Inland Waters & Catchment Ecology

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Distribution and Abundance of *Ruppia tuberosa* in the Coorong, December 2014

Kate Frahn and Susan Gehrig

SARDI Publication No. F2012/000074-2
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SARDI Aquatics Sciences
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PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

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EXECUTIVE SUMMARY

Ruppia tuberosa is an extremely salt tolerant, submergent macrophyte, which historically formed extensive beds in the South Lagoon of the Coorong; in areas inundated over the winter and spring and dry in the summer and autumn. Since 2000, *Ruppia tuberosa* abundance in the South Lagoon declined to the point where it was not observed in the system by 2009. The decline was related to decreased water levels and increased salinity during the drought, which lasted from 1996 to 2010. Floods in 2010/11 resulted in rising water levels, decreasing salinity (in the South Lagoon) and limited re-colonisation of *Ruppia tuberosa* in the South Lagoon and southern end of the North Lagoon. The aim of this study was to quantify the distribution and abundance of *Ruppia tuberosa* (including the propagule bank) and filamentous algae in December 2014 and compare it to assessments of the same sites and parameters made in December 2011.

In the Coorong, a total of 14 sites (13 in the South Lagoon and one in the southern end of the North Lagoon) were sampled using a grab sampler to a maximum depth of 1 m, at 20 cm intervals. Grab samples were sieved and *Ruppia tuberosa* shoots were counted and dried to determine biomass. Filamentous algae percent cover was visually estimated, separated from *Ruppia tuberosa* shoots in the grab samples and also dried to determine filamentous algae biomass. This study focused on the presence of *Ruppia tuberosa* and *Ulva paradoxa* (filamentous algae) in the South Lagoon of the Coorong.

The 2014 survey indicates that *Ruppia tuberosa* was present in the South Lagoon with a wider distribution and slightly greater abundance, as opposed to larger, more dispersed patches in 2011. In particular, *Ruppia tuberosa* biomass and shoot numbers tended to be highest at sites on the western shoreline of the South Lagoon in both 2011 and 2014, suggesting a more persistent population in this area. There was also a slight increase in the distribution of *Ruppia tuberosa* on the eastern shoreline between 2011 and 2014, indicating a degree of successful recruitment and establishment. Overall, while the differences in *Ruppia tuberosa* abundance were significant between survey years, the low shoot numbers and abundances compared to those reported prior to the drought suggest recovery is not, as yet, biologically significant.

Alternatively, the filamentous algae was as widespread as it had been in 2011, but there was a noticeable decrease in abundance and percent cover in the 2014 surveys. In 2011, the

abundance and percent cover of *Ulva paradoxa* was greatest at the southern end of the North Lagoon and to some extent on the western ocean shoreline of the South Lagoon, whereas in 2014, filamentous algae tended to fringe both sides of the South Lagoon.

Ruppia tuberosa appears restricted to the shallow water depths (20 – 60 cm depth) therefore the risk of desiccation before life cycle is completed (i.e. turions and/or seeds develop) and the propagule bank is replenished (usually mid-November to late December) is considerable. Results also suggest a reduced presence of *Ruppia tuberosa* in areas with higher salinities (>60‰ TDS), which may further impact distribution and abundance as lower salinities (<100‰ TDS) are also required for the production of seed and replenishment of the sediment propagule bank. The observed decrease in filamentous algae may also influence the increase in *Ruppia tuberosa* abundance as it has been known to outcompete *Ruppia tuberosa* and smother flower stalks, causing them to break off before seeds are developed. Most of the *Ruppia tuberosa* plants that were sampled had not flowered or developed turions in the 2014 survey, although some reproductive propagules (19 seeds and five turions) were recorded from the southern end of the South Lagoon.

The suitability of Lake George as an alternative translocation source site of *Ruppia tuberosa* seeds for the Coorong system was also assessed. Sediment samples were collected along five transects within Little Lake (southern most basin of Lake George) to determine the presence and number of viable seeds present per sample (seed density m^{-2}). Three species of seeds, namely *Ruppia tuberosa*, *Ruppia megacarpa* and *Lepilaena* sp. were present. The number of *Ruppia tuberosa* seeds within Lake George ranged from 0 – 2325 seeds m^{-2}; *Lepilaena* sp. seeds ranged from ~50 – 3500 seeds m^{-2} and there was one *Ruppia megacarpa* seed. Potential hot spots (where the number of *Ruppia tuberosa* seeds per sediment sample are greatest) exist within Lake George, but the mean density of viable seeds is still markedly less than the number of viable seeds found within hot spots in Lake Cantara (~4500 seeds m^{-2}).

Results from this study indicate that *Ruppia tuberosa* populations in the South Lagoon are increasing; however the low shoot numbers and abundances reported in this study were still much lower than those observed prior to the drought, suggesting recovery is slow. Furthermore, potential hot spots of viable *Ruppia tuberosa* seeds exist within Lake George, but densities were too low to warrant use as a suitable donor site. Continued monitoring of existing populations and the development of additional investigations are still required to help determine the factors that are limiting establishment and recruitment of *Ruppia tuberosa*.

1. INTRODUCTION

Ruppia tuberosa is a highly salt tolerant submergent macrophyte (Brock 1982), commonly found in temporary saline lakes throughout southern Australia (Brock 1981; Brock and Shiel 1983). From the 1970s to the early 2000s, *Ruppia tuberosa* was the dominant submergent plant in the South Lagoon of the Coorong (Geddes and Brock 1977; Brock 1979; Brock 1981; Paton 1982; Geddes and Butler 1984; Geddes 1987; Paton 1996) where it formed extensive beds between Salt Creek and Hells Gate (Figure 1), growing in water depths ranging from 30 to 80 cm in the winter and spring (Womersley 1975; Geddes and Brock 1977; Gilbertson and Foale 1977; Paton 1982; Leary 1993; Paton 1996; Paton 2000; Paton 2001; Paton *et al.* 2001; Paton and Bolton 2001; Paton 2002; Paton 2003; Nicol 2005).

Ruppia tuberosa is a key component of the biota of the South Lagoon of the Coorong. It is one of only two submergent macrophytes (the other being the charophyte *Lamprothamnium macropogon*) that have been recorded in the hypermarine South Lagoon. It is thought to provide habitat for small mouth hardyhead (*Atherinosoma microstoma*) and the shoots, rhizomes, seeds and turions are an important component of the diet of herbivorous waterfowl and waders (Paton *et al.* 2001; Paton 2005). *Ruppia tuberosa* was historically restricted to the higher salinities of the South Lagoon of the Coorong because at lower salinities (such as those found in the North Lagoon) it was smothered by the filamentous green algae (identified as *Enteromorpha* sp. at the time, but most likely *Ulva paradoxa*) despite exhibiting higher growth rates in the absence of competition (Paton 1996).

However, between 2000 and 2010, *Ruppia tuberosa* declined in abundance in the South Lagoon and before the 2010/11 flood was no longer observed, only being present in the southern end of the North Lagoon of the Coorong (Brookes *et al.* 2009; Whipp 2010). The decline in abundance and subsequent disappearance from the South Lagoon was attributed to the steady increase in salinity between 2000 and 2010, which at times exceeded 160‰ TDS (Paton and Rogers 2008; Brookes *et al.* 2009; Whipp 2010). Since the 2010/11 flood, water levels have risen and salinities have decreased and *Ruppia tuberosa* has recolonised limited areas of the South Lagoon (Frahn *et al.* 2012) and remained present in the North Lagoon.

Nevertheless, recolonisation has been slow (Frahn *et al.* 2012) and water levels in the South Lagoon in mid-November to late December (i.e. towards the end of its growing season) remain insufficient to maintain life cycle completion (D. Paton, Adelaide University, *pers. comm*). Furthermore, an increased presence of filamentous green algae has been observed in recent years in the Coorong (Frahn *et al.* 2012), which may outcompete *Ruppia tuberosa* and/or smother flower stalks, causing them to break (Paton 1996). The increased presence of filamentous algae is most likely related to the lowering of salinity (Paton 1996) in the Coorong since 2010/11 providing suitable conditions for its establishment in the North Lagoon and northern end of the South Lagoon.

Since the 2010/11 flood there is limited quantitative information regarding the current distribution, abundance and biomass of *Ruppia tuberosa* or filamentous algae in the Coorong. Furthermore, there is limited information regarding propagule (seed and turion) abundance and current abiotic conditions (e.g. water quality). This information is important for the short to medium-term management of the Coorong as it will provide information about the conditions required for establishment of *Ruppia tuberosa*, help to monitor changes and determine whether there is a viable propagule bank.

The key objectives of this project were therefore to:

i) record and map the current distribution, abundance and biomass of *Ruppia tuberosa* and filamentous green algae in the Coorong,
ii) compare populations of *Ruppia tuberosa* and filamentous algae between December 2011 and December 2014 and determine any changes to abundance, and
iii) assess whether *Ruppia tuberosa* has established a viable propagule bank.

To assist recolonisation of *Ruppia tuberosa* and promote the formation of a propagule bank in the Coorong South Lagoon, the Department of Environment, Water and Natural Resources (DEWNR) Coorong, Lower Lakes and Murray Mouth Recovery Project established a translocation project in 2012. Specifically, sediment from Lake Cantara, which was found to contain large numbers of *Ruppia tuberosa* seeds (~4,500 seeds m^{-2}) was translocated to the South Lagoon of the Coorong. To determine if any alternative source sites exist in the region, this project also assessed the *Ruppia tuberosa* seed bank in Lake George for its suitability as donor site for translocation.

2. METHODS

2.1. Study site

The Coorong is a shallow, elongate coastal lagoon confined by the coastal dune barrier of the Younghusband and Sir Richard Peninsulas. The Coorong stretches for 140 km (Geddes 1987; Seaman 2003) (Figure 1) and is comprised of two main lagoons (the North and South Lagoons) of similar size, almost separated by a spit of land (Hells Gate) (Lothian and Williams 1988) (Figure 1).

