Seed Bank Assessment of Dunn's and Shadow's Lagoons

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Cover Photograph: 11,000 EC salinity treatment with *Typha domingensis, Myriophyllum salsugineum, Chara* sp. and *Potamogeton pectinatus* seedlings (Adrienne Frears).

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Executive Summary

The seed bank is the only desiccation resistant life history stage for many submergent aquatic plant species and plays an important role in the recovery of the plant community after disturbance, such as desiccation or elevated salinity, has removed the extant vegetation. Dunn's and Shadow's Lagoons were permanent freshwater wetlands that, until 2007, were hydrologically connected to Lake Alexandrina and contained highly diverse aquatic plant communities. Since 2007 low lake levels have resulted in the disconnection and desiccation of Dunn's and Shadow's Lagoons and subsequent loss of the aquatic plant communities.

Due to the high conservation values of Dunn's and Shadow's Lagoons they were short-listed to receive environmental water from either Goolwa Channel or Lake Alexandrina. However, information was required to determine whether there was in situ potential for regeneration of aquatic plant communities (a seed bank) and the response of the seed bank to the modelled salinity of the potential sources of the environmental water (Lake Alexandrina 6,400 μS.cm^{-1}, electrical conductivity and Goolwa Channel 11,000 μS.cm^{-1}). This information will be used to determine where to use and source the environmental water to gain the maximum benefit.

The submergent seed banks of Dunn's and Shadow's Lagoon's were assessed using the seedling emergence technique. Sediment samples were collected from each wetland, dried and submerged to a depth of 20 cm in freshwater and water with the predicted (using hydrological modelling) salinities of Lake Alexandrina (6,400 μS.cm^{-1}) and Goolwa Channel (11,000 μS.cm^{-1}) in August 2010. Seedlings were identified and removed fortnightly and seed density and floristic composition between treatments and wetlands were compared.

A total of 11 taxa germinated from the seed bank under all salinity treatments. The species composition of the seed bank was significantly different between wetlands, with the Shadow's Lagoon seed bank possessing higher a seed density and species richness (all 11 species were present; nine were present in the Dunn's Lagoon seed bank). Salinity had no effect on the species composition that germinated from the seed bank of either wetland.

Results suggest that Shadow's Lagoon would likely exhibit a greater response to environmental watering than Dunn's Lagoon because it had a larger and more species rich seed bank. However, both wetlands have the capacity to recover under hydrological restoration because they both have a submergent plant seed bank and the species present reproduce asexually and under favourable conditions can rapidly expand their distribution once they reach maturity.

Results also suggest that the source of the environmental water is not important because there was no significant difference between salinity treatments. However, salinity remained constant throughout this study whereas under natural conditions it would increase through time in a disconnected system due to evapoconcentration and salinity may exceed the tolerances of some of the species present. Therefore, to gain the maximum environmental benefit, the water with the lowest salinity should be used (if possible).

1. Introduction

The seed bank is defined as the reserves of viable seeds (and spores) in and on the soil surface and associated litter (e.g. van der Valk and Davis 1976; Thompson and Grime 1979; Roberts 1981; Thompson 1987) and is part of the flora of the system although it is not readily evident (Major and Pyott 1966). The primary role of the seed bank is to ensure the continuation of species after disturbance or natural mortality has killed the extant vegetation; however, population maintenance (Simpson *et al.* 1989) and a reserve of genetic diversity (Templeton and Levin 1979) are also functions. The role of the seed bank in vegetation dynamics varies from system to system. Seeds are the only desiccation resistant stage of the life cycle of most submergent species; hence, the seed bank plays an important role in their persistence during unfavourable periods such as drought or elevated salinities (e.g. Casanova and Brock 1990; Brock and Britton 1995; Brock and Rogers 1998; Leck and Brock 2000; Nielsen *et al.* 2007; Aponte *et al.* 2010). Therefore, the seed bank contributes to the resilience of a system by providing an in situ (there is no reliance on dispersal into the area) source of propagules for recolonisation after disturbance has killed, reduced or significantly changed the extant plant community.

