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Water quality influences on the distribution of *Ruppia tuberosa* in the Coorong, South Australia

A pilot study using multivariate analyses of the Coorong water quality monitoring data

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Executive summary

The submerged aquatic macrophyte, *Ruppia tuberosa* plays a central role in the Coorong ecosystem by providing physical habitat for a range of aquatic organisms from epiphytes to fish; by modifying the physicochemical environment through its effects on water quality and water movement; and as a direct source of food for a number of water birds (Rogers and Paton 2009). Historically the distribution of *R. tuberosa* was restricted to the South Lagoon of the Coorong where the hypersaline conditions were suited to its growth. In more recent times significant changes to the surface hydrology of the region, largely as a result of increased regulation of flows in the River Murray, have impacted on the water exchange, seasonal water level patterns, and water quality within the Coorong (Brookes et al 2009). These changes were exacerbated during the prolonged Millennium Drought (2000 to 2010) causing major shifts in the water quality and microalgae composition of the Coorong (Oliver et al 2014).

These changing conditions also influenced the abundance and distribution of *R. tuberosa* in the Coorong, and this was monitored at a set of sites over the period 1998 to 2011 (Paton and Bailey 2013; 2014). *R. tuberosa* progressively declined from the southern end of the South Lagoon northwards so that by July 2008 no plants were detected growing in the South Lagoon. However, during this time beds of *R. tuberosa* became established in the North Lagoon where, as freshwater flows declined, salinity increased (Paton and Bailey 2013; 2014).

The water quality monitoring data that had been collected from the Coorong, Lower Lakes and Murray Mouth region for many years was recently collated and multivariate analyses used to describe changes across sites and times (Oliver et al 2013; 2014). The collated data set provided the opportunity to investigate influences of changing water quality on other biota for which monitoring data was available. This pilot study was undertaken to investigate whether the application of multivariate statistical analyses to data from the Coorong monitoring programs could help identify major changes in water quality and water depth that influenced the abundance and distribution of *R. tuberosa*.

Multivariate analyses were undertaken using the software programs Primer 6 and PERMANOVA+ (Anderson, Gorley & Clarke 2008). Results are largely shown graphically and in the nMDS and PCA plots points that are closer together are more similar to each other while points increasingly further apart are more dissimilar. The statistical significance of differences between points was determined using PERMANOVA.

R. tuberosa sampling sites included in the analyses were Noonameena, Villa de Yumpa, Policeman's Point, Salt Creek and Teatree Crossing (Figure 1). These extended from the southern end of the Coorong to half way along the Northern Coorong and provided a representative spatial coverage, although the majority of sites were in the Southern Coorong (Figure 2). *R. tuberosa* shoot density measured in core samples (shoots per core) was used in the analyses. Regular water quality monitoring stations were situated at or near to each of the *R. tuberosa* sites. In this study the water quality data set was restricted to: temperature, water level, pH, conductivity (EC), Total Iron, Ammonia, nitrogen oxides (NO_X), Total Kjeldahl Nitrogen (TKN), Total Nitrogen (TN), Total Inorganic Nitrogen (TIN), Total Organic Nitrogen (TON), Filterable Reactive Phosphorus (FRP) and Total Phosphorus (TP), as these parameters provided the longest times series for analyses (Figure 3).

Multivariate analyses successfully identified changing water quality conditions that were correlated with changes in *R. tuberosa* shoot density. These were the same associations proposed by other researchers providing support for the analyses. Conductivity was strongly correlated with *R. tuberosa* shoot density at Noonameena (Figure 6), Villa de Yumpa (Figure 7) and Policeman's Point (Figure 8). Phosphorus concentrations were also correlated with *R. tuberosa* shoot density at these three sites but were more strongly associated with the *R. tuberosa* at Salt Creek (Figure 9) and Teatree Crossing (Figure 10). Also water depth was significantly associated with *R. tuberosa* shoot density at Teatree Crossing (Figure 10), and pH was associated with the *R. tuberosa* density at Villa de Yumpa (Figure 7).

At each of the sites more than one water quality attribute correlated strongly with the changes in *R. tuberosa* and interpretation was further complicated by correlations between the water quality parameters themselves (Table 1). Statistical analyses alone could not distinguish the direct influences of these attributes on *R. tuberosa* shoot density and further analyses of the data attempted to identify "natural" experiments where separate fluctuations in the individual water quality parameters over time might assist in delineating their individual influences. Interpretation also included expert knowledge to assess whether water quality changes were of a magnitude expected to be influential, and consideration of consistencies in responses across sites and times that supported the proposed relationships.

At Noonameena, Villa de Yumpa and Policeman's Point, average conductivity between January and July was significantly correlated with the changes in *R. tuberosa* shoot density. The average conductivity was a consistent indicator of unsuitable conditions across sites and times, increasing the confidence in its role, and enabling identification of the concentration ranges affecting *R. tuberosa* shoot density (Figure 11). The apparent range of suitable conductivities was between 60,000 and 125,000 µS cm^{-1} (salinities of 44.5 to 106.5 g L^{-1}) with shoot densities decreasing when conductivity increased or decreased outside this range. This is significantly less than the published salinity range for *R. tuberosa* of 12 to 230 g L^{-1} (Brock and Lane 1983; Boon and Morris 2002) which is likely based on spot observations of *R. tuberosa* plants occurring at their salinity extremes where their growth and reproductive condition is uncertain.

Changes in pH at Villa de Yumpa were associated with changes in *R. tuberosa* shoot density (Figure 7). Inspection of pH monitoring data from the South Lagoon showed that it varied between 7.8 and 8.4 over this time, but had varied within this range for many years. From a general understanding of pH impacts on submerged macrophytes, and the similarity of the pH range over times of high and low *R. tuberosa* numbers, the direct influence of pH on *R. tuberosa* shoot density was not considered major.

Phosphorous, either as FRP or TP was strongly correlated with the *R. tuberosa* changes at most of the sites. It has been proposed that this is due to increased phosphorus supporting the growth of attached algae that smother the *R. tuberosa* (Paton and Bailey 2013; 2014). If this was the case then microalgae chlorophyll concentrations might be expected to increase, reflecting this connection. The water quality data is available to investigate this link but was not included in the current project.

Water depth was identified as influencing *R. tuberosa* shoot density at Salt Creek and Teatree Crossing, but the monitored vertical depth data was not considered to correspond well with changes in the shallow water regions where *R. tuberosa* grows as this depends on shoreline bathymetry. Consequently, the direct influence of depth could not be readily assessed. With further analyses the measured depths could be connected to shallow water areas using the Coorong bathymetric map, but was beyond the scope of this project.

Further exploration of the data, especially with respect to pH, FRP, water depth and other correlates, is possible and is recommended as a useful way forward to further identify the environmental influences on *R. tuberosa* in the Coorong. These analyses should be regularly updated as monitoring continues.

The analyses used only the shoot density data from the monitoring program as time did not allow investigation of the other measurements, including the numbers of *R. tuberosa* turions and seeds. It would be worthwhile incorporating these in future analyses to provide a more complete assessment of the lifecycle of the plant.

The water quality and water depth characteristics of the Coorong are closely linked to the interactions between barrage flow regimes and connectedness to the ocean through the River Murray mouth. There are models available that describe these links (eg. Webster 2007) and it would be very beneficial to use these models in conjunction with the multivariate analyses to develop a dynamic model of the responses of *R. tuberosa* to conditions generated by the changing flow regimes. Linking flows, water depths, water quality and *R. tuberosa* distribution and abundance would provide managers of the Coorong with a powerful tool to assess the environmental flow requirements and the likely responses to changing water allocations.

Recommendations for progressing these studies are presented in Section 5.

1 Introduction

In 1998 a monitoring program was established in the Coorong to measure changes in the distribution and abundance of *R. tuberosa* (Rogers and Paton 2009; Paton and Bailey 2013; 2014). This submerged aquatic macrophyte is considered to play a central role in the Coorong ecosystem by providing physical habitat for a range of aquatic organisms from epiphytes to fish, by modifying the physicochemical environment through its effects on water quality and water movement, and as a direct source of food for a number of water birds (Rogers and Paton 2009). Historically the distribution of *R. tuberosa* was restricted to the South Lagoon of the Coorong where the hypersaline conditions were suited to its growth. In more recent times significant changes to the surface hydrology of the region, largely as a result of increased regulation of flows in the River Murray, have impacted on the hydrology and water quality of the Coorong. In particular these changes have altered the seasonal water level patterns within the Coorong and resulted in particularly high salinities (Brookes et al 2009). The largest changes in conditions were associated with the prolonged Millennium Drought (2000-2010) with impacts evident in the water quality and microalgae composition of the Coorong (Oliver et al 2014). Over this period the *R. tuberosa* monitoring program documented significant changes in the distribution and abundance of the plant which progressively declined from the southern end of the South Lagoon northwards so that by July 2008 no plants were detected growing in the South Lagoon (Paton and Bailey 2013; 2014). However, beds of *R. tuberosa* became established in parts of the North Lagoon where salinity had increased above sea water concentrations as freshwater flows declined (Paton and Bailey 2013; 2014).

The monitoring program instituted by Prof. Paton and his team provides an invaluable data set that describes the changes in plant distribution in relation to changing environmental conditions (Rogers and Paton 2009; Paton and Bailey 2013; 2014). Many water quality parameters change in unison during periods of altered flows but to date there has not been an attempt to assess more broadly the relative influence of possible drivers of the changes in *R. tuberosa* abundance. The water quality and microalgae monitoring data that has been collected for many years from the Coorong, Lower Lakes and Murray Mouth regions was recently collated and multivariate analyses used to describe changes across sites and times (Oliver et al 2013; 2014). This project also focuses on multivariate analysis of the collated water quality data set to help identify the influences of key water quality attributes on the distribution and abundance of *R. tuberosa,* and in particular, provide a statistical basis to help assess the significance of the influences of salinity and water depth which are thought to be major drivers. This pilot study was undertaken to explore the potential of such analyses using water quality monitoring data collated by CSIRO from DEWNR monitoring programs, and *R. tuberosa* data provided by Assoc. Prof. D. Paton and his colleagues. The principle aim was to use multivariate analyses to try and provide an initial description of the influence of a range of selected water quality parameters, including conductivity and water depth, on the distribution and abundance of *R. tuberosa* and to determine if the outcomes suggested that further development of these approaches was warranted.

2 Methods

2.1 Introduction

The *R. tuberosa* monitoring data consisted of a set of measurements taken once each year in early July at selected sites along the length of the Coorong commencing in 1998 (Rogers and Paton 2009; Paton and Bailey 2013; 2014). In the data provided, five sites were monitored from July 1998 through to July 2011, while at four additional sites monitoring commenced in July 2009 with data through to July 2011 (Figure 1). Data for more recent years were not available. In this pilot study, analyses were restricted to the five longer-term sampling sites (Figure 1). These sampling sites ranged from the southern end of the Coorong to half way along the Northern Coorong and so provided a representative spatial coverage, although the majority of sites were in the Southern Coorong (Figure 2). Manually monitored water quality stations were situated at, or near to each of the *R. tuberosa* sites, as were telemetred stations that measured water level and in some cases semi-continuously monitored temperature and conductivity (Figure 2).

