The waters in the lagoon are separated from the ocean by the narrow sand dune system of the Younghusband Peninsula. The region is critically important to First Nations communities, including the Ngarrindjeri Nation, their *Yarluwar-Ruwe* (Sea-Country), with ongoing connection and use of this Country (Ngarrindjeri Nation 2018). This region is culturally, environmentally and economically important: in addition to its *Yarluwar-Ruwe*, the Coorong, as part of the 'Coorong, and Lakes Alexandrina and Albert Wetland', was recognised as a Wetland of International Importance under the Ramsar Convention in 1985. The Coorong has experienced long-term declines in its ecological condition due to changing water availability, which was exacerbated in recent decades through drought, most recently the Millennium Drought (2001 to 2010), the worst drought and one of the driest periods ever recorded across south-eastern Australia (Van Dijk et al. 2013).
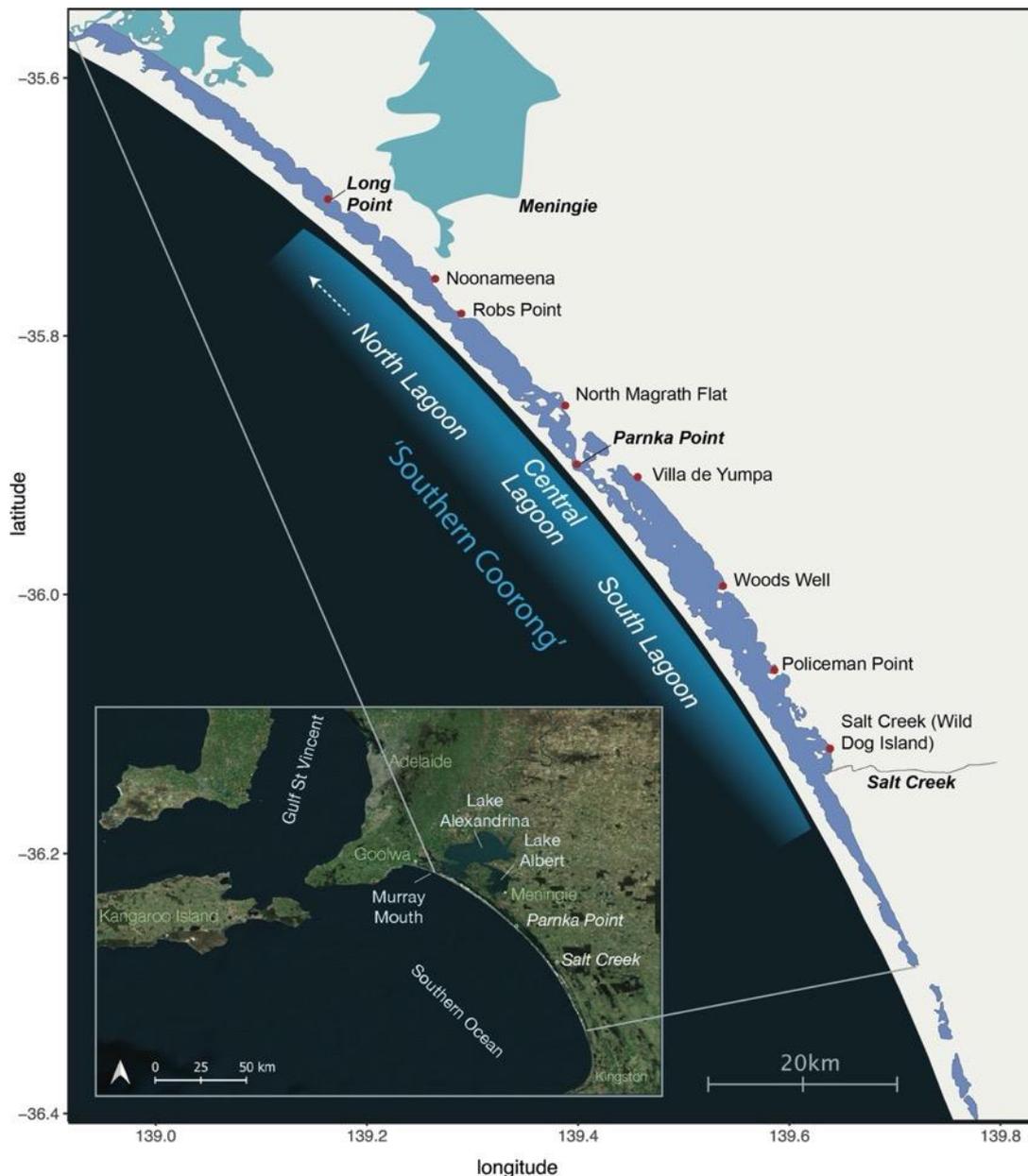
Figure 1. Region over which sampling was conducted for experimental testing of the aquatic macrophyte and filamentous algal communities. Additional site access details are available in Lewis et al. (2022) and exact site locations are listed in tables within this report, named places are listed in relevant sections.

The aquatic macrophyte community that, for the last 30 years, has been dominated by *Ruppia tuberosa* but can also contain *Althenia cylindrocarpa* and another unknown species of *Ruppia* (hereafter referred to as the Ruppia Community; Lewis et al. 2022), is a keystone component of the southern Coorong (Paton 1986, Phillips and Muller 2006, Lewis et al. 2022). The loss of this community during the Millennium Drought (in particular the period from 2007 to 2010) and its subsequent slow recovery since the drought ended has contributed to a shift in food web complexity, with the system becoming algal dominated with blooms of filamentous algae and phytoplankton over large areas (Paton 2010, Brookes et al. 2018, Jamieson et al. 2022, Waycott and Lewis 2022). A review of knowledge available to support restoration of the Coorong ecosystem identified a range of critical ecological knowledge gaps (Brookes et al. 2018) with an emphasis on recovery of the Coorong South Lagoon. This review identified that a high research priority was to investigate the dynamics of the Ruppia Community dominated by hypersaline tolerant species such as *Ruppia tuberosa* and the filamentous algal community that has become prevalent across large areas of the southern Coorong.

Surveys of Ruppia Community distribution, community composition and diversity during the *Healthy Coorong, Healthy Basin* (HCHB) program, have shown that the Ruppia Community lost during the Millennium Drought has recovered to its pre-drought extent of its distribution but not its pre-drought condition (Lewis et al. 2022). The southern Coorong ecosystem remains hypersaline to extremely hypersaline with annual salinities over 100 g L^{-1}; and has recently been classified as hyper-eutrophic (Mosley and Hipsey 2019, Mosley et al. 2020). In this hyper-eutrophic state, the Coorong exhibits very high nutrient loads, characterised by frequent and severe algal blooms, a shift towards rapid turnover biota.

In addition to the widespread extent of the Ruppia Community in the southern Coorong, Lewis et al. (2022) also documented the current range of depths occupied by plants, relative to the sediment height above sea level (m AHD; Hobbs et al. 2019). This depth range was predominantly ±0.4 m AHD, however plants were also found in some areas up to -0.8 m AHD (Lewis et al. 2022). These depths reflect the water availability during the growth and reproductive period (May–December) for the aquatic macrophytes present. Water levels are particularly important during the final stages of the reproductive cycle as flowering occurs through spring and into early summer. If water levels drop before this process is complete, failure to set seed can occur (Paton et al. 2015, Collier et al. 2017, Asanopoulos and Waycott 2020, Waycott and Lewis 2022).

A considerable proportion of the detailed knowledge of the aquatic macrophyte community in the Coorong including life cycle stages was conducted during or immediately following the Millennium Drought (Phillips and Muller 2006, Paton and Bailey 2010, Paton et al. 2011, Kim 2014, Paton et al. 2015, Paton et al. 2017b). Companion studies to this one, (Auricht et al. 2019, Waycott et al. 2019, Asanopoulos and Waycott 2020, Mosley et al. 2020, Jamieson et al. 2022, Lewis et al. 2022, Waycott and Lewis 2022) have documented the extant distribution of the Ruppia Community and other microbial communities present within the southern Coorong and the current state of these components of the biota. These reports provide evidence for: a widespread *Ruppia* Community; significant extent of filamentous algae capable of forming high biomass dense, surface mats during the warmer months, and; diverse microbiota of extreme hypersaline adapted phytoplankton and other microbiota. This current community composition is reflective of the hyper-eutrophic and hyper-saline conditions present in the southern Coorong, and, in the case of the Ruppia Community, the recovery period from loss in the system following the Millennium Drought, to present conditions.

The current ecological state of the southern Coorong has been characterised as degraded such that waterbirds, fish, plants and invertebrates have been affected by prolonged hyper-saline and hyper-eutrophic conditions (see discussion paper; Department for Environment and Water 2021b). Furthermore, the desired ecological state of the southern Coorong would be a naturally variable system with only short periods of restricted connectivity and hyper-salinity (see discussion paper; Department for Environment and Water 2021b). Under this desired state, the southern Coorong would also exhibit moderate levels of nutrients that are found in the tissues of persistent aquatic plants along with a diverse invertebrate community that promote healthy nutrient cycling. The aquatic macrophyte community would provide habitat and food for waterbirds and fish, currently limited in the southern Coorong ecosystem. To achieve this desired state, the emerging research has found that salt and nutrients loads need to be significantly reduced across the southern Coorong (Mosley et al. 2020, Priestley et al. 2022a). Restoration of the ecological functions

associated with primary production in the southern Coorong will include restoration of a vigorous aquatic macrophyte community (Waycott and Lewis 2022) supporting nutrient sequestration in persistent biomass.

Rehabilitation of the primary producer community in the southern Coorong to a desired state of macrophyte dominated, low algal bloom conditions (Figure 2) will require the return of some critical ecological functions and the reduction of hyper-eutrophic conditions (Department for Environment and Water 2021b). Recovery of high vigour, extensive populations of the Ruppia Community that act as a keystone resource for the food web of the Coorong (Phillips and Muller 2006, Brookes et al. 2018, Department for Environment and Water 2021a) will be essential for rehabilitation and long term restoration efforts (Waycott and Lewis 2022).
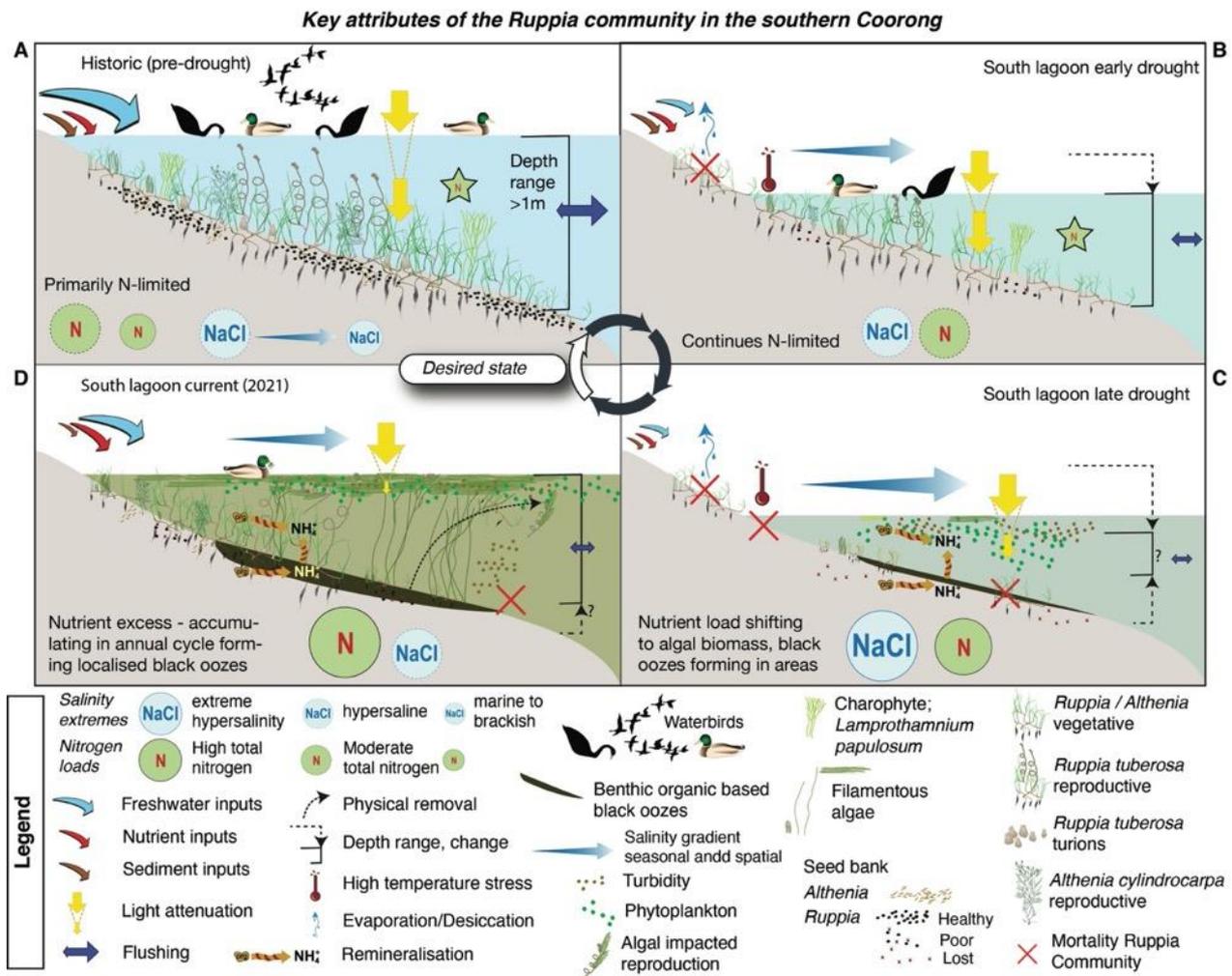
Figure 2. Conceptual diagram summarising alternative states for the southern Coorong, emphasising primary producers, based on observed ecological conditions and documented critical ecological processes (Waycott and Lewis 2022).

The development of the toolkit to inform options for improving outcomes to the Coorong includes at its centre a quantitative modelling resource based on detailed hydrological, sediment, nutrient and biological components of the Coorong ecosystem (Department for Environment and Water 2022d). The approach has been to develop and apply a suite of models, referred to as the Coorong Dynamics Model (CDM), that can be used to quantify nutrient cycling and habitat changes under different environmental conditions (Hipsey et al. 2022). The biological models have taken the form of evaluating Habitat Suitability through determining the parameters that limit survival and growth. The availability of the CDM has enabled different management scenarios to be evaluated, informing options for changing the environmental conditions in the Coorong (Department for Environment and Water 2022b, Hipsey et al. 2022).

Previous studies have undertaken the first stage of developing a Ruppia Habitat Suitability Index (Ruppia HSI) that has been incorporated into the Coorong Dynamics Model (Collier et al. 2017, Hipsey et al. 2022). The Ruppia HSI required further data sets to test its accuracy and to incorporate the deeper, emerging

understanding of the dynamics of the aquatic macrophyte community in the Coorong (Asanopoulos and Waycott 2020, Hipsey et al. 2020, Mosley et al. 2020). The basis of habitat suitability includes parameters associated with hydrodynamic and other physical environmental features and compared to a series of thresholds that limit the conditions suitable for Ruppia Community growth as described in Collier et al. (2017) and updated in Hipsey et al. (2022). The application of habitat suitability evaluation using this model, alongside the Ruppia Restoration Strategy (Waycott and Lewis 2022), provide tools to inform the options available to improve the health of the Coorong ecosystem. Improving data availability to inform the Ruppia HSI model and test the model against more detailed current field data will improve modelling outcomes and enhance scenario evaluation for future management options development.

1.1 Aim

The Phase One Trials and Investigations project of the *Healthy Coorong, Healthy Basin* 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 Trials and Investigations 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 distribution and abundance of aquatic macrophytes in the southern Coorong has been measured, along with an evaluation of the environmental drivers for the performance of the Ruppia Community for broad seasonal periods (Lewis et al. 2022, Waycott and Lewis 2022). In addition, techniques to improve measuring the filamentous algal mats were developed (Auricht et al. 2019), and the seasonal variation in microbiota documented (Jamieson et al. 2022). These studies were conducted at a broad spatial and temporal scale in the seasonal cycle across the distribution of the southern Coorong region. The studies incorporated into this report aim to:

- Utilise experimental approaches to determine the growth and reproductive performance, and thresholds for both the Ruppia Community and the filamentous algal community present in the southern Coorong.
- Improve knowledge of temperature and light availability changes in the shallow water littoral zone occupied by the aquatic macrophytes of the southern Coorong ecosystem using environmental data loggers.
- Record detailed information on the phenology of the Ruppia Community across multiple monitoring locations including the life cycle of aquatic macrophytes, quantifying growth variability and application to habitat modelling used in system scale decision making, specifically the Coorong Dynamics Model.
- Evaluate the status of seedbanks, and hence inferred resilience of the Ruppia Community in the southern Coorong.
- Conduct an evaluation, through ad hoc regional surveys across a range of different regions of South Australia, for potential seed donor sites for aquatic macrophytes that could be used for translocation into the Coorong in the future.
- Document the current nutrient status of locations occupied by the Ruppia Community in the southern Coorong, including measuring the nutrient content of the water column, sediment, aquatic macrophyte and filamentous algae in the littoral zone.
- Provide comprehensive data sets for use in modelling, evaluation of current Coorong ecosystem status and monitoring (see Appendix A for data management system).
- Results from the phenology assessments were used to inform the Ruppia Habitat Suitability Index of the Coorong Dynamics Model. Provide advice, based on evidence collected throughout these investigations, on the options and strategies available to rehabilitate the Coorong ecosystem defined by a system scale desired state.

2 Aquatic macrophyte and filamentous algal growth and thresholds

2.1 Introduction

The current ecological state of the Coorong includes the annual formation of large areas of filamentous algal mats on the surface of the water in late spring that can persist well into summer (Paton et al. 2021, Lewis et al. 2022). Algal blooms can be caused by excess nutrients in an ecosystem, which can adversely affect seagrass meadows, such as the Ruppia Community, due to overlapping ecological niches and the physical structure of the seagrass plants providing a substrate for bloom formation (Tweedley et al. 2008). The relative performance of seagrasses is associated with the competition that occurs between algae and seagrass for space, nutrients and light. As algal nutrient uptake rates are typically faster than that of seagrasses, their rapidly growing biomass intercepts sunlight and reduce seagrass photosynthetic rates (Coffaro and Bocci 1997, Cummins et al. 2004, Han et al. 2016). As a result of light limitation, seagrass growth rates and meadow density decline, usually leading to contraction of the seagrass colonisation depth (Han and Liu 2014) and overall biomass in the ecosystem. Small-statured seagrass species, such as *Ruppia*, are particularly vulnerable to the shading and smothering impacts of algal bloom formation due to their limited energy stores (Holmer and Nielsen 2007) reducing the time they can persist under low light conditions.

Seasonal blooms of the filamentous green algae community comprising *Ulva paradoxa*, *Cladophora* sp. and *Rhizoclonium* sp. have become typical of the late spring and summer aquatic plant community in the southern Coorong (Paton et al. 2011, Frahn et al. 2012, Collier et al. 2017, Paton et al. 2021, Lewis et al. 2022). These studies have also found that filamentous algal blooms have been observed to directly interfere with reproductive outputs of the members of the Ruppia Community in the Coorong, i.e. *Ruppia tuberosa* and *Althenia cylindrocarpa* (synonymous name for *Lepilaena cylindrocarpa*). The loss of ecological functions supported by the Ruppia Community has been identified as a motivation for system-scale intervention through restoration. The key functions associated with this were summarised by (Brookes et al. 2018):

> *"The decrease in abundance and distribution of aquatic plants from the South Lagoon is a reduction of food and habitat for waterbirds" (Paton et al. 2017a).*

> *"It is the experience globally that a lack of aquatic plants in wetlands leads to high nutrient availability, which then encourages algal growth" (Collier et al. 2017).*

> *"Aquatic plants utilise nutrients as they grow and so reseeding areas of the system may also help address reducing available nutrients" (Collier et al. 2017).*

> *"Ruppia tuberosa populations are currently unable to complete their life-cycles due to competition with filamentous algae" (Paton et al. 2017a).*

Despite occurring as a natural component in regional estuarine environments (Womersley 1975, Collier et al. 2017), these filamentous green algae are rapidly-growing, opportunistic species that typically have a high demand for nitrogen (Lavery and McComb 1991, Pedersen and Borum 1996). Indeed, nitrogen was found to be the primary limiting factor both *in situ* in the Coorong and under experimental conditions to test plant responses to salinity (Collier et al. 2017). Filamentous algal blooms dominated by *Ulva* are dependent on a constant high supply of nutrients (Coffaro and Bocci 1997) and nutrient reduction is a primary management option for preventing bloom formation. It is plausible that phosphorus may also become limiting under high nitrogen concentrations (Teichberg et al. 2010). Globally, the presence of filamentous algal blooms are often symptomatic of excessive system-scale nutrient loads (Nelson 2017). In addition to the high nutrient conditions, the extreme hypersalinity experienced in the southern Coorong during the growing period means few comparative data from elsewhere are available for predicting growth responses to varying conditions (e.g. Collier et al. 2017).

Previous research has been applied to the development of a Habitat Suitability Index (HSI) for *Ruppia* in the Coorong that includes the parameterisation of filamentous algal growth rates and distribution as a modifier

of *Ruppia* survival (Collier et al. 2017). In this model it was identified analytically that there is a critical role of filamentous algae for determining the success of seagrass community development. The 'Coorong Dynamics Model' for the Coorong (Hipsey et al. 2020, Hipsey et al. 2022) refines the hydrological, geological and chemical parameters of the Coorong to enable reconstruction of field measured characteristics such as flow rates or water levels. Biological parameters such as the growth of filamentous algae and phytoplankton are also included in the model and this chapter provides additional data for the improved development of this model that is used to inform decision making for rehabilitation and restoration of the Coorong (Hipsey et al. 2022). Experimental data have been collected to improve this model (e.g. Waycott et al. 2019) and in addition, a new approach to understanding the formation and growth of the filamentous algal community in the Coorong is identified, along with trials in the field undertaken to assess the viability of physical removal of filamentous algae that may contribute to direct reduction in the nutrient load of the system. Extended analyses of some previous experiments were conducted and supplemented by field based measures of filamentous algal and Ruppia Community productivity, improving their application to the Coorong Dynamics Model.

2.2 Methods

2.2.1 Study area

The focus area of this study was the southern Coorong at locations specific to each experiment or data set (Figure 1), between Long Point and Salt Creek. A number of sites were included in data collection activities in other studies (Waycott et al. 2019, Jamieson et al. 2022, Lewis et al. 2022, Waycott and Lewis 2022) enabling future comparisons to be conducted. To enable such comparisons, where possible, samples, data loggers and *in situ* experiments were conducted at one or more of the following sites; Noonameena (site 29), North Magrath Flats (site 30), Parnka Point (site 24), Villa de Yumpa (site 22), Woods Well (site 17), Policeman Point (site 14), Wild Dog Island (site 10) and a saline lake, to the South of Salt Creek, which is isolated from the main water body of the Coorong, Lake Cantara (LC) (Table 1).

2.2.2 Experimental, mesocosm based growth responses of the Ruppia Community to shading

Materials

Plant material was harvested as cores taken from Lake Cantara (LC), south of the Coorong (-36.331545°S 139.745409°E) (refer to Collier et al. 2017), a site that was previously utilised as the donor site for *Ruppia tuberosa* restoration in 2012–2014 (Waycott and Lewis 2022). Cores were collected by inserting a 150 × 75 mm PVC pipe into the sediment to a depth of at least 6 cm (below the root zone). Cores were stored in PVC pipe and placed in large plastic bins covered with a wet hessian bag to prevent desiccation whilst in transit.

Ruppia tuberosa and *Althenia cylindrocarpa*, along with another species of as yet unresolved *Ruppia*, grow in a mixed community (the Ruppia Community) in the Coorong and Lake Cantara, and are essentially indistinguishable when not flowering (see Lewis et al. 2022). This report also builds on experimental results published previously (i.e. Collier et al. 2017) by undertaking additional extended analysis of these data and incorporating additional information.

The experimental setup undertaken during previous data collection (2016–2017) (Collier et al. 2017) included cores of the Ruppia Community translocated into mesocosms based at the South Australian Research and Development Institute (SARDI) West Beach facilities in identical, open air tanks. Extracted cores were 'planted' in 25 × 25 × 10 cm square pots lined with polyester fabric to allow water flow but prevent sediment loss. Cores were surrounded by sand at a density of six cores per pot. The experimental pots containing translocated cores were kept in 1000 L tanks under natural light and temperatures, and the salinity that initially matched that of the water they were collected from (18 g L^{-1}, August 2016). From there, salinity was

slowly increased until seawater salinity was reached (35 g L^{-1}). Thereafter the tanks were kept in seawater on a flow-through system that exchanged the water in each tank approximately twice per day.

Experiments were conducted under two salinities (i.e. (1) marine salinity (35 g L^{-1}), and (2) high salinity (80 g L^{-1})) and six light treatments representing the gradient of salinity plants exposed to during their growth season up to fruit set. Six light treatments were applied by placing a wire dome shaped frame over each pot and attaching shade cloth (Figure 3). The light treatments were generated using different densities of shade cloth resulting in 100%, 86%, 63%, 38%, 16% and 5% of full sunlight. Shading treatments were conducted to identify ecologically significant thresholds leading to changes in the morphology or abundance of the Ruppia Community. Due to limitation in the number of experimental tank units, the marine salinity experiment was conducted first, September–November 2016, across five replicate tanks, each tank contained two replicates of each light treatment (n = 10) and the experiment was run for 8 weeks. The salinity was then increased over a period of 7 days, then the high salinity experiment was conducted over 4 weeks (November–December 2016). Shoot and flower density were counted in each pot once a week for 8 weeks (35 g L^{-1}) and 4 weeks (80 g L^{-1}). Leaf length of 10 randomly selected leaves was measured (mm) from the base of the leaf. After 8 weeks one replicate of each light treatment was destructively harvested and the above-ground biomass was dried at 60ºC for 48 hours and weighed. As an additional measure, fresh leaf samples were harvested from each treatment for microscopic evaluation of leaf width (µm) and leaf cell length for 10 cells in the lower section of the leaf (i.e. emerging from leaf sheath and most recently formed). Statistical analysis was conducted using SPSS™ v28.0.1.0, applying Bayesian ANOVA at 95% confidence interval.
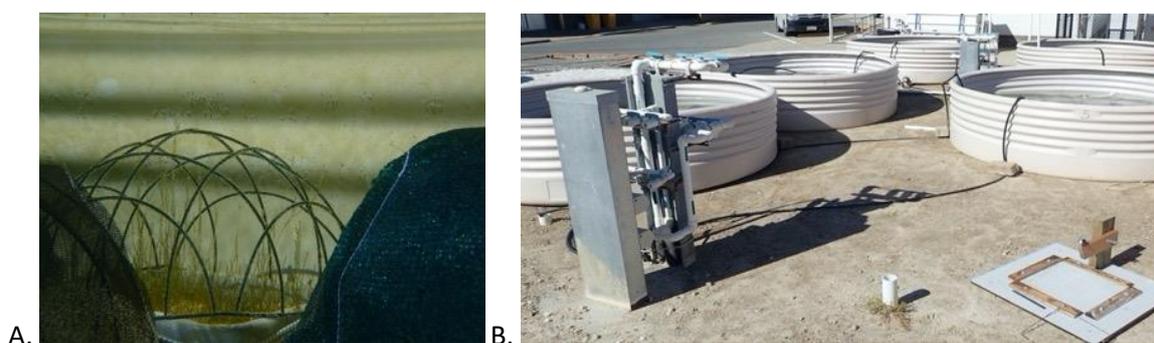
Figure 3. A. Growth experimental units in shallow open air tanks, each unit with different shading exposure. Central unit shows the zero shading (control) treatment. B. Outdoor experimental tank setup at SARDI, filtered seawater system, low-sided 1000 L tanks with aeration and circulation.

2.2.3 Local and landscape scale growth of filamentous algal mats and trials of their physical removal

2.3 Methods

Field observations on the presence of filamentous algae were made whenever samples were taken from field sites from September 2019 to December 2021 (see Auricht et al. 2019, Asanopoulos and Waycott 2020, Lewis et al. 2022). Detailed observations of algal growth were made at six sites (Table 1); Wild Dog Island (site 10), Policeman Point (site 14), Villa de Yumpa (site 22), Parnka Point (site 24), North Magrath Flats (site 30), Noonameena (site 29) associated with Ruppia Community and Microbial Community repeat sampling (Chapter 3; Jamieson et al. 2022; Lewis et al. 2022). In addition, more detailed survey data were collected for remote sensing validation analysis on 20 December 2019 in the Needles Island section of the central Coorong (Figure 4). Across this area, validation surveys of the Ruppia Community and filamentous algae were undertaken through the central Coorong (Figure 1; North of Parnka Point to The Needles) using a hovercraft launched from 35°50'5.74"S 139°21'22.06E (Table 2, Figure 4).

Table 1. Sampling and observation locations where plant material was collected, or observations made, for detailed investigations.

SITE	LATITUDE °S	LONGITUDE °E
Noonameena (site 29)	-35.755899	139.261625
North Magrath Flat (site 30)	-35.852757	139.385565
Parnka Point (site 24)	-35.902333	139.398445
Villa de Yumpa (site 22)	-35.909933	139.452095
Policeman Point (site 14)	-36.058623	139.586122
Salt Creek (Wild Dog Islands, site 10)	-36.120136	139.636639
Lake Cantara (LC)	-36.331545	139.745409

Samples for filamentous algal biomass estimation were taken using a plastic tube with a diameter of 400 mm (0.125 m^2). 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 inside the water inside the tube and put into a mesh bag for drying. Algae were dried at 60°C for 48 hrs and then weighed.

Table 2. Specific sites referred to in validation field survey, listed north to south, plotted on Figure 4. Notes associated with specific locations are included clarifying site characteristics.

SITE CODE	LATITUDE AND LONGITUDE (DECIMAL DEGREES)	FIELD NOTES
Val1	S35° 51.041' E139° 21.435'	Ankle deep water - algae everywhere on surface. *Althenia* flowers caught in the algae on the surface, only a few *Ruppia* flowers.
Val2	S35° 51.040' E139° 21.435'	Channel approximately 1.2m deep, sandy bottom no seagrass or algae.
Val3	S35° 51.126' E139° 21.395'	Dense *Ruppia* and *Althenia* covered in algae but not on the surface, water is very murky.
Val4	S35° 51.121' E139° 21.404'	Large mats of algae on the bottom on the Coorong side of the channel. On the ocean side of the channel there is a large algal mat on the surface.
Val5	S35° 51.121' E139° 21.392'	Channel with hard clay like sediment on the bottom, no *Ruppia* or *Althenia* growing.
Val6	S35° 51.360' E139° 21.806'	Black sediment ooze on edge of algal mat which is on the surface and seagrass patches.

Preliminary physical removal trials were conducted at two sites, North Magrath Flat (site 30) and Parnka Point (site 24) in October 2019. Larger areas of accessible algal mat formation were observed at North Magrath Flat and this site was chosen to conduct more extensive removal trials. A series of hand-held apparatus were used to evaluate the efficiency of algal removal and the potential limitations to their application. Aerial survey by drone of the areas cleared 3 weeks after the removal actions was used to monitor outcomes.
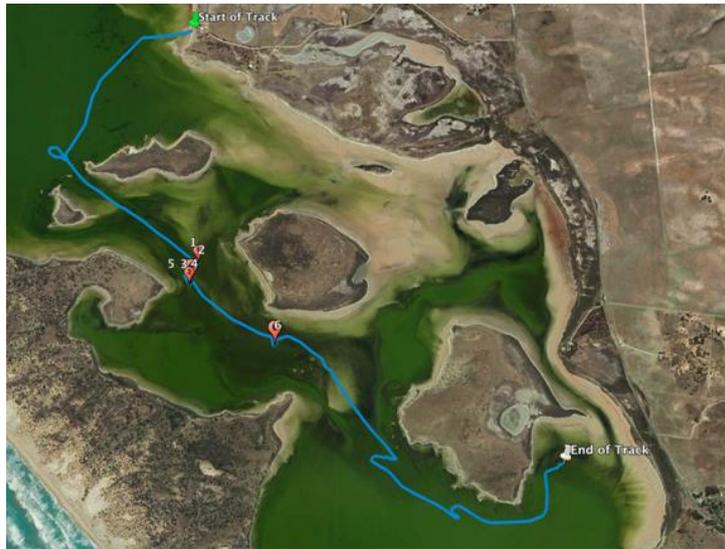
Figure 4. Remote sensing image of the northern section of the central Coorong, north of Needles Island (35°50'5.74"S 139°21'22.06") to south of Rabbit Island. The blue path visualises the GPS recorded track of the survey followed for undertaking field validation of algal mat production. Locations where sediment and plants were observed (red numbered markers) relate to specific site data and notes recorded in Table 2. Note dark areas in the water are a sparse Ruppia Community densely covered in filamentous algal growth in early summer.

2.3.1 Growth rates of algae in experimental culture

Mesocosm based experimental testing of filamentous algal mat growth rate for resistance to salinity had previously been undertaken to develop salinity thresholds (Collier et al. 2017) and methods therein. Based on these data a net daily growth rate for biomass was calculated to provide an estimate for comparative purposes.

2.3.2 Environmental data using shallow short term deployed loggers and *in situ* photosynthetic efficiency

Data loggers

Light loggers (HOBO) were attached to a stake and placed 50–100 mm from sediment, facing north at an angle of approximately 20 degrees. Temperature loggers were attached to a stake as close as possible to the sediment interface and were deployed from October 2019 at Salt Creek, Policeman Point and North Magrath Flat, see Table 3 for deployment dates at other sites. Individual units were cleaned during site visits and replacement units placed Loggers were deployed for periods described above.

Data collected from loggers were processed, trimmed and aligned to sequential periods of deployment. Temperature logger data were collected at 15 minute intervals. Light logger data were collected at 30 minute intervals, and these values were used to calculate daily average illuminance (kilolux) of daylight hours where daylight hour values are those over 0.4 kilolux (approximately the illuminance at sunrise/sunset).

Table 3. Locations across the Coorong where environmental monitoring loggers were deployed and periods of deployment.

SITE	LATITUDE	LONGITUDE	LOGGER TYPE	START DATE	END DATE
Salt Creek	-36.120183°	139.636667°	Temperature	October 2019	January 2022
			Light	October 2021	January 2022
Seagull Island	-36.079583°	139.599700°	Temperature	March 2021	February 2022
Policeman Point	-36.058700°	139.585733°	Temperature	October 2019	January 2022
			Light	September 2021	January 2022
Jack Point	-36.031817°	139.568883°	Temperature	March 2021	November 2021*
Woods Well South	-36.007967°	139.554850°	Temperature	March 2021	February 2022
Villa de Yumpa	-35.911405°	139.450225°	Temperature and light	November 2021	February 2022
Parnka Point	-35.902335°	139.398935°	Temperature and light	November 2021	January 2022
North Magrath Flat	-35.853017°	139.384500°	Temperature	October 2019	February 2022
			Light	October 2021	February 2022

*battery dead and data incomplete

Photosynthetic efficiency (PAM)

Field based measurement of photosynthetic efficiency measured as the effective quantum yield of photosystem II was conducted using a Diving Pulse Amplitude Modulation (PAM) fluorometer (WALZ GmBH) following Collier et al. (2017). Effective quantum yield ($\Delta F/F_m'$) indicates the efficiency that the photosynthetic apparatus can convert sunlight into chemical energy for carbon fixation, and ultimately growth. Replicate measurements were taken for each comparison of Ruppia Community $\Delta F/F_m'$ for plants (leaves) at four sites and where available (Table 4); surface algal mats, basal filamentous algal clump (no Ruppia Community) and Ruppia Community with basal filamentous algal clumps. This experiment was conducted at Noonameena, North Magrath Flats, Woods Well and Salt Creek (site 10) on 23 November 2021 in water depths of 0.25 m to 0.32 m (Table 4).

Table 4. Locations and site characteristics in the Coorong where measurements of photosynthetic efficiency was conducted on 23 November 2021 using a diving PAM fluorometer.

SITE	LATITUDE °S	LONGITUDE °E	TEMPERATURE °C	WATER DEPTH M	CATEGORIES MEASURED	NO. REPLICATE MEASUREMENTS
Noonameena	-35.754847	139.262	18.87	0.25	1 Ruppia Community	27
					2 Ruppia Community plus basal algal clump	13
					3 Basal algal clump alone	22
					4 Surface algal mat	15
North Magrath Flat	-35.852369	139.385	18.36	0.2	1 Ruppia Community	20
					2 Ruppia Community plus basal algal clump	20
					4 Surface algal mat	15
Woods Well	-35.99401	139.537	18.49	0.3	1 Ruppia Community	28
Salt Creek (site 10)	-36.119919	139.638	19.86	0.32	1 Ruppia Community	24

2.4 Results

2.4.1 Experimental, mesocosm based growth responses of the Ruppia Community to shading

The comparison of overall performance in mesocosm trials of translocated material of the Ruppia Community indicated that the plants grew more vigorously (shoot counts and biomass) under marine salinities than high salinities ($p < 0.05$), despite no difference in mean leaf length (Figure 5) ($p = >0.05$). Under marine salinities, the biomass of shaded Ruppia Community was significantly affected when shading was reduced to 16% surface light ($p < 0.01$) (Figure 6 A.). Interestingly, the translocated cores exhibited significant growth of charophytes (*Lamprothamnium* sp.) when placed in clean, lower salinity water, and sufficient growth was harvestable at the conclusion of the marine salinity shading experiment to determine that it had significantly reduced biomass when shading was reduced to 16% surface light (Figure 6 B.) ($p < 0.05$).

The growth responses to shading of the Ruppia Community aquatic macrophytes in mesocosms at two salinities (Figure 7) saw a significant reduction in shoot counts at higher salinity ($p < 0.01$) (Figure 7 A.), and a reduction in shoot length at marine salinity at full sunlight (Figure 7 B.) (due to increased exposure to light) and higher biomass (Figure 7 C.) although not significantly. Comparing the results from the marine salinity 8 week treatment alone, under significant light reduction (<16% full sun) shoot density and biomass significantly differed in their responses: shoot density increased at lower light exposure and biomass decreased under lower light. At the end of the 8 week experiments, the treatments that were significantly different to higher light treatments, 16% and 5% of full sunlight, mean biomass was ~70% of the full sun treatments for 16% and 5% full sunlight were ~50% mean biomass of the full sun.
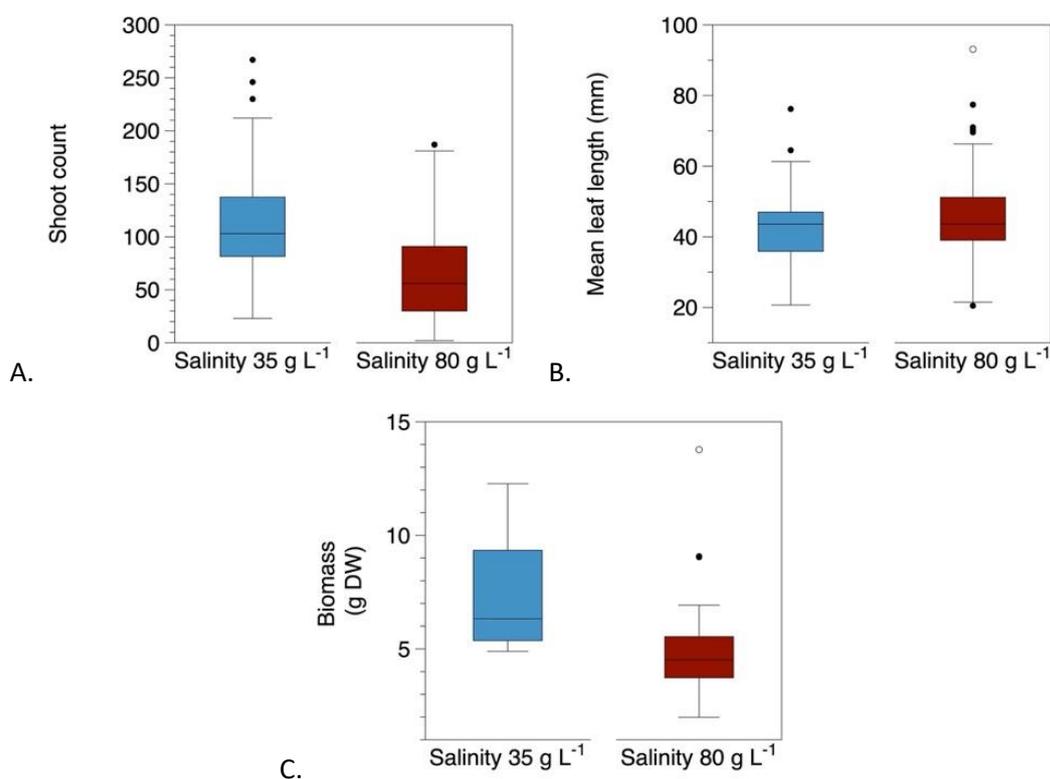
Figure 5. Box plots summary results of experimental testing of the growth responses of Ruppia Community mesocosm at two salinities 35 g L^{-1} (blue) and 80 g L^{-1} (red) (all shading treatments combined). A. shoot number, B. leaf length and C. biomass.

A.

B.
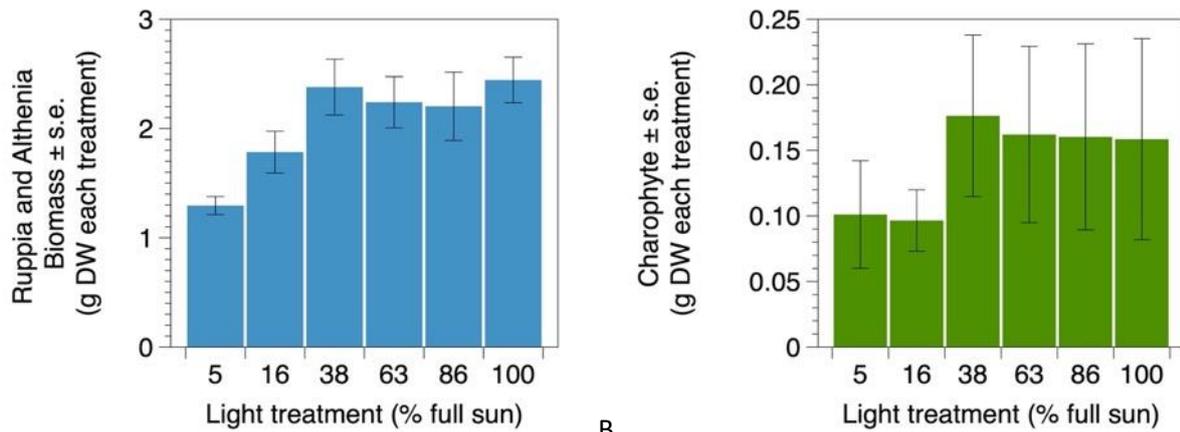
Figure 6. Response of A. Ruppia Community biomass (g DW) and B. Charophyte Biomass (g DW) when grown under different levels of mesocosm based shading at salinity 35 g L^{-1} over an 8 week period.

A.

B.

C.
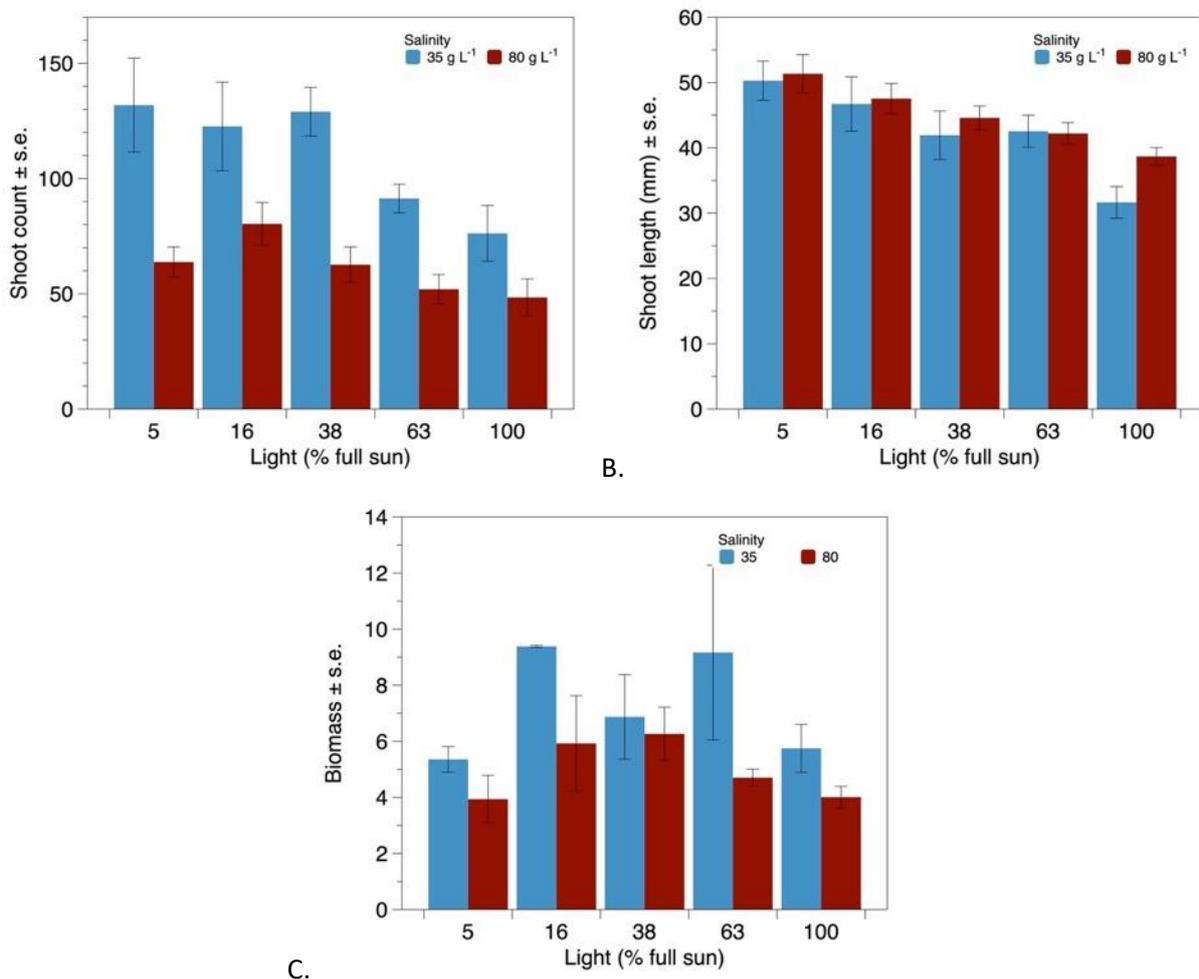
Figure 7. Growth responses to shading of translocated cores of Coorong Ruppia Community based on A. mean shoot number (per treatment), B. mean shoot length and C. biomass growth responses to different levels of shading and salinity.