Extended drought combined with abstraction for domestic, agricultural and industrial purposes in the Murray-Darling Basin has resulted in reduced inflows into South Australia since 2007, which inturn has resulted in flows over Lock and Weir number 1 being insufficient to maintain pool level downstream. Subsequently water levels in Lakes Alexandrina and Albert have fallen to unprecedented lows (<-0.75 m AHD), which has disconnected all of the fringing wetland habitats. Disconnection and subsequent desiccation of fringing habitats has resulted in areas that historically (pre 2007) were permanent freshwater wetlands that often contained diverse submergent, emergent and amphibious plant communities (Renfrey *et al.* 1989; Holt *et al.* 2005; Nicol *et al.* 2006) changing to communities dominated by terrestrial species or bare soil (Marsland and Nicol 2009; Gehrig *et al.* in prep).

Dunn's and Shadow's Lagoons were identified as high conservation value wetlands by the South Australian Murray-Darling Basin Natural Resources Management Board. Historically (pre 2007) they were permanent freshwater wetlands with a high diversity of aquatic plant species (Renfrey *et al.* 1989; Holt *et al.* 2005), which provided habitat for numerous species including threatened fish (Bice *et al.* 2008). Due to the historical high conservation values and proximity to Goolwa Channel, Dunn's and Shadow's Lagoons were shortlisted to receive environmental water (if available). Environmental water would be sourced from either Goolwa Channel or Lake

Alexandrina; however, the predicted salinity of the aforementioned water bodies in August 2010 (Lake Alexandrina 6,400 $\mu S.cm^{-1}$ and Goolwa Channel 11,000 $\mu S.cm^{-1}$) will exceed 5,000 $\mu S.cm^{-1}$. Nicol and Ward (in prep.) reported that the species richness and number of seeds germinating from the seed bank of Goolwa Channel was significantly lower in treatments where the salinity exceeded 5,000 $\mu S.cm^{-1}$.

Information regarding the capacity of the submergent plant community to regenerate (the seed bank) and the germination response of the seed bank to the predicted salinity of the environmental water is required to determine where to source and use environmental water for maximum benefit. Therefore, the aims of this study were threefold:

- To investigate seed density and species composition of the submergent plant seed banks of Dunn's and Shadow's Lagoons.
- To determine the effect of two levels of salinity (modelled Lake Alexandrina salinity: 6,400 and modelled Goolwa Channel salinity: 11,000 MS.CM-1) on germination from the seed bank.
- To use this information to inform managers regarding where to use and source environmental water.

2. Methods

2.1. Field Site

Dunn's Lagoon is a fringing wetland located on the western shoreline of Lake Alexandrina near the township of Clayton (Figure 1). Shadow's Lagoon is a shallow wetland that connects Lake Alexandrina (via Holmes' Creek) with Hunter's Creek on Hindmarsh Island (Figure 1). Historically Dunn's and Shadow's Lagoons were permanent wetlands connected to Lake Alexandrina; however, since 2007 water levels have receded resulting in the disconnection and subsequent desiccation of the aforementioned wetlands.