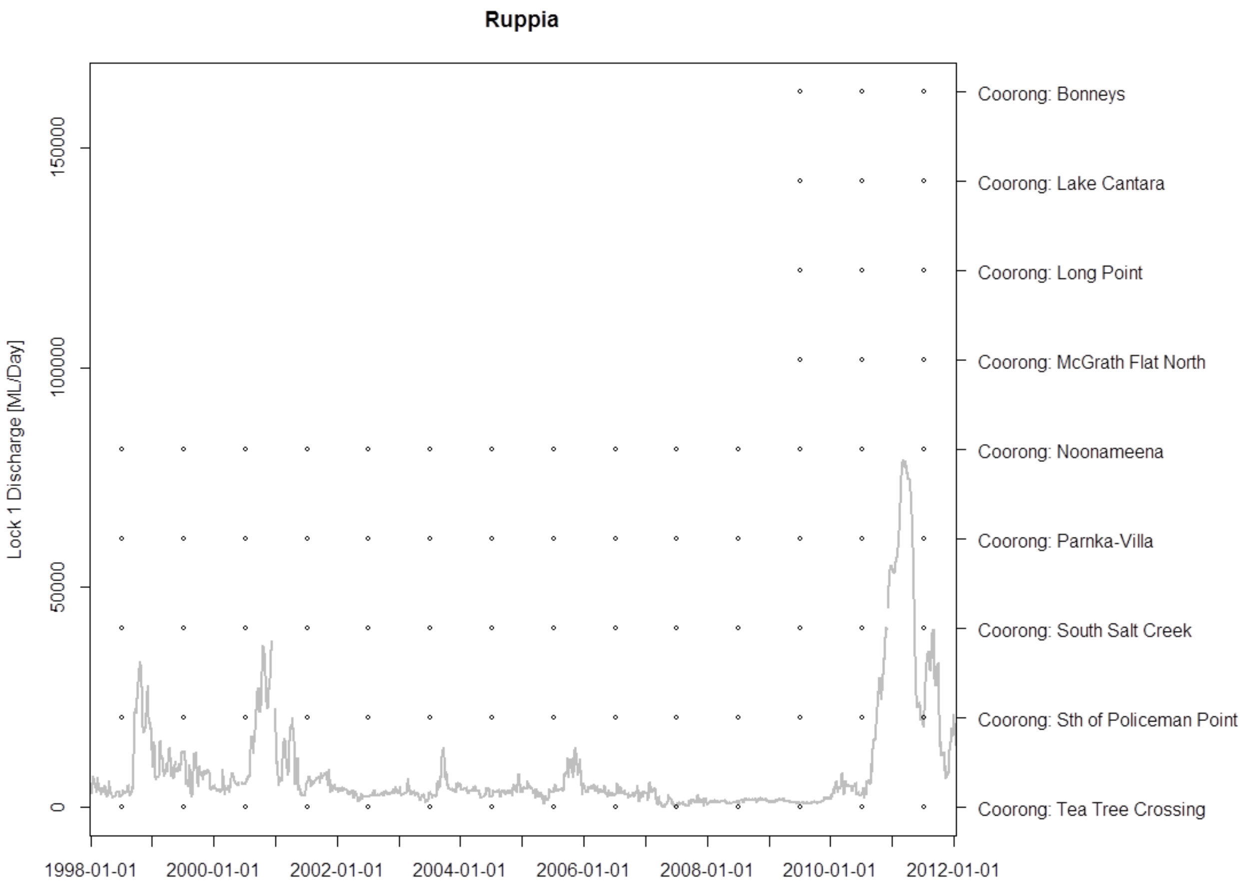


Figure 1 Sampling dates (circles) for the *R. tuberosa* monitoring program at nine sites in the Coorong. The River Murray inflow to the region measured at Lock 1 is shown as a continuous line.

2.2 *R. tuberosa* sampling

At each of the *R. tuberosa* sampling sites, five parallel transects were established 25m apart that ran perpendicularly out into the water from a baseline along the shore (Rogers and Paton 2009). To estimate shoot density, 50 cores of 7.5cm diameter were collected from each of the four regions between transects within water depths of ca. 0.4 to 0.6m, and shoots recorded as the number per core. Other sets of measurements were made at particular depths along each of the transect lines and included in addition to shoot density per core, the numbers of *R. tuberosa* turions and seeds, as well as chironomid larvae and polychaetes. In this pilot study the analyses were restricted to shoot density measurements in the 50 sampling cores between each transect.

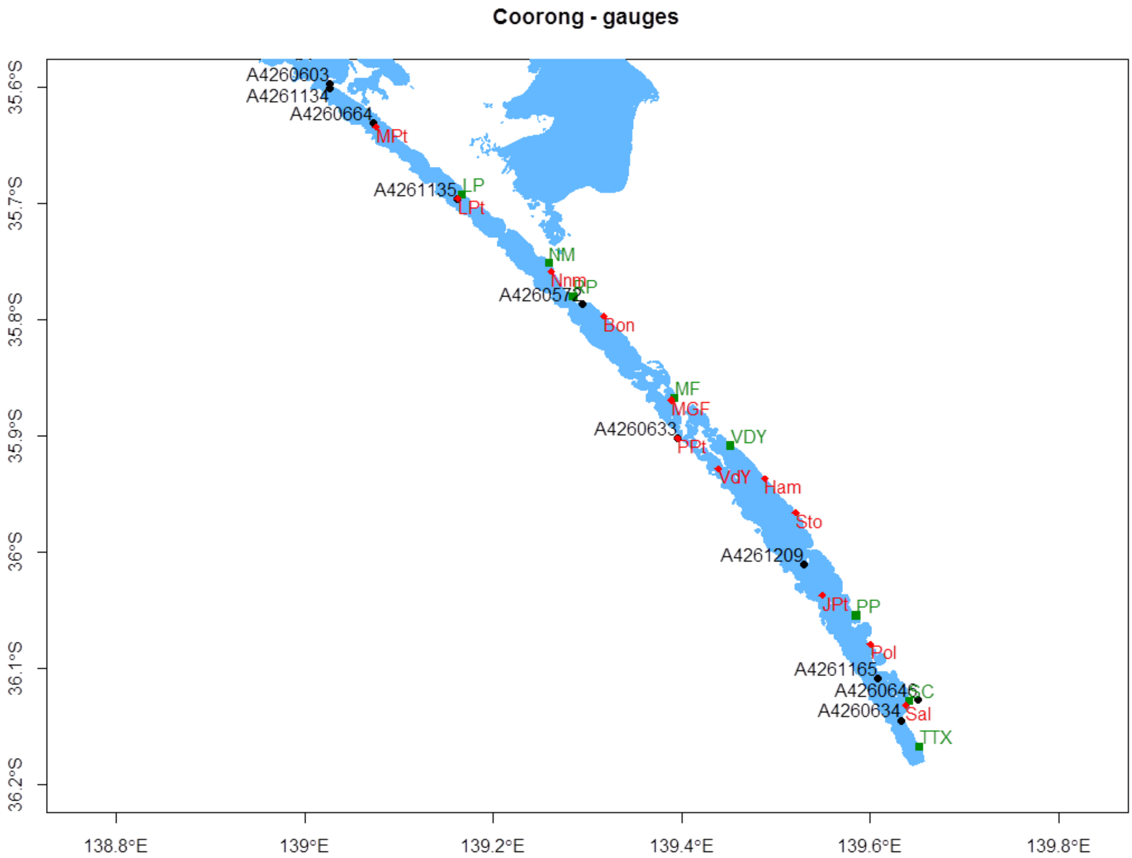


Figure 2 The section of the Coorong containing the selected *R. tuberosa* monitoring sites (green), manual water quality monitoring stations (red) and telemetered stations (black). Named sites are: Mark Point (MPt), Long Point (LP/LPt), Noonameena (NM/Nnm), Rob's Point (RP), Bonney's (Bon), McGarth Flat (MF/MGF), Parnka Point (PPt), Villa de Yumpa (VDY/VdY), Hamilla Downs (Ham), Stony Well (Sto), Jack Point (JPt), Policeman's Point (PP/Pol), Salt Creek (SC/Sal) and Teatree Crossing (TTX)

2.3 Water quality monitoring

The water quality parameters monitored, the monitoring frequency, and their association with the extended *R. tuberosa* sampling are given in Figure 3 using Long Point as an example. Water quality monitoring characteristics were the same at all of the manually sampled sites. In this pilot study the water quality data set was restricted to temperature , water level, pH, conductivity (EC), Total Iron, Ammonia, nitrogen oxides (NO_X), Total Kjeldahl Nitrogen (TKN), Total Nitrogen (TN), Total Inorganic Nitrogen (TIN), Total Organic Nitrogen (TON), Filterable Reactive Phosphorus (FRP) and Total Phosphorus (TP), as these parameters supported the longest times series for analyses. It is evident from Figure 3 that other water quality attributes could be tested but over more restricted time periods.

In most cases water quality parameters were taken from the nearest, relevant sampling site, but in general these were not co-located with the *R. tuberosa* monitoring sites. Consequently, small-scale patchiness in water quality could be an issue, but it is assumed here that the water quality sites are representative of the associated *R. tuberosa* sites. In the case of water depth, data were taken from gauging stations as depth was not recorded at the individual *R. tuberosa* sites. As gauging stations tend to be in deeper parts of the lagoons it is uncertain how well the vertical changes in depth that are used in these analyses relate to changes in the shallow waters where *R. tuberosa* grows (-0.1 to 0.2m water depth; Brock 1982), as this will depend on the shoreline bathymetry.

2.4 Aligning *R. tuberosa* and Water Quality data

In most years water quality samples were taken at approximately 3 monthly intervals, although this varied between years (Figure 3). In order to compare changes in water quality and *R. tuberosa* shoot densities the data sets were aligned by selecting two periods of water quality data leading up to the *R. tuberosa* sampling in early July that were considered to influence particular parts of the *R. tuberosa* growth cycle. These data sets consisted of the average of the water quality conditions for the spring-summer period of September 1st to March 1st (SM), and the average water quality conditions for the period January 1st to June 1st (JJ). The first set was chosen as the ephemeral mud flats where *R. tuberosa* grows are covered with water during spring and early summer (Paton and Bailey 2013) and this might influence the growth and development of *R. tuberosa* plants and the abundance of new shoots appearing in July when seeds and turions germinate. The second set was chosen on the basis that it captures the influence of the dry period on the number of shoots in July, as seeds and turions formed during the wet phase remain on the frequently dry mud surface until late autumn and winter and then germinate when water levels rise (Paton and Bailey 2013). As the *R. tuberosa* annual growth cycle occurs across calendar years, the analyses were performed for functional growth years (FYears) which run from July 1st one year to June 30th the following year.