The translocated cores produced flowers in the mesocosms during the 4 week salinity comparison experiments, the response of *Althenia cylindrocarpa* was to continue producing flowers throughout the experiment in no discernible pattern (Figure 9 A.). *Ruppia tuberosa* only produced flowers in the first week of the trials, perhaps indicating flowering was limited in the cores collected due to experimental treatment conditions being relatively mild and vegetative growth dominated (Figure 9 B.).

Leaf widths did not exhibit any discernible pattern except being narrower at full sunlight although not significantly so ($p < 0.08$) (Figure 10 A.). At the cellular level, shading stimulated a significant increase in cell length under the lowest light treatment ($p < 0.01$) but only at marine salinity (Figure 10 B.). There was no discernible pattern in the cell lengths for the hypersalinity treatment (Figure 10 B.).
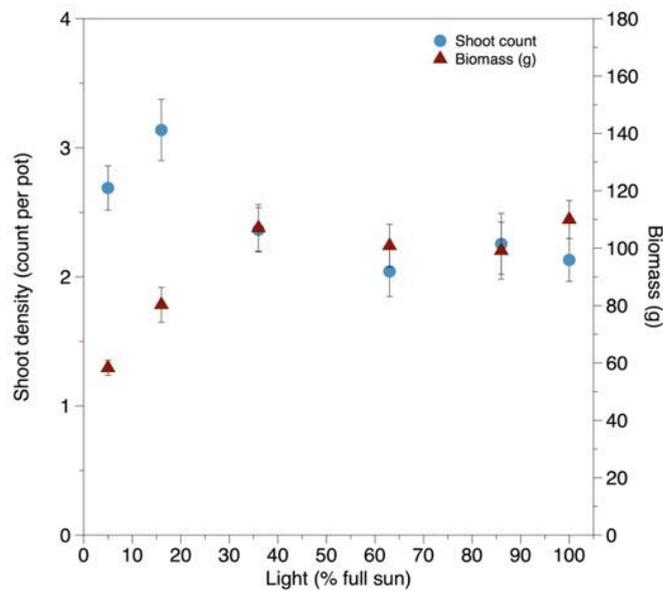
Figure 8. Biomass and shoot count of Ruppia Community translocated cores after 8 weeks of exposure to shade treatments ranging from 5 to 100% of full sunlight (n = 10 ± S.E.) (adapted from Collier et al. 2017).
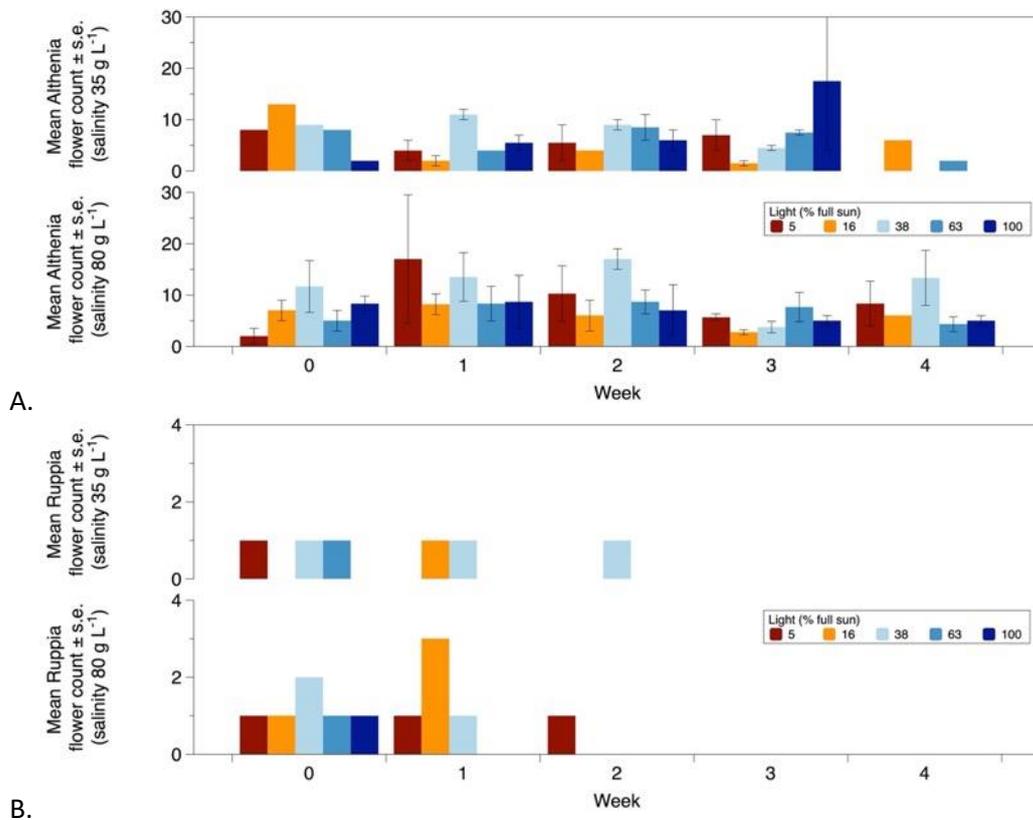
Figure 9. Plots of Mean ± s.e. Ruppia Community flowering frequency for A. _Althenia cylindrocarpa_ (number of flowers) and B. _Ruppia tuberosa_ (number of inflorescences) grown under different shading conditions (legend, proportion of surface light) and salinity (upper panel 35 g L^{-1}, lower panel 80 g L^{-1}). Note, no error due to occurrence in single pot.
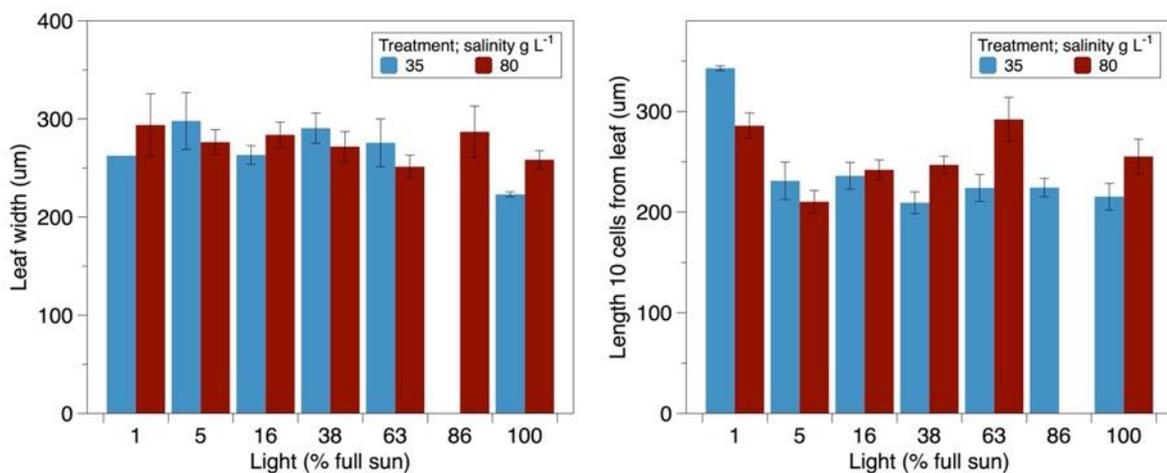
Figure 10. Leaf width and cellular level growth responses to shading of plants growing in translocated cores of Coorong Ruppia Community based on A. mean shoot number (per treatment) and B. mean shoot length growth responses to different levels of shading and salinity.

2.4.2 Establishing the filamentous green algal community life cycle

The interaction between the mat forming filamentous green algae and the Ruppia Community was observed as a strong direct relationship. Filamentous algae were found attached to Ruppia/Althenia plants at all field sites sampled from September-December 2019. In the surveys conducted by Lewis et al. (2022) from October 2020–December 2021, filamentous algal mat formation was observed to be associated with the Ruppia Community at 112 of the 114 sites surveyed. The two sites surveyed without the Ruppia Community had substrates unfavourable to growth, such as rough, rocky benthic cover. Observations made during the survey in December 2019 (Figure 4, Table 2) determined that later in the growing season, once salinity levels and growth rates increase due to increasing temperatures (Waycott et al. 2019), the algal mats begin to collapse, remaining associated in some part with their substrate. This algal mat biomass then degrades and in the conditions present at site Val1, Val3, Val6, (Table 2) they start to form murky, black organic oozes (Figure 12).

Initial observations were used to develop the scoring matrix for broader field surveys (Table 5). These observations were able to confirm a consistent algal mat development life cycle for filamentous algae observed across the Coorong. This life cycle included the following components (refer to Figure 11 for illustration) noting the dominant substrate are plants of the Ruppia Community:

1. Predominantly as water levels rise early in the growing season, filamentous algae appear to start growing attached to benthic substrate (typically the Ruppia Community). Early stages are only visible as strands or small clumps at sediment surface.

2. Filamentous algae form conspicuous clumps and grow to envelope the substrate above the sediment.

3. Filamentous algal clumps begin filling the water column, at this stage the clumps are not yet reaching the water's surface.

4. Filamentous algae now reach the water's surface, attached to the substrate but filling the water column, spreading over the surface forming surface mats which fully obscure the Ruppia Community below.

5. The algal mat spreads beyond the immediate area of benthic substrate it was initially attached to. The mat can cover 100% of the water surface and is at this stage weakly attached to its substrate. These mats become detached with minimal energy such as high winds.

Table 5. Filamentous algal scoring matrix used in Lewis et al. (2022) based on observations of the proposed seasonal lifecycle for the formation of surface mats of filamentous green algae in the Coorong.

ALGAL SEVERITY SCORE	DESCRIPTION OF ALGAL FORM AND QUANTITY	CATEGORY	REFERENCE TO PANEL IN FIGURE 11 PHOTOGRAPHS
1	No filamentous algae observed in area sampled	*none*	-
2	Only as drift (i.e. floating on surface, unattached)	*drift*	-
3	Attached to sediment only (not attached to plants)	*sediment*	-
4	Attached to plants at sediment level (basal)	*basal*	E. & F.
5	Attached to plants in water column (not just basal)	*column*	-
6	Attached to plants in water column, obscuring plants	*obscuring*	D.
7	Attached to plants as a surface mat	*surface*	B. & C.
Other	Drift on shoreline, exposed	other	A.
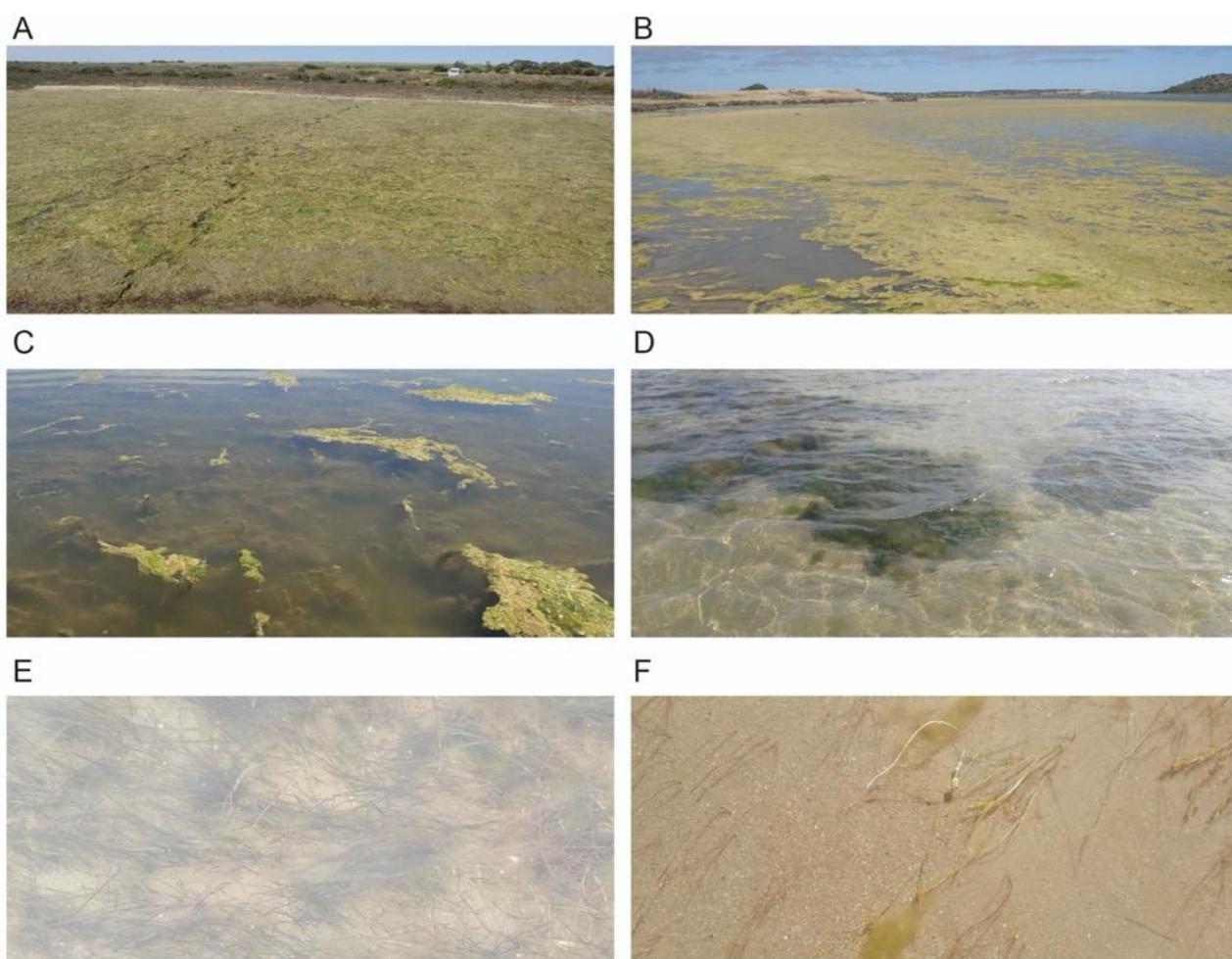
Figure 11. Different stages of filamentous algal mat formation and release in the Coorong November 2020–December 2021: A. mounded wrack of filamentous algae drift (near Noonameena); B. Dense cover of surface mat (Parnka Point); C. Patchy surface mat late in growing season, dense dead algal mat below water surface (near The Needles); D. Evidence of attached filamentous algae growing over the top of the Ruppia Community but below the water surface; E. Small basal clumps of algae attached to Ruppia Community plants; F. Sparse algal clumps forming or catching onto Ruppia Community plants (*Althenia* flowering seen here and drift *Ruppia* flowers).

Figure 12. Algal mat degradation forming black sediments validation survey site Val6 (Table 2, Figure 4).

2.4.3 Growth rates of algae in experimental culture

Mesocosm based experimental testing of filamentous algal mat growth rate across a broad salinity range at standard conditions (Collier et al. 2017) estimated that a mean maximum daily growth in biomass of 25.6 ± 2.0 (% mg DW day^{-1}) at the lowest salinity (10 g L^{-1}) and negative growth (i.e. loss of biomass) at salinities of 90 g L^{-1} and higher to maximum daily loss of -18.7 ± 1.3 (% mg DW day^{-1}) at 150 gL^{-1} (Table 6, Figure 13).

Table 6. Filamentous algal mat net change in biomass per day (% mg DW day^{-1}) for mesocosm based experiments to test salinity tolerance (Collier et al. 2017).

SALINITY (G L^{-1})	MEAN ALGAL GROWTH RATE % PER DAY (% MG DW DAY^{-1})
10	25.6 ± 2.0
30	20.6 ± 4.0
40	24.9 ± 2.9
50	8.6 ± 1.3
60	9.2 ± 4.1
70	1.5 ± 2.9
80	2.2 ± 3.6
90	-5.5 ± 2.6
100	-7.3 ± 1.3
110	-6.6 ± 1.2
120	-7.9 ± 1.2
130	-12.3 ± 1.9
140	-15.7 ± 1.6
150	-18.7 ± 1.3
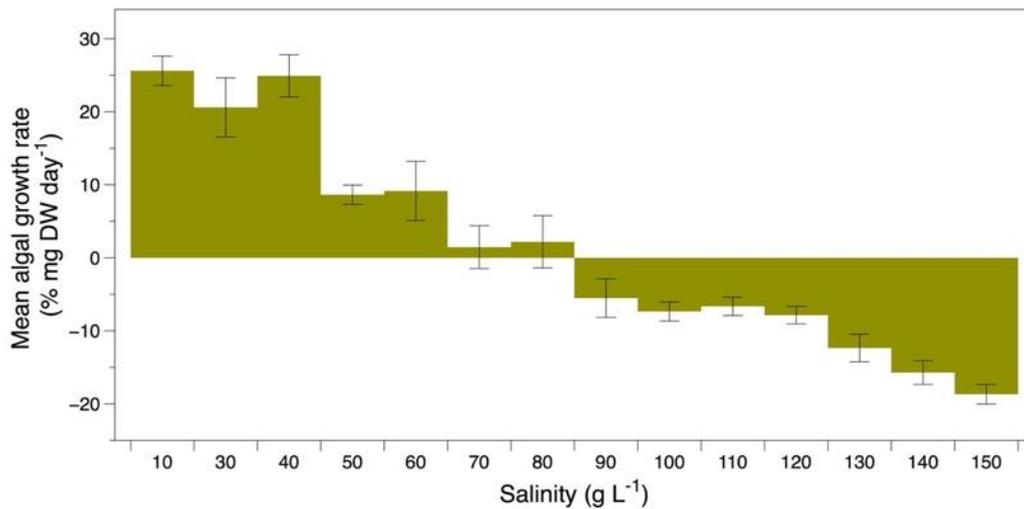
Figure 13. Plot of filamentous algal mat net change in biomass per day (% mg DW day⁻¹) for mesocosm based experiments to test salinity tolerance (Collier et al. 2017).

2.4.4 Removal trials of the filamentous green algal mats

Removal of algal mats once they reach the water's surface (e.g. Figure 14) in a general sense was relatively straight forward although disruptive to the site for all three different types of manual algal mat removal applied.

Option 1: single person algal removal by scooping

This method was immediately dismissed, as were other options based on a single person needing to carry algae any distance. The volume and weight of scooped algae was very high and anything other than applying this to a 1-2 m² scale would be unmanageable.

Option 2: two person mesh covered frame

The application of a mesh frame was relatively straight forward, although more difficult in water greater than 1 m deep. The fine lightweight nylon mesh over a poly-pipe frame and secured with zip-ties was very manageable and could scoop up and relocate the algae easily (Figure 15).

Option 3: two person pulling rod

An approximately 3.5 m long metal rod pulled by lightweight acrylic rope threaded through the centre of the rod was easy to handle and removed the larger area more rapidly and easily (Figure 16).

Of the two field removal options implemented (i.e. options 2 and 3), option 3 (the pulling rod) was the most efficient. The algal removal trial site (North McGrath Flats, site 30) had a large expansive area of algae to undertake trials that would not interfere with each option being tested. The trials consisted of clearing approximately 0.5 ha of algae with hand held apparatus after having taken reference samples of the algal biomass, *Ruppia* shoot density and scoring the presence of flowering *Ruppia* or *Althenia*. Follow up shoot counts were made approximately 1 month later (Figure 17). The algal mat biomass removed was transported to the shoreline where it was left to decay with the large quantities of already stranded algal mat material.

Figure 14. Algal removal trial location at North McGrath Flats (site 30), where the majority of algal removal trials were conducted 1–2 October 2019 facing deeper water areas showing continuous algal cover.

A.   B.

Figure 15. Algal removal trial for method in option 2, two person mesh covered frame, at North McGrath Flats (site 30) 1–2 October 2019. A) Mesh frame scooping. B) Biomass of algae scooped in one 50 m pass.

Figure 16. Algal removal trial using method in option 3, two person pulling rod trialled 1–2 October 2019.

Using aerial imagery provided by local Coorong community member Geoff Gallasch, it is clear that three weeks after the clearing there was still evidence of the larger cleared area generated by option 3 (Figure 17). The option 2 area appeared to have been re-covered by patchy surface mat algae. A wind event shortly after the aerial drone survey resulted in more than 50% of the surface algae mats being 'blown' away from the site, likely landing on the shoreline where algal mat deposits become stranded and dry out, creating a barrier to the sediment.
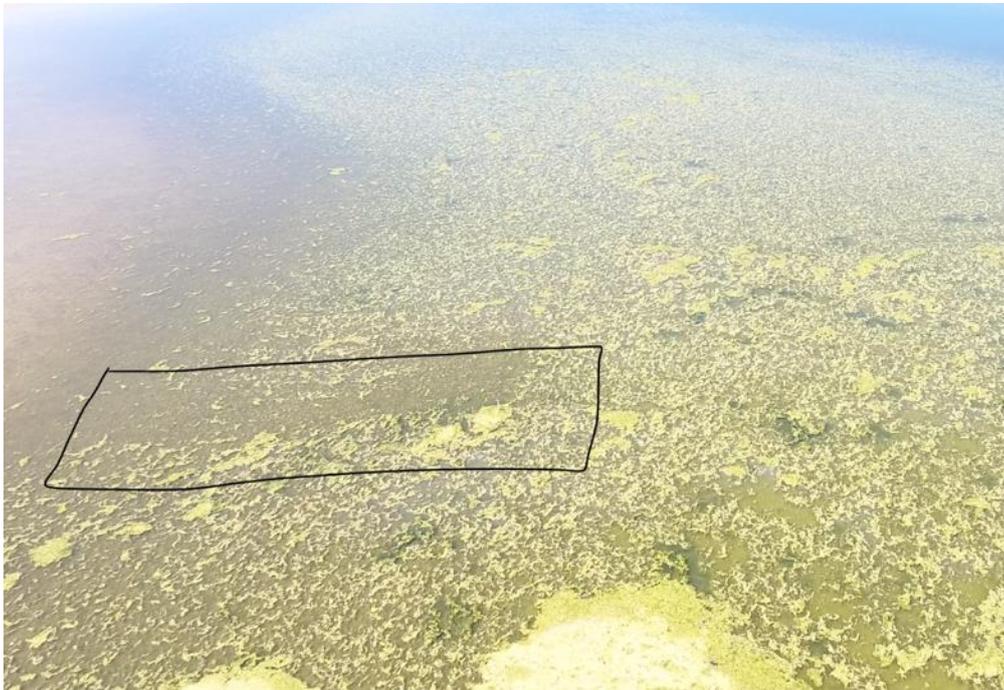
Figure 17. Aerial image of the algal removal trial area for option 3 at North McGrath Flats (site 30). The image was taken on 22 October 2019, with the black box indicating the approximate area cleared by the pulling rod method on 2 October 2019. Imagery provided for use with permission by Geoff Gallasch.

2.4.5 Logger data – variation in temperature and light

Temperature

Temperature loggers deployed at up to eight sites in shallow water depths (< 0.25 m in October–November 2019, i.e. spring) showed strong seasonal temperature regime (Figure 18), with warmer temperatures associated with spring–autumn months. As water levels drop, loggers detect stronger daily variation in temperature, due to warmer air temperatures and shallower water or exposure. Months of the year where temperature loggers recorded > 22°C to 30°C were during spring and summer months, coincident with the presence of algal blooms. Maximum temperatures were highest at the Salt Creek (site 10) and Policeman Point locations, both areas having broad shallow areas with shallow depth over the warmer months. The temperature range varied between 22°C and 44°C at different sites (Table 7). Three sites (Salt Creek, Policeman Point and Villa de Yumpa) exceeded a relatively high temperature resistance threshold value of 43°C for seagrass leaves (Figure 19) (compared to; Moore et al. 2013, Collier and Waycott 2014, Rasmusson et al. 2020). Temperature data is being incorporated into the Ruppia Community Habitat Suitability Index (Hipsey et al. 2022) model to evaluate the importance of temperature to plant habitat suitability.
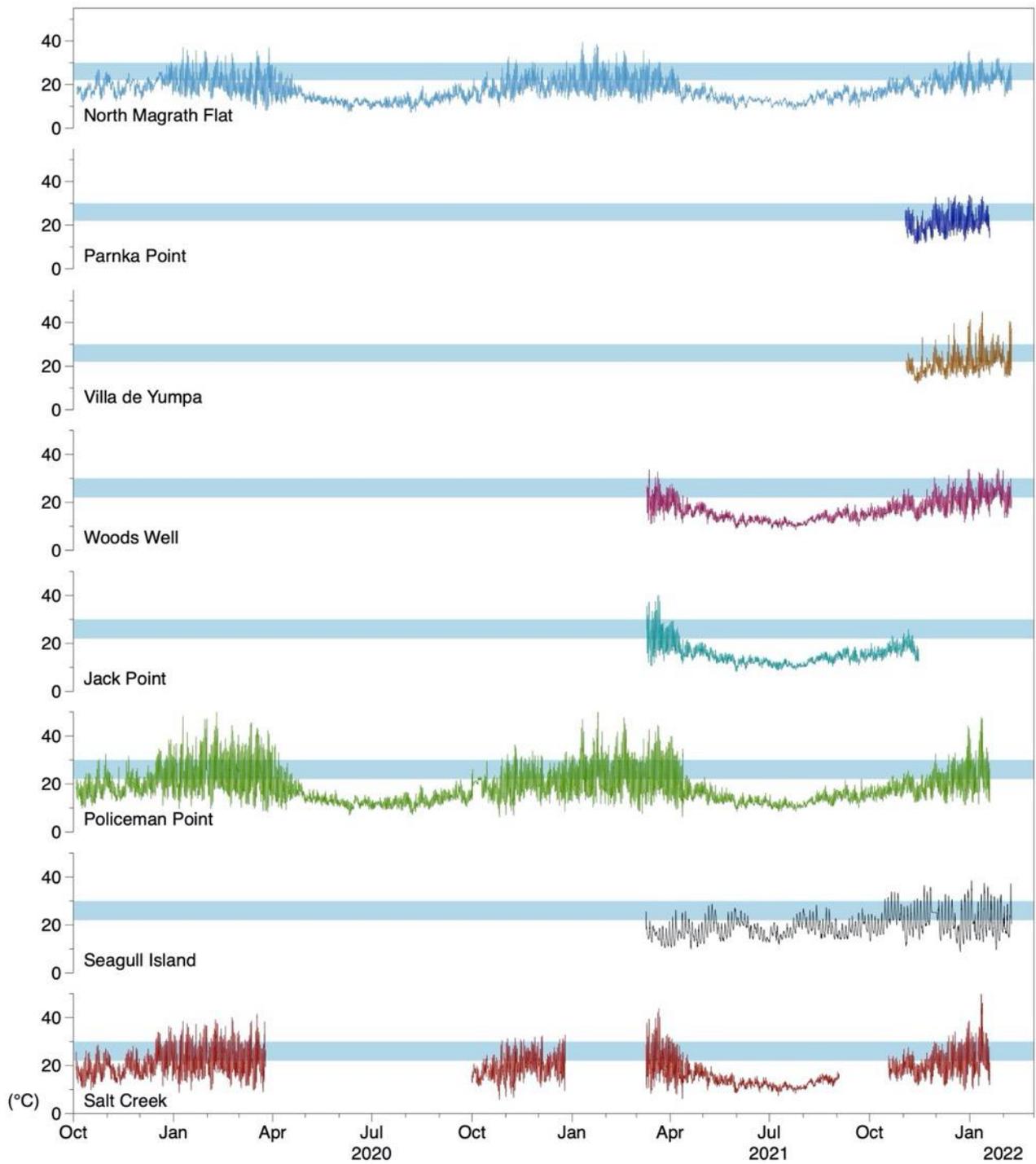
Figure 18. Temperatures measured using HOBO MX2201 Temperature or MX2202 Light/Temperature loggers in shallow water (location data Table 3). Blue band highlighting the 22°C–30°C temperature range across all sites.

Table 7. Summary of data recorded by temperature loggers over the periods deployed (see Table 3 for site location and deployment details; °C).

SITE	SITE NO.	MIN °C	MAX °C	RANGE °C	AVERAGE °C	SE
Salt Creek	1	5.79	50.02	44.23	18.81	0.02
Seagull Island	2	8.58	40.41	31.83	16.48	0.03
Policeman Point	3	6.26	50.96	44.70	18.71	0.02
Jack Point	4	8.15	40.07	31.92	15.06	0.03
Woods Well	5	8.49	34.27	25.78	16.55	0.03
Villa de Yumpa	6	12.01	45.04	33.03	21.38	0.07
Parnka Point	7	11.45	33.8	22.35	20.71	0.07
North Magrath Flat	8	6.99	39.47	32.48	18.01	0.01
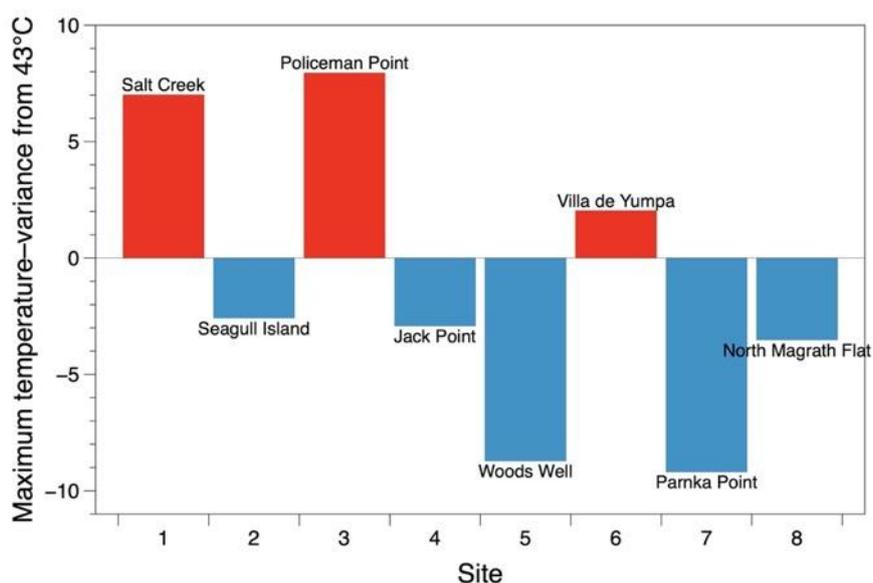
Figure 19. Site maximum values recorded by temperature loggers over the periods deployed (see Table 3 for site location and deployment details; °C) compared to a threshold of 43°C for seagrass leaf survival. Positive values indicate the site maximum values recorded exceeded 43°C.

Light

Daily average illuminance (kilolux) was seasonally variable, with mid-summer reaching full sunlight, likely due to the exposure of loggers to very shallow water or even periods of exposure to air (Figure 20). Two sites, North Magrath Flat and Parnka Point experienced light levels below the threshold estimated in experimental shading of Ruppia Community in mesocosms for multiple, periods of varied duration (< 16% full sunlight); November–December, North Magrath Flat, and December–January, Parnka Point (Figure 20). These levels for the period observed would have likely negatively impacted the Ruppia Community. These observations of light levels over the Ruppia Community are being incorporated into the Ruppia Community Habitat Suitability Index (Hipsey et al. 2022) model to improve the ability of the model to reflect growth of the Ruppia Community and filamentous algae based on the site conditions, in particular light availability, a primary limiting factor to growth, as recorded in the field in this study.
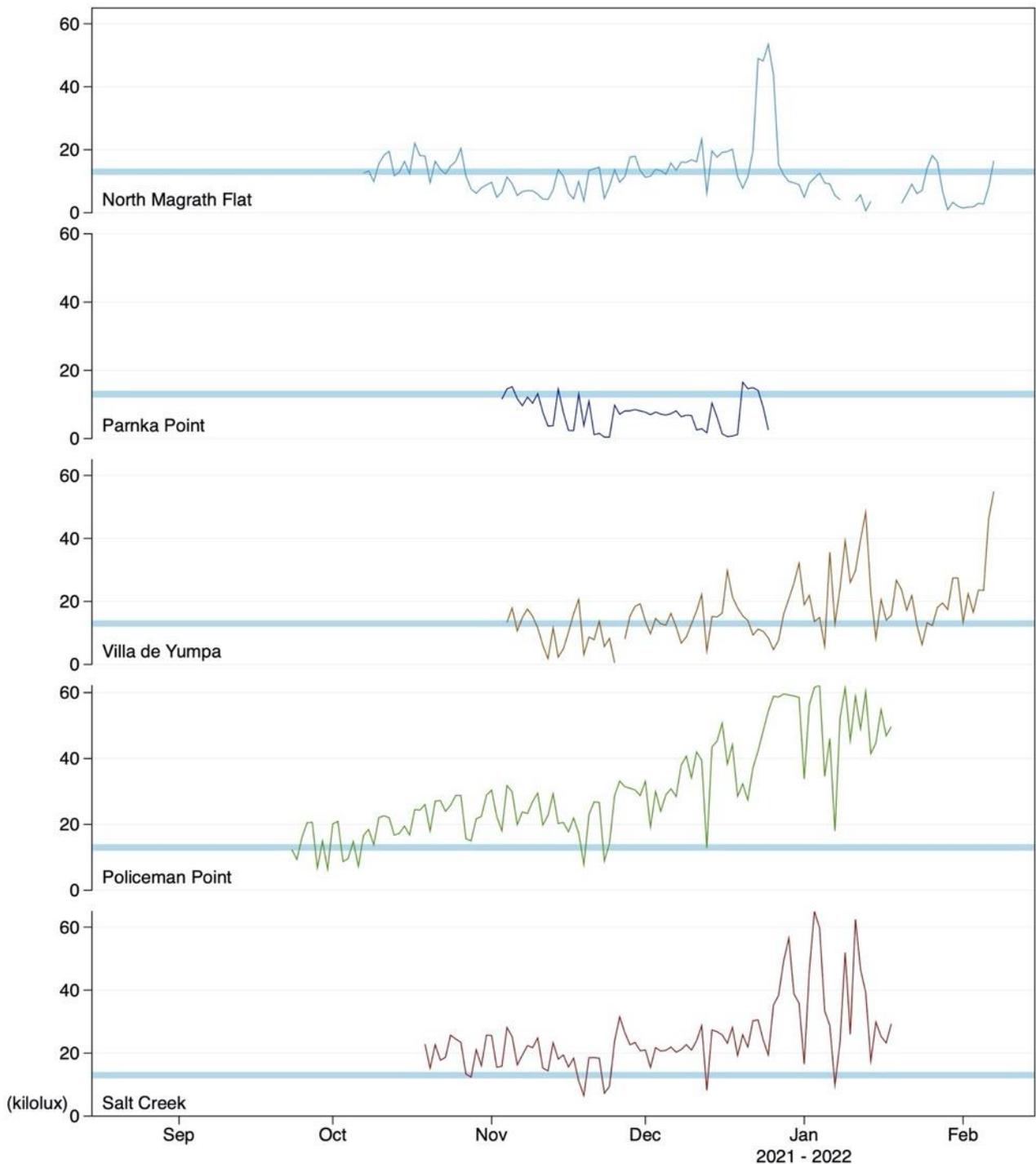
Figure 20. Daily average illuminance (kilolux) of daylight hours (i.e. values > 0.4 kilolux) measured using HOBO MX2202 Light/Temperature loggers in shallow water (location data Table 3). Blue band highlights the 16-20% of full sunlight for the maximum measured across any site, below this, plant condition would be impacted.

2.4.6 Field based measurement of photosynthetic efficiency

Field measurements of effective quantum yield (i.e. photosynthetic efficiency) varied at the different sites substantially but not significantly (p < 0.213) (Figure 21), with Noonameena exhibiting highest mean values for the Ruppia Community. This was possibly associated with overall better water clarity at Noonameena compared to the other sites where higher turbidity and water column algal content was anecdotally observed. At Noonameena, the basal algal clumps were able to be measured independently of the Ruppia Community plants and indicated a consistently high positive yield (Figure 21 category 3, Noonameena). The

measured Ruppia Community yield ranges were poorest at North Magrath Flat where the responses were also the most variable (Figure 21). When these measurements were taken, the North Magrath Flat sampling area was covered with a dense filamentous algal mat that was beginning to decay. The decaying algal mat started to form a black ooze layer over the sediment, and this is likely reflected in the difference between the yield measured in the surface algal mats at Noonameena and North Magrath Flat (Figure 21). Photosynthetic efficiency is being incorporated into the Ruppia Community Habitat Suitability Index (Hipsey et al. 2022) model to improve the ability of the model to reflect growth of the Ruppia Community and filamentous algae based on the site conditions recorded in the field in this study.
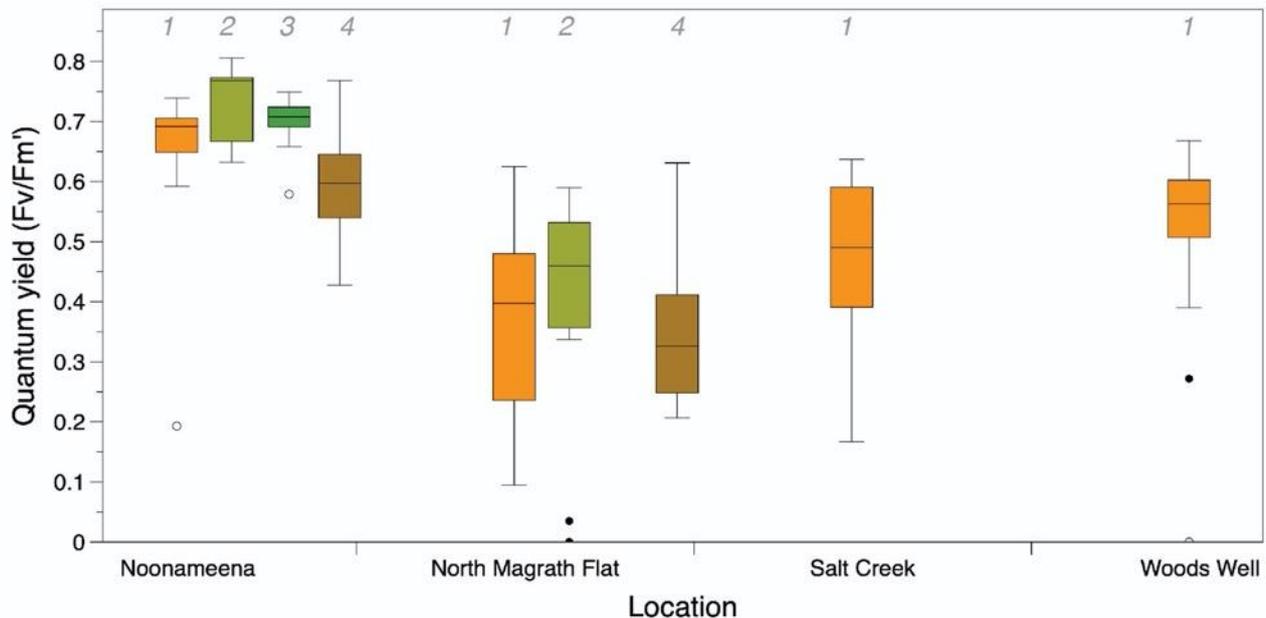
Figure 21. Box plots (inter quartile range, IQR) of quantum yield measured in four categories; 1 Ruppia Community (orange), 2 Ruppia Community plus basal algal clump (olive green), 3 Basal algal clump alone (mid green), 4 Surface algal mat (brown) (site numbers indicated above graphs), across four southern Coorong locations 23 November 2021.

2.5 Discussion

2.5.1 Ruppia Community thresholds

The results of these experiments provide further evidence that the Ruppia Community responds to its salinity and light environment using multiple adaptive strategies, enabling it to grow over an extremely wide range of environmental conditions. The Ruppia Community of the Coorong has been well documented to have extreme salinity tolerance, with plants reportedly observed to survive in less than marine salinities to more than 200 g L^{-1} (Asanopoulos and Waycott 2020). In mesocosm based experimental testing under variable shading conditions the Ruppia Community had higher biomass and shoot production in marine salinity (35 g L^{-1}) treatments suggesting more optimal conditions than hypersaline conditions (80 g L^{-1}). At marine salinities, experimental shading of plants led to a significant reduction in biomass at light levels below 16% full sunlight for both the Ruppia Community and charophytes that emerged from sediments during the experiment. The measured morphology of plants in the Ruppia Community responded to shading: overall leaf length elongation occurred with light reduction, although treatments were not significant. At the cellular level there was a significant response in cell elongation, but only at the most extreme light reduction (1% of full sunlight). Conversely, leaf width was narrower in full sunlight, and there was a trend for leaf length to be shorter in the lower salinity treatment, although this effect was not significant in these experiments. These responses of the Ruppia Community to stress caused by low light, can also be inferred from these results including shorter narrower leaves, as has been measured in other seagrasses (Zimmerman 2006). Under hypersaline conditions

there was no observed trend in leaf or cell morphology likely due to the already stressed conditions due to the high salinity.

Field observations of light conditions, based on benthic deployed loggers, identified periods of >8 weeks of <16% full sunlight at Parnka Point and North Magrath Flat, due to the shading effects of dense filamentous algae mats. Such durations of low light were associated with significant reductions in the biomass of the Ruppia Community. In the 2021 Ruppia Community growth season, significant periods of shading occurred at two locations: Parnka Point and North Magrath Flat, in the central section of the Coorong (e.g. filamentous algae mat forming in Figure 22). Such extended periods of shading are likely to result in significantly reduced biomass during the spring-summer period in the field where these dense algal mats formed. In addition, logger data recorded water temperatures at these two locations, along with Villa de Yumpa, that exceeded a threshold of 43°C which has been reported to lead to shoot death in other seagrass species from more tropical regions (Collier and Waycott 2014).

Experimental measurements of photosynthetic efficiency (effective quantum yield) as a proxy for plant performance, was conducted at four locations in the Coorong. This data indicated that overall, water quality, in particular water clarity, may be critical to enable the Ruppia Community to tolerate impact filamentous algal mat, or shrouding algal clumps, shading may have on plant growth.

Although the Ruppia Community continues to persist at the sites where algal blooms frequently form (limiting light, and increasing the organic loads of sediments), these populations remain highly vulnerable. Where other water quality factors inhibit light penetration such as suspended sediments or phytoplankton (chlorophyll a) blooms, plants will be at a significantly higher risk of decline or loss and measures of these could be included in future studies.
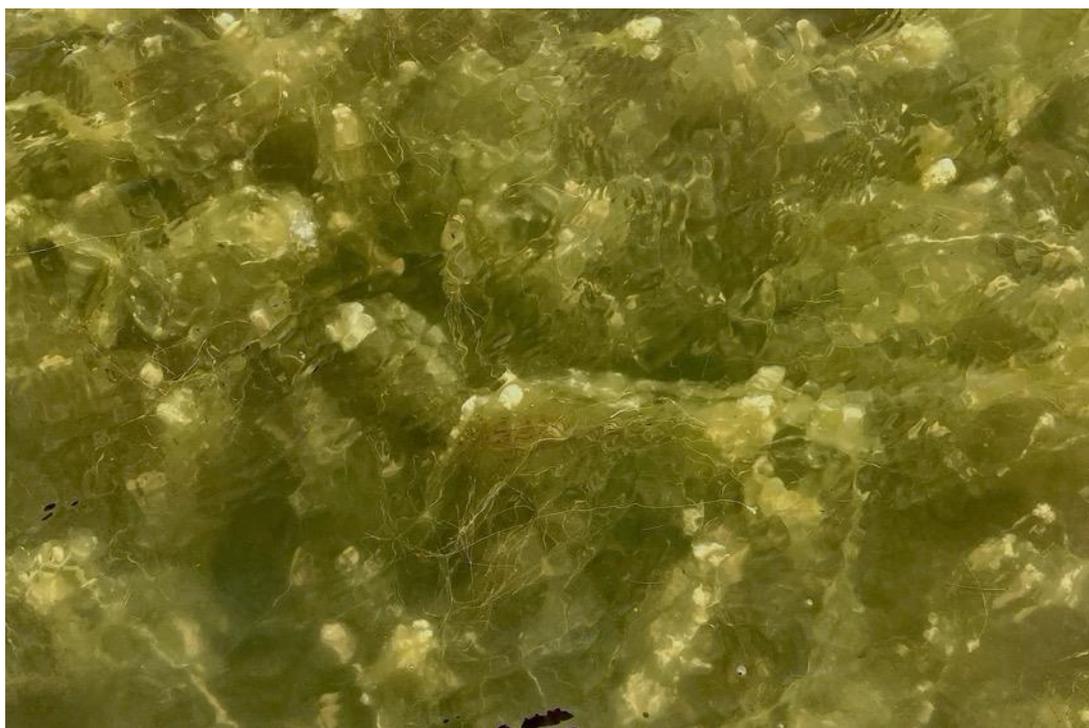
Figure 22. Filamentous algal mat forming at the surface in small patches, algal clumps shrouding the aquatic macrophytes of the Ruppia community in the water column, entangling with reproductive structures (flowering) of both *Ruppia* sp. and *Althenia cylindrocarpa*, November 2021, North Magrath Flat. Dead algal biomass is already forming high organic content/black ooze layer at the base of the Ruppia Community plants.

