Resilience of Biota in wetlands of intermediate salinity

by

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Abstract

Wetlands of intermediate salinity (10 g/L to 45 g/L) are known to have lower biodiversity than their fresher counterparts but are also known to be highly productive providing habitat for resilient plant, invertebrate and fish species. However, many of these species are unable to tolerate hypersaline conditions (> 45 g/L) and if these wetlands become hypersaline their ecological and conservation value rapidly decreases. This study investigated the changes in salinity and watering regimes of wetlands of the northwestern Victoria overtime, the salinity thresholds and sub lethal effects of increased salinity for the loss of aquatic marcophyte and invertebrate communities, the effect of drying periods on the propagule banks of wetlands of intermediate salinity.

Historical and current distributions of wetlands of intermediate salinity in the Kerang, Lake Charm and Lake Boga regions were examined. Results indicated that the abundance of these wetlands and their biota has decreased since European settlement, in response to increased salinity and changes to watering regimes. The remaining permanent wetlands of intermediate salinity in the region were surveyed and results showed that the diversity of aquatic macrophyte and fish species was low. However three wetlands supported populations of the threatened fish species *Craterocephalus fluviatilis* McCulloch 1912 (Murray hardyhead).

Propagule bank experiments were conducted on the sediments of an ephemeral wetland of an intermediate salinity in the region to investigate the effect of increasing salinity on the emergence of aquatic macrophyte and invertebrate species. Results showed that species present in the propagule bank were resilient to short term salinity increases and were able to re-establish at lower salinities. The majority of aquatic macrophyte and invertebrate species present were tolerant of salinity treatments up to and including 37.7 g/L.

Studies were conducted on *Ruppia megacarpa* seeds to investigate the effect of salinity, photoperiod, temperature, seed source and drying periods on their germination. While germination rates were low (< 35%), the presence of substrate, increased temperature and lower salinities (< 45 g/L) had a significant positive effect on germination of *R. megacarpa*. The information gained from these studies will assist managers in designing improved watering regimes and management plans for these remaining wetlands of intermediate salinity to ensure maximum biodiversity is maintained in the region.

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1.0 Salinity and its effects on biodiversity

1.1 Introduction

There are two types of saline wetland systems found within Australia. Those wetland systems that are naturally saline (primary salinisation), and those that are affected from rising water tables caused by anthroprogenic changes to the landscape (secondary salinisation) (Davis *et al.*, 2003; Strehlow *et al.*, 2005). Wetlands that are naturally saline (primary salinisation) are often very productive and can be areas of high ecological and conservation value, whereas secondary salnised wetlands are often degraded (Timms, 1993; Williams, 1993b; Williams, 1993a; Timms, 1997; Timms, 1998b; Timms, 1998a; Strehlow *et al.*, 2005; Timms, 2005; Bailey *et al.*, 2006). Secondary salinity has been recognised as an increasing problem throughout Australia and it has been reported that currently around 252 700 hectares in Victoria are effected by dyland salinity and that by 2050, almost 14% of the total area of Victoria will be affected by increased salinity (Morgan, 2001; Blinn *et al.*, 2004). Salinity has been shown to have adverse effects on aquatic biodiversity in many regions across Australia including Victoria, and the southwest of Western Australia (Brock and Lane, 1983; Brock and Sheil, 1983; Hart *et al.*, 1990; Hart *et al.*, 1991; James *et al.*, 2003; Nielsen *et al.*, 2003b; Nielsen and Brock, 2009; Beatty *et al.*, 2011).

There are two forms of secondary salinity that can affect landscapes: dryland salinity and irrigation salinity. Dryland salinity is caused by the loss of deep rooted vegetation, often as a result of landclearing. Widespread clearing of deep rooted trees and vegetation in agricultural areas of Australia has occurred and native vegetation has been replaced with shallow rooted grasses. These shallow rooted pastures do not absorb as much water as the deep rooted native vegetation therefore excess water enters the water table and causes the water table to rise towards the surface. Water tables of much of the interior regions of Australia are naturally salty, and as such can cause widespread salinity issues as the water table rises (Aplin, 1998). Irrigation salinity is caused by the build of up salts at or near the surface of the soil in irrigated areas and is often caused by the application of large volumes of water to areas without adequate drainage. This again can cause a rise in the water table and an accumulation of salts near the surface. Run off from irrigated areas can also contain

high salt loads, thus increasing the salinity concentrations of streams and other waterways (Aplin, 1998).

Salinity can have differnt types of adverse effects on aquatic biota, the first being direct toxic effects through physiological changes, particularly changes caused by the stress placed on osmoregulation. The second being indirect effects, caused by the modification of ecosystem's species composition and the loss of species that the community relies on for food as well as habitat (ANZECC and ARMCANZ, 2000; Nielsen *et al.*, 2003b). Changes to the environment caused by salinity can also impact biota (Bailey *et al.*, 2006; Boon, 2006). For example with increased salinity, suspended clays tend to fall out of suspension in the water column causing increased water clarity. Salinity is also known to reduce dissolved oxygen concentations in water and is also known to be associated with lower pH. Secondary salinity is often associated with higher loads of sulphates and can lead to the production of acid sulphate sediments (Bailey *et al.*, 2006; Boon, 2006).

The impact of the effects of salinity on freshwater biota has been extensively reviewed (Hart *et al.*, 1990; Hart *et al.*, 1991; Metzeling *et al.*, 1995; James *et al.*, 2003; Nielsen *et al.*, 2003b; Nielsen and Brock, 2009). However our knowledge of the ecological consequences of increased salinisation in Australian freshwater systems and the sublethal effects of salinity is limited to some knowledge on few species and few studies have been completed investigating the effects of salinity on ecosystem functioning (Nielsen *et al.*, 2003b) (Table 1.1).

Table 1.1 Known effects of salinity on the major biotic taxa of freshwater systems in Australia, in taxonomic order

Taxa	Salinity Tolerance	References
Algae	Majority of algae do not appear to be tolerant of salt concentrations > 10 g/L, although there are exceptions e.g. *Dunaliella salina* and many diatom species	Blinn *et al.,* (2004), Neilsen *et al.,* (2003b), James *et al.,* (2013)
Benthic microbial mats	Tend to dominate at salinities > 50 g/L and be can be found in wetlands with lower salinities (approximately 12 g/L).	Herst and Blinn (1998) Sim *et al.,* (2006b), Sim *et al.,* (2006c)
Macrophytes	Many submerged freshwater macrophyte species experience lethal or sublethal effects at salt concentrations of 1 to 2 g/L and most freshwater species disappear from aquatic systems at salinities > 4 g/L. Exceptions to this include *Lepilaena* spp. and *Ruppia* spp. suggesting that halophyte species have an upper tolerance around 45 g/L	Bailey (1998), Bailey and James (2000), James *et al.*, (2013) Hart *et al.,* (1991), Metzeling *et al.,* (1995), Neilsen *et al.,* (2003b), Sim *et al.,* (2006a)
Riparian Vegetation	Affected at salinities > 3 g/L Increased salinity will affect non-halophytic plants. Increased salinity decreases riparian plant diversity	Hart *et al.,* (1991), Lymbery *et al.,* (2003)
Macro-invertebrates	The effect of salinity on this group is well researched using both field observations and toxicity tests. Reductions in the abundance of many animals within this group becomes apparent once salinity is > 1 g/L. Each phyla of invertebrates contain species that are highly sensitive to increases in salinity. However substantial changes in the diversity of wetland macroinvertebates only occurs in salinities ≥ 10 g/L.	Bailey (1998), Bailey and James (2000), Halse *et al.,* (1998), Hart *et al.,* (1991), Kefford *et al.,* (2007) Metzeling *et al.,* (1995)
Fish	Tolerant between 7 and 13 g/L. Adults of most fish associated with lowland rivers appear to be tolerant of high salinities, but juveniles and eggs of some species are known to be susceptible to concentrations > 10 g/L	Beatty *et al.,* (2011) Hart *et al.,* (1991), James *et al.,* (2003), Metzeling *et al.,* (1995)
Amphibians	Little information on the impact of salinity on amphibians, however one study on tadpoles reports that no tadpoles were found in waters > 3.84 g/L.	Hart *et al.,* (1991), Smith *et al.,* (2007)
Waterbirds	May not be directly affected. Indirectly, the loss of riparian vegetation, macrophytes and invertebrates may change the distribution of many birds. Many species are able to feed in saline wetlands but need freshwater nearby to drink. Salinities > 3 g/L may affect breeding success.	Hart *et al.,* (1991), Kingsford *et al.,* (1994), Timms (2009)

Given the nature of salinity in Australia there have been many studies on the effects salinity has on freshwater biota focusing on the impact of toxicity on plants and macroinvertebates (Hart *et al.*, 1990). Studies have observed that with increased salinity there is a decrease in biodiversity (Brock and Lane, 1983; Hart *et al.*, 1990;

Williams *et al.*, 1990; Hart *et al.*, 1991; Williams, 1998a; Brendonck and Williams, 2000; Williams, 2001; Williams, 2002). While an increase in salinity may reduce overall biodiversity, the effects of salinity on particular taxa can be very different. Hart *et al.,* (1990) found that micro-algae, plants, and macroinvertebrates were the taxa most sensitive to salinity changes. There are however some species within these taxa, that are a very salt tolerant, Hart *et al.,* (1991) and James *et al.,* (2003), reported that the aquatic macrophytes *Ruppia* (Widgeon grass)*, Lepilaena* (Watermat) and the charophyte *Lamprothamnium* (Stonewort) genera can tolerate concentrations of salinity in excess of 10 g/L. Fish and birds are also less affected by salinity increases because of their mobility; thus enabling them to swim or fly away from areas of high salinity. In the case of waterbirds they have a distinct advantage over other genera in that they are able to move easily from one water body to the next. It is generally accepted that fish, for example *Bidyanus bidyanus* Mitchell 1838 (Silver perch), *Hypseleotris klunzingeri* Ogilby 1898 (Western carp gudgeon) *Maccullochella macquariensis* Cuvier 1829 (Trout cod), *Macquaria australasica* Cuvier 1830 (Macquarie perch) and *Macquaria ambigua* Richardson 1845 (Goldern perch) can tolerate salinity concentrations up to 10 g/L (Hart *et al.*, 1991; Metzeling *et al.*, 1995; Clunie *et al.*, 2002; Nielsen *et al.*, 2003b).

But these salinity tolerance values need to be treated with caution, as there has been little research on the sublethal effects of salinity on both plants and animals and further research is required in this area (Hart *et al.*, 1991; James *et al.*, 2003). The majority of studies have only focused on salinity thresholds and tolerance levels of adult life stages and have not considered juvenile life stages, seeds or the effects of salinity on plant growth and vigour (Hart *et al.*, 1991; James *et al.*, 2003). O'Brian and Ryan (1997), found that the early stages of development in *M. australasica* were more susceptible to increases in salinity than adult life stages. Adult fish have a salinity tolerance of more than 30 g/L, but egg survivorship was reduced by 100% at a salinity of only 4 g/L. Therefore while some biota may appear to be tolerant of salinity above 10 g/L, early life forms are potentially at risk at lower salinity concentrations (O'Brian and Ryan, 1999). Sublethal effects have also been reported in plant species, for example Hart *et al.,* (1991), found that increases in salinity effected the germination and growth of *Phragmites australis* (Cav.) Trin ex Steud (Common reed). While James and Hart (1993), reported different sublethal effects on four macrophyte species: *Myriophyllum crispaturn* Orchard (Upright water milfoil),

Eleocharis acuta R.Br. (Common spike-sedge), *Potamogeton tricarinatus* F.Muell and A. Benn ex A. Benn (Floating pondweed), and *Triglochin procera* R.Br. (Water ribbons), from the same freshwater community as a result of increased salinity concentrations.

While the lethal, and in some cases sublethal effects of salinity are well known for many individual species, researchers are now focusing on defining the thresholds of salinity tolerance at a community level (James *et al.*, 2003; Sim *et al.*, 2006a). As salinity concentrations rise, the biotic communities respond in two ways: the first being that the most sensitive species are lost from ecosystems, and secondly that tolerant species become dominant (Hart *et al.*, 1991; James *et al.*, 2003). Researchers have long known that the relationship between the loss of biodiversity in response to increased salinity is not linear (Williams *et al.*, 1990; Williams, 1998c). Williams *et al.,* (1990), found that the loss of biodiversity and increased salinity was not significant across intermediate salinity concentrations as many species have a broad salinity tolerance. Williams *et al.,* (1990) also noted that the relationship between salinity and biodiversity at a community level might not necessarily be matched by the responses of individual taxa to increased salinity. It has been observed that with an increase in salinity, freshwater communities with a diverse range of species change to a system dominated by a few macrophyte species (James *et al.*, 2003). With further increases in salinity it has also been suggested that phytoplankton or macrophyte dominated wetlands may change to systems dominated by microbial mats, composed mainly of cyanobacteria and halophytic bacteria (Strehlow *et al.*, 2005; Sim *et al.*, 2006b; Sim *et al.*, 2006c).

Many studies have focused on how to classify waterways on the basis of their salinities and terms such as “freshwater”, “”, “saline”, “hyposaline”, “mesosaline” and “hypersaline” have been used, yet the salinity range for each category often differs between studies and can be arbitary (Hammer, 1986). For the purposes of this study, the classification of waters follows Davis *et al.,* (2003) and Sim et al., (2003a) (Table 1.2). Intermediate saline wetlands are those with salinity concentrations between 10 g/L and 45 g/L where submerged aquatic macrophyte communities are able to exist and support a variety of invertebrate and vertebrate species.

Table 1.2 Classification of wetlands on the basis of salinity concentrations (Davis *et al.*, 2003; Sim *et al.*, 2006a)

Category	Salinity (g/L)
Freshwater	< 3
Hyposaline	3 to 10
Saline (Intermediate)	10 to 45
Hypersaline	> 45

1.2 Models for predicting the effects of increased salinity on biodiversity

The response of ecosystems to changing conditions can vary from smooth and continual to discontinuous (Scheffer and Carpenter, 2003; Gordon *et al.*, 2008; Davis *et al.*, 2010), depending on the type of ecosystem and the condition being investigated. Figure 1.1 illustrates how ecosystems can respond differently to changes in a particular condition: Figure 1.1 (1) shows a continual smooth response to a change in conditions, if the stress is removed the ecosystem returns to its original state with a continual smooth response. Figure 1.1 (2) shows how an ecosystem may change abruptly from one stable state to the next at a given threshold, if the stress is removed, again the ecosystem can return its original state, but recovery will only occur if the level of stress is lower than the threshold. Figure 1.1 (3) again shows how an ecosystem may change abruptly at a given threshold, however unlike Figure 1.1 (2), the ecosystem cannot return to its original state. Figure 1.1 (4) also shows how an ecosystem can change abruptly at a given threshold, but unlike Figures 1.1 (2) and 1.1(3), no recovery to any improved state is possible once the ecosystem has collapsed.

Much research in past years has focused on determining if the alternative stable states model is an appropriate way of describing how shallow wetlands in Australia respond to fluctuations in salinity (Davis *et al.*, 2003; Strehlow *et al.*, 2005; Sim *et al.*, 2006a; Sim *et al.*, 2006b; Sim *et al.*, 2006c; Gordon *et al.*, 2008; Davis *et al.*, 2010).

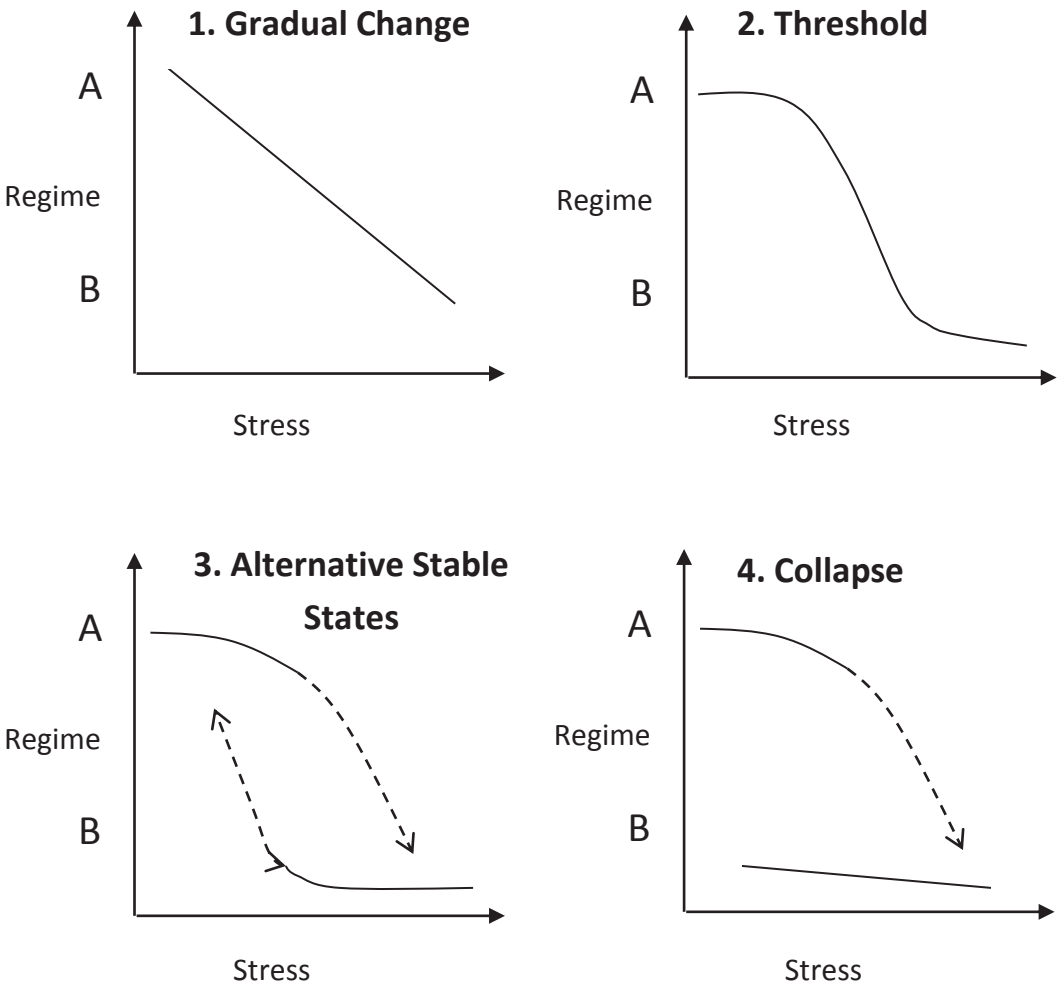


Figure 1.1 Differing models to show ways in which ecosystems can respond to external stressors such as salinity – modified from Gordon *et al.,* (2008) and Davis *et al.,* (2010). A and B refer to different ecological regimes

The alternative stable states theory is used to explain how a community or ecosystem changes dramatically from one state to another (May, 1977; Carpenter, 2003). For example, in aquatic ecosystems it has been commonly used to show how a macrophyte dominated wetland can become a eutrophic phytoplankton dominated wetland when nutrients from the catchment are added (Carpenter, 2003; Scheffer and Carpenter, 2003; Strehlow *et al.*, 2005). Usually, a population or even an ecosystem fluctuates around a trend or stable average so not all changes in ecosystems can be attributed to the alternative stable states theory (Scheffer and Carpenter, 2003). However, ecosystems can be impacted by an abrupt change resulting in a shift to a different state (Scheffer and Carpenter, 2003). The alternative stable states theory has also been used to explain how wetlands and coral reefs change dramatically in response to eutrophication (McClanahan *et al.*, 2002; Mumby *et al.*, 2007). Additionally it has been applied to the way in which freshwater fish populations

respond to overfishing, and terrestrial ecosystems where slow changes have resulted in the loss of vegetation in grazed ecosystems (May, 1977; Rietkerk and van de Koppel, 1997; Folke *et al.*, 2004). In Australia the alternative stable states model has been used to explain the change from submerged macrophytes in wetlands to phytoplankton dominated weltands as a result of increased nutrients to systems (Boon and Bailey, 1998; Morris *et al.*, 2003a; Morris *et al.*, 2003b; Morris *et al.*, 2004). More recently alternative-states models have been considered useful tools for describing stepped rather than linear threshold relationships between the loss of biodiversity and increasing salinity in wetlands (Davis *et al.*, 2003; James *et al.*, 2003).