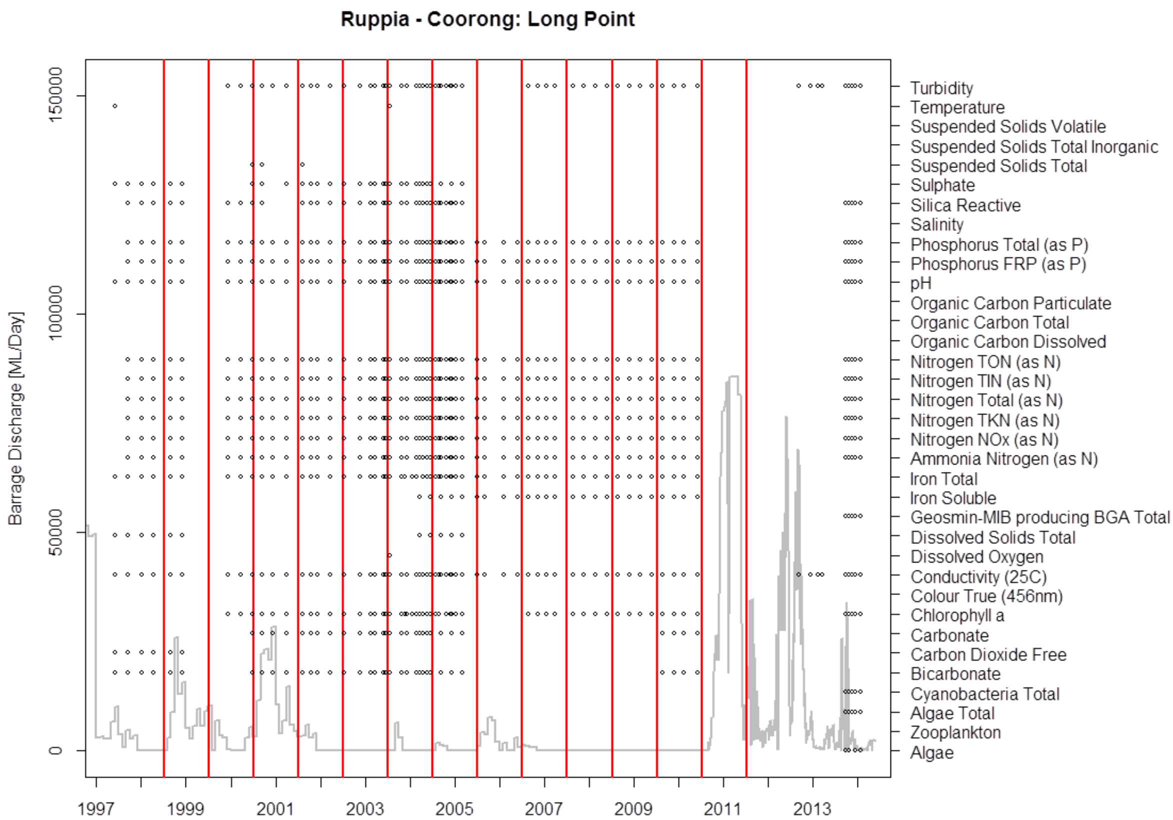


Figure 3 *R. tuberosa* sampling dates are shown by vertical red lines while circles indicate the water quality sampling dates for the listed parameters, represented here by the Long Point monitoring station. The continuous solid line in the background shows barrage discharge for comparison with Murray River discharge in Figure 1.

2.5 Visualizing water quality assessment periods

As a result of seasonal changes in river flows (Figure 1), barrage releases (Figure 3), and evaporation rates, water quality conditions in the Coorong showed strong seasonal patterns. The significance, in relation to the seasonal cycles, of the two time periods chosen to assess the influence of water quality on *R. tuberosa*, can be demonstrated using average salinity data for the North and South Lagoons based on telemetered data (Figure 4). The continuous data shows that seasonal patterns in salinity are very regular, particularly in the South Coorong, where salinity annually follows a sinusoidal change in concentration. This pattern is less distinct in the North Coorong but still evident. Depicted on Figure 4 are the two time periods over which water quality data were averaged. The first period, September to March (SM), captures the period of increasing salinity due to evaporative concentration. The mean of this is a measure of the salinity encountered by *R. tuberosa* during plant growth and development in the wet phase of the growth cycle when seeds and turions are forming (Brock 1982). The second period, January to June (JJ), captures the rise and fall of salinity through its peak concentration during the late growth cycle (Figure 4). This is the period when plant growth is minimal and seeds and turions are the major components of the plant matter and will germinate and commence to grow in June-July if conditions are suitable (Brock 1982). Due to the timing and pattern of salinity changes, the average of the JJ period provides an estimate of the integral salinity over the late growth cycle (Figure 4). Although the two overlapping 6 month periods for averaging water quality are associated with different stages of the *R. tuberosa* life cycle, the regularity of the seasonal salinity fluctuations resulted in the two average salinities being strongly correlated (Figure 5). Consequently, either was considered suitable as an indicator of salinity differences between sites and years for the multivariate analyses. Although correlated, the two measures show sufficient variation to suggest that further investigation of the influence of salinity at different stages of the *R. tuberosa* life cycle might be worthwhile. In the multivariate analyses described later the manual water quality monitoring measurements were used as these provided better opportunities to identify site differences.

2.6 Conductivity and salinity

A debate continues between those who prefer data expressed as conductivity and those who prefer data expressed as salinity. It should be noted that conductivity is the actual water quality parameter most frequently measured in the Coorong monitoring data for assessing salinity. The approach followed in this report was that analyses were based on actual measured values to minimise distortions that could be caused by data transformations that were either inappropriate or not universally accepted. However, the impact on organisms is due to salinity rather than conductivity so in places conductivities were converted to salinities to provide a broader perspective to the data. It is unlikely that these will meet everyone’s expectations and so the conversion formula is provided to enable recalculation. The expression used here is that of Thomas and Lang (2003) which was derived specifically for the Coorong based on comparisons of dissolved solid concentrations with conductivities. The equation is:

$$Salinity\ (g/L) = (0.5865 * Cond + 3 \times 10^{-6} * Cond^2 - 7 \times 10^{-12} * Cond^3)/1000$$

where *Cond* is the specific conductivity in µS/cm.

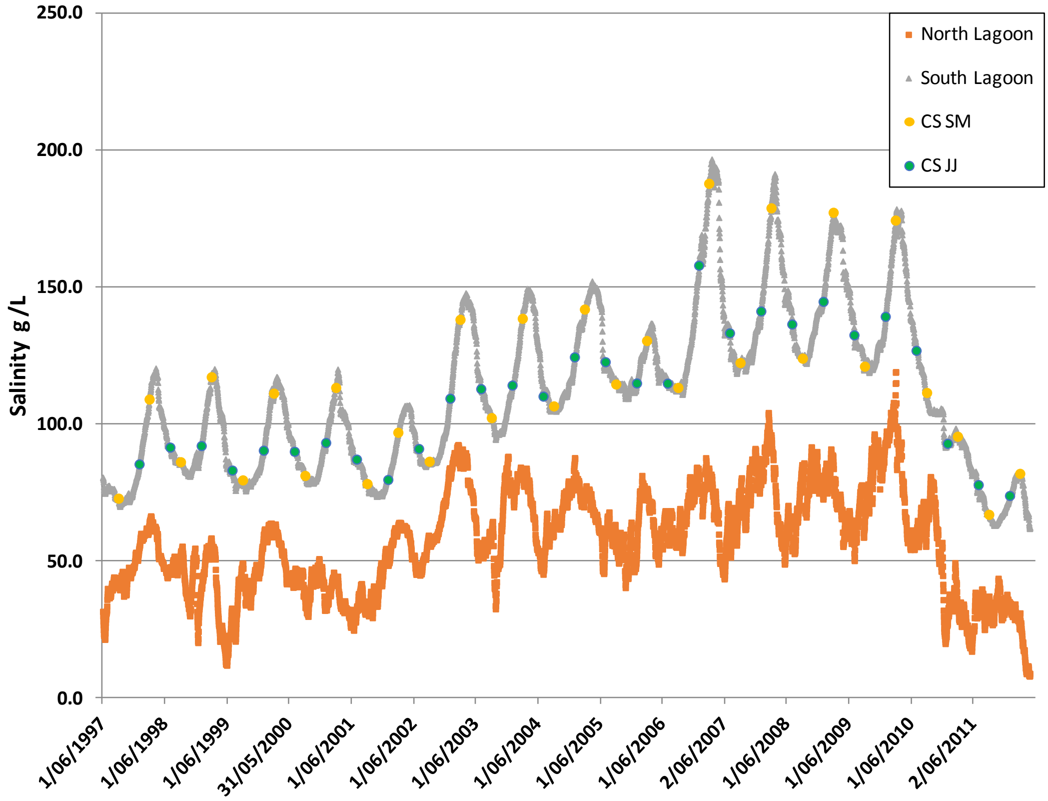


Figure 4 Time series of average salinity in the South and North Coorong over the period of *R. tuberosa* monitoring. On the South Coorong times series yellow circles indicate the September to March water quality averaging period (SM) while green circles indicate the January to June averaging period (JJ).

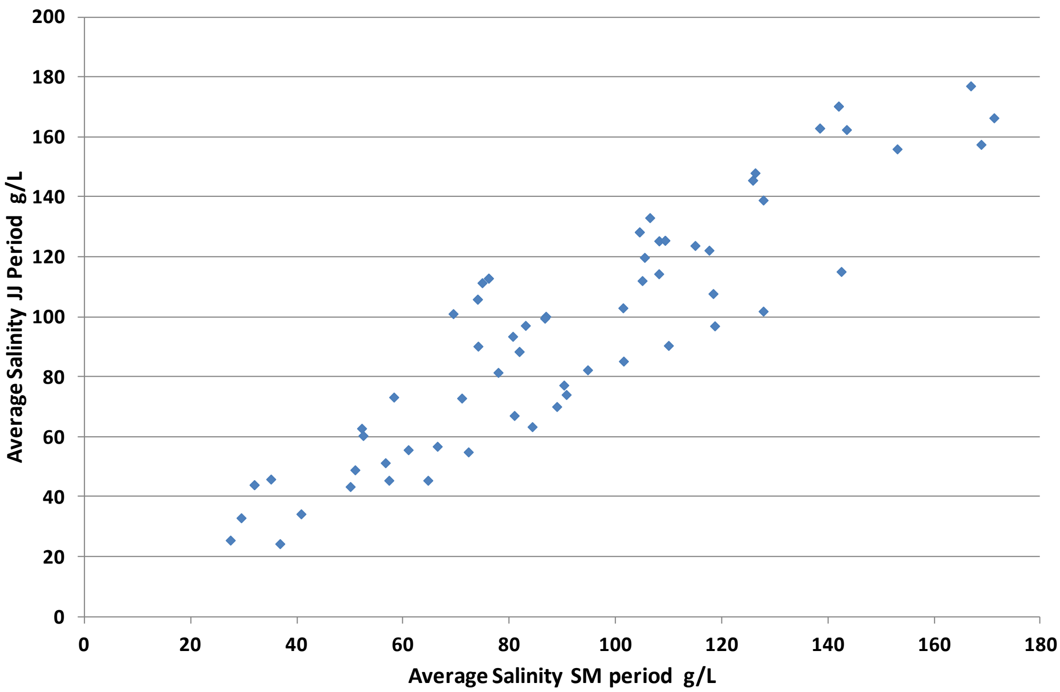


Figure 5 Scatter plot comparing the average salinity in the September to March (SM) period with the average salinity in the January to June (JJ) period.

2.7 Statistical analyses

All analyses were undertaken using the methods within the statistical program Primer 6 and PERMANOVA+ (Anderson, Gorley & Clarke 2008). Patterns in *R. tuberosa* shoot densities were displayed using non-metric multi-dimensional scaling (nMDS) and unless otherwise stated these were derived from a Bray-Curtis similarity matrix based on non-transformed abundance data.

Environmental and water quality parameters were transformed using log (1+x) except for water level which was not transformed. The environmental parameters were normalised and Euclidean distance used as the resemblance measure with patterns in water quality displayed using Principal Components Analysis (PCA).

PERMANOVA (a multivariate equivalent of ANOVA) was used to test if there were significant differences between *R. tuberosa* data sets that were selected *a priori* (e.g. years). To gain an appropriate amount of replication for the analyses, *R. tuberosa* samples collected from each of the four areas at a site were considered replicates, both within years and across years and the average of each of the four areas included in the analyses. Environmental data was averaged either for the September-March period or for the January-June period of the financial year preceding the *R. tuberosa* sampling.