2.5.2 Filamentous algal community growth and thresholds

We propose that the filamentous algal community in the southern Coorong develops in direct association with the Ruppia Community. The Ruppia Community acts as a physical substrate enabling the algae to attach

and develop larger clumping units. Due to the seasonality of water levels, the presence of substrate, temperature and salinity, the filamentous algal community has developed a life cycle that is associated with the changes in the system during the annual cycle. Based on this life cycle, the absence of filamentous algae developing large 3-dimensional clumps and surface mats in certain areas is likely due to the lack of alternative substrates. The observed benthic substrates throughout the southern Coorong are predominantly sand to clay sediments that are relatively fine grained, although some rocky areas occur and it may be possible for the filamentous algal community to establish there if they can entangle or attach.

The consequence of this algal mat formation life cycle is that physical removal of algae will directly disturb the Ruppia Community (see section 2.4.3 for discussion). The surface algal mats, and even the shrouding clumps, appear to be readily detached from the plants, primarily through strong winds or hydrological action (fast flowing water or waves). During warmer months when the surface algal mats form extensive areas and coincide with the Ruppia Community developing reproductive structures (flowers and fruits), strong wind events can lead to very large areas of filamentous algal biomass on the shoreline (Figure 23). These shoreline deposits were anecdotally observed to persist, sometimes until the next season, creating high organic load sediments, that can be anoxic smelling of sulphur and retain the black colour.

Extensive areas of dense surface filamentous algal mats (Figure 24) were observed between mid-September and mid-December (Waycott et al. 2020b). In November 2019, preliminary surveys and analysis of aerial imagery (Waycott et al. 2020b) indicated an estimated 13.6 km^2 of the 26.9 km^2 (51%) mapped was covered in filamentous algae growing on Ruppia Community. In this benthic habitat area from Needles Island to Hack Point, i.e. the central Coorong, this algal biomass could represent between 1,700 tonnes and 6,400 tonnes dry weight in the 26.9 km^2 areas of this section of the southern Coorong.

Salinity and temperature also have a significant impact on algal mat formation (Waycott et al. 2019), another seasonally driven process as both salinity and temperature increase throughout the growing season. The results of this previous experimental testing in mesocosms of the filamentous algal community to a range of salinity and temperature conditions (Waycott et al. 2019), supports the upper threshold of 90 g L^{-1} for filamentous algal growth to stop, however death was not observed until higher salinities (Figure 25). In the field, these salinities can occur locally, and logger data also documented (Figure 18) that temperatures in the Ruppia Community growing areas commonly reach 30°C where maximum growth rates were observed (Figure 25A) at lower salinities (Figure 25B).

A.   B.

Figure 23. A. Filamentous algal mat washed onshore from adjacent areas, November 2021, North Magrath Flat. B. closer view of decaying algal mat, upper dried crust layer peeled away to see sediment underneath, the black ooze layer obvious and evidence of Ruppia Community tissue interspersed in the upper layer.

Figure 24. Extensive filamentous algal surface aggregations forming a mat over the Ruppia Community below across several km² in the central Coorong, upper right image indication of the location of the photo.

A.

B.
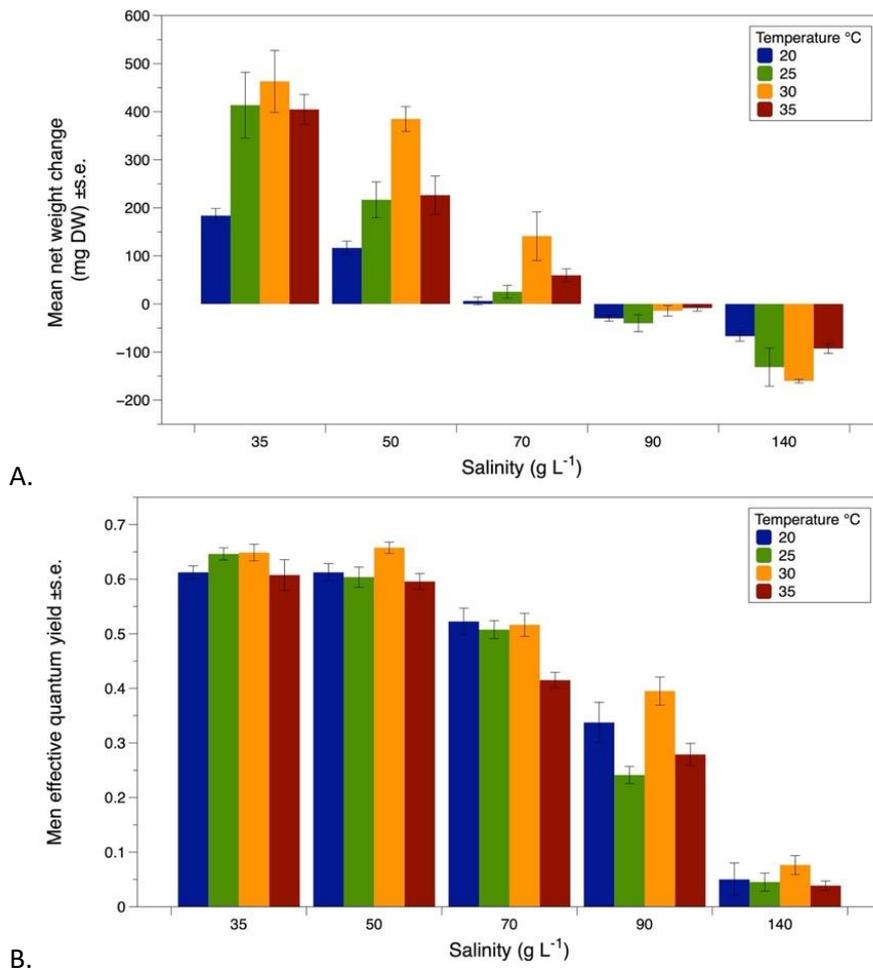
Figure 25. Growth responses of filamentous algal community grown in mesocosms. A. net change in weight of the algal clump, B. effective quantum yield to different salinity and temperature conditions (adapted from; Waycott et al. 2019).

The results of measured growth rates and tested survival thresholds are being incorporated into a revised version of the Coorong Dynamics Model, specifically with reference to the Ruppia Habitat Suitability Index (Ruppia HSI) (Hipsey et al. 2022). Further resolution of the relationships of the various co-factors to filamentous algal growth, including the ability to form large aggregate mats, is ongoing.

2.5.3 Algal removal intervention trial

Undertaking algal removal trials was intended to support efforts to reduce nutrient loads in the southern Coorong at high priority sites. Prior to this study, the close association between filamentous algal mat formation and the presence of the Ruppia Community was not understood (Lewis et al. 2022). The removal of surface algal mats before the onset of flowering and fruiting was thought to be a potential benefit to enhancing seed set opportunities, even if only possible at sentinel sites. The trials immediately identified several limitations with the tested methodologies for filamentous algal removal. Firstly, a high degree of localised impact was observed when disturbing the sediment, which resulted in a high degree of disruption at the *Ruppia* – sediment interface. The resulting impact would be an unacceptably high negative impact on *Ruppia* meadow integrity. The second significant limitation was that the *Ruppia tuberosa* and *Althenia cylindrocarpa* plants were fully flowering at the time the algal biomass was sufficiently developed to be cleared. Although there may be interannual differences in this timing it is likely that rapid algal growth and flowering will consistently be coincident with the increasingly warmer conditions during spring and early summer. As a result, clearing of filamentous algae would incur the same problem of disruption to reproductive success as the algae itself does.

In addition to experimental testing, local residents and researchers have observed wind to move surface algae mats and change their coverage in particular areas (pers obs. Geoff Gallasch, 22 October 2019), although this requires the algal mat to have sufficiently formed at the water surface. The wind driven algal mats are mobilised and become deposited on shorelines (Figure 11 A.), waterlogged and or detritus in deeper water appear susceptible to forming anoxic sediments as the algal biomass degrades (Figure 24).

Local mechanical removal of filamentous algae in proximity to the growing Ruppia Community, even with simple and easy methods, would disrupt these ecosystems. However, mechanical removal of the shoreline deposits would have considerable benefits preventing degradation of sediment quality and preventing foraging or colonising by animals.

3 Phenology and seasonal growth of the Ruppia Community in southern Coorong populations

3.1 Introduction

Phenology is the study of the lifecycle phases or activities of plants in their temporal occurrence throughout the year. Phenological studies can present a simple way to track changes in the behaviour of species (Menzel 2002). In temperate ecosystems, the reproductive cycle of plants is primarily controlled by temperature and day length, as well as a number of environmental and physical factors including; light, photoperiod, water and nutrient availability (Madsen 1991, Menzel 2002). Aquatic macrophytes are critical for the ecological and biogeochemical processes of aquatic ecosystems, however, little is known about their phenological cycles (Calero et al. 2015).

Phenological data have been used for a variety of applications, including; management of invasive species (Taylor et al. 2020), improving knowledge of pollen releases to reduce health issues (Sapkota et al. 2020), exploring relationships between phytoplankton blooms and fish spawning (Asch et al. 2019) and to enhance plant restoration techniques for maximum success (Buisson et al. 2017). Due to climate change, the phenological cycles of plants have received increasing attention in recent decades and are increasingly used as an indicator of species responses to changes in climatic and environmental variables (Wolkovich and Cleland 2011).

Lewis et al. (2022) observed that the condition and resilience of the Ruppia Community of the southern Coorong is heavily influenced by environmental factors, such as water availability, salinity, temperature and sediment condition. The responses of aquatic macrophytes, such as the Ruppia Community, can be highly plastic and change within individual populations (Madsen 1991). It is therefore important to understand variation within the phenological cycle for managing critical species assemblages such as the Ruppia Community.

Brock (1983) studied the resource allocation of *Ruppia* spp. and other submerged aquatic macrophytes in saline lakes of south-eastern South Australia, however this study did not include the Ruppia Community of the Coorong. Brock (1983) found that perennial and annual populations of *Ruppia* spp. differed in their phenological response (resource allocation), which was also observed by Lewis et al. (2022).

This study aimed to investigate the phenological variation in the life cycles of the Ruppia Community across the southern Coorong. We conducted a series of field surveys at a high temporal frequency (fortnightly) at six sites, in conjunction with the large-scale, seasonal surveys conducted by Lewis et al. (2022). This aimed to fill knowledge gaps which are not able to be detected by seasonal sampling methods, such as changes in biomass allocation and growth across the Ruppia Community life cycle and timing of events such as reproduction (flowering and turion production) and senescence. This data will also assist in filling knowledge gaps and building the Ruppia Community Habitat Suitability Index (Hipsey et al. 2022).

3.2 Methods

To quantify the growth and phenology of the Ruppia Community, we used a series of field surveys at semi-regular intervals (fortnightly) to measure change in biomass across the growth cycle. Due to the dependence of the Ruppia Community on environmental variables to determine length and timing of their life cycle (Lewis et al. 2022), it was necessary to undertake field observations regularly so that we could start sampling close to growth commencement.

Six sites were selected based on Ruppia Community presence and the ability to easily access for surveys (Table 8, Figure 26). The survey sites focussed on the southern Coorong and were based on the surveys conducted by (Lewis et al. 2022). The northernmost (Noonameena) and southernmost (Salt Creek, site 30)

sites were located at 40 km and 93 km from the Murray Mouth, respectively (Figure 26). The selected sites aimed to give an indication of how life cycles differ across the southern Coorong.

A field sampling program began in December 2020 with samples taken on 19 occasions over 12 months to determine when the Ruppia Community began its lifecycle (i.e. germination from seeds or vegetative growth from turions). These samples were returned to the laboratory and examined for their contents but not included in data analyses (see below).

Table 8. Locations of survey sites where phenology samples were collected.

SITE	LATITUDE °S	LONGITUDE °E
Noonameena	-35.755899	139.261625
North Magrath Flat	-35.852757	139.385565
Parnka Point	-35.902333	139.398445
Villa de Yumpa	-35.909933	139.452095
Policeman Point	-36.058623	139.586122
Salt Creek (site 10)	-36.120136	139.636639
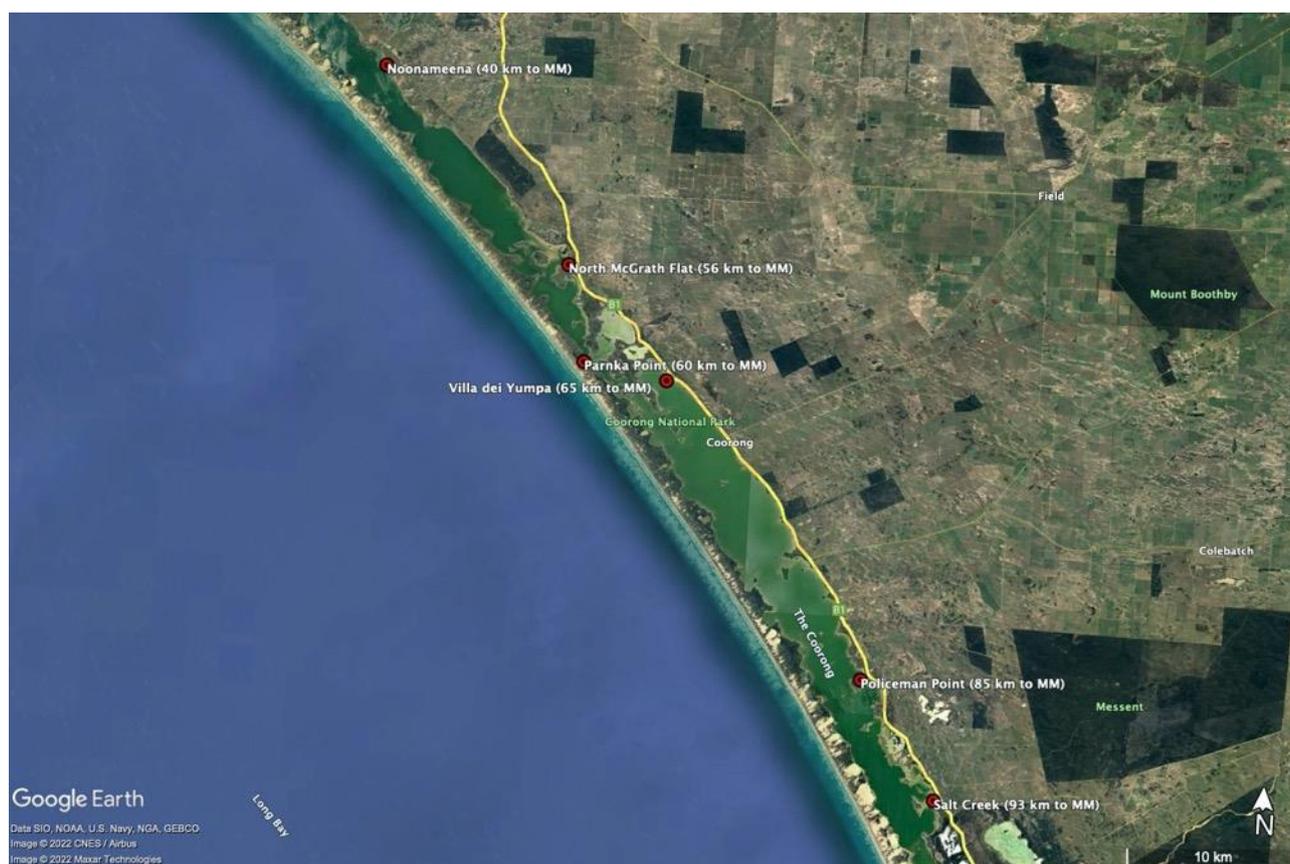
Figure 26. Map of survey site locations

Sampling for data collection began on 11 June 2021 when Ruppia Community was present and continued fortnightly until the 3 December 2021 (although some Ruppia Community was still present, sample processing needed to be taken into account). Surveys were conducted using sediment core sampling for aquatic plants using 75 mm diameter cores, aligned to The Living Murray Icon Site condition monitoring methodology (Paton et al. 2017b, Murray–Darling Basin Authority 2022).

Pilot sampling was conducted to determine the optimal sampling design for capturing the Ruppia Community lifecycle prior to June 2021. At each site, 15 replicate samples of 75 mm diameter cores were taken across 3 transects, the transects were approximately 50 m long with 5 cores taken 10 m apart and ran parallel to shore, each transect was approximately 20-30 m from the previous, from shallow to deep water depth (~20-80 cm depth relative to current conditions).

Each site was georeferenced, the position of each core taken was recorded by GPS for each sampling event. In addition to the replicate shoot count cores, water depth was recorded at the start and end of each transect, and water samples were collected in 25 mL Falcon Tubes and salinity determined later using a Milwaukee digital refractometer (in Parts Per Thousand, PPT, equivalent to g L^{-1}).

Each individual core was placed in a numbered calico bag with each site kept together for ease of processing and transported to Adelaide. All cores were frozen upon return to Adelaide for laboratory processing.

Table 9. The dates and sites included in phenology surveys.

DATE	NOONAMEENA	NORTH MAGRATH FLAT	PARNKA POINT	VILLA DE YUMPA	POLICEMAN POINT	SALT CREEK
16-Dec-20	✓	✓	✓	✓	✓	✓
19-Jan-21	✓	✓	✓	-	✓	✓
21-Feb-21	✓	✗	✓	-	✗	✗
24-Mar-21	✓	✓	✓	-	✗	✗
23-Apr-21	✓	✓	✓	-	✗	✗
21-May-21	✓	✓	✓	-	✗	✗
31-May-21	✓	✓	✓	-	✗	✗
11-Jun-21	✓	✓	✓	✓	✓	✓
25-Jun-21	✓	✓	✓	✓	✓	✓
09-Jul-21	✓	✓	✓	✓	✓	✓
05-Aug-21	✓	✓	✓	-	✓	✓
25-Aug-21	✓	✓	✓	✓	✓	✓
09-Sep-21	✓	✓	✓	✓	✓	✓
22-Sep-21	✓	✓	✓	✓	✓	✓
06-Oct-21	✓	✓	✓	✓	✓	✓
18-Oct-21	✓	✓	✓	✓	✓	✓
02-Nov-21	✓	✓	✓	✓	✓	✓
17-Nov-21	✓	✓	✓	✓	✓	✓
03-Dec-21	✓	✓	✓	✓	✓	✓

✓ = samples collected (**bold** where data were collected for analysis)

✗ = site visited but no samples collected

- = site not visited

For laboratory processing, samples were defrosted from storage and sample numbers cross-referenced with site-wide data to ensure quality. Samples were placed in a sieve and the sample bag was rinsed inside-out into the sieve to ensure all sample material was caught. The sample was then sieved through 500 µm and

200 μm mesh to retain seeds and all plant material, using mechanical action and running water. All sediment was collected for return to the Coorong as per permit requirements.

Once thoroughly sieved, the remaining material was rinsed and emptied into a plastic tray for further sorting. The tray was elevated on one side, and its contents slowly spread across and up the tray, such that seeds, turions and rhizomes could be separated from sediment, and the entire contents of the tray inspected thoroughly. If the sample being processed was the first of its site, then photos were taken of its contents pre and post-sieving with its corresponding sample number, to provide additional context for future reference.

Seagrass seeds, shoots, turions, flowers and fruits were removed and placed into separate trays to be counted. In order for seagrass material to be recognised as living, and included in data collection, its general structure of rhizome, roots and shoots was required to be present. For each sample, each of these variables was recorded on a physical data sheet, in addition to the sample number, the date, the research assistant's name, and any additional notes. Loose, unstructured and otherwise dead biomass was not included.

Seeds were enclosed together in teabags, which were labelled with a sample number, sealed in a container with silica and kept for future reference. Small aluminium foil containers were then produced for the weighing of sample biomass, one per sample. These were labelled twice with their sample number for redundancy, and subsequently weighed empty to five decimal places, so that dry seagrass biomass may later be determined. This weight was also recorded on the data sheet. Once counted, all remaining seagrass biomass (turions, shoots, flowers, fruits, rhizome) was placed into its corresponding container, then placed into an oven at 60° C for 48 hours in order to dry fully. Once dry, the sample was weighed in its container to five decimal places. The original weight of the container was then subtracted from this to determine the dry weight of the sample biomass. Likewise, both these weights were recorded on the data sheet.

The aluminium foil container was then tightly folded shut with its label clearly visible and stored in a vacuum-sealed bag among other biomass samples for future study. Once finalised, data sheets were digitised and their contents manually recorded in the corresponding database. This provided strong data provenance, and allowed clerical errors or inconsistencies to be rectified through cross-referencing the database with the sample processing data sheets and the original field collection data sheets.

Statistical analysis of growth rate data was conducted undertaken using the software package IBM SPSS Statistics V. 11. A One-way ANOVA was conducted on log transformed data to analyse the growth rates of different plant structures.

3.3 Results

Overall, Ruppia Community biomass was present across the whole survey period (June – December 2021) with peak total biomass across all sites in November 2021 with 16.97 ± 0.93 g of dry weight (DW) biomass per m^{-2} (Figure 27), and a small reduction in December 2021, which would be expected to have continued as water levels receded. In June 2021 we observed higher total biomass than in the following months of July and August, which was primarily in root production, total biomass in June 2021 was 7.95 ± 1.12 g DW m^{-2}, which declined to 6.24 ± 0.58 and 4.08 ± 0.44 in July and June respectively (Figure 27). Post-August, total biomass continually increased towards the peak in November. With the exception of turion biomass, all other plant structures peaked in overall biomass overall in November 2021 and began to decline in December.

Shoot biomass was overall lowest in June 2021 at 1.27 ± 0.14 g DW m^{-2} accounting for 16.03 % of total mean biomass and increased monthly to its peak in November 2021 of 3.6 ± 0.27 g DW m^{-2} (21.44 % of total mean

biomass). However, although there was less biomass than at the peak in November, in August 2021, shoots accounted for 33.7 % of total mean biomass (Figure 27).

Mean rhizome biomass was also lowest in June 2021 (0.5 ± 0.08 g DW m^{-2}) and increased consistently through to November 2021 where it peaked at 4.22 ± 0.30 g DW m^{-2} and accounted for 24.9 % of total mean biomass. Rhizome biomass also began to decline in December 2021 with a mean of 3.50 ± 0.64 g DW m^{-2}, however still accounted for 22.7 % of total mean biomass (Figure 27).

Root biomass did not follow the same trend as overall mean biomass and other non-reproductive plant structures (i.e. not turions or flowers/fruits). In June 2021, when sampling commenced, root biomass accounted for 71.40 % of total biomass (5.70 ± 0.94 g DW m^{-2}). With the exception of August 2021 where a mean of 2.1 ± 0.22 g DW m^{-2} was observed, growth of other structures accounted for increasingly more of the total mean biomass as months progressed, while roots remained between $4 - 6 \pm 0.40 - 1.4$ g DW m^{-2}, by December, root biomass had declined to 28 % of total mean biomass (Figure 27).

Overall, turion biomass was low until an increase in November. From June to September turion biomass ranged from $0.23 - 0.5 \pm 0.024 - 0.052$ g DW m^{-2}, however increased to 1.14 ± 0.20 g DW m^{-2} in October and 3.6 ± 0.38 and 5.26 ± 0.63 g DW m^{-2} in November and December respectively (Figure 27). Mean turion biomass ranged from 3.3 % of total mean biomass in August 2021 to 34.40 % in December 2021.

Reproductive biomass was low in comparison to other plant structures, whilst it was present in samples from all months with the exception of July 2021, mean reproductive biomass only accounted for 0.73 % (0.12 ± 0.040 g DW m^{-2}) of total mean biomass at peak flowering in November 2021 (Figure 27).
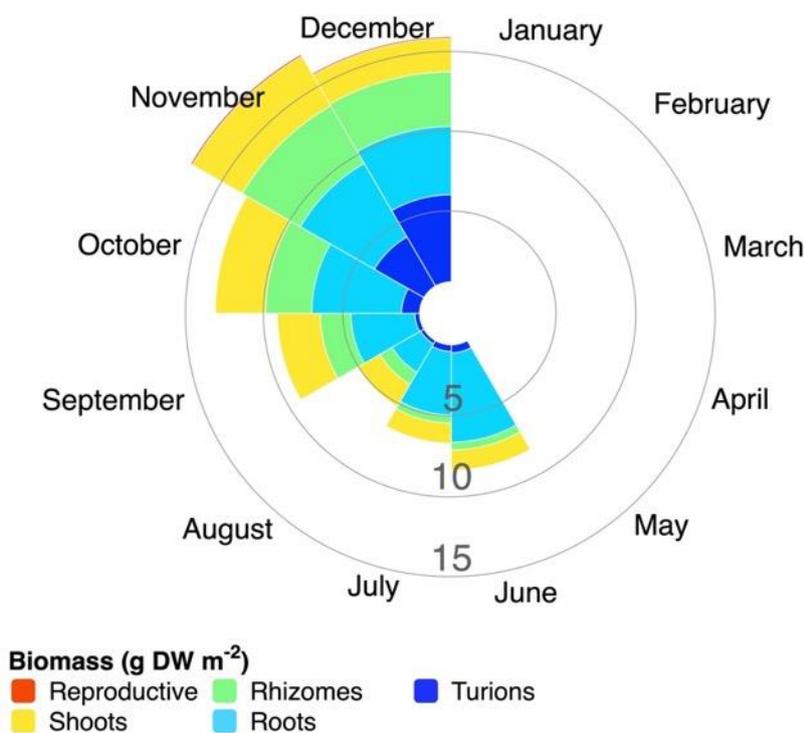
Figure 27. Phenological rose plot of mean biomass for the different plant structures combining data for all sites from June to December 2021 (no data included from January-May 2021) measured in grams of dry weight (DW) biomass per m^{-2}. Reproductive biomass present October - December

High levels of variation in growth were observed between the six sites (Figure 28). The northernmost site, Noonameena was different to all other sites, with an apparent peak in total biomass in June 2021 (18.4 $\pm$ 4.07 g DW m^{-2}), a reduction in July (7.3 $\pm$ 2.52 g DW m^{-2}), then fluctuation between 2 - 5 g DW m^{-2} until December where biomass reduced to 1.20 $\pm$ 1.10 g DW m^{-2} (Figure 28A). Mean biomass was lower overall at Noonameena where outside of the peak in June 2021, mean total biomass ranged from 1.20 – 7.27 $\pm$ 1.10 – 2.52 g DW m^{-2}. At the peak in June 2021, root biomass accounted for 80.70 % of total biomass (14.8 $\pm$ 3.46 g DW m^{-2}) (Figure 28A). Turion production was lowest at Noonameena with mean turion biomass between 0.001 – 0.08 $\pm$ 0.001 – 0.08 g DW m^{-2} (Figure 28A). Reproductive biomass was only observed via sampling in June 2021, with a mean biomass of 0.008 $\pm$ 0.008 g DW m^{-2}.

At North Magrath Flat, peak biomass was observed in October (20.9 $\pm$ 2.27 g DW m^{-2}), earlier than the southern sites, at which peak biomass was observed in November and December (Figure 28). Similar to Noonameena, North Magrath Flat maintained relatively high levels of biomass throughout the survey period ranging from 4.70 – 20.9 $\pm$ 0.90 – 2.30 g DW m^{-2} (Figure 28B). Turion biomass was low (< 0.6 g DW m^{-2}) until October 2021 where it increased to 4.50 $\pm$ 0.71 g DW m^{-2} and was highest in December 2021 at 6.54 $\pm$ 1.2 g DW m^{-2} (Figure 28B). Reproductive biomass was present between August and December 2021, but also peaked in October 2021 at 0.30 $\pm$ 0.11 g DW m^{-2} (Figure 28B).

Parnka Point had the highest total mean biomass across the survey period, ranging from 11.22 $\pm$ 1.90 g DW m^{-2} through to peak biomass in November 2021 of 26.13 $\pm$ 3.50 g DW m^{-2} (Figure 28C). Across all sites, reproductive biomass was not present in samples until November and December 2021, where mean reproductive biomass of 0.49 $\pm$ 0.17 and 0.51 $\pm$ 0.20 g DW m^{-2} were observed, the highest across all sites (Figure 28C). Parnka Point also had the highest mean turion biomass in December 2021, at 9.60 $\pm$ 1.44 g DW m^{-2}.

Total mean biomass peaked in November 2021 at Villa de Yumpa at 16.6 $\pm$ 3.01 g DW m^{-2}, and across the survey period was lower than at all other sites. Mean total biomass remained low until October 2021 where an increase from 1.03 $\pm$ 0.40 to 4.42 g DW m^{-2} was observed. Post-peak mean biomass in November 2021, mean total biomass decreased to 6.20 $\pm$ 2.70 g DW m^{-2} (Figure 28D). Turion biomass peaked in November 2021 but reduced in December 2021 with mean biomass of 5.90 $\pm$ 1.30 and 3.70 $\pm$ 1.9 g DW m^{-2} respectively. No reproductive biomass was observed at Villa de Yumpa throughout the survey period (Figure 28D).

At the Policeman Point site, mean biomass peaked in December 2021 with the highest total observed across all sites and months, of 31.8 $\pm$ 9.4 g DW m^{-2} (Figure 28E). Turion biomass increased in November and December 2021, with mean biomass of 3.11 $\pm$ 0.71 and 8.0 $\pm$ 1.31 g DW m^{-2} respectively, less than 1 g DW m^{-2} was observed each month from June to October 2021. As with Villa de Yumpa, no reproductive biomass was observed in core samples across the survey period (Figure 28E).

Salt Creek followed the trend of peak mean biomass in November 2021 of 22.63 $\pm$ 3.33 g DW m^{-2}, November was also the only month in which reproductive materials were observed in sampling with a mean of 0.14 $\pm$ 0.10 g DW m^{-2} (Figure 28F). Turion biomass was relatively low in comparison with other sites, with mean biomass from June – October $\leq$ 0.30 $\pm$ 0.06 g DW m^{-2}, with an increase to 5.90 $\pm$ 1.30 g DW m^{-2} in November, followed by a reduction in December 2021 (3.62 $\pm$ 1.90 g DW m^{-2}).
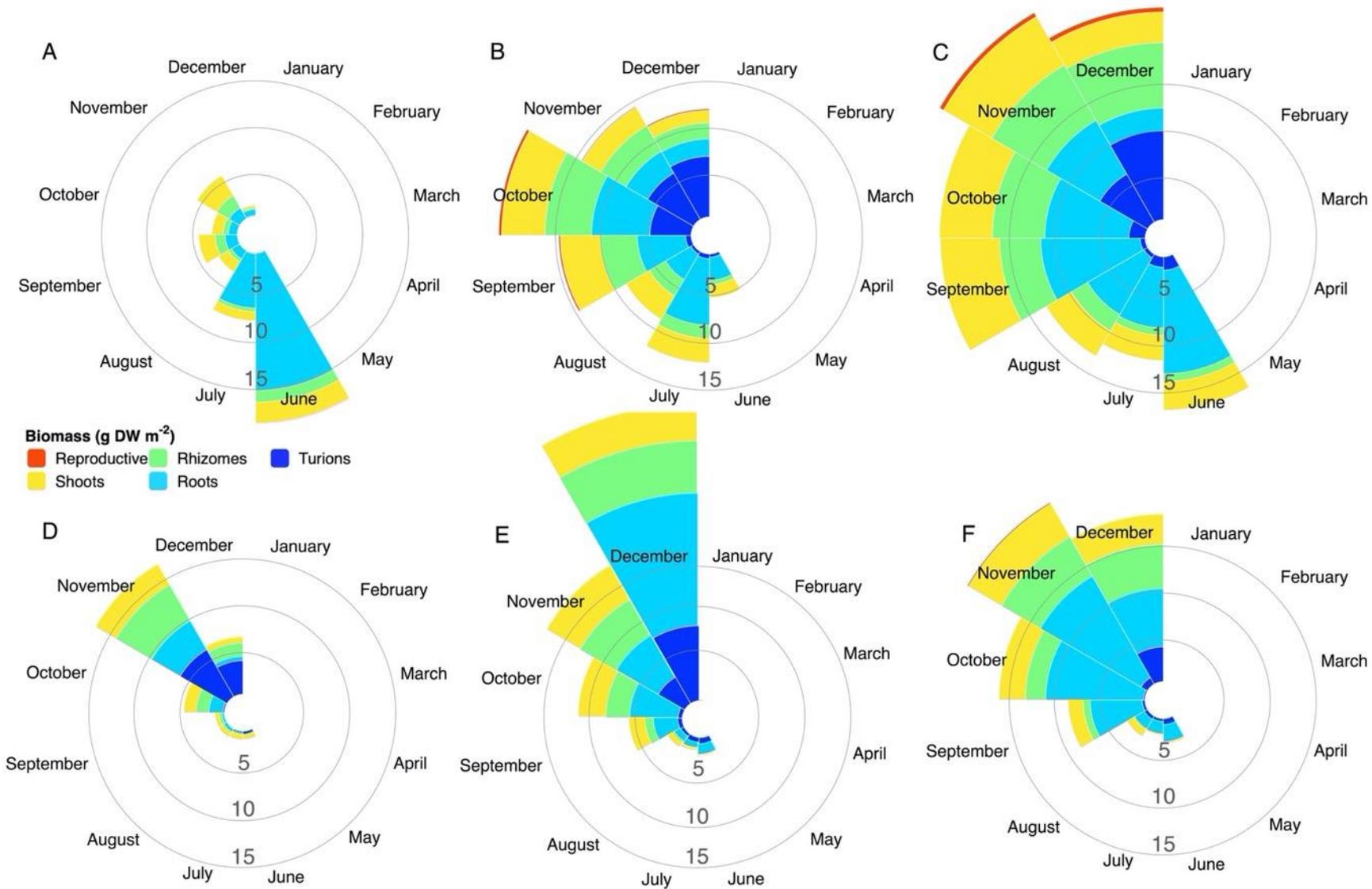
Figure 28. Phenological rose plots of mean biomass by plant structure/component from June to December 2021 (no data included from January-May 2021) measured in grams of dry weight biomass per m^{-2}. A. Noonameena, B. North Magrath Flat, C. Parnka Point, D. Villa de Yumpa, E. Policeman Point, F. Salt Creek (site 10).

Biomass allocation across the survey period varied between sites. Most sites (except Villa de Yumpa, which had low overall biomass until September 2021) showed root biomass development initially, before increasing rhizome and shoot production, with, as described above, small amounts of reproductive biomass being observed from October through to December 2021 (Figure 29). Turion biomass was overall low until ~October, however at all sites some turion biomass was present year-round (Figure 29).
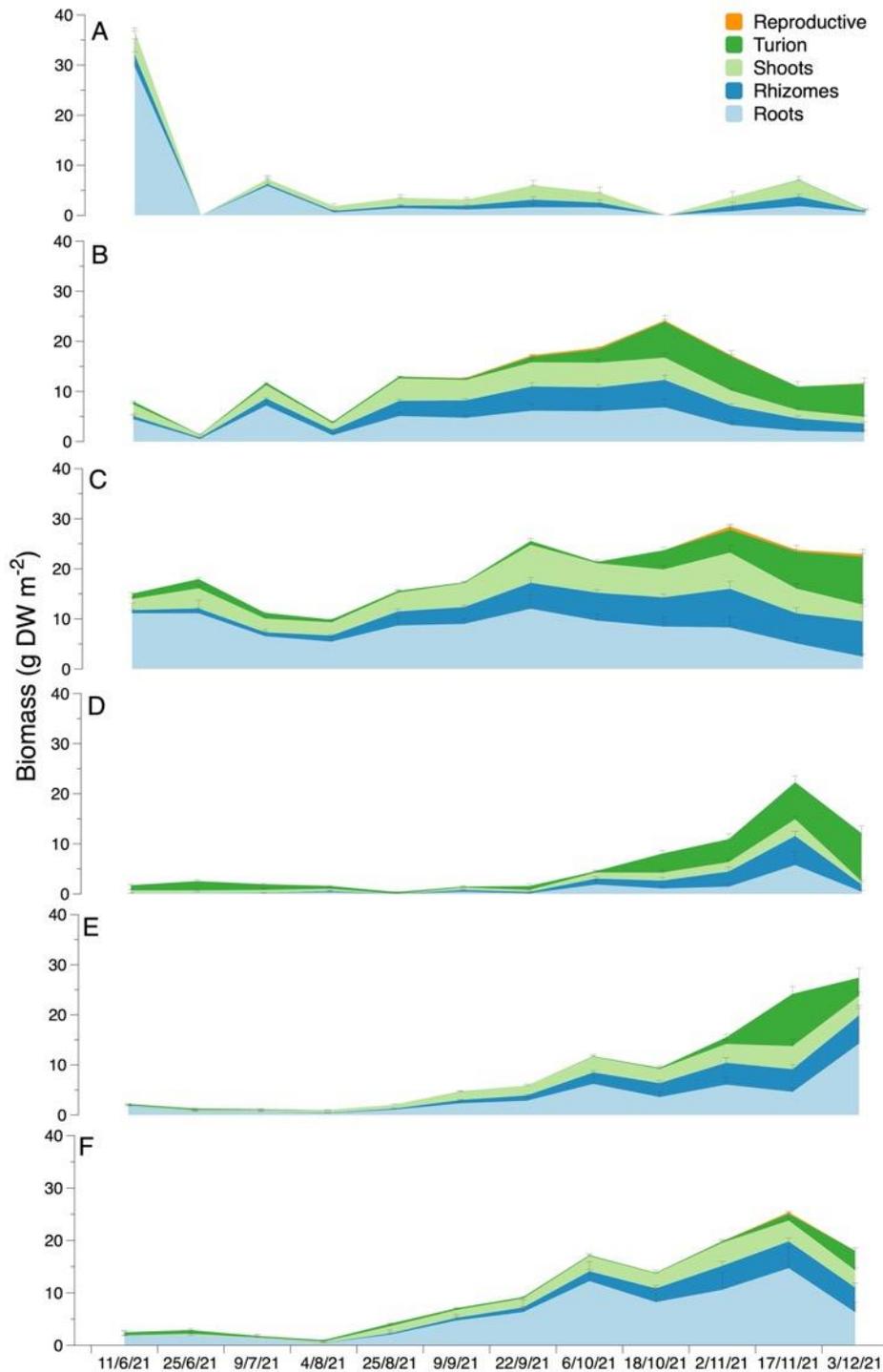
Figure 29. Biomass (g DW m^{-2} ± s.e.) allocation to different plant parts over time for, A. Noonameena, B. North Magrath Flat, C. Parnka Point, D. Villa de Yumpa, E. Policeman's Point, F. Salt Creek (site 10).

Overall, growth rates of the different plant structures did not differ significantly (p=0.68), although early June 2021 appeared to have slightly higher rhizome growth (Figure 30A).

The majority of non-reproductive, vegetative growth (shoots, rhizomes and roots) occurred between June and September 2021, whilst reproductive biomass experienced increased growth in October and November 2021. Turion growth was highest in mid-October (0.892 g DW m^{-2}) but continued into December 2021 (Figure 30B). Reproductive growth began in September 2021 and peaked in October 2021 at 2.5 g DW m^{-2} (Figure 30B).

Mean seed density at the start of the survey period in June 2021 was 452.7 $\pm$ 153.72 seeds per m^{-2}, which varied through to a peak of 583.80 $\pm$ 160.48 seeds per m^{-2}, before declining during the period of highest vegetative growth in July-August 2021. Seed density was low from ~September, through until December 2021 where an increase from 7.55 $\pm$ 5.30 to 52.81 $\pm$ 27.63 mean seeds per m^{-2} was observed (Figure 30C).
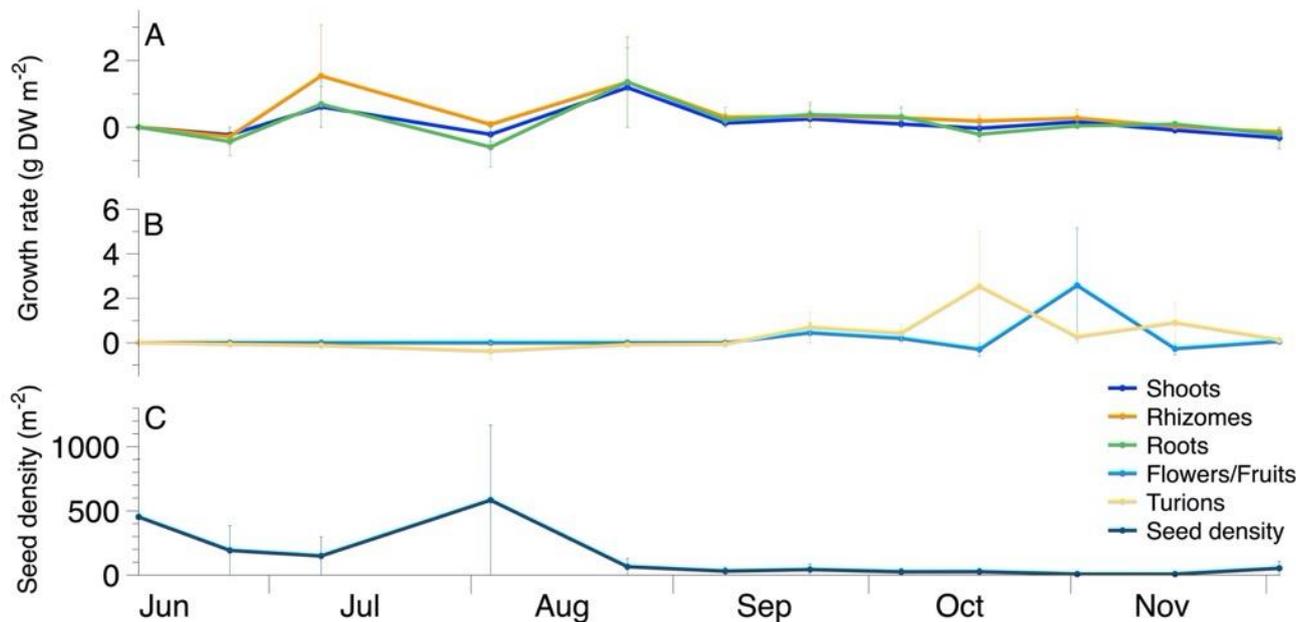
Figure 30. Change over time during 2021 for A) growth rates in g DW m^{-2} of vegetative structures, including shoots, rhizomes and roots; and B) growth rates in g DW m^{-2} reproductive structures, including flowers/fruits and turions, and C) seed density. Note, different y-axes scales.

3.4 Discussion

This study was one of the first to closely follow the Ruppia Community phenology and growth throughout the majority of the growth period in a strongly seasonal system. We selected six sites, which were sampled as part of large-scale field assessments conducted by Lewis et al. (2022) and followed them fortnightly from 11 June through to 3 December 2021. The Ruppia Community can be difficult to sample due to the plants' small size and somewhat fragile structure, which makes dissecting the plants to measure the biomass of different structures time consuming and technical. This study may have benefited from larger sample sizes and more sampling times, but this was not feasible given time, staffing and resources. However, this study also describes for the first time, to our knowledge, the growth rates and detailed phenological cycles of the Ruppia Community in the southern Coorong.

Although conditions in the southern Coorong are highly variable from year to year, as described in Lewis et al. (2022), in 2021, water levels and the duration of inundation were increased by the volume of water entering the Coorong from the River Murray and Salt Creek. In 2020, water levels receded in October leaving most of our survey sites (excluding Noonameena) dry and the Ruppia Community largely absent (Lewis et al.

2022). For this study, there was still some Ruppia Community biomass present at all sites into January 2022 (M. Waycott unpublished data) although due to project constraints, we needed to cease surveys in December 2021. Sites at which the Ruppia Community were present into 2022 were those that remained inundated. It is likely that once these sites dry, the Ruppia Community biomass will quickly decline through desiccation.

Developed flowers are located on a long stalk which reaches to the air-water interface for pollination, which would likely be excluded from the sample by our corers. Our measurements of reproductive biomass only counted the flowers and fruits which were collected in core samples, so may mainly reflect flowers which are post-emergence or only just emerged from the plant. Field observations also suggested that more flowering was occurring than reflected in the results of this study. This discrepancy may be due to the difficulty in quantifying flower density, as environmental conditions, such as wind or water depth appear to influence whether flowers are submerged or on the water's surface, and therefore their detectability.

At most sites we observed higher root biomass (proportional to total biomass) in June than going forward towards peak biomass in October-December. This suggests that the Ruppia Community develops strong root structures prior to allocating resources to other structures, which may be a response to growing conditions in the southern Coorong (i.e. unconsolidated sediments and variable water levels). There could be several advantages to this strategy, including increasing sediment oxygenation and therefore improving sediment condition, sediment stabilisation, increased nutrient and water availability in the event of variable water levels and secure attachment to substrate.

Generally, it appeared that biomass peaked later as distance from the Murray Mouth increased, likely due to environmental conditions. The environmental variables which heavily influence ecosystem health in the southern Coorong (water availability, salinity, water temperature, desiccation, turbid water) were largely absent from Noonameena, and this was reflected in the results of this study. All sites appeared to follow a trend of increasing biomass from June to November 2021 with turion and reproduction in October to December. This was not the case at Noonameena where our surveys indicated high biomass in June 2021, then a decline to levels which persisted for the rest of the survey period. Lewis et al. (2022) comment that more permanent water which is closer to the salinity and clarity of seawater than further south in the Coorong, potentially influences the Ruppia Community composition or lifecycle as a result.

Field observations at Noonameena noted that the Ruppia Community was patchy, often with large spans of sandy sediment between patches of Ruppia Community. Consequently the sampling design used in this study may not have been optimal for capturing the community at this site. However, it was necessary to capture data at Noonameena as a contrast with environmental conditions and Ruppia Community presence in comparison to other sites in the Coorong South Lagoon. The persistence of the Ruppia Community at Noonameena throughout the study period and the quantities found in a series of four seasonal surveys in Lewis et al. (2022) might also suggest that this population is perennial, rather than the annual persistence observed at other sites.

North Magrath Flat, like Noonameena, is located in the southern North Lagoon, however the two sites did not follow the same seasonal patterns. The Ruppia Community at North Magrath Flat followed growth patterns similar to Parnka Point and the sites situated in the Coorong South Lagoon, with high growth in July to September, leading to a defined peak in biomass in October 2021, although peak biomass at the southern sites occurred in November. Lewis et al. (2022) found that Ruppia Community abundance and biomass peaked around the central, Parnka Point region and declined with distance from the Murray Mouth, which this study supports.