Salinity is one of the primary factors that influences the plant community in the Coorong (Womersley 1975; Noye and Walsh 1976; Geddes and Brock 1977; Gilbertson and Foale 1977; Geddes 1987; Geddes and Hall 1990; Webster 2005a; Webster 2005b; Brookes *et al.* 2009; Lester and Fairweather 2009). Salinity levels in the Coorong vary substantially at spatial and temporal scales, ranging from fresh near the barrages (when large quantities of water are being released from Lake Alexandrina) through brackish, to the salinity of seawater (35‰ TDS) in the Murray Mouth area, grading to hypersaline (>35-115‰ TDS) in the southern end of the North Lagoon and South Lagoon (Geddes 1987; Lothian and Williams 1988; Department of Environment and Heritage 2000; Paton 2000; Paton 2001; Paton and Bolton 2001; Paton 2003; Seaman 2003). Within the last five years the south lagoon of the Coorong underwent elevated salinity (EC) levels (up to 180,000 µS cm^{-1} in 2008; Figure 2) and extreme low water levels and exposure (Figure 2). Water levels are an important factor in the South Lagoon where water levels can fluctuate up to 1 m at longer seasonal and temporal scales from winter/spring highs to late summer/autumn lows (Geddes 1987; Seaman 2003) and over shorter temporal scales due to seiching caused by the speed and direction of the wind (Noye and Walsh 1976). Within the last three years, water levels tend to decline in late winter/early spring, and increase in late summer (Figure 2).

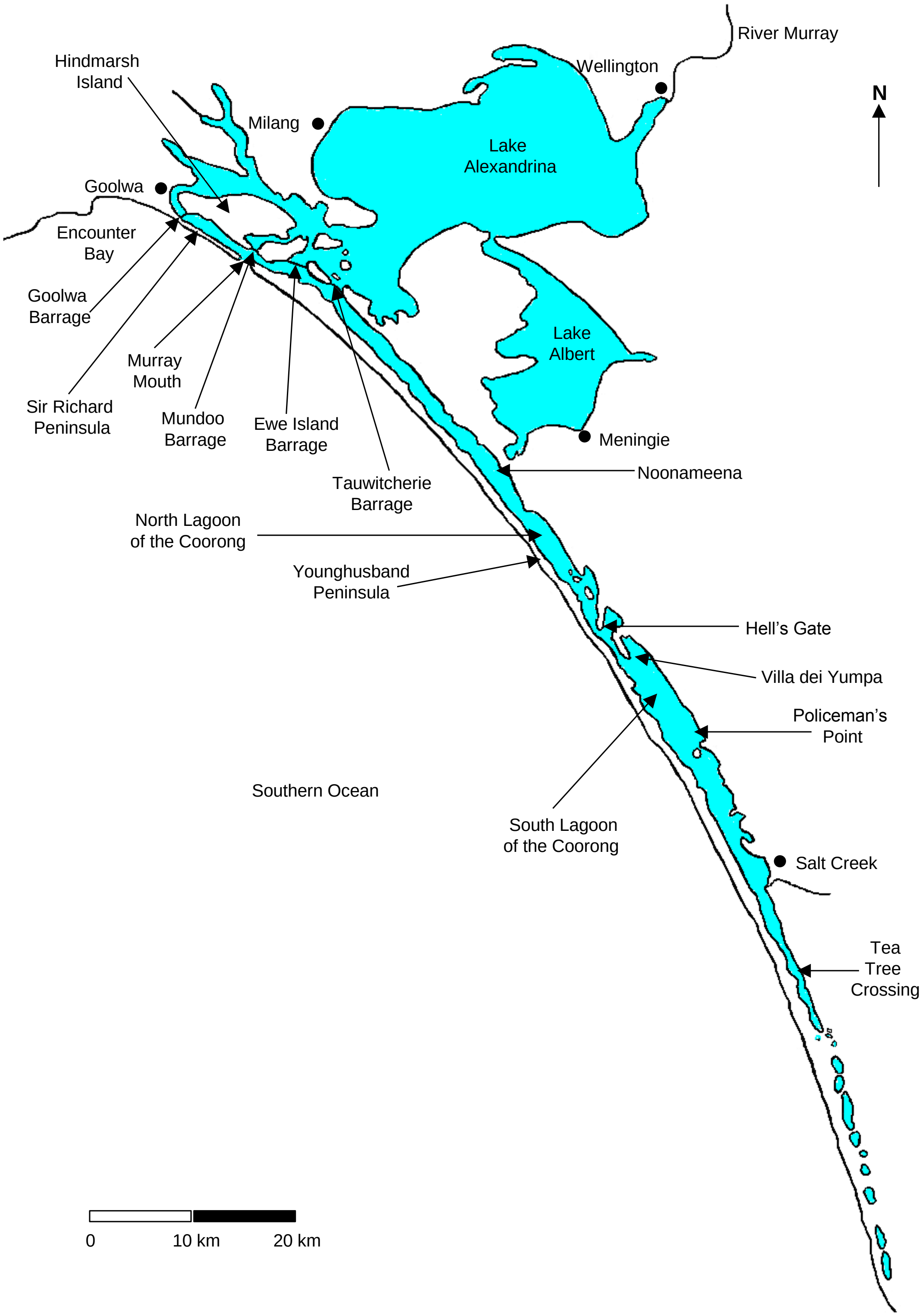

Figure 1: The Lower Lakes and Coorong.

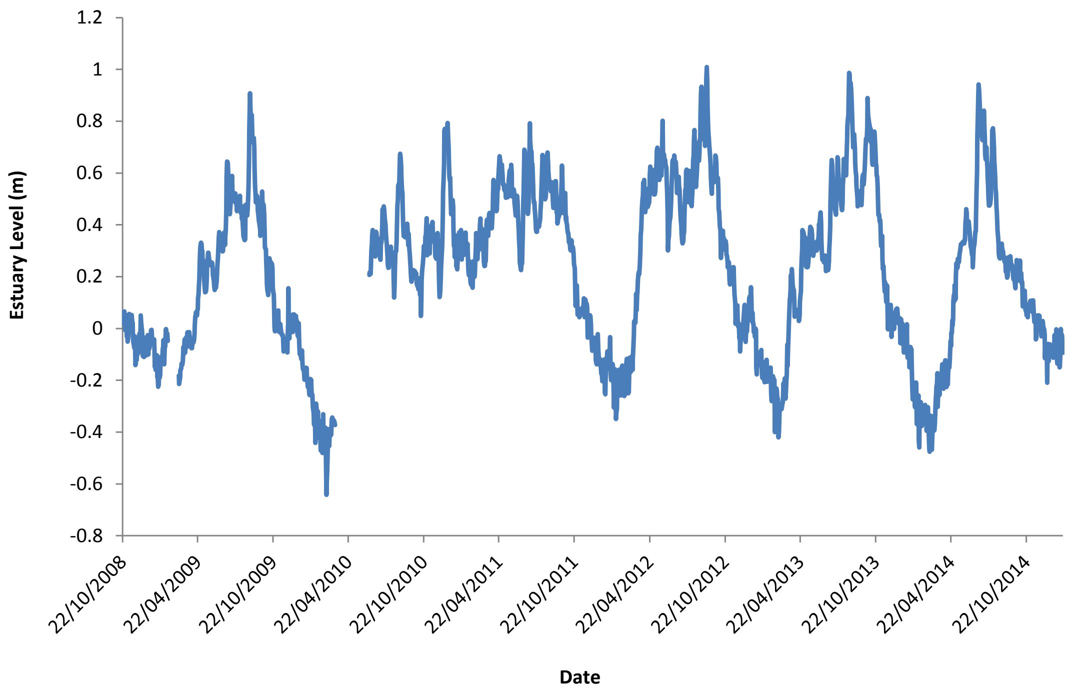

Figure 2: Mean surface water Level (m) at the Coorong NW Snipe Island station (site A4261165) in the Southern Lagoon of the Coorong from 2008-2014. Data courtesy of Water Connect (www.waterconnect.sa.gov.au).

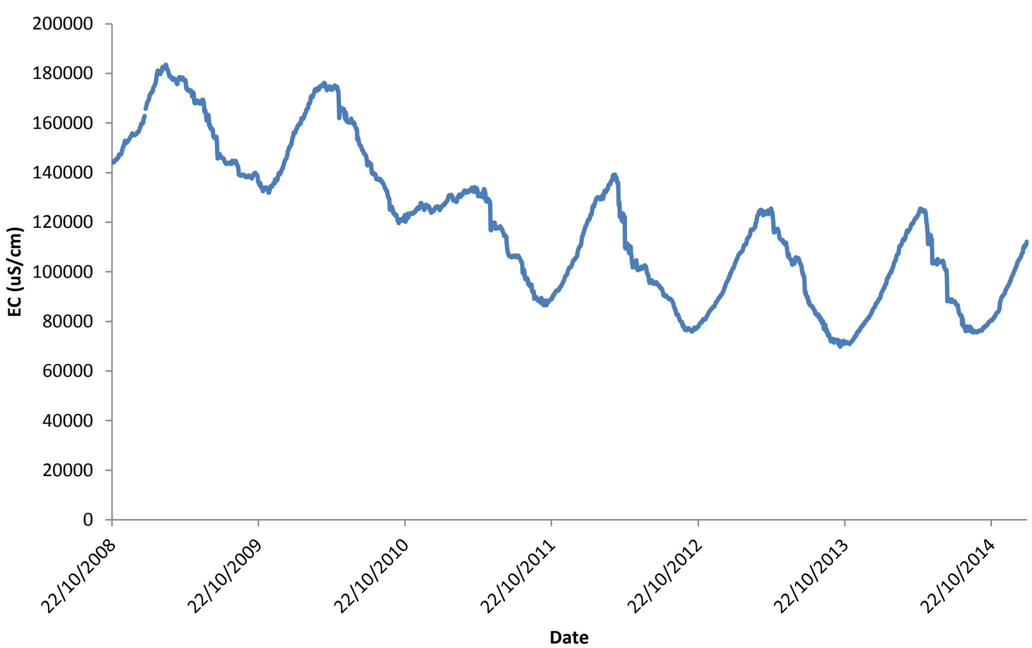

Figure 3: EC (uS cm^{-1}) recorded at the Coorong NW Snipe Island station (site A4261165) in the Southern Lagoon of the Coorong from 2008-2014. Data courtesy of Water Connect (www.waterconnect.sa.gov.au).