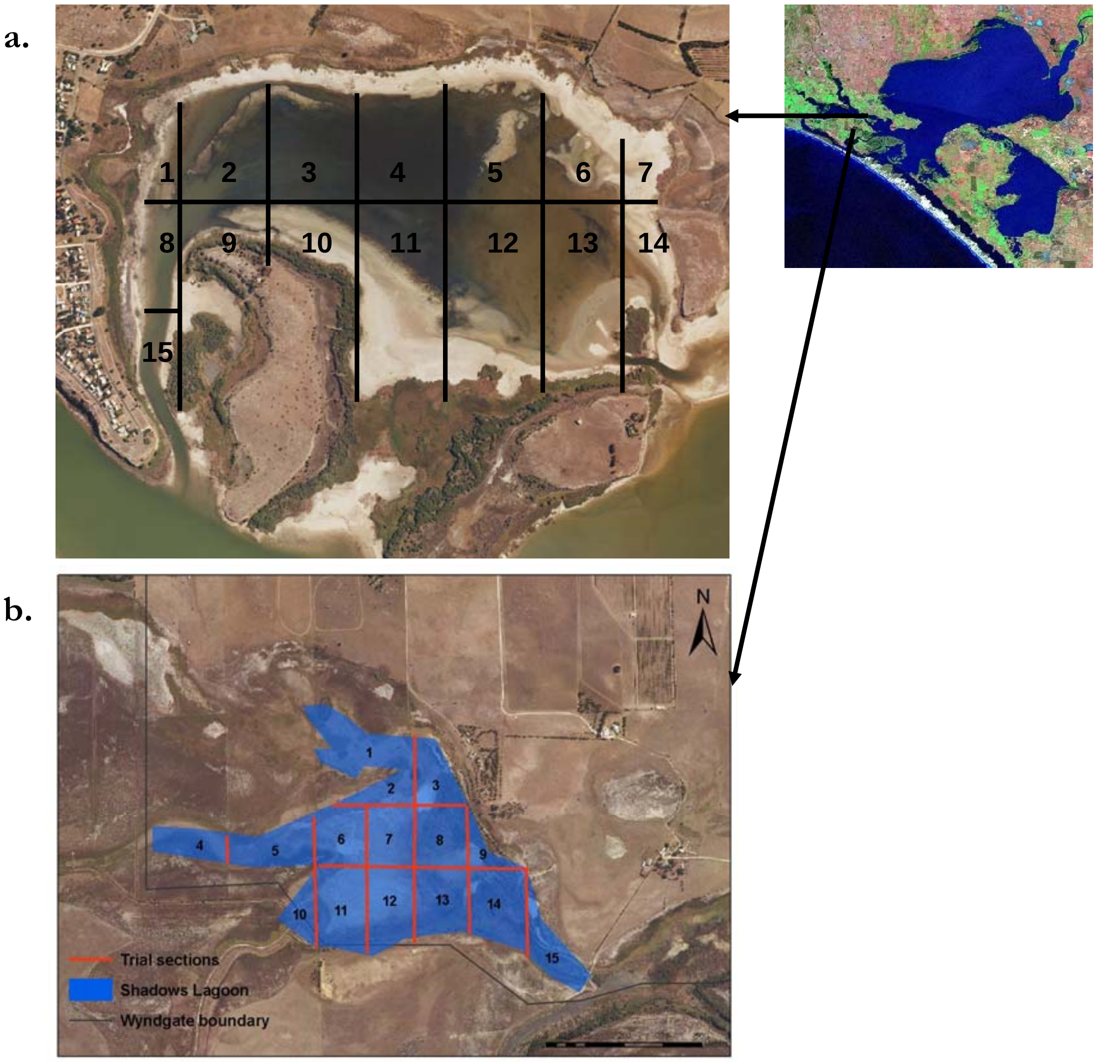

Figure 1: Aerial photographs of a. Dunn's and b. Shadow's Lagoons showing sediment sampling sites.

2.2. Sediment Sampling and Pre-treatment

The area below 0.4 m AHD in each wetland was divided into 15 sections (Figure 1). In each section 20 samples of the top 5 cm of sediment were collected using a hand trowel and pooled. Sediment samples were transported to South Australian Aquatic Sciences Centre, air dried (40°C) to a constant weight and gross organic matter (roots, rhizomes and stems) removed. Subsamples (from each section, for each salinity treatment and from each wetland *n*=15) of a known mass were spread over sterile 80:20 sandy loam contained in 30 cm diameter pots (*sensu* Nicol *et al.* 2003). In addition, a subsample of soil from each section was taken to calculate soil bulk density to determine seed densities (seeds m^{-2}).

2.3. Experimental Design and Protocol

Seed bank composition was determined using the seedling emergence technique (e.g. Leck and Graveline 1979; Haag 1983; Roberts 1986; Thompson 1987; Poiani and Johnson 1988; Gross 1990). A known mass of sediment was spread evenly over a base of 15 cm of sterilised 80:20 sandy loam contained in 22.5 cm (height) x 30 cm (diameter) nursery bags. Prior to addition of the seed bank, a slow release fertiliser (Osmocote N:P:K=17.4:4.4:8.8 plus micronutrients, Scott's Sierra Horticultural Products, Marysville, OH) was added to the sandy loam to give a loading rate of 100 kg N m^{-2} $year^{-1}$ to ensure there was no nutrient limitation for plant growth (*sensu* Nicol *et al.* 2003). In addition to the seed bank samples, three pots containing only sandy loam were placed in each treatment to determine if there was any contamination of the soil or external seed inputs. Sediment samples were submerged in three outdoor tanks to a depth of 20 cm with water at the August 2010 modelled electrical conductivity of Lake Alexandrina (6,400 $\mu S.cm^{-1}$), Goolwa Channel (11,000 $\mu S.cm^{-1}$) and a freshwater control (<1,000 $\mu S.cm^{-1}$). The desired conductivity for each treatment was achieved by mixing the appropriate volumes of seawater and freshwater in separate tanks then adding it to the experimental tanks. Water level was kept static for the duration of the experiment. Water quality was measured weekly (more frequently during periods of hot weather and after rain) and adjusted to maintain appropriate conductivities for each treatment throughout the study period. The trial ran for 16 weeks from February 1st to May 17th 2010.