These types of non-parametric statistical analyses explore the similarity of community composition or sets of water quality parameters across locations and time. The nMDS and PCA plots are in general easily interpreted in that points that are closer together are more similar to each other while points increasingly further apart are more dissimilar. A caveat to this is that multivariate data is being displayed on a two dimensional plot and at times this is not easily achieved, so the plots do not always clearly indicate the significance of differences between points. This is determined directly from the data using PERMANOVA.

3 Results

3.1 Correlations between environmental parameters

A potential problem with using multivariate analyses to identify the water quality parameters that influence *R. tuberosa* abundance is the inherent assumption that each parameter is changing independently. This is not always true, and in the current case correlations were expected between some environmental parameters because they are physically linked through processes that are operating in the Coorong. For example, during periods of low or no-flows, evaporation has a strong influence on conditions in the Southern Coorong. Evaporation leads to increased concentrations of dissolved nutrients and to increased conductivity so that these changes are strongly correlated, potentially making it difficult to separate individual effects. However, if the parameters are also differentially altered by other physical or chemical processes, for example sedimentation, then correlations caused as a result of connected processes can be weakened and individual influences more readily discerned. Correlation analyses between environmental parameters for the January to June data sets across all of the *R. tuberosa* sampling sites indicated some strong correlations (Table 1). This was especially so with respect to the various forms of nitrogen where the data indicated that changes in TN, TON and TKN were indistinguishable (Table 1). The multivariate analyses needed to be cautiously interpreted because of these correlations. Further investigation of the correlations between water quality characteristics should be undertaken to develop a better conceptual understanding of these interactions.

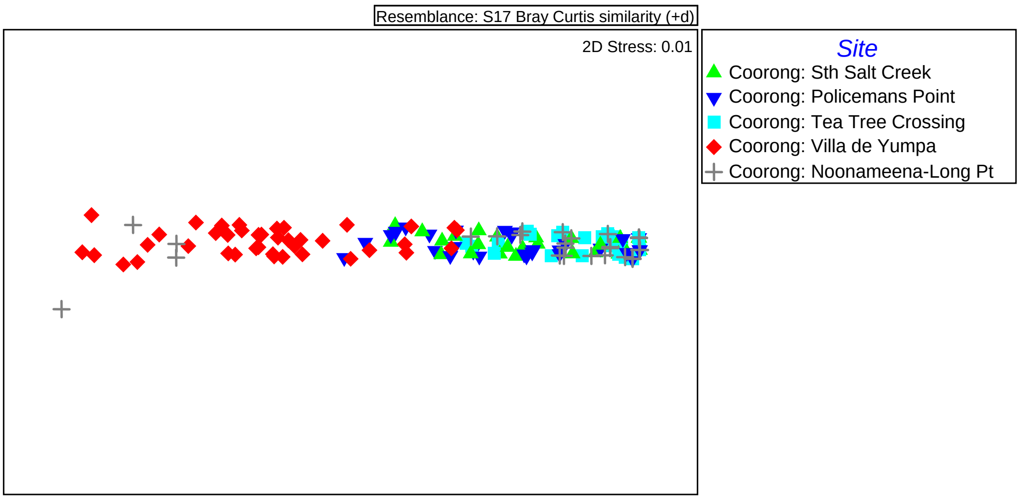
Table 1 Pearson correlations between water quality and environmental parameters for the average January to June measurements along the Coorong. Shaded numbers show high correlations associated with some of the nitrogen compounds.

	Temp	Level	Conduct	Iron Total	Ammonia	Nox	TKN	TN	TIN	TON	pH	FRP	TP
Temp													
Level	0.02												
Conductivity	0.04	-0.57											
Iron Total	0.26	-0.16	-0.30										
Ammonia	-0.25	-0.13	0.10	-0.02									
NOx	0.35	-0.19	-0.31	0.87	0.08								
TKN	-0.23	-0.58	0.85	-0.18	0.14	-0.20							
TN	-0.23	-0.58	0.85	-0.17	0.14	-0.19	1.00						
TIN	-0.20	-0.15	0.06	0.09	0.99	0.20	0.11	0.11					
TON	-0.22	-0.57	0.85	-0.18	0.09	-0.20	1.00	1.00	0.06				
pH	0.04	0.44	-0.62	0.10	-0.14	0.20	-0.50	-0.50	-0.12	-0.49			
FRP	0.38	-0.58	0.53	0.07	0.01	0.08	0.30	0.30	0.02	0.30	-0.41		
TP	0.23	-0.41	0.68	-0.22	-0.21	-0.21	0.58	0.58	-0.23	0.60	-0.44	0.37	

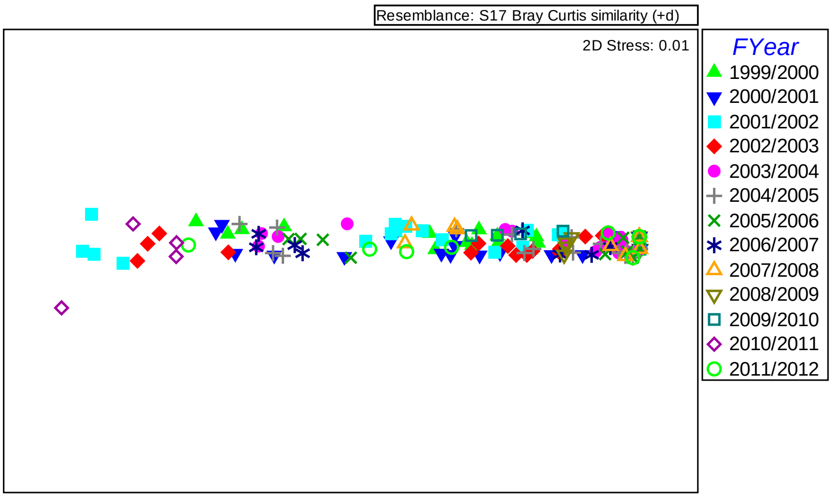
3.2 Distributions of *R. tuberosa* shoots within and between sites

Data on *R. tuberosa* shoot density from the five long-term monitoring sites was collated and analysed together to compare patterns within and between sites. The 50 individual core counts taken between each of the five transects were averaged for each sampling, resulting in four replicate estimates of average shoot density at each site, in each year, for the thirteen FYears that data was available. An nMDS resulted in a sorting of the data on the basis of shoot density and site (Figure 6). Throughout the report some nMDS figures are repeated but with the data points re-coded to describe differences between particular attributes such as sites, years, and shoot densities. This enables comparisons to be made by aligning the points between figures. The analyses of all sites showed separation between Villa de Yumpa (Figure 2) and the three more southerly locations (Figure 6a). In contrast, the more northerly Noonameena site had a

(a)



(b)



(c)

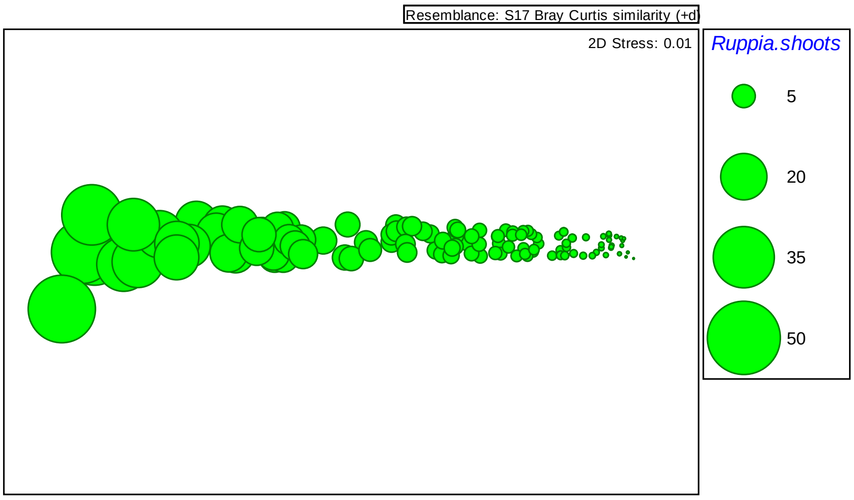


Figure 6 A nMDS of changing *R. tuberosa* shoot density comparing five sites along the Coorong (a) Sites: South Salt Creek, Policeman's Point, Tea Tree Crossing, Villa de Yumpa and Noonameena; (b) FYears when *R. tuberosa* shoot density was measured (c) Average *R. tuberosa* shoot density.

bivariate density overlapping the Villa de Yumpa site when shoot density was high and the southerly sites when shoot density was low. The distribution of average shoot densities ranged from 0 to 33 shoots per core (Figure 6c). Annual differences across all sites suggested that, apart from Noonameena, there was a general reduction in shoot density over time, especially between 2007 and 2010 when most data points in the nMDS were to the right hand side of the figure and aligned with low or zero shoot densities (Figure 6b). In contrast the density at Noonameena increased over time, causing the bivariate distribution.

A pair wise comparison of sites using PERMANOVA indicated that most were significantly different from each other (Table 2). Surprisingly, those sites that were similar were not always closely adjacent. Salt Creek was not significantly different from Policeman's Point which is adjacent to it on the northerly side, but it was significantly different from Tea Tree Crossing which is adjacent on the southerly side. Although Salt Creek and Tea Tree Crossing were significantly different from each other, neither was significantly different from Noonameena which is in the Northern Coorong at the opposite end of the sampling series. Despite intermittent similarities between sites at the ends of the sampling series, Villa de Yumpa, which is approximately mid-way along the longitudinal range, was always significantly different from other sites. These results suggest that changes in the distributions of *R. tuberosa* shoots were not related to uniform changes in environmental conditions along the sampling series, and that there were probably site specific influences. This is in agreement with the time series of shoot abundances that indicated there was a pattern of change along the length of the Coorong (Figure 7). Sites deteriorated in chronological order from the southern end northwards to Villa de Yumpa, while at Noonameena, the northernmost site, shoot density increased up until 2010 (Rogers and Paton 2009).

Table 2 PERMANOVA of pair wise site comparisons of *R. tuberosa* shoot densities for the years 1999/2000 to 2011/2012

Groups	t	P(perm)
SC, PP	1.0962	0.256
SC, TTX	2.1059	0.027
SC, VDY	7.5058	0.001
SC, NML	1.54	0.102
PP, TTX	3.1369	0.002
PP, VDY	6.5695	0.001
PP, NML	2.1673	0.014
TTX, VDY	9.1133	0.001
TTX, NML	1.4845	0.112
VDY, NML	7.2111	0.001

Changes in the distribution of *R. tuberosa* shoots were compared with changes in water quality from the matching SM water quality data, as this supported the largest data collation. The process of data matching did reduce the periods for analyses as water quality measurements were not available in all years (Figure 3). However, an nMDS of the reduced *R. tuberosa* data set matched the results obtained from the full data set with sites similarly delineated on the basis of shoot density. Analyses of the SM water quality and *R. tuberosa* shoot density did not produce a Pearson Correlation above a value of 0.3, the strongest associations being with water level (Pearson 0.26) and total iron (Pearson 0.29). This suggested either that none of the measured water quality parameters were strongly influencing the density of *R. tuberosa* shoots, or as suggested previously, that there were inconsistent interactions across sites. To investigate this possibility each site was analysed separately.