2.2. Sampling protocol

A total of 14 sites were established by Frahn *et al.* (2012) in December 2011 in the North and South Lagoons of the Coorong and resurveyed in December 2014 (Figure 4). The sites were determined in consultation with DEWNR, generally in areas where *Ruppia tuberosa* has been reported since the 2010/11 flood. Several sites where *Ruppia tuberosa* was historically present, but now absent were also selected to gain information about potential abiotic factors that may be unfavorable for recruitment.

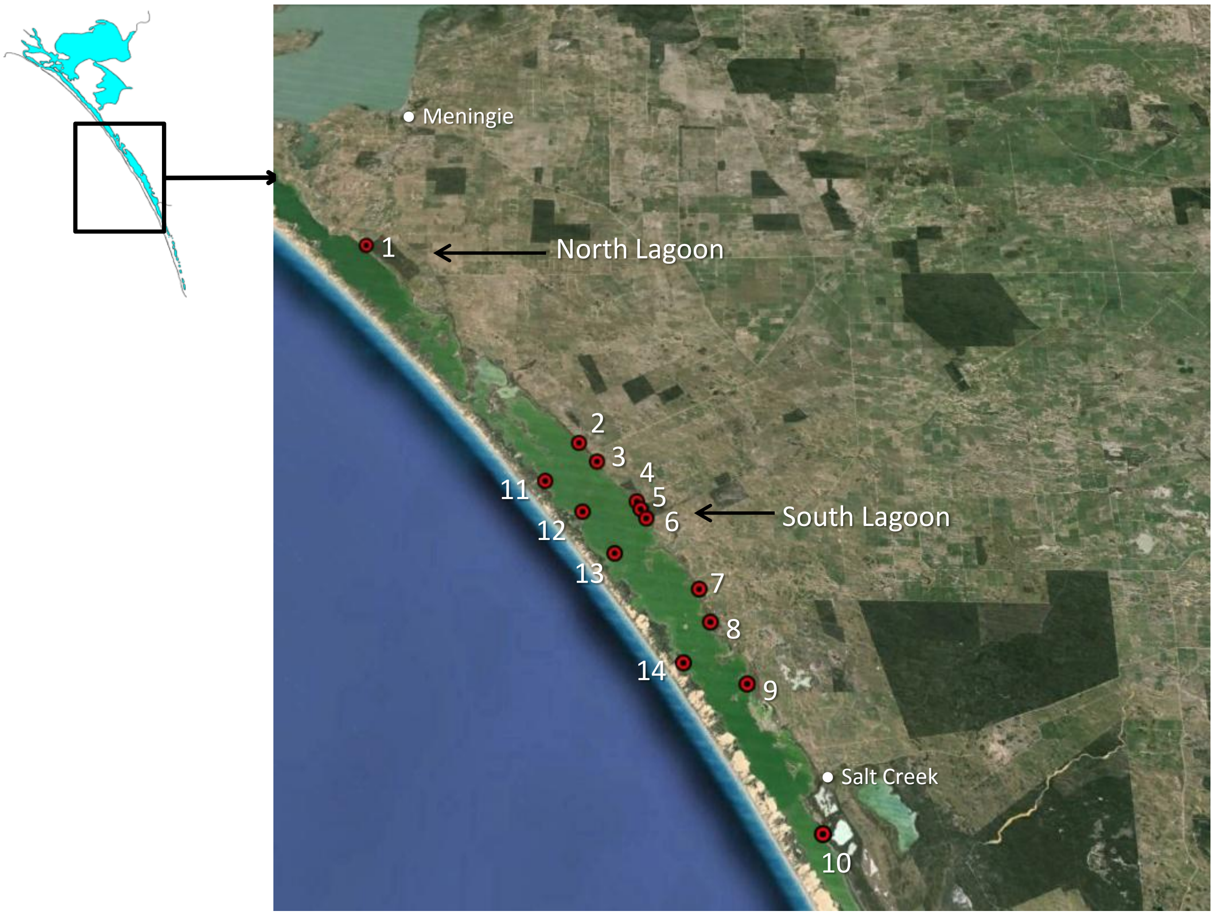

Figure 4: Aerial photo of the southern end of the North Lagoon and South Lagoon of the Coorong showing the vegetation sampling sites in relation the Lower Lakes and Coorong.

2.3. *Ruppia tuberosa* surveying protocol

Three transects, running perpendicular to the shoreline and separated by 50 m, were established at 14 sites (Figure 4). Along each transect, grab samples (total of five at each location) were taken using a small van Veen grab (i.e. area sampled per grab 12.5 x 12.5 cm; 156.25 cm^2) at water depths of 20, 40, 60, 80 and 100 cm. The same locations were sampled in December 2014 to allow for comparisons of the changes through time at each site. At each site, a visual estimate of percent cover of filamentous green algae was also made. Grab samples at each site (n = 5) were pooled and then washed through a 500 µm sieve and the number of live shoots, turions and viable seeds of *Ruppia tuberosa* were counted. All *Ruppia tuberosa* shoot and rhizome material was transported back to SARDI, Aquatic Sciences laboratories, West Beach. In addition, any filamentous algae present in the grab sample were separated from the *Ruppia tuberosa* and also collected, then transported back to SARDI, Aquatic Sciences laboratories. Shoots and rhizomes of *Ruppia tuberosa* and filamentous green algae were dried at 40ºC to a constant weight to determine biomass (dry weight, grams). At each site spot measurements of water quality (depth, salinity, pH, temperature, and turbidity) were taken with a U-50 series HORIBA multi-parameter meter.

2.4. *Ruppia tuberosa* survey data analysis

Biomass of *Ruppia tuberosa* and filamentous algae, plus the number of *Ruppia tuberosa* shoots, seeds and turions were converted to grams dry weight m^{-2}. This information was used to produce GIS layers using ArcGIS (v. 10.1). Maps were produced from the GIS layers to depict the spatial patterns of *Ruppia tuberosa* and filamentous algae biomass, shoot number and propagule bank (seed and turion) density across sites and years. Multivariate analyses were undertaken using statistical software packages PRIMER v. 6.1.15 (Clarke and Gorley 2006) and PERMANOVA+ v. 1.0.5 (Anderson 2005; Anderson *et al*. 2008). Differences in parameters such as the biomass of *Ruppia tuberosa* and filamentous algae were compared between survey times (December 2011 and 2014), sites (1 – 14 sites) and depths (20 – 100 cm) and analysed using a univariate three-factor PERMANOVA in PRIMER (Anderson 2001; Anderson and Ter Braak 2003). Similarly, changes in percent cover of filamentous algae and *Ruppia tuberosa* shoot number were analysed using a univariate three-factor PERMANOVA in PRIMER (Anderson 2001; Anderson and Ter Braak 2003). Euclidean distances were used to calculate the similarity matrices for PERMANOVA analyses and $\alpha = 0.05$ for all analyses. Multiple comparisons (where appropriate) were conducted using Bonferroni correction (Quinn and Keogh 2002).

2.5. Seed bank assessment in Lake George

A seed bank assessment of Lake George was conducted in December 2014 in Little Lake (the most southerly and shallowest basin of Lake George) (Figure 5). This assessment was used to determine Lake George's suitablity as a source site of *Ruppia tuberosa* seed for translocation purposes to the Coorong system. Surveys were also designed to assess whether there are any areas of high seed bank density (i.e. 'hot spots') present in Lake George.

Within 50 – 100 m from the shoreline in Little Lake, five transects were estalished toward the centre of the Lake (transect length ranged 450 – 600 m; Figure 5). Sediment samples (dimensions: 20 × 20 cm; 5 cm depth; weight ~3 kg) were collected every 20 - 30 m along each transect, and the sampling position was recorded using a GPS. Sediment samples were bagged immediately and placed into storage containers to minimise spread and/or contamination on site. Samples were then transported to SARDI, Aquatic Sciences laboratories, West Beach and processed by wet seiving thorugh a range of seives (2 mm, 1 mm and 500 µm) to collect viable *Ruppia tuberosa* seeds. The presence and approximate number of any other seeds present per sample was also recorded. Images of the seed species found were taken using a Zeiss AxioCam camera for stereomicroscope Discovery V12 with motorised base and processed in AxioVision software. The number of viable *Ruppia tuberosa* seeds per sediment sample were then counted and calculated to provide a measure of seed density m^{-2}. This information was then used to produce a GIS layer to determine whether there are any seed bank "hot spots" in Lake George that can be used as donor sites for translocation.

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Figure 5: Aerial photo of Little Lake (southern basin of Lake George) showing the soil collection sites.

3. RESULTS

3.1. Water quality

Water quality parameters were variable from site to site (Table 1). The salinity at sampled sites ranged from 51.5 – ≥60‰ TDS; pH from 8.37 – 8.52; temperatures from 21.1 – 27.5°C and turbidity from 9.8 to 168 NTU, with the turbidity in the North Lagoon (9.8 NTU) considerably less than values recorded in the South Lagoon (40.3 – 168 NTU). The most northern (Site 1) and southern sites (Sites 10 and 14) recorded the lowest salinity values (<60‰ TDS), while the central sites of the South Lagoon recorded the highest salinity values.

Table 1: Water quality (salinity, pH, turbidity and temperature) at each sampling site in December 2014.

Site #	Salinity ‰ (TDS)	pH	Turbidity (NTU)	Temperature
1	51.5	8.46	9.8	21.5
2	≥60	8.45	96	21.9
3	≥60	8.4	58.1	24.5
4	≥60	8.48	43.5	26.3
5	≥60	8.47	45.7	27
6	≥60	8.44	45.8	27.5
7	≥60	8.37	50.3	21.1
8	59.1	8.39	44.3	21.9
9	55.9	8.5	40.3	26.8
10	56.2	8.52	41.9	21.5
11	≥60	8.45	168	21.1
12	≥60	8.5	65.1	23.1
13	≥60	8.39	65.5	24.5
14	54.7	8.47	56.1	24.9

3.2. *Ruppia tuberosa* distribution and abundance

In 2014, mean surface estuary water levels were similar to the mean surface water levels at the time of sampling in 2011 (approximately -0.02 m; Figure 3). Three species, namely *Ruppia tuberosa*, *Ulva paradoxa* (filamentous algae) and *Lepilaena* sp. were recorded during the 2014 surveys. It was confirmed that *Lepilaena* sp. forms a close association with *Ruppia tuberosa*, making it difficult to distinguish between the two plants when no reproductive organs are present (Michelle Waycott, University of Adelaide, *pers. comm.*). Due to this close association the presence of *Lepilaena* sp. were recorded (sites 1 and 3), but their biomass was pooled with *Ruppia tuberosa*.