The highest reproductive (flowers and fruits) biomass was observed at North Magrath Flat and Parnka Point, however these sites also had high turion biomass. The higher reproductive biomass at these sites may reflect

the greater duration of suitable conditions (i.e. consistent water levels) to develop reproductive structures than occurred at Noonameena and South Lagoon sites. Interestingly, reproductive biomass was only recorded in samples from Noonameena in June 2021, however, in field observations, flowers and fruit were common at Noonameena but likely not often sampled due to the patchy nature of the Ruppia Community at this site. The highest reproductive biomass always occurred when plant biomass was highest, as would be expected. If surveys had continued further into 2022, we expect we would have also observed an increase in viable seed bank.

This study has contributed to our knowledge of the lifecycle and phenology of the Ruppia Community in the southern Coorong and informed the Ruppia Habitat Suitability Model allowing for more informed management of this important ecosystem component (Hipsey et al. 2022).

4 Seedbanks of the Ruppia Community and regional aquatic macrophyte resources

4.1 Background

South Australia has at least three native aquatic macrophyte, flowering plant, genera adapted to grow in brackish to hypersaline water (flora.sa.gov.au). The most widespread of these (Figure 31) is *Ruppia*, which has four accepted species according to the Flora of South Australia (flora.sa.gov.au/census.shtml). Based on vouchered herbarium records (avh.ala.org.au), *Ruppia* spp. occur predominantly in coastal areas, but also are present within inland marshes and lakes (Robertson 1984). *Althenia* has six accepted species and is more commonly found on the Fleurieu Peninsula and in saline wetlands along the Coorong, although several species are more common in inland wetlands with lower salinity. *Zannichellia,* known from a single species, *Z. palustris*, is found inland along the Murray River to the border of Victoria and New South Wales, but also occurs on the northern Adelaide Plain where it grows in seasonal, brackish wetlands. In addition to these three widespread genera, *Stuckenia pectinata* is another taxon that has the potential to grow in the Coorong if conditions were suitable. *Stuckenia* is found throughout many of the hypersaline lakes, however, its ability to grow under the environmental extremes experienced in the Coorong is unknown.
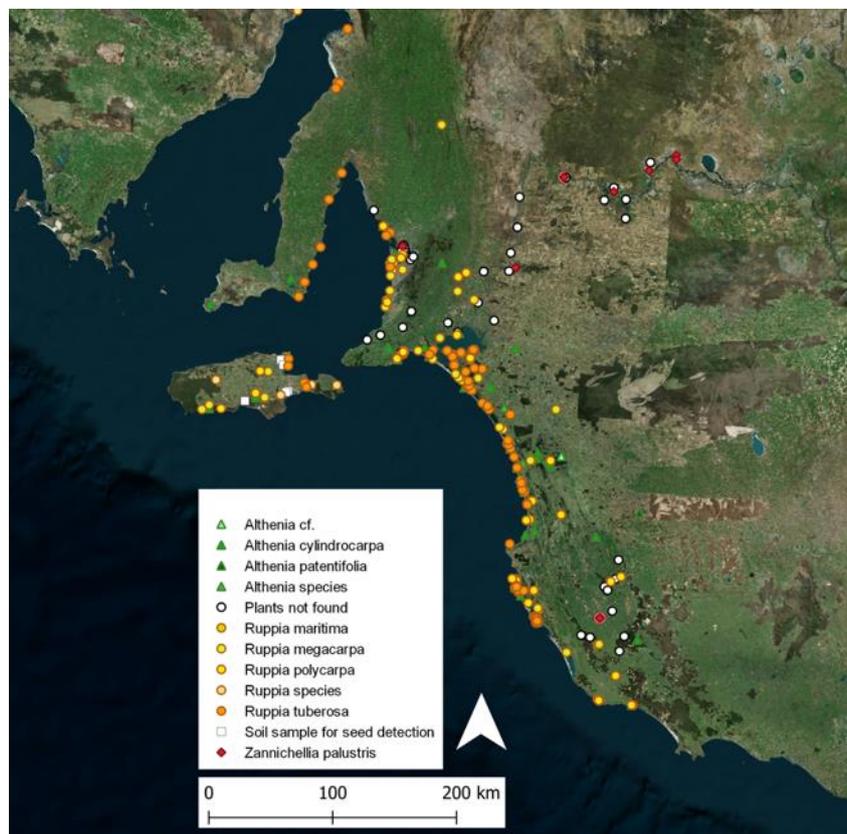
Figure 31. An occurrence map of submerged aquatic macrophyte of the genera *Althenia*, *Ruppia* and *Zannichellia* based on South Australian herbarium specimens collected in South Australia between 1852-2022.

Field observations suggest that these different macrophytes appear to display both regional and seasonal responses within South Australia. The vast majority are annuals in seasonally inundated wetlands, but several *Althenia* spp. can also behave as opportunistic perennials if standing water is present long-term. As a rule, most wetlands will have a mixture of taxa, but generally only one or two will dominate. Germination occurs mainly following the onset of autumn rains, with flowering in most cases from winter onwards until the lakes dry out or the plants die from increasing salinity as the season progresses. This means that some wetlands can be completely dominated by these macrophytes in late winter and spring, when they are grazed heavily

by waterfowl, but by summer, there may be little evidence for their presence, other than abundant fruits or seeds in the seedbank.

4.1.1 Aims

This survey aimed to identify populations of *Ruppia* species and other submerged aquatic macrophytes capable of tolerating conditions in the Coorong South Lagoon. Identifying locations capable of producing seed in large quantities can be used to inform modelling for future restoration success if Coorong sites do not produce enough seed for restoration or disappear completely. Thus this study aimed to identify potential seed donor sites for use in future translocation efforts if the Ruppia Community declines to a critical effective population size. This supporting information for the development of future restoration actions is critical as such interventions rely on a large quantity of seed being available at highly resilient sites such as outlined in the Ruppia Community Restoration Strategy (Waycott and Lewis 2022). The locations *Ruppia* and the other submerged aquatic macrophyte genera occur in would be an important knowledge resource for future planning (Waycott and Lewis 2022).

4.2 Methods

4.2.1 Field surveys

We surveyed sites across the distribution of *Ruppia* and other submerged aquatic macrophytes capable of tolerating conditions in the Coorong South Lagoon in a range of locations across South Australia. The field-based surveys of the Coorong were conducted four times over a 15-month period (Lewis et al. 2022) and focussed on 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 and for a subset of sites confirmed with DNA analysis (Lewis et al. 2022). Further field-trips around South Australia were conducted to collect voucher specimens and also recorded where aquatic macrophytes were not found. Note at this stage only potential locations were identified, seed bank collections were not made to assess donor suitability just the presence of the range of species.

4.2.2 Vouchered collections and observations

Numerous field trips to identify extant submerged aquatic macrophyte populations were made between 2019-22 to regions where species had been known to occur in the past or were expected to be based on conditions (Table 10). All trips were made in the months between June to February and mostly in the peak flowering and growing time of September to December. Herbarium specimens were made at sites where plants occurred and sites where macrophytes were absent were noted.

Table 10. Summary of macrophyte collection trips made around South Australia.

SOUTH AUSTRALIAN REGION	NUMBER OF TRIPS
Flinders Ranges/Arid Zone	6
Riverland	5
Eyre and Yorke Peninsulas	2
Kangaroo Island	1
Fleurieu Peninsula and Adelaide	3
South East and Coorong	10

4.2.3 Coorong survey observations

The primary survey area stretched the length of the main body of the Coorong from Long Point (-35.695404°S, 139.161932°E) to just south of Salt Creek (-36.140916°S, 139.628937°E). Surveys to document the aquatic macrophyte community distribution were undertaken four times. 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°S, 'central' between -35.905129°S and -35.838217°S 'south' greater than -35.905129°S.

All core samples taken for seasonal surveys (Lewis et al. 2022) or phenology (this report) were included in the assessment of seed banks and biomass. Samples were collected at sites as previously described in (Lewis et al. 2022). This included 15 replicates of 75 mm diameter sediment cores being taken to a depth of up to 8 cm. This is consistent with long term monitoring (Paton et al. 2017b) and was adopted so that results were consistent with existing *Ruppia* The Living Murray Icon Site condition monitoring data sets at the core sampling level. 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. Georeferencing of sites and samples was undertaken, the position of each core taken was recorded by GPS. Environmental co-variates were also collected at sites including water depth and salinity at time of sampling along with a sediment condition score at some sites (Lewis et al. 2022). For each core, species composition, shoot count, biomass and seed counts were recorded and tallied. Summary results were graphed for seed bank data versus linear distance from Murray Mouth and biomass, binned to 20 g biomass groups and plotted in Datagraph v. 5.0.

4.2.4 Genetic diversity of regionally collected *Ruppia* samples and the Coorong

Samples of *Ruppia tuberosa* were collected to assess genetic diversity of *Ruppia* in the Coorong prior to the translocation studies in 2012–2014 at 5 different locations (Figure 32), including a total of 12 sampling sites (Table 11). DNA analysis was conducted using Next Generation Sequencing (NGS) method following established protocols (Cross et al. 2016). Single nucleotide polymorphisms (SNPs) for 77 loci were scored and standard measures of genetic diversity were generated using Genodive and a Principal Components Analysis (PCA) conducted on the 43 samples from 5 locations with adequate DNA concentration and final DNA sequence coverage. PCA results were plotted for the first four partitions of variation.

Figure 32. Map of *Ruppia tuberosa* 2013 collected samples used in genetic analysis (for location details see Table 11).

The results from genetic screening of samples to determine community composition in the seasonal surveys for the Coorong were compared to the genetic diversity results from 2013 collected samples above. To conduct this comparison, genetic diversity based on SNPs generated in Lewis et al. (2022) were compared with additional samples from sites in South Australia and Western Australia to ascertain regional and species

diversification. Genetic distances were generated from this data and a PCA conducted on 111 samples. PCA results were plotted for the first two partitions of variation.

Table 11. Locations of samples taken for genetic analysis prior to translocation activities across South Australia (see Waycott and Lewis 2022 for additional details on translocation activities).

SITE	LOCATION DESCRIPTION	HABITAT DETAILS	ZONE	EASTING	NORTHING	DATE COLLECTED
Innes National Park 1	Lagoon ~800 m south of Browns Beach carpark, Innes National Park.	Common with ~50% area cover. Growing in shallow lagoon ~60 cm deep. Shallow sandy clay with calcrete.	53	672681	6104176	22/07/2013
Innes National Park 2	Lagoon ~850 m southeast of Browns Beach carpark, Innes National Park.	Common with ~70% area cover. Growing in shallow lagoon ~50 cm deep. Sandy with a bit of clay.	53	672963	6103971	22/07/2013
Noonameena 1	North Lagoon, Coorong National Park adjacent to Noonameena Park office, ~15 m into the water.	Scattered patches 1-2 m wide. Growing in shallow saline water up to 40 cm deep and out to ~30-40 m from the shore. Present of filamentous green algae. Sandy bottom with exposed calcrete.	54	342602	6042209	8/07/2013
Noonameena 2	North Lagoon, Coorong National Park, south of Noonameena Park office ~40 m into the water.	Scattered, ~20% cover. Growing in shallow lagoon to 10 cm deep. Brackish water, good visibility. Sandy base	54	342867	6041861	10/07/2013
Villa de Yumpa	Villa de Yumpa south Lagoon, Coorong National Park, ~29 km south of Meningie along Princes Hwy.	Scattered, ~30% cover. Growing in shallow lagoon 20 cm deep. Brackish water, low visibility, can't see bottom. Black clay.	54	360383	6024916	10/07/2013
Lake Cantara 1	Lake Cantara south, Coorong National Park, east of homestead ~50 m into the water.	Common, everywhere 1000's. Growing in shallow lagoon, 15 cm deep in brackish water, very clear. Sandy on top with black clay underneath.	54	387174	5978085	9/07/2013
Lake Cantara 2	Lake Cantara south, Coorong National Park, ~700 m from end of causeway.	Common, 1000's. Growing in shallow lagoon, 10 cm deep in blackish water, very clear, see to bottom. Sandy on top with black clay underneath.	54	387206	5978457	9/07/2013
Lake Cantara 3	Lake Cantara north, Coorong National Park, end of causeway, northside 100 m into water from track.	Common, 1000's of plants. Growing in shallow lagoon, 15-20 cm deep, water not clear, low visibility and brackish. Very high clay content.	54	387502	5978980	9/07/2013
Lake Cantara 4	Lake Cantara south, Coorong National Park, ~100 m from end of causeway and 10 m into water.	Common, 1000's. Growing in shallow lagoon, 5-10 cm deep in blackish water, high visibility and very clear, see to bottom. Sandy base with black clay underneath.	54	387553	5978644	9/07/2013

Lake Cantara 5	Lake Cantara north, Coorong National Park, at causeway gate and ~50 m into water.	Common, 1000's. Growing in shallow lagoon, 15 cm deep in blackish water, low visibility. Very high clay content.	54	387746	5978982	9/07/2013
Lake George - Beachport 1	Track to Lake George on the right of main entrance to Beachport Conservation Park. ~20 m into Lake George .	Scattered patches with ~60% area cover. Growing in shallow lagoon at ~40 cm deep. Brackish water. Sandy clay soil.	54	411766	5852905	7/08/2013
Lake George - Beachport 2	Track to Lake George on the right of main entrance to Beachport Conservation Park. ~400 m into Lake George.	Scattered patches with ~40% area cover. Growing in shallow lagoon at ~50 cm deep. Brackish water. Sandy clay with lots of shell fragments.	54	412178	5853110	7/08/2013

4.3 Results

4.3.1 Vouchered collections and field observations

Separate to the southern Coorong distribution studied in this project (Lewis et al. 2022), there were 52 macrophyte collections made in South Australia between 2019-2022 (Figure 33). *Ruppia tuberosa* was predominantly collected in small isolated locations adjacent to Lake Alexandrina and near the Murray Mouth. *Althenia* species were collected in the Coorong and further inland. *Zannichellia* was collected from Adelaide and Port Elliot. Many sites in Adelaide, in the Murray and on Kangaroo Island did not have aquatic macrophytes growing in them.
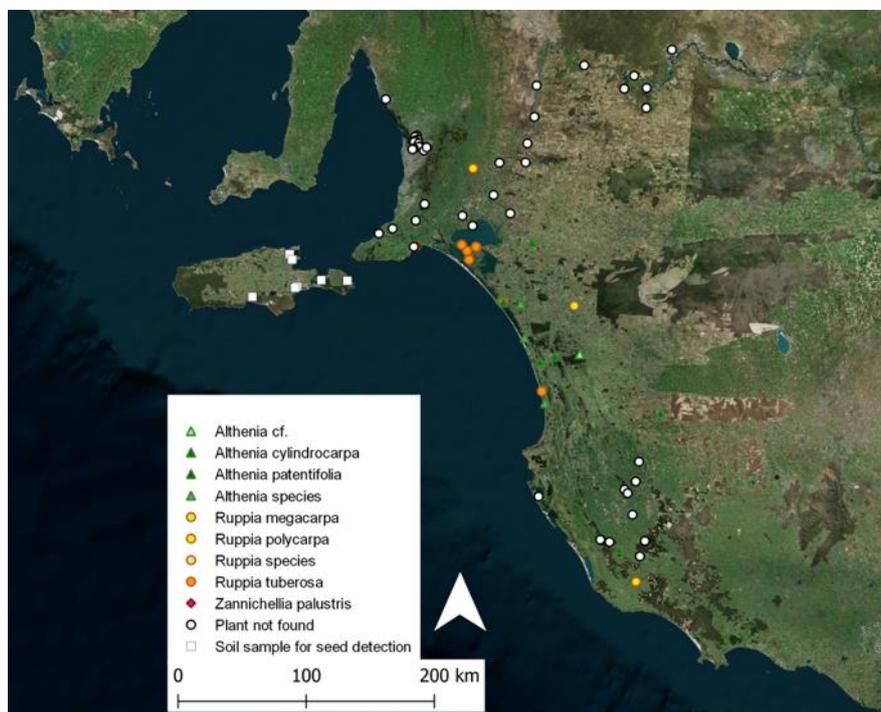
Figure 33. An occurrence map of macrophyte specimens collected in South Australia between 2019-2022.

Both *Althenia* and *Ruppia* were observed in the Coorong (Figure 34) with their frequency higher in the southern section of the survey area (Lewis et al. 2022). *Ruppia* species were more common than *Althenia* which was rarely observed in the southern end of the Coorong South Lagoon.

Figure 34. Presence of aquatic macrophyte species in surveys observed across HCHB sites (Lewis et al. 2022; this study). White dot points represent areas where no plants were found.

4.3.2 Seed bank of *Ruppia* species in the Coorong

There was widespread distribution of the seed bank of *Ruppia* species across the surveyed region in the Coorong, extending from Long Point in the North Lagoon to south of Salt Creek in the South Lagoon, on both sides of the lagoon. In most areas there were a greater number of seeds in the seed bank in 2020 than 2021 (Figure 35), which when compared with the production of turions (Figure 36) was opposite to the number of turions produced. In 2021, the area on the western side of the Coorong, south of Parnka Point to just south of Swan Island appeared to have more substantial seed banks (Distance from the Murray Mouth (DFMM); 65–69 km) (Figure 35). Interestingly some of these sites also possessed high biomass and were growing in deeper water (Lewis et al. 2022).
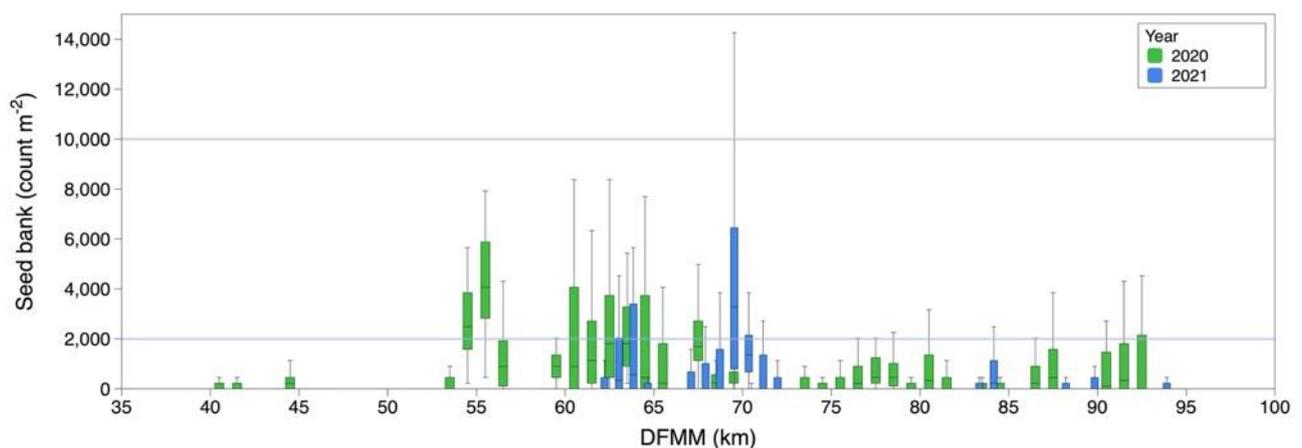
Figure 35. Boxplots of seed bank as number of seeds estimated (m^{-2}) based on replicate cores taken at each location at all sampling times (Lewis et al. 2022; this study) plotted by linear distance from the Murray Mouth (DFMM, km) grouped into 1 km bins for comparative purposes.
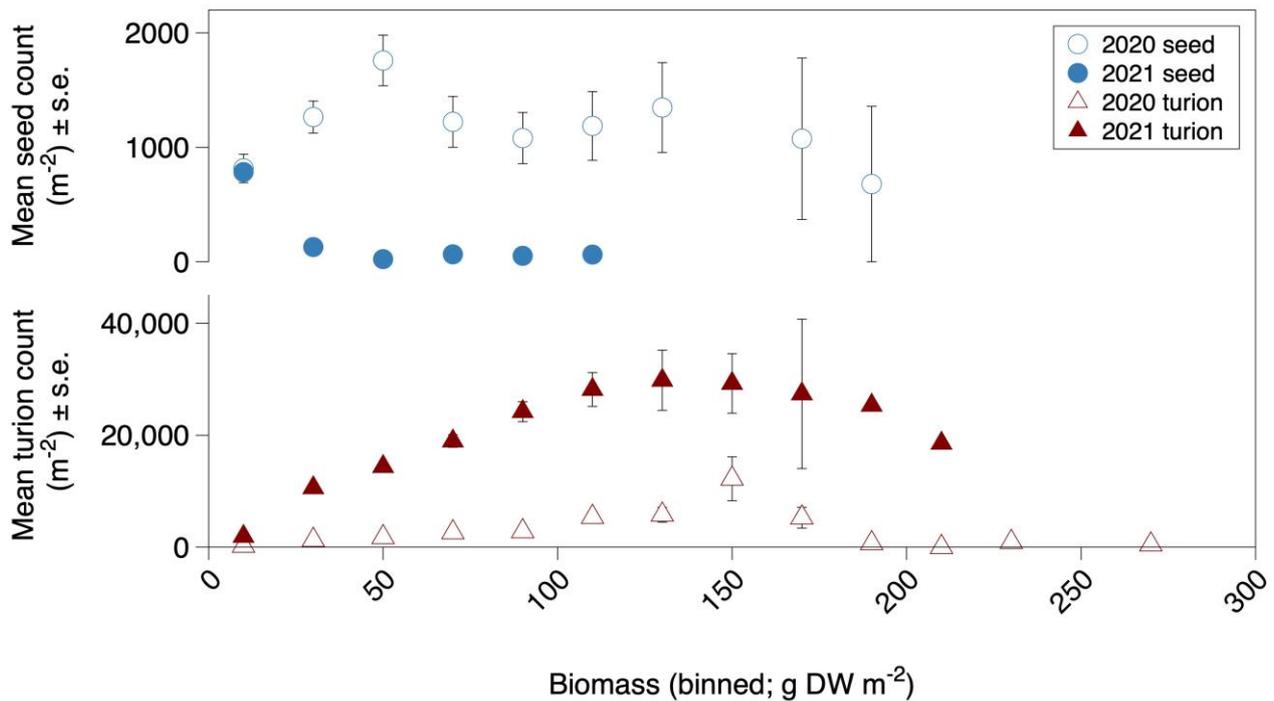
Figure 36. Mean seed bank density as number of seeds m^{-2} (top) and turion density as number of turions developing (m^{-2}) based on replicate cores taken at each location at all sampling times (Lewis et al. 2022; this study) plotted compared to total biomass (g DW m^{-2}), biomass data grouped into 20 g DW m^{-2} bins for comparative purposes.

4.3.3 Regional variation in genetic connectivity and Coorong *Ruppia* species

Genetic variation comparisons based on the PCA of the 5 regions surveyed in 2013 samples from each of the sampling locations demonstrated regional groupings, with overlaps in the distinctiveness of the genetic variation documented (Figure 37). The greatest proportion of variation (PCA axis 1, 40.5%) weakly separated the populations and incompletely resolved clusters indicative of connectivity across the 5 locations. Similarly, from the recent surveys, samples from the Coorong and other known *Ruppia tuberosa* samples were clearly grouped together indicative of high connectivity; even samples from Western Australia aligned with the highly clustered group (Figure 38). Herbarium specimens of *Ruppia megacarpa* were highly differentiated, both from Western Australia and South Australia. All populations sampled exhibited a moderate to high number of genotypes present (i.e. no evidence of clonality among the 111 samples screened in this survey).
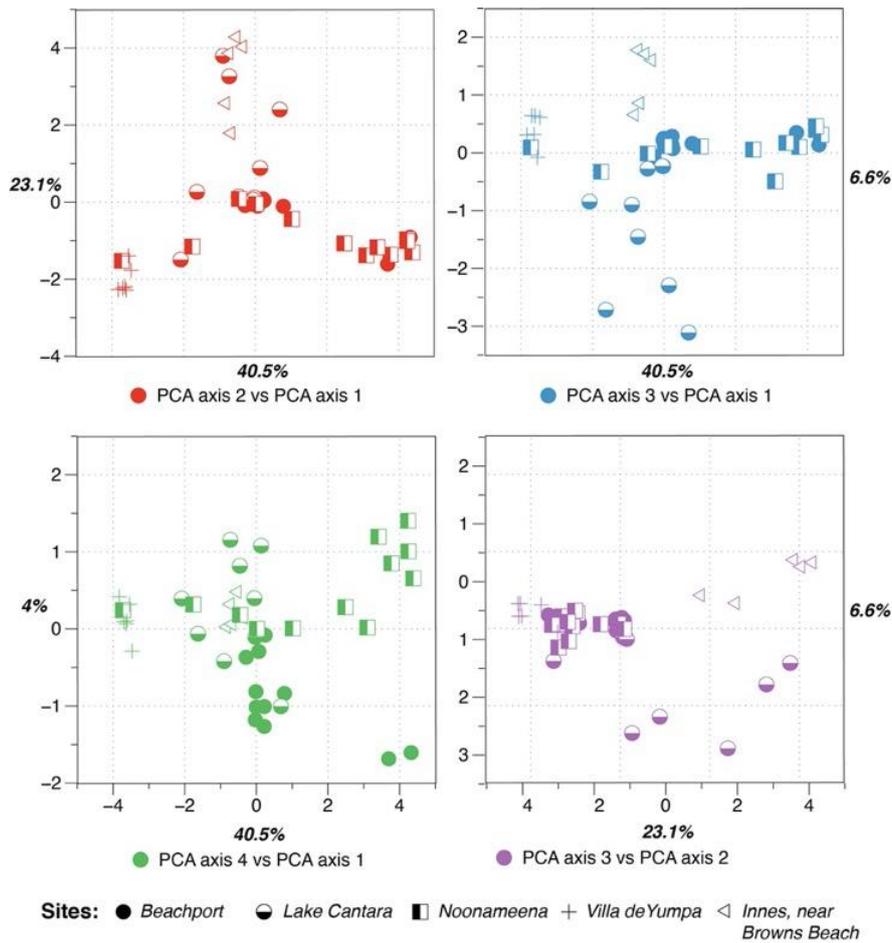
Figure 37. Principal Components Analysis (PCA) of SNP data based on population samples from five regions collected prior to translocation actions 2013–2014 data analysed for this study. The proportion (%) of variation attributed to the PCA axis is noted on the relevant axis. Sites details are found in Table 11.
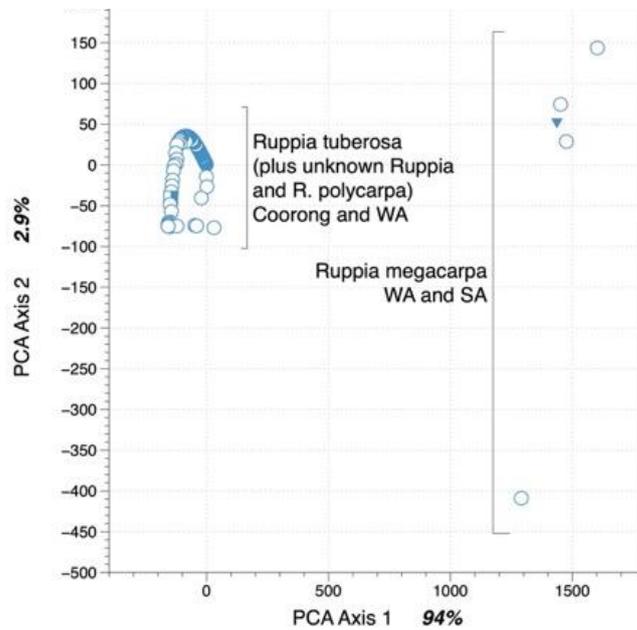
Figure 38. Principal Components Analysis (PCA) of SNP data based on samples collected during surveys (Lewis et al. 2022) and from herbarium specimens. The proportion (%) of variation attributed to the PCA axis is noted on the relevant axis.

4.4 Discussion

Among the more than 50 field locations across South Australia visited from 2019-2022 there are populations of submerged aquatic macrophytes outside the Coorong and adjacent areas. The conditions in some of these locations may contain plants that could potentially survive in the Coorong, although our current knowledge suggests most require lower maximum salinities to survive. Interrogating the South Australian herbarium data for longer term historical records identified collections date back to 1852 when this region remained unmodified. Given how much of the landscape has changed since the 19th century recovering these species in the current ecosystem would be unlikely.

If restoration of submerged aquatic macrophytes is needed in the Coorong then identifying additional potential donor sites from existing populations that produce a suitable quantity of seed is critical (Waycott and Lewis 2022). If found, these populations should be protected as a matter of priority. If a wider range of source donor sites is not possible then efforts to generate translocation sources in situ will be needed. We surveyed the Coorong and other parts of the state and found some locations where other aquatic macrophytes occur, although data on the condition or seed banks at these sites was not obtained outside the Coorong. In addition, these surveys determined there are areas where aquatic macrophytes were expected but were not found. The lack of good potential sites to explore as donors for translocation activities leaves Lake Cantara and a few other small hypersaline salt lagoons nearby as the remaining potential donor sites. If rehabilitation and restoration of the Coorong is required in the future, there will be a need to develop alternative strategies such as seed nurseries. The restoration strategy for the Ruppia Community in the Coorong (Figure 39) highlights the importance of seed sources to be available for continued ecological restoration options (Figure 39B) as this would represent a road block step to future restoration actions for the site (Waycott and Lewis 2022) to maintain similar ecological community characteristics.

A.
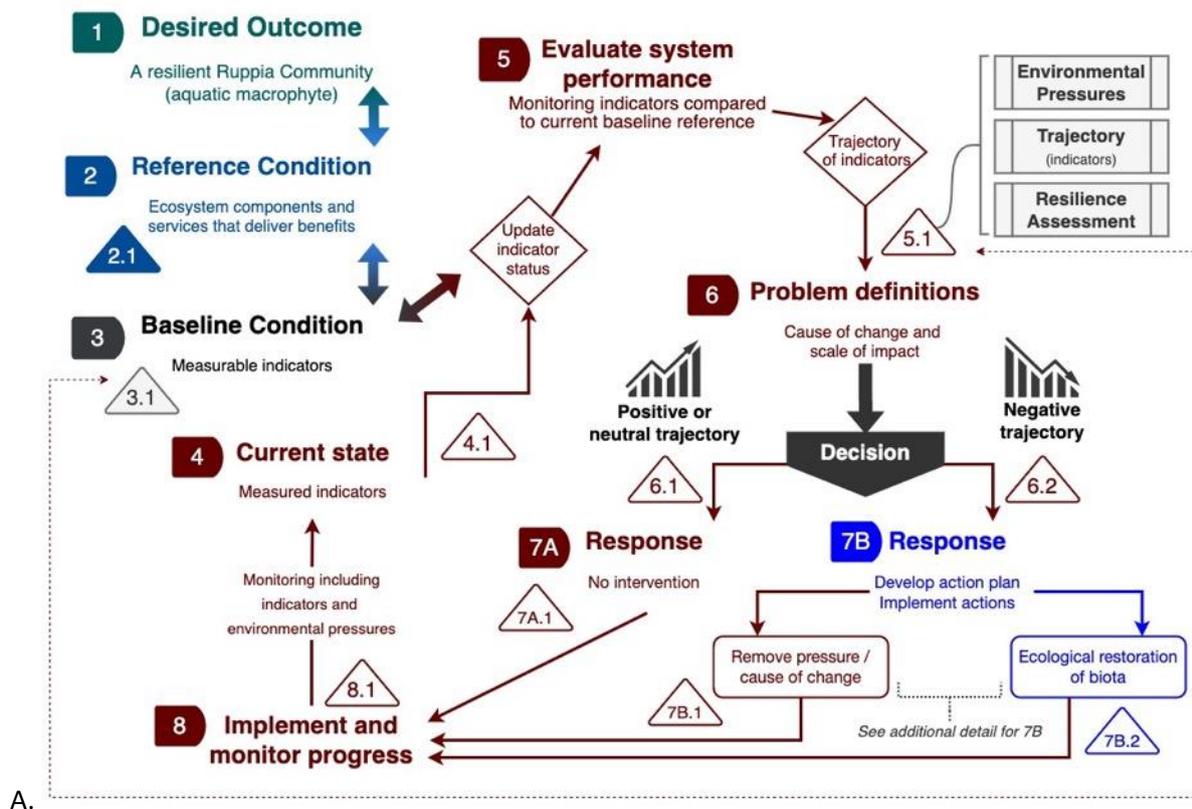
Continued over page.

B.
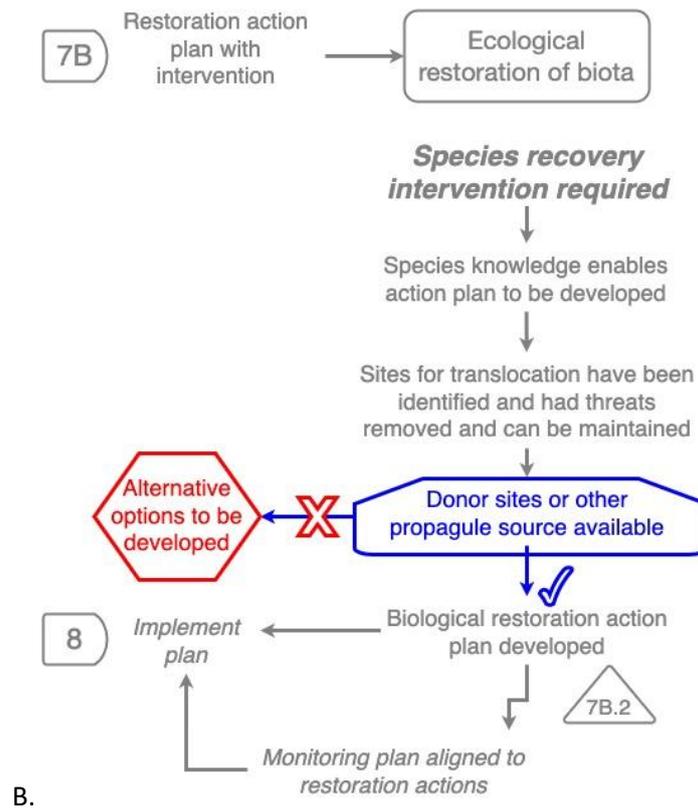
Figure 39. Excerpt from the restoration strategy for the Coorong Ruppia Community (Waycott and Lewis 2022) A. overall process proposed for future evaluation and decision making strategy, B. part 7B, detail on the Ecological Restoration of biota (7B, highlighted blue in A.) and the critical nature of the need for seed sources to the outcome.

Observations of the overall presence of plants and *Ruppia* species seed banks at sites in the Coorong showed that there are widespread submerged aquatic macrophytes across the surveyed area. This is important both to maintaining existing recovering populations but also for the potential to use plants from these populations as a seed source if conditions decline in the future. It is preferable to maintain a local source of translocation material for restoration from locations similar to the Coorong, as these plants are more likely to have local adaptations. These adaptations will give any new restored communities an advantage and in this area include any cryptic genetic variation that is still unknown (Lewis et al. 2022, Waycott and Lewis 2022). Previous surveys of genetic diversity from samples collected in 2013 supported moderate to high genetic diversity among 5 regions in South Australia analysed prior to translocation of Ruppia into the Coorong. The lack of discrete clustering among samples in PCA supports the concept of connectivity among the major regions of *Ruppia tuberosa* in South Australia, likely due to bird mediated gene flow. Future strategies to restore the Ruppia Community may include sourcing materials from widespread locations.

5 Chemical composition of sediment, water, algae and plants in the southern Coorong

5.1 Introduction

The Coorong is characterised by a salinity gradient that is estuarine to marine at the Murray Mouth becoming hypersaline and even extreme hypersaline towards the southern end of the South Lagoon (Mosley et al. 2020). The Coorong South Lagoon has been exceeding >100 g L^{-1} seasonally, for several decades (Mosley et al. 2020) and this salinity gradient is now documented to have an almost linear relationship between increasing total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) with increasing salinity towards the southern end of the Coorong South Lagoon (Priestley et al. 2022a). High nutrient concentrations are now known to be associated with the organic material in the Coorong (Priestley et al. 2022a) and the Coorong South Lagoon annual nutrient cycle is driven by the loads present in sediments and the growth of fast turnover organisms including microbiota such as phytoplankton (Huang et al. 2022, Priestley et al. 2022a). The water column microbial community in the hypersaline areas of the Coorong is dominated seasonally by picophytoplankton which include groups adapted to hypersaline, high nutrient conditions (Jamieson et al. 2022). The new combined evidence supports the hypothesis that nutrients are accumulating within the Coorong along with salinity and the characterisation of the Coorong South Lagoon as hyper-eutrophic (Mosley and Hipsey 2019, Mosley et al. 2020).

The seasonal change in dissolved and particulate nutrients occurs through their concentration and accumulation over time from early winter, when salinity is reduced, to a peak at the end of summer, associated with a lack of flushing and evaporation (Priestley et al. 2022a). The strong correlation between nutrient concentrations and salinity, where higher nutrient concentrations occur in the areas of higher salinity, indicates the importance of this cyclic process (Mosley et al. 2020). In addition, further studies of nutrient dynamics in the *Healthy Coorong, Healthy Basin* program document a significant effect of locally derived nutrients bound up in sediments influencing nutrient cycles for the Coorong South Lagoon overall (Mosley and Hipsey 2019, Mosley et al. 2020, Huang et al. 2022, Priestley et al. 2022a).

In Spring 2021, following widespread and high winter rainfall in the south-east of Australia, flows into the Coorong from the South East drainage scheme through Salt Creek were increased (from 9 August). Salt Creek is the source of direct freshwater input to the Coorong South Lagoon and provides an important opportunity to reduce hypersalinity and improve water quality. Simultaneous releases of water from the Lower Lakes barrages also occurred during Spring 2021. This led to higher water levels than recent years in the South Lagoon and could be expected to alter the nutrient inputs, overall nutrient dynamics and water quality of the system that the Ruppia Community occupies.

The nutrient environment influences the manner in which the growth of the Ruppia Community, as has been found in other seagrasses, can be limited, through limitation or stressed through excess (Hemminga and Duarte 2000). Nutrients that are in short supply or excess have been well established to result in poorer performance and lead to lower overall plant biomass, and there is a strong relationship between the dominant form of nutrient, particularly nitrogen, in an ecosystem and also shows in the seagrass tissue nutrient content measured as a proportion of tissue biomass (Duarte 1990, Udy et al. 1999, Collier et al. 2017). The application of the Redfield Ratio (a ratio of carbon, nitrogen and phosphorus, C:N:P) tending towards 550:30:1 for seagrasses (Atkinson and Smith 1983) may vary considerably depending on species and the water quality environment (Duarte 1990). The ratio is indicative of the nutrient balance in an ecosystem and has been used to infer nutrient limitation, eutrophication or changes in nutrient balance over time globally (e.g. Duarte 1990). Plants residing in nutrient poor waters show significantly high C:N and/or C:P ratios compared with plants from nutrient rich conditions (Atkinson and Smith 1983). However, the total carbon content of seagrass tissue is primarily associated with structural carbon and light limitation and the interpretation of nutrient conditions relative to this have led to the proposition that the N:P ratios may better indicate ambient nutrients as a more accurate summary of recent water quality than C:N and C:P (Johnson et al. 2006). A simplified model that explains the nutrient limitation of plants to nitrogen and phosphorus

availability can be used to compare plant tissue nutrient levels (Figure 40). Given the diversity in plant form changing resource allocation, longevity of tissues and mobilisation of nutrients with reproduction and senescence (Kilminster et al. 2015) it is unsurprising the variation among species can be very high (e.g. Duarte 1990). However, these ratios represent a reference point for comparison with global studies if comparisons made are done with these factors in mind. The changes in these ratios will be more useful when applied at the same locations for the same species of seagrass to evaluate trends over time. Thus using comparison of nutrient ratios over time and space within the same ecosystem we can use nutrient ratios and other attributes such as isotope ratios to facilitate understanding as to how the nutrient environment of the Coorong may be influencing Ruppia Community growth or at least the trends they exhibit. In this chapter we consider the plant nutrient status along with local environmental nutrients in the water column and sediments.
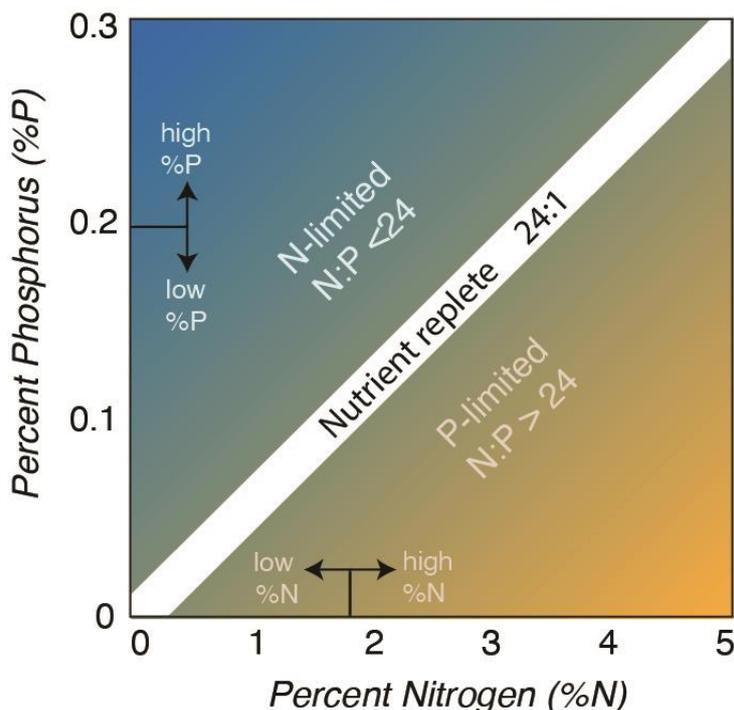
Figure 40. Framework for interpreting relative availability of nutrients for seagrasses (Collier et al. 2017). When the ratio of nitrogen to phosphorus (N:P) is 24:1, the seagrass is likely to be 'replete' and when N:P is less than 24 then they are N-limited (Duarte 1990). The primary limiting nutrient is independent of N and P concentrations. Median tissue N concentration of seagrasses globally is 1.8% and values above this indicate high N availability; high N can occur when either N or P are limiting. Equivalent thresholds are different in plankton and macroalgae.

5.2 Methods

Collections for nutrient content analysis of sediment, water and plant material of the Ruppia Community and filamentous algae where present were made at 16 locations (Figure 41) over a period of six months (Table 12). Locations were distributed from the lower section of the Coorong South Lagoon near Tea Tree Crossing, included the Salt Creek outlet (Loop Road) to Parnka Point and sites in the central section, the most northern location at Noonameena in the Coorong North Lagoon. These locations span the majority of distribution where surveys of the Ruppia Community have been conducted previously (Paton et al. 2017b, 2021, Lewis et al. 2022). In addition to sampling the spatial extent of the region occupied by the Ruppia Community in the southern Coorong sampling at different stages of the annual growth cycle were undertaken, in particular early growth in winter (August), during rapid growth in Spring (October) and during senescence in Summer (January) (Table 13).

To detect possible changes in nutrient regime with the additional water releases that occurred in 2021 (due to extensive weather driven runoff across the wider region) led to accumulation of a large volume of water in Morella Basin which allowed for a release of water through Salt Creek in August 2021 (Mosley et al. 2022). Sampling began prior to the water release from the Salt Creek outlet on 8 August 2021. As per planned operations of Morella and Salt Creek, flow from the Salt Creek regulator to the Coorong South Lagoon was gradually reduced from around 200 ML day^{-1} on 5 October 2021 to fishway-only flow of around 10–30 ML day^{-1} by 8 October 2021.

Samples were collected prior to commencement of increased flows (3-5 August 2021) and fortnightly until flows were reduced, with the last collection being undertaken on 5 January 2022 (Table 13). Each of the 16 sites was sampled on 3-5 August and 18 October 2021, pre and post increased flows; however, a subset were chosen for more regular sampling, from which samples were collected fortnightly (which were the same as the phenology survey sites, see Chapter 3). Samples from the Salt Creek regulator were collected fortnightly from 3 August to 18 October 2021 while increased flows were occurring.

Sediments were collected using a 75 mm diameter core sampler (the same size as used in Ruppia Community sampling), five cores were taken to 100 mm depth, mixed in a bucket then a subsample was collected and stored in a 50 mL Falcon tube. Time taken to mix sediments was kept to a minimum and immediately placed in the 50 mL tube and capped.

Plant tissues (for the Ruppia Community) were collected for nutrient analysis with a 75 mm diameter core sampler being scraped along approximately 300 mm of sediment where seagrass was present. This was replicated five times to obtain enough material for analysis (1-3 g DW requested by laboratory).

Table 12. Locations of nutrient collections in this study, approximate locations shown on the map in Figure 41.

SITE	SITE NUMBER IN THIS CHAPTER	LATITUDE °S	LONGITUDE °E
Tea Tree Crossing	20	-36.185111	139.659781
Loop road 1	19	-36.164061	139.649459
Loop road 2	19	-36.157398	139.645713
Salt Creek outlet	17.5	-36.132266	139.640712
Salt Creek Phenology Site (site 10, Wild Dog Island)	16	-36.119467	139.638808
Snipe Island	15	-36.109075	139.629365
Seagull Island	14.5	-36.078975	139.600486
Policeman Point	13	-36.059014	139.586525
South Jack Point	11	-36.050587	139.578618
Jack Point	12	-36.031925	139.569755
Fat Cattle Point	11.5	-36.014657	139.561787
Woods Well	10	-35.994037	139.538751
Villa de Yumpa	9	-35.909919	139.452697
Parnka Point	7	-35.902328	139.398428
North Magrath Flat	3	-35.852698	139.386004
Noonameena	1	-35.755128	139.262041

Water samples were collected using a triple-rinsed 50 mm Falcon Tube, which was filled approximately 100 mm below the water surface. Two Falcon tubes of unfiltered water were collected for total nutrient analyses. One Falcon tube of filtered water was collected for dissolved nutrients.

Algae was collected where present in the same way as for biomass measurements (see Lewis et al. (2022) from Parnka Point on 3 and 17 November 2021. At this time there was 100 % algal surface cover which was made up of a mixed community of filamentous green algae species forming clumps and mats containing species confirmed to be *Ulva paradoxa*, *Rhizoclonium sp*. and *Cladophora sp*. (as described in Collier et al. 2017), samples were taken using a plastic tube with a diameter of 400 mm (0.125 m^2 surface area). 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 using a scoop of 0.8-1 mm mesh size, while removing as much macrophyte material as possible and placed 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). These samples were submitted to the laboratory for nutrient analysis.