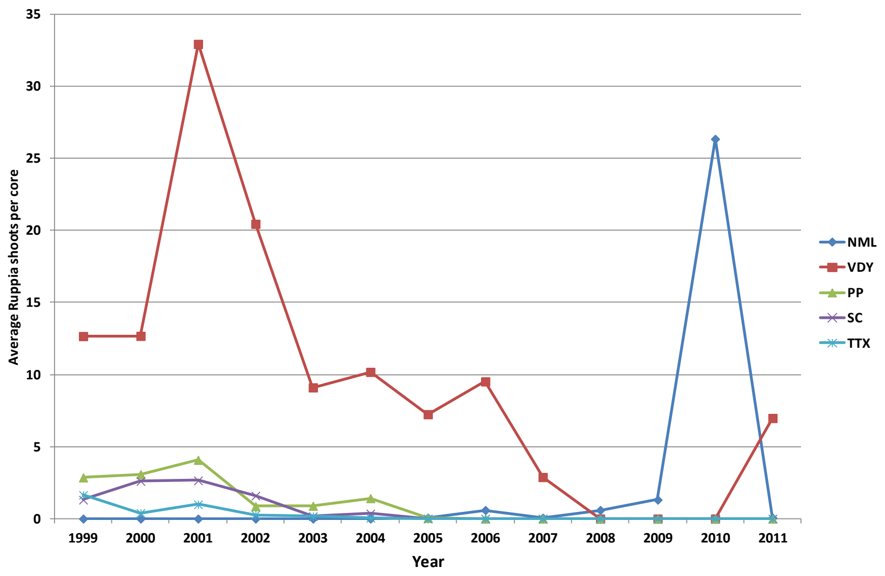


Figure 7 Mean *R. tuberosa* shoot density in July of each year at five sites along the Coorong, Noonameena (NML), Villa de Yumpa (VDY), Policeman's Point (PP), Salt Creek (SC) and Teatree Crossing (TTX).

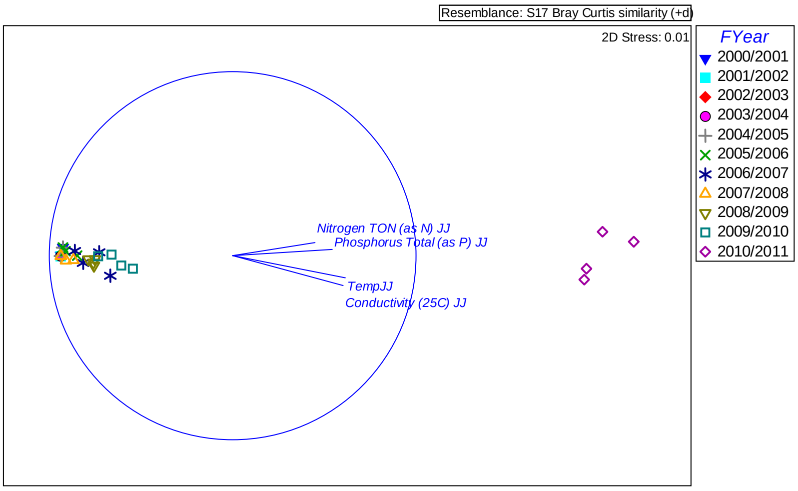
3.3 Site specific changes in *R. tuberosa* shoot densities

Each of the five longer term monitoring sites was analysed with matching water quality data from either the preceding SM or JJ period. Correlations between average water quality conditions and *R. tuberosa* shoot density were similar using either of these water quality data sets, but influences were often more distinct, and in some cases more significant, using the water quality data from the January-June period. For this pilot study the average water quality for the January-June period was predominantly used in the analyses. The years 1999/2000 and 2011/2012 were excluded from analyses due to a lack of suitable matching January-June water quality data, although 1999/2000 could be included if the matching water quality sets were based on the SM data (Figure 3).

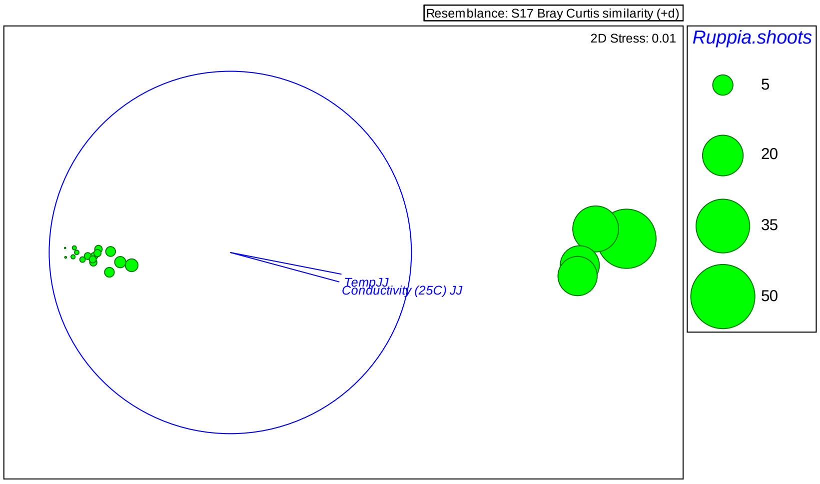
3.3.1 *R. TUBEROSA* SHOOT DENSITIES AND WATER QUALITY AT NOONAMEENA

The *R. tuberosa* shoot density data from Noonameena was matched with water quality data from Long Point which, although not the closest water quality station, provided the longest data set for analysis (Figure 2). Water quality data was sporadically available from other nearby stations but was not tested to check if it differed significantly enough to influence the findings, something that should be done as water quality can be patchy in this region. The 1999/2000 and 2011/12 FYears were excluded by the lack of water quality data, but a continuous sequence of years was available from 2000/2001 to 2010/2011. An nMDS of the shoot data indicated low and similar densities for all years except the final year when shoot density increased significantly (Figure 8). Pearson correlations between associated water quality and shoot density changes indicated that the most strongly correlated characteristics were conductivity (Pearson 0.6) and temperature (Pearson 0.61). The next most significant correlations were with Total Phosphorus (Pearson 0.54) and Total Organic Nitrogen (Pearson 0.45). These correlations are shown as overlays on the nMDS coded for FYears (Figure 8a) while the most strongly correlated are shown on the nMDS coded for *R. tuberosa* densities (Figure 8b). The centroids of the four data points in each FYear were used to help visualize the time sequence of changes. The Noonameena time sequence is shown for all *R. tuberosa* shoot data up to 2010/11 (Figure 8c). Although 2011/12 was not included in the nMDS as the full water quality data was not available, *R. tuberosa* shoot densities declined again to almost zero (Figure 7).

(a)



(b)



(c)

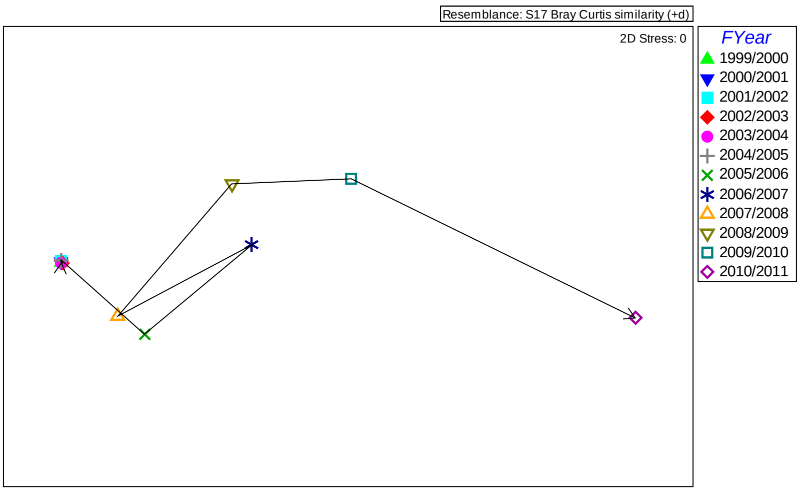


Figure 8 A nMDS of changes in *R. tuberosa* shoot density at Noonameena with correlated water quality changes at Long Point (a) All data points for FYear (b) Average *R. tuberosa* shoot density (c) Time sequence of changing *R. tuberosa* shoot density

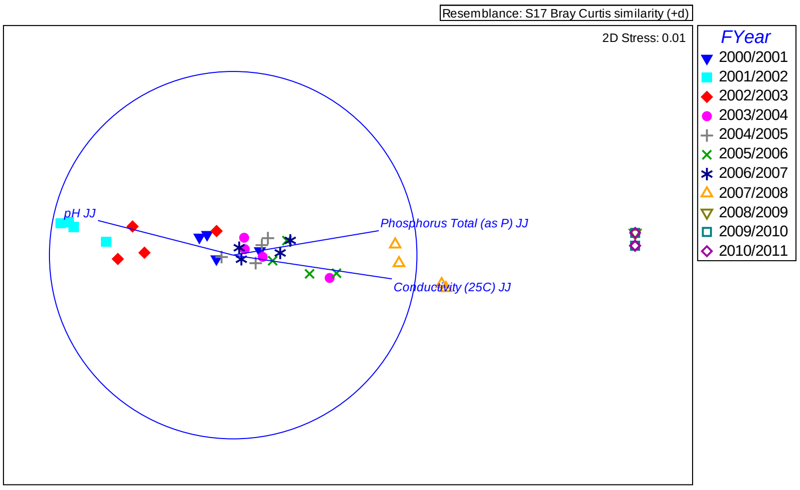
3.3.2 *R. TUBEROSA* SHOOT DENSITIES AND WATER QUALITY AT VILLA DE YUMPA

The *R. tuberosa* shoot density data from Villa de Yumpa was matched with combined water quality measurements from Parnka Point and Villa de Yumpa (Figure 2). These two water quality stations were considered close enough to combine in order to maximise occurrences of matching water quality and shoot density with the artificial construct depicted as Parnka-Villa. The 1999/2000 FYear was excluded due to a lack of water quality data but a continuous sequence of FYears was available from 2000/2001 up to 2010/2011. The sequence of changes in shoot densities was quite different from those observed at Noonameena (Figure 8 and Figure 9). An nMDS of the shoot data indicated moderate shoot densities in 2000/2001, an increase in 2001/2002 and 2002/2003, a return to moderate densities from 2003/2004 until 2005/2006, and then a decline in 2007/2008 and a total loss of shoots in the following years until the end of the data in 2010/2011 (Figure 7 and Figure 9). Pearson correlations between associated water quality and shoot density changes indicated that the most strongly correlated characteristic was conductivity (Pearson 0.86). The next most significant correlations were with Total Phosphorus (Pearson 0.79) and pH (Pearson 0.74). These correlations are shown as overlays on the nMDS (Figure 9). The centroids of the four points in each FYear were used to help visualize the time sequence of changes. The Villa de Yumpa time sequence is shown for all *R. tuberosa* shoot data up to 2010/11 (Figure 9c). Although not shown on the nMDS, shoot density returned to moderate levels in 2011/12 (Figure 7).