Seedlings were identified, counted and removed fortnightly. Seedlings that could not be identified were transplanted and grown to a stage at which they could be identified. Seed density (seeds m^{-2}) was calculated using the following equation (Nicol 2004):

$$\text{total number of seeds}\left(m^{-2}\right) = \frac{\text{number of germinants}\left(m^{-2}\right) \times \text{mass of sediment (g)}}{\text{sample mass (g)}}$$

2.4. Plant Identification and Nomenclature

Plants were identified using keys in Cunningham *et al.* (1981), Jessop and Tolken (1986), Prescott (1988), Sainty and Jacobs (1981; 2003), Dashorst and Jessop (1998) and Romanowski (1998). Nomenclature follows Barker *et al.* (2005).

2.5. Data Analysis

Differences in the number of germinants and floristic composition that germinated from the submergent plant seed bank between wetlands and salinities were analysed using NMS ordination (McCune *et al.* 2002), two-factor PERMANOVA (Anderson 2001; Anderson and Ter Braak 2003) and indicator species analysis (Dufrene and Legendre 1997) using the packages PRIMER version 6.1.12 (Clarke and Gorley 2006) and PCOrd version 5.12 (McCune and Mefford 2006). Euclidean distances were used to calculate the similarity matrix for PERMANOVA analysis of the number of germinants and Bray-Curtis (1957) similarities were used to calculate the similarity matrices for all other multivariate analyses. α for all statistical analyses =0.05.

3. Results

A total of 11 taxa from six families were present in the submergent plant seed banks of Dunn's and Shadow's Lagoons (Figure 2, Appendix 1). All taxa germinated from the Shadow's Lagoon seed bank in all salinity treatments (Figure 2, Appendix 1). *Vallisneria australis* and *Ruppia megacarpa* were absent from Dunn's Lagoon seed bank (in all treatments) and *Crassula helmsii* did not germinate in the 6,400 μS.cm^{-1} and 11,000 μS.cm^{-1} treatments (Figure 2, Appendix 1).

PERMANOVA results showed that a significantly higher number of seeds germinated from Shadows Lagoon sediments compared with Dunn's Lagoon (Table 1, Figure 3). In addition, there was a significant difference in the number of seeds that germinated between salinity treatments but there was no significant interaction between wetland and salinity (Table 1). However, the differences in the number of germinants between salinity treatments were not consistent between wetlands. In Dunn's Lagoon there was no significant difference between the number of germinants from the fresh and 11,000 μS.cm^{-1} treatments but a significantly smaller number of seeds germinated in the 6,400 μS.cm^{-1} treatment (<1,000 μS.cm^{-1}=11,000 μS.cm^{-1}>6,400 μS.cm^{-1}). In Shadow's Lagoon a significantly higher number of seeds germinated in the 11,000 MS.CM-1 treatment compared with the fresh and 6,400 μS.cm^{-1} treatment. There was no significant difference in the number of germinants between the fresh and 6,400 μS.cm^{-1} treatments (11,000 μS.cm^{-1}>6,400 μS.cm^{-1}=<1,000 μS.cm^{-1}).

Table 1: PERMANOVA results comparing the number of germinants from both wetlands and all salinity treatments.