While catastrophic changes are often attributed to the alternative stable states theory, theoreticians have stressed that even small incremental and often gradual changes in conditions can trigger a dramatic shift in some ecosystems (Folke *et al.*, 2004). This change can occur at a threshold level and if the threshold level is known, accurate models can be developed, predictions made, and these ecosystems managed accordingly (Folke *et al.*, 2004). Threshold levels associated with the alternative-stable states theory should be used with some caution as ecosystems may respond dramatically to abiotic or biotic factors other than those suggested in a model. Whether the change in stable states has been due to a small or dramatic change in conditions, the movement back to the original state (i.e. to reverse the change in the ecosystem) is very difficult. Also systems are not guaranteed to return to the conditions experienced in the previous stable state (Beisner *et al.*, 2003; Folke *et al.*, 2004).

Another consideration in the use of these models is that rarely is an ecosystem state driven by one single abiotic factor. Davis *et al.,* (2010) hypothesized that several factors including hydrology, salinity, acidification, and eutrophication are all environmental factors that could potentially cause a shift in stable states in wetlands in Western Australia. Davis *et al.,* (2010) also identified that a shift from one stable state to another may be a result of compounding effects of environmental factors, thus making the modelling of such complex relationships difficult.

Limitations of the alternative states model include the fact that in reality ecosystems are rarely stable, populations tend to fluctuate and environmental conditions are

seldom constant. This can make it hard to establish if a change is due to natural fluctuations or is a shift in stable states. Schröder et al., (2005) distinguishes four experimental approaches to tesing for alternative stable states in ecological systesms being:

- Discontinuity in the response to an environmental driving parameter
- Lack of recovery potential after a perturbation
- Divergence due to different initial conditions
- Random divergence

Research into how alternative stable states relate to salinity in aquatic ecosystems is relatively recent. Davis *et al.,* (2003) suggested that a discontinuous alternative stable states model similar to the one posed by Scheffer (2001) for increasing nutrients, may be how wetlands in south Western Australia respond to increasing salinity, particularly secondary salinity.

Further studies conducted by Strehlow *et al.,* (2005), Sim *et al.,* (2006a) Sim *et al.,* (2006b) and Davis *et al.,* (2010) suggest that the relationship between changes in alternative ecological regimes within saline wetlands in Australia, may be more complicated than the alternative stable states model first posed. Strehlow *et al.,* (2005) stated that there were four ecological regimes in saline wetlands, and that shifts from one regime to another may be caused by increases in nutrients as well as changes in salinity (Table 1.3).

Table 1.3 Criteria defining the four ecological regimes found by Strehlow *et al.,* (2005) for saline wetlands in southwest Australia

Ecological Regime		Turbidity (NTU)	Chorolophyll *a* (μgL^{-1})	Cover of submerged macrophytes (%)	Cover of benthic microbial community (%)
I	**Clear water, macrophyte dominated**	<10	<30	>50	
II	**Clear water, benthic microbial community dominated**	<10	<30		>50
III	**Turbid water, phytoplankton dominated**	>10	>30	<50	<50
IV	**Turbid water, sediment dominated**	>10	<30	<50	<50

Adapted from (Strehlow *et al.*, 2005)

The clear water, macrophyte dominated regime was identified by Sim *et al.,* (2006c) as the most desirable regime in salinising wetlands in Western Australia, as these wetlands support a more diverse range of ecological functions and greater biodiversity.

The criteria used by Strehlow *et al.,* (2005) to define these four regimes include: turbidity, chlorophyll *a* concentration, percentage cover of aquatic macrophytes and percentage cover of benthic microbial communities (Table 1.3). Strehlow *et al.,* (2005) predicted that shifts from a clear water macrophyte dominated regime to a clear water benthic microbial community dominated regime was driven by an increase in salinity. However, shifts from clear water macrophyte dominated regimes to turbid water phytoplankton dominated regimes were driven by increased nutrients. Sim *et al.,* (2006a) concurred that salinity drives the shift from a clear water macrophyte dominated to clear water benthic microbial community dominated regime. Sim *et al.,* (2006a) also found that to maintain a clear water macrophyte dominated regime, salinity concentrations should remain below 45 g/L.

Another study by Sim *et al.,* (2006b) found that hydrology could also affect which regime was present in a wetland, by influencing the formation of benthic microbial mats in temporary wetlands.

1.3 Resilience

Resilience is defined as the ability of the biotic components of the ecosystem to maintain ecological function in the face of disturbance and variability, in this case, salinity concentrations (James *et al.*, 2003; Jin, 2008). Resilience and tolerance are important concepts when considering the alternative stable states and other modelling theories. The resilience of the community determines if the system is able to maintain ecological function during or after a disturbance or disturbances have occurred (Scheffer *et al.*, 2001; Carpenter, 2003). Carpenter (2003), defined resilience as having three different properties: the amount of change a system can undergo, the degree to which the system is self-organising and the degree to which the system can adapt. The tolerance of the community defines the amount of change the ecosystem can withstand before there is a change in stable states or ecological regimes (Scheffer *et al.*, 2001; Carpenter, 2003).

Often an ecosystem can tolerate some change in conditions without significantly altering states and therefore in this scenario, when and if conditions revert back to those first experienced, the ecosystem remains in its original condition (Figure 1.2A). Once the threshold of the system is passed and the ecosystem changes states, a return to the original conditions does not necessarily mean a return to the original state depending on how resilient the ecosystem is (Figure 1.2B).

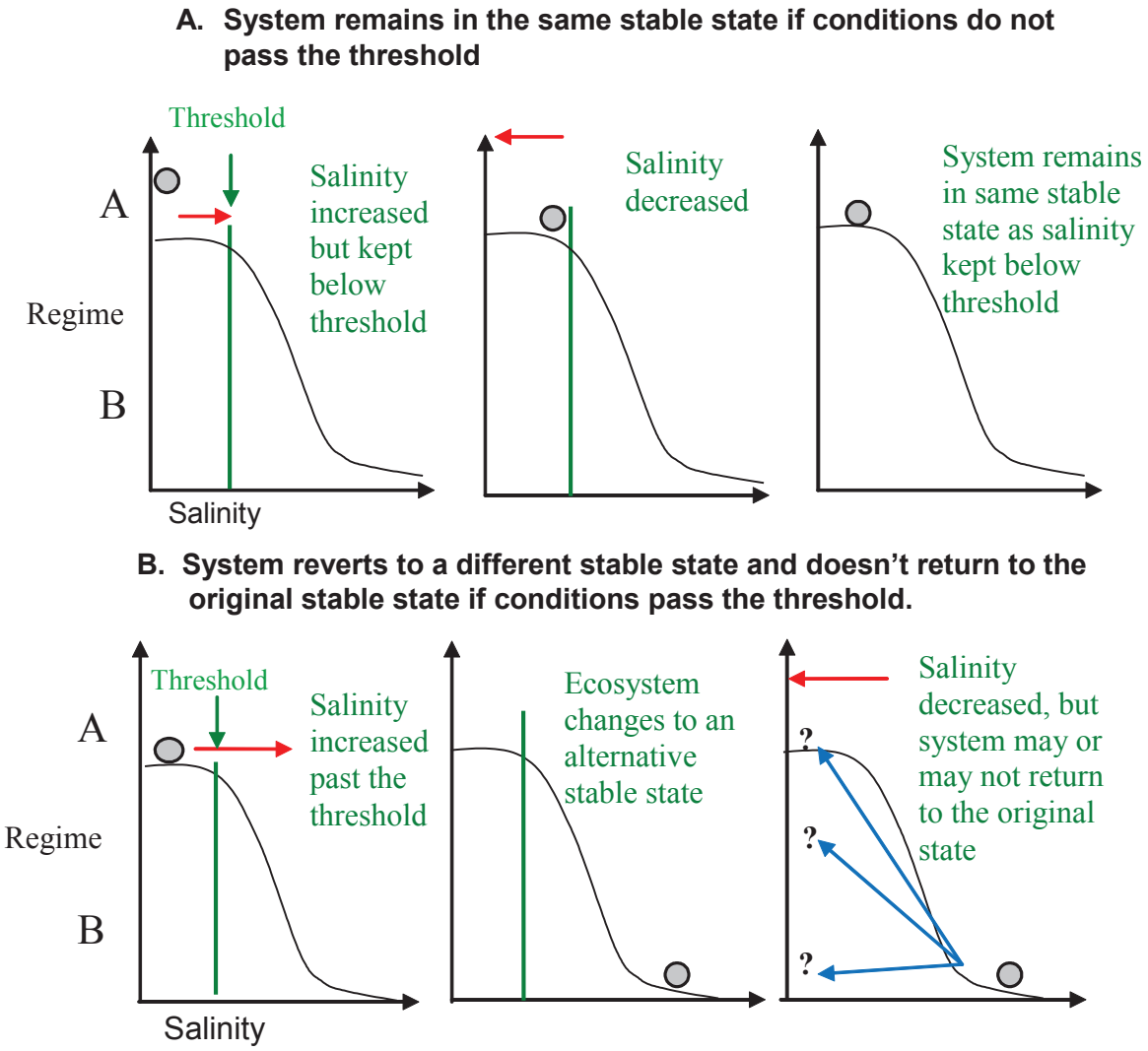


Figure 1.2 Resilience in ecosystems and the shift between stable states. Adapted from Scheffer *et al.,* (2001) and Levin (2009)

By understanding the resilience and thresholds of a community, ecosystems may be effectively managed to reduce the risk of change from one stable to state to the next, particularly if one stable state is more desirable than another. For example, a clear water macrophyte dominant state is generally considered to be more desirable that a benthic microbial mat dominated state as the former supports a higher diversity of biota. Resilience and thresholds of ecosystems are hard to measure and quantify, although Davis *et al.,* (2003) first hypothesized that the threshold for a transition between a macrophyte community and a benthic microbial mat community in Western Australia may be at a salinity concentration of 100 g/L. A more recent study by Sim *et al.,* (2006a) found that the threshold for this transition in saline wetlands in

Western Australia is probably much lower than this and has suggested an upper salinity threshold for macrophyte communities at 45 g/L.

Resilience is also an important concept when considering individual species responses to increased salinity concentrations in aquatic ecosystems. The resilience of a species can differ between populations depending on their past exposure to environmental conditions. Studies on *Eucalyptus camaldulensis* Dehnh have found that seeds obtained from differing soil salinities showed differing resilience to salinity treatments tested, with those seeds from low soil salinity sources having a lower tolerance to raised salinity concentrations (Sands, 1981). Similar results have been found in other Australian plants including members from *Eucalyptus*, *Melaleuca* and *Casuarina* genera (Sands, 1981; Van der Moezel *et al.*, 1989; Van der Moezel *et al.*, 1991). Dixon (2007), also reported that populations of the fish species *Craterocephalus fluviatilis* (Murray Hardyhead) McCulloch, 1913 from different lakes had differing tolerances of raised salinity concentrations. It is important to note that for plants that seeds are not the only method for plant survival and dispersal, especially in weltands. A number of wetland plant species exhibit clonial growth in many different ways including turons, stolons, tubers, rhizomes and plantlets. These methods provide an alternative to seeds which may not always be produced from wetland plants (Grace, 1993).

Aspects of resilience traits that enable organisms to exist in high salinity environments include acclimation and avoidance. When salinity increases gradually within an aquatic system, some organisms are able to acclimatise to the elevated salt concentrations. But these same organisms may not be able to tolerate such elevated salt concentrations if the increases occurred rapidly (Rai and Rai, 1998).

Other species use a range of avoidance strategies including, dispersal to less saline habitats, the use of a less saline microhabitat within a salinising patch, or remaining in a salinising area in a dormant phase until conditions become less saline (for example seeds, asexual progagules and invertebrate eggs that remain in the propagule bank) (James *et al.*, 2003). James *et al.* (2003) in their review of the literature reported that both acclimation and the avoidance mechanisms used by individuals can make it hard to generalise and quantify the tolerance and resilience of an ecosystem because different populations of a particular species may have differing

threshold limits, depending on their location and past exposure to elevated salinity concentrations.

1.4 Biota of wetlands of intermediate salinity

Saline wetlands are often association as being of low value, however many studies have shown that weltands of intermediate salinity do have a number of economic, social, environmental, educational and scientific values (Lugg et al., 1989; Williams, 1993a; 1993b; 1998b; 2001). The flora and fauna of wetlands of intermediate salinity are often characterized by low diversity yet high productivity leading to systems that can support numerous water birds and fish populations (Brock, 1986; Timms, 1993; Kingsford and Porter, 1994).

1.4.1 Aquatic macrophytes

Aquatic macrophytes are an important food source and provide habitat for many species in wetland systems including invertebrates, fish and water birds. Hart *et al.,* (1991), identified plant communities as being the most sensitive of wetland biota to salinity increases. However, as previously mentioned while most aquatic macrophytes are salt sensitive, there are a few species that can tolerate wide salinity ranges. The salt tolerant submerged aquatic macrophytes include *Potamogeton pectinatus* L. (Sago pondweed) which can tolerate salinities of above 10 g/L, many species of *Ruppia* (Wigeongrass) *Lepilaena* (Watermats) some species of which are able to tolerate salinities of above 100 g/L and the charophyte species *Lamprothamnium macropogon* (A. Braun) L.Ophel (Stonewort) which has a tolerance range of 2 to 58 g/L (Brock, 1986; Hart *et al.*, 1991; Garcia, 1999). The salt tolerant species are usually found as a component of macrophyte communities with other species in fresh to hyposaline wetlands (up to 10 g/L). Wetlands of intermediate salinity (10 – 45 g/L) are often characterised by these salt tolerant macrophyte species. Two genera of submerged aquatic macrophytes (*Ruppia* and *Lamprothamnium*), and two gerera of emergent macrophytes were found in wetlands of intermediate salinity in north western Victoria throughout this study.

Four different species of *Ruppia* occur within Australia, three of which are endemic. All species are tolerant of hyposaline to saline waters (3 g/L to 100 g/L), but can also occur in freshwater habitats (Jacobs and Brock, 1982). There have been many studies on the *Ruppia* genus with many focusing on *Ruppia maritima* L. (Wigeongrass).

Ruppia maritima is the most salt tolerant of the angiosperms and it has been suggested that this species can tolerate salinities of over 100 g/L (Hart *et al.*, 1991; Murphy *et al.*, 2003). Studies by La Peyre and Rowe (2003) and Murphy *et al.,* (2003) focussed on the short-term effects of elevated salinity concentrations on this species. Both studies found that short-term changes in salinity concentrations, either increased or decreased concentrations, had few negative effects on *R. maritima*. Murphy *et al.,* (2003) noted that while the initial change in salinity was stressful, this species was able to physiologically adapt after several days. *Ruppia maritima* is able to osmoregulate in low and high salinities by adjusting the amount of proline accumulated within the plant cells, and can more easily adjust when allowed to acclimate at intermediate concentrations, rather than when exposed to more extreme changes in salinity (Murphy *et al.*, 2003). Little work has been done on the impact of salinity on the sensitive life stages of plants (Nielsen *et al.*, 2003b), however it has been identified that salt sensitivity of various life stages of a species may differ (Bailey and James, 2000). Brock (1982a) found that the germination of *Ruppia megacarpa* R. Mason (Large Fruit Tassel) decreased with increasing salinity concentrations, but for *Ruppia tuberosa* J.S. Davis and Toml. (Tuberous Tassel), increased salinity concentrations produced increased germination. This further shows that biota within a taxa can vary in their tolerance to increased salinity concentrations.

There are two *Lamprothamnium* species growing in Australia, but until recently all species in Australia were listed as *Lamprothamnium papulosum* (Wallroth) J. Groves. (Stonewort), and as such there is little ecological information for each individual species (Garcia and Chivas, 2004; Sim *et al.*, 2006a). The two species that occur in Australia: *Lamprothamnium succinctum* (A. Braun) R.D. Woods (Stonewort), is found in coastal lagoons, and *L. macropogan*, is widespread in saline wetlands, particularly in Victoria (Garcia and Chivas, 2004). *Lamprothamnium macropogan* is found in shallow alkaline waters in salinities ranging from 2 to 76 g/L. Generally in inland wetlands with salinity > 5 g/L, *L. macropogan* exists in monospecific stands with no other charophyte species. A propagule bank study by Sim *et al.,* (2006a) found that *L. succinctum* was able to germinate in salinities up to and including 45 g/L, and *L. macropogan* was able to germinate in salinities up to and including 30 g/L.

Emergent macrophytes and riparian vegetation are also an important food source and provide habitat for many species in wetland systems. A number of emergent macrophytes have been associated with hyposaline waters including *Phragmites* spp., *Typha* spp. and *Juncus* spp (Hart *et al.*, 1991). *Typha domingensis* is known to only tolerate small increases in salinity and reduced growth in this species have been recorded between salinities of 2.8 g/L and 5.9 g/L (Hocking, 1981). *Juncus acutus* is known to tolerate higher salinity increases and can be found in waters of 10g/L (Greenwood and MacFarlane, 2009). Riparian vegetation associated with weltands of intermediate salinity often includes *Eucalyptus largiflorens* F. Muell, which is the more salt tolerant of riparian species and can tolerate salinities of up to 3.2 g/L (Roberts and Marston, 2011).

1.4.2 Macroinvertebrates

Macroinvertebrates, like aquatic macrophytes, are also an important food source for fish and waterfowl in saline wetlands. As a group, macroinvertebrates contain species that represent the most salt sensitive to the most salt tolerant of taxa. For example Timms (1993; 1998) found that members of Class Crustacea (e.g. copepods) have very salt sensitive species (with upper tolerance level of around 0.67 g/L) and also have very salt tolerant species (with an upper tolerance level of approximately 177.5 g/L). Unlike many salt tolerant macrophyte species, macroinvertebrates with a salinity tolerance of above 18 g/L are unable to tolerate low concentrations of salinity (Clunie *et al.*, 2002). Also, it is hard to generalise as to which species of aquatic macroinvertebrates are commonly found in saline wetlands, as there are strong regional differences in macroinvertebrate community species composition (Williams, 1984). However, some species of chironomids, ostracods e.g. *Mytilocypris henricae* Chapman 1966, amphipods e.g. *Austrochiltonia subtenuis* Sayce 1902, and shrimps are known to be saline water specialists (James *et al.*, 2003; Nielsen *et al.*, 2003b; Shelley, 2008).

While the salinity tolerances of adult life stages of macroinvertebrates are well documented, there has been very little research on the sub-lethal effects of salinity on the larval stages of many species. One study on salinity tolerance of the early life stages of selected macroinvertebrates found that the early life stages (eggs) for 60 to 70% of freshwater macroinvertebrates have a lower salinity tolerance than their adult

life stage (Kefford *et al.*, 2007). Kefford *et al.,* (2007) also found that young crustaceans, e.g. *Paratya australiensis* (Cherry shrimp) Kemp 1917 and *Caridina nilotica* (Freshwater shrimp) Roux 1833, are more tolerant of elevated salinity concentrations than the early life stages of other macroinvertebrate groups studied.

1.4.3 Fish

A review of Australian adult freshwater fish found that they tend to be tolerant of salinities from 7 g/L to 13 g/L (James *et al.*, 2003). While data on many species are lacking, few species are able to tolerate salinity concentrations > 13 g/L (James *et al.*, 2003). Some fish species are tolerant of salinities above that of seawater (35 g/L), and these include but are not limited to, *Craterocephalus stercusmuscarum fulvus* Günther 1867 (Unspecked Hardyhead), *Craterocephalus fluviatilis* (Murray Hardyhead), *Galaxias maculatus* Jenyns 1842 (Common galaxias), *Hypseleotris* sp. (Carp Gudgeons) and *Retropinna semoni* Weber 1895 (Australian smelt). There are introduced species that are tolerant of salinities above 10 g/L, the most tolerant being the *Gambusia holbrooki* Girard 1859 (Eastern mosquito fish) which has a reported direct acute LC_{50} (Lethal Concentration 50% - the concentration of a toxicant (salinity) that results the death of half the individuals in a population tested) of 25 g/L (Nordlie and Mirandi, 1996). The adults of most species are able to acclimatise to elevated salinity concentrations, however juveniles appear to be less tolerant (James *et al.*, 2003).