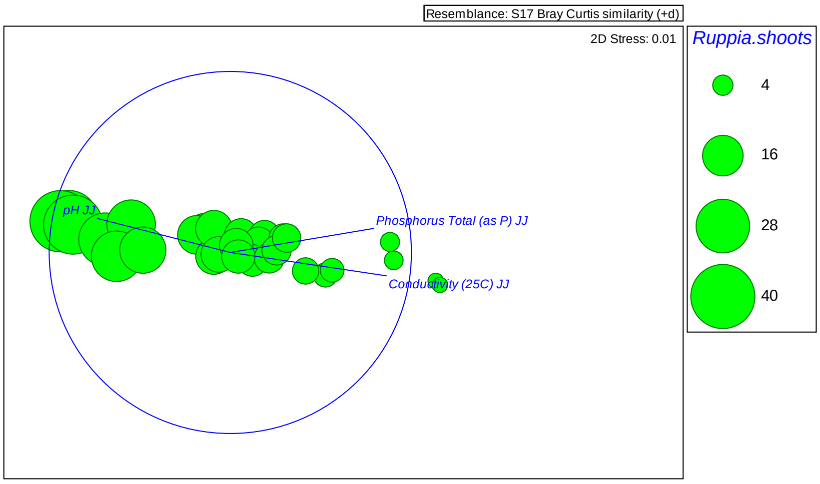
3.3.3 *R. TUBEROSA* SHOOT DENSITIES AND WATER QUALITY AT POLICEMAN'S POINT

The *R. tuberosa* shoot density data from Policeman's Point was matched with water quality data from North Jack Point which is the closest water quality station (Figure 2). The 1999/2000 FYear was excluded by a lack of water quality data, but a continuous sequence of FYears was available from 2000/2001 up to 2010/2011. The sequence of changes in shoot densities was similar to that observed at Villa de Yumpa but maximum shoot densities were substantially lower (Figure 9 and Figure 10). An nMDS of the shoot data indicated low shoot densities in 2000/2001 then a small increase in 2001/2002, but this was not sustained like the much larger increase at Villa de Yumpa, instead shoot densities declined again in 2002/2003 (Figure 7 and Figure 10). Densities remained at a similar level in 2004/05, reduced further in 2005/06 and then there was a total loss of shoots in the following years until the end of the data series in 2010/2011. Pearson correlations between associated water quality and shoot density changes indicated that the most strongly correlated characteristic was conductivity (Pearson 0.81). The next most significant correlation was with Filterable Reactive Phosphorus (Pearson 0.66). These correlations are shown as overlays on the nMDS's (Figure 10). The centroids of the four points in each FYear were used to help visualize the time sequence of changes. The Policeman's Point time sequence is shown for all *R. tuberosa* shoot data up to 2010/11 (Figure 10c). Although not shown on the nMDS, shoot density remained at zero in 2011/12 (Figure 7).

(a)



(b)



(c)

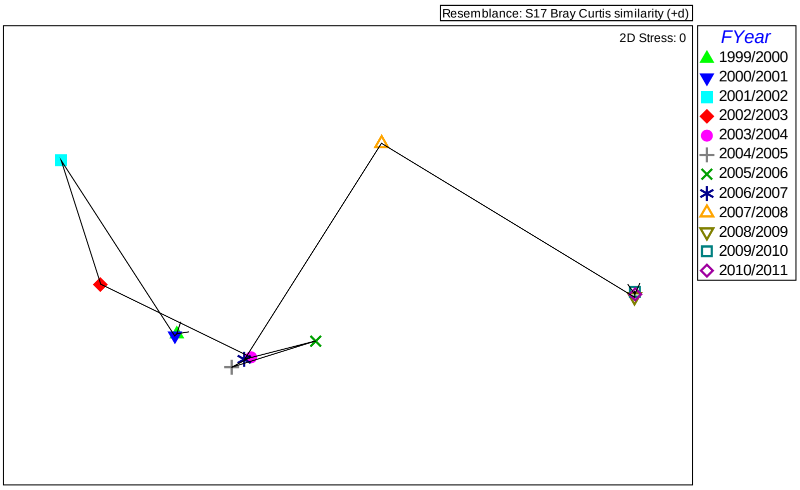
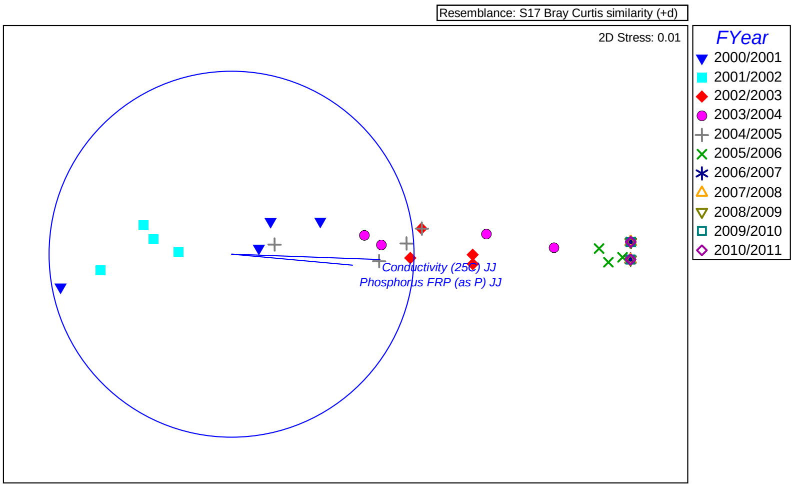
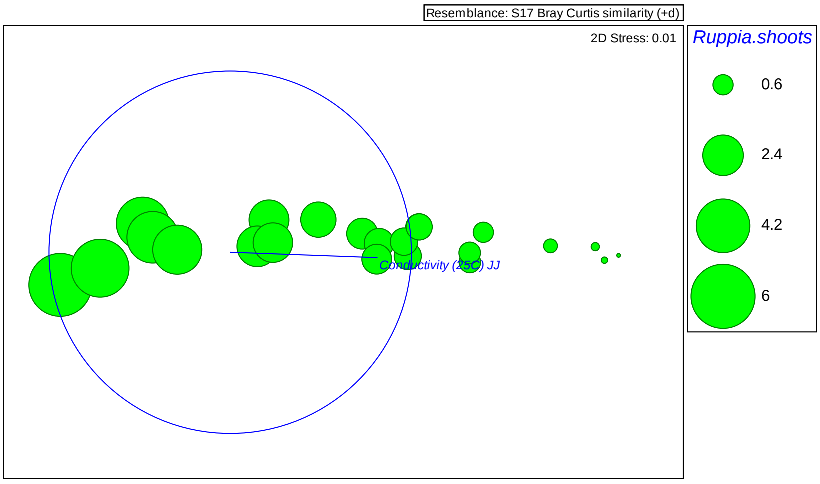


Figure 9 A nMDS of changes in *R. tuberosa* shoot density with correlated water quality changes at Villa de Yumpa (a) All data points for each FYear (b) Average *R. tuberosa* shoot density (c) Time sequence of changing *R. tuberosa* shoot density

(a)



(b)



(c)

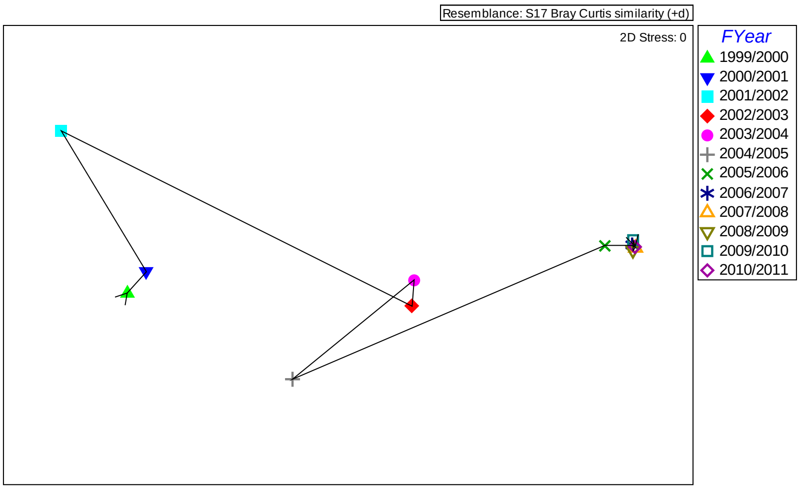


Figure 10 A nMDS of changes in *R. tuberosa* shoot density with correlated water quality changes at Policeman's Point (a) All data points for each FYear (b) Average *R. tuberosa* shoot density (c) Time sequence of changing *R. tuberosa* shoot density

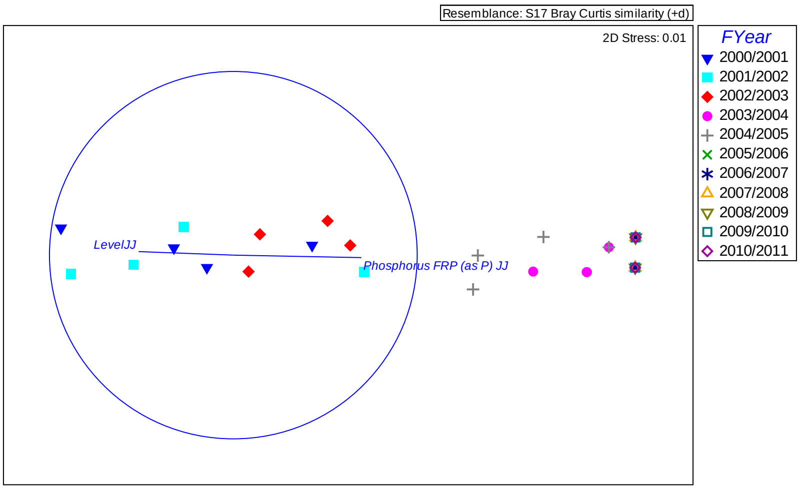
3.3.4 *R. TUBEROSA* SHOOT DENSITIES AND WATER QUALITY AT SALT CREEK

The *R. tuberosa* shoot density data from Salt Creek was matched with water quality data from the nearby South Salt Creek water quality station (Figure 2). The 1999/2000 FYear was excluded by a lack of water quality data, but a continuous sequence of FYears was available from 2000/2001 up to 2010/2011. The sequence of changes in shoot densities was similar to that observed at Policeman's Point with similarly low maximum shoot densities (Figure 10 and Figure 11). An nMDS of the shoot data indicated low shoot densities from 2000/2001 through to 2002/2003, a significant decline in shoot density in 2003/2004, a slight recovery in 2004/05, and then a loss of shoots in following years until the end of the data series in 2010/2011 (Figure 7 and Figure 11). Pearson correlations between associated water quality and shoot density changes indicated that the most strongly correlated characteristic was Filterable Reactive Phosphorus (Pearson 0.70) indicating a reduction in shoot density as FRP increased. The next most significant correlation was with water level (Pearson 0.51) suggesting a decline in shoot density as water level fell. These correlations are shown as overlays on the nMDS (Figure 11). The centroids of the four points in each FYear were used to help visualize the time sequence of changes. The Salt Creek time sequence is shown for all *R. tuberosa* shoot data up to 2010/11 (Figure 11c). Although not shown on the nMDS, shoot density remained at zero in 2011/12 (Figure 7).