All samples were kept in an esky on ice in transit to the laboratory where they were frozen before transportation frozen on ice by courier to laboratory for analysis. Laboratory analysis was conducted by Environment Analysis Laboratory (EAL), Southern Cross University, New South Wales. The range of analyses conducted including testing nutrients on sediments, plant tissue and water column (Table 14).
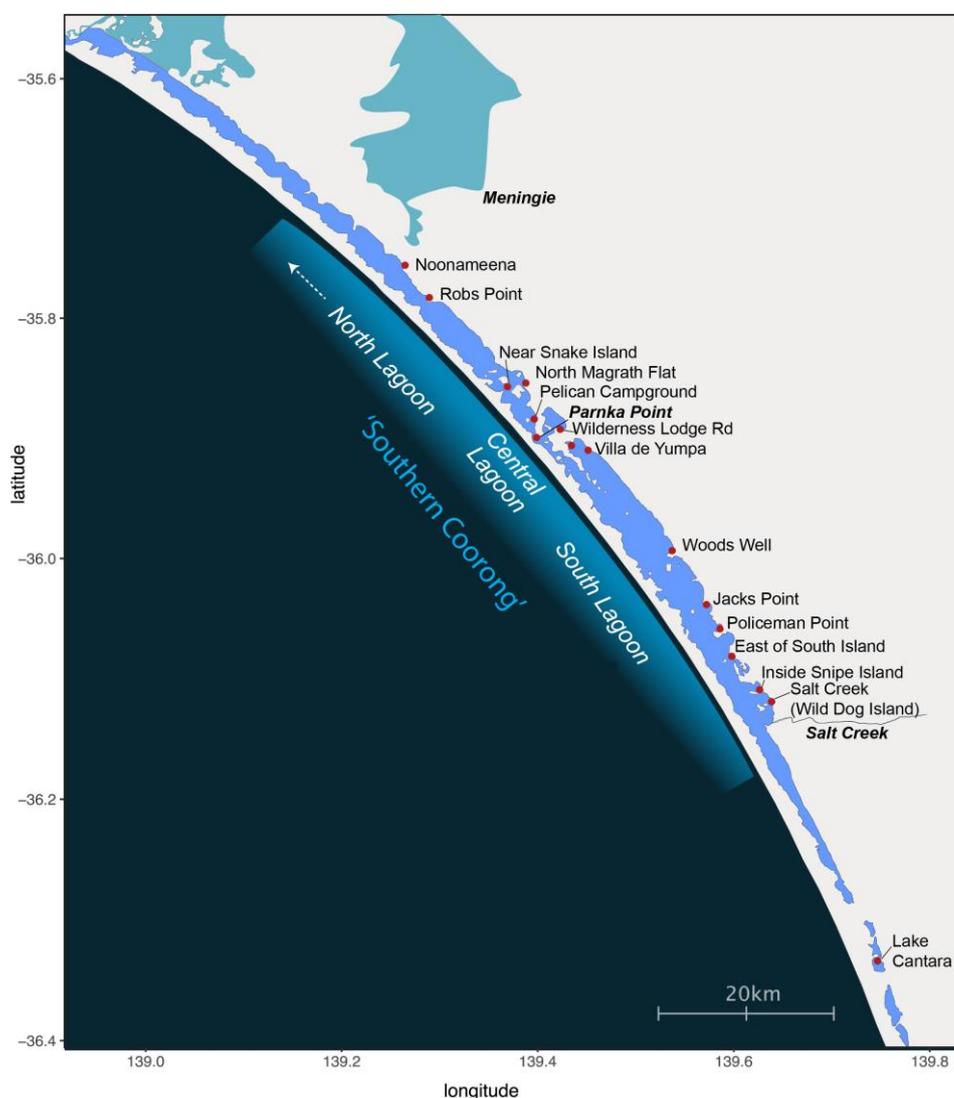
Figure 41. Map of nutrient collection sites in the southern Coorong included in this study, for site details see Table 12.

Statistical analysis of plant tissue nutrient data was undertaken using the software package IBM SPSS Statistics V. 11. A series of One-way ANOVA tests were conducted on untransformed data to analyse the different sampling times, locations and tissue nutrient composition. Multivariate analysis of cofactors was conducted using generalised linear model approach to test site level differences.

Table 13. Locations and dates of when nutrient sampling was undertaken in this study (locations refer to Table 12 and Figure 41).

SITE	3-5 AUG 2021	25 AUG 2021	9 SEPT 2021	22 SEPT 2021	6 OCT 2021	18 OCT 2021	2 NOV 2021	17 NOV 2021	14 DEC 2021	5 JAN 2022
Tea Tree Crossing	✓					✓				
Loop road 1	✓					✓				
Loop road 2	✓					✓				
Salt Creek outlet	✓	✓	✓	✓	✓	✓				
Salt Creek Phenology Site	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Snipe Island	✓					✓				
Seagull Island	✓					✓				
Policeman Point	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
South Jack Point	✓					✓				
Jack Point	✓					✓				
Fat Cattle Point	✓					✓				
Woods Well	✓	✓	✓	✓	✓	✓				
Villa de Yumpa	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Parnka Point	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
North Magrath Flat	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Noonameena	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 14. List of analyses conducted by Environment Analysis Laboratory for Coorong sediment, plant (seagrass and filamentous algae) and water samples.

SAMPLE TYPE	ANALYSIS
Sediment	Particle size analysis – ISSS hydrometer plus 0.2 and 2.0 mm sieving
	Particle sizing of soils and sediments for ISSS classification: < 2 µm, 2–20 µm and 20 µm–2 mm by hydrometer; 0.2–2.0 mm and > 2 mm by sieving
	Phosphorus - total acid extractable
	Total nitrogen
	Carbon total and organic
	Acid volatile sulfide (AVS) (S-AV)
	Total nitrogen in bulk solids - δ15N isotopes and % total nitrogen (TN)
	Organic carbon in bulk solids - δ13C Isotopes and % C
Plant (seagrass and algae)	Phosphorus - total acid extractable
	Total carbon, total organic carbon and total nitrogen in bulk solids - δ13C and δ15N Isotopes % TC %TCorg and % TN
Water	Nutrients - Total Total nitrogen (TN), total phosphorus (TP).
	Nutrients - dissolved nitrate, nitrite, phosphate, ammonium.
	Carbon - total organic (TORG)

5.3 Results

5.3.1 Bulk sediment quality

Bulk sediment quality results collected in this study were combined with a dataset collected 2020–2021 as part of the *Healthy Coorong, Healthy Basin* Program (Huang et al. 2022). Generally, total nitrogen (TN) (Figure 43), total phosphorus (TP) (Figure 44), total organic carbon (TORG) (Figure 45) and Acid Volatile Sulfide (AVS) concentrations increased in a gradient towards the South Lagoon (Figure 46). The sediment in deeper basins (>2 m water depth) of the South Lagoon had high total nutrient, organic carbon and AVS concentrations (~5–7% TORG, 0.5–0.7% TN, 0.5–0.6% TP, >0.004% AVS). The southern region of the North Lagoon (i.e. 7 Mile Basin to the north of The Needles) also has high total nutrient, TORG and AVS concentrations and is also dominated by a black ooze sediment type. Samples collected from the North Lagoon site Noonameena, and immediately adjacent to the inflow of water at the Salt Creek Outlet, showed lower sediment nutrient and TORG concentrations.

Sediment nutrient concentrations over the 6 month sampling period varied depending on location and an increase in overall organic carbon and total nitrogen into warmer months although the scale of this increase was variable and clearly site dependent. Sampling sites for the sediments collected in these analyses are from shallow depth, near shore locations, typically where the Ruppia Community grows. Overall increase in values sediment nutrient values measured at the Villa de Yumpa location for many nutrient parameters including total nitrogen, phosphorus, organic carbon, and Acid Volatile Sulfide (AVS), were higher than elsewhere during the period sampled.
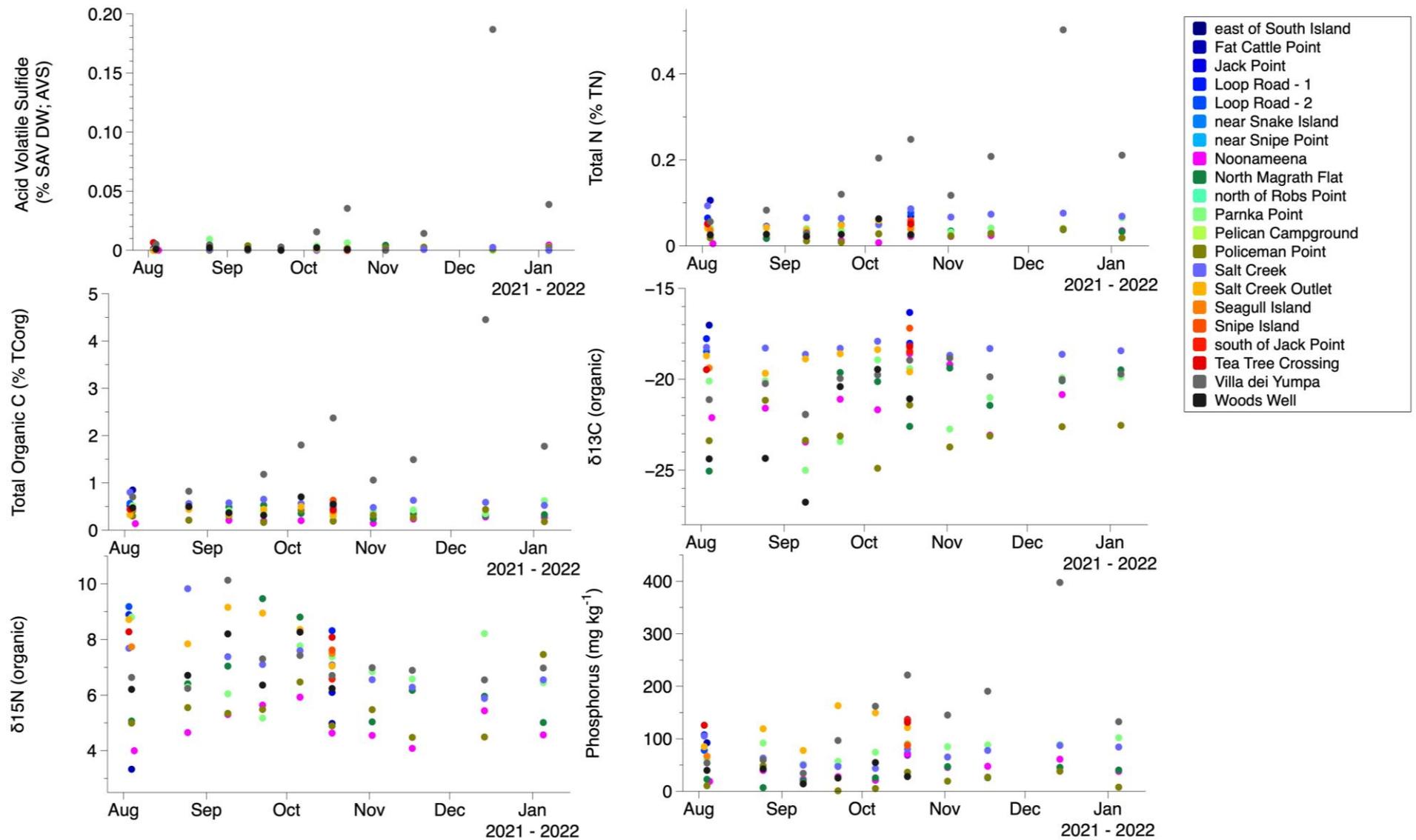
Figure 42. Sediment nutrient measurements across locations (see Table 12) and all sampling periods (Table 13). Each point represents an individual analysis.
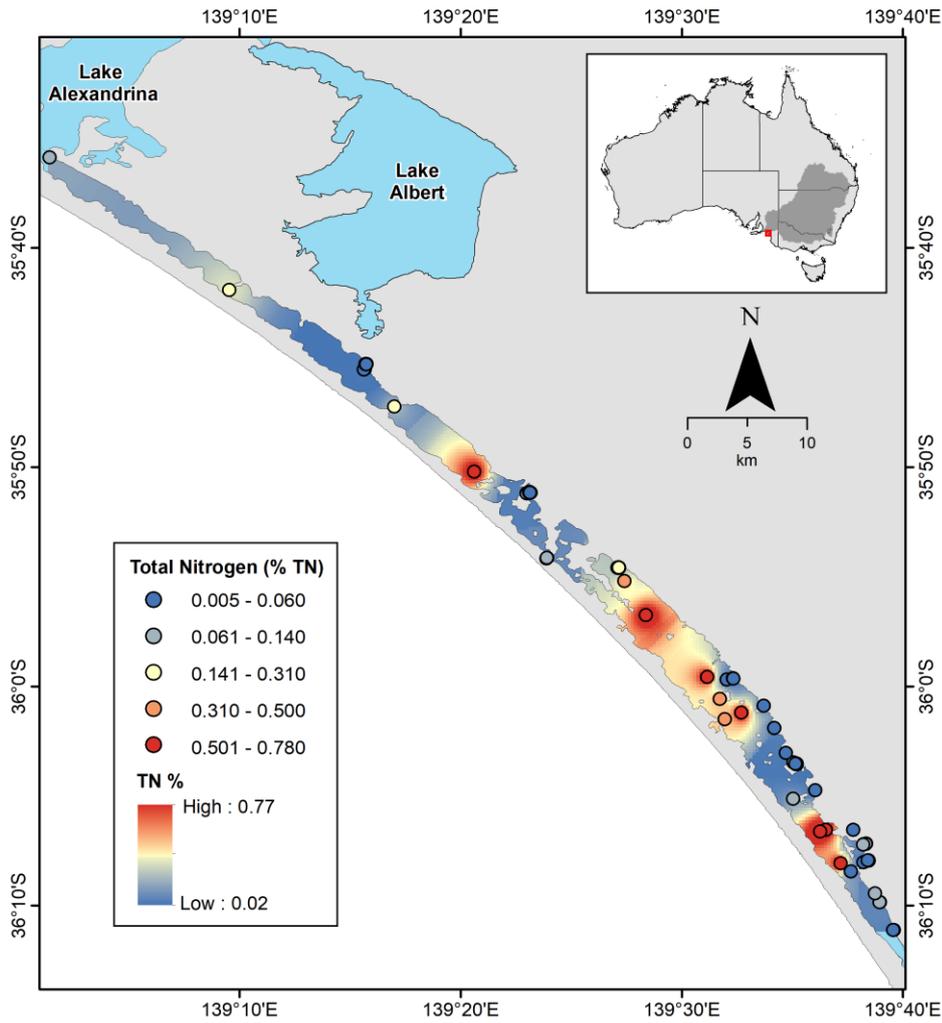
Figure 43. Map of total nitrogen (TN) in the Coorong sediment. Interpolation between sampling points was performed via Inverse Distance Weighting (IDW). There is lower accuracy of interpolation where there is a lower sampling density.
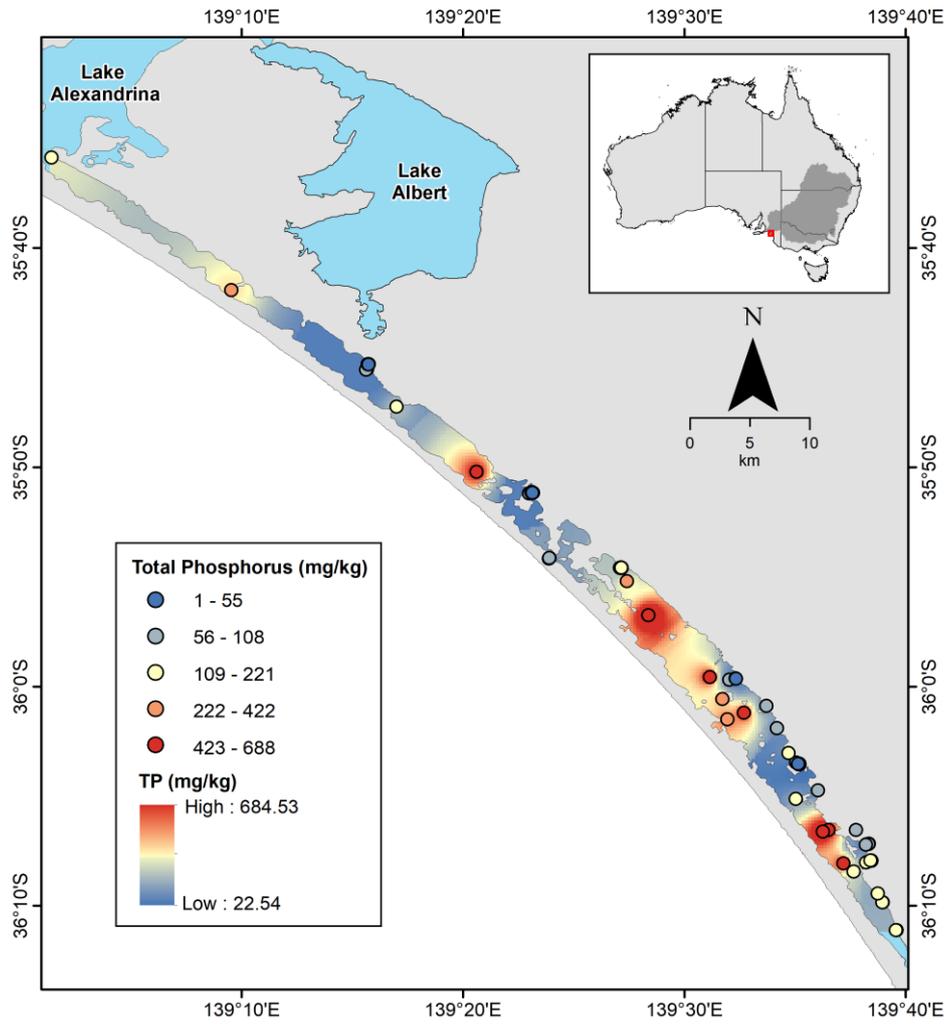
Figure 44. Map of total phosphorus (TP) in the Coorong sediment. Interpolation between sampling points was performed via Inverse Distance Weighting (IDW). There is lower accuracy of interpolation where there is a lower sampling density.
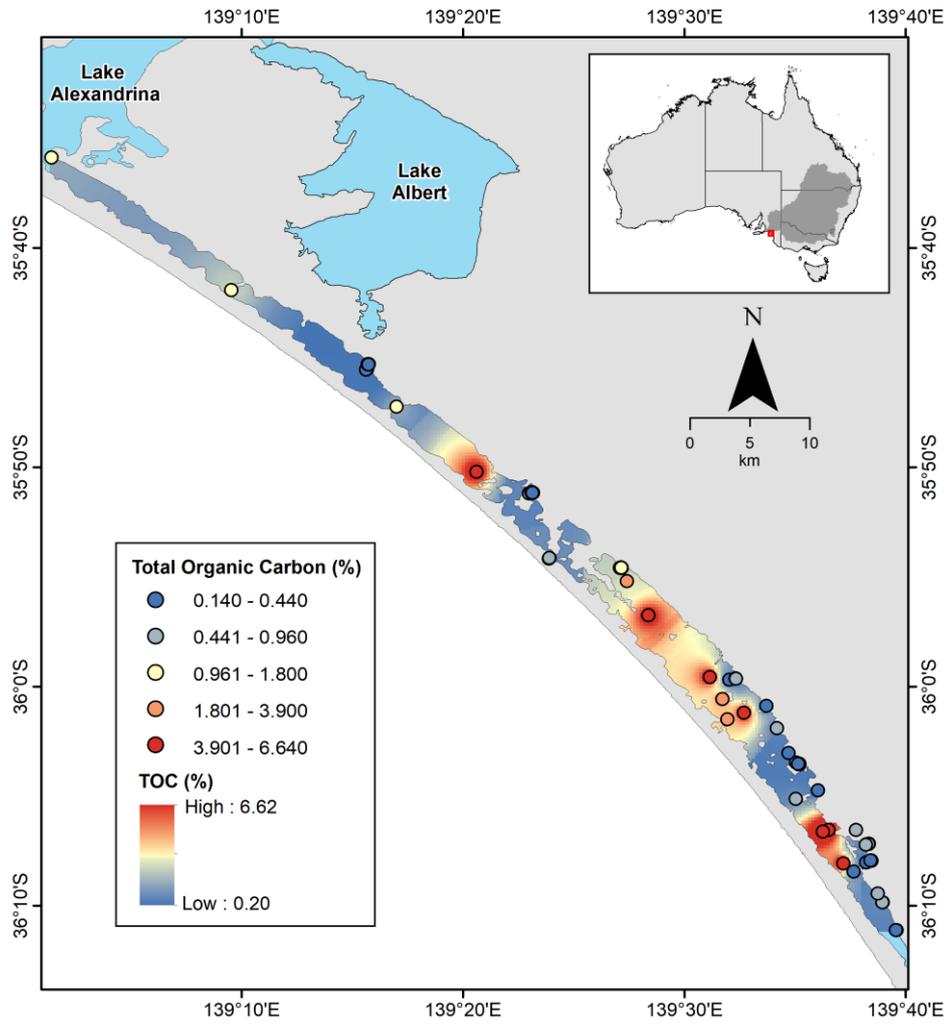
Figure 45. Map of total organic carbon (TORG) in the Coorong sediment. Interpolation between sampling points was performed via Inverse Distance Weighting (IDW). There is lower accuracy of interpolation where there is a lower sampling density.
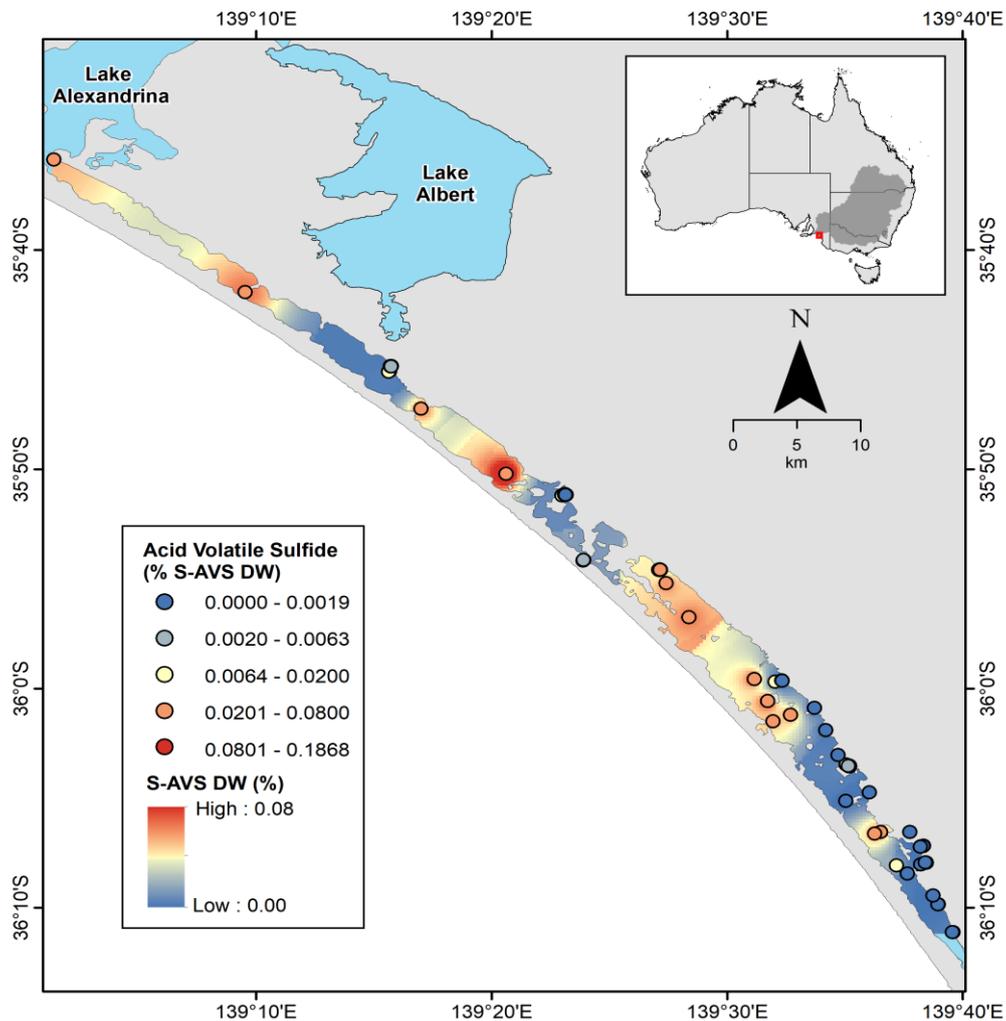
Figure 46. Map of Acid Volatile Sulfide (AVS, dry weight basis) in the Coorong sediment. Interpolation between sampling points was performed via Inverse Distance Weighting (IDW). There is lower accuracy of interpolation where there is a lower sampling density.

5.3.2 Comparison of bulk sediment nutrient content between 2017 and 2021

Samples from collections made in 2016-2017 along with plant material were available that had been analysed with the same methods and some of the same locations so could be used in direct comparison with samples collected in 2021-2022. In these comparisons (Figure 47) the range of total nitrogen (%N) was lower in the 2017 samples (Figure 47 A. and E.). Total phosphorus was similar although some sites were considerably higher (Figure 47 F.) most notably Villa dei Yumpa which had higher sediment nutrient concentrations than other sites in all analyses. The sediment N:P ratios were significantly higher in 2021 (Figure 47 C.) as would be expected with more nitrogen in the sediment overall in many locations (Figure 47 E.).
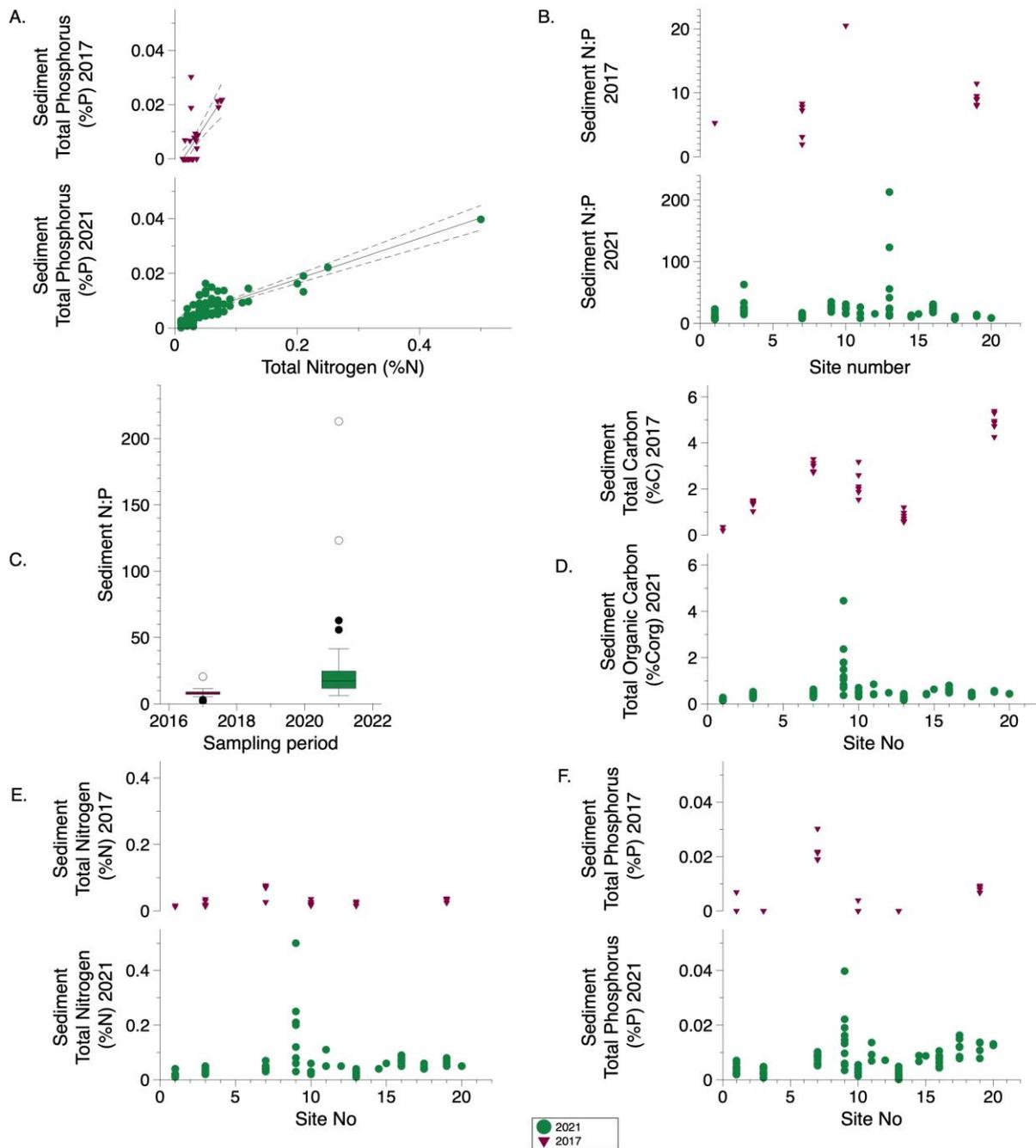
Figure 47. Concentrations of nutrients in sediment from bulk samples and from locations sampled in 2016-2017 and 2021-2022, for details of locations associated with site numbers refer to Table 12, site number order 1–20 is from the North Lagoon site Noonameena to Tea Tree Crossing south of Salt Creek. Each nutrient plot has a separate axis for samples collected in each year. A. Direct comparison of %P and %N and a line of best fit and 95% confidence interval. B. N;P ratio plotted for each site. C. Box plots for the overall N:P ratio for the two major sampling periods. D. Total carbon (%C and %Corg) for each site. E. Total nitrogen (%N) for each site. F. Total phosphorus (%P) for each site.

5.3.3 Comparison of bulk sediment quality to Ruppia Community biomass and Rapid Assessment Protocol scores

Based on the mean total Rapid Assessment Protocol (RAP) score (Hallett et al. 2019) from each site, three classes of RAP sediment quality were developed; 'Poor'≤5, 'Moderate' 6-10, and 'Good' 11-15. Sites in the

poor, moderate and good sediment quality classes exhibited consistent patterns across their TN, TORG, TP and AVS values, with the median values of the poor class being 4–5 times higher than those of the good class (Figure 48). Many of the poor sediment quality results were from deeper basin areas of the South Lagoon and southern region of the North Lagoon (see Figure 43 and Figure 46). The Ruppia Community is generally absent from these sites and it is likely sediment stability and light availability limits colonisation at these sites. The Ruppia Community can enhance sediment quality where it is present through promoting oxygenation of the rhizosphere in the sediment which would lead to enhanced nitrification-denitrification (nitrogen loss mechanism) and aerobic mineralisation of organic carbon (see Huang et al. 2022). The strong positive association of biomass with good sediment RAP scores (Figure 49 A) is accompanied by a positive relationship with turion development (Figure 49 B). Note that the relatively poor seed set in 2021 sampling results appears associated with high turion production (see Figure 36).
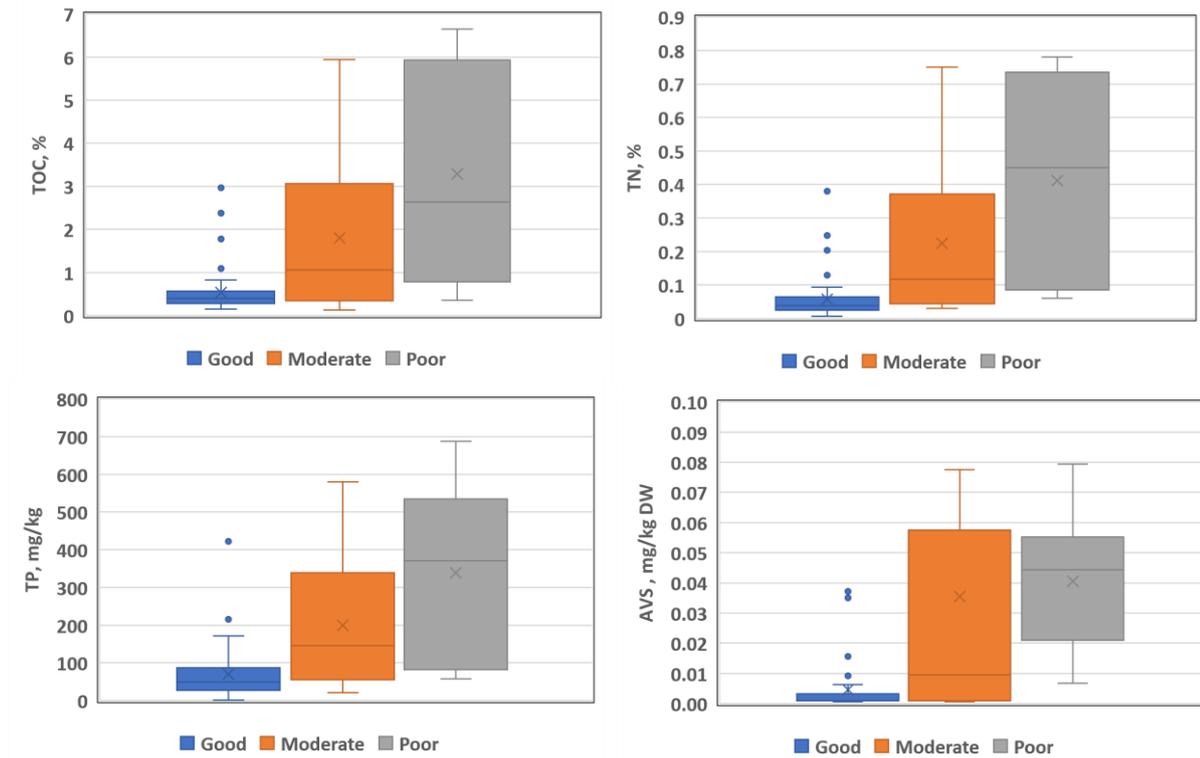
Figure 48. Box and whisker plots of concentrations of key sediment quality parameters (TORG, TN, TP, AVS) classified against Rapid Assessment Protocol (RAP) total scores (RAP score ≤ 5 Poor, 6-10 Moderate, 11-15 Good). Boxes are inter-quartile ranges; horizontal lines within boxes are medians; crosses within boxes are means, Whisker endpoints are high/low extremes with outliers as single points.
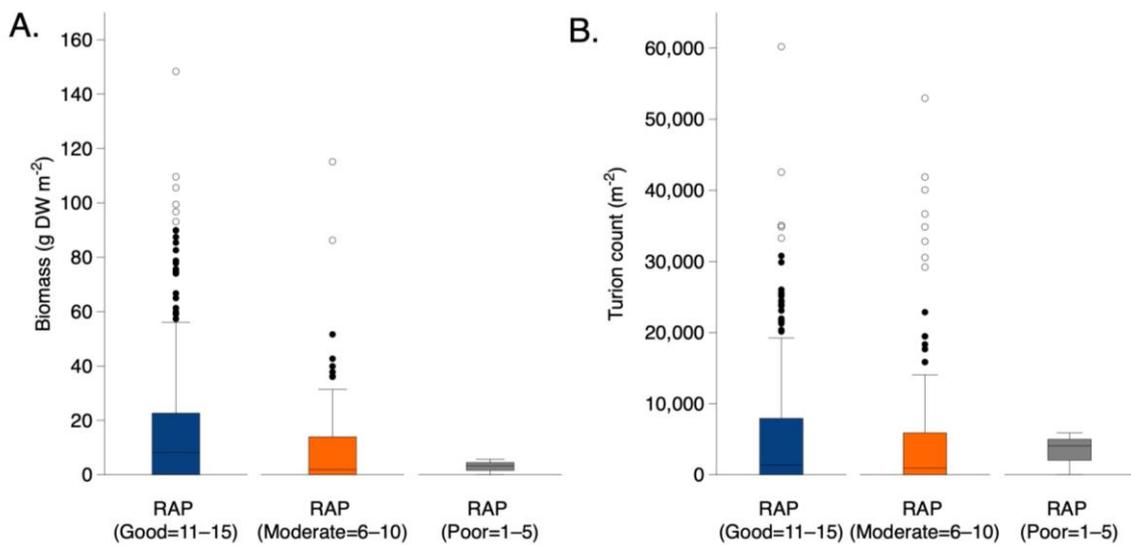
Figure 49. Box and whisker plots of A. plant total biomass (g DW m⁻²) and B. turion count (no. m⁻²) against Rapid Assessment Protocol (RAP; Hallett et al. 2019) total scores and Ruppia Community biomass across the southern Coorong.

5.3.4 Plant tissue nutrients

The tissue nutrient concentrations for the three major components (TC, TN, TP) were compared across sites and sampling periods in 2020–2021 (this study) and 2016–2017 (from Collier et al. (2017)) (Figure 50). The results of testing Ruppia Community derived plant tissues for total carbon (TC) varied from a mean of 15.7%C ± 0.65% in 2017 to 15.8%C ± 1.3% in 2021, with no significant differences between years nor sampling locations in 2021, although there were significant differences between sampling locations in 2017 ($p = 0.002$). Mean total nitrogen (TN) varied from 0.7%N ± 0.08% in 2017 to 1.17%N ± 0.08% in 2021, a significant difference between years ($p < 0.001$) and sampling locations in 2017 ($p = 0.012$) but not significant at 95% confidence for sampling locations in 2021 ($p = 0.06$). Mean total phosphorus (TP) varied from 292 ± 39 mg/kg (0.03%P ± 0.004%) in 2017 to 784 ± 67 mg/kg (0.08%P ± 0.007%) in 2021, a significant difference between years ($p < 0.001$) and between sampling locations in 2021 ($p = 0.035$) but not 2017 ($p = 0.215$).

Algal derived tissue varied little for TCtot (total organic carbon) and TCorg (total organic carbon) between years although not significantly: 2021 mean 14.9%Corg ± 0.61%; 2017 mean 16.8%Corg ± 2.5% and 2021 mean 14.6%Ctot ± 0.77%. TN varied between years although not significantly: 2021 mean 0.92%N ± 0.04%; 2017 mean 0.80%N ± 0.1%. TP varied significantly between years: 2021 mean 592 ± 29 mg/kg (0.058%P ± 0.002%); 2017 mean 263 ± 88 mg/kg (0.03%P ± 0.009%) ($p < 0.001$). Sampling locations were limited for algae so statistical comparisons of sites were not conducted. The Ruppia Community tissue nutrient concentrations overall were higher for nitrogen and phosphorus in the 2020–2021 sampling period and lower proportion of carbon in the plant tissues (Figure 50).
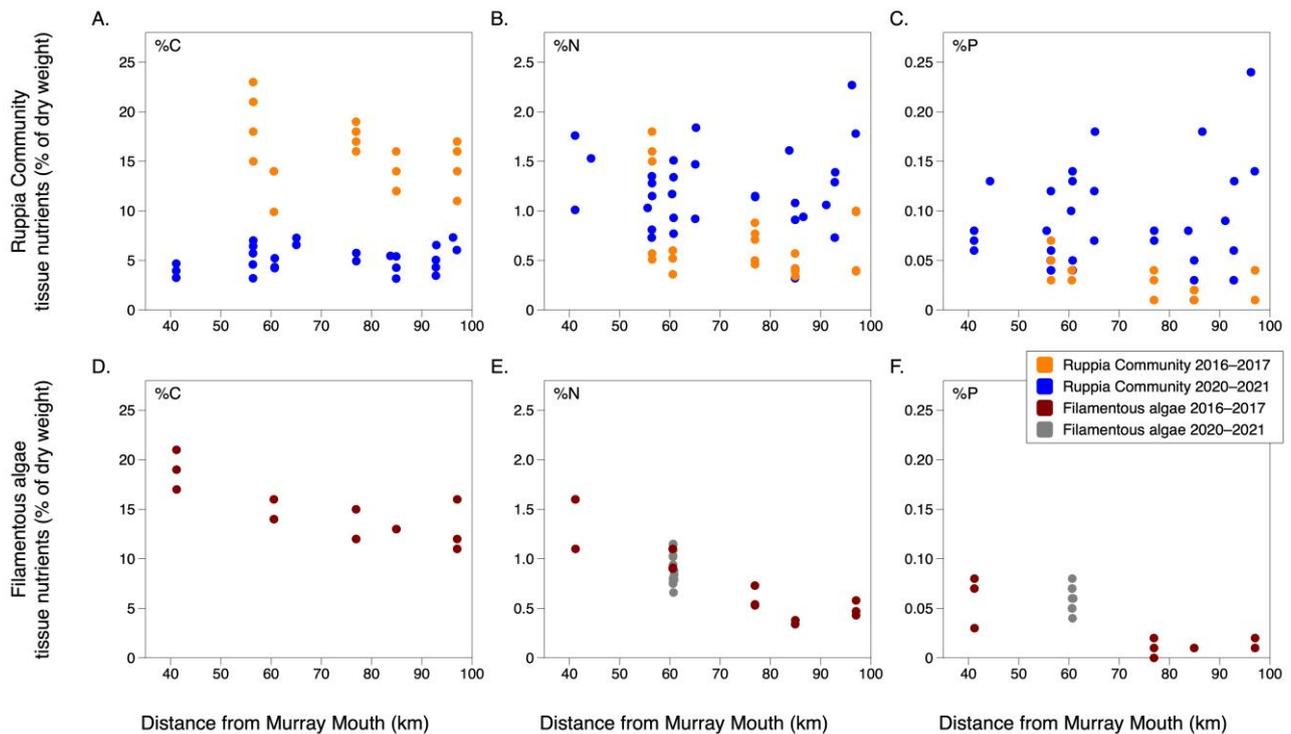
Figure 50. Direct comparisons of plant tissue nutrient concentrations as percent of tissue dry weight for Ruppia Community plant tissue (A.–C.) and filamentous algae tissue (D.–F.). Samples collected and analysed in 2016– 2017 and 2020–2021 are highlighted with different colours.

Comparison of pairwise trends in the relative concentrations of tissue TN, TC and TP nutrients for tissues derived from the Ruppia Community (Figure 51) and filamentous algae (Figure 52) identified correlated linear positive relationships within each year's sampling. For the Ruppia Community, increasing concentrations of nitrogen were associated with increasing concentrations phosphorus (Figure 51). Total nitrogen and total phosphorus were significantly higher in 2021 and reflected a lack of low concentrations of TP in particular. The trends between sampled years for filamentous algae was less consistent, likely reflecting the more ephemeral growth of these plants although again, TP was higher. The significant increase in tissue phosphorus concentrations indicate further changes in the nutrient availability and cycling processes in the Coorong South Lagoon in 2021 compared to 2017.