Factor	df	*Pseudo-F*	*P*
Wetland	1,89	62.27	0.001
Salinity	2,89	3.31	0.04
Wetland x Salinity	2,89	1.53	0.24

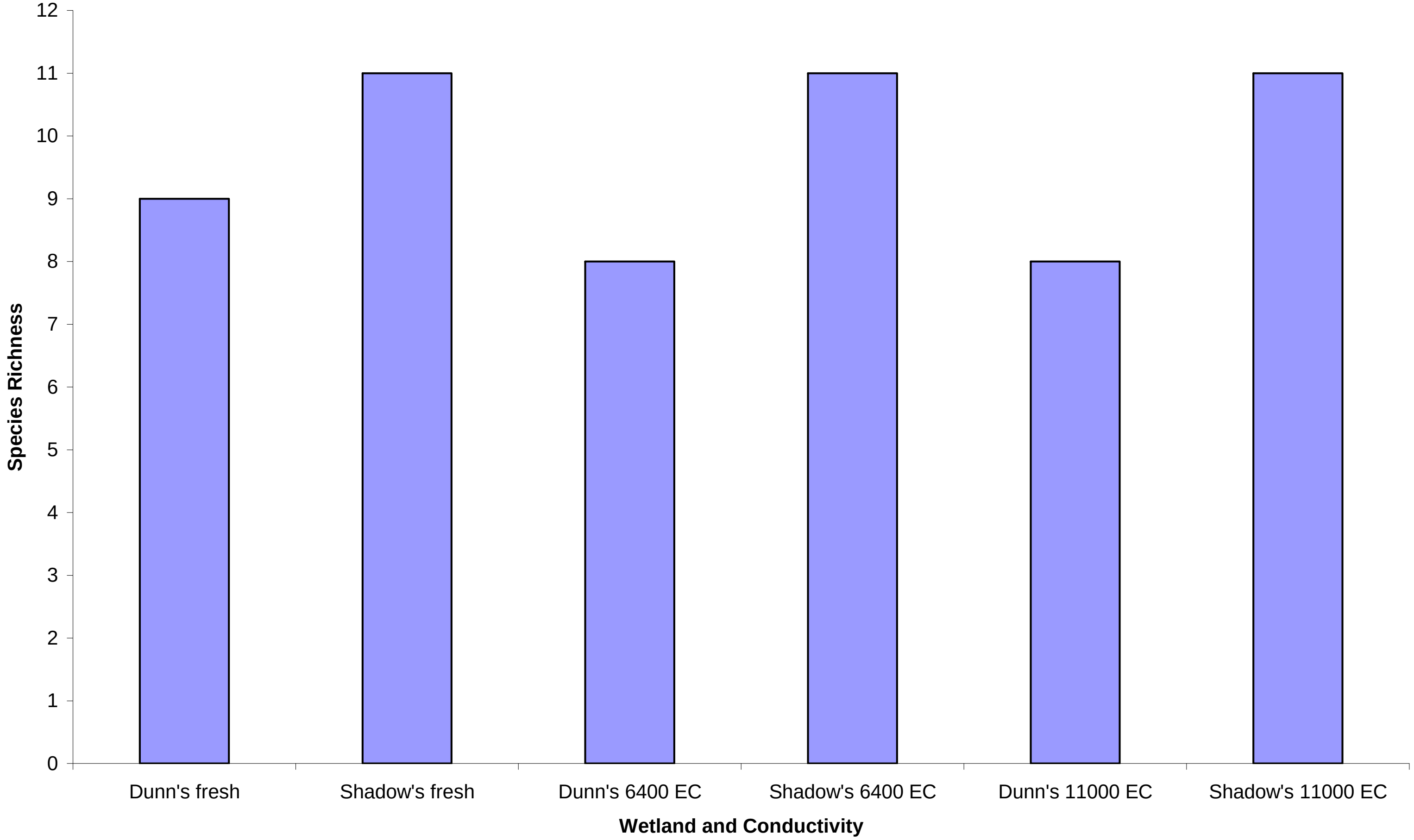

Figure 2: Submergent plant seed bank species richness for each wetland and salinity treatment.