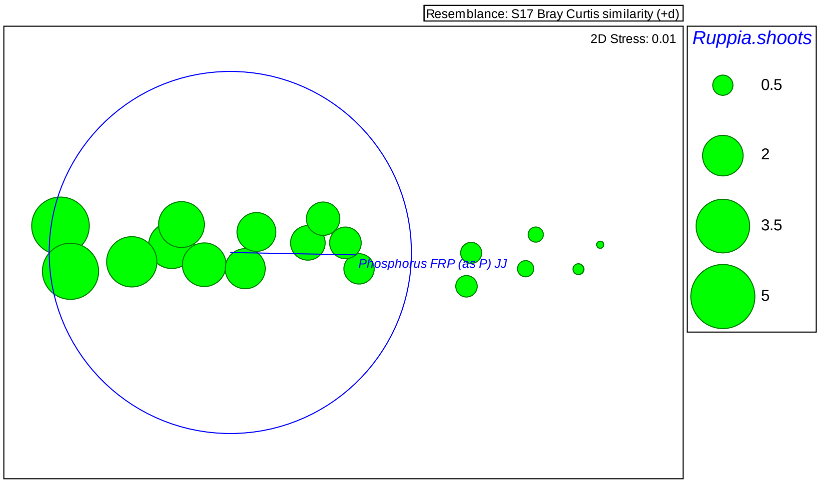
3.3.5 *R. TUBEROSA* SHOOT DENSITIES AND WATER QUALITY AT TEA TREE CROSSING

The *R. tuberosa* shoot density data from Tea Tree Crossing was matched with water quality data from the South Salt Creek water quality station, the same water quality data that was used for the Salt Creek *R. tuberosa* analyses (Figure 2). The 1999/2000 FYear was excluded by a lack of water quality data, but a continuous sequence of FYears was available from 2000/2001 up to 2010/2011. The sequence of changes in shoot densities was similar to that observed at Policeman's Point and Salt Creek, but with even lower maximum shoot densities (Figure 11 and Figure 12). An nMDS of the shoot data indicates low shoot densities in 2000/2001, a small increase in 2001/2002, then a return to low densities until 2003/04, followed by a decline in shoot density in 2004/2005 and then a loss of shoots until the end of the data series in 2010/2011 (Figure 7 and Figure 12). Pearson correlations between water quality and shoot density changes indicated that the most strongly correlated characteristic was Filterable Reactive Phosphorus (Pearson 0.63) indicating a reduction in shoot density as FRP increased. The next most significant correlation was with water level (Pearson 0.52) suggesting a decline in shoot density as water level fell. These correlations are shown as overlays on the nMDS and are similar to the results for Salt Creek. The centroids of the four points in each FYear were used to help visualize the time sequence of changes. The Tea Tree Crossing time sequence is shown for all *R. tuberosa* shoot data up to 2010/11 (Figure 12c). Although not shown on the nMDS, shoot density remained at zero in 2011/12 (Figure 7).

(a)



(b)



(c)

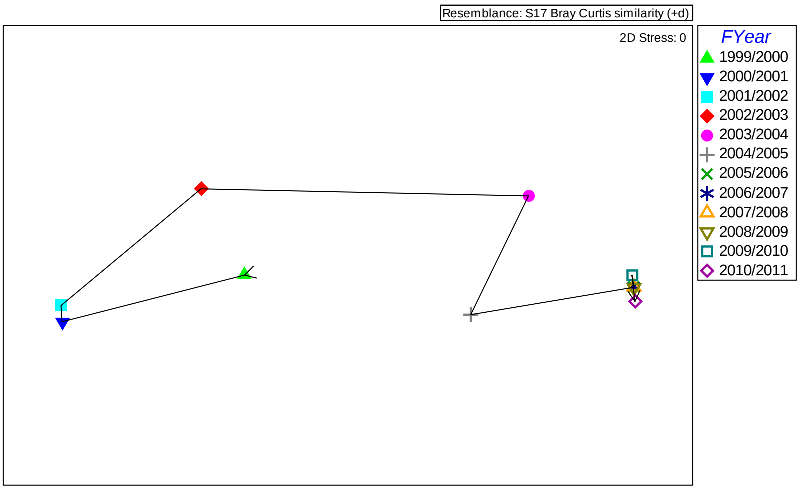
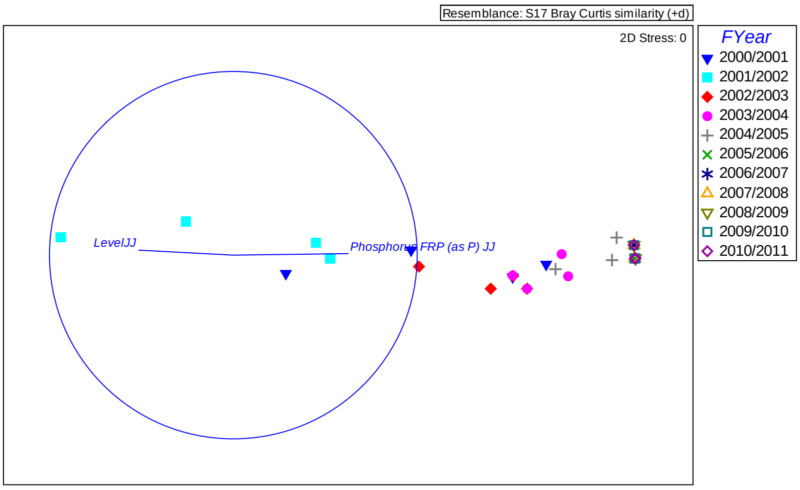
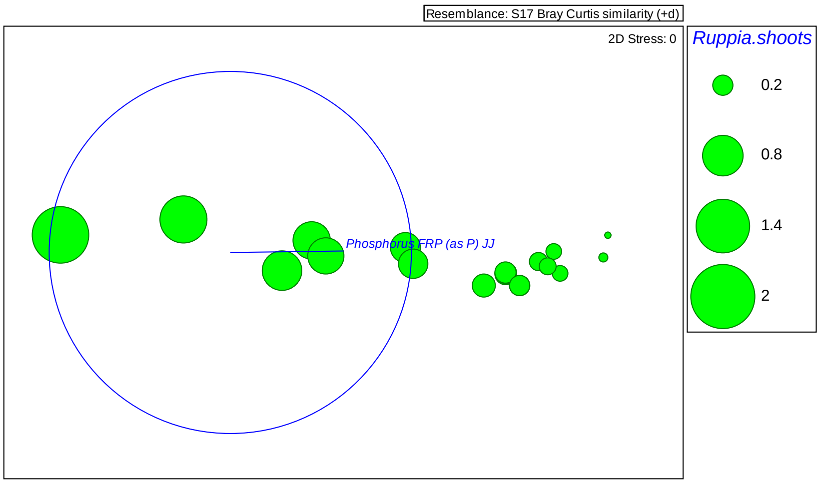


Figure 11 A nMDS of changes in *R. tuberosa* shoot density with correlated water quality changes at Salt Creek (a) All data points for each FYear (b) Average *R. tuberosa* shoot density (c) Time sequence of changing *R. tuberosa* shoot density

(a)



(b)



(c)

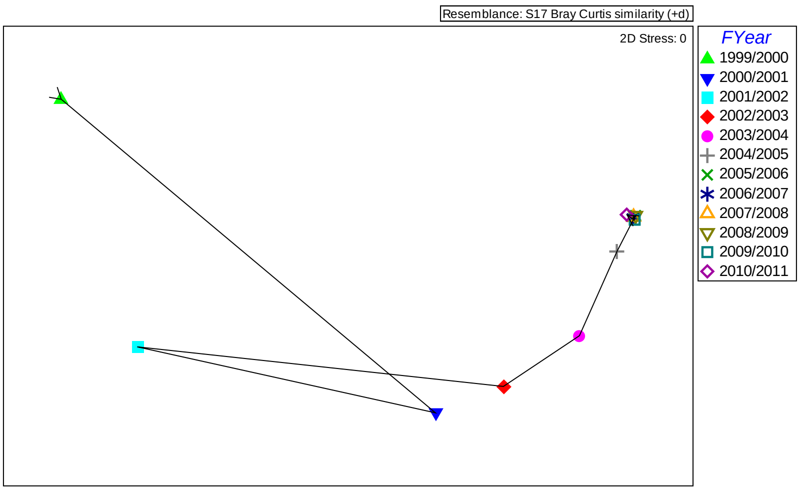


Figure 12 A nMDS of changes in *R. tuberosa* shoot density with correlated water quality changes at Tea Tree Crossing (a) All data points for each FYear (b) Average *R. tuberosa* shoot density (c) Time sequence of changing *R. tuberosa* shoot density

3.3.6 CONDUCTIVITY

Conductivity had a major influence on *R. tuberosa* shoot density at Noonameena (Figure 8), Villa de Yumpa (Figure 9) and Policeman's Point (Figure 10). The effects of conductivity on shoot density are depicted diagrammatically in Figure 13 where the average January-July conductivity for each year and the directional change in shoot density measured in July are compared. It can be estimated from these diagrams that, if conductivity is the driving influence on *R. tuberosa* shoot density, then the apparent range of suitable average conductivities for the January to July period lies between 60,000 and 125,000 µS cm^{-1} (salinities of 44.5 to 106.5 g L^{-1}) with shoot densities decreasing when average conductivity increased or decreased outside this range. These values were estimated by visual inspection of the average data but statistical classification applied to the detailed conductivity measurements would better define these limits.

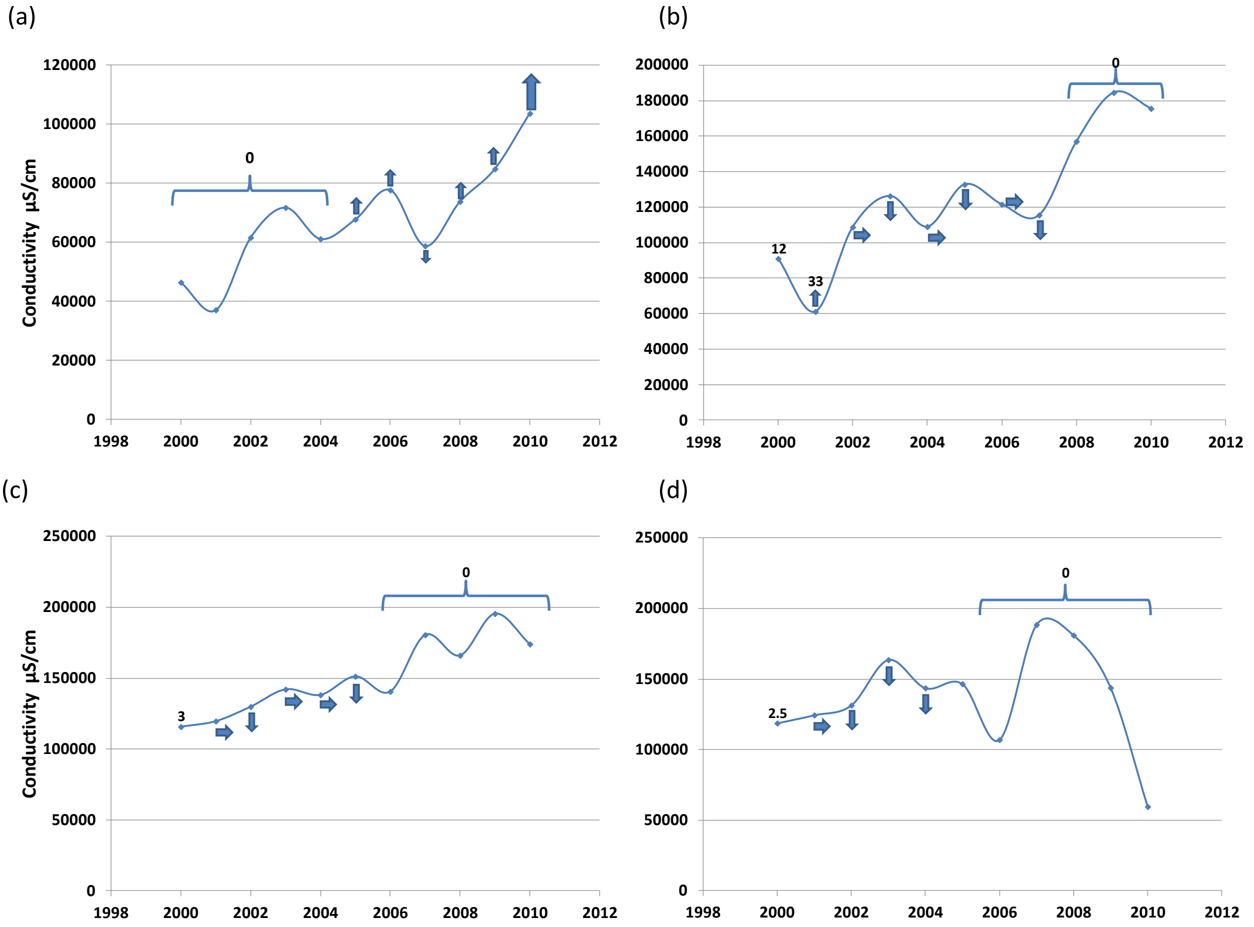


Figure 13 Changes in the January-July average conductivity at each site with numbers indicating *R. tuberosa* shoot densities in particular years and arrows indicating the change in shoot density from the previous year (a) Noonameena (b) Villa de Yumpa (c) Policeman's Point (d) Salt Creek (the response at Tea Tree Crossing was similar to Salt Creek and is not shown)