Tissue nutrient concentrations expressed as carbon:nitrogen:phosphorus ratios (C:N:P; Redfield ratio e.g. N:P < 24:1 indicative of nitrogen limitation in plant tissues Figure 40) have been widely used as an indicator in seagrasses for which nutrients are limiting to growth. The relationship of the atomic ratio of these elements in plant tissues are indicative of the plants (Atkinson and Smith 1983). The ratio of C:N:P across this study indicated a change in relative availability of nitrogen and phosphorous (Figure 53) resulting in strong nitrogen excess and therefore a relative phosphorus limitation. Virtually all samples reflected an N:P >24 for both the Ruppia Community and filamentous algae (Figure 54) indicative of P Limitation. It is noteworthy that at one site, Villa de Yumpa (site 4), in 2017 plants appeared to have relatively high amount of P indicative the site may have been N limited at that time. There was a significant difference in nutrient ratios between years for the Ruppia Community (C:N $p < 0.001$, C:P $p < 0.001$, N:P $p = 0.002$) and the filamentous algae (C:N $p = 0.022$, C:P $p = 0.006$, N:P $p = 0.013$) (Figure 54).
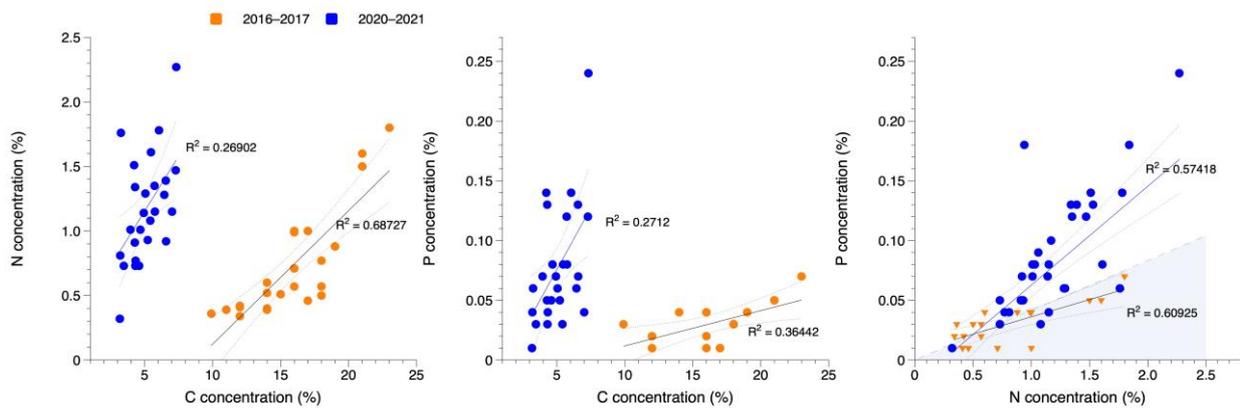
Figure 51. Plant tissue concentrations for the Ruppia Community of total nitrogen (TN %), carbon (TC %) and phosphorus (TP%). Left, TN versus TC, Centre, TP versus TC, Right TN versus TP. Data from previous analysis (2016–2017) is plotted for reference with 2020–2021 samples. Line of best fit with 95% confidence interval is shown in dashed lines either side for the two sampling periods.
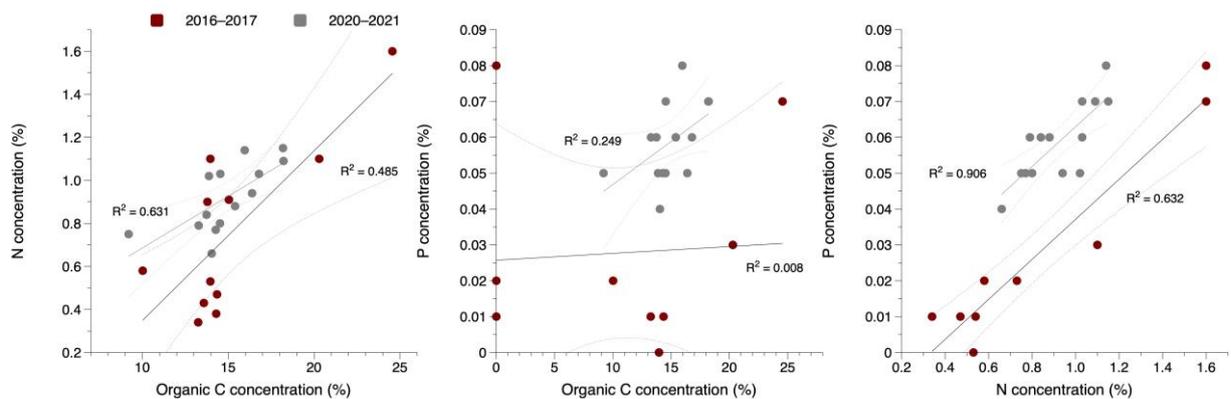
Figure 52. Plant tissue concentrations for filamentous algae of total nitrogen (TN %), organic carbon (TCorg %) and phosphorus (TP mg/kg). A. Left, TN versus TCorg, Centre, TP versus TCorg, Right TN versus TP. Data from previous analysis (2016–2017) is plotted for reference with 2020–2021 samples. Line of best fit with 95% confidence interval is shown in dashed lines either side for the two data sets.
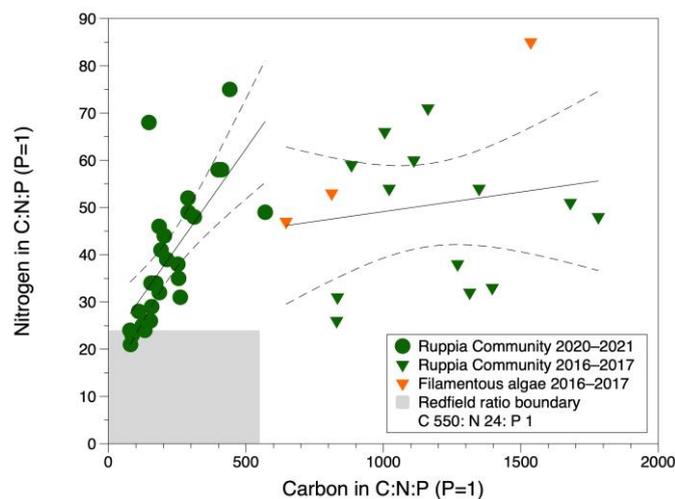
Figure 53. Overall C:N:P ratio (P=1) for Ruppia Community (green) and filamentous algae (orange) plant tissues the sampled at two different times, 2016–2017 (inverse triangles) and 2020–2021 (circles) (note: total carbon and not organic carbon). Shaded area reflects the boundary of N limited growth.
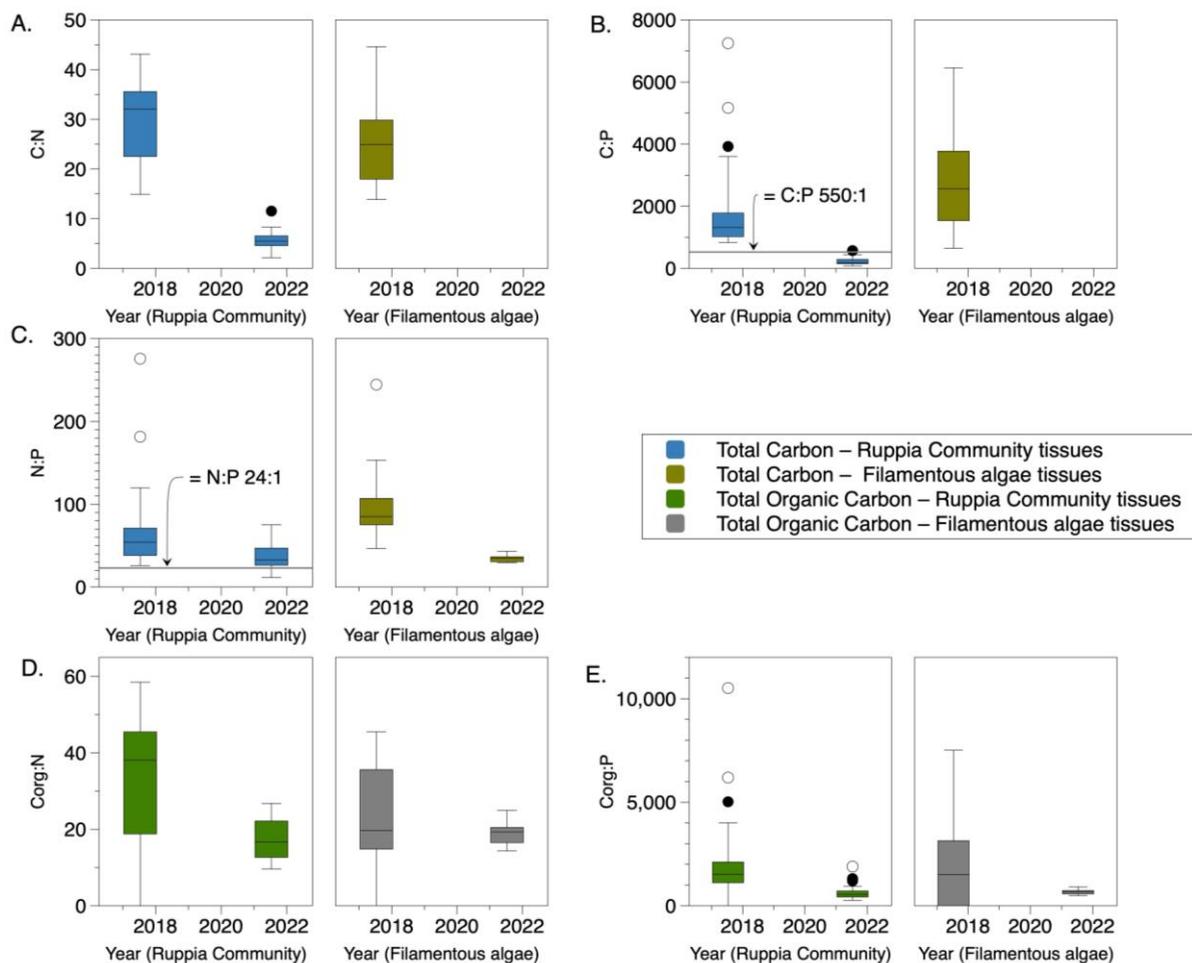
Figure 54. Box plots for plant tissue nutrient ratios as overall annual data presented as the inter-quartile range (IQR) for sites and seasonal samples of Ruppia Community plant tissue and filamentous algae tissue, outliers plotted (>95% confidence interval as circles). A. C:N atomic mass ratio (total carbon); B. C:P atomic mass ratio (total carbon); C. N:P atomic mass ratio; D. Corg:N atomic mass ratio (organic carbon); E. Corg:P atomic mass ratio (organic carbon). For reference N:P ratios of <16:1 in algae, and <24 in seagrass represent nitrogen limited plants. Note the different scales on each axis.

5.3.5 Nitrogen isotopes

Current δ^{15}N values in sediment, seagrass and algal tissue (Figure 55), which have been combined with the data collected by Priestley et al. (2022a) shows the very high δ^{15}N values in the Ruppia Community derived seagrass tissue compared with other sources. There is otherwise good alignment of the sediment δ^{15}N values collected in the Ruppia Community locations with those measured by Priestley et al. (2022b), and these would be expected to remain relatively stable throughout temporal scale of the project. Algal tissue δ^{15}N values generally align with particulate organic nitrogen (PON) δ^{15}N values confirming that the majority of the PON in the Coorong is algal material. Priestley et al. (2022) discussed sediment nitrogen and δ^{15}N values being the result of deposition and retention of nitrogen from deposition of algal (PON) material. They also hypothesised that higher δ^{15}N values in the South Lagoon (Figure 57) are due to increased cycling and loss of lighter ^{14}N, with one possible mechanism being loss of ^{14}N via ammonia production and then loss from the sediment due to the dominance of dissimilatory nitrate reduction to ammonium (DNRA). This would increase the δ^{15}N of ammonium assimilated by phytoplankton, thereby increasing the δ^{15}N within suspended organic matter and surface sediment in the South Lagoon. There is some preliminary evidence for the above hypothesis with measured ammonium concentrations increasing slightly in the South Lagoon (Figure 56 A.) (Huang et al. 2022). Moreover, high ammonium concentrations (NH$_4^+$ >2 mg/L N) were detected in samples

where the δ^{15}N-NH$_4^+$ values increase to up to +59‰ in a couple of samples collected in summer (Figure 56 B). Seagrass tissue δ^{15}N values (noting whole plants were analysed) were higher than sediment and algal tissue (Figure 55). Seagrass δ^{15}N values from around the world are typically less than 20‰ (Christiaen et al. 2014), more aligned with PON and algal δ^{15}N values.
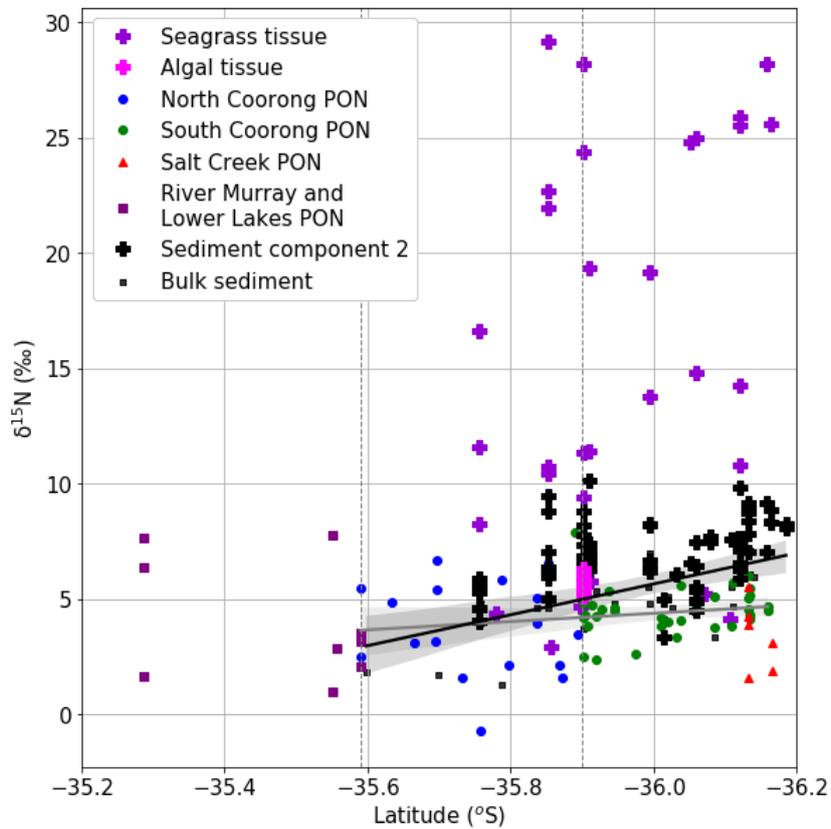
Figure 55. A cross-plot of Particulate organic nitrogen (PON) δ^{15}N against sample latitude including bulk sediment δ^{15}N results, as well as those incorporated into Priestley et al. (2022a) tissue and sediment results. The grey regression line represents all Coorong PON isotope values and black regression line represents the bulk sediment isotope values.
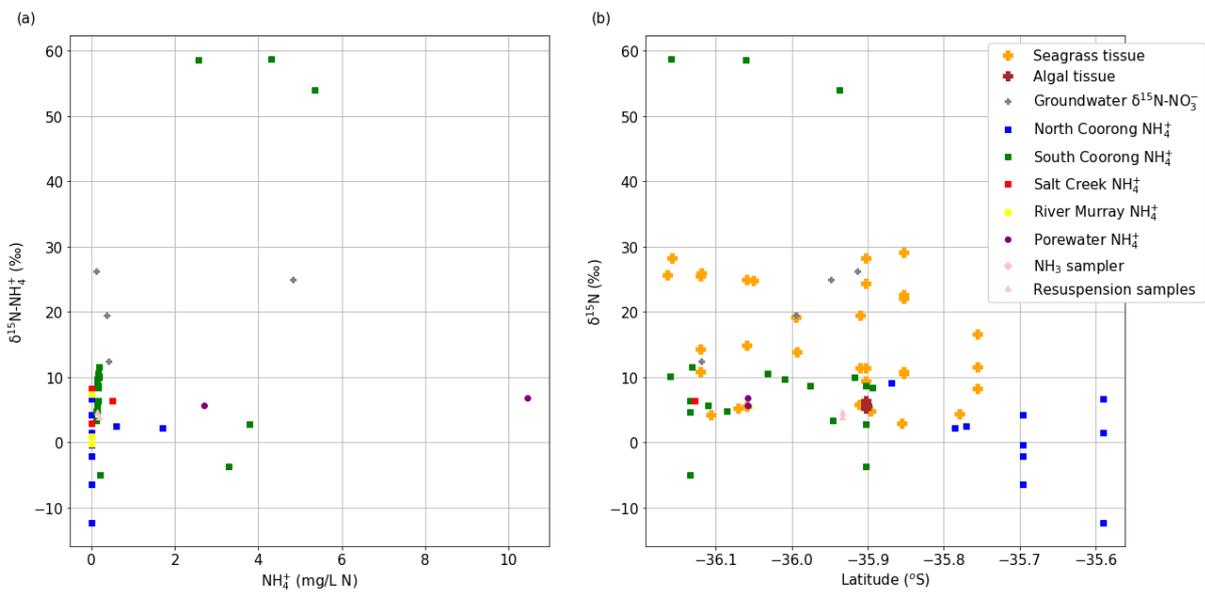
Figure 56. A cross-plot of (a) δ^{15}N-NH$_4^+$ against NH$_4^+$ concentration and (b) δ^{15}N against sample latitude for NH$_4^+$, as well as those incorporated into Priestley et al. (2022a) tissue results
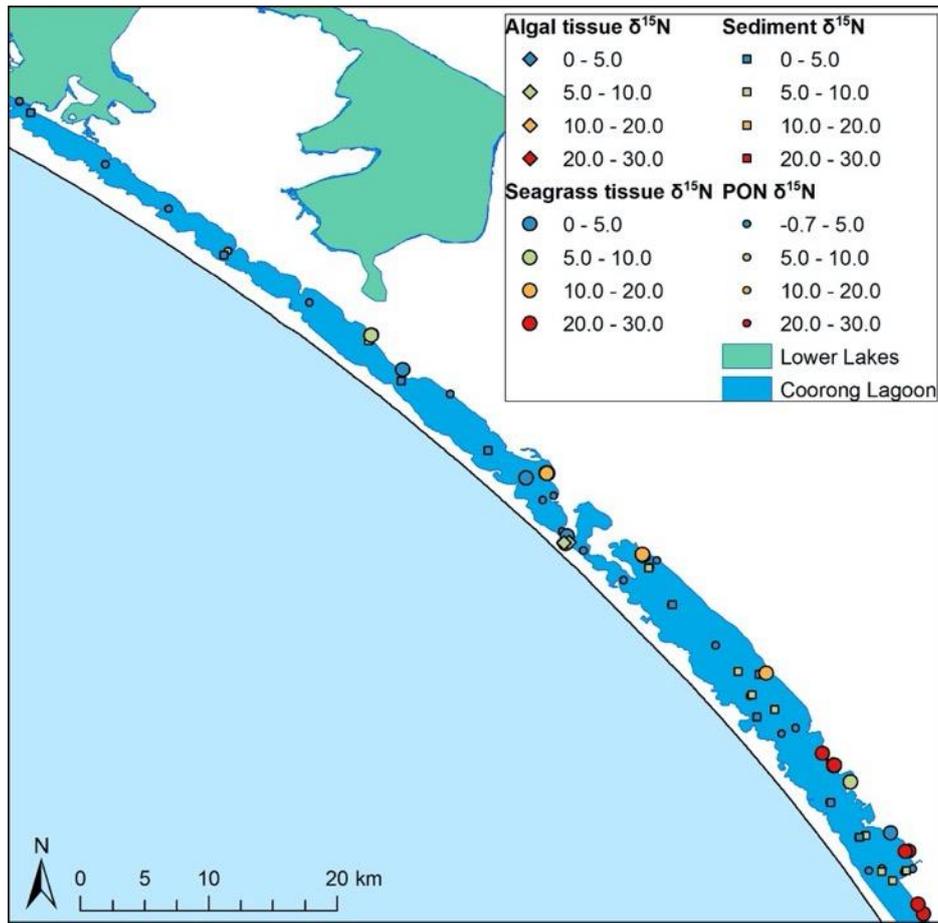
Figure 57. Map of δ¹⁵N in Coorong sediment, PON, seagrass and algae.

5.3.6 Carbon isotopes

Sediment $\delta^{13}C$ values (Figure 58) also align with those measured by Priestley et al. (2022), showing consistent sediment carbon isotope values. Algal and seagrass tissue $\delta^{13}C$ values range from -10 to -15‰. The differentiation in nutrient status between sampling periods was significant (Figure 59) where $\delta^{13}C$ values were low (closer to zero) in the Ruppia Community plant tissue from the 2021 seasons samples. The highest values for $\delta^{13}C$ were found at the southernmost end of the main Coorong South Lagoon (Figure 60).
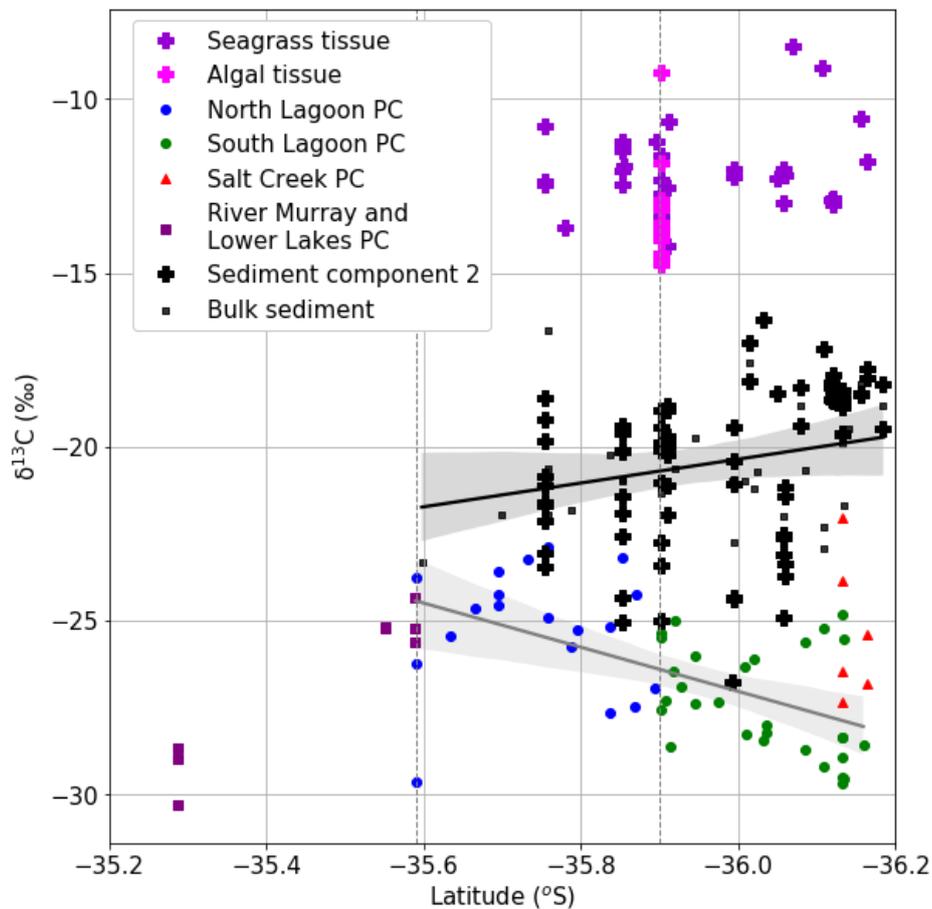
Figure 58. A cross-plot of Particulate carbon (PC) δ^{13}C against sample latitude including bulk sediment δ^{13}C results, as well as those incorporated into Priestley et al. (2022a) tissue and sediment results. The grey regression line represents all Coorong PON isotope values and black regression line represents the bulk sediment isotope values.

5.3.1 Carbon and nitrogen isotopes

Comparing the trend in δ^{13}C and δ^{15}N across both sampling years (2016–2017 and 2020–2021) and plant tissue derived from the Ruppia Community (seagrass) and filamentous algae (Figure 59 left panel) the greatest proportion of variation occurred among the samples of Ruppia Community sampled in 2020–2021. The elevated tissue % nitrogen and high δ^{15}N values in this plant material were largely co-associated (Figure 59 right panel).
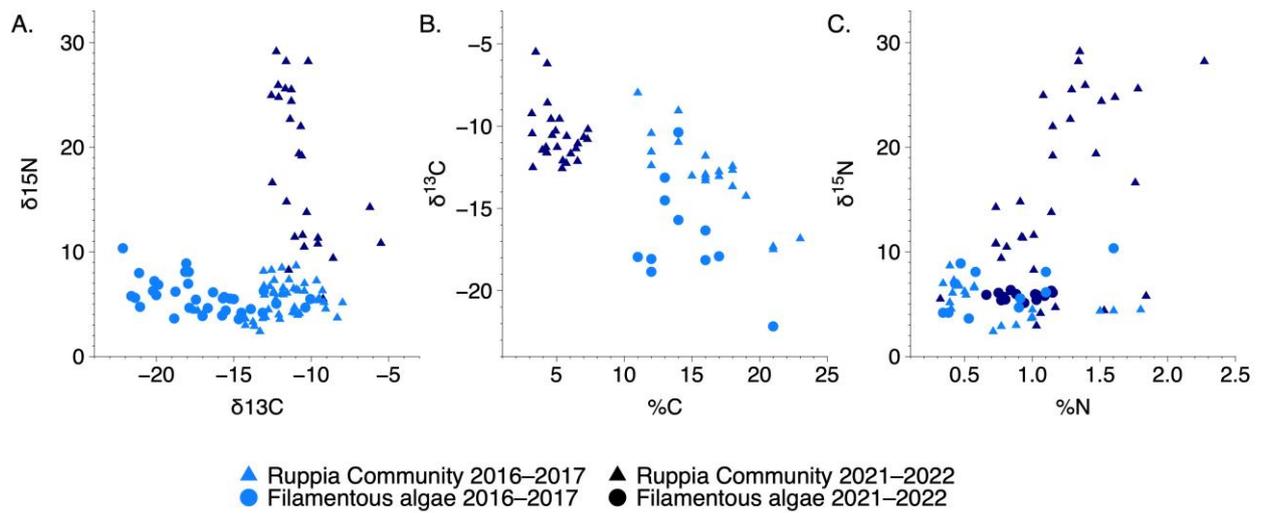
Figure 59 Plant tissue-based estimates of A. δ¹³C and δ¹⁵N content (‰) values and comparisons among seagrass (Ruppia Community) and filamentous algae, B. total proportion of carbon (%C) and C. nitrogen (%N) over two sampling periods (2016–2017 and 2020–2021).
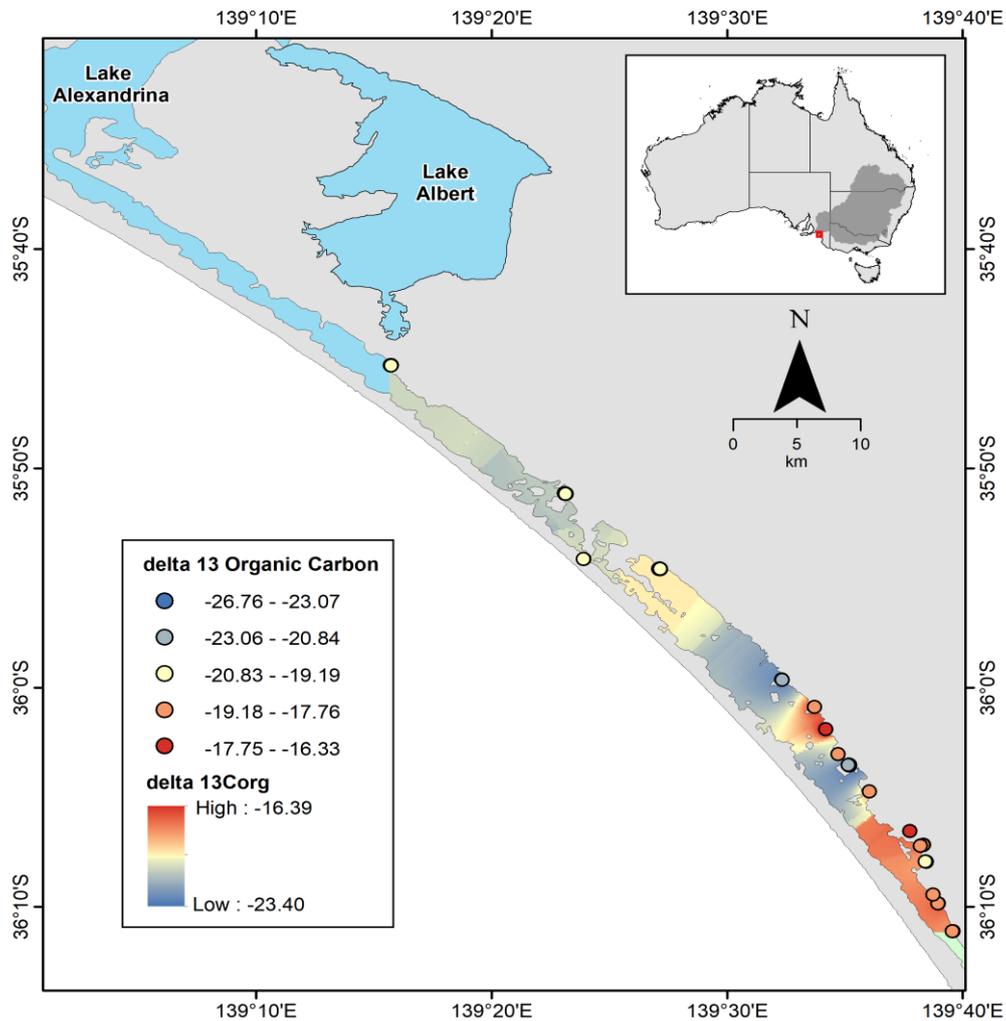
Figure 60 A spatially interpolated plot of δ¹³C in Coorong sediment.

5.3.2 Water quality

The highest total nitrogen (TN) and total phosphorous (TP) levels were detected in the narrow and flow constricted zone at the juncture between the North and South Lagoon and Villa dei Yumpa at the top of the South Lagoon (Figure 61 A. and D. sites 3, 7, 9). These locations have large shallow sediment banks where the Ruppia Community colonises extensive areas (Lewis et al. 2021). Repeated sampling across sites throughout the season demonstrated a seasonal increase in total nitrogen and organic carbon (Figure 62). Other nutrients varied with location time, most notably, the total phosphorus increasing sharply at southern sites in September 2021 (Figure 62). Water quality at the aquatic plant sites at the time of sampling also detected most of the relatively high concentrations of nitrogen in the water column (Figure 61 A.) is in organic form, with much lower levels of dissolved nutrients. However, there were several instances where ammonium was detected (Figure 61 B.) which is consistent with the results in Mosley et al. (2020) and Priestley et al. (2022). The presence of ammonium is likely due to flux out of anoxic sediment, based on results in Huang et al. (2022).

Total phosphorus is high in the water column relative to dissolved phosphorus (Figure 61 D. and E.). These low dissolved and high total nutrient results appear due to a high sediment flux of dissolved nutrients (Huang et al. 2022), and then rapid uptake by aquatic plants and algae. The role of bacterial mineralisation of organic nutrients in the water column, releasing dissolved nutrients for rapid uptake, is unclear at present.
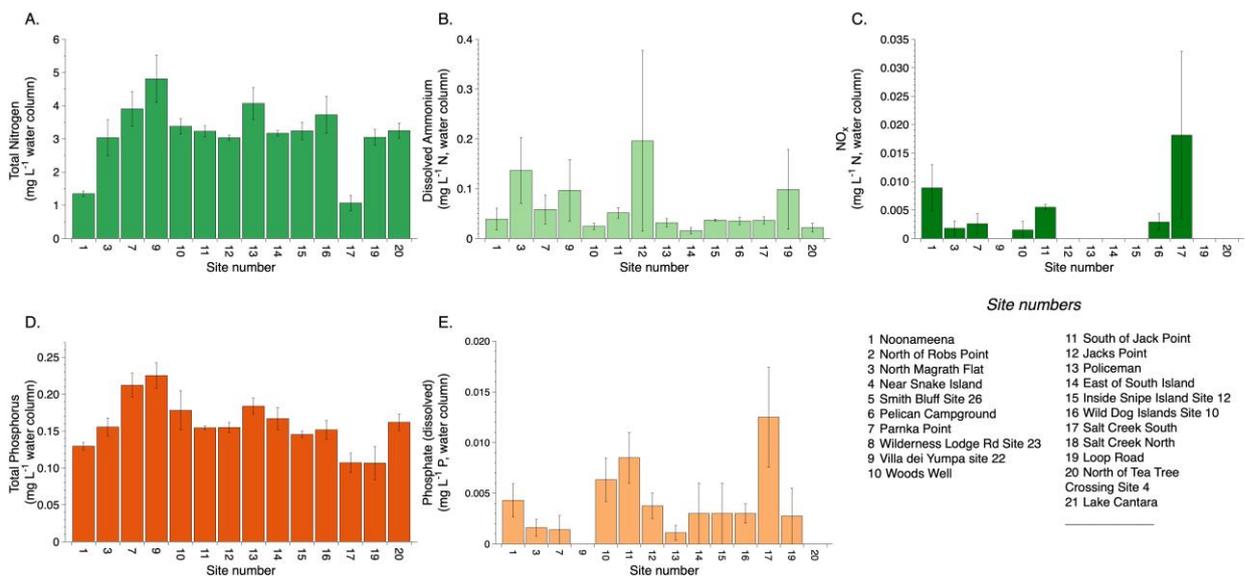
Figure 61. Water quality of water column samples collected at aquatic plant sites showing mean ± s.e. for repeated samples collected at same site and over time; A. total nitrogen, B. dissolved ammonium, C. NO$_x$ (total nitrate and nitrite), D. total phosphorus, E. total phosphorus (dissolved, filterable reactive). Site numbers are listed and correspond to those in

Ruppia Community biomass and salinity changed during the sampling period where as expected it was lower earlier in the growing season (Figure 63 A.) increasing to highest biomass in early Summer. Salinity was low adjacent to the Salt Creek outlet where water releases were concentrated and was highest on the shallow flats in the central section of the Coorong in summer such as at North Magrath Flat, January 2022 (Figure 63 B.). Plant tissue nutrient concentrations varied across sites and seasons for the Ruppia Community (Figure 64).

Table 15. Descriptive statistics for algae nutrient analyses conducted on filamentous algae samples collected from Parnka Point, 2021.

ANALYTE	MEAN	MIN	MAX	SD
Delta nitrogen (δ15N)	5.81	5.12	6.35	0.36
Total nitrogen (% N $_{TN}$)	0.92	0.66	1.15	0.16
Total organic carbon (% C$_{TORG}$)	14.90	9.20	18.22	2.30
Delta organic carbon (δ13C)	-13.33	-14.75	-9.22	1.41
Total phosphorus (mg kg^{-1})	592.11	397.70	826.40	111.50
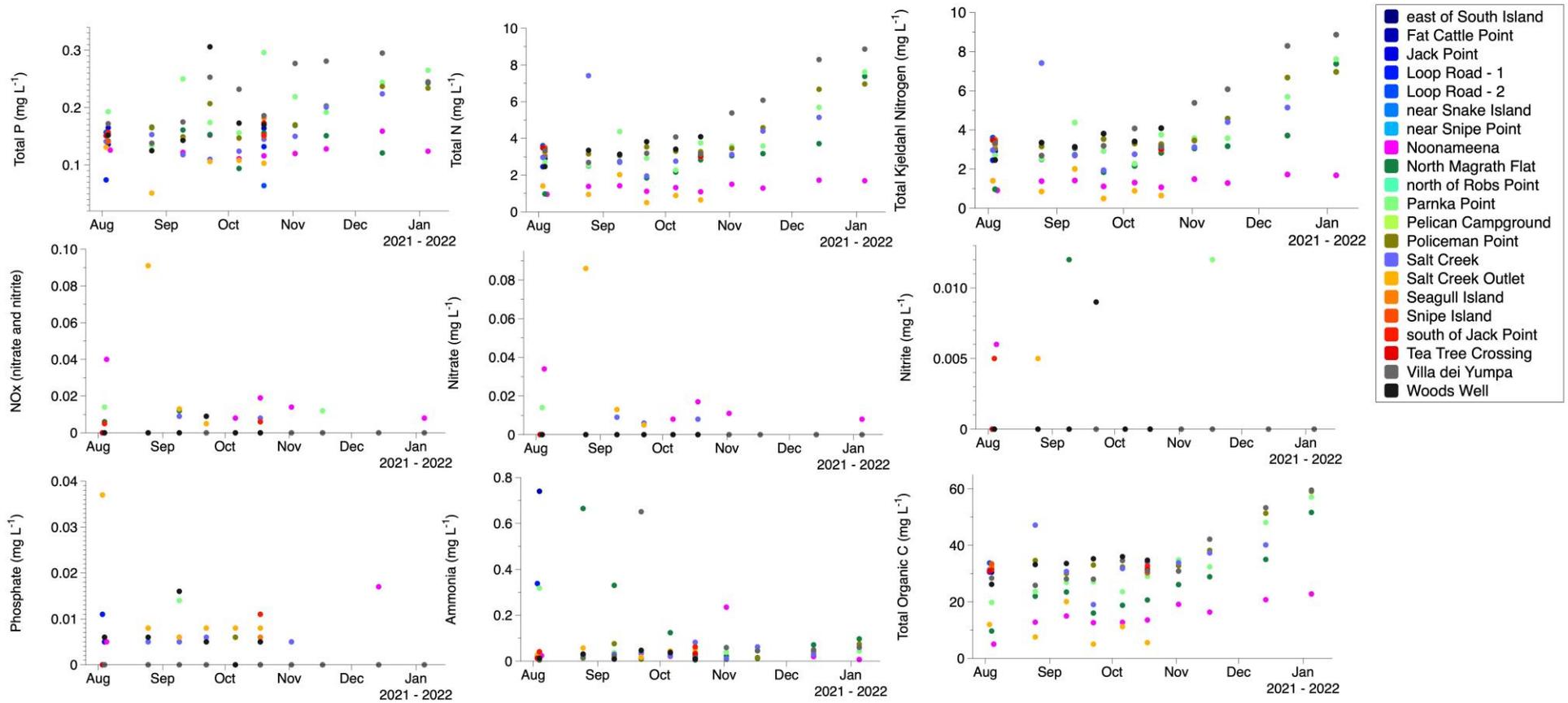
Figure 62. Water quality nutrients plotted for each sampling event and all locations sampled in the southern Coorong.
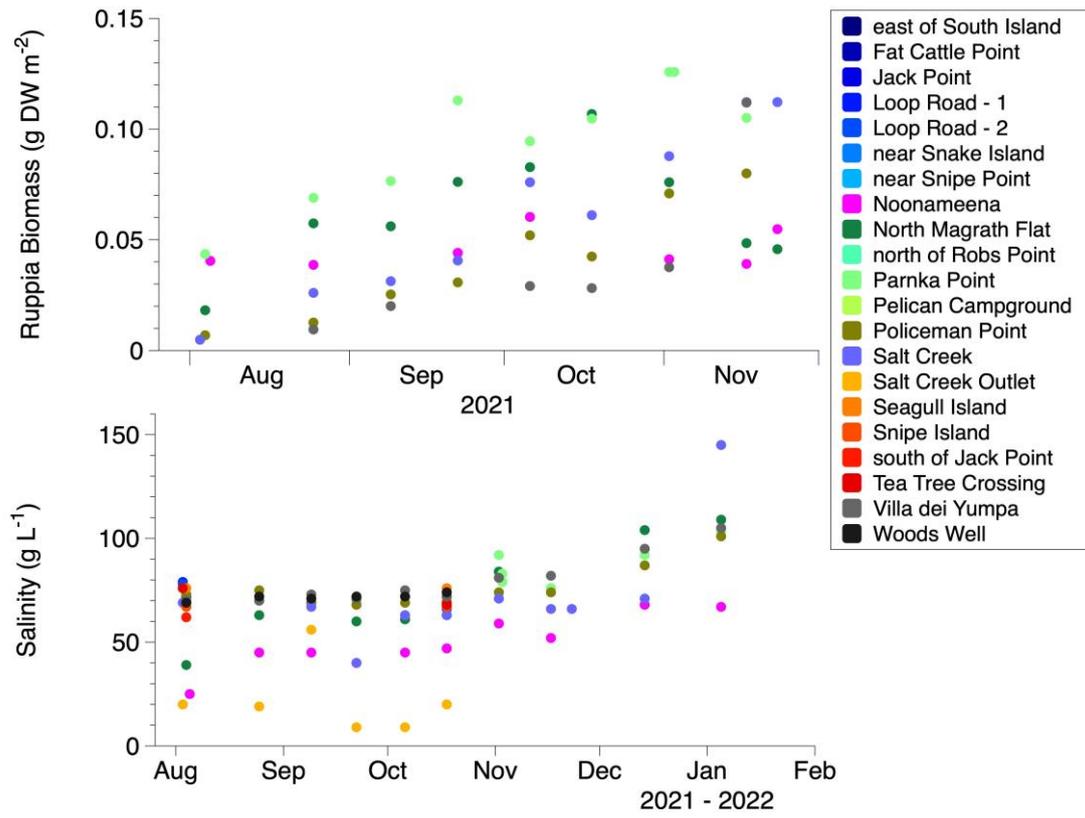
Figure 63. Changes in plant biomass (Ruppia Community; g DW m^{-2}) and salinity (g L^{-1}) for multiple sampling events during 2021/22 and each collecting location in the southern Coorong (see Table 13 for details).
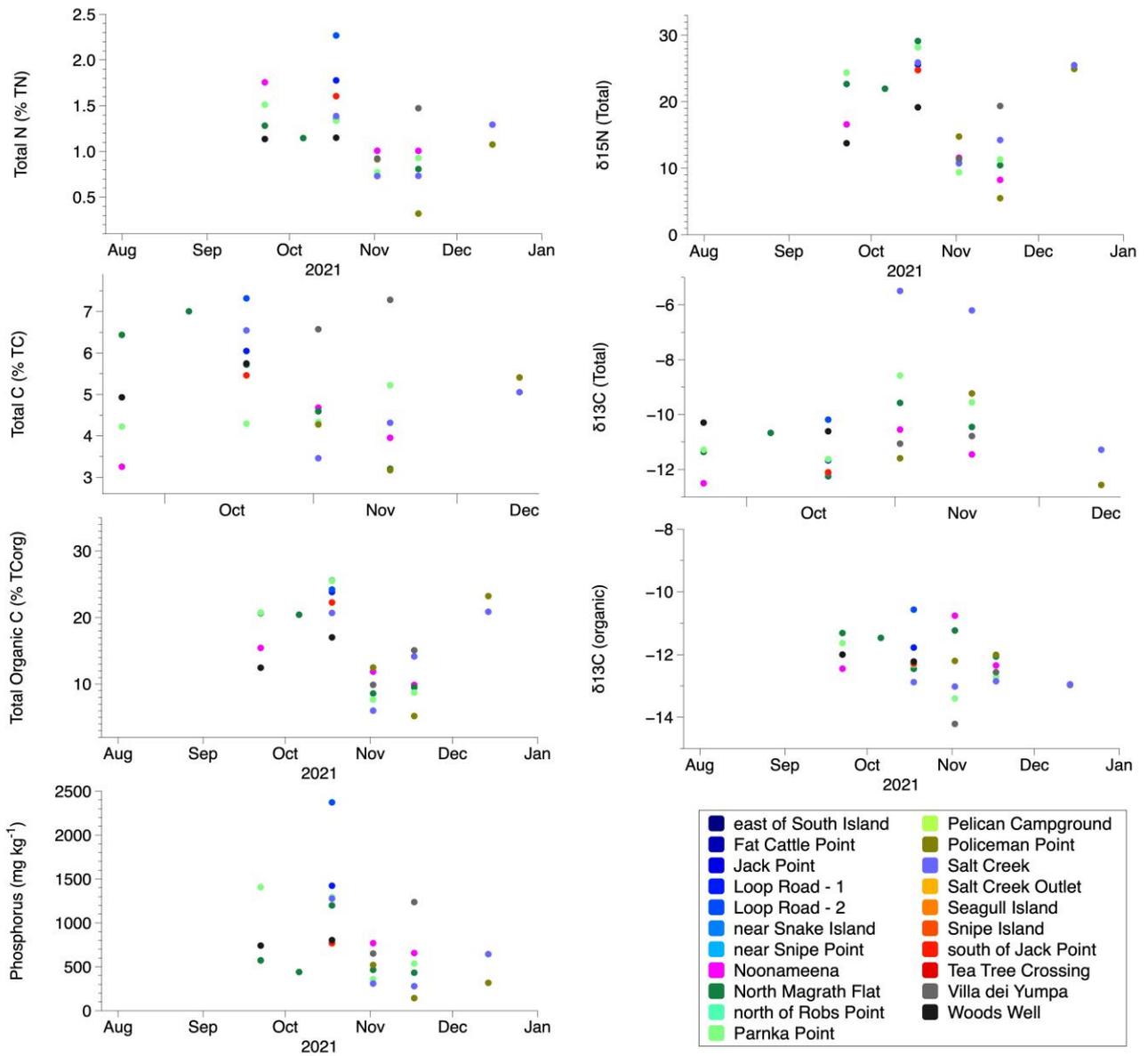
Figure 64. Plant tissue nutrient concentrations for 7 different measures for multiple sampling events during the 2021 season and each collecting location in the southern Coorong. Total nutrients for carbon, nitrogen and phosphorus as well as total organic carbon and stable isotope values measuring $\delta^{13}C$, $\delta^{15}N$.

5.4 Discussion

Nutrient content in the water column, sediments and the plants themselves provided data documenting the nutrient environment of the aquatic macrophyte community in the southern Coorong littoral zone during the 2021/2022 growth season. Over the ~6 months from August 2021 to early January 2022, nutrient concentrations followed a seasonal pattern where in general the water column concentrations of nitrogen, organic carbon and phosphorus increased in the warmer months as salinity increased. Likewise, sediment, concentrations of nitrogen and phosphorus increased into the warmer months; organic carbon exhibited this relationship in the southern part of the South Lagoon and at Parnka Point and Villa dei Yumpa, these latter two locations exhibiting higher organic content and stable isotope values overall. Where higher concentration sources of nutrients were associated with black ooze sediments, aquatic plant growth was inhibited and other studies observed a lack of bioturbating macroinvertebrates in the South Lagoon (see Lam-Gordillo et al. 2022).

Sediment nutrient concentrations of total organic carbon (Torg), total nitrogen (TN) and total phosphorus (TP) were relatively consistent among sites, with Parnka Point exhibiting the highest concentrations, likely due to its location at the constriction point between the two lagoons. Very high values detected at the Villa dei Yumpa site in late December 2021 were also associated with a good biomass of plants and very high organic load at that site. The δ^{13}C values were variable across sites and δ^{15}N values across time, with the highest measured values observed in early spring. As found by companion studies (Mosley et al. 2020, Priestley et al. 2022a), the overall values for sediment nutrients were high and water column nutrients from the same locations indicate a strong organic matter-nutrient coupling between the water column and sediment. Stable isotope concentrations in Coorong sediments and water column also indicated their source was likely algal derived organic matter supporting earlier inferences (Priestley et al. 2022a). Ammonium is considered to be the dominant form of N (inorganic) at some locations (Huang et al. 2022, Priestley et al. 2022a) and seagrasses have a preference for ammonium uptake (Touchette and Burkholder 2000, Nayar et al. 2018). This contrasts to land plants, which do not have a water-saturated sediment, that may be experiencing hypoxic or anoxic conditions that often characterise these seagrass habitats (Touchette and Burkholder 2000, Nayar et al. 2018). Lower δ^{15}N values may be associated with the locations that ammonium uptake and indicative of altered nutrient cycling pathways.

Total nutrient and organic carbon concentrations were lower in sediments where there are freshwater inputs relative to the Coorong lagoon water, i.e. Salt Creek outlet and the site sampled in the main body of the North Lagoon (Noonameena), as has been observed previously by Mosley et al. (2020), and flushing is higher at these locations. TN, TP and TORG concentrations in the water column compared to sediments suggest strong organic matter-nutrient coupling between the water column and sediment, as supported by stable isotope measurements in the Coorong sediment and water column suggesting an algal derived organic matter sources. The deposition of organic carbon to the sediment promotes sulfate reducing bacteria activity, which forms AVS and leads to development of the monosulfidic black ooze (MBO) sediment.

The evidence of a strong, significant relationship between sediment condition and plant performance (as biomass) was measured using the rapid assessment protocol (RAP) for sediment condition (Hallett et al. 2019) (see Figure 49). Sediments associated with high TORG (mean values >3%), high TN (mean values >0.45%) and high TP (mean values > 400 mg kg^{-1}) were categorised as poor with a RAP score of less than 5. Moderate RAP scores (6–10) were highly variable across nutrient levels although means were lower than those of poor RAP scores. The nutrient conditions associated with good RAP scores were TORG <1% (mean ~0.5%), TN <1% (mean ~0.4%) and TP is < 100 mg kg^{-1} (mean 50 mg kg^{-1}). This relationship supports the notion that adoption of the RAP score methodology for monitoring would provide a relatively simple, easily applied tool for fine scale assessment of habitat condition, and with modest monitoring of sediment nutrient concentrations the prohibitive costs for replicate nutrient analysis in such systems can be managed.