It is important to note that the majority of studies on the salinity tolerance of fish in Australia are based on laboratory studies involving the development of LC_{50} values. Kefford *et al.,* (2004) found that direct acute LC_{50} values and LC_{50} values for early life stages in freshwater fish tended to be a poor estimate of maximum field observations for many species. This was attributed to the fact that changes in salinity in the field (or *in situ*) are often gradual, which allows the individuals to acclimatise to increased salinity concentrations. Also adult fish, being highly mobile organisms, are able move away from saltier environments that their early life stages are unable to tolerate or easily avoid. Kefford *et al.,* (2004) found that gradual increases in salinity concentrations, LC_{50} values were a more accurate measure in the prediction if a species maximum field observation salinity tolerance level.

The diversity of fish species in Australia is low (Boulton and Brock, 1999). Table 1.4 summarises data on the native and exotic fish species that are salt tolerant and found in the northwestern Victorian wetlands of intermediate salinity studied as a part of this project.

Table 1.4 Native and introduced fish of Victorian inland waters in the Murray Darling Basin that are tolerant of salinities above 10 g/L

Common Name	Scientific Name	Adult Salinity Tolerance levels, direct acute, LC_{50} g/L	Max. Length (mm)
Native Species Murray Hardyhead	*Craterocephalus fluviatilis*	32.6 g/L	60
Unspecked Hardyhead	*Craterocephalus sterc. fulvus*	43.7[(1)]	78
Flat Headed Gudgeon	*Philypnodon grandiceps*	23.7[(2)]	115
Introduced Species Eastern Mosquito Fish	*Gambusia holbrooki*	19.5 [(3)] and 25[(4)]	60

Modified from (Clunie *et al.*, 2002; James *et al.*, 2003)
[(1)] Williams and Williams (1991); [(2)] Jackson and Pierce (1992); [(3)] Chessman and Williams (1974); [(4)] Nordlie and Mirandi (1996)

The Murray Hardyhead (*Craterocephalus fluviatilis*), is a small, moderately deep-bodied species, endemic to the lowlands of the Murray and Murrumbidgee River systems in southeastern Australia (Ebner *et al.*, 2003) (Table 1.4). It is recognised as an endangered species under the Flora and Fauna Guarantee Act in Victoria (1988) and is on the threatened species list of the New South Wales Fisheries Management Act (1994). The Murray Hardyhead is also recognised as threatened under the Federal Environment Protection and Biodiversity Conservation Act (1999) and has been listed as a potentially threatened species by the ICUN (Lyon *et al.*, 2002). Past distributions of this species are hard to determine as the identification and taxonomy is not clear. It has been confused with other species of hardyhead such as *Craterocephalus eyresii* Steindachiner 1883 (Lake Eyre Hardyhead), *C. amniculus* Crowly and Ivantsoff 1990 (Darling River Hardyhead) and *C. sterc. fulvus* (Crowley and Ivantsoff, 1990). It is believed however that the current distribution for Murray Hardyhead in Victoria is restricted to a few wetlands connected to the Murray River in the Kerang, Swan Hill and Mildura (Flemming, 1990; Allen *et al.*, 2002; Lyon *et al.*, 2002; Ebner *et al.*, 2003). This species also occurs in the Riverina district of

South Australia. There are no known populations in New South Wales with no recorded sightings since the 1970s despite efforts to locate the species over the past 20 years (Ebner *et al.*, 2003).

The Unspecked Hardyhead (*Craterocephalus stercusmuscarum fulvus*), is a small slender fish (Table 1.4). It is generally only found in lowland areas of river systems throughout eastern Australia (Lintermans, 2007). It was formerly an abundant species but its distribution has reduced and it is now considered a rare species in the southern part of its range (Lintermans, 2007). In Victoria, the Unspecked Hardyhead is listed as threatened under the Flora and Fauna Guarantee Act (1988). This species is generally found in slow flowing lowland rivers, lakes, backwaters and billabongs and prefers habitats with aquatic vegetation. It is a carnivorous species feeding on small insects such as mosquito larvae and micro crustaceans (Lintermans, 2007). Scientists often have trouble distinguishing between the Murray and the Unspecked Hardyhead and both are known to co-exist in waterbodies such as Lake Hawthorn in Mildura, Victoria, which makes studies of the distribution of both species unreliable (Ellis, 2005b).

The Flat Headed Gudgeon *Philypnodon grandiceps* Krefft 1864 (Flat Headed Gudgeon), is a small fish with a broad head and large mouth (Lintermans, 2007) (Table 1.4). It is common in wetlands and tributaries of the lower Murray River (New South Wales, Victoria and South Australia), along the edges of the lower lakes of South Australia and it also occurs in coastal streams in Victoria, New South Wales, South Australia and Queensland (Lintermans, 2007). It is a carnivorous species that feeds on aquatic insects, molluscs, tadpoles, micro crustaceans and smaller fish (Lintermans, 2007).

The introduced Eastern Mosquito fish, (*G. holbrooki*), is a small fish that is abundant and common in wetlands and still or slow-flowing streams, particularly around aquatic vegetation (Lintermans, 2007) (Table 1.4). The Eastern Mosquito fish is an aggressive species that is believed to prey on the eggs of native fish and frogs, and the juvenile stages of native fish. This species has been implicated in the decline of 9 species of Australian fish and 10 species of frog (Lintermans, 2007).

1.4.4 Waterfowl

As waterfowl are highly mobile they are able to use productive saline water bodies for feeding if fresh drinking water is nearby and as such they are often tolerant of saline conditions (Kingsford and Porter, 1994). Waterfowl chicks however may be more vulnerable due to their reduced mobility (James *et al.*, 2003). Given the mobility of waterfowl, their presence on a wetland is often regarded as being as influenced as much by conditions elsewhere as by those on the particular wetland (Lyons *et al.*, 2007).

Saline wetlands with a macrophyte-dominated ecosystem support a larger number of birds than freshwater lakes, as food sources (both macrophytes and macro and microinvertebrates) are more abundant in these productive saline lakes (Kingsford and Porter, 1994; Kingsford, 1995). Two species of waterfowl that are particularly tolerant of highly saline environments are *Tadorna tadornoides* Jardine and Selby 1828 (Australian shelduck) which has been reported to tolerate salinity concentrations up to 125 g/L and the *Anas gracilis* Buller 1869 (Grey teal) which can tolerate salinity concentrations up to 64 g/L (Chapman and Lane, 1997).

1.4.5 Benthic microbial mats

At high salinities the biological communities of wetlands can be dominated by benthic microbes which form a thick or thin, cohesive or non-cohesive layers on the substrate (Bauld, 1981). Benthic microbial mats are defined as 'layered microbial communities made up of accretionary, cohesive microbial populations' (Guerrero *et al.*, 2002) and are comprised of phototrophic and chemotropic bacteria, fungi and micro algae (Kushner 1993). They range in thickness from several millimetres to a few centimetres (Guerrero *et al.,* 2002). Benthic microbial mats have been recorded in a number of different environments including: intertidal coastal sediments, marine salterns, hypersaline lakes, thermal springs, dry and hot deserts and Antarctic lakes (Jorgensen *et al.*, 1983; Taton *et al.*, 2006; McGregor and Rasmussen, 2007).

These mats are often associated with extreme environmental conditions such as high salinities, high temperatures, very clear waters and extreme light conditions. They are found in a variety of habitats including hypersaline lagoons, alkaline lakes, hot springs, sulphur springs and deep-sea hydrothermal vents (Guerrero *et al.,* 2002).

While microbial communities are known to occur at low as well as high salinities, they are often out-competed by aquatic macrophytes at low salinities and therefore only become dominant in highly saline systems (Kushner, 1993). It has also been suggested by Pinckney *et al.,* (1995), that microbial mats grow best during seasonal periods of reduced salinity of around 45 g/L and that when salinities rise to concentrations > 90 g/L, microbial mats do not grow and instead exist in a state of near dormancy.

1.4.6 Phytoplankton

There have been few studies on the phytoplankton of saline lakes. Oren (2006) reported that the main planktonic primary producers in saline to hypersaline lakes were species from the genus *Dunaliella*. Borowitska (1981) reported that the species *Dunaliella salina* (Dunal) Teodoresco, was tolerant of a broad salinity range. Skinner *et al.*, (2001) also found that some phytoplankton were able to emerge in substantial numbers from the dry sediments of a wetland when exposed to saline water, but the diversity was reduced. Studies have also reported that diatom communities are sensitive to increases in salinity (Blinn *et al.*, 2004; James *et al.*, 2009).

1.5 Hypotheses

This study aims to investigate the resilience of biota exposed to increased salinity concentrations, in wetlands of intermediate salinity in northwestern Victoria. The hypothesis tested in this study were

- Have wetlands of intermediate salinity and their associated biota that occur within the Kerang, Mildura and Swan Hill areas of Victoria changed in salinity and watering regimes overtime?
- What are the salinity thresholds for submerged aquatic macrophyte communities in these regions?
- Are submerged aquatic macrophyte communities found in wetlands of intermediate salinities able to tolerate periods of drying?
- Do the salinity tolerance thresholds of the invertebrate species present correspond closely to the salinity and watering regime thresholds determined for submerged aquatic macrophyte communities
- Do the sub lethal and indirect effects of increasing salinity affect the biota found in wetlands of intermediate salinity?

2.0 The distribution of intermediate saline wetlands in northwest Victoria and their associated biota including the threatened fish *Craterocephalus fluviatilis* (Murray Hardyhead)

2.1 Introduction

Saline wetlands make up a significant part of the Australian landscape particularly in arid and semi arid regions (Williams, 1993b). They are often undervalued as they are areas with low diversity, but if salinity concentrations are not excessive, the individuals present are numerous and thus saline wetlands tend to be areas of high productivity (Brock, 1986; Timms, 1993; Williams, 1993a; Kingsford and Porter, 1994). Many of the saline wetlands in northwestern Victoria have been engineered for the delivery of irrigation waters from the Torrumbarry Irrigation Scheme to farms and many are at risk from a number of threats including increased salinisation, altered flow regimes, saline water disposal, mineral harvesting, and the effects of introduced species (KLAWG, 1992; Williams, 1993b).

This chapter contains two sections; the first explores the complexity of the management of the wetlands in this region through a case study. This case study focuses on the management and consequent changes in salinity and biota, and was prepared by undertaking an extensive literature review, covering the major wetlands of the Kerang - Swan Hill region. The second section details the results of a fieldword study focusing on four wetlands found in the northwest Vitocia region.

2.1.1 Case study – Changes in salinity and distribution of key biota of selected wetlands in the Kerang to Swan Hill region of northwest Victoria.

This case study focuses on wetlands in three regions of northwestern Victoria: the Kerang region, the Lake Charm region, and the Lake Boga region, (Figure 2.1). There are hundreds of wetlands in the Kerang to Swan Hill region forming what is commonly known as the Kerang Lakes Area. The Kerang Lakes area located approximately 300 km northwest of Melbourne along the Loddon River and is made up of wetlands ranging from fresh to hypersaline with a range of hydrological regimes. Wetlands within this region have been recognised as significant areas for

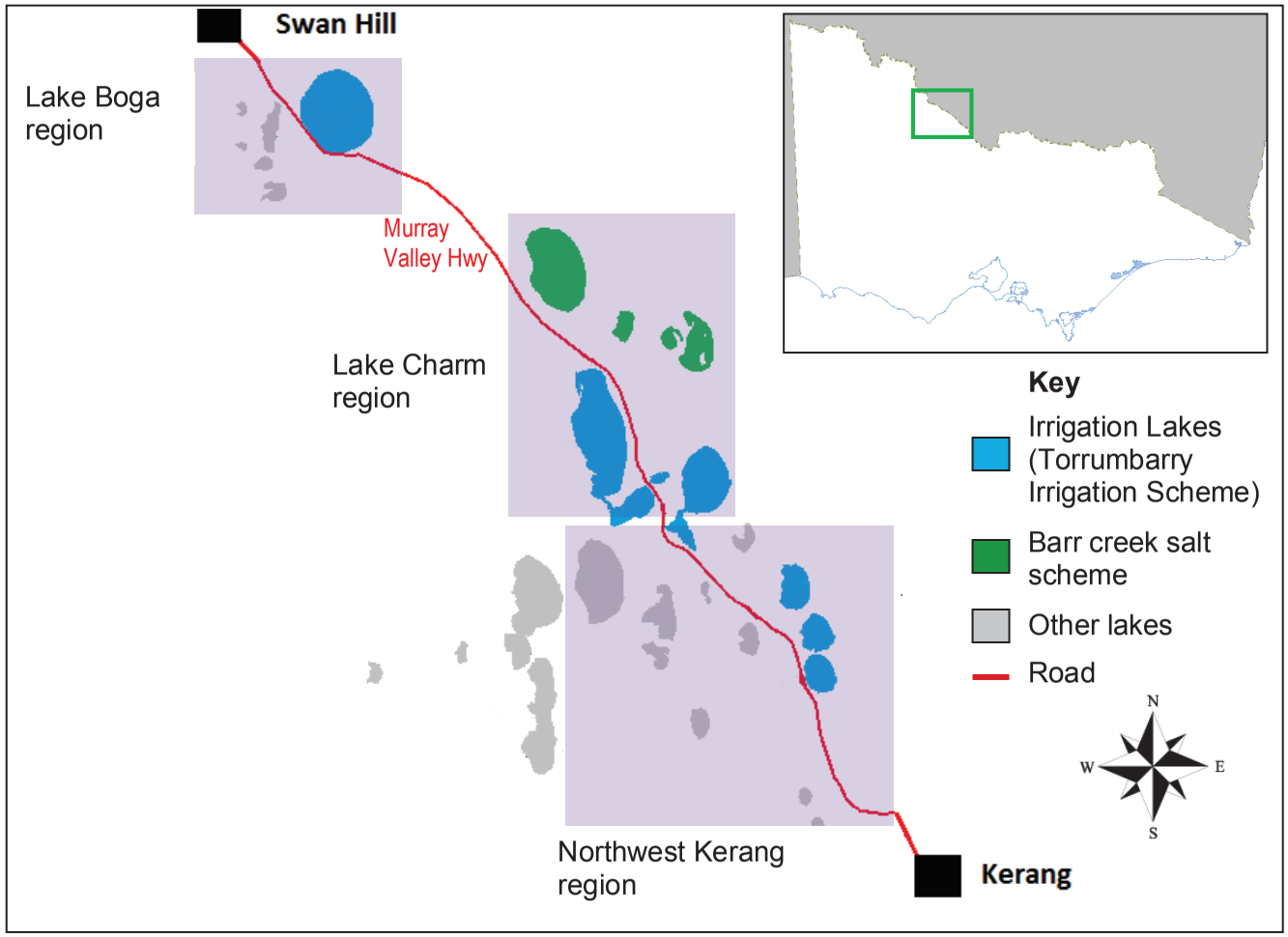


Figure 2.1 Case Study – Locations of the northwest Kerang, Lake Charm and Lake Boga regions and selected associated wetlands, not to scale. Modified from Department of Sustainability and Environment (2010).

water bird habitat and as a result have been listed under the Ramsar convention, JAMBA and CAMBA treaties (KLAWG, 1992).

Many of the wetlands in each of these regions have been altered since European settlement to assist in the storage, transport and distribution of irrigation waters throughout the Torrumbarry Irrigation Scheme (KLAWG, 1992). The Torrumbarry Irrigation Scheme consists of a series of engineered and natural channels, rivers, weirs, streams and wetlands that transport water from the Murray River for irrigators in various districts including Tresco, Mystic Park, Woorinen and Fish Point, all of which are located around Kerang and Swan Hill (KLAWG, 1992). Changes in the hydrological regimes and salinity concentrations of wetlands in this area have resulted in changes to the biota that these wetlands support (KLAWG, 1992; Shelley, 2008).

The areas selected for this study, do not contain all of the wetlands of the region but instead focus on those where historical data about the biota were available. This case study investigates fluctuations in salinity and changes in the distribution of the fish species, Murray Hardyhead, *Craterocephalus fluviatilis*, from 1975 to 2003, based on information gathered in an extensive literature review. As discussed in Chapter 1, *C. fluviatilis* is of particular interest in the region as it is an endangered species which was once common in the area, but is now restricted to a few wetlands within the Kerang – Swan Hill region. Historically *C. fluviatilis* has been recorded in the Cardross Lakes, Lake Boga, Lake Cullen, Lake Elizabeth, Lake Golf Course, Lake Hawthorn, Lake Wandella, Lake Woorinen North, Long Lake and Round Lake. All of these locations have been included in this case study except Lake Hawthorn, Lake Woorinen North and the Cardross Lakes. These lakes were omitted due to a lack of historical information about the salinity and fish populations present over time (Hardie, 2000; Ebner and Raadik, 2001; Lyon *et al.*, 2002; Ebner *et al.*, 2003; Backhouse *et al.*, 2006).

Northwest Kerang region

The wetlands studied include Lake Wandella, Pelican Lake, Lake Elizabeth, Duck Lake North, Duck Lake South, Cranes Lake and Lake Cullen. Cranes Lake, Duck

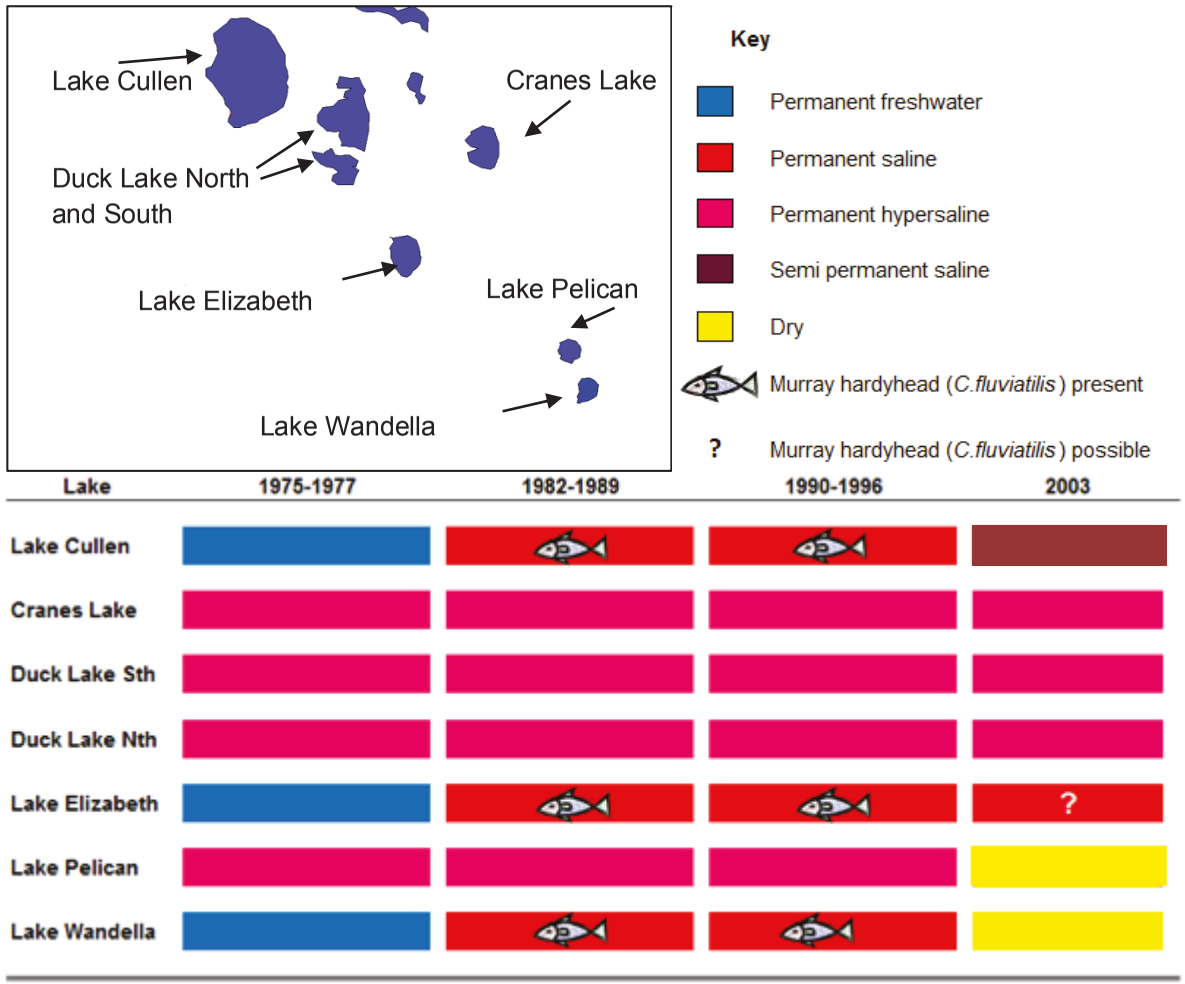


Figure 2.2 Northwest Kerang region – changes in salinity and distribution of *Craterocephalus fluviatilis* (Murray Hardyhead) in selected wetlands between 1975 to 2003

Lake North and South (Figure 2.2) have been hypersaline lakes since the mid 1970s and have changed very little in terms of their salinity since then (Corrick and Cowling, 1975; Lugg *et al.*, 1989; Flemming, 1990; O'Donnell, 1990; Anderson, 1991; KLAWG, 1992). Two of these wetlands, Cranes Lake and Duck Lake South are used for commercial salt and gypsum harvesting respectively. Given the high salinity of these lakes, no macrophyte or fish species were recorded from 1975 to 2003 (Corrick and Cowling, 1975; Lugg *et al.*, 1989; Flemming, 1990; O'Donnell, 1990; Anderson, 1991; KLAWG, 1992) (Figure 2.2).