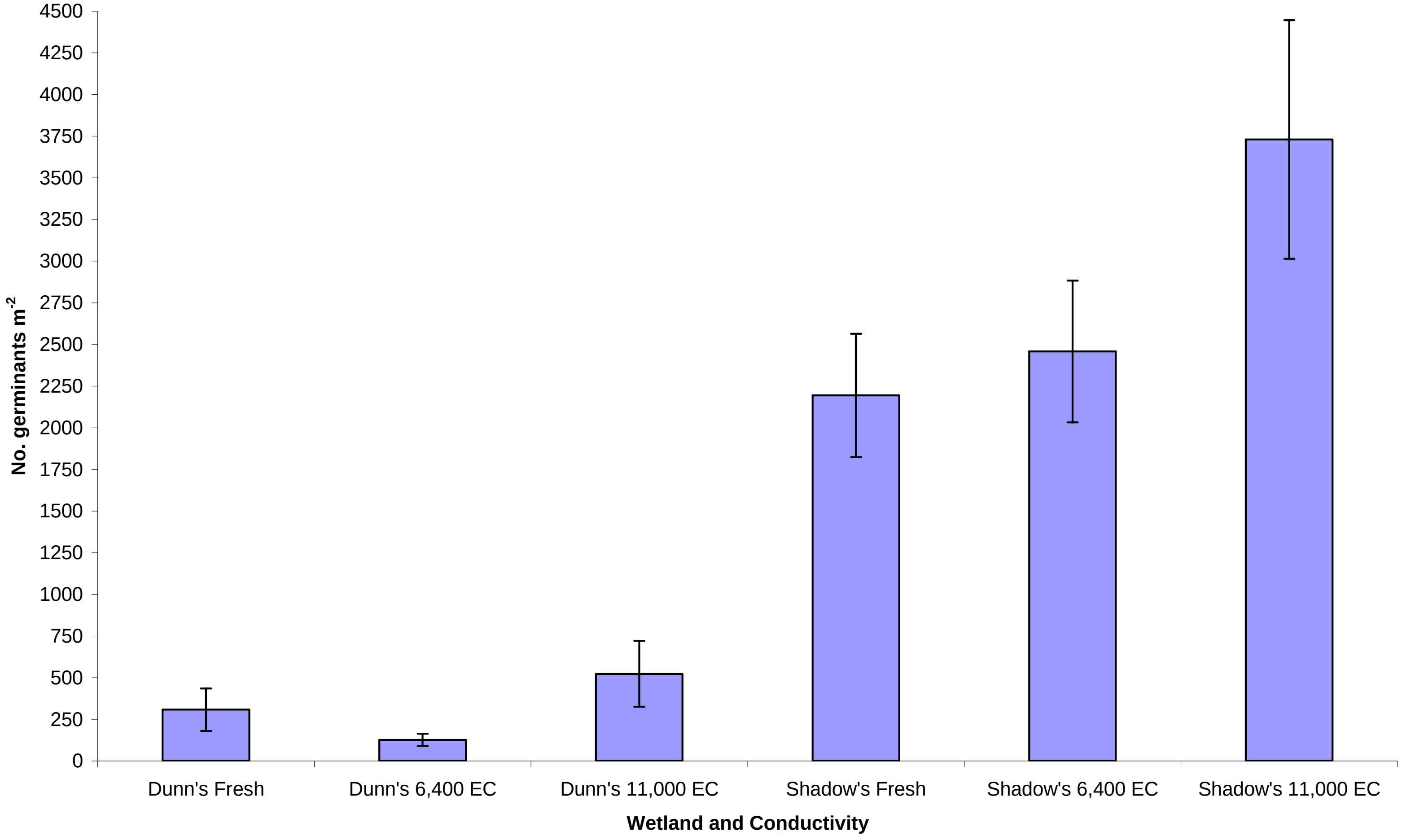

Figure 3: Mean number of germinants (germinants m^{-2}) for each wetland and salinity treatment (error bars=± 1 S.E.).

The species composition that germinated from the submergent seed bank of Dunn's and Shadow's Lagoons was significantly different but there was no significant difference between salinity treatments or a significant interaction (Table 2, Figure 4).

Table 2: PERMANOVA results comparing the species composition that germinated from the submergent seed bank from Dunn's and Shadow's Lagoons under different salinity treatments.