Pearson correlations indicated that water level and filterable reactive phosphorus were major influencers of changes in *R. tuberosa* shoot density at Salt Creek and Teatree Crossing. Average conductivity at these sites was initially at the upper limit of the *R. tuberosa* range, as defined from the Coorong data, and shoot density was low (Figure 13d). Conductivity increased above this range only two years into the data set and shoot density fell to zero. This suggested that conductivity had an influence on shoot density but that its effect may have been reduced in the nMDS's because of the limited years with shoot counts (Figures 11 and 12). Also, both of these sites were linked to the same water quality station. It is not certain that this single station adequately described the water quality at both sites, and using the same data for both sites minimised the differences between them. Further analyses of other nearby water quality stations are warranted to assess the spatial variation in water quality. The reliability of water level measures is also questionable as these sites are at the far end of the southern lagoon and measurements at the gauging stations may not adequately represent the changes in the shallow margins. Further investigations of the changes in water depth, conductivity and phosphorus concentrations at these sites would help improve the conceptual understanding of water quality influences on *R. tuberosa*.

4 Discussion and conclusions

4.1 Multivariate analyses

The primary purpose of this project was to investigate whether the application of multivariate statistical analyses using data from the Coorong monitoring programs could help identify changes in water quality and water regimes that influenced the abundance and distribution of *R. tuberosa*. Overall the approach was successful in that water quality changes were identified that correlated with changes in *R. tuberosa* shoot density, and these corresponded with interactions proposed by other researchers (Rogers and Paton 2009; Paton and Bailey 2013; 2014). The multivariate analyses provided statistical measures of the correlations enabling an assessment of their relative importance. However care was required with the interpretation because even strong correlations do not necessarily indicate a causal connection, and also because of significant correlations between the water quality parameters themselves. Despite these difficulties reasonable interpretations were possible, especially in relation to the effects of salinity on *R. tuberosa* shoot density which were sufficiently consistent across sites and years to enable the limits of influence to be estimated. The published salinity range of *R. tuberosa* is between 12 to 230 g L^{-1} (Brock and Lane 1983; Boon and Morris 2002), but it is likely that this range is based on spot observations of *R. tuberosa* plants occurring at their salinity extremes where their growth and reproductive condition is uncertain. The Coorong data suggested that *R. tuberosa* shoot density decreased to zero if the average January to July salinity moved outside the range of 44.5 to 106.5 g L^{-1}, with higher shoot densities occurring between these concentrations. This data significantly reduces the expected range for *R. tuberosa*, and will be of importance in better understanding the ecological conditions required by the plant. Although it is possible that other environmental influences in the Coorong reduced the capacity of *R. tuberosa* to utilise a wider range of salinities, the data appears to be robust. Further analyses of the Coorong data, in particular consideration of salinity maxima, could help identify salinity extremes that might consolidate these observations with those reported in the literature.

Although multivariate techniques were used to investigate the influence of water quality conditions on *R. tuberosa*, the *R. tuberosa* data was restricted to an assessment of shoot density at the time of germination which is a univariate measure. One consequence of this was that clustering produced a monotonic series across sites and times reflecting shoot abundance alone. Although site specific analyses indicated that conductivity had a major influence on shoot abundance, this was not reflected in analyses using pooled data from all sites. The reason for this was that at some sites shoot density declined because of conductivity increasing above an upper limit, while at others, specifically Noonameena, shoot density was low when conductivity fell below the zone of suitability. Consequently the pooled data contained sites with low shoot abundance due to either high or low conductivity, and this could not be discriminated in the monotonic series of sites based on shoot abundance alone. It was fortunate for the analyses applied here that at each individual site reductions in shoot density were due to either high or low conductivities, but not both.

The analyses in this pilot study were based only on *R. tuberosa* shoot density data but inclusion of the measurements of turions and seeds would provide a more complete assessment of the life cycle of the plant, and a multivariate basis for the analyses. Similarly the inclusion of a broader range of parameters, such as the occurrence of microalgae, chironomid larvae and polychaetes might provide better discrimination between sites and times, for example where *R. tuberosa* shoot densities were similar but due to different conductivity levels. Although multivariate analyses are a powerful method for investigating and identifying interactions, they need to be augmented with more focused statistical methods to explore the specific interactions that are identified.

4.2 Multiple Environmental influences

It is to be expected that the abundance and distribution of aquatic organisms will be affected by a wide range of environmental conditions that alter over time and space. The purpose of the multivariate analyses applied in this pilot study was not to determine the functional role of all the many factors that are likely to influence the density and distribution of *R. tuberosa*, but rather through correlations between water quality and *R. tuberosa* shoot density to identify major influences that might be usefully managed to improve the conditions for *R. tuberosa* growth. However, the occurrence of a significant correlation does not necessarily mean that the identified characteristic is directly causing changes in *R. tuberosa* shoot density as the effect may be due to correlated variations in other influential factors. At each of the sites interpretation of the data was complicated by the presence of more than one water quality attribute that correlated strongly with the changes in *R. tuberosa,* and also by correlations between water quality parameters themselves (Table 1). The best approach to tease apart these interactions is to experimentally manipulate systems, changing a single characteristic at a time to assess the influence on *R. tuberosa*, but this experimental approach is a long term activity. Additional options include further exploration of the field data looking for "natural" experiments where particular conditions occur that are suitable for discriminating between attributes, applying expert knowledge to assess whether water quality changes are of a magnitude expected to be influential, and looking for consistencies in responses across sites and times that support the proposed interactions. These approaches generate hypotheses that can then be continually tested as new field data is collected. These approaches were used to help interpret the multivariate analyses in the following examples.

At Noonameena, Villa de Yumpa and Policeman's Point, conductivity was significantly correlated with the changes in *R. tuberosa* shoot density. Conductivity was found to be a consistent indicator of unsuitable conditions across sites and times enabling repeated identification of a concentration range affecting *R. tuberosa* shoot density (Figure 13). This consistency of response increased confidence that an effect on *R. tuberosa* had been reliably identified. At Villa de Yumpa changes in *R. tuberosa* shoot density also appeared to be associated with changes in pH (Figure 9). Inspection of monitoring data from the South Lagoon showed that pH varied between 7.8 and 8.4 during the period when the correlation was identified, but that pH had varied within this range for many years, including periods when *R. tuberosa* was widely distributed. From a general understanding of pH impacts on submerged macrophytes, and the similarity of the pH range over times of high and low *R. tuberosa* abundance, it is concluded that a major, direct pH affect on *R. tuberosa* shoot density was unlikely. This is not to exclude a possible pH influence, for example the distribution of the forms of inorganic carbon used by plants in photosynthesis changes significantly within these pH ranges and further investigation of the data is required. Future analyses would benefit from a more comprehensive assessment of the historical record of water quality conditions to determine if these had changed substantially, as this will contribute to knowledge of the general growth conditions experienced by *R. tuberosa* in the Coorong.

Phosphorous, either as FRP or TP, was strongly correlated with the *R. tuberosa* changes at most of the sites. It has been proposed that this is due to increased phosphorus supporting the growth of attached algae that smother the *R. tuberosa* (Paton and Bailey 2013; 2014). If this was the case then microalgae chlorophyll concentrations might be expected to increase, reflecting this connection. The water quality data is available to test this link but analyses were not included in the current project. However, it appeared from the analyses that the influence of phosphorus was greatest at more saline sites where smothering algae might be less expected and where salinity impacts were already occurring. A more critical assessment of the phosphorus data is required.

At Salt Creek and Tea Tree Crossing water depth was also identified as influencing *R. tuberosa* shoot density, but as described previously the available depth data was not expected to reliably represent changing water depths in the shallow *R. tuberosa* sites. With further analyses the measured vertical depth changes could be related to alterations in shallow water areas using a bathymetric map, but this was beyond the scope of the current project. A focus should be placed on addressing this shortcoming as water level in the Coorong has a major impact on the extensive shallows, affects a wide range of important organisms and is a manageable attribute.

Teasing apart some of these multiple interactions will require more detailed and critical analyses of changes in conditions over space and time in order to identify consistent and reliable responses. The water quality and water depth characteristics of the Coorong are closely linked to the interactions between barrage flow regimes and connectedness to the ocean through the River Murray mouth. There are models available that describe these links (eg. Webster 2007) and it would be very beneficial to use these models in conjunction with the multivariate analyses to develop a dynamic model of the responses of *R. tuberosa* to conditions generated by the changing flow regimes. Linking flows, water depths, water quality and *R. tuberosa* distribution and abundance would provide managers of the Coorong with a powerful tool to assess the environmental flow requirements and the likely responses to changing water allocations.

5 Recommendations

1. Correlations between changes in water quality characteristics should be further investigated as this would help to further understanding of the physical and chemical functioning of the system.
2. Improved indicators are required for some of the environmental parameters, for example water depth. Vertical changes in depth could be related to alterations in the area and locations of shallows where *R. tuberosa* grows using the Coorong bathymetric map.
3. Analyses were restricted to assessing the abundance of shoots during germination but should be broadened to include data on seeds and turions to provide a more comprehensive analysis of the *R. tuberosa* annual growth cycle. Further testing of within and between site differences in *R. tuberosa* measurements is required to confirm statistical significance.
4. *R. tuberosa* shoot abundance was assessed against average water quality conditions in the September to March and January to July periods. These were chosen based on stages of the annual growth cycle of the plant. There is sufficient data to attempt to statistically identify more refined periods when water quality conditions most reliably indicate responses. This may help identify growth stages sensitive to particular water quality conditions.
5. Water quality was determined from a selected set of monitoring sites. Comparisons with other monitoring sites is needed to describe the scale of variation across space and time in order to extract the most reliable estimates of water quality conditions encountered by the *R. tuberosa*.
6. A selected set of water quality parameters was used in the analyses and consideration should be given to other measurements that were not utilised in this pilot study.
7. The multivariate analyses should be expanded to include more recent data gathered through the *R. tuberosa* monitoring program as there has been another period of significant change.
8. Multivariate analyses are useful for identifying major linkages between components in complex systems, but further investigation of the linkages requires more specific conceptual, empirical and statistical approaches. This would include separation of samples into consistent groupings that inform on specific interactions eg. conductivity changes associated with the upper and lower bounds of suitability for *R. tuberosa*.
9. This pilot study has shown that multivariate analyses of the Coorong water quality monitoring data, augmented by other conceptual, empirical and statistical approaches, can enhance the interpretation of the data collected in the *R. tuberosa* monitoring program. These preliminary analyses suggest that further insights would be achieved by a more sustained analytical effort. Considering the potential value of these insights to management of the Coorong, and the relative cost compared to the monitoring programs, it is recommended that further analyses are undertaken.

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