Lake Pelican is a semi-permanent hypersaline lake, whilst Lake Wandella was considered a saline lake from the mid 1970s to 1990s (Corrick and Cowling, 1975; Lugg *et al.*, 1989; Flemming, 1990; O'Donnell, 1990; Anderson, 1991; KLAWG, 1992). Given its high salinity, Lake Pelican has not supported any macrophytes or fish species since 1975, whereas Lake Wandella has supported *Ruppia* spp. and *Chara* spp. as well as *Gambusia holbrooki* Girard 1859 (Eastern mosquito fish) and *C. fluviatilis* populations (Corrick and Cowling, 1975; Lugg *et al.*, 1989; Flemming, 1990; O'Donnell, 1990; Anderson, 1991; KLAWG, 1992). Both wetlands are completely disconnected from the Loddon River and as a result do not receive any irrigation outfalls and cannot receive any environmental water allocations. In 2003 both wetlands dried out, apart from a groundwater intrusion filling a small part of Lake Wandella (KLAWG, 1992; Hardie, 2000) (Figure 2.2).

In the past, Lake Cullen and Lake Elizabeth were the only freshwater wetlands within this region connected to the Torrumbarry Irrigation Scheme. Whilst both wetlands are still connected to the irrigation scheme, neither lake is currently used for the storage of irrigation waters and both have become progressively more saline over time (Lugg *et al.*, 1989; Nolan ITU *et al.*, 2000). Lake Cullen was removed from the irrigation system in 1969 and was initially managed as a permanent saline wetland filled by excess flood waters (State Rivers and Water Supply Commission, 1982). Being a terminal lake, increasing salinity became an issue in the late 1980s after which it was allowed to dry and was then managed as a semi-permanent saline lake to control the salinity concentrations (Lugg *et al.*, 1989; Nolan ITU *et al.*, 2000). Since the 1980, Lake Cullen has been filled by environmental water allocations and allowed to dry (Department of Sustainability and Environment, 2004). In the past Lake Cullen supported aquatic macrophytes including: *Potamogeton pectinatus* L.

(Sago Pondweed), *Ruppia* spp., *Nitella* spp., *Lepilaena cylindrocarpa* (Muell. Stuttg.) Benth. (Long Fruit Watermat) and *Valisneria spiralis* L. (Eelgrass). It is important to note here that past identification of charophytes (Nitella spp, Chara spp and Lamprothamnium spp.) in Austrlian lakes have often been misidentified (Garcia and Chivas, 2004) thus I have included here the species that were reported in the literature, however the accuracy of these identifications is unknown. The wetland also provided habitat for *C. fluviatilis* (Murray Hardyhead)*, Philypnodon grandiceps* Krefft 1864 (Flat Headed Gudgeon), *Carassius carassius* Linneaus 1758 (Crucian Carp) and *Cyprinis carpio* Linnaeus, 1758 (European Carp) (Bennison, 1978; Flemming, 1990). Since being managed as a semi permanent wetland, Lake Cullen supports *Ruppia megacarpa* R. Mason (Large Fruit Tassel) and *Lamprothamnium macropogon* (A. Braun) I.L. Ophel (Stonewort) (Bradbury, 2002). *Cuprinis carpio* (European Carp) were also observed in 2002, but any fish carried into the wetland with the environmental water allocation or flood waters, ultimately die when the lake dries out. This wetland is a terminal one, and as such, fish populations are unsustainable under the current flow management regime (Figure 2.2).

Lake Elizabeth was originally described as a permanent freshwater wetland, but salinity in the area increased in response to the rising water table, and groundwater intrusions into the lake resulted in dramatic increases in salinity concentrations. Consequently since the 1970s, Lake Elizabeth has been classed as a permanent saline wetland (Corrick and Cowling, 1975; Lugg *et al.*, 1989; Flemming, 1990; O'Donnell, 1990; Anderson, 1991; KLAWG, 1992; Kelly, 1996; Delany, 2004). Like Lake Cullen, Lake Elizabeth is a terminal lake that has become increasingly saline as the lake no longer receives regular flows of fresh water (Lugg *et al.*, 1989; KLAWG, 1992; Kelly, 1996; Delany, 2004). In the past Lake Elizabeth originally supported *Ruppia* spp. and *Chara* spp. and the fish species *C. fluviatilis* (Lugg *et al.*, 1989; Flemming, 1990; O'Donnell, 1990; Anderson, 1991; KLAWG, 1992; Kelly, 1996; Delany, 2004). Given the lake's increasing salinity it is doubtful that it still supports populations of *C. fluviatilis* (Ellis, 2005c; 2005b; Backhouse *et al.*, 2006; Ellis 2006) (Figure 2.2).

Lake Charm region

The wetlands in this case study include: Lake Charm, Little Lake Charm, Lake Racecourse, Lake Kangaroo, Lake Kelly, Little Lake Kelly, Lake William and Lake

Tutchewop (Figure 2.3). The Lake Charm region forms a part of the Torrumbarry Irrigation Scheme delivering water from the Murray River to farms in the Kerang – Swan Hill area, and as a result many of these wetlands have experienced dramatic changes in their management since the scheme was designed (Lugg *et al.*, 1989; KLAWG, 1992). Wetlands of this region can be separated into two distinct groups, Lake Charm, Little Lake Charm, Lake Racecourse and Lake Kangaroo are currently, or have been used for storage and transport of irrigation waters and as such most of these wetlands contain fresh water. These lakes will be referred to as the 'irrigation lakes'. Lake Charm was removed from the irrigation scheme in 1964 and since then has only received top up fresh water from Little Lake Charm during floods (KLAWG, 1992). As a result, this wetland has become a terminal lake, gradually increasing in salinity and is now hyposaline (State Rivers and Water Supply Commission, 1982; KLAWG, 1992) (Figure 2.3).

The second group of wetlands in this area include Lake Kelly, Little Lake Kelly, Lake William and Lake Tutchewop which together form the Barr Creek Salt Disposal Scheme (KLAWG, 1992). This salt disposal scheme was implemented in 1960 when a pumping channel was constructed to carry saline water from Barr Creek, through Lake Kelly, Little Lake Kelly, Lake William and finally to Lake Tutchewop. The scheme was seen as an important way of stopping saline water entering the Murray River, but has severely impacted the flora of Lake Tutchewop (KLAWG, 1992) in particular. As a result of the scheme these lakes have become permanent and more saline over time (KLAWG, 1992) (Figure 2.3).

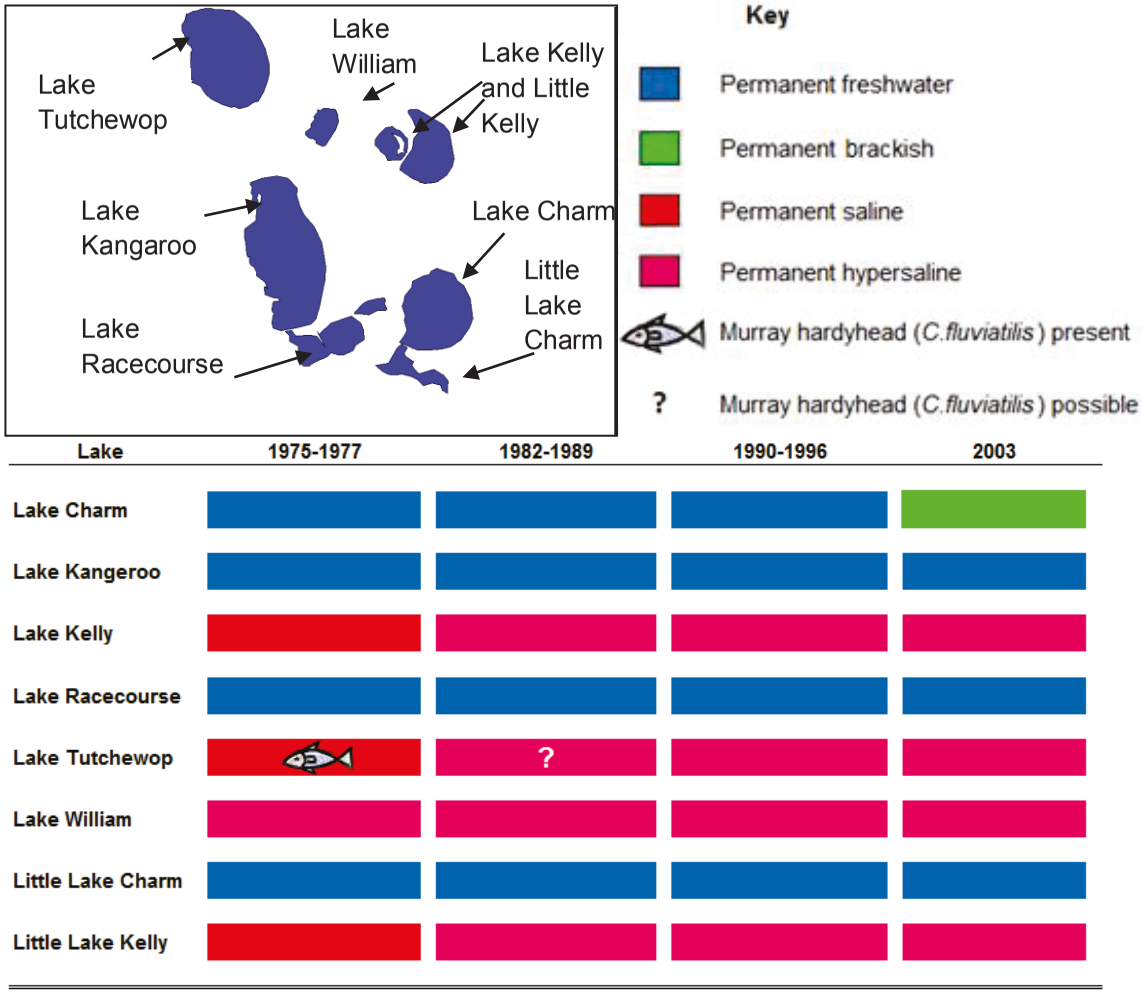


Figure 2.3 Case study Lake Charm region – changes in salinity and distribution of *Craterocephalus fluviatilis* (Murray Hardyhead) in selected wetlands from 1975 to 2003.

In response to salinity changes, the biota of the irrigation lakes and the wetlands of the Barr Creek Salt Disposal Scheme have altered dramatically over time. Species such as *V. spiralis*, *P. pectinatus* and *Potamogeton ochreatus* Roul (Blunt pondweed) were often found in Lake Kangaroo, while Lake Charm supported these species in addition to *Lepilaena biloularis* Kirk (Small fruit watermat)*, Chara* spp.*., Nitella* spp. and *Ruppia* spp. (Corrick and Cowling, 1975); macrophytes such as *Myriophyllum elatiniodes* Gaudich (Upright Water milfoil), which was also found at Lake Racecourse in addition to *Myriophyllum propinquum* A.Cunn. (Water milfoil), and *V. spiralis*. Since the mid 1970s it has been noted that macrophyte populations have been in decline, particularly in Lake Kangaroo. It has been suggested that the introduced fish species *C. carpio* (European Carp), have negatively impacted submerged marcophytes (Lugg *et al.*, 1989) and over time macrophyte populations have been reduced or completely lost from these wetlands.

The irrigation lakes were known to support a variety of fish species including *Retropinna semoni* (Australian smelt), *Nematolosa erebi* Günther 1868 (Bony bream), *Perca fluviatilis* L. 1758 (Redfin), *Tinca tinca* L. 1758 (Tench), *Maccullochella peelii peelii* Mitchell 1838 (Murray cod), *Bidyanus bidyanus* Mitchell 1838 (Silver Perch), *Macquaria ambigua* Richardson 1845 (Golden Perch), *P. grandiceps*, *Carassius auratus* L. 1758 (Goldfish) and *C. carpio* (Flemming, 1990).

Lake Tutchewop, Lake Kelly and Little Lake Kelly were always more saline than the irrigation lakes (Corrick and Cowling, 1975; Lugg *et al.*, 1989; KLAWG, 1992). In the past Lake Tutchewop was described as a permanent saline lake, receiving flood waters from the Avoca River (Corrick and Cowling, 1975; Lugg *et al.*, 1989). During this time (1970's) Lake Tutchewop supported fish species *C. fluviatilis* (Murray Hardyhead) and *G. holbrooki* (Eastern Mosquito fish) and macrophytes including *Ruppia* spp., *L. bilocolaris, Nitella* spp. and *Chara* spp. (Powling, 1977; Lugg *et al.*, 1989; O'Donnell, 1990). Lake Kelly and Little Lake Kelly were semi-permanent saline lakes prior to being included in the Barr Creek Salt Disposal Scheme. During this time (1970's) these lakes had beds of *R. megacarpa* (Powling, 1977; Lugg *et al.*, 1989; O'Donnell, 1990). Over time these three lakes have become permanent hyper-saline wetlands and as a result, by 2000 they no longer supported any macrophyte or

fish species (Hardie, 2000). In contrast, Lake William, the fourth wetland of the Barr creek salt disposal scheme, has always been a hypersaline with no macrophyte or fish species present in the past and has changed very little over time (Powling, 1977; Lugg *et al.*, 1989; O'Donnell, 1990).

Lake Boga region

The wetlands included in this case study include: Lake Boga, Lake Golf Course, Round Lake, and Long Lake. Lake Boga was the only wetland connected to the Torrumbarry Irrigation Scheme and as such was classed as a permanent freshwater lake (1975 to 2003) (KLAWG, 1992). Little is known of the macrophytes present in this wetland, but it supports a range of fish species including *C. fluviatilis*, *R. semoni*, *N. erebi*, *P. fluviatilis*, *T. tinca*, *M. peelii peelii*, *B. bidyanus*, *M. ambigua*, *C. auratus* and *C. carpio*. It no longer supports *C. fluviatilis,* and the reason for its disappearance is not known (Lugg *et al.*, 1989; Flemming, 1990; O'Donnell, 1990; KLAWG, 1992; Cottingham, 1996) (Figure 2.4).

In the past Long Lake, Lake Golf Course and Round Lakes were used for storing saline tile drain waters from the nearby Tresco farming region (KLAWG, 1992). Tile drains are networks of small drains designed to collect and drain sub surface waters away from irrigated fields (Swinton *et al.*, 2000). All three lakes were known to support macrophytes such as, *Ruppia* spp. and the fish species *C. fluviatilis.* It should be noted that fish were recorded at Long and Golf Course Lakes as *Craterocephalus eyresii* Steindachner 1883 (Lake Eyre Hardyhead). Given advances in taxonomy however it is more likely that these populations were *C. fluviatilis* (Crowley and Ivantsoff, 1990). More recently both Long Lake and Golf Course Lake have dried out. Long Lake is considered to habe become a semi-permanent hypersaline wetland and Gold Course Lake has become a semi-permanent, saline wetland resulting in the loss of this fish species (Tunbridge and Glennane, 1984; Flemming, 1990).

In 2003, Round Lake was still classed as a permanent saline wetland and continued to receive tile drain water run off. Unlike many other saline wetlands in the region, Round Lake is not a terminal lake and excess water flows into Lake Golf Course, so salinity concentrations within Round Lake remain relatively stable. It is believed that populations of *Ruppia* spp. and *C. fluviatilis* still exist within this lake (Lyon *et al.*, 2002; Backhouse *et al.*, 2006) (Figure 2.4).

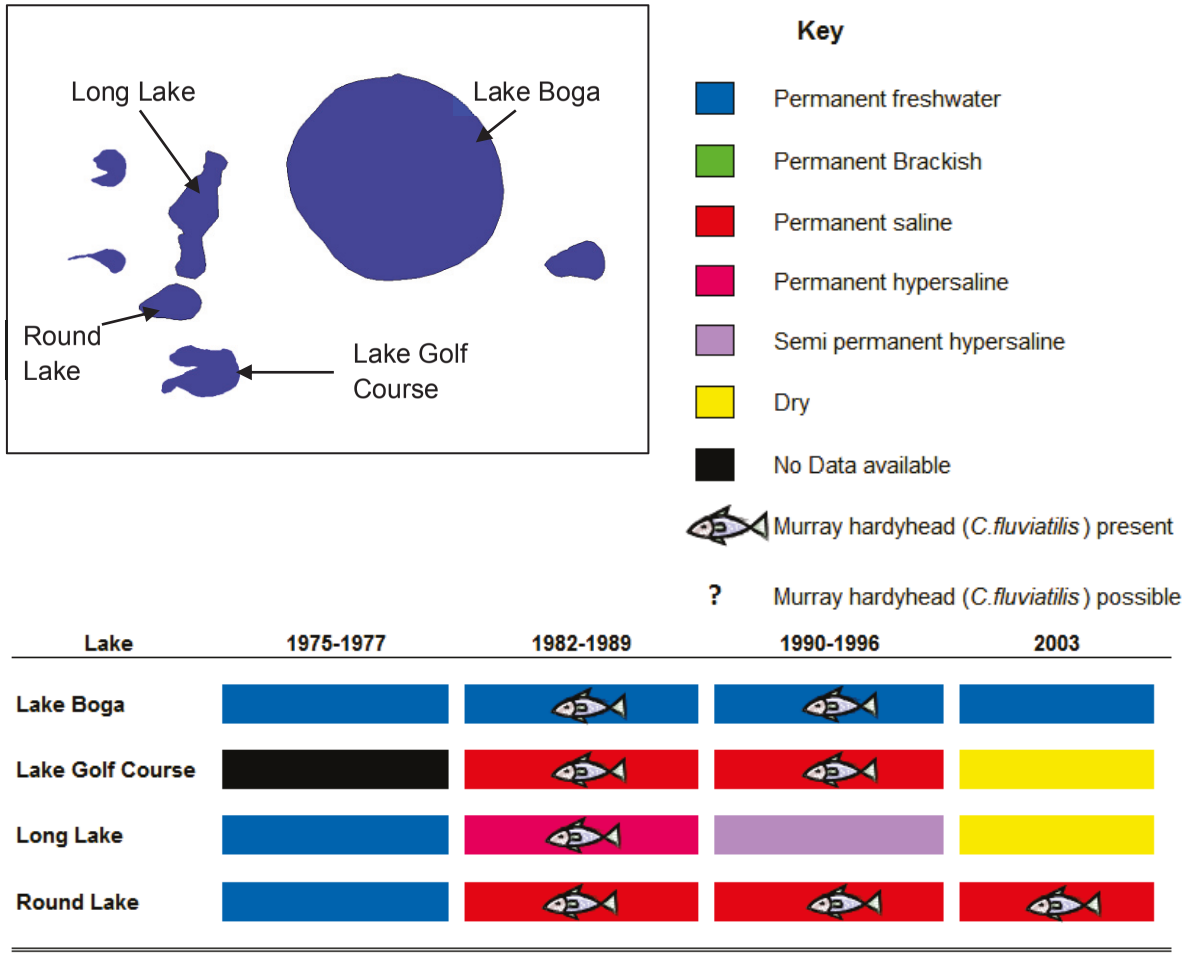


Figure 2.4 Case study Lake Boga region - changes in salinity and distribution of *Craterocephalus fluviatilis* (Murray Hardyhead) in selected wetlands from 1975 - 2003.