There was a significant difference between *Ruppia tuberosa* biomass sampled between years (Table 2), with a greater mean *Ruppia tuberosa* biomass in 2014 (9.472 ± 2.816 g m^{-2}; mean ± S.E.) t 2011 (1.528 ± 1.074 g m^{-2}; mean ± S.E). In 2011, *Ruppia tuberosa* was only present at seven out of 14 sites, but by 2014 it was present in 11 of the 14 sites, namely recorded in sites 1, 4 and 5, but still absent from sites 3, 6 and 7 (Figure 6; Figure 7). There were no significant differences in *Ruppia tuberosa* biomass detected between sites (Table 2), but in general, sites located on the western (ocean) side of the South Lagoon tended to have the higher biomass in both 2011 and 2014 (Figure 6; Figure 7). Similarly, there were no significant differences in *Ruppia tuberosa* biomass between depths, although *Ruppia tuberosa* occurred more frequently at depths of 20, 40, 60 and 80 cm in 2014, than it did in 2011 (Figure 7; Figure 6). In 2014, *Ruppia tuberosa* was present at 100 cm depth, but was absent at this depth in 2011.

A significant Year × Site × Depth interaction (Table 3) indicated that shoot number varied across years, sites and depths. In 2011, *Ruppia tuberosa* shoot number was greater at sites on the western shoreline of the South Lagoon (Sites 11 and 12; at the shallow depth of 20 cm and site 14 at 60 cm) (Figure 8; Figure 9). Similarly, in 2014, shoot number was also generally greater within sites along the western shoreline of the South Lagoon (Sites 11 – 14, inclusive) at the shallower depths ranging 20 – 60 cm (Figure 8; Figure 9).

In 2014, a greater number of propagules (19 seeds and five turions) of *Ruppia tuberosa* were recorded, whereas in 2011 only one seed was recorded. The majority of seeds and turions were present at Site 10 (most southern site in the South Lagoon), whereas only one turion was recorded at Site 8 (Figure 4).

In general, a lower *Ruppia tuberosa* biomass was also observed at sites where higher salinity (i.e. TDS ≥ 60‰) measurements were recorded (Table 1).

Table 2: Multivariate three-factor PERMANOVA results for comparing *Ruppia tuberosa* biomass between survey times (years), site locations and depths (cm), (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	df	Pseudo-F statistic	p-value
Year	1, 420	3.96	0.038
Site	13, 420	1.69	0.068
Depth	4, 420	0.32	0.9
Year × Site	13, 420	1.65	0.08
Year × Depth	4, 420	0.21	0.94
Site × Depth	52, 420	0.56	0.99
Year × Site ×Depth	52, 420	0.31	1

Table 3: Multivariate three-factor PERMANOVA results for comparing *Ruppia tuberosa* shoot number between survey times (years), site locations and depths (cm), (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	df	Pseudo-F statistic	p-value
Year	1, 420	36.71	0.001
Site	13, 420	7.34	0.001
Depth	4, 420	13.14	0.001
Year × Site	13, 420	3.64	0.001
Year × Depth	4, 420	5.46	0.001
Site × Depth	52, 420	5.29	0.001
Year × Site ×Depth	52, 420	1.89	0.001

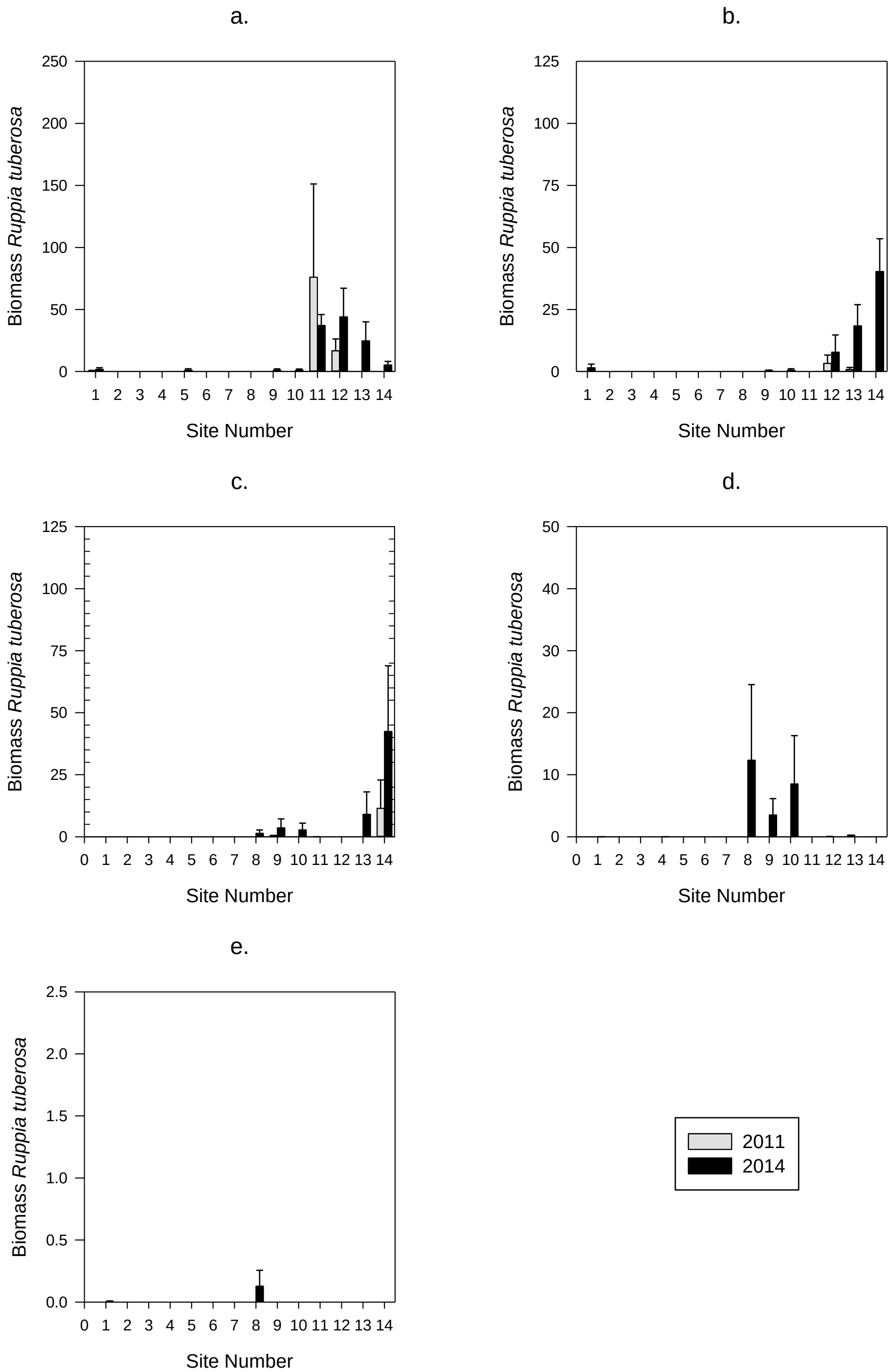

Figure 6: *Ruppia tuberosa* biomass (g dry weight m^{-2}) at each site in December 2011 and 2014 at a. 20 cm, b. 40 cm, c. 60 cm , d. 80 cm and e. 100 cm (error bars =+1 S.E.).