Factor	df	*Pseudo-F*	*P*
Wetland	1,89	13.91	0.001
Salinity	2,89	0.67	0.817
Wetland x Salinity	2,89	1.25	0.212

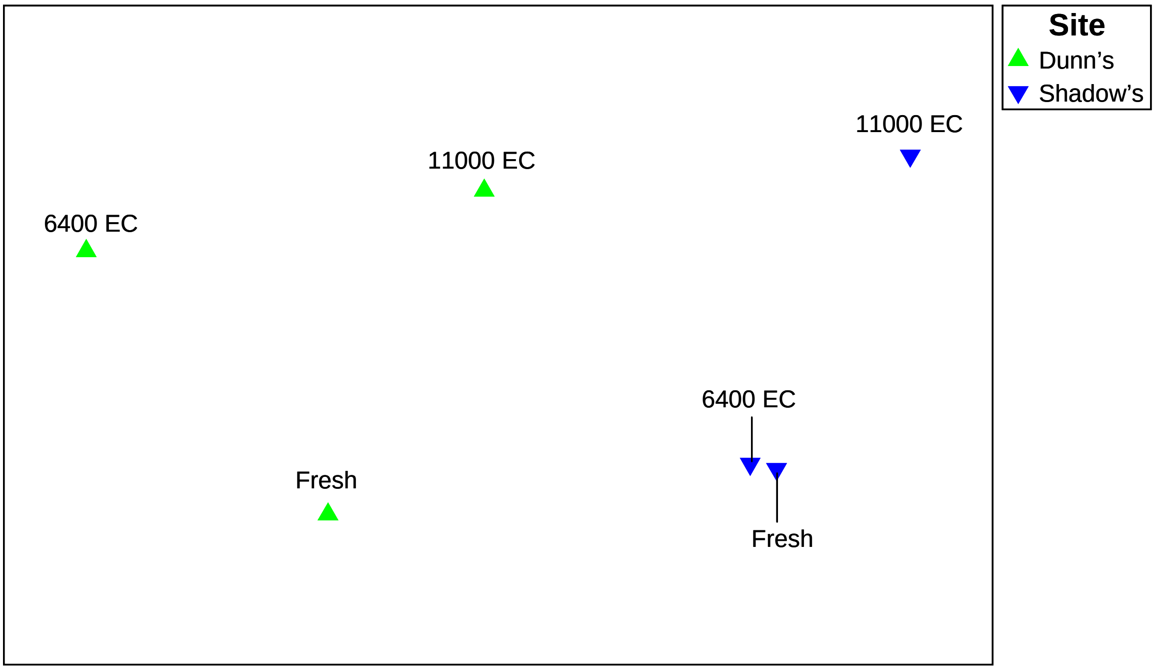

Figure 4: NMS ordination comparing the floristic composition of the submergent seed bank of Dunn's and Shadow's Lagoons under three salinity treatments (stress=0.03).

The differences between the submergent seed banks of Dunn's and Shadow's Lagoons was due to significantly higher abundances of all species present except *Crassula helmsii* in Shadow's Lagoon (Table 3).

Table 3: Indicator species analysis results comparing the species composition that germinated from the submergent seed bank from Dunn's and Shadow's Lagoons under different salinity treatments (statistically significant values in bold).

Species	Wetland	*Monte Carlo P*
Chara fibrosa	Shadow's Lagoon	**<0.001**
Chara sp.	Shadow's Lagoon	**<0.001**
Crassula helmsii	Shadow's Lagoon	0.113
Myriophyllum salsugineum	Shadow's Lagoon	**0.046**
Nitella sp.	Shadow's Lagoon	**<0.001**
Potamogeton pectinatus	Shadow's Lagoon	**0.019**
Ruppia megacarpa	Shadow's Lagoon	**<0.001**
Ruppia polycarpa	Shadow's Lagoon	**<0.001**
Ruppia tuberosa	Shadow's Lagoon	**<0.001**
Typha domingensis	Shadow's Lagoon	**<0.001**
Vallisneria australis	Shadow's Lagoon	**0.013**

There was no effect of salinity on the timing of species germinating from the seed bank (and no difference between wetlands); nevertheless, different species required different periods of inundation to break dormancy. *Typha domingensis* was generally the first species to germinate, with seedlings present within two weeks (germination continued throughout the study period). Charophytes, *Ruppia* spp. and *Potamogeton pectinatus* germinated after being inundated for four to six weeks and *Myriophyllum salsugineum* and *Vallisneria australis* were not detected until week eight. *Crassula helmsii* was present in low numbers and not recorded until week 16 (this species may have germinated earlier but plants were small and not noticed until pots were removed from the water).