As shown in Figures 2.2 to 2.4, many of the wetlands discussed have changed dramatically since European settlement and in particular the construction of the Torrumbarry Irrigation Scheme (Corrick and Cowling, 1975; Powling, 1977; Tunbridge and Glennane, 1984; Lugg *et al.*, 1989; Flemming, 1990; O'Donnell, 1990; KLAWG, 1992; Cottingham, 1996; Hardie, 2000). Given that these wetlands are now completely cut off from their floodplain and associated rivers, and are managed for a number of anthropogenic uses, it is imperative that we understand the biota of these systems and their response to disturbance, in order to maintain biodiversity whilst balancing the needs of surrounding landholders. For *C. fluviatilis* in particular, the appropriate management of the few lakes that still support populations of this fish is vital for their survival within Victoria and even perhaps Australia (Backhouse *et al.*, 2006).

2.1.2 Hypotheses

This chapter investigates the biota of four wetlands of intermediate salinity in north western Victoria, these are: Lake Elizabeth, Round Lake, Woorinen North Lake, and Lake Hawthorn, all of which are known to support populations of *C. fluviatilis*. The hypotheses tested in this study were:

- Are *C. fluviatilis* (Murray Hardyhead) populations present within these wetlands of intermediate salinity?
- Which submerged aquatic macrophytes are associated with *C. fluviatilis* (Murray Hardyhead) populations?
- What are the salinity thresholds for *C. fluviatilis* (Murray Hardyhead) populations

2.2 Methods

Aquatic flora and fauna were investigated in four lakes in northwestern Victoria: Lake Elizabeth (near Kerang), Round Lake (near Lake, Boga), Lake Woorinen (near Swan Hill), and Lake Hawthorn (near Mildura) (Figure 2.5). These lakes were selected for this study because: they contained water, were of intermediate salinity (between 10 g/L and 50 g/L) and were sites where *C. fluviatilis* (Murray Hardyhead) and submerged aquatic macrophyte populations had been recorded since the hydrology of the lakes was modified by their incorporation into the various irrigation schemes. All field studies were conducted from July to August 2005.

2.2.1 Site descriptions

Lake Elizabeth

Lake Elizabeth is approximately 10 km northwest of Kerang in Victoria and is around 94 hectares in size (Lugg *et al.*, 1989) (Figure 2.6). Lake Elizabeth is a terminal lake, connected to the Torrumbarry Irrigation Scheme via the Macorna channel (Lugg *et al.*, 1989). This lake was used for storing irrigation waters for surrounding farms until in the 1970s. Subsequently the underlying saline water table rose as a result and the lake changed from permanent freshwater to being a permanent saline lake (Kelly, 1996). Lake Elizabeth is 94 hectares in size and has an average depth of > 2 meters (Kelly, 1996).

Lake Elizabeth is surrounded by scattered areas of *Eucalyptus largiflorens* F. Muell (Black box) and extensive areas of chenopod shrub land and a reed bed community dominated by the introduced *Juncus acutus* L. (Spiny rush) (Figure 2.6). The aquatic plant community of this lake has been surveyed in the past and *Ruppia* spp. and charophytes were recorded (Lugg *et al.*, 1989; Kelly, 1996).

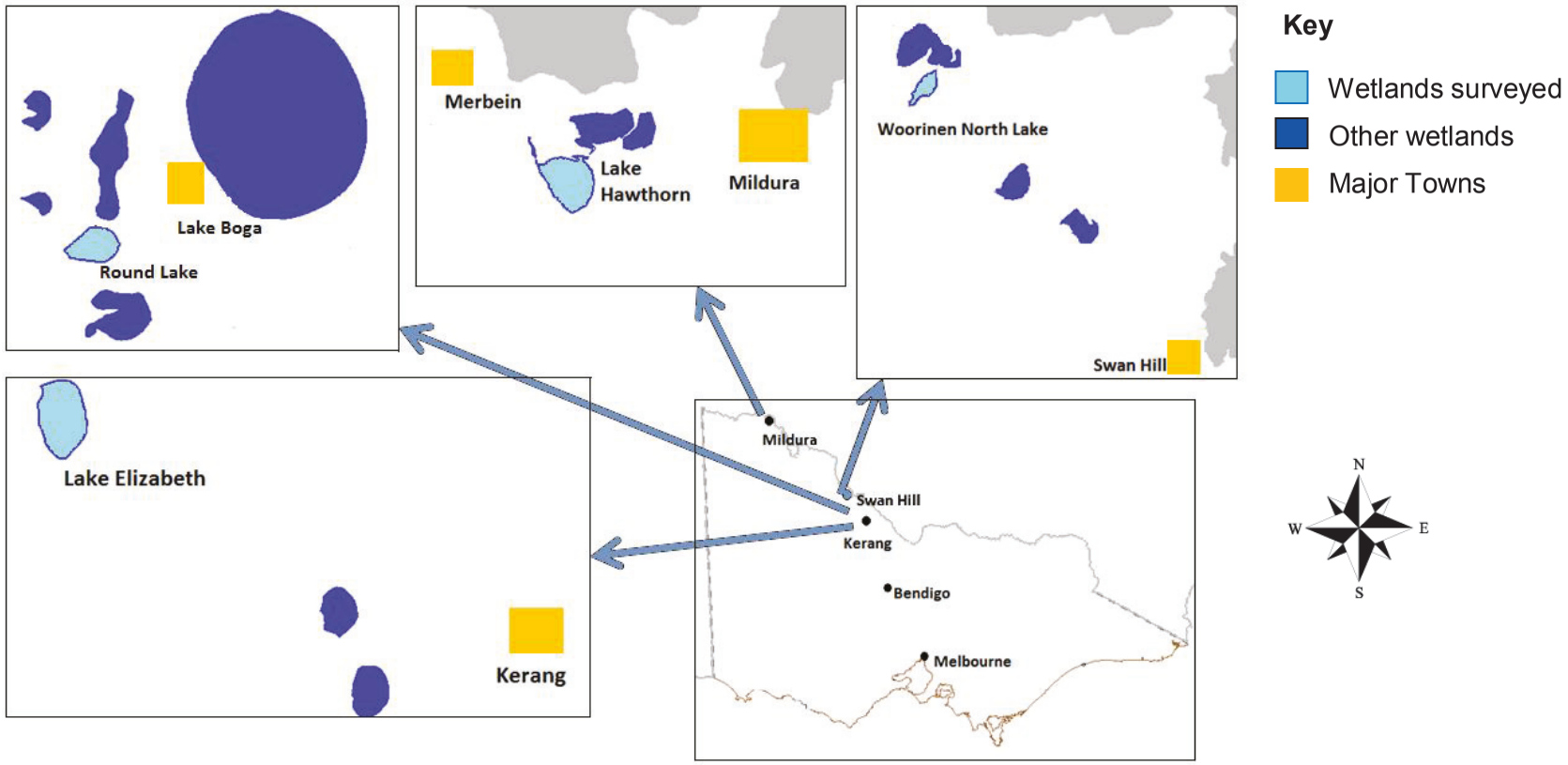


Figure 2.5 Location of Lake Elizabeth, Round Lake, Woorinen North Lake and Lake Hawthorn in north western Victoria (not to scale), modified from Department of Sustainability and Environment (2010)

Figure 2.6 Lake Elizabeth, **A.** Aerial photo showing surrounding farmland (Google, 2012), **B.** Photo (looking northwest across the lake) showing *Junus acutus* (Spiny rush) in foreground and *Cygnus atratus* (Black swans) on the lake.

This lake is also supports a variety of birds and is habitat for species listed under the JAMBA and CAMBA agreements (Kelly, 1996). Populations of the fish *C. fluviatilis* (Murray Hardyhead) have been recorded in the lake over the past 30 years to 2005 (Lugg *et al.*, 1989; Flemming, 1990; Kelly, 1996; Lyon *et al.*, 2002).

Round Lake

Round Lake is located about 3km west of Lake Boga and is approximately 40 hectares in size (Lugg *et al.*, 1989) (Figure 2.7). This lake is connected to Long Lake and Lake Golf Course by pipelines and regulators and is not a terminal lake. Round Lake receives tile drainage waters from the nearby Tresco irrigation district but has the capacity to receive environmental flows (Lugg *et al.*, 1989; KLAWG, 1992). This lake was described as being freshwater by Langtry in the 1940s (Cadwallader, 1977) and in the 1970s by Corrick and Cowling (1975), but it has become a permanent saline lake since the late 1980s (Lugg *et al.*, 1989; KLAWG, 1992). Round Lake is 42 hectares in size and has an average depth of > 2 meters (KLAWG, 1992).

Round Lake has been considered as a wetland of moderate value for waterbirds and has been known to support *Ruppia* spp. since the 1980s (Lugg *et al.*, 1989; O'Donnell, 1990; Hardie, 2000), but its value as habitat for *C. fluviatilis* was later

recognised when this species was recorded there in 1999 and 2000 (Hardie, 2000). A previous study by Flemming (1990) found no fish species in the lake.

Figure 2.7 Round Lake, **A.** Aerial photo showing surrounding farmland (Google, 2012), **B.** Photo (looking southwest across the lake) showing *Junus acutus* (Spiny rush) in foreground and numerous *Cygnus atratus* (Black Swans) on the lake.

Woorinen North Lake

Woorinen North Lake is located between Swan Hill and Nyah in northwest Victoria, and in the past was used a drainage lake for the surrounding irrigated farms of the Woorinen area (Lugg *et al.*, 1989; Lyon *et al.*, 2002) (Figure 2.8). This lake once received runoff from surrounding farms as well as surplus irrigation flows. But in 2003 a pipeline was constructed in the area to reduce water loss by evaporation from existing open irrigation channels. Since then an annual environmental water allocation has been pumped to Woorinen North Lake. This lake is not a terminal lake and when water concentrations are high, water flows through to another nearby drainage lake (Hollway's basin) (Lugg *et al.*, 1989; Lyon *et al.*, 2002). Woorinen North Lake is 63 hectares in size and has an average depth of less than 2 meters (Lyon *et al.,* 2002).

There is very little riparian vegetation surrounding Woorinen North Lake, but the lake has been surveyed and a submerged aquatic vegetation community consisting of *Ruppia* spp. has been recorded (Lugg *et al.*, 1989; Lyon *et al.*, 2002). This lake is also an important habitat for waterbirds and bird species listed under the JAMBA and CAMBA agreements have been recorded there. Populations of *C. fluviatilis* have also been recorded at Woorinen North Lake in the past 20 years (Lugg *et al.*, 1989; Lyon *et al.*, 2002).

Figure 2.8 Woorinen North Lake, **A.** Aerial photo showing surrounding farmland (Google, 2012), **B.** Photo (looking southwest across the lake) showing two *Pelecanus conspicillatus* Temminck, 1824 (Australian Pelicans) and riparian vegetation surrounding the lake.

Lake Hawthorn

Lake Hawthorn is located southeast of Merebin approximately 7 km west of Mildura (Lloyd Lloyd Environmental, 2007) (Figure 2.9). This lake was once a freshwater wetland filled by floodwaters from the nearby Murray River, but since 1968, it has been used as an irrigation drainage basin. Lake Hawthorn is now cut off from floodwaters by levee banks and only receives water as runoff from nearby farming areas, except after very high flow events (Lloyd Lloyd Environmental, 2007). The salinity of the lake before 2000 was relatively stable at 3.4 g/L but in the period from 2000 to 2004 the salinity rose to approximately 6.1 g/L (Lloyd Lloyd Environmental, 2007). Lake Hawthorn is 222 hectares in size and a maximum depth of 5 meters (Lloyd Environmental, 2007).

Remnant *Eucalyptus largiflorens* and chenopod vegetation surrounds Lake Hawthorn. These areas are affected by salt and lack of water flow due to the nearby levee banks. Other species present around the lake include *Suaeda* spp. (Seablite), *Enchylaena tomentosa* R.Br (Ruby saltbush), *Lycium ferocissimum* Miers (African boxthorn) and various species of *Atriplex* spp. (Saltbush) and *Maireana* spp. (Bluebush) (ECOS Environmental Consulting, 2001). The submerged aquatic species *Ruppia* spp. has also been observed in the lake (Lloyd Lloyd Environmental, 2007).

This lake provides habitat for native fish including *C. fluviatilis* (Murray Hardyhead), *C. sterc. fulvus* (Unspeked Hardyhead) *Hypseleotris* spp. (Carp Gudgeons), *N. erebi* (Boney bream), *P. grandiceps* (Flat Headed Gudgeon), and *M. ambigua* (Golden Perch). Lake Hawthorn also supports the introduced fish species *G. holbooki* (Eastern Mosquito Fish) and *C. carpio* (Common Carp) (ECOS Environmental Consulting, 2001). Other vertebrates including three turtle species; *Chelodina expansa* Gray 1857 (Broad Shelled Turtle), *Chelodina longicollis* Shaw 1794 (Eastern Long-Necked Turtle) and *Emydura macquarii* Gray 1830 (Murray Turtle), as well as *Cherax* spp. (Yabbies), shrimp, and prawns. Up to 60 species of waterbirds are also found in and around this lake (ECOS Environmental Consulting, 2001).

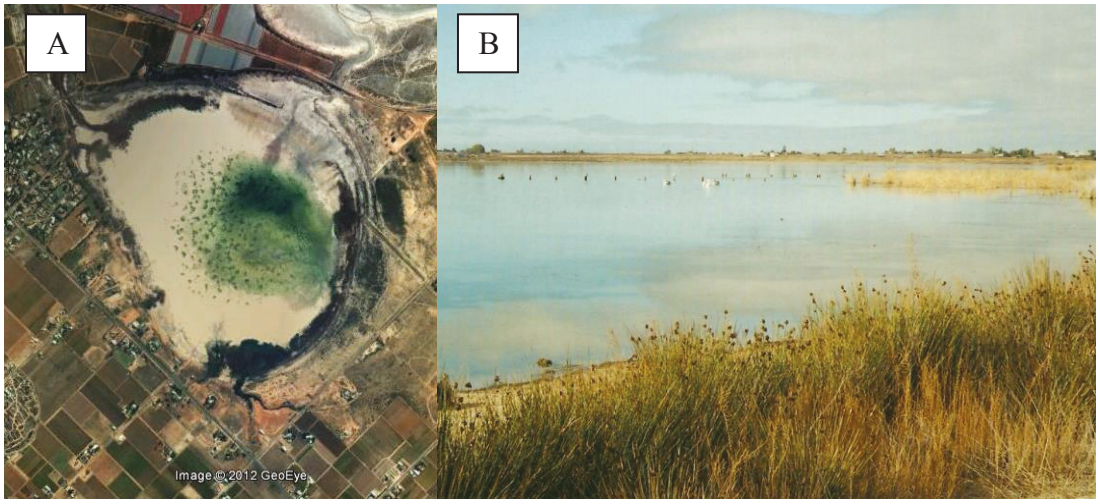


Figure 2.9 Lake Hawthorn, **A**. Aerial photo showing surrounding farmland (Google, 2012), **B.** Photo (looking southwest across the lake) showing *Junus acutus* (Spiny rush) in foreground.

2.2.2 Water quality

Water quality parameters were assessed at four sites around each wetland. Sites were selected by marking out 12 points on a map of each lake like a clock face (Figure 2.10) and using four random numbers (from 1 to 12, Appendix 1) to specify the sites. At each of these sites salinity (g/L), dissolved oxygen (mg/L), water temperature (^{o}C), and pH were measured using an Orion Multimeter Model No. 1230. Turbidity (NTU) was measured using a turbidity tube. Nutrient analysis was also carried out to at each site to determine the total phosphates (mg/L) and total nitrates (mg/L) of the water using a Palintest Nutrient Analysis kit, following manufacturer's instructions.

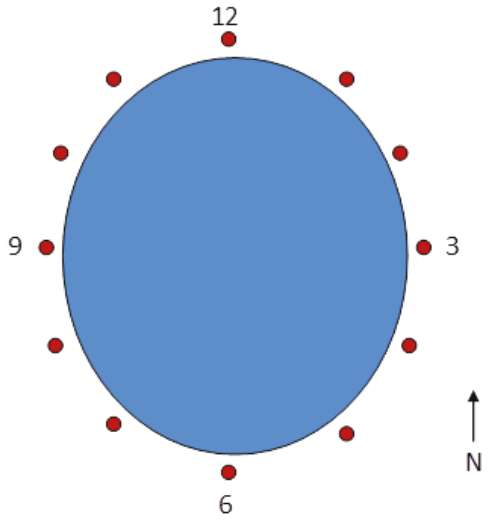


Figure 2.10 Random selection of sites for water quality measurements using a table of random numbers and a clock face method.

2.2.3 Aquatic macrophytes – belt transects

Percentage cover, dry weight biomass (g) and biovolume (mL), of aquatic macrophytes were assessed along belt transects within the lakes. The size of quadrats used in the belt transects was determined by completing a species area curve at 8 random sites within each lake. Quadrat sizes tested were 0.25m^2, 1m^2, 4m^2, 9 m^2, 16 m^2, and 25 m^2. A 0.5 meter by 0.5 metre (0.25m^2) quadrat proved to be most appropriate for all lakes surveyed (Appendix 2).

Four 0.5m wide and 15m long belt transects were established perpendicular to the shoreline at the same sites where water quality was assessed at each lake to investigate how plant density responded to water depth (transects A, B, C and D). A further two 0.5 meter wide and 15m long belt transects were run parallel to the shore to assess how plant density varied across the lake (transects E and F). The sites for the paraellel transects were next to two of the perpendicular transects (transects A and C) (Figure 2.11).