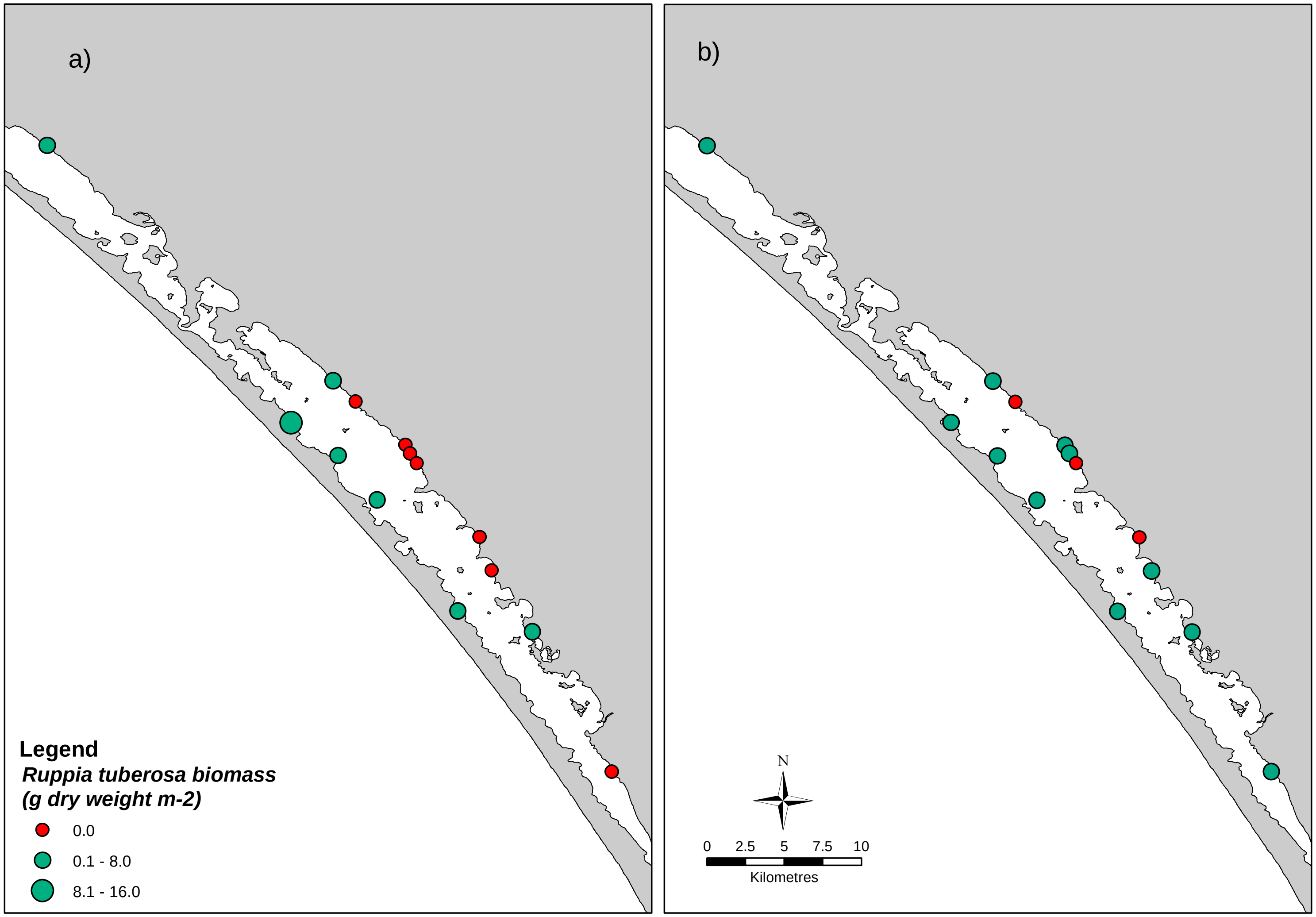

Figure 7: Map of the Coorong showing mean *Ruppia tuberosa* biomass (g dry weight m^{-2} for all depths) at each site in a) 2011 and b) 2014. Red dots symbolise where *Ruppia tuberosa* was not recorded.

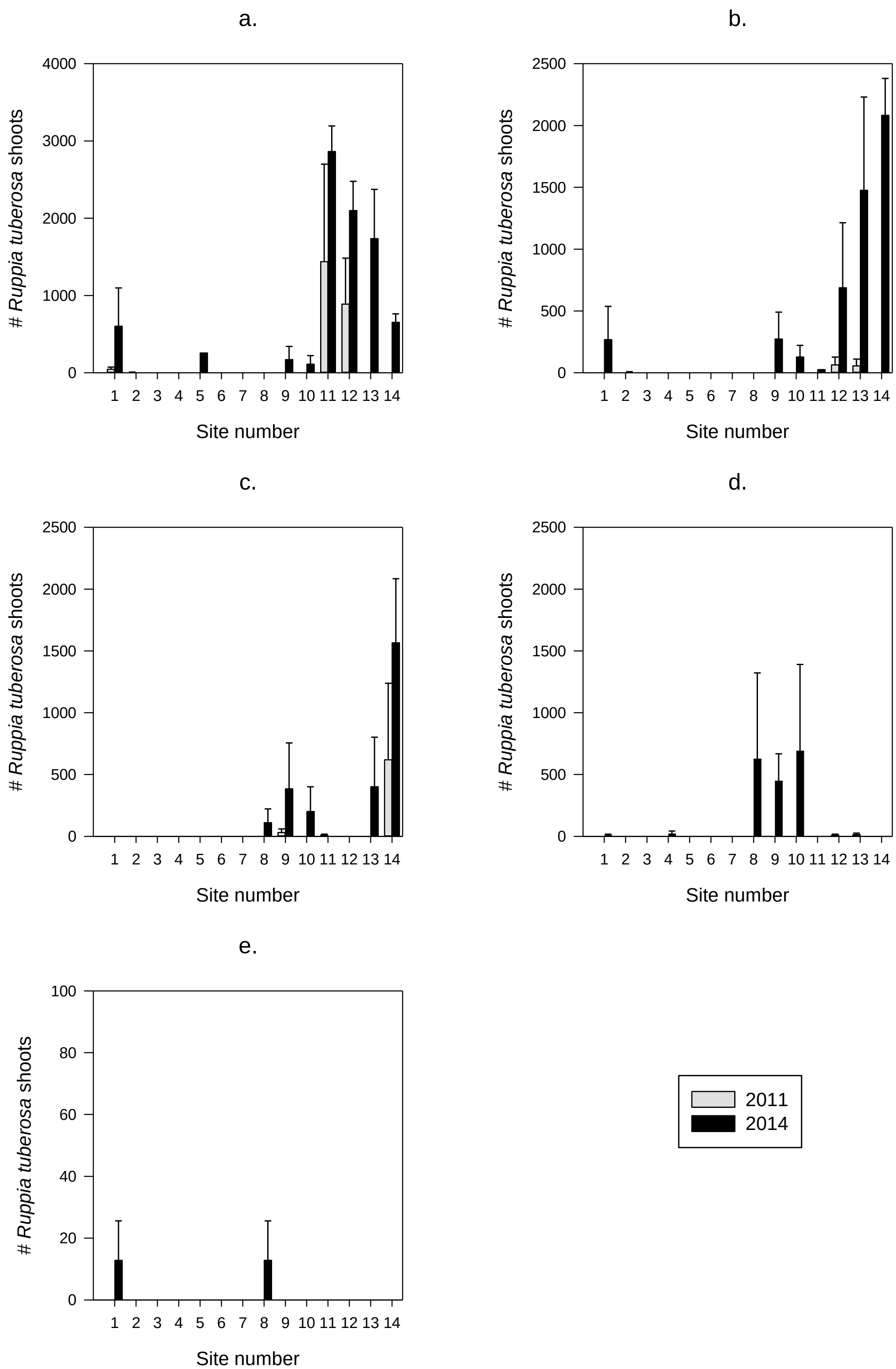

Figure 8: *Ruppia tuberosa* shoot abundance (no. shoots m^{-2}) at each site in December 2011 and 2014 at depths a. 20 cm, b. 40 cm, c. 60 cm, d. 80 cm and e. 100 cm (error bars =+1 S.E.).

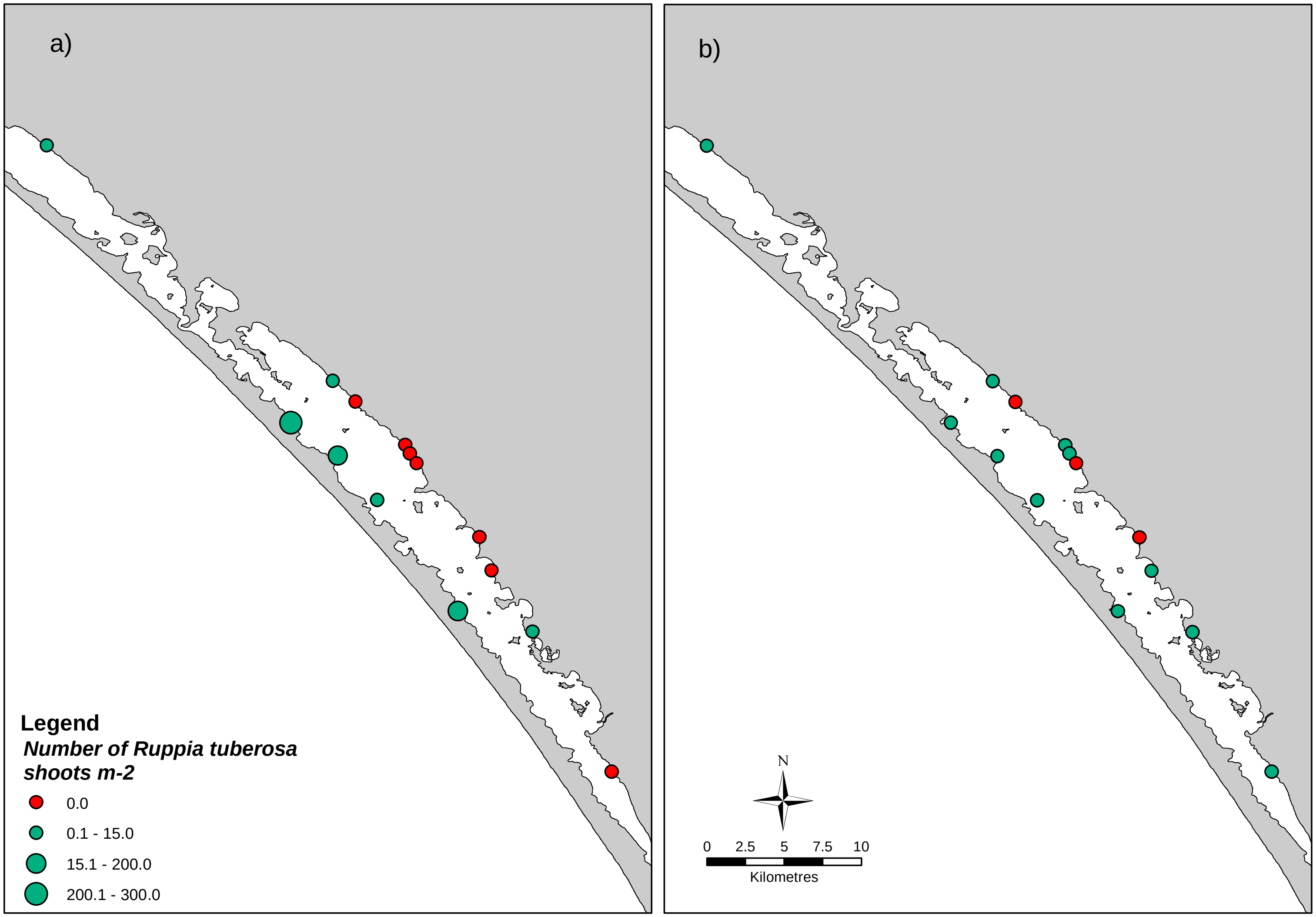

Figure 9: Map of the Coorong showing mean *Ruppia tuberosa* shoot abundance (shoots m^{-2} for all depths) at each site in a) 2011 and b) 2014. Red dots symbolise where *Ruppia tuberosa* was not recorded.