4. Discussion and Management Implications

Results from this study showed that there is a larger and more species rich submergent (and emergent) plant seed bank in Shadow's Lagoon compared with Dunn's Lagoon and there was no significant effect of salinity (up to 11,000 µS.cm^{-1}) on germination from the seed bank. The submergent plant seed bank in Shadow's Lagoon is significantly larger and more species rich than Dunn's Lagoon and in the current situation of low water availability (especially for environmental outcomes) the maximum benefit would be gained by providing water for Shadow's Lagoon. Furthermore, minimal engineering works are required for Shadow's Lagoon to hold water (an upgrade the culvert on the inlet channel to enable stop logs or a sluice gate to be fitted is all that is required). In contrast, for Dunns Lagoon to hold water, banks will need to be constructed at the upstream and downstream ends of the wetland that will need to be removed when lake levels rise.

Results show that the source of the environmental water may not be important because a greater number of seeds germinated in the 11,000 µS.cm^{-1} treatment (the modelled salinity in Goolwa

Channel in August 2010) for both wetlands. However, results from the Goolwa Channel seed bank showed that there was a significant decrease in the number of germinants and species richness when salinities exceeded 5,000 $\mu S.cm^{-1}$ (Nicol and Ward in prep.). Brock *et al.* (2005) reported that the impact of elevated salinity on germination from the seed bank was significantly lower in submerged treatments compared with waterlogged treatments because salt accumulated in the waterlogged treatments, which resulted in increase soil salinity, but was flushed from the soil in submerged treatments where salinity remained constant. Nevertheless, salinity does not remain constant in nature especially in wetlands that are disconnected. As there is no flushing to remove salt from the system through time salinity will rise due to evapoconcentration, which may exceed the salinity tolerance of some species before they have competed their life cycle. Furthermore, as salinity increases the time required for germination of many submergent species increases (Sim *et al.* 2006; Nicol and Ward in prep.) and a longer hydroperiod is required for plants to complete their life cycle and replenish the seed bank. Therefore, the lowest salinity water available is the preferable option for the source of the environmental water.

Regardless of the salinity of the environmental water, the wetland will need to be inundated for at least six but preferably nine months to enable plants to complete their life cycle and replenish the seed bank. *Typha domingensis*, charophytes and *Ruppia* spp. have the ability to colonise areas more rapidly than *Myriophyllum salsugineum* and *Vallisneria australis*, which are adapted to permanent (or semi-permanent) water regimes (Cunningham et al. 1981; Sainty and Jacobs 1981; Sainty and Jacobs 2003). Hydroperiods of less than six months may result in seed bank depletion of some species and compromise recovery when water levels return to historical levels.

Nevertheless, there is potential for submergent (and emergent) species to recolonise both wetlands from the in situ seed bank if water levels are reinstated to historical levels. Submergent species are present in the seed banks of both wetlands and whilst initial colonisation of Dunn's Lagoon will probably be slower than Shadow's Lagoon, all of the species in the seed bank are capable of asexual reproduction (Sainty and Jacobs 1981; Sainty and Jacobs 2003). Once established from seed, plants are capable of rapidly increasing their numbers by asexual reproduction (*sensu* Grace 1993) and through time the cover of submergent plants may not differ between wetlands.

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6. Appendices

Appendix 1: Species list of the submergent plant seed banks of Dunn's and Shadow's Lagoons from all salinity treatments.

Species	Family	Dunn's Lagoon	Shadows Lagoon
Chara fibrosa	Characeae	*	*
Chara sp.	Characeae	*	*
Crassula helmsii	Crassulaceae	*	*
Myriophyllum salsugineum	Haloragaceae	*	*
Nitella sp.	Characeae	*	*
Potamogeton pectinatus	Potamogetonaceae	*	*
Ruppia megacarpa	Potamogetonaceae		*
Ruppia polycarpa	Potamogetonaceae	*	*
Ruppia tuberosa	Potamogetonaceae	*	*
Typha domingensis	Typhaceae	*	*
Vallisneria australis	Hdyrocharitaceae		*