The seagrass tissue δ^{13}C values measured in this study are similar to the *Ruppia megacarpa* (absent from the Coorong at the time of this study (Krull et al. 2008, Lewis et al. 2022)), δ^{13}C values of 13.2‰ (Boyce et al. 2001) and are similar to seagrass values elsewhere (Duarte 1990, Fourqurean et al. 2007). The general range of seagrass δ^{15}N values (Fourqurean et al. 1997, Johnson et al. 2006, Fourqurean et al. 2007, McGlathery et

al. 2007) is similar to the observed PON values measured in this study. This suggests high fractionation of N in the plant or the possibility that the seagrasses of the Ruppia Community use a nutrient source with higher δ^{15}N values. As ammonium has been shown to be preferred nitrogen source for seagrasses when available (Touchette and Burkholder 2000, Nayar et al. 2018) the observed δ^{15}N variation may be associated with conditions that reflect the source of nitrogen the seagrass has as a nutrient source is prevalent in some locations and not others. The very high seagrass δ^{15}N values are only found in the South Lagoon (Figure 57), in areas with poorest sediment quality based on RAP scores, which further corresponds with a regionally available nutrient source or nutrient cycling process such as de-nitrification cycles. High ammonium levels were detected in sediments at some locations by Huang et al. (2022). Generally accepted marine algal estimates of δ^{13}C (Valiela et al. 2018) appear consistent with the filamentous algae δ^{13}C values in this study and align with the 13.9‰ measured in the Coorong previously (Krull et al. 2008).

Comparisons with Priestley et al. (2022) particulate carbon (PC) δ^{13}C values, which were consistently lower, may not be appropriate as it is possible that they are influenced by dissolved inorganic carbon (DIC). Our samples were not acidified, due to the primary focus of our study being the nitrogen isotope system, so it is possible that the PC δ^{13}C values are a mixture of both organic and inorganic carbon. Nevertheless, previous measurements of particulate organic carbon (POC) δ^{13}C values are between -20 and -24‰, and are predominantly representative of phytoplankton (Krull et al. 2008). Thus the sediment δ^{13}C values of -20 ± 2‰ are consistent with a predominantly phytoplankton source (Krull et al. 2008).

High nutrient levels that increase over the annual cycle of changing water levels and salinity further emphasise the variable nutrient environment that the aquatic macrophytes of the Ruppia Community occupy and adaptability required to survive. The concentrations of nutrients in the tissues of seagrasses and algae are globally well known to reflect the growth of tissues integrating short to medium term nutrients availability into their tissues. The relative proportion of tissue nutrient concentrations for carbon, nitrogen and phosphorus has been used as an indicator of the limitations to plant growth. This has been defined as the "Redfield" ratio of 106C:16N:P for phytoplankton (Redfield et al. 1963). ''Seagrass Redfield ratios'' have been estimated to be between 550:30:1 (Atkinson and Smith 1983) and 474:24:1 (Duarte 1990) due to the large quantities of structural carbon in their tissues. Seagrasses growing in different nutrient environments will have C:N:P ratios that reflect elevated nitrogen and phosphorus levels (Duarte 1990). In addition, the growth form of the dominant biomass component of the seagrass community, in this case colonising, fast growing species of *Ruppia* and *Althenia* (Kilminster et al. 2015), will contain relatively less structural carbon than persistent species and as a result influence the ratio (i.e. have relatively less carbon). Nutrient poor waters result in significantly lower N:P ratios than those from nutrient rich waters (Atkinson and Smith 1983). Shifts away from these ratios of carbon, nitrogen and phosphorus (C:N:P), in particular expected algae N:P ratios <16:1 and seagrass <24, that are retained within plant tissue are used as an alternative means of evaluating the nutrient status of coastal waters (Duarte 1990).

The relatively rapid growth of the algal tissue under salinities of 40 g L^{-1} or less (Chapter 2, Figure 13; Collier et al. (2017)) would double in biomass in 3-4 days, although this may occur faster depending on the temperature and nutrient availability (Waycott et al. 2019). Equally, under poor conditions of very high salinity (130 g L^{-1} or more) and moderate temperature (22°C), algal mat decline could occur in 3–4 days and faster at higher temperatures. This rapid uptake followed by a crash of biomass is acting to create a feedback between sediment-water column nutrient concentrations, the fluxes between them triggering new cycles of high turnover algal growth. These fluxes, in experiments conducted by Huang et al. (2022), demonstrate changing day and night processes, including higher porewater values for phosphate at night. These fluxes can modify the local environment and, if conditions favour phosphorus bioavailability, the plant tissue nutrients would reflect this.

The high biomass algal blooms in early summer when salinities rise and water levels fall eventually crash and this leads to the deposition of organic carbon onto the sediment which promotes sulfate reducing bacteria activity, formation of AVS and the development of the monosulfidic black ooze (MBO) sediment. We observed this in the field at all of the high filamentous algal cover sites, and these sites also experienced periods of shading during the growth season when flowering occurs (Chapter 2, section 2.3.1). The link between these biochemical and biological processes continues to provide the feedback for the hyper-eutrophic cycle through the action of the filamentous algae.

In this study, we were able to compare tissue nutrient concentrations directly between samples analysed in 2017 (Collier et al. 2017) and 2021 (this study). We were also able to make a direct comparison of tissue nutrient concentrations from the Ruppia Community plants and from filamentous algae in both years. There was a substantial difference between years driven by higher total phosphorus and total nitrogen concentrations in the plant tissue and the sediments. The primary cause for these values is unknown at this time but speculatively the higher values detected may reflect an ongoing change in the nutrient dynamics of the Coorong Lagoons including increasingly elevated water levels in the system between 2019–2023. Future evaluation of the status of nutrient in the Coorong Ruppia Community will be on considerable value, in particular in the upcoming seasons where water levels are likely to be lower and the dynamics of the plant community consequently will change.

6 Key findings on the condition of the Ruppia Community in the southern Coorong

These scientific investigations, conducted between 2019 and 2022, had a focus on improving our understanding of the Ruppia Community in the southern Coorong and, the filamentous algal community that has become dominant in many areas (i.e. Auricht et al. 2019, Waycott et al. 2019, Asanopoulos and Waycott 2020, Jamieson et al. 2022, Lewis et al. 2022, Waycott and Lewis 2022, this study, i.e. Waycott et al. 2022) Alongside ongoing routine monitoring of the Ruppia Community for The Living Murray Program (e.g. Paton et al. 2021), these studies add to already available knowledge (e.g. Rogers and Paton 2009, Paton et al. 2011, Paton et al. 2015, Collier et al. 2017, Paton et al. 2017b, Nicol et al. 2018, Paton et al. 2021), improving our understanding of the main features of the Ruppia Community referred to as keystone in the Coorong ecosystem.

The Ruppia Community was observed to have recovered to the extent documented prior to the Millennium Drought and, is able to survive the extreme range of conditions that occur in the Coorong intra- and inter-annually (Lewis et al. 2022). The distribution of the Ruppia Community reflects the time taken for this severely impacted system to recover after the catastrophic losses from the Millennium Drought (Lewis et al. 2022). However, the resilience of the populations that are occupying the Coorong remains low in almost all regions (Waycott and Lewis 2022).

The ecological community itself has two dominant aquatic macrophyte species currently present, *Ruppia tuberosa* and *Althenia cylindrocarpa*, which appear to have similar tolerances. However, given that the species are not readily able to be identified unless flowering or with the help of DNA identification tools, the majority of experiments conducted throughout these studies, field observations made in The Living Murray Program (see citations above) and the research results presented by Collier et al. (2017) are likely to be, at least in part, mixed species results. Due to this and the variability of the system itself (e.g. Rogers and Paton 2009), documenting the range of conditions these species are able to grow and their productivity, can be challenging, particularly when there are highly variable between-season conditions.

The factors that affect growth of the Ruppia Community, its distribution and abundance, can be viewed as overlapping requirements (Figure 65) and include three major elements and a number of secondary, sometimes critical, factors:

- Water level
- Salinity
- Light availability
- Others
    - Temperature (specifically where the plants are growing)
    - Nutrient load (e.g. total bioavailable nitrogen, phosphorus, carbon)
    - Sediment conditions including particle size, organic content and oxygenation
    - Microbial community and microbial biochemical cycles
    - Direct grazing on plants
    - Physical disruption including filamentous algal interference

Utilising a mix of experimental and field-based observations, data collected is applied to informing thresholds in the Ruppia Habitat Suitability component of the Coorong Dynamics Model (CDM) (Collier et al. 2017, Hipsey et al. 2020, Hipsey et al. 2022). The modelling of Ruppia Habitat Suitability as an Index (Ruppia HSI) associated with the Coorong Dynamics Model (Hipsey et al. 2022) was originally developed to utilise the sophisticated hydrodynamic, physical environmental features and climate data to assess the likelihood of the Ruppia Community surviving as conditions change (Collier et al. 2017, Hipsey et al. 2022). Specifically, the application of habitat suitability evaluation using this model, alongside the Ruppia Restoration Strategy (Waycott and Lewis 2022), is proposed to be able to provide tools to inform the options available to improve the health of the Coorong ecosystem. In fact data from this study, and the others mentioned above, are being

used in this context to assess strategies and options to restore the ecological state of the Coorong South Lagoon (Department for Environment and Water 2018, 2019, 2021b, 2022e, c, a, Hipsey et al. 2022).
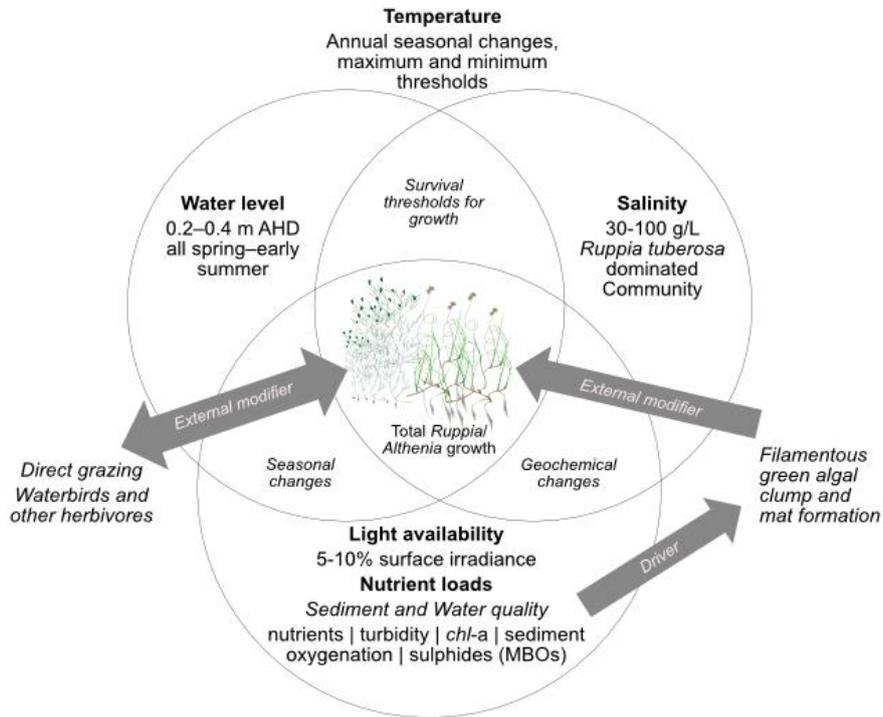
Figure 65. Overview of the interactions between the conditions that control the growth of the dominant aquatic macrophytes in the southern Coorong, *Ruppia tuberosa* and *Althenia cylindrocarpa* (adapted from Asanopoulos and Waycott 2020).

In this final chapter, key findings are summarised and a number of combined data sets are presented to inform these key ecological parameters where available. The presentation of these results was enabled by having highly structured data systems (Appendix A) and adopting a critical review of data through quality assurance procedures. The focus is the Ruppia Community along with the associated filamentous algal community, although limited observations that were able to be made on the charophyte *Lamprothamnium* spp. that had reduced significantly and is now uncommonly observed in the southern Coorong.

6.1 Key findings

Throughout this chapter there are references to Distance from Murray Mouth an several key sites, for reference we provide a guide to those numbers and sites (Figure 66).
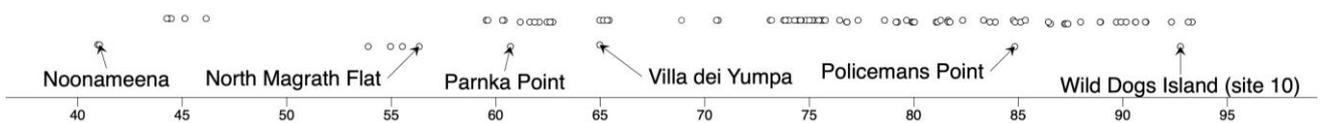
Figure 66. Distribution of samples made across studies presented as liner distance (km) from Murray Mouth (DFMM). Sites labelled with location names are those sampled repeatedly for Phenology (Chapter 3).

6.1.1 The Ruppia Community

The trends in distribution and abundance of the Ruppia Community are described overall, and then assessed against depth, salinity, light availability, sediment condition, nutrient loads, plant growth strategies and the formation of a seed bank and/or turions. Results from the detailed site survey (Lewis et al. 2022) and phenology (section 2.4.1) data collections are considered together in most cases here, as described in the figure captions of this chapter.

Overall distribution and abundance

Populations of the Ruppia Community were found across the majority of locations surveyed between Long Point and Tea Tree Crossing, as has been described as the outcomes of detailed site surveys (Lewis et al. 2022) (Figure 67). In addition, repeated sampling at 6 locations (see Chapter 3) to determine phenology, gave insights into the changes in biomass between the 2020 and 2021 growing season (Figure 67). Viewed monthly, the mean biomass across all sites was lowest in late summer months and increased towards peak biomass in 2020 in Spring and in 2021 early Summer. These differences were associated with inter-annual variation in water levels and associated salinity (e.g. Figure 69 A.). It is worth noting that some biomass persisted at sites throughout the year and some perennial plants were observed to persist. This was markedly the case at Noonameena in the Coorong North Lagoon, this site clearly responds to the different environmental conditions experienced (Chapter 3). Abundance, as biomass, was highest in different regions in different years (Figure 67). The higher values in the North Lagoon and central section locations in 2020 aligns with the lower water levels in the later months of the growing season than in 2021 where higher water levels supported higher biomass in the Woods Well to Policeman's Point region (Figure 69 A.).
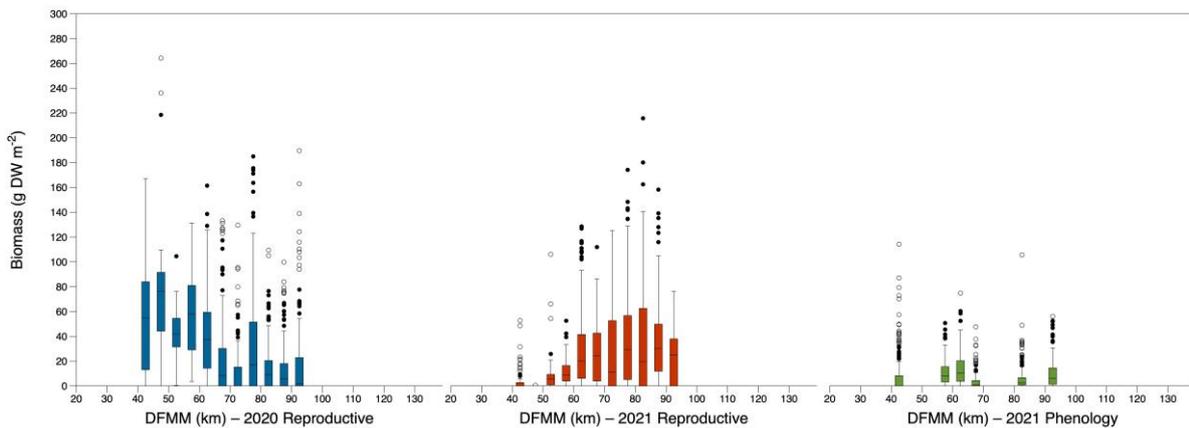
Figure 67. Boxplots (IQR) of the plant tissue biomass during the Reproductive period (Lewis et al. 2022) for each of the 2 years sampled, and the phenology sampling 2021, sites binned into 5 km groups for locations defined by the distance (km) from Murray Mouth (DFMM). Note phenology sampling extended over a 6 month period, reproductive period sampling was over a 2–4 week period.
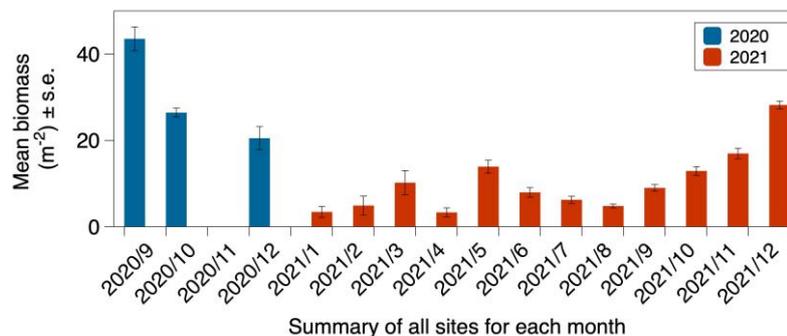
Figure 68. Plot of plant tissue biomass over the whole year for each of the 2 years sampled including Reproductive and Phenology sampling, sample data binned into monthly groups for all sites.

A.

B.
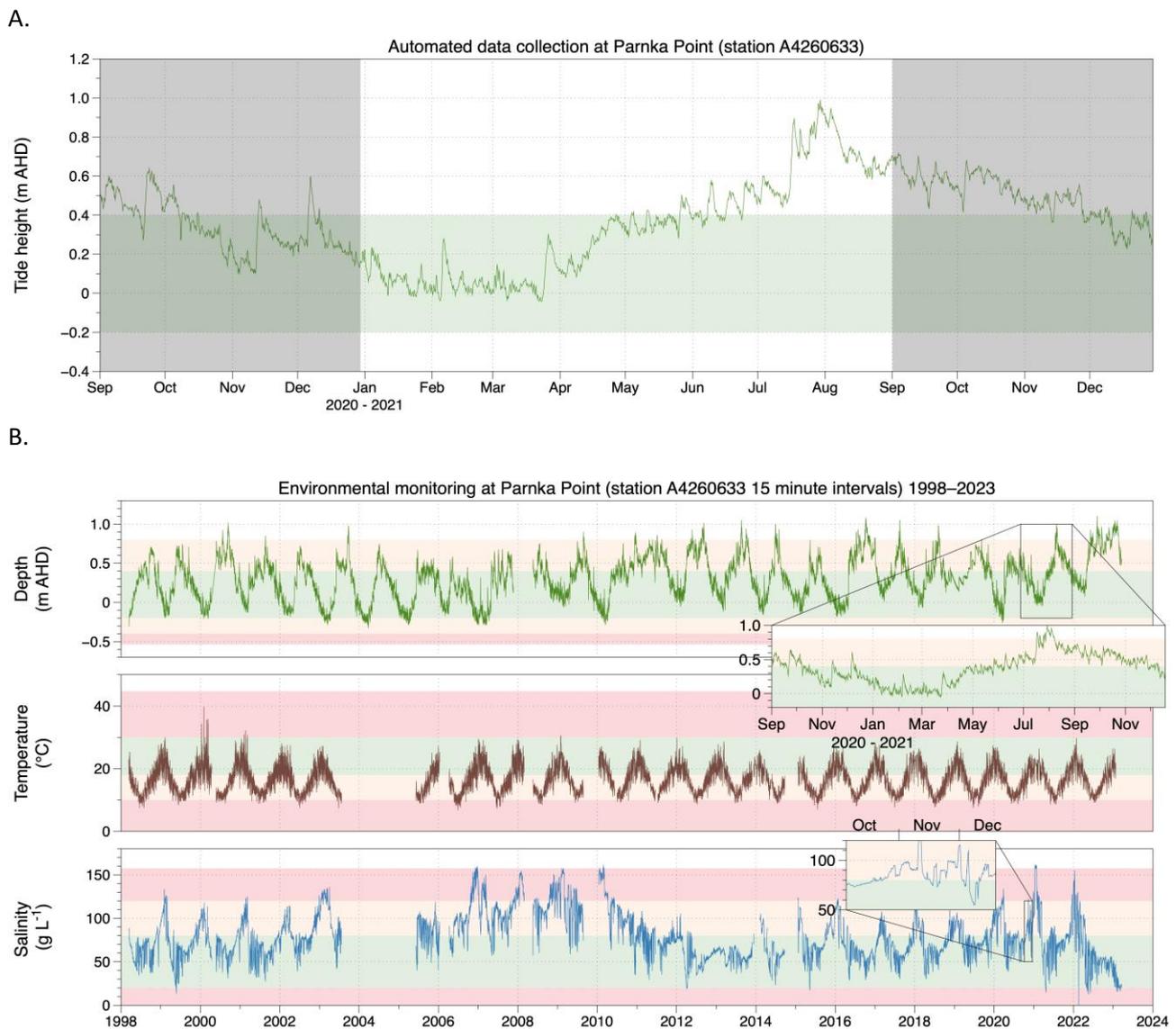
Figure 69. Key environmental parameters measured 4 times hourly plotted over time for the in-water monitoring station located at Parnka Point (station number A4260633). A. Tide height as an indication of depth (m AHD) for the Reproductive seasons 2020 and 2021. The grey shaded months are those critical to having water levels available for the completion of reproduction and the green band at depths of -0.2–0.4 m AHD reflect the zone where the majority of the Ruppia Community is found growing (Lewis et al. 2022). B. Long term data (1998–2023) for depth, temperature and salinity including a magnified window to highlight the Spring-Summer 2020-21 season in greater detail for salinity and depth. The coloured bands on each graph reflect suggested optimal (green), suboptimal (yellow) and unsuitable (red) conditions for Ruppia Community adult plant growth based on recent observations. Salinity data extracted from TDS.

In summary, the Ruppia Community in 2020 and 2021 was continuous between Noonameena and the Salt Creek outlet. The highest biomass differed in each season which is likely related to the higher water levels in 2021. Other factors will influence the abundance of Ruppia Community productivity including water depth, salinity, sediment condition and light availability.

Depth and abundance

The depth range of where the Ruppia Community were observed can be described in two ways. First, biomass for categories of the water depth above plants when samples were collected (Figure 70 A.). Second, the height of sediment based on the digital elevation model (DEM) of Hobbs (2019) which is referenced against

sea level (Figure 70 B.). More up to date and improved resolution data is needed to improve the digital elevation model as the shallow areas where key biota such as the Ruppia Community are present were likely underestimated by the current models.

A.

B.
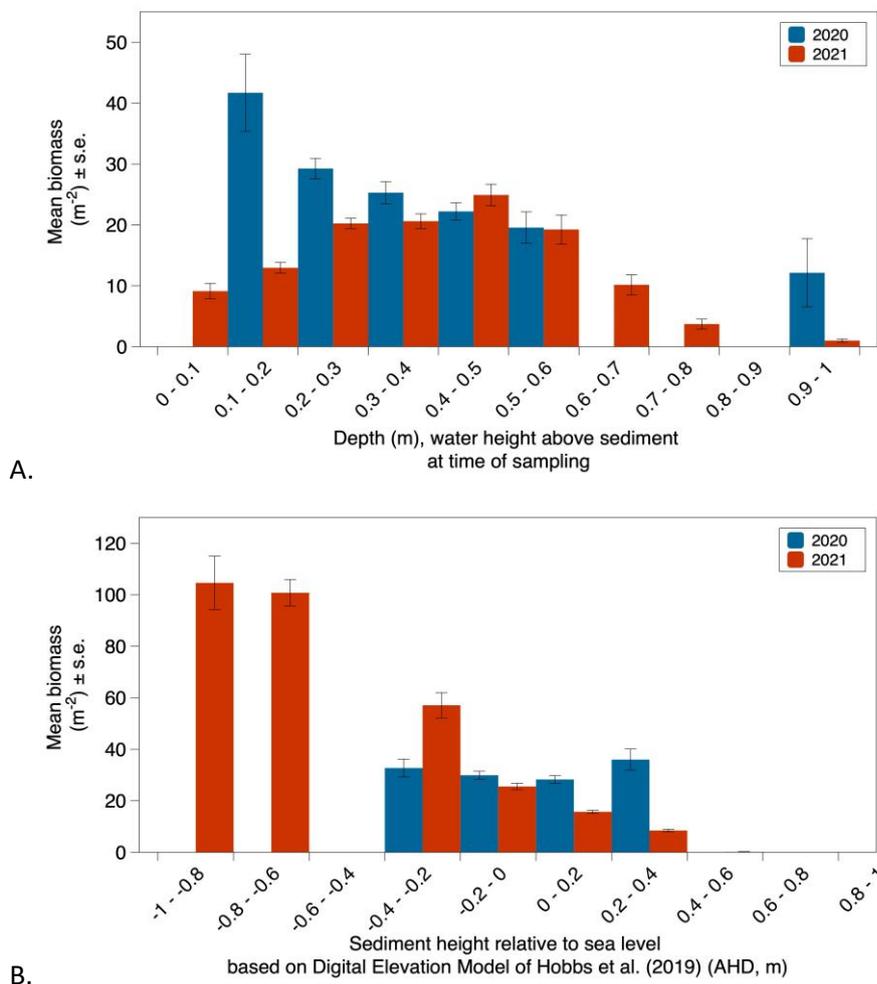
Figure 70. Plots of mean biomass (g DW m^{-2}) ± s.e. of Ruppia Community plant tissue for each of the 2 years sampled, excluding Aestivation samples, including Reproductive and Phenology samples (see Lewis et al. 2022) for; A. the depths of water observed (m) above plants at the time sampled in the field, B. the sediment height relative to sea level (m AHD).

Sampling in both years were in the same approximate locations (i.e. within 100 m described in Lewis et al. 2022) thus the detection of higher biomass plants growing at depths of around 1 m (Figure 70 B.) that were undetected in 2020 is evidence of the significant interannual variability that can occur with this ecological community. However, over both years the plants were predominantly detected in the range of water depths of ±0.4 m AHD.

Salinity and abundance

Experimental evidence from mesocosm trials demonstrated that plants from the Ruppia Community grew more vigorously (shoot counts and biomass) under marine salinities (35 g L^{-1}) than high (hypersaline) salinities (80 g L^{-1}) over a range of light availability (section 2.4.1; Figure 5 C.). During field sampling, salinities were measured at the time of sample collection and comparing the range of salinities between sampling years indicated a wider salinity range in 2021 than 2020 (Figure 71). Due to the higher water levels in 2021 (Figure 69 A. and B. top panel) sampling occurred later in the year although collections spanned a similar range of

observed water depths at that time (Figure 70 A.). Salinity had already begun to increase with the warmer weather in the shallow waters of the aquatic macrophyte littoral zone (e.g. Figure 69 B. lower panel).
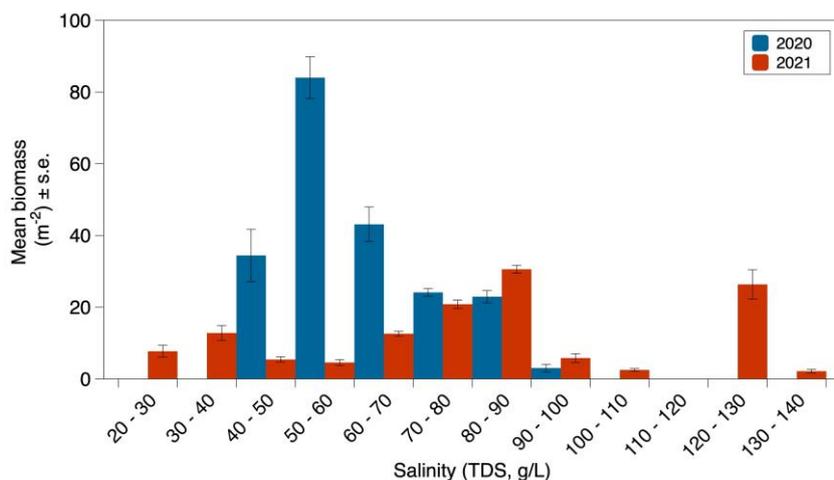
Figure 71. Mean biomass (g DW m^{-2}) ± s.e. of Ruppia Community plant tissue for each of the 2 years sampled, excluding Aestivation samples, including Reproductive and Phenology samples (see Lewis et al. 2022) for salinity in 10 g L^{-1} categories for comparative purposes.

The higher mean biomass samples observed to occur at higher salinities (Figure 71) appear to be associated with sites that were forming turions, a widespread occurrence in 2021 and is discussed in the section below on reproduction.

It is worth noting that in the literature, aquatic macrophytes that are tolerant of these extremes are limited to those that typically colonise salt lakes, including species of *Ruppia* around the world. Most are incapable of surviving salinities greater the 50-60 g L^{-1} further emphasising the adaptability of these species to productively occupy this ecosystem. Lower salinities would enable a wider range of hypersalinity tolerant aquatic macrophytes to occupy the Coorong, although there would likely be periods where the system would reach higher values.

Sediment condition and abundance

The application of a Rapid Assessment Protocol (RAP; Hallett et al. 2019) to evaluate current sediment condition was an effective in-situ field method. There was a relationship between sediments that scored poorly i.e. a RAP score ≤ 5 Poor and low Ruppia Community biomass (see Figure 48). In contrast RAP scores of 6-10, classified as Moderate and RAP scores of 11-15, classified as Good, exhibited increasingly high biomass as scores increased (Figure 72).

Using the 'Good / Moderate / Poor' classification scheme, sediments classified as Good were observed to have low total nutrients and AVS whereas the sediments classified as Poor had high total nutrients and AVS although variable (see Figure 48). The growth of aquatic macrophytes in Moderate sediments and some Poor sediments is important as their presence in denser and higher biomass areas will support enhanced sediment quality by promoting oxygenation in the sediment and lead to nitrification-denitrification that can act as a nitrogen loss mechanism (see Huang et al. 2022). The recovery of seagrasses has been well documented to enhance denitrification pathways (e.g. Aoki and McGlathery 2018) leading to nitrogen mobilisation and loss to the sediment and water column.

Another outcome from the application of RAP scores is the potential for these scores to act as a proxy to the more detailed investigations otherwise required to characterise Ruppia Community sediment habitat conditions. This significantly enhances the options for monitoring detailed changes in sediment condition at a fine scale when complemented by a modest monitoring of sediment nutrient concentrations, in order to overcome prohibitive costs associated with replicate nutrient analysis.
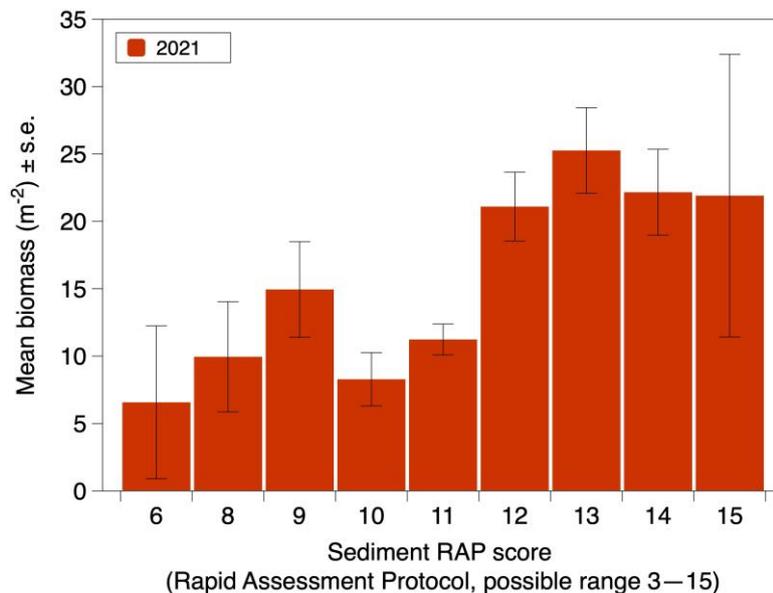
Figure 72. Mean biomass (g DW m^{-2}) ± s.e. of Ruppia Community plant tissue for samples with sediments scored using the Rapid Assessment Protocol (RAP; Hallett et al. 2019) including 2021 Reproductive samples (see section 5.3.3 for more details).

The formation of black ooze dominated sediments, in particular monosulfidic black ooze (MBO), in areas occupied by the Ruppia Community will result in ongoing plant community stressors associated with sediment based organic nutrient cycles that persist. Although the Ruppia Community has slowly recovered its extent since its loss during the Millennium Drought, the stressful conditions associated with poor sediment quality and light limitation, often associated with poor quality sediments, will combine leading to future declines in the system if not reversed.

Light availability and abundance

In the field, data from light loggers (see Figure 20), demonstrated that there were extended periods of light levels below the threshold estimated in experimental shading of Ruppia Community in mesocosms (<16% full sunlight for 8 weeks) during the 2020–2021 seasons (see section 2.4.1). Two sites, North Magrath Flat and Parnka Point, experienced extended periods of low light and based on experimental data conditions (<16% full sunlight; see Figure 20), this would translate to a 30%–50% reduction in biomass. If plants were already stressed, such as due to higher salinities, this could lead to plant death.

Field measurements of instantaneous photosynthetic efficiency (effective quantum yield) at four locations in the Coorong indicated that overall water quality, in particular water clarity was important to performance. The inhibition of aquatic macrophyte photosynthesis will reduce the ability of the Ruppia Community to tolerate the impacts of filamentous algal mats through direct shading. This suggests that where compounding water quality factors inhibit light penetration such as suspended sediments or phytoplankton (chlorophyll a) blooms, plants will be at a significantly higher risk of decline or loss.

Nutrient loads and abundance

The nutrient environment was found to be hostile where sediments were associated with high total organic matter (mean values >3%), high TN (mean values >0.45%) and high TP (mean values > 400 mg kg^{-1}) were categorised as poor with a RAP score of less than 5. Moderate RAP scores (6–10) were highly variable across nutrient levels although means were lower than those for Poor RAP scores (see 5.3.3). The nutrient conditions for good RAP scores were total organic matter <1% (mean ~0.5%), TN <1% (mean ~0.4%) and TP < 100 mg kg^{-1} (mean 50 mg kg^{-1}). It is highly desirable for any infrastructure or intervention options to reduce

the ambient sediment loads in the upper 10 cm highlighted in studies on the Ruppia Community and supported by those of Mosley and collaborators (Mosley et al. 2020, Huang et al. 2022, Priestley et al. 2022a).

There was a high ratio of nitrogen to phosphorus in the tissues of aquatic plants in the southern Coorong in 2021 (referred to as P limited), samples from 2017 were also phosphorus limited, but not to the extent observed in 2021 (see section 5.3.4). The difference between years is due to a significantly higher concentration in nitrogen relative to phosphorus in the plant tissue although P levels were also higher. The higher concentration of nitrogen supports excessive growth of filamentous and micro algae. These combined, along with the presence of ammonium in some locations are likely contributing to a high δ^{15}N values (see 5.3.5).

The nutrient driven presence of excessive filamentous algae in the southern Coorong is leading to the formation of dense, nutrient rich, organic Monosulfidic Black Oozes that deposit on top the Ruppia Community. In addition, wind driven movement of detached algal mats result in the detritus covering areas of the shoreline and decaying. These processes are more prevalent in the warmer months when water temperature and salinity rise. This leads to a nutrient cycle feedback supporting the dominance of high turnover species prevents nutrient cycling that reduces nutrient loads such as denitrification. The detection of increased nutrient loads since 2017 support the need for intervention to reduce the likelihood of this cycle continuing.

Plant growth strategies and abundance

The dominant genus of the Ruppia Community of the southern Coorong is currently *Ruppia*, however the species *Althenia cylindrocarpa* is also common (Lewis et al. 2022). These species are morphologically similar when plants are not reproductive and have small shoots, they also have similar life cycles (Figure 73) following the annual cycle of water levels dropping and higher salinity in the Spring and Summer months. In the past, the majority of the Ruppia Community was considered annual; plants must have completed their flowering and fruiting before conditions became too inhospitable (outlined in detail in Asanopoulos and Waycott 2020). The early life stages are critical, seed germination and initial colonisation in particular. Good data on seed germination characteristics is available (Kim et al. 2013, Kim 2014, Kim et al. 2015). However, resource allocation changes over time, phenology and reproductive effort may vary due to diverse factors.

Observations to determine phenology and biomass allocation were made for six sites, the most northern site, Noonameena, which is located at the southern end of the Coorong North Lagoon, clearly behaved differently to other sites (see section 3.3; Figure 28). Plants at Noonameena exhibited a perennial habit in 2020-2021 season likely due to the regime of water levels, salinity and nutrients that differed at this site compared to the South Lagoon sites. The main Coorong South Lagoon locations, Villa de Yumpa, Policeman Point, and Wild Dog Islands (Salt Creek, site 10) were consistent exhibiting a slow build of biomass following germination of the seed bank. Biomass increase was slow until late August 2021 which then continued to increase until sampling was completed in December 2021. These phenology studies also determined that the Ruppia Community develops strong root structures prior to allocating resources to other structures. This results in a high root:shoot ratio suggesting colonising behaviour (e.g. Kilminster et al. 2015) which aligns with the annual cycle of the whole community. Such an investment in below ground biomass provides advantages including increased sediment oxygenation reducing anoxia, sediment stabilisation, increased nutrient and water availability in the event of variable water levels and secure attachment to substrate.
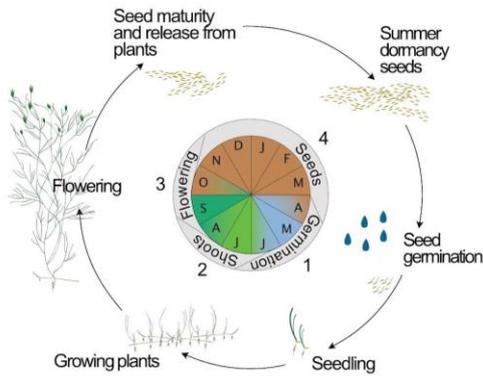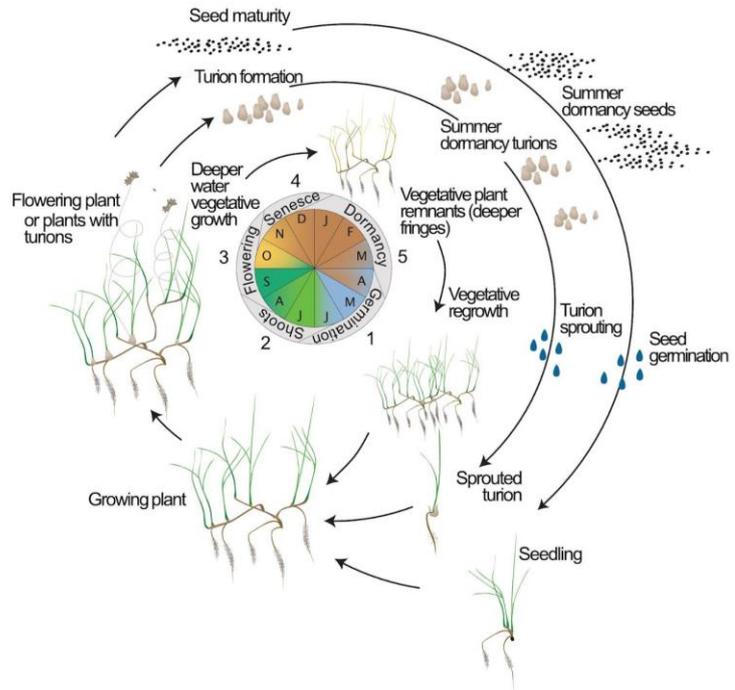
Figure 73. Summary schematic of the lifecycles for *Althenia cylindrocarpa* and *Ruppia tuberosa* depicting seasonal changes in the stages of growth, reproduction and senescence and broad timing of each life stage. Attribution: Michelle Waycott, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons.

Reproductive effort

Seed bank

The completion of the reproductive lifecycle through the production of propagules enables the plants to colonise the next season (Brock 2011). Evidence of this process in the Ruppia Community of the Coorong is the formation of a seed bank. The seeds of *Ruppia* spp. are of a form that can build up a long term seed bank, and able to be extracted from sediments using manual and mechanical sorting. Although small, typically <1 mm diameter, the seeds of *Ruppia tuberosa* are able to be identified and an estimate of the seed bank made.

Seed density followed similar trends to adult plants with respect to water depth (Figure 74), a wider range of depths in 2021 (Figure 74 A.) and the depth range for the majority of the seed bank being ± 0.4 m AHD, slightly deeper in 2021 (Figure 74 B.). The mean depth of -0.6 to -0.4 m AHD in 2020 (Figure 74 B.) contained the highest number of seeds, more than 3000 seeds/m^{-2}. Seeds are more likely to accumulate in the areas occupied by the aquatic plants in particular zones that sediment and water movement favour resulting in the heterogeneous distribution of seeds locally.

A.

B.
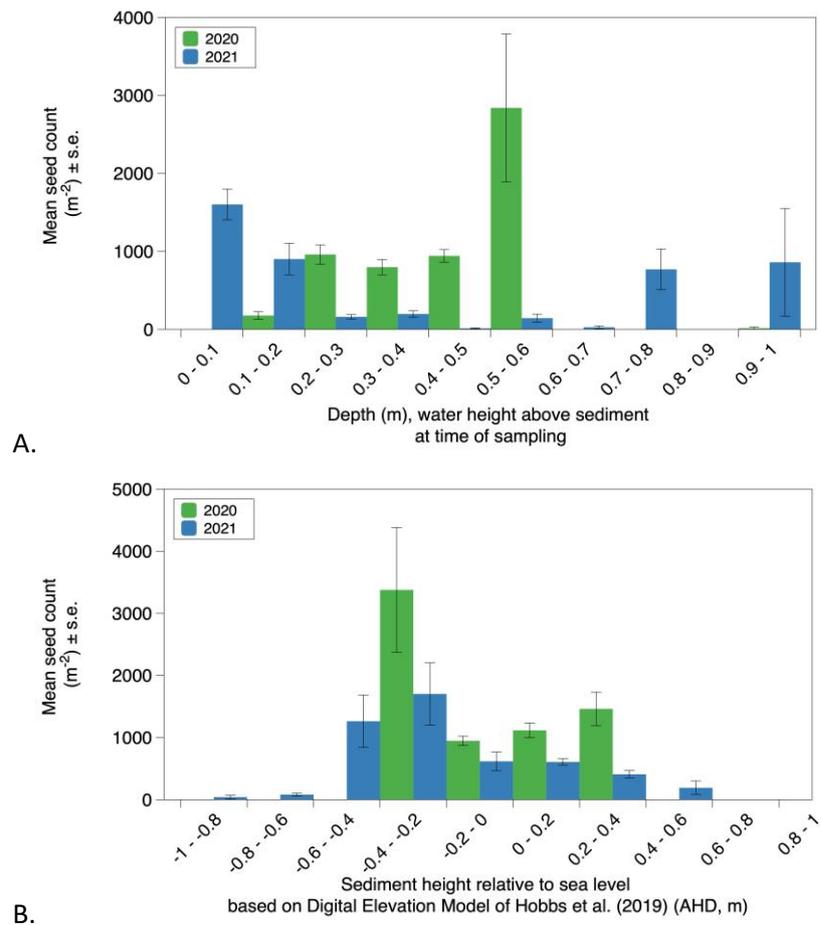
Figure 74. Seed count (Mean ± s.e. m⁻²) associated with, A. the depth of water above the sediment height at time of sampling in categories of 0.1 m depth, B. the sediment height above sea level as categories of 0.2 m AHD bands. Data included across all sites and the peak growing season collection dates (Reproductive period) and the two years of collections.

The distribution of seed banks at sites along the gradient from the more northern sites (i.e. closer to the Murray Mouth) to Salt Creek at the southern end of the South Lagoon differed between years (Figure 75) In 2020 there were denser seed banks in the central section between North Magrath Flat and south of Parnka Point (50–65 km from Murray Mouth, refer to Figure 66 for distribution of sites). However, in 2021 the denser seed banks were found in the section from Parnka Point then South (60–75 km from Murray Mouth).
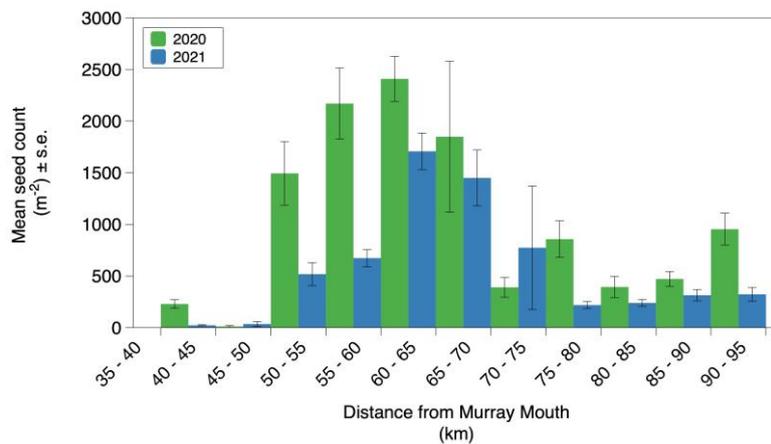
Figure 75. Seed count (Mean ± s.e. m⁻²) compared to the sampling sites distance from the Murray Mouth (DFMM) categorised into 5 km groups and the two years of collections and all sites.