3.3. Filamentous algae distribution and abundance

A significant Year × Site interaction (Table 4) indicates that filamentous algae (*Ulva paradoxa*) varied between sites and years (Figure 10; Figure 11). There was a greater mean of filamentous algae biomass in 2011 (10.88 ± 3.62 g m^{-2}; mean ± S.E) than 2014 (0.98 ± 0.376 g m^{-2}; mean ± S.E.). In 2011, *Ulva paradoxa* was present in 11 out of 14 sites surveyed in both 2011 and 2014, but its presence within certain sites varied between years (Figure 7: Figure 11). Similarly, significant Year × Site and Site × Depth interactions (Table 5) indicate percent cover of *Ulva paradoxa* was not consistent across site and depth. In 2011, percent cover of *Ulva paradoxa* was greatest (up to 40% cover) at the southern end of the North Lagoon (Site 1) and Site 14 on the western shoreline of the South Lagoon at depths ranging 20 – 80 cm (Figure 12: Figure 13), however in 2014, the greatest percent cover of *Ulva paradoxa* was more widespread across the South Lagoon and at shallower depths, ranging 20 – 40 cm (Figure 12: Figure 13).

Table 4: Multivariate three-factor PERMANOVA results for comparing filamentous algae (*Ulva paradoxa*) biomass between survey times (years), site locations and depths (cm), (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	**df**	**Pseudo-F statistic**	**p-value**
Year	1, 420	9.90	0.003
Site	13, 420	7.55	0.001
Depth	4, 420	1.04	0.40
Year × Site	13, 420	0.001	0.001
Year × Depth	4, 420	0.52	0.44
Site × Depth	52, 420	0.90	0.79
Year × Site ×Depth	52, 420	0.91	0.83

Table 5: Multivariate three-factor PERMANOVA results for comparing filamentous algae (*Ulva paradoxa*) percent cover between survey times (years), site locations and depths (cm), (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	**df**	**Pseudo-F statistic**	**p-value**
Year	1, 420	5.89	0.021
Site	13, 420	7.57	0.001
Depth	4, 420	2.55	0.041
Year × Site	13, 420	5.18	0.001
Year × Depth	4, 420	2.29	0.76
Site × Depth	52, 420	1.72	0.002
Year × Site ×Depth	52, 420	1.004	0.46

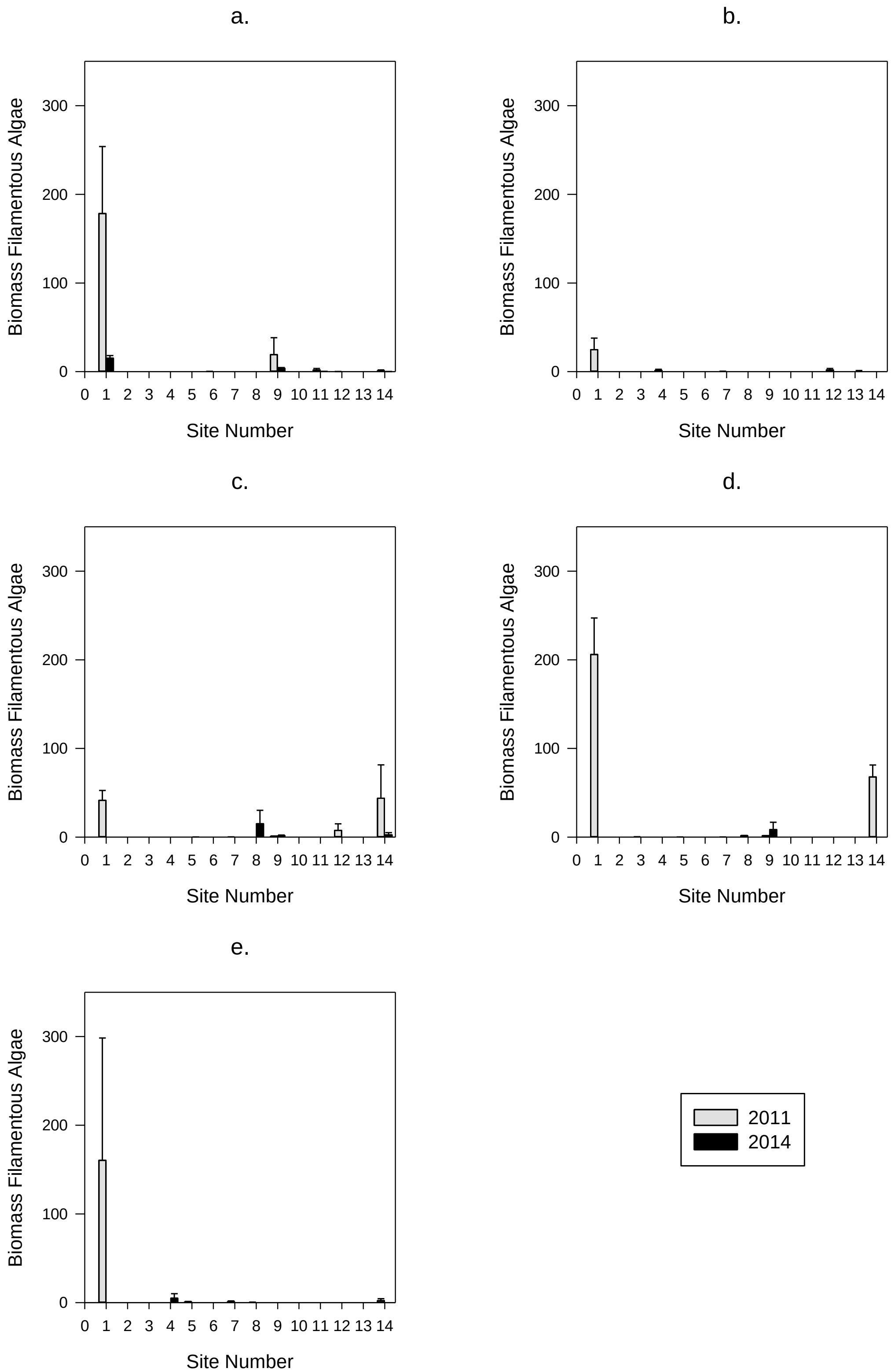

Figure 10: Biomass of filamentous algae, *Ulva paradoxa* (g dry weight m^{-2}) at each site in December 2011 and 2014 at depths a. 20 cm, b. 40 cm, c. 60 cm, d. 80 cm and e. 100 cm (error bars =+1 S.E.).

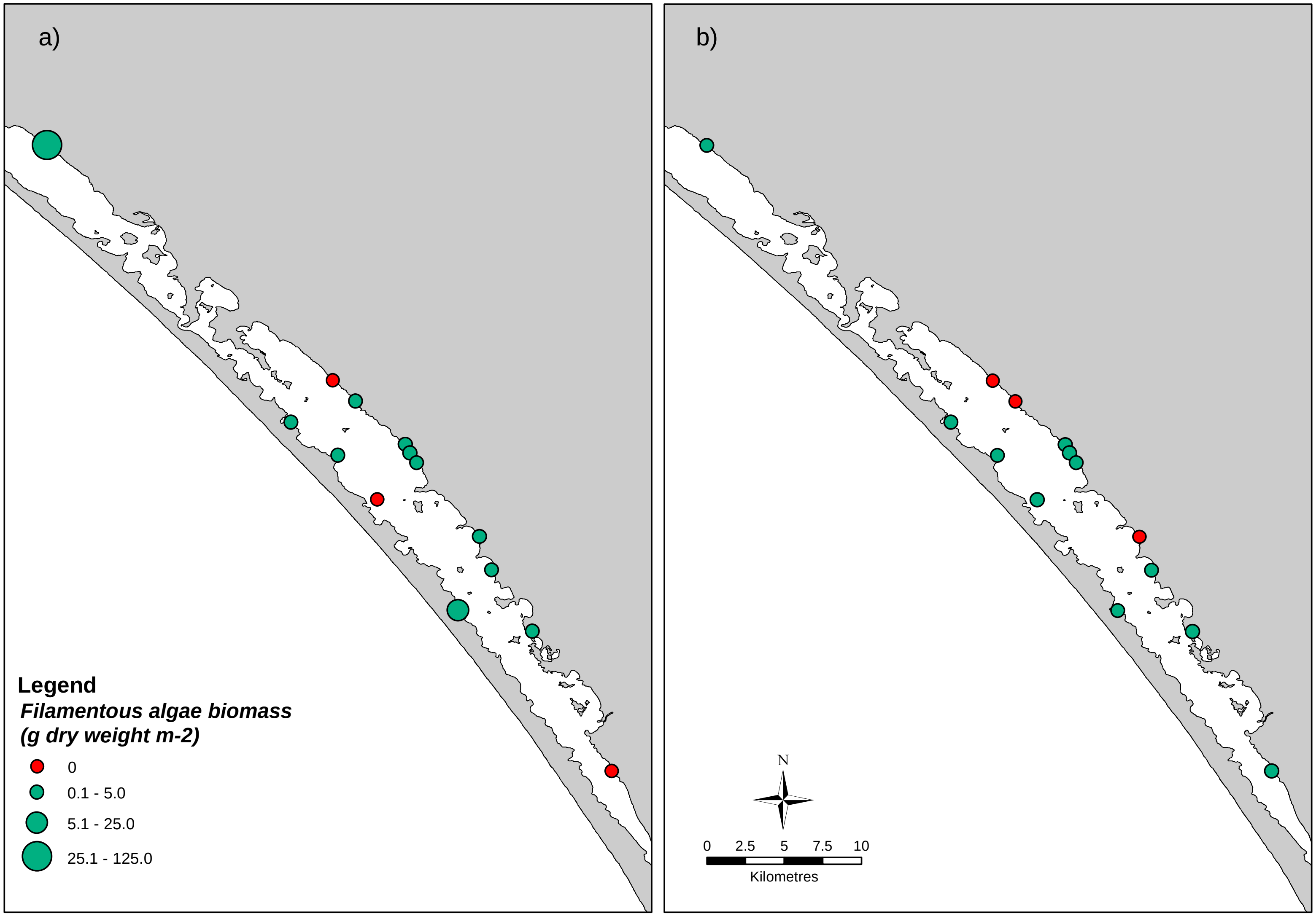

Figure 11: Map of the Coorong showing mean biomass of filamentous algae, *Ulva paradoxa* (g dry weight m^{-2}) for all depths, at each site in a) 2011 and b) 2014. Red dots symbolise where filamentous algae was not recorded.