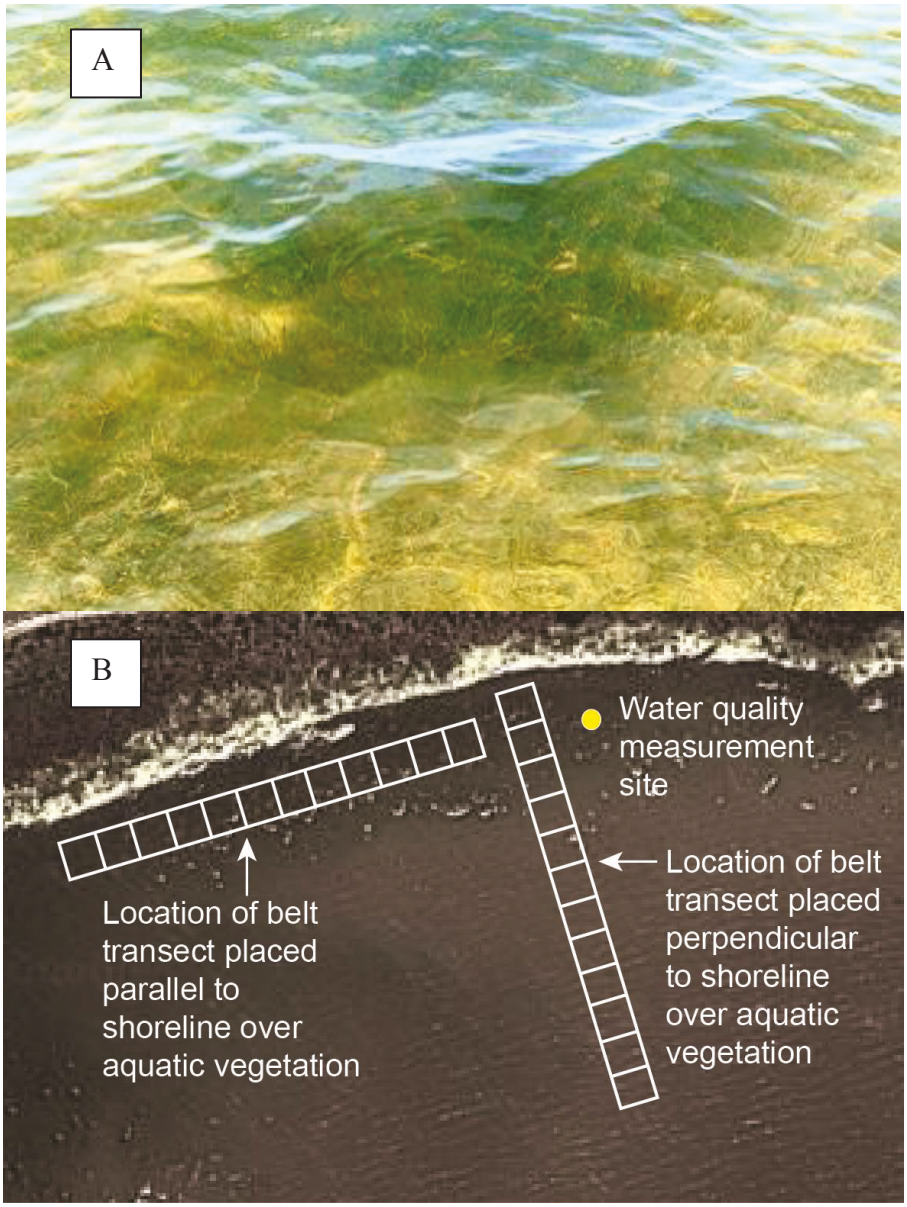


Figure 2.11 Selection of belt transect sites, **A**. Photo showing the patch like nature of macrophyte growth in Round Lake, near Lake Boga; **B.** Diagram showing selection of perpendicular and parallel belt transects

Quadrats along belt transects were set up by floating plastic 0.5m by 0.5m quadrats in the water secured by four stakes. A weighted (using fishing sinkers) cloth curtain was attached to the sides of the quadrats to delineate the quadrat boundaries on the lake bed and to ensure that plant material removed from within the quadrats was not lost. Water depth (cm) was recorded using a 1m ruler in each quadrat along the belt transects. Visual percentage cover of aquatic macrophyte species within each quadrat was recorded before all above ground vegetation was removed. Plant material was transported back to the laboratory where it was identified, sorted and assessed for biovolume (mL) and dry weight biomass (g). Biovolume was determined using the

Archimedes' Principle of the displacement of liquid wich states that the amount of water displaced by a submerged object is equal to the volume of that object in water (Pickover, 2008). The biovolume measurement gives an indication of the space (volume) that submerged aquatic take up in the water column and has been used in previous studies of aquatic macrophytes (Valley and Drake, 2007; Valley *et al.*, 2010). The biovolume of each species was determined by placing the plant material in a measuring cylinder of appropriate volume, depending on the amount of vegetation present. Then a known amount of water was added to the cylinder. The total volume of water and vegetation was recorded and the biovolume of the plant sample was determined using the following equation:

Plant Biovolume (mL) = Total volume of plants and water – Volume of water added to measuring cylinder

To determine the dry weight of the biomass (g), vegetation was removed from the cylinder and left to drain. Drained plant material was placed in a paper bag of known weight that had been dried in an oven 70^{o}C for 48 hours. Bags were reweighed and the dry weight biomass was determined using the equation below:

Dry weight biomass (g) = Total dry weight of plants and bag – Weight of pre dried bag

2.2.4 Aquatic macrophytes – boat survey

While the belt transects are useful for determining the biovolume and biomass of aquatic macrophytes, they can only be used in shallow waters. It is much more difficult to determine the density and biomass of plants in deeper waters, a visual assessment of plant cover was conducted from a boat. A 0.5m by 0.5m quadrat was floated on the side of an anchored boat at 100 random points in each lake. At each point total percentage cover of all macrophytes was recorded, because it was not possible to distinguish individual species in deep water.

2.2.5 Murray Hardyhead fish survey

A fish survey was conducted at the first three water quality monitoring sites in each lake. At each site 3 seine net hauls were conducted using a 10m long, 1mm mesh seine net. The live fish were caught by extending the entire length of the net from the

shore out into the water. The net was pulled approximately 10 metres parallel to the shore, over submerged macrophytes, before being pulled in a horseshoe shape until both ends met back at the shore. The net was then removed from the water and any fish caught were transferred to an aerated tank, where they were sedated using Alphaxon Quatazone, injected into the tank (1.5mL per 10 L of water). The first 100 Murray Hardyhead (*C. fluviatilis*) individuals caught at each wetland were measured for fork length (Figure 2.12). In accordance with animal ethics, no more than 100 Murray Hardyhead from any wetland were measured during this survey. Any other fish species caught were identified and the total length of the fish measured (Figure 2.12). Forked length is the preferred measurement in fish species with a forked tail, as the measurement is not biased by any damage the individual fish may sustain to the end of the tail (Jennings *et al.*, 2001).

Figure 2.12 Diagram showing measurement methods used in fish survey for forked tailed fish (left), and those without a forked tail (right)

All fish were returned live to the lake at the site where they were caught. Fish were handled using "wet" gloves to prevent any stress or damage to their scales. They were also given an antifungal wash to reduce the chance of infection, prior to being released at the site where they were captured.

2.2.6 Data Analysis

The mean percentage cover of awautic macrophytes determined in the boat survey was analysed using a One way ANOVA using the following model:

ModelDV = constant + Lake Location

Where the results of the ANOVA indicated that there was a significant difference between lakes, a *post hoc* Tukeys test was undertaken to determine which lakes differed significantly in percentage cover of aquatic macrophytes. All data analysis was undertaken using the PASW 18 statistical software (previously known as SPSS).

2.3 Results

2.3.1 Water quality

As shown in Table 2.1, the mean salinity of the four lakes varied greatly with the freshest wetland being Lake Hawthorn (mean salinity 8.2 g/L ± 0.023 g/L S.E) and the saltiest Lake Elizabeth (mean salinity 29.3 g/L ± 0.48 g/L S.E). The turbidity of the wetlands also varied with the saltier lakes (Round Lake and Lake Elizabeth) being clearer (mean turbidity concentrations 0 to 3 NTU respectively) than the fresher lakes (Lake Hawthorn and Woorinen North Lake) with mean turbidity concentrations of 29 and 34 NTUs respectively. The pH and dissolved oxygen results were similar across the four lakes with means ranging from pH of 8.2 to 8.8 and dissolved oxygen 8.6 mg/L to 9.6 mg/L. Mean phosphate concentrations were quite low at each lake, with mean phosphate concentrations ≤ 0.1 mg/L, with the exception of Woorinen North (0.3 mg/L ± 0.1 mg/L S.E). Mean nitrate concentrations varied across the different lakes with lower concentrations recorded at Lake Hawthorn and Lake Elizabeth and mean concentrations > 1.5 mg/L recorded at Round Lake and Woorinen North Lake (Table 2.1).

Table 2.1 Water quality results for four lakes of intermediate salinity in northwest Victoria, figures shown are means (± standard error).

Lake	Salinity (g/L)	Turbidity (NTU)	pH	DO (mg/L)	Phosphates (mg/L)	Nitrates (mg/L)
Hawthorn	8.2 (±<0.1)	28.8 (± 4.19)	8.6 (± 0.1)	8.6 (± 0.5)	< 0.1 (±<0.1)	0.93 (± 0.18)
Woorinen North	15.2 (±0.3)	33.5 (± 8.46)	8.2 (± 0.3)	9.4 (± 0.2)	0.3 (± 0.1)	1.56 (± 0.32)
Round	22.0 (±0.2)	0.0 (± 0)	8.6 (± 0.1)	9.1 (± 0.3)	0.1 (±<0.1)	2.17 (± 0.57)
Elizabeth	29.3 (±0.5)	2.5 (± 2.5)	8.8 (± 0.1)	9.6 (± 0.2)	<0.1 (± <0.1)	0.98 (±0.32)

2.3.2 Aquatic macrophytes

Lake Elizabeth

Two species of aquatic plants were present at Lake Elizabeth, both being submerged macrophytes *R. megacarpa* and *L. macropogon*. Lake vegetation in all transects except Transect C, was found to be continuous with vegetation present in the majority of quadrats with the exception of those close to the shore (Table 2.2 and Table 2.3). Although the vegetation was thick and continuous in many parts of the lake, the biovolume and dry weight biomass of both species was found to be "patchy" with no obvious pattern between increasing depth and the amount of vegetation present (Appendix 3). This lack of relationship was also found with the parallel transects where vegetation in terms of biomass and biovolume varied across very similar depths and percentage cover (Appendix 3). The lake vegetation was very thick in Transect A, with up to 10 000 mL of biovolume and > 700 g of dry weight biomass being recorded in some quadrats. *R. megacarpa* was the dominant species in the lake, contributing approximately 90% of overall biovolume and biomass in many quadrats (Table 2.4).

Table 2.2 Lake Elizabeth belt transects – presence/absence of *Ruppia megacarpa* in the lake

Key ☐ Absence of species ■ Presence of species

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A - perpendicular			■	■	■	■	■	■	■	■	■	■	■	■	■
B - perpendicular		■	■	■	■	■	■	■	■	■	■	■	■	■	■
C - perpendicular						■	■	■		■	■		■	■	■
D - perpendicular				■	■	■	■	■	■	■	■	■	■	■	■
E – parallel	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
F – parallel	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Table 2.3 Lake Elizabeth belt transects – presence/absence of *Lamprothamnium macropogon* in the lake

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A - perpendicular							■	■	■	■	■	■	■	■	■
B - perpendicular				■	■	■	■	■	■	■	■	■	■	■	■
C - perpendicular							■	■	■	■	■	■	■	■	■
D - perpendicular				■	■	■	■	■	■	■	■	■	■	■	■
E – parallel	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
F – parallel	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Table 2.4 Summary of macrophyte biovolume and dry weight biomass results from belt transects surveyed at Lake Elizabeth

	Plant Species							
	Ruppia megacarpa		*Lamprothamnium macropogon*		*Juncus acutus*		*Typha domingensis*	
	Min	Max	Min	Max	Min	Max	Min	Max
Dryweight biomass (g)	0	755	0	5	Not present	Not present	Not present	Not present
Biovolume (mL)	0	10 007	0	60	Not present	Not present	Not present	Not present

Round Lake

Four species of aquatic plants were found in the belt transect survey of Round Lake. These species included the emergent macrophytes, *Juncus acutus* L. (Spiny Rush) and *Typha domingensis* Pers. (Narrow-leaved Cumbungi) as well as submerged macrophytes *R. megacarpa* and *L. macropogon*. The vegetation of Round Lake was very "patchy" with quadrats without vegetation between areas of high plant biomass and biovolume (Table 2.5 to 2.8, Appendix 3). Two species of emergent macrophytes (*J. acutus* and *T. domingensis*) were only found in the littoral zone along transects on the north and east sides of the lake, whereas the submerged species (*R. megacarpa* and *L. macropogon*) were found in deeper waters towards the centre of the lake. No patterns were found between depth and biovolume, or dry weight biomass on either the perpendicular or parallel transects. The vegetation in Round Lake was not as dense as that in Lake Elizabeth, with the most vegetation in a quadrat found in Transect D > 3000 mL of biovolume and > 700 g of dry weight biomass. *Ruppia megacarpa* was the dominant species contributing to over 90% of overall biovolume and biomass in many quadrats (Table 2.9).

Table 2.5 Round Lake belt transects – presence/absence of *Ruppia megacarpa* in the lake

Key: Absence of species; Presence of species

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A – perpendicular															
B – perpendicular															
C – perpendicular															
D – perpendicular															
E – parallel															
F – parallel															

Table 2.6 Round Lake belt transects – presence/absence of *Lamprothamnium macropogon* in the lake

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A – perpendicular															
B – perpendicular															
C – perpendicular															
D – perpendicular															
E – parallel															
F – parallel															

Table 2.7 Round Lake belt transects – presence/absence of *Typha domingensis* in the lake

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A – perpendicular															
B – perpendicular															
C – perpendicular															
D – perpendicular															
E – parallel															
F – parallel															

Table 2.8 Round Lake belt transects – presence/absence of *Juncus acutus* in the lake

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A – perpendicular															
B – perpendicular															
C – perpendicular															
D – perpendicular															
E – parallel															
F – parallel															

Table 2.9 Summary of macrophyte biovolume and dry weight biomass results from belt transects surveyed at Round Lake

	Plant Species							
	Ruppia megacarpa		*Lamprothamnium macropogon*		*Juncus acuta*		*Typha domingensis*	
	Min	Max	Min	Max	Min	Max	Min	Max
Dryweight biomass (g)	0	702	0	6	0	86	0	392
Biovolume (mL)	0	2995	0	64	0	290	0	1174

Woorinen North Lake

Only one aquatic macrophyte was present at Woorinen North Lake, the submerged species *R. megacarpa*. Density and percentage cover of vegetation at this lake was very patchy and much lower than all other lakes. In fact many quadrats were devoid of vegetation between areas of *R.megacarpa* growth (Table 2.10, Appendix 3). No patterns were found between depth and biovolume or dry weight biomass on either the perpendicular or parallel transects. The biovolume and dry weight biomass found in this lake was much lower than all other lakes with a maximum of 1064 mL biovolume and a maximum of 755g dry weight biomass measures in any quadrat (Table 2.11).

Table 2.10 Lake Woorinen North belt transects – presence/absence of *Ruppia megacarpa* in the lake

Key ☐ Absence of species ■ Presence of species

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A – perpendicular													■	■	
B – perpendicular													■	■	■
C – perpendicular													■	■	
D – perpendicular			■	■											
E – parallel					■				■	■	■	■	■	■	■
F – parallel	■	■					■	■	■						

Table 2.11 Summary of macrophyte biovolume and dry weight biomass results from belt transects surveyed at Lake Woorinen North

	Plant Species							
	Ruppia megacarpa		*Lamprothamnium macropogon*		*Juncus acutus*		*Typha domingensis*	
	Min	Max	Min	Max	Min	Max	Min	Max
Dryweight biomass (g)	0	755	Not present	Not present	Not present	Not present	Not present	Not present
Biovolume (mL)	0	1064	Not present	Not present	Not present	Not present	Not present	Not present

Lake Hawthorn

Three macrophytes were found in the belt transect survey of Lake Hawthorn. Species included the emergent macrophyte, *T. domingensis* and submerged macrophytes *R. megacarpa* and *L. macropogon*. The vegetation of Lake Hawthorn was very "patchy" with quadrats without vegetation between areas of dense vegetation (Table 2.12 to 2.14, Appendix 3). The emergent species *T. domingensis* was only found in the littoral zone of the lake on Transect A, (on the east side of the lake), whereas the submerged species were found in deeper waters towards the centre of the lake. No patterns were found between depth and biovolume or dry weight biomass on either the perpendicular or parallel transects. The vegetation was not as dense as that of Lake Elizabeth, with the greatest vegetation cover found on Transect B with > 2100 mL of biovolume and > 500g of dry weight biomass. Again *R. megacarpa* was the dominant species contributing to over 90% of overall biovolume and biomass in many quadrats (Table 2.15).

Table 2.12 Lake Hawthorn belt transects – presence/absence of *Ruppia megacarpa* in the lake

Key ☐ Absence of species ■ Presence of species

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A – perpendicular					■	■	■			■	■	■	■		
B – perpendicular												■	■	■	■
C – perpendicular			■	■	■							■	■	■	■
D – perpendicular											■	■	■		
E – parallel				■	■										
F – parallel		■	■						■		■	■	■		

Table 2.13 Lake Hawthorn belt transects – presence/absence of *Lamprothamnium macropogon* in the lake

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A – perpendicular					■	■	■			■	■	■	■		
B – perpendicular												■	■	■	■
C – perpendicular			■	■	■							■	■	■	■
D – perpendicular											■	■	■		
E – parallel				■	■										
F – parallel		■	■						■		■	■	■		

Table 2.14 Lake Hawthorn belt transects – presence/absence of *Typha domingensis* in the lake

Transect	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A – perpendicular	■	■													
B – perpendicular															
C – perpendicular															
D – perpendicular															
E – parallel															
F – parallel															

Table 2.15 Summary of macrophyte biovolume and dry weight biomass results from belt transects surveyed at Lake Hawthorn

	Plant Species							
	Ruppia megacarpa		*Lamprothamnium macropogon*		*Juncus acutus*		*Typha domingensis*	
	Min	Max	Min	Max	Min	Max	Min	Max
Dryweight biomass (g)	0	506	0	9	Not present	Not present	0	189
Biovolume (mL)	0	2080	0	66	Not present	Not present	0	548

Results from the boat based macrophyte survey showed that the mean percentage cover varied for each lake, with the lowest mean cover recorded at Woorinen North Lake and the highest at Round Lake (Figure 2.13). Results of a one way ANOVA showed that there was a significant ($p < 0.01$) difference in the mean percentage cover of aquatic macrophytes between the lakes ($p < 0.001$, $F = 37.281$, $df = 3, 396$). A *post hoc* Tukey's test showed that there was a significant difference ($p < 0.001$) between all the lakes surveyed with the exception of Lake Hawthorn and Lake Elizabeth (Table 2.16).

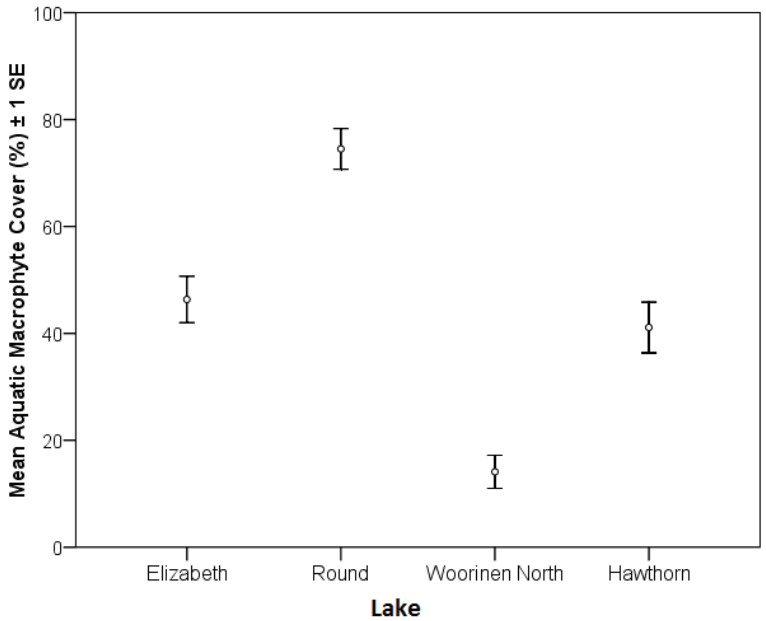


Figure 2.13 Mean aquatic macrophyte % cover as observed from boat at the four wetlands – Lake Elizabeth, Round Lake, Woorinen North Lake and Lake Hawthorn (n = 100).

Table 2.16 Results of *post hoc* Tukey's test for % cover of aquatic macrophytes at the four lakes

	Lake Elizabeth	Round Lake	Woorinen North Lake	Lake Hawthorn
Lake Elizabeth				
Round Lake	<0.001			
Woorinen North Lake	<0.001	<0.001		
Lake Hawthorn	0.789	<0.001	<0.001	

2.3.3 Fish survey

Three different fish species were caught in the four lakes, namely *C. fluviatilis* (Murray Hardyhead), *G. holbrooki* (Eastern Mosquito fish) and *P. grandiceps* (Flat Headed Gudgeon). It should be noted that individuals in Lake Hawthorn could be either *C. fluviatilis* or *Craterocephalus sterc. fulvus* Ivantsoff, Crowley and Allen 1987 (Unspecked Hardyhead), which are both known to inhabit the lake. These two species are difficult to distinguish between, especially the juvenile fish and thus have been counted together.

The greatest number of individuals was caught in Round Lake (*C. fluviatilis* =277 and *G. holbrooki* = 1) with large numbers of fish also caught in Lake Hawthorn (*C. fluviatilis* = 182 and *G. holbrooki* = 14). Low numbers of all three species were recorded from Woorinen North Lake and no fish were caught from Lake Elizabeth (Table 2.17)

The mean catch per unit effort (mean number of individuals per seine net haul) was variable across Lake Hawthorn and Round Lake for individuals of *C. fluviatilis*. Little can be determined from the catch per unit effort data for *G. holbrooki* and *P. grandiceps* as overall few individuals were caught for these species (Table 2.17).

Table 2.17 Total number of individuals caught for each fish species and catch per unit effort for each species, found in the four lakes.