The relationship between salinity and the presence of a seed bank surprisingly determined that in 2021 the highest density of seeds was found in the 100-110 g L^{-1} salinity category (Figure 76). Given the later timing of sampling in 2021 it is likely this reflects the later stage of the population maturing than any other specific feature of seed production.
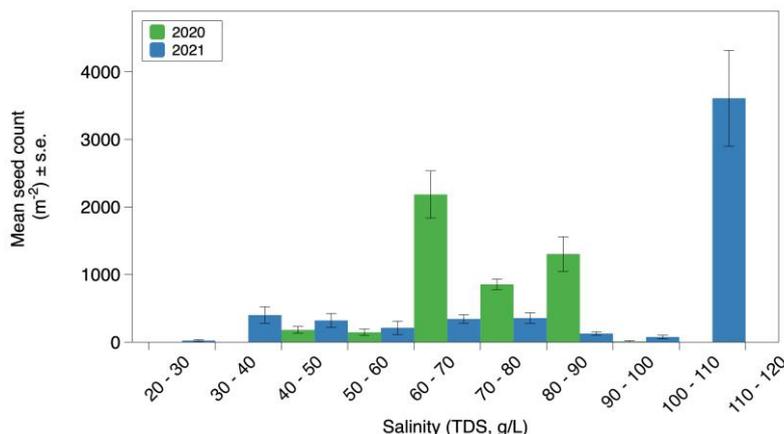
Figure 76. Seed count (Mean ± s.e. m^{-2}) compared to the salinity measured at time of sampling each site categorised into 10 g L^{-1} salinity groups for the two years of collections and all sites.

The density of the seed bank in locations and regions is highly variable and of the majority of spatial scales examined, few achieved the desired outcome of >2000 seeds m^{-2} as evidence of a resilient Ruppia Community (refer to Paton et al. 2017b, Department for Environment and Water 2020). Individual sites, or subgroups of sites (e.g. Figure 76) achieved a seed bank greater than this number, however, as noted the seed bank is highly heterogeneous and thus this specific target will be very difficult to achieve under typical conditions.

Turion formation

The numbers of turions presented here includes fully formed (type II) turions as well as those in the process of forming (Lewis et al. 2022). Therefore these summary results will reflect the observations that turions were forming or fully formed as a single data set.

The production of turions overall and the relative depths that turions formed (i.e. sediment height relative to sea level based on Digital Elevation Model of Hobbs et al. (2019, m, AHD) differed significantly between years (Figure 77). Notably very few sites developed turions in the 2020 season compared to the 2021 season and shallower sites (>0.2 m AHD) developed very few turions at all, likely due to their more exposed locations.
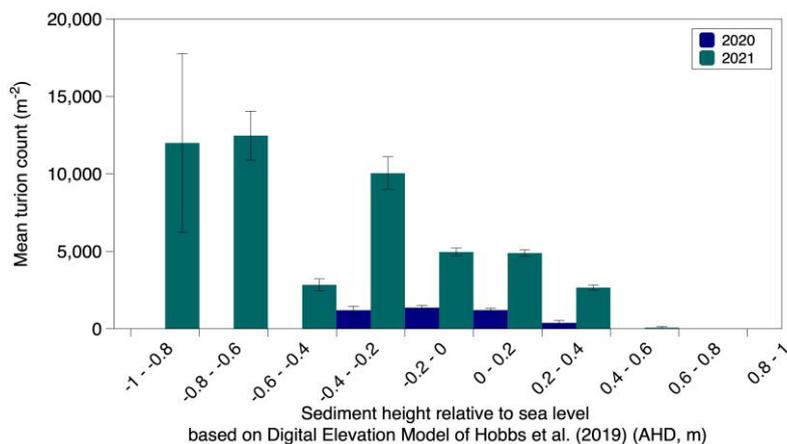
Figure 77. Turion count (Mean ± s.e. m^{-2}) associated with the sediment height above sea level as categories of 0.2 m AHD bands. Data included across all sites and the peak growing season collection dates (Reproductive period) and the two years of collections.

The distribution of turion density along the gradient from the more northern sites (i.e. closer to the Murray Mouth) to Salt Creek at the southern end of the South Lagoon differed between years (Figure 78). In 2020 there was higher turion density in the most northern region (including Noonameena, refer to Figure 66 for distribution of sites). However, in 2021 the denser seed banks were found in the section from the central section north of North Magrath Flat to south of Parnka Point then south past Villa dei Yumpa (60–75 km from Murray Mouth) (Figure 78). In 2021 there were large numbers of turions found developing across the Coorong South Lagoon and based on the data collected we suggest this is likely due to the variation in salinity levels in the warmer months, as 2021 had extended period of higher water in the South Lagoon due to the release of water from Salt Creek in August-October. On site, we observed differences in the conditions across the sampling seasons (Figure 79) that included a rapid change to the higher salinities in observed late 2021, presumably as water levels receded and temperatures increased and the highest turion numbers per core were associated with those higher salinities (Figure 80).
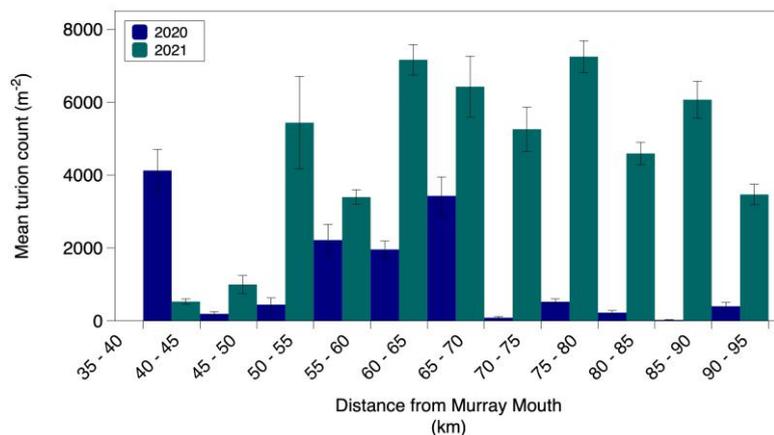
Figure 78. Turion count (Mean ± s.e. m^{-2}) compared to the sampling sites distance from the Murray Mouth (DFMM) categorised into 5 km groups and the two years of collections and all sites.
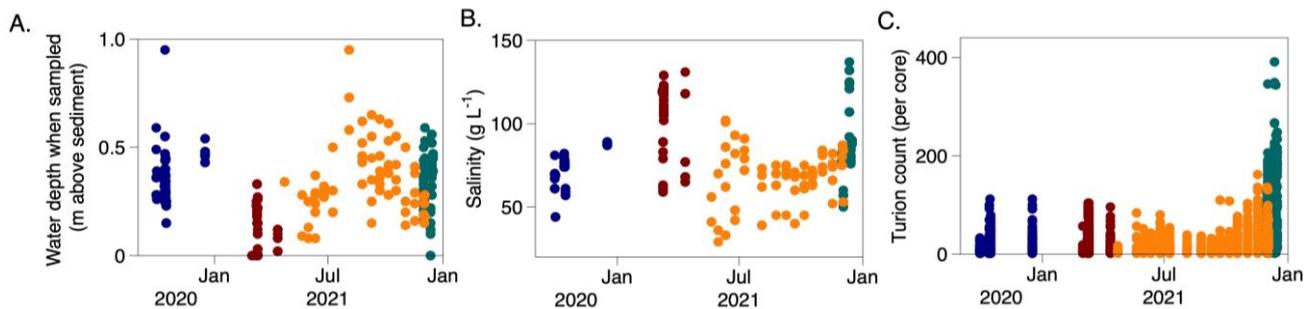
Figure 79. On site environmental conditions measured when samples taken for turion counts and only include samples that had more than one turion, A. water depth at site as samples were being taken (m above sediment), B. salinity (g L^{-1}) at site as samples were being taken, C. turion counts per core (7.5 cm diameter core). Colours represent seasonal category; dark blue = 2020 Reproductive, dark red = 2021 Aestivation (lowest water levels at sites), dark green = 2021 Reproductive and orange = phenology samples taken throughout the 2021 growing season.
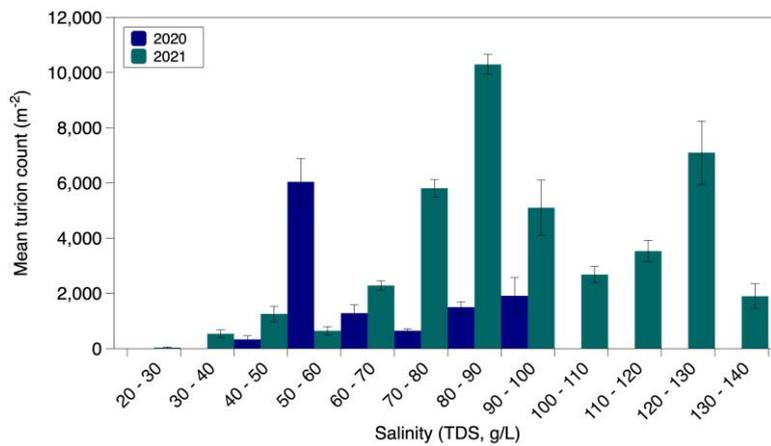
Figure 80. Turion count (Mean ± s.e. m^{-2}) compared to the salinity measured at time of sampling, each site categorised into 10 g L^{-1} salinity groups the two years of collections and all sites.

There was an observed relationship between turion formation and biomass, high numbers of turions being formed may result in higher biomass (Figure 81). As already noted, the turion counts presented here may reflect different stages of development (refer to Figure 79 C.). Over the growing season some locations may initiate turion formation, they are usually classified as type I turions (i.e. appear relatively small and immature) and may never reach a mature stage, where they can persist without living plants to support their growth. In the comparison of turion density with categorised biomass classes for the two Reproductive sampling seasons (Figure 82), the maximum density of turions is associated with the moderate biomass classes and both sampling periods exhibited the same tendency despite the differences in total turion numbers.
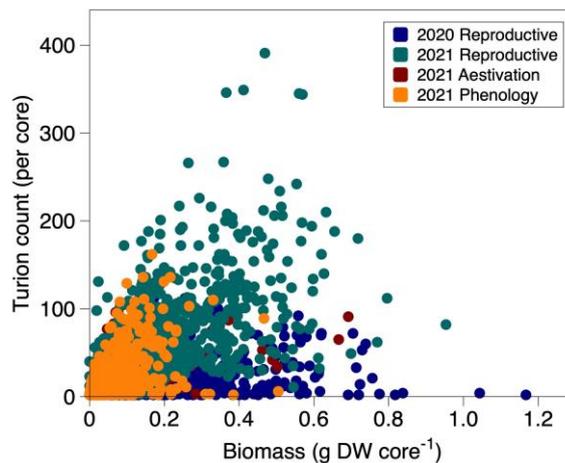
Figure 81. Turion count (per core) compared to the total biomass (g DW per core) for the two years of collections and all sites coloured by sampling season.
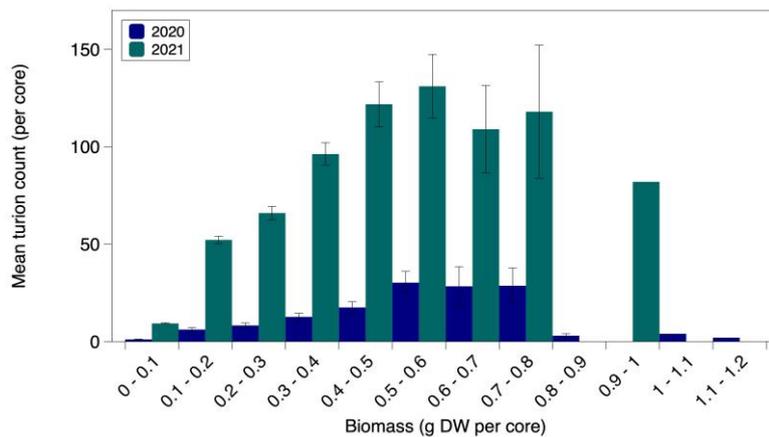
Figure 82. Turion count (Mean ± s.e. per core) compared to the total biomass (g DW per core) for the two years of Reproductive period collections and all sites.

There has been a clear switch in the production of seeds versus turions in the two reproductive seasons sampled (see section 4.2.3, Figure 36). The relationship between late reproductive season, higher salinity in 2021 and increased turion formation could explain this difference. However, the occurrence of an extended growing season may be associated with this response or, more likely a combination of these conditions. Turions were detected rarely in the years immediately following the Millennium Drought (Paton and Bailey 2014, Paton et al. 2016, 2021) although more commonly in recent years. The recovery of aquatic macrophyte communities following large scale losses can take considerable time and may involve different stages of meadow reformation (Kilminster et al. 2015, O'Brien et al. 2018). Large scale losses of a foundational benthic community, such as the Ruppia Community in the Coorong, can be associated with a structural change to habitats, such as the loss of sediment cohesion. Given the loss of the Ruppia Community from the southern Coorong during the Millennium Drought, the early recovery stages may not have provided adequate plant growth conditions for turion formation. As a result, if this is occurring, only locations with more mature Ruppia Community populations will begin forming turions. Future monitoring efforts may be able to assess this if locations are able to be categorised in this way.

6.1.2   Other aquatic macrophyte biota

In regional surveys, outside the Coorong, of more than 50 field locations across South Australia visited from 2019-2022 there were populations of submerged aquatic macrophytes outside the Coorong and adjacent areas, some of which are likely to survive in the Coorong, although to our current knowledge most require lower maximum salinities to survive. Future work on the remnant populations of aquatic macrophytes around the region will be needed as restoration options are considered.

Within the Coorong main lagoon, between October 2019 and March 2023, we have not observed the charophyte, *Lamprothamnium* sp., an algal-like species previously common in the main lagoon (Delroy 1974, Womersley 1975). Water quality of the system is likely to have a large influence on the ability of these aquatic macrophytes to survive. However, the propagules of *Lamprothamnium* sp. must remain viable in the sediments for long periods as they have emerged from translocated cores when placed in clean, lower salinity water. Sufficient numbers of *Lamprothamnium* sp. emerged in culture and were able to be tested at marine salinity under a range of different shading conditions. Like the Ruppia Community, *Lamprothamnium* sp. demonstrated significantly reduced biomass when shading was reduced to 16% surface light. The presence of charophytes in the sediments as propagules, implies that if the condition of the system improves, in particular if salinities lower and water clarity improves, these charophytes should be able to recover rapidly in the system.

6.1.3 Filamentous algae

We have proposed the filamentous algal community that develops in the southern Coorong is directly associated with the Ruppia Community as an attachment substrate (see section 2.5.2). This is based on a strong, direct relationship being observed between the mat forming filamentous green algae and the Ruppia Community. The association between filamentous algae and the Ruppia Community was observed during field sampling between September – December 2019 (Lewis et al. 2022). All locations where filamentous green algae occurred in visible clumps or mats were generally attached to *Ruppia/Althenia* plants. Prior to this study, the close association between filamentous algal mat formation and the presence of the Ruppia Community was not well understood.

The formation of the mat forming filamentous algae community of the Coorong follows the annual cycle of water levels, temperature and salinity (Figure 83). The species of algae that occur in the mat forming community can also grow in free floating or benthic forms that do not aggregate. However, when the filamentous algae become attached to the aquatic macrophyte plants they can use this physical substrate to develop larger clumped masses. Growth of the algal clumps can be rapid as temperature and nutrient availability increases (see section 2.5.2) and can completely shroud the plants of the Ruppia Community, forming surface mats covering the surface of the water. When water movement and wind are mild, these mats can form large areas while still attached and shade the Ruppia Community. Once salinity levels get too high (> 90 g L^{-1}) and temperatures are high (>30°C), the algal mats begin to decay rapidly and eventually deposits onto the sediment below where they grow, or if physically removed from their substrate of attachment they will likely form a detrital layer on the shoreline or sink into the deeper water of the main lagoon. This life cycle of this algal community is intimately associated with the Ruppia Community. Such a strong relationship between the formation of filamentous algal mats and the likelihood of the mats interacting with the plants growing in the Ruppia Community reduces the likelihood of successful mechanical removal of the algal mats without significant impact to the Ruppia Community. Although there may be interannual differences in timing, it is likely that rapid algal growth and flowering will continue to be coincident with the increasingly warmer conditions during spring and early summer.
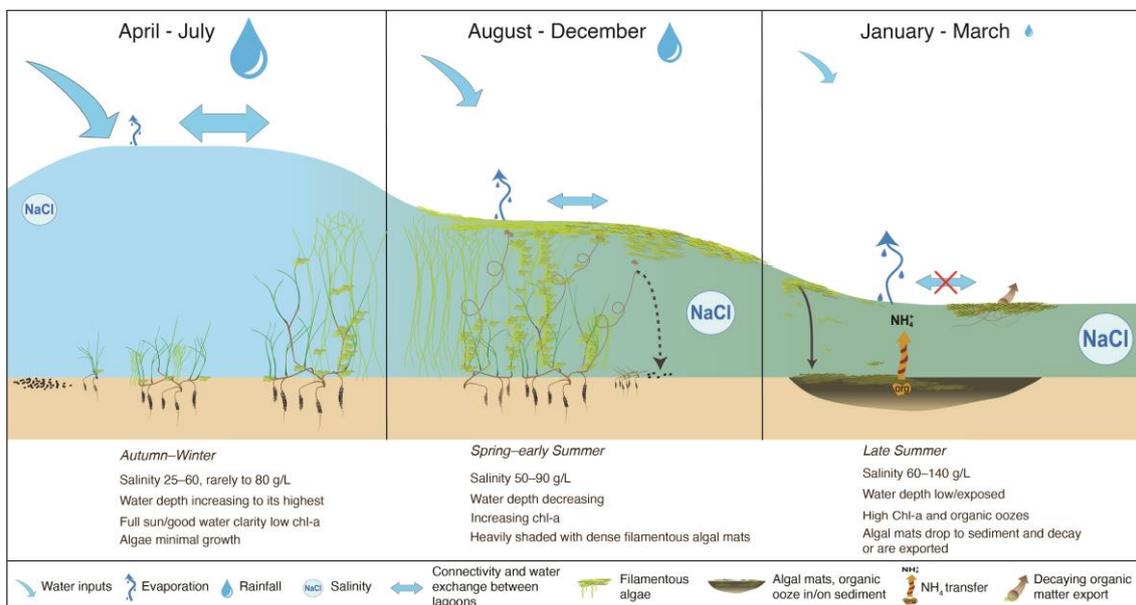
Figure 83. Conceptual diagram summarising the seasonal development of the filamentous algal community and indicative conditions observed or measured during the 2019–2021 field surveys reported here and by Lewis et al. (2022). The different stages of filamentous algal mat formation are summarised in Table 5 and Figure 11 and associated text.

The observation by Lewis et al. (2022) that filamentous algae are largely absent from the area south of Woods Well may relate to the hydrodynamic regime of this area. However, this is speculative and will require

modelling to determine if the movement of water and the direction of that movement may be associated with reduced filamentous algal biomass. If there is an association between increased hydrodynamic energy and reduced filamentous algal mat formation, this may provide solutions for managing the interactions between these two communities.

The potential for clearing filamentous algae manually from the Coorong waterbody was not supported based on the trials in this study. Manual removal activities would disrupt the Ruppia Community and adversely impact reproductive success. Local mechanical removal in the growing Ruppia Community, even with simple and easy methods, would disrupt these ecosystems. However, mechanical removal of the shoreline algae deposits would have considerable benefits, preventing degradation of sediment quality and enabling more effective foraging or colonisation by higher order species.

Parameters that limit growth of the filamentous algae community

Salinity and temperature seasonally fluctuate and are highest throughout the growing season of the Ruppia Community. Previous experimental testing in mesocosms (Collier et al. 2017, Waycott et al. 2019) of the filamentous algal community, in response to a range of environmental (salinity and temperature) conditions supports the upper threshold of 90 g L^{-1} for filamentous algal growth to stop, however algae death was not observed until higher salinities (>100 g L^{-1}). It is worth noting that under experimental conditions, maximum daily growth in biomass was observed at lower salinities (< 40 g L^{-1}). Maximum growth rates have also been observed to occur when water temperatures reach ~30°C (Waycott et al. 2019). In the field, salinities, based on point measurements, in these ranges occurred locally and at locations where water temperatures reach 30°C, leading to maximum growth rates during periods of mid–late summer, conditions ideal for algal growth.

The high biomass of algal blooms in many areas has led to the deposition of organic carbon into the sediment, which promotes sulfate-reducing bacteria activity, formation of AVS and the development of the Monosulfidic Black Ooze (MBO) sediment. We observed this in the field at all of the high filamentous algal cover sites, most obviously Parnka Point and North Magrath Flats. These two sites also experienced periods of shading during the growth season when flowering occurs. The link of these biochemical and biological processes continues to provide the feedback for the hyper-eutrophic cycle through the presence of filamentous algae.

The biomass of filamentous algae and phytoplankton is highly dependent on the presence of high turnover nutrients present in the environment. Experimental results demonstrate that higher nutrient loads in the ecosystem also assist in making filamentous algae more resistant to higher salinities (Collier et al. 2017). The excess of nitrogen present in the system in 2021 compared to 2017 led to more algal biomass overall. While the higher nutrients are part of a high turnover, organic cycles as opposed to where nutrients are bound up in the longer-lived species such as seagrasses, or exported via alternative hydrological (e.g. flushing), ecological (removal through food web) and biogeochemical (e.g. denitrification).

List of shortened forms and glossary

Acid Volatile Sulfide (AVS) When treated with acid sediments that release H_2S are known as acid volatile sulfides or AVS.

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/Score A scale of algae cover and level of interference with *Ruppia* 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.

Box plot method In this report the box plots are drawn around the Inner Quartile Range (IQR), where the IQR is the difference between the first and third quartiles. Outliers (1.5 times beyond the IQR) are drawn as filled-in circles, and extreme outliers (3 times beyond the IQR) are open circles. The whiskers are drawn to the smallest/largest non-outlier.

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, Virideplantae) are the extant group of green algae that are most closely related to modern land plants.

Chlorophyll a (C*hl* a) 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 a.

Coorong Dynamics Model A spatially resolved model to simulate the environmental conditions within the Coorong at varying spatial and temporal resolutions. The core of the Coorong Dynamics Model is the fine resolution Coorong TULOW-FV hydrodynamic model, and Aquatic EcoDynamics (AED) comprising a library of modules and algorithms for simulation of water quality, aquatic biogeochemistry, biotic habitat, and aquatic ecosystem dynamics. These models can be optionally linked with the Simulating WAves Nearshore (SWAN) wave model.

DFMM Distance from the Murray Mouth

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 *Ulva paradoxa*, *Rhizoclonium* sp. and *Cladophora* sp. defined in Collier et al. 2017.

HCHB *Healthy Coorong, Healthy Basin* program

Hyper-eutrophic Very nutrient-rich waters characterised by frequent and severe nuisance algal blooms and low transparency.

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 that an organism goes through during its lifetime. i.e. for *Ruppia tuberosa*, seed, seedling, vegetative growth of mature plant, flowering, fruiting, turion formation, senescing plant.

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.
Monosulfidic black ooze	Black gel-like materials that are enriched in sulfides.
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.
NO$_x$	**Collective abbreviation for nitrate and nitrite (dissolved) forms of nitrogen.**
PCA	Principal Components Analysis
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
PON	Particulate organic nitrogen
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).
Ruppia Community	The multi species assemblage identified in this study that has become established across the southern Coorong and includes *Ruppia tuberosa*, *Althenia cylindrocarpa* along with an as yet unresolved species of *Ruppia*.
Salinity	Measure of the concentration of salt in the water.
	Commonly reported at ppt (parts per thousand) equivalent to g/kg or g/L water. Historically salinity was calculated as
	salinity=1.80655*chlorinity (ppt or g/kg)
Sampling core 'Cores'	A standard core in this study is a 7.5 cm diameter (0.0044 m^2) and 8 cm deep sample, 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 plumbing pipe) with a mark made at 8 cm to indicate the depth that can be pushed into the sediment to isolate the area to be sampled.
SNP	Single nucleotide polymorphisms
Southern Coorong	In this report, the region south of Long Point to south of Salt Creek (Figure 1).
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.
TC	Total carbon
TDS	Total dissolved solids. The total concentration of dissolved solids in water.
TN	Total nitrogen
TORG	Total organic carbon
TP	Total phosphorus
Trophic	Feeding and nutrition of plants and animals and where they fit into niches and levels of the food web.
Turion	Reproductive structure that *Ruppia polycarpa* (Type I) and *Ruppia tuberosa* (Type I and Type II) produce underground that is capable of forming into a new plant.

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Appendix A – Data management system

Aquatic plants and algae data management

The '**Ruppia Habitat and Filamentous Algae Database**' (the database) was developed for this project based on the central principle that all samples would be able to refer to a georeferenced site (with unique identifiers), collection methods were equivalent (same core size and site data collected) and that derived data sets would be traceable to the collection site, date, collector and subproject. Metadata were developed for each data set within the database.

Data management system

Introduction

The data management process included a comprehensive cross referencing protocol that centred around the implementation of a data schema (Figure A1) with unique identifiers (a Primary Key) for each record. The process was implemented in Filemaker as a system that can be used with a simple user interface equivalent to spreadsheets or with sophisticated Graphical User Interface (GUI) input options (Figure A.2). We implemented a detailed data management workflow where each sample had unique codes that were recorded and linked to spatial data and physical collections made. All the required reference data was collected while out in the field using a comprehensive field data sheet that was developed ensuring it was easy to fill out and extract the required data for entry into the database later. The database interface incorporated an image of each Field Datasheet stored in the database for cross referencing and fields can also be transcribed directly from the image (Figure A.3). Hard copy Field Datasheets were then archived in the project repository at the University of Adelaide, curated by the Activity coordinator. Spatial georeferencing using GPS data of all sites is linked (Figure A.4), and once sample processing of cores collected is completed the core level information was also linked, i.e. shoot numbers, biomass, flower numbers, seed numbers, species and later DNA profiles and samples for nutrient data analysis.

At project start-up a Data Management Plan (Waycott et al. 2020a) was developed to guide the development of data management system functions (database interfaces visualised in Figures A.1 to A.9).
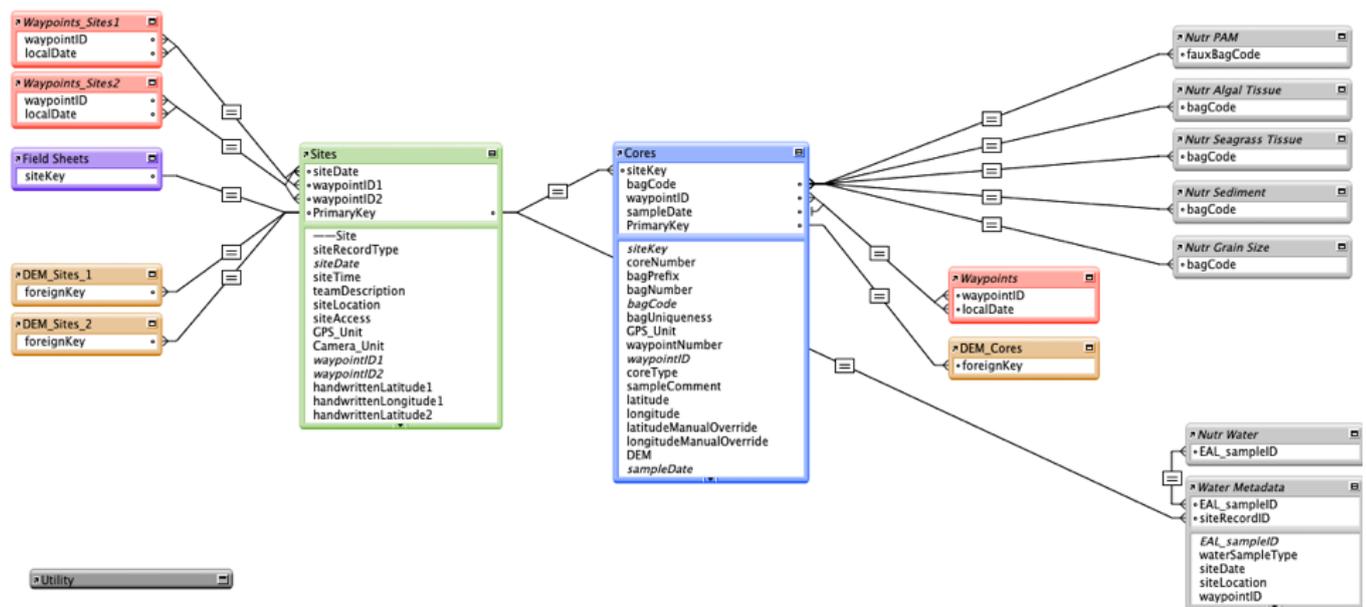
Figure A.1. Data relationships for Ruppia Community and filamentous algae surveys and analyses
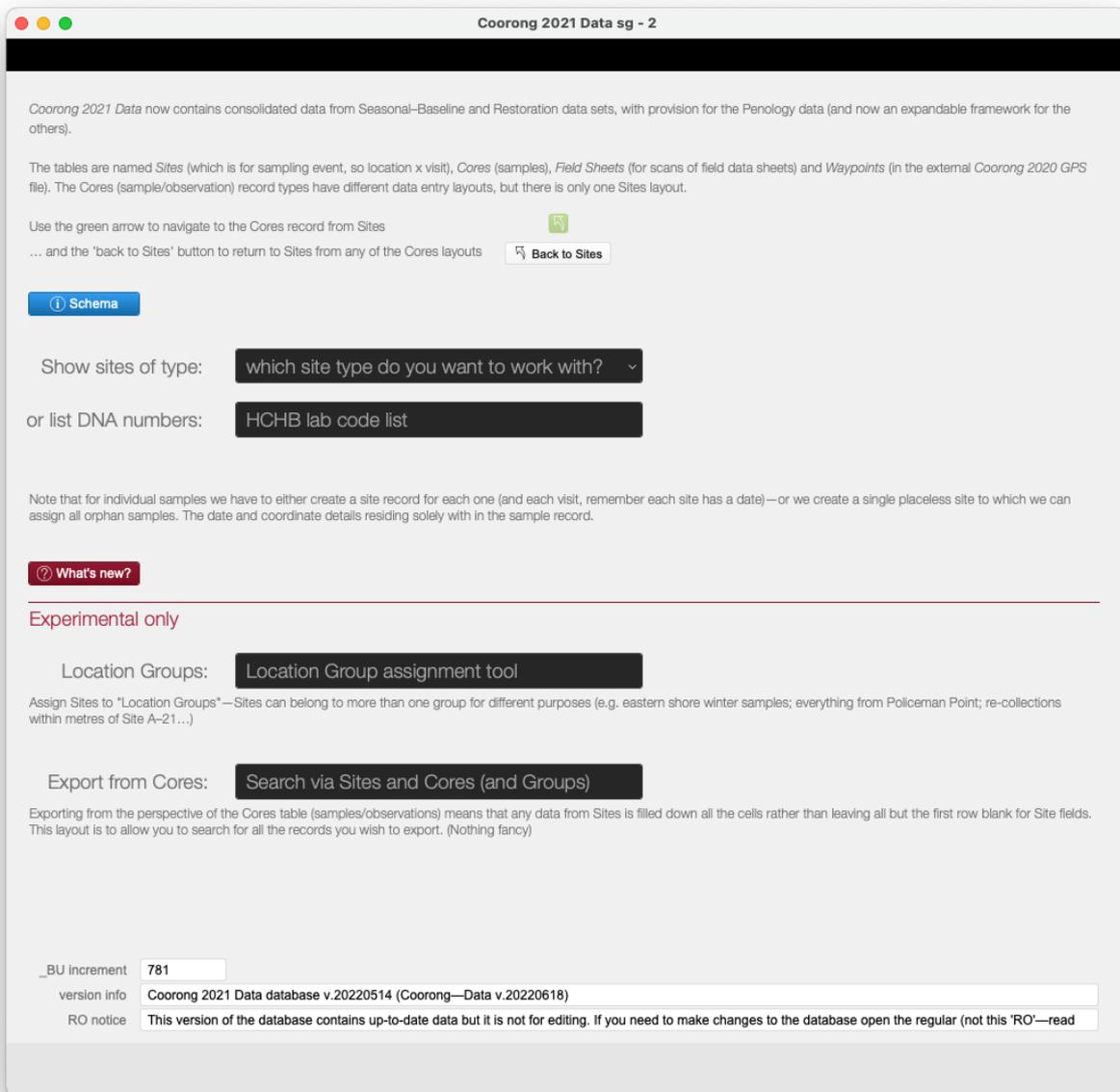
Figure A.2. Start-up screen for data entry
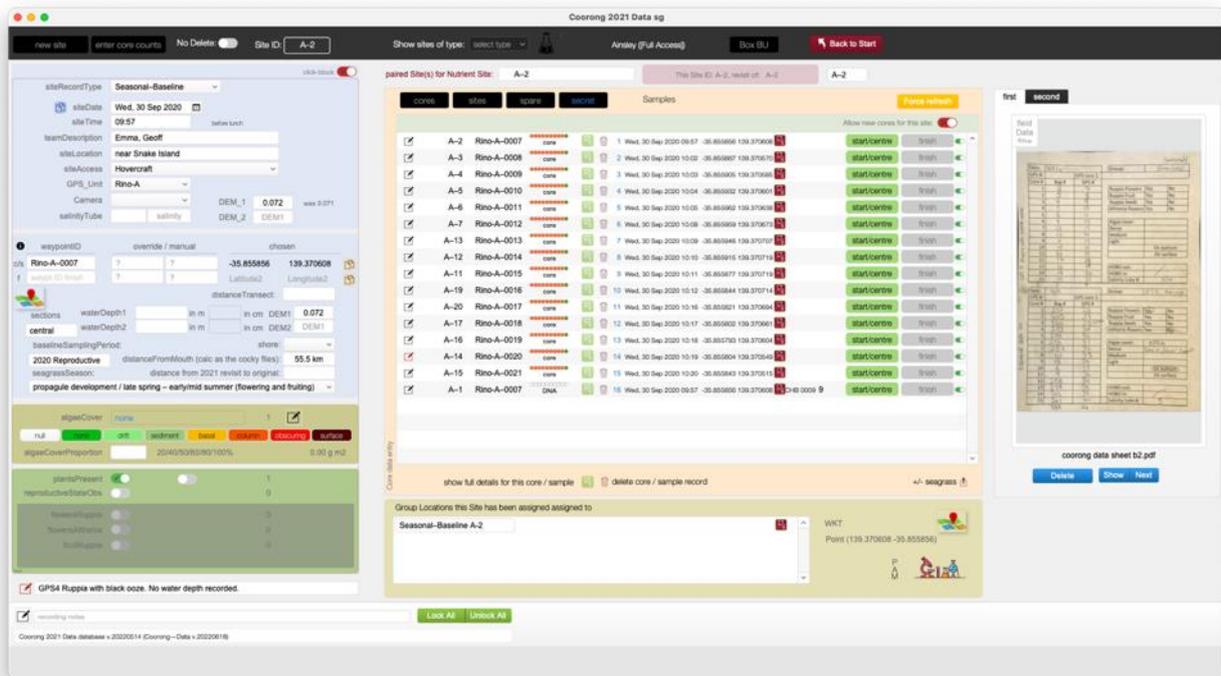
Figure A.3. Sites baseline (seasonal) survey screen for data entry
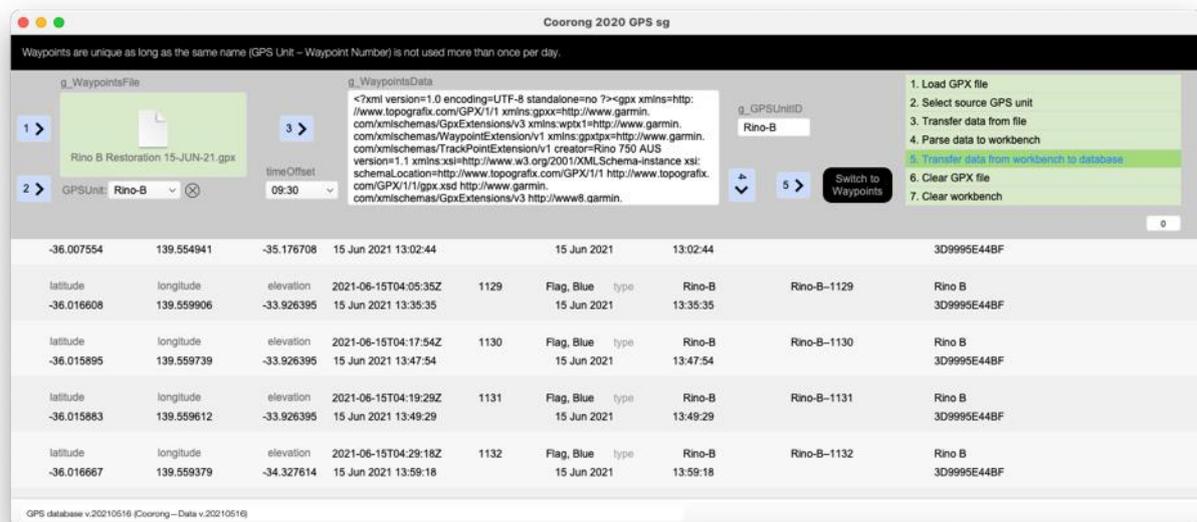
Figure A.4. GPS data entry workbench

Data capture

The overall data structure included information that was associated with each 'site', defined as the place a set of samples would be collected at a particular time. Each site has a suite of metadata attributes; georeferenced location, site observations and other data such as water depth and salinity. These attributes have a data system layout for entry that can reference field notes and visualise locations (google maps) (Figure A.5).

Additional details can be added through a series of data entry layouts that are linked to each site (Figures A.6–9).
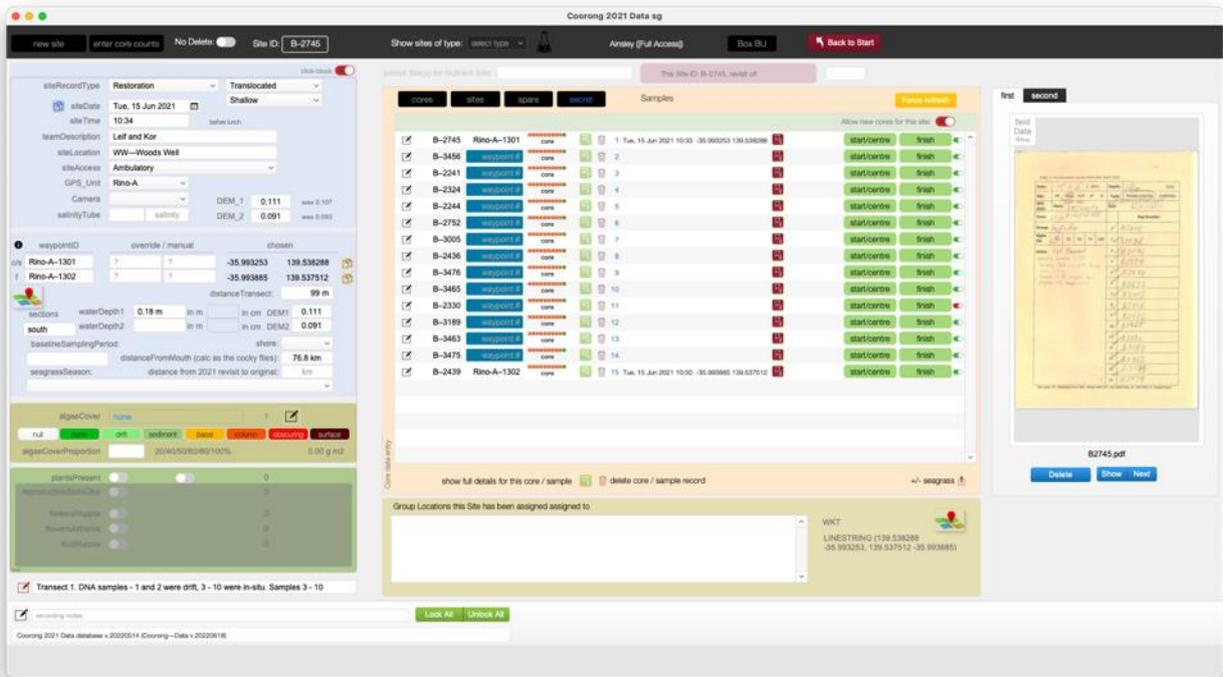
Figure A.5. Sites transect based survey screen for data entry
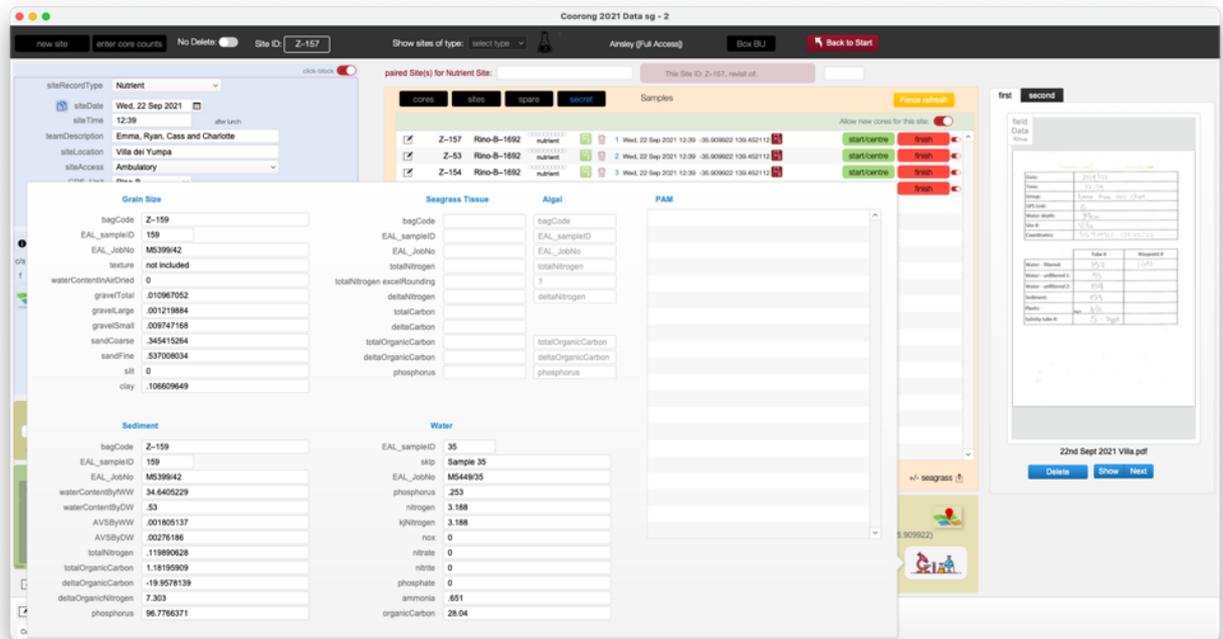
Figure A.6. Sites popover screen, data entry for nutrient samples and environmental parameters

Figure A.7. Cores baseline (seasonal) and restoration survey screen for data entry of individual core data

Figure A.8. Cores phenology popover screen for data entry of individual core data
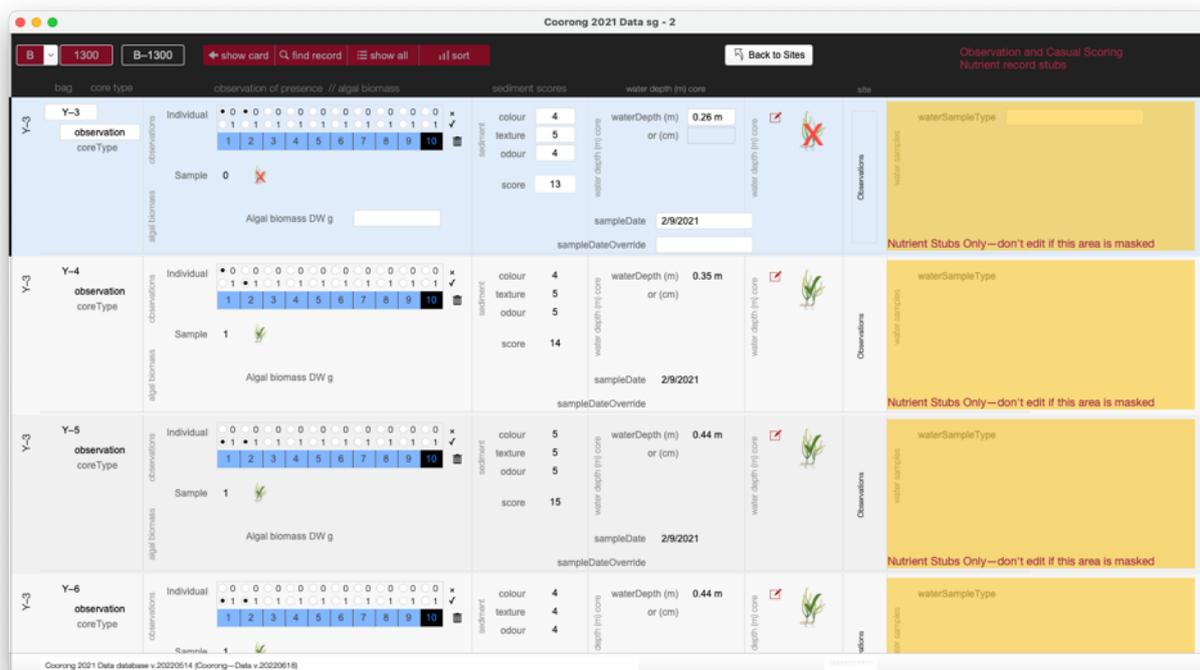
Figure A.9. Observations and transect depth survey screen for data entry of individual point data (equivalent to cores)

Quality control

Data entry was conducted by staff who had been involved in field data collections then checked by the field coordinator specifically to check that the correct data sheet was associated with the data entered, that there was no sign of typographical entry errors and to cross reference with manually recorded GPS locations and the imported data. Once endorsed by the coordinator the database manager then conducted a review to establish the data formats conformed and were linked.

Preliminary analyses identified a few data transcription errors by graphing results to find outliers. The coordinator then checked to establish the error and made corrections directly.

Data export and use

Data sets were extracted from the database through linked searches inclusive of metadata and delivered to staff for use in analysis. All unprocessed data was delivered to project partners as each data set was made available.

Future applications for the database

Additional surveys, based on the same, or similar methodologies can be added to this database readily. When sampling methodologies align, links between data sets can be identified and resolved for analyses appropriate to combine. The metadata for each site where collections of cores occur or observations are made would be equivalent and allow location based comparisons to be readily made.