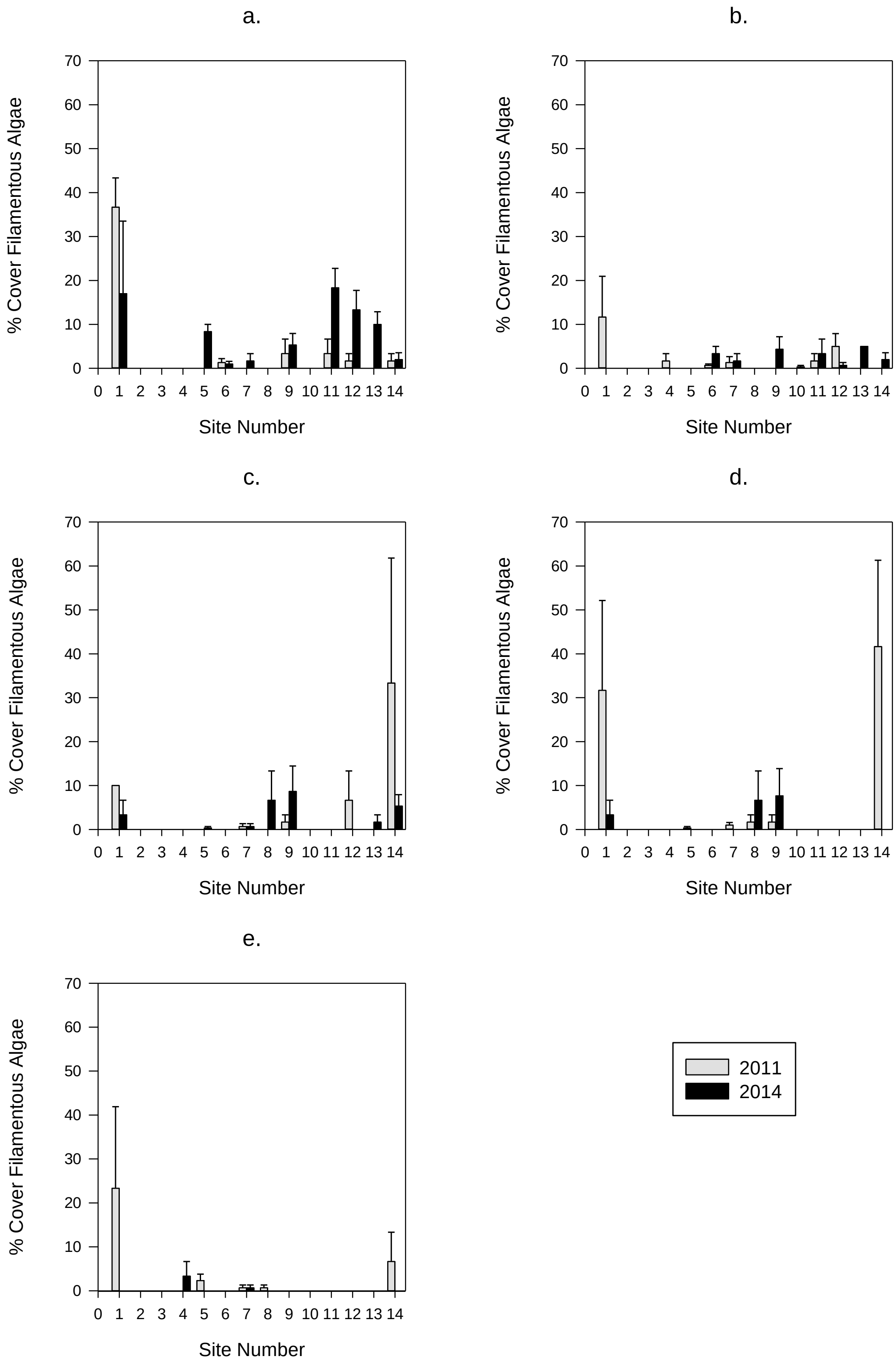

Figure 12: Percent cover of filamentous algae (*Ulva paradoxa*) at each site in December 2011 and 2014 at depths a. 20 cm, b. 40 cm, c. 60 cm , d. 80 cm and e. 100 cm (error bars =+1 S.E.).

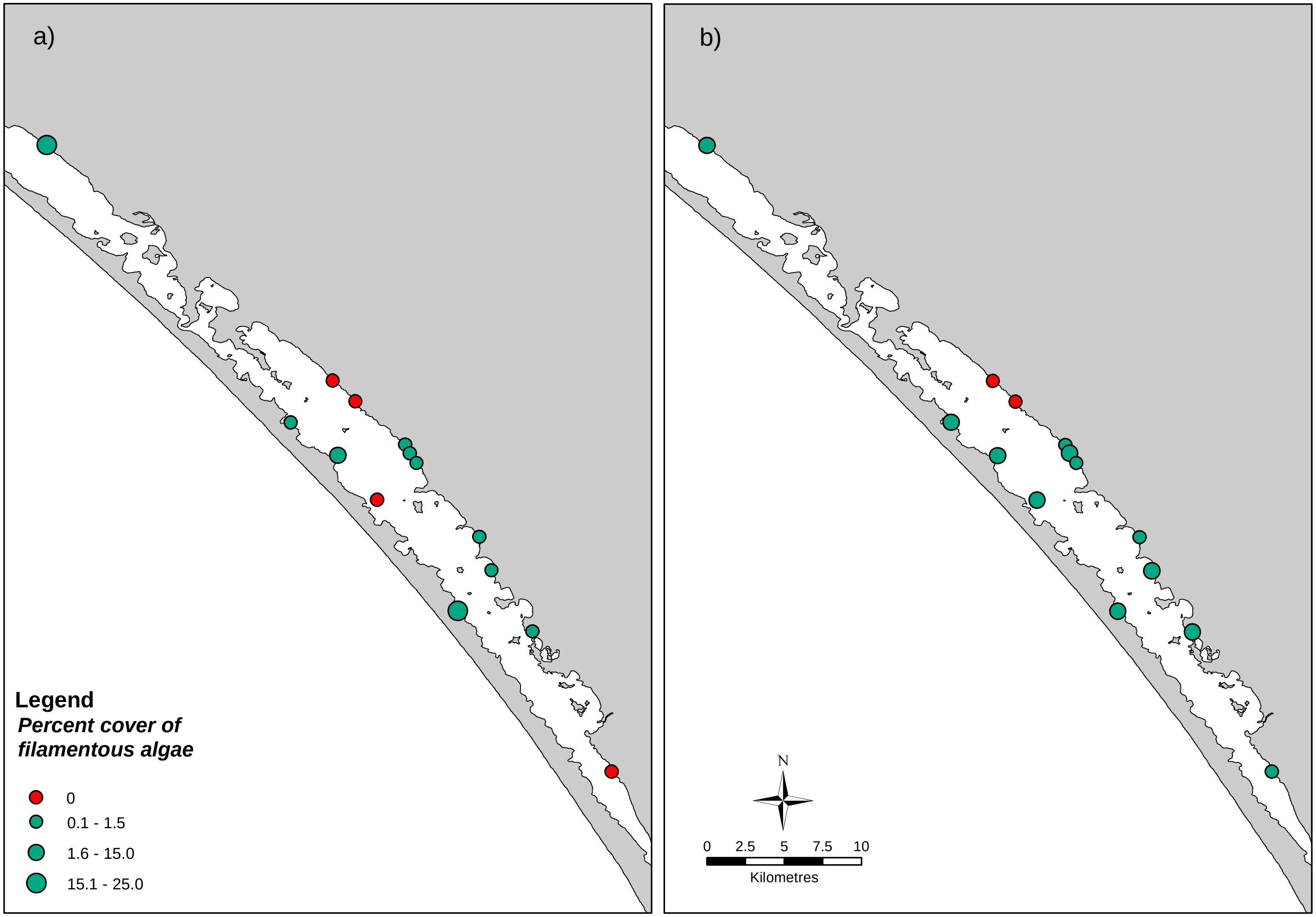

Figure 13: Map of the Coorong showing mean percent cover of filamentous algae, *Ulva paradoxa* for all depths, at each site in a) 2011 and b) 2014. Red dots symbolise where filamentous algae was not recorded.

3.4. Ruppia tuberosa seed bank assessment at Lake George

Three species of seeds; *Ruppia tuberosa*, *Ruppia megacarpa* and *Lepileana* sp. were present in the Little Lake (southern basin of Lake George) sediment samples (Figure 14). The number of *Ruppia tuberosa* seeds in the sediment samples ranged from 0 – 2325 seeds m^{-2}, whereas estimates of the number of *Lepilaena* sp. seeds ranged from 50 – 3600+ seeds m^{-2}. Transects 3 and 5 had the greatest mean density (m^{-2}) of *Ruppia tuberosa* seed (Table 6); however there were spots within Transects 1, 2 and 3 that had the greatest number of seeds per sediment sample (range: 1350 – 2325 *Ruppia tuberosa* seed m^{-2}) (Figure 15).

Table 6: Mean density (m^{-2}) of *Ruppia tuberosa* seeds per transect within Little Lake (southern basin of Lake George).