Lake	*Craterocephalus fluviatilis*		*Gambusia holbrooki*		*Philypnodon grandiceps*	
	Total	**Catch per unit effort**	**Total**	**Catch per unit effort**	**Total**	**Catch per unit effort**
Hawthorn	182	46	14	4	0	0
Woorinen North	8	< 1	1	< 1	2	< 1
Round	277	40	1	< 1	0	0
Elizabeth	0	0	0	0	0	0

Little information can be drawn from the results of the fish size classes from Lake Woorinen or the fish species *G. holbrooki* and *P. grandiceps* given the few individuals caught (Appendix 4). Nevertheless, from the *C. fluviatilis* and *Craterocephalus* spp. data recorded from Round Lake and Lake Hawthorn, the catch was dominated by small fish of < 35mm in length and fewer fish > 35mm (Figure 2.14).

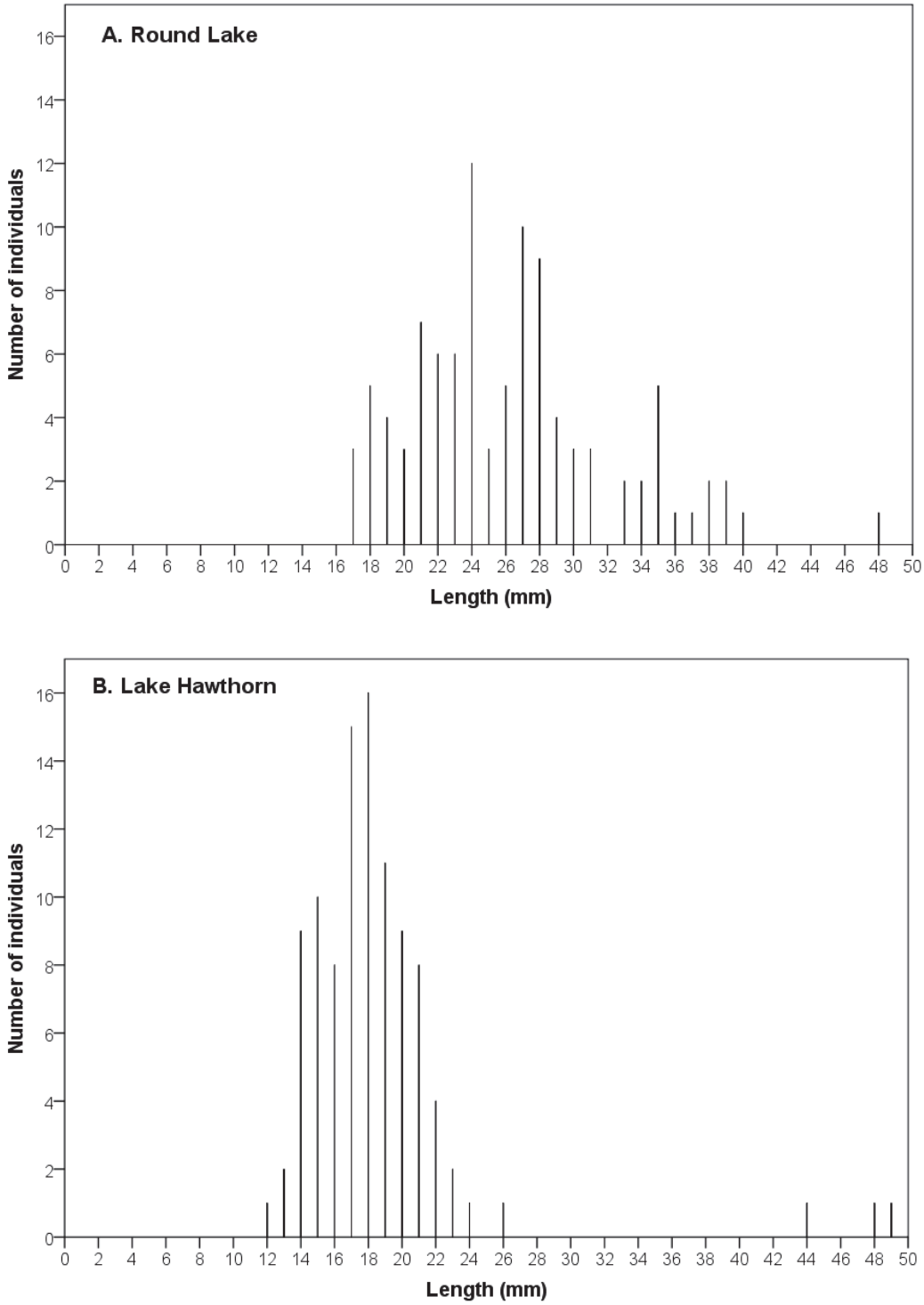


Figure 2.14 A. Size of *Craterocephalus fluviatilis* (Murray Hardyhead) individuals caught from Round Lake **B.** Size of *C. fluviatilis* individuals caught from Lake Hawthorn

2.4 Discussion

All four lakes surveyed in this study were saline (ranging from 8.2 g/L ± <0.1 g/L S.E to 29.3 g/L ± 0.5 g/L S.E) and supported few aquatic plants and fish species, which is typical of saline lakes in Australia (Brock, 1986; Timms, 1993; Kingsford and Porter, 1994). Whilst these lakes have low diversity of biota, they were found to be similar to many other saline lakes in that they very productive with extensive cover of submerged aquatic macrophytes (Timms, 1997).

2.4.1 Water quality

The water quality results were similar to those recorded in other saline wetlands in Australia with low turbidity (mean <34 NTU) and high pH (mean pH >8). High pH levels have been recorded in lakes in western Victoria (Williams, 1981; Khan, 2003) and in other desert salt lakes in New South Wales (Timms, 1993). Alkaline pH levels have been linked to increased photosynthetic activity in inland waters (López-Archilla *et al.*, 2004), and given the high productivity of the wetlands is a plausible explanation for the high pH levels recorded.

Turbidity concentrations < 30 NTU, are often found in saline lakes and have been recorded in previous studies of saline lakes by Davis *et al.,* (2003) and Timms (1997). It should be noted that a number of factors including wind and time of day of the testing can impact on turbidity readings and as such no conclusions can be drawn from one assessment. But given the extensive cover of macrophytes in the wetlands surveyed, especially Lake Elizabeth and Round Lake, light is probably not a limiting factor for macrophyte distribution and growth.

Dissolved oxygen concentrations were not limiting to fish populations with mean dissolved oxygen concentrations ranging between 8.6 mg/L to 9.6 mg/L across the four lakes which is well above the ANZECC recommended minimum of 6 mg/L (ANZECC and ARMCANZ, 2000).

Nutrient analysis of the wetlands indicated that phosphorus concentrations for Lake Elizabeth, Lake Hawthorn and Round Lake were all within acceptable concentrations

as recommended for Australian waters with concentrations less than 0.05 mg/L (ANZECC and ARMCANZ, 2000). Mean phosphate concentrations in Lake Woorinen were 0.3 mg/L (± 0.1 mg/L S.E). This high concentration is probably due to one sample, with a phosphate concentration of 0.7 mg/L being recorded. Although it should be noted that many other samples had high phosphate concentrations of between 0.1 and 0.3 mg/L.

Nitrate concentrations in these lakes were much higher than those recommended for Australian waters (between 0.1 mg/L and 0.5 mg/L) (ANZECC and ARMCANZ, 2000). Results of this study varied between 1.0 mg/L (± 0.3 mg/L S.E) and 2.1 mg/L (± 0.6 mg/L S.E) nitrate. There are a number of factors that may influence nutrient concentrations in wetlands, particularly runoff from surrounding farms through agricultural fertilizers or via sewage (Boulton and Brock, 1999) and also from waterbirds that utilize the wetlands. Mitchell and Wass (1995) found that when Black Swans (*Cygnus atratus* Latham 1790), were present in high numbers, they contribute a large amount of nutrients to the waters. Many *C. atratus* were observed on all wetlands included in this study and their contribution to nutrient concentrations warrants further study.

2.4.2 Aquatic macrophyte composition and abundance

Clear water is often associated with saline lakes and thus light is generally not considered a limiting factor for macrophyte growth in shallow saline lakes (Davis *et al.*, 2003; Sim *et al.*, 2006a). Previous studies have shown that saline lakes have low plant diversity, but tend to have high productivity and the lakes surveyed in this study exhibit this (Brock, 1986; Timms, 1993; Kingsford and Porter, 1994). Round Lake and Lake Elizabeth in particular supported extensive areas of submerged aquatic macrophyte beds throughout the lake, with an average of 74% (± 4% S.E), and 73% (± 17% S.E) cover across the lakes respectively. In comparison, less macrophyte cover was found in Lake Hawthorn and Woorinen North Lake with average of 48% (± 5% S.E) and 14% (± 3% S.E) cover respectively. The percentage cover of aquatic macrophytes in all lakes (in particular those with a lower overall percentage cover) was heterogeneous across the wetlands with some areas showing 100% cover of an aquatic macrophytes, and other areas with 0% vegetation cover. No relationship was found between the average percentage cover of aquatic

macrophytes and salinity. But wetlands with lower percentage cover of macrophytes, did have higher turbidity suggesting that turbidity influences macrophyte growth. Lakes with higher turbidity will have higher suspended sediments in the water column thus reducing light and covering macrophyte leaves leading to reduced photosynthesis (Groves, 1994). More detailed field surveys need to be conducted to investigate the effect of turbidity on plant growth, as this pattern was observed in a simple field assessment.

Plant species composition was very similar across the four lakes with *R. megacarpa* found in all lakes, and *L. macropogan* found in all lakes except Lake Woorinen North. These species are known for their tolerance of salinities ranging from fresh to intermediate salinity concentraion (Brock and Lane, 1983; Brock, 1986; Hart *et al.*, 1991; Garcia and Chivas, 2004; Sim *et al.*, 2006a). The emergent macrophytes *J. acutus* and *T. domingensis* were also recorded at Lake Hawthorn and Round Lake. These emergent macrophytes were dominant in the littoral zone and were not found in deeper waters, which is characteristic of these species. All of these species were recorded at these locations in previous surveys (Anderson, 1991).

Submerged macrophytes recorded had higher biomass within the deeper parts (>1m) of the lake, where plants were tall compared to the short individuals growing in the shallower sections of the lakes. Some very high dry weight biomass and biovolume were recorded in Lake Elizabeth and Round Lake with over 700g per $0.5m^2$ dryweight biomass recorded in some quadrats. Hartke *et al.*, (2009) studied *Ruppia* biomass along the Texas Gulf coast and recorded biomass of 202 g/m^2 which is considerably lower than maximums recorded in this study, indicating that Lake Elizabeth and Round Lake in particular are extremely productive. Productive lakes provide more food resources and habitat for invertebrates, fish and waterbird communities, making these lakes of high ecological value in the landscape. No relationship was found between salinity concentration and biomass, indicating that salinity had little impact on biomass weights observed in this study.

2.4.3 Fish community composition and abundance

Three species of fish (*C. fluviatilis, G. holbrooki* and *P. grandiceps*) were found in three of the four wetlands. The only lake where no fish were recorded was Lake Elizabeth which was the saltiest of the four wetlands in this study. These three fish species have been identified by Wedderburn *et al.,* (2007), as being present in wetlands of the Murray Darling Basin. Populations of *C. fluviatilis* were found in Round Lake and Lake Hawthorn (over 100 individuals caught in each lake, with mean catch per unit effort of over 30 individuals per seine net) with much lower numbers recorded Woorinen North Lake.

Philypnodon grandiceps was only recorded in Woorinen North Lake which was expected, as the salinity concentation of the other lakes in this study were too high for this species to tolerate (Jackson and Pierce, 1992), whilst the introduced species *G. holbrooki* was recorded at all locations where *C. fluviatilis* was present. The effect of *G. holbrooki* on *C. fluviatilis* populations is unknown but has been suggested as a possible reason for the decline of this species (Ellis, 2005a; Backhouse *et al.*, 2006). In particular it has been suggested that these two species may compete for food resources thus the effect of *G. holbrooki* on *C. fluviatilis* warrants further study (Ellis, 2005b; Backhouse *et al.*, 2006). Of the three species found in this study, *C. fluviatilis* was the most abundant; whilst the catch per unit effort results were high for *C. fluviatilis*, they were also extremely variable with high standard errors. This is due to *C. fluviatilis* being a schooling species, so if fish were caught in the nets, they were caught in large numbers. A range of sizes were present in fish populations in Round Lake and Lake Hawthorn, but very little information can be gained from the results in Woorinen North Lake given the low number of individuals caught. For most fish species, size is a good indicator of age (Pitcher, 2002), but as discussed by Ellis (2006) *C. flvuviatilis* is an annual species with individuals rarely surviving more than one year. Size class range data for this species may be used to monitor the success of spawning seasons in these lakes.

Loss of *Craterocephalus fluviatilis* populations from Lake Elizabeth

Fish populations have been recorded in Lake Elizabeth in past studies (Lugg *et al.*, 1989; Flemming, 1990; Anderson, 1991; Kelly, 1996; Delany, 2004; Ellis, 2005b), but no fish were found in this study, or another completed by the Department of

Sustainability and Environment in 2005 (Ellis, 2005b). Much discussion has focused on reasons for the loss of the *C. fluviatilis* from this lake. Possible suggestions include increased salinity concentations and acid sulphate soils. Many studies have looked at the formation of acid sulphate soils, where sulphidic materials such as pyrite (FeS_2) and monosulphides (FeS) have accumulated in the sediments. When these materials are oxidised, a range of water quality issues can arise, including the development of acidic waters and low dissolved oxygen concentration (Environment Protection and Heritage Council and the Natural Resources Management Ministerial Council, 2011). An acidic pH (4.83) was recorded in Lake Elizabeth in September 2004 by the Department of Sustainability and Environment (Ellis, 2006). Interestingly results in this study were in direct contrast with high pH and dissolved oxygen concentations recorded (Table 2.1). The possibility of acid sulphate soils at Lake Elizabeth requires further investigation.

As suggested by Dixon (2007), high salinity concentrations are a more plausible reason for the loss of fish from Lake Elizabeth, where salinity concentrations peaked at 40.8 g/L in 2001. Fish were able to tolerate this because surveys in 2002 found a large population of *C. fluviatilis* living in the lake (Lyon *et al.*, 2002). It is important to note that all individuals found in this survey were comparatively large, indicating that they were adults with few juveniles in the population. Between 2002 and 2005 salinity concentrations were maintained at lower concentrtations, yet the fish population did not survive. One possible reason for the loss of this population is that whilst the high salinity concentrations in 2002 may not have been lethal for the adult fish, it may have prevented reproduction of this species. Given that *C. fluviatilis* is an annual species (Ellis, 2006), the lack of reproduction in one year could lead to the loss of populations.

Little research has been conducted on the sublethal effects of salinity on *C. fluviatilis*, however research of sublethal effects on other fish have been conducted (Guo *et al.*, 1993). Guo *et al.*, (1993) reported that the LC_{50} for eggs and larvae of *Macquarua australasica* Cuvier, 1930 (Macquarie perch) and *Maccullochella macquariensis* Cuvier, 1829 (Trout cod) was as low as 2.1 g/L and that in general, pre hardened fish eggs have an upper salinity threshold of approximately 2 g/L to 4.5 g/L and juvenile fish have an upper salinity tolerance of 3 g/L to 5 g/L.

Ellis (2006) studied the reproduction of *C. fluviatilis* and found that this species is an annual species (with life span of about 1 year) meaning that it is quite short lived so spawning is required each year in order for the populations to exist long term. Whilst this species can tolerate high salinities, even one season of salinities past the threshold (yet to be determined) can have dramatic impacts on the survival of the population within a wetland.

The salinity range that *C. fluviatilis* tolerates is very wide; from fresh water locations in South Australia (between 0.5 g/L to 2.7 g/L) to the saline wetlands in Victoria (over 40.8 g/L). Studies by Wedderburn *et al.*, (2008), found that *C. fluviatilis* are extremely good osmoregulators, thus allowing them to inhabit a range of saline environments. The exact salinity tolerance range for this species has been tested using both field and laboratory studies. The upper salinity ranges where *C. fluviatilis* have been recorded include 45.9 g/L at Lake Golf Course (McGuckin, 1999), and populations were known to exist after a salinity peak of 40.8 g/L in Lake Elizabeth (Ellis, 2006). Laboratory testing has shown that the salinity tolerance of adults tends to be lower than these reported concentrations. Dixon (2007) conducted laboratory tests on the salinity tolerance range of *C. fluviatilis* individuals taken from Lake Woorinen North and found that individuals of this species from Lake Woorinen North had an LC_{50} acute salinity tolerance of 59.5 g/L. Dixon (2007), indicating that individual populations of *C. fluivatilis* may have varying salinity tolerance ranges, suggesting that acclimation of the species to its environment may be important and that gradual increases in salinity may have less of an impact on populations than sudden marked increases. It is important to note that these tests have been conducted only on adult life stages and that larval stages and the eggs of this species are probably more susceptible to increases in salinity. In particular, the loss of the species from Lake Elizabeth when adults had survived a high salinity peak of 40.8 g/L and yet the population did not survive the subsequent lower salinity concentrations suggests that reproduction, spawning and early more sensitive life stages have a much lower tolerance of increased salinity concentrations.

2.4.4 Management implications for saline wetlands in northwest Victoria

It has been reported that there has been a decline in submerged aquatic macrophyte density in wetlands in the Kerang Lakes area since the introduction of the Torrumbarry Irrigation Scheme (KLAWG, 1992). The loss of submerged aquatic macrophytes is thought to be a consequence of a number of anthropogenic changes including increased turbidity in freshwater wetlands used for irrigation water storage, increased salinity due to land clearing, irrigation and salt disposal practises. Additionally, drying out, lack of connectivity to the floodplain and reduced water availability as a result of climate change and irrigation practices in the region have also reduced macrophyte density and diversity (KLAWG, 1992). Therefore the management of saline wetlands with a clear water, macrophyte dominated regime would benefit from the conservation of submerged aquatic plant communities in the area. This would also provide lakes with high productivity enabling them to support invertebrate, fish and waterbird communities often associated with saline lakes (Timms, 1993; Kingsford and Porter, 1994; Kingsford, 1995; Timms, 1997).

In wetlands were *C. fluviatilis* populations are no longer present, permanent hydrological regimes are not required as both *R. megacarpa* and *L. macropogon* have propagules that can withstand drying (Brock, 1982a; Brock, 1982b; Bradbury, 2002; Sim *et al.*, 2006a). There are also invertebrate species that can produce eggs that survive by entering diapause within the substrate and emerge upon flooding (Nielsen *et al.*, 2002; Brock *et al.*, 2003; Nielsen *et al.*, 2003a; Brock *et al.*, 2005; Nielsen *et al.*, 2007; Nielsen *et al.*, 2008). There is some evidence to suggest that Lake Elizabeth in particular would be best managed as a semi-permanent lake. Interactions between the water in the lake and the underlying water table have been thought to cause potential salinity issues in surrounding farmland (Delany, 2004) and thus this lake may be better managed as a semi-permanent lake. However, this requires further investigation.

The water regime in lakes with populations of *C. fluviatilis* need to be monitored carefully to ensure the survival of this endangered species. Early studies of *C. fluviatilis* in Victoria suggested that the presence of *Ruppia* spp. maybe a good indicator of the presence of *C. fluvatilis* populations (Lyon *et al.*, 2002). *Craterocephalus fluviatilis* was thought to be reliant on *Ruppia* for survival, as all populations of this fish were found in wetlands in Victoria that supported *Ruppia*

growth. In contrast studies in South Australia (Wedderburn *et al.*, 2007; Wedderburn *et al.*, 2008), have found *C. fluviatilis* in areas without *Ruppia*, but have been found where *Myriophyllum* is present. *Craterocephalus fluviatilis* are known to deposit eggs on submerged vegetation, so the plant species it is not as important, as the presence of submerged vegetation is for the survival of this species.

Whilst maintaining salinity at acceptable concentrations is extremely important, lack of water seems to also be a major risk to the survival of this fish species. During the recent drought (2003 to 2012) some of the lakes studied completely dried out. Populations of *C. fluviatilis* were kept in captive breeding programs in Mildura and South Australia to ensure the species was not lost completely, and reintroductions of *C. fluviatilis* have taken place at sites around Victoria (Brock, 2011). Since this study was completed the Kerang Lakes area was also extensively flooded (Seabloom *et al.*, 1998; Darvas, 2007). Recent investigations of irrigation lakes in the Kerang area have found populations of *C. fluviatilis* in Middle Reedy lake, a site that previously did not support this species (Williams, 1966). Further studies on the wetlands in the region may be useful in seeing if refuge populations have migrated to new locations that had previously been dry or disconnected from the flood plain. It may also be possible for *C. fluviatilis* to be re-introduced back to wetlands that in the past had become too saline and/or were dry.