Transect #	# *Ruppia tuberosa* seeds m^{-2} (mean ± S.E.)
T1	189 ± 73.5
T2	313 ± 78.71
T3	417 ± 111.17
T4	315.22 ± 68.09
T5	398.86 ± 50.17

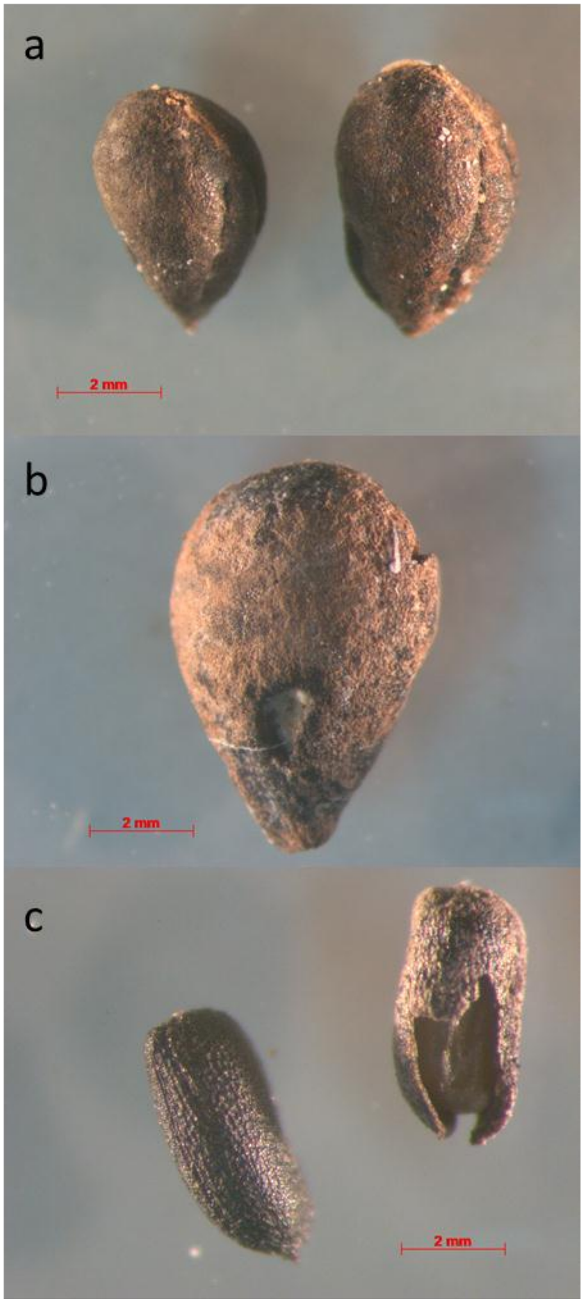

Figure 14: Stereoimage of a) *Ruppia tuberosa*, b) *Ruppia megacarpa* and c) *Lepilaena* sp. seeds found in Little Lake (south basin of Lake George) sediment samples (Little Lake, South Australia) using Zeiss AxioCam camera for stereomicroscope Discovery V12 with motorised base. Images processed in AxioVision software.

Figure 15: Map of Little Lake (southern basin of Lake George) showing *Ruppia tuberosa* seed density per m^2 per sample area along each of the five (T1 -T5) transects sampled. White circles symbolise where no *Ruppia tuberosa* seeds were found.

4. DISCUSSION

There was a slightly higher abundance of *Ruppia tuberosa* present in the southern Coorong in 2014 compared to the previous survey in 2011; where *Ruppia tuberosa* tended to be more widespread; as opposed to larger, more dispersed patches in 2011. In particular, *Ruppia tuberosa* was recorded in the southern most location of the South Lagoon, (south of the Salt Creek outlet), where it was not observed in 2011. *Ruppia tuberosa* was also sampled across a wider range of depths in 2014 than the 2011 survey. The increase in distribution and abundance between surveys indicates a level of successful recruitment and establishment. Prior to the drought; however, *Ruppia tuberosa* distribution and abundance was highly variable across the South Lagoon, but Paton (2000) reported mean densities of *Ruppia tuberosa* shoots as high as 6000 per m^{-2} in the northern most section of the South Lagoon in July 1999. *Ruppia tuberosa* is an annual species, which grows in early winter or late autumn, producing seeds and/or turions during spring and early summer (September to December in Australia) (Brock 1982); hence the observation of mean shoot densities <3500 m^{-2} in the South Lagoon in this study in December 2014 suggests re-establishment and recruitment continues to be limited. While this study detected significant differences in *Ruppia tuberosa* abundance between survey years, the low shoot numbers and abundances compared to those reported prior to the drought suggest recovery is not, as yet, biologically significant.

To some extent the patchy distribution may be due to difficulty of designing a sampling protocol that allows adequate replication across the extensive region for an adequate assessment of change (Paton 2000). The historically patchy and clumped distribution of *Ruppia tuberosa*; however, is in part also due to differences in sediment texture and/or the chemistry within the system (Brookes *et al.* 2009). For instance, the highest abundance and biomass of *Ruppia tuberosa* was on the western shore of the South Lagoon in both the 2011 and 2014 survey. The persistence of this particular population on the western shoreline suggests that it may be a perennial population, where sediments on this shore are generally coarser and the substrate is less rocky than the eastern side. The evidence of whether *Ruppia* species prefers coarser sediment is conflicting, but this species was historically abundant in areas with fine sediments (Nicol 2005).

This study also found the highest abundances of *Ruppia tuberosa* at sites with lower salinities and a lack of presence in sites with the highest salinity levels, which may also drive the patchy distribution and abundance. *Ruppia tuberosa* is one of the most salt tolerant angiosperms with a maximum salinity tolerance of 230 g L^{-1} for adult plants (Brock 1982a; determined under controlled greenhouse conditions) with a preferred salinity range for *Ruppia tuberosa* in the Coorong between 40 – 80‰ TDS (Whipp 2010). Brock (1982b) noted that at elevated salinities *Ruppia tuberosa* did not flower and was restricted to reproducing asexually; therefore, lower salinities are required for the production of seed and subsequent replenishing of the sediment propagule bank. Much lower salinities are required for life cycle completion where Kim *et al.* (2013) reported that salinities lower than 85 g L^{-1} for 15 days are required for germination from seeds and 125 g L^{-1} for sprouting from turions. Exposure to elevated salinity, followed by lower salinity, stimulated germination in seeds, but reduced viability of turions by over 90% (Kim *et al.* 2013). It may be that the *Ruppia tuberosa* populations present in deeper water on the western shore of the South Lagoon predominantly reproduce asexually, hence their persistence. Alternatively populations on the eastern shoreline grow in shallower water and are reliant on sexual reproduction for persistence; therefore dispersal may be sporadic due to a depleted propagule bank.

Modelling of salinity levels in the Coorong system by Kim *et al.* (2013) suggests that seed germination of *Ruppia tuberosa* is likely to be restricted to less saline areas, such as the northern half of the Coorong. In this study, there was an observed trend towards a higher abundance of *Ruppia tuberosa* at the most southern sites in the South Lagoon (Sites 8, 9, 10 and 14), which had lower salinity levels (< 55‰ TDS) during the 2014 surveys. There were also a total of 19 seeds and five turions recorded in the 2014 survey at 2 sites in close proximity to the Salt Creek Outlet. The lower salinities in this region may be due to inputs of freshwater into the Coorong South Lagoon (Salt Creek outlet) from the South East drainage scheme (SE Flows Restoration Project). Monthly salinity (EC) values from the salt creek outlet highlight that water at this site is much fresher (0 – <35 000 µS cm^{-1}) (https://waterconnect.sa.gov.au; Salt Creek Outlet station A2390568) than measurements made upstream of the outlet at Snipe Island (65, 000 – 180, 000 µS cm^{-1}; Figure 3); however, the influence of this incoming water on salinity (and other physio-chemical properties) within the South Lagoon are as yet unknown and require further investigation.

Flower abundance and seed density are also influenced by water depth (Kim *et al.* 2013). *Ruppia tuberosa* is highly sensitive to desiccation but has high light requirements; therefore it is believed there is only a narrow band where the species can occur in the highly turbid South Lagoon (Nicol 2005). *Ruppia tuberosa* colonises areas between 0 and -0.5 m AHD in May to June in the South Lagoon; areas below -0.5 m AHD are below the euphotic zone and areas above 0 m AHD are prone to desiccation due to wind driven water level fluctuations (seiching) (Nicol 2005). These water levels need to be maintained until at least mid-November, preferably mid to late December, to ensure the life cycle is completed and the seed bank replenished. Nevertheless, the early water level drawdown associated with reduced barrage outflows in recent years means that many of the mudflats that were previously considered to be good *Ruppia* habitat are now exposed before plants have a chance to mature and produce seeds or turions can germinate (Kim *et al.* 2013).

Shoot numbers and abundance of flowers may also be affected by the presence of filamentous algae, which can outcompete *Ruppia tuberosa* by smothering flowering stalks (causing them to detach) and limit seed production (Paton 1996). The observed increase in *Ruppia tuberosa* abundance may partially be due to lower filamentous algae abundance. Furthermore *Ruppia tuberosa* establishment across a greater range of depths in 2014 may lower its risk of desiccation and provide opportunities to replenish the propagule bank.

The suitablity of using Lake George as an alternative translocation source site of *Ruppia tuberosa* seeds for the Coorong system was also investigated. Lake George is intermittently open to the sea and consequently salinities and water levels fluctuate, and high temperatures and low dissolved oxygen levels are prevalent during the summer months (Department for Environment Heritage and Aboriginal Affairs 1997). Lake George typically has low species richness of submergent plants; however, *Ruppia tuberosa* has been recorded in recent years (J. Nicol, *pers. comm*). In our study, *Ruppia tuberosa* seeds were present in the sediment samples (0 – 2325 seeds m^{-2}), but *Lepilaena* sp. seeds were the most abundant (~50 – 3500 seeds m^{-2}). As *Lepilaena* sp. was found growing in close association with *Ruppia tuberosa* samples in the Coorong South Lagoon, this would not exclude the use of sediment from this system and no other seeds, besides one *Ruppia megacarpa* seed were recorded. Little Lake is the first basin of Lake George to dry out; hence it is often subject to increased public access, so the risk of introducing pest species through translocation needs to be taken into consideration. Nonetheless, the mean density of viable seeds in Lake George sediments was markedly less than the number of viable seeds found within hot spots in Lake Cantara (~4500 seeds m^{-2}), which means at this time it is potentially unsuitable as a source site.

Overall, the results suggest *Ruppia tuberosa* populations are increasing since the 2010/11 flood; albeit at a slow rate. Continued monitoring of existing populations and the development of additional investigations are required to help determine the factors that are limiting establishment and recruitment of *Ruppia tuberosa*. In addition, the density of viable *Ruppia tuberosa* seeds in Lake George sediments is low (~1500 seeds m^{-2}), which, along with other factors, such as high public access and possibility of contaminants means it is probably unsuitable as a translocation donor site.

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