3.0 Effect of salinity on the egg and propagule bank of Lake Cullen, an ephemeral saline wetland of northwestern Victoria

3.1 Introduction

Secondary salinisation is recognized as an increasing problem throughout Australia and it has been reported that by 2050 almost 14% of the total area of Victoria will be affected by increased salinity (Morgan, 2001). Whilst salt is considered a natural part of the Australian landscape and much of the inland groundwater is of a saline nature, secondary salinisation has caused major changes to the landscape across parts of Australia. Secondary salinity is a consequence of the large scale clearing of deep rooted native vegetation to make way for shallow rooted grass crops. Without the deep rooted native plants to soak up the rainfall, excess water has entered these inland salty groundwater systems causing water tables to rise towards the surface. The rise of groundwater tables has caused excess salt to accumulate in the soil and in some low lying areas the water table actually intersects the beds of rivers, creeks and lakes causing groundwater intrusions which raise salinity concentrations dramatically (Aplin, 1998). Causes of secondary salinisation also include irrigation practices where salty waters are discharged into freshwater systems (Williams, 2001), which subjects aquatic ecosystems are subjected to the negative effects of increasing salinity. Recently some studies have also focused on the effects of climate change on wetlands and have identified that decreased rainfall and increased temperatures may increase the risk of rising salinity in many waterways across Australia (Herbst and Blinn, 1998; James, 2005; Nielsen and Brock, 2009).

Saline wetlands with submerged aquatic macrophyte species tend to have less biodiversity than freshwater systems. They can be very productive with few species, producing a large amount of aquatic plant biomass. These macrophytes support large numbers of salt tolerant invertebrate and vertebrate taxa (Brock, 1986; Timms, 1993; Kingsford and Porter, 1994). However once salinity exceeds the threshold for germination and growth, the loss of macrophytes from the wetland results in a hypersaline system that is less complex (and may be dominated by microbial mats) that can no longer support a diverse range of animal species (Bauld, 1981; Davis, 2002; Davis *et al.*, 2003; Strehlow *et al.*, 2005; Sim *et al.*, 2006a; Sim *et al.*, 2006b; Sim *et al.*, 2006c; Davis *et al.*, 2010). Many studies have suggested an upper salinity

threshold for macrophyte dominated systems (Hammer, 1986; Davis *et al.*, 2003; James *et al.*, 2003). Sim *et al.,* (2006a) suggested that the upper salinity threshold for macrophyte dominated saline wetlands, is approximately 45 g/L.

3.1.1 Lake Cullen

Lake Cullen is a large (632 ha), but shallow (maximum depth 2m) wetland, located 330 km northwest of Melbourne, near Kerang, Victoria (Figure 3.1). It is part of the Kerang Wetlands Ramsar site and the Torrumbarry Irrigation Scheme, which delivers water from the Murray River along engineered and natural channels, weirs and wetlands. It receives water through an irrigation channel connecting it to Racecourse and Kangaroo Lakes. Lake Cullen is surrounded on three sides by lunettes as well as engineered structures such as a railway line, levee banks and roads. As a result natural water flows into the lake are completely cut off and water management authorities currently control the flooding regime (KLAWG, 1992).

Before 1970, Lake Cullen was used for storing irrigation waters which were drawn directly from this lake to nearby farms. After this time the lake was no longer used for irrigation water storage because high evaporation rates from the site deemed the practice wasteful. The lake progressively became salty through evaporation, changing it from permanent freshwater to a permanent saline wetland, topped up by floodwaters (KLAWG, 1992). The increased salinity of Lake Cullen became a concern in the early 1980s, and as a result, possible management options were investigated in an attempt to reduce salinity. It was recommended that the lake be allowed to dry and then flushed with freshwater to force salt back into the water table in a process called “lake bed flushing” (Department of Sustainability and Environment, 2004). The lake dried out in 1996 and since then has been managed to receive water on a one in five year regime (Department of Sustainability and Environment, 2004).

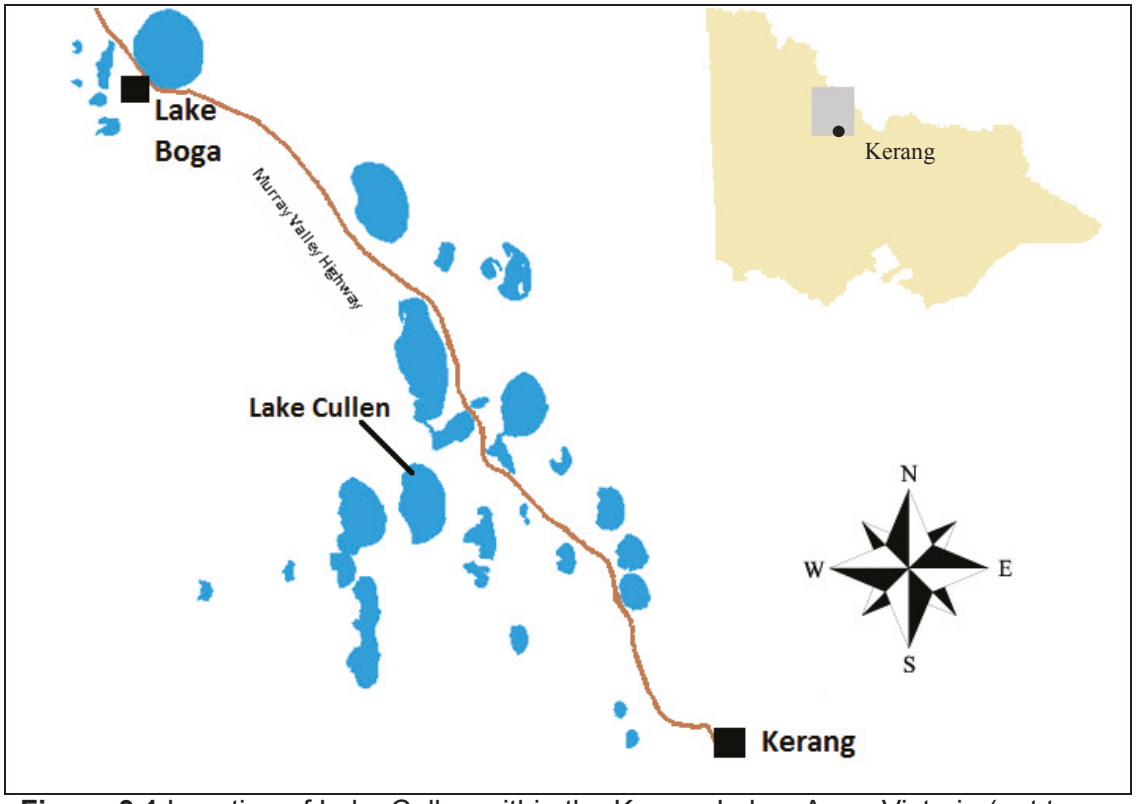


Figure 3.1 Location of Lake Cullen within the Kerang Lakes Area, Victoria (not to scale)

3.1.2 Hypotheses

This study is the first of two studies investigating the effect of salinity on macrophyte and invertebrate communities emerging from the egg and propagule bank of Lake Cullen. The hypotheses tested in this study were

- What are the salinity thresholds for the emergence of aquatic macrophyte and invertebrate communities found in Lake Cullen?
- Is there any evidence for indirect effects of salinity on the emergence of aquatic macrophyte and invertebrate communities found in Lake Cullen?
- Is there any evidence for indirect effects of salinity on growth and reproduction of aquatic macrophytes?

Establishing salinity thresholds for the survival of vertebrate adult life stages has been the focus of many studies. Some studies identified that juveniles are more sensitive to increases in salinity concnetrations (James *et al.*, 2003). Studies on species of Australian fish have focused on the effects of salinity concentrations on different life stages such as juvenile survivorship and egg hatching. Others have identified the sub lethal effects e.g. reduced growth rates or stunting of plants (Hogan

and Nicholson, 1987; Williams, 1987; Bacher and O'Brien, 1989; Hart *et al.*, 1991; Guo *et al.*, 1993; Guo *et al.*, 1995; O'Brian and Ryan, 1999). It is important to consider that any sub lethal effects and salinity thresholds identified for adults may not be the same for all life stages of plants and animals. For example, James *et al.,* (2003) found in their review of the literature that whilst adult Australian fish are often able to tolerate salinities of up to 8.8 g/L, sub lethal and adverse effects on fish eggs, juvenile growth rates and survivorship as well as sperm motility were observed at salinities of between 4.5 to 5.0 g/L. For invertebrates Kefford *et al.,* (2007) found that early larval stages had lower salinity tolerance concentrations than adult life stages in 60% to 70% of freshwater species studied. There were some species e.g., Glossiponiidae species (Leeches), however where the salinity tolerance concentrations for earlier life stages were very similar to that of the adults.

The sub lethal and lethal effect of salinity on the life stages of plants has not been widely studied. This study aims to investigate if salinity affects the reproductive success and growth of plant species establishing from the propagule bank. Aquatic plant reproduction is an important issue because it is not known how long propagules in a propagule bank can remain viable. Given that Lake Cullen is filled only once every 5 years under the current management regime, it is essential that aquatic plant propagules and invertebrates are able to remain viable in the propagule bank during dry periods and that these species are able to contribute to the seed and egg bank of the lake before it dries out (Department of Sustainability and Environment, 2004). This study also aims to provide information to enable adaptive management of the flooding regime of this lake.

3.2 Methods

The effect of increasing salinity on the propagule bank and invertebrate egg bank of Lake Cullen was investigated by subjecting lake substrate to nine salinity treatments. Saline lakes of Australia are mostly dominated by sodium chloride and often mimic the ionic concentration of sea water (Bayly and Williams, 1966; Williams, 1966; Williams, 1998c), thus "Ocean Nature" sea salt was used throughout this experiment. The salinity of the treatments (measured in g/L) was determined using a partial Fibonacci sequence, so the treatments represented a natural logarithmic relationship (Table 3.1). Fibonacci sequences are known numeric patterns to occur in the living world with many animal population models, plant structures and animal shells following Fibonacci number patterns (Darvas, 2007).

Table 3.1 Salinity treatments tested in this study, using Lake Cullen substrate samples.

Treatment Number	Concentration g/L
1	3.4
2	5.5
3	8.9
4	14.4
5	23.3
6	37.7
7	61.0
8	98.7
9	159.7** (136)

** Due to difficulties in dissolving salts to the required 159.7 g/L concentration, treatment 9 was set at 136.0 g/L.

Sediment from Lake Cullen was collected in September 2005, when the wetland was dry, prior to an environmental water allocation in October 2005. Lake sediments were collected from the top 5cm of the wetland substrate using a spade. Sites for sediment collection were chosen from randomly selected locations within 50 meters of the wetland edge. Sediments were then transported back to the laboratory where they were stored in dark conditions at 4^{o} for two months. The soil was dried thoroughly under a hessian cover in a glasshouse (to prevent contamination from airborne propagules), and then passed through a soil crusher set at a diameter of 6mm (Jaw Crusher PEX 60 x 100). Any dead vegetation was removed during this process. Crushed samples were mixed thoroughly to minimise the influence of spatial variation in the wetland sediment propagule bank.

A total of fifty-five 20 L clear plastic tubs were used in this experiment. There were 5 replicates set up for each of the 9 treatments plus the control treatment which consisted of 10 tubs. The tubs were arranged randomly throughout an air conditioned glasshouse. Their position was determined by a table of random numbers (Appendix 5 and Figure 3.2). Each tub contained a total of 1kg of sediment from Lake Cullen, distributed between two 500 mL plastic trays (120mm x 175mm x 60mm). The 10 control tubs contained 1 kg of sterilised sand distributed between two 500mL trays, to test for contamination by airborne propagules throughout the experiment. The control tubs contained 3.4 g/L saline solution and no plants or animals were found in the tubs at the conclusion of the experiments. Tubs were filled with 15 L of solution of the required salinity and the depth of the liquid was marked. Tubs were checked three times a week and topped up with tap water if necessary over a 14 week period to ensure that salinity concentrations were maintained close to the treatment concentrations (± 16%).

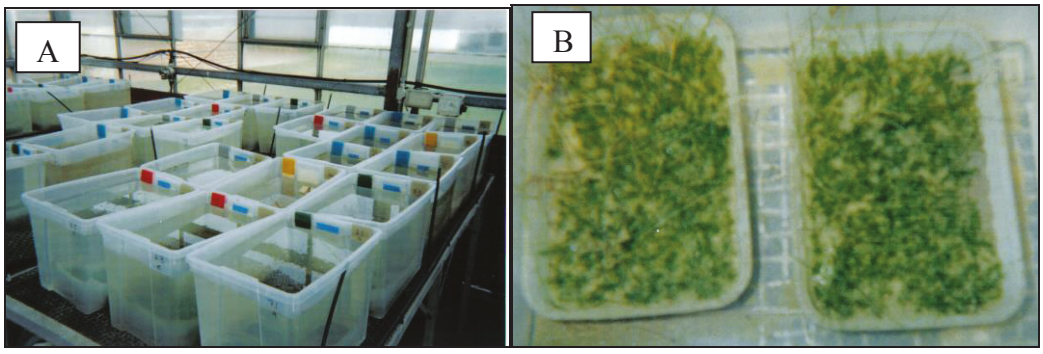


Figure 3.2 A. Glasshouse set up showing random allocation of tubs. **B**. Close up of one of the tubs showing plant growth in two plastic trays.

3.2.1 Air temperature and water quality monitoring

Maximum air temperature of the glasshouse was regulated using an air conditioner set to come on at 25 ^{o}C to reduce maximum air temperatures. Maximum and minimum air temperature (^{o}C) was monitored weekly at two locations in the glasshouse (Appendix 5). A digital max/min thermometer (Temp Tec Max-Min thermometer) was used and reset every week after the maximum and minimum temperatures were recorded. Water temperature (^{o}C) was also monitored on a weekly basis in four of the control treatment tubs (Appendix 5). Temperatures (^{o}C) were monitored using standard manual max/min thermometers that were reset after the

temperatures were recorded each week. Salinity (g/L) and pH of the water in each tub was also monitored on a fortnightly basis using an Orion Multimeter (Model No. 1230).

3.2.2 Monitoring of aquatic macrophytes and invertebrate emergence from the propagule bank, plus algal blooms

Plant growth and invertebrate hatching from the propagule bank was monitored each week for 14 weeks. For aquatic angiosperms the following was recorded:

- species presence
- number of shoots (only possible up to 25 as after this point it was difficult to determine the number of individual shoots in the tubs due to crowding in the trays)
- number of flowers
- fruit production

For any charophytes the following was recorded:

- species presence
- production of antheridia

The presence of invertebrates and algal blooms was also recorded for each tub on a weekly basis.

3.2.3 Invertebrate emergence and identification

At the end of the 14 week experiment invertebrates that had hatched from the sediments were collected by filtering the liquid in the tubs through graded sieves (40 mm 10 mm, and 108 μm). The larger sieves were checked for invertebrates and added to the smallest sieve, before the contents of the smallest sieve were rinsed into a sample jar and preserved using 70% ethanol. In the laboratory the invertebrates collected by the 108 μm sieve were sorted according to size by sieving the sample through another series of graded sieves (500 μm, 250 μm and 108 μm).

To assist in removing invertebrates from detritus in the greater than 500 μm size cohort, a flotation method was used, using a sucrose solution (Anderson, 1959;

Britton and Greeson, 1987; Rosillon, 1987). This method was used as there was a copious amount of detritus present in these samples which made counting difficult. A sugar solution with a specific gravity of 1.12 (360g per litre of water) was added to the samples and stirred. This solution was left to stand for 2 minutes before invertebrates were collected from the surface. All invertebrates collected were counted and identified to species level where possible using published keys (De Deckker, 1974; De Deckker, 1978; De Deckker, 1981b; De Deckker, 1981c; De Deckker, 1981a)and dissecting microscope (Nikon optical, model SMZ-1B). The sampling efficiency of this method was also checked using a QA/QC (Quality Assurance/Quality Control) check, where 5 samples (10% of the total number of samples) were subjected to the sugar floatation method, and then the detritus was searched. Any extra invertebrates remaining in the sample were counted to determine the percentage of invertebrates captured by this method.

For the 500 µm, 250 µm and the 108 µm size classes, no flotation method was necessary and the invertebrates were identified to genus concentration and counted using the same dissecting microscope.

3.2.4 Harvesting of aquatic macrophytes

Above ground aquatic macrophyte material was harvested and sorted to species, species were identified using Jacobs and Brock (1982). For each aquatic angiosperm species the following characteristics were recorded, the number of:

- shoots
- reproductive individuals
- flowers budding
- mature flowers
- fruit
- seeds

For each charophyte species (identified by Michelle Casanova) the following characteristics were recorded, the number of:

- individuals
- reproductive individuals

- individuals containing antheridia
- individuals containing immature oospores
- individuals containing mature oospores
- individuals containing aborted oogonis

The biovolume (mL) and dry weight biomass (g) of the aboveground plant material of each species was measured. Biovolume was determined using the Archimedes' Principle of the displacement of liquid which states that the amount of water displaced by a submerged object is equal to the volume of that object in water (Pickover, 2008). The biovolume (mL) of each species was determined by placing the plant material in a measuring cylinder of appropriate volume, depending on the amount of vegetation present. Then a known amount of water was added to the cylinder. The total volume of water and vegetation was recorded and the biovolume of the plant sample was determined using the following equation:

Plant biovolume (mL) = Total volume of plants and water – Volume of water added to measuring cylinder

To determine the dry weight biomass (g) of each species, plant material was removed from the cylinder and drained. Drained plant material was placed in a paper bag of known weight that had been dried in an oven at 70^{o}C for 48 hours. The bag of plant material was then placed in an oven at 70^{o}C for 48 hours. Bags were reweighed and the dry weight biomass was determined using the equation below:

Dry weight biomass (g) = Total dry weight of plants and bag – Weight of pre-dried bag

3.2.5 Results of sugar floatation method for invertebrate sorting

Results of a quality assurance/quality control (QA/QC) to check of the effectiveness of the sugar floatation method in 10% of replicates showed that a minimum of 94% of invertebrates for each species were successfully extracted from the organic material present using this method (Appendix 6).

3.2.6 Data analysis

Data was analysed for linear, quadratic, and cubic regressions to determine how the species responded to increased salinity concentrations and to ascertain an upper salinity threshold. Results were also analysed using one way ANOVA's to determine if there was any significant difference ($p < 0.05$) between salinity treatments, the model for the ANOVA's was:

DV = constant + salinity treatment

Where the results of the one way ANOVA's indicated that there was a significant difference between treatmetns, a *post hoc* Tukey's test was conducted to determine which treatments were significantly different. Square root transformations of the dependent variable were undertaken when the data from this experiment did not meet the assumptions of normality or homogeneity of variances for parametric tests which were analysed using a Levenes test. The one way ANOVA's and *post hoc* Tukey's tests were conducted using the PASW 18 (previously known as SPSS statistics) software package.

Multivariate analysis of the total number of individuals for each species for each replicate using Plymouth Routines in Multivariate Ecological Research (PRIMER) statistical software (Version 6.1.6). The data were square root transformed before analysis. A non-metric multi-dimensional scaling (MDS) plot and a hierarchical clustering analysis was constructed from a Bray-Curtis similarity matrix.

3.3 Results

3.3.1 Air temperature and water quality monitoring

Maximum air temperatures were between 28.5^{o}C and 40.5^{o}C, while minimum air temperatures ranged from 10.7 ^{o}C to 14.7 ^{o}C. Maximum water temperatures ranged from 20^{o}C to 32^{o}C, while minimum water temperatures were between 11^{o}C and 25^{o}C (Appendix 7). Monitoring results of salinity concentrations for each replicate showed that on average, maximum salinity concentrations were maintained within 16% of target treatment concentration. The monitoring results for pH showed that solutions remained alkaline, between 7.4 and 9.9 (Appendix 7).

3.3.2 Emergence of aquatic macrophyte and invertebrate taxa

Only two aquatic macrophyte and two invertebrate species emerged from the sediments of Lake Cullen in this experiment. The two macrophytes were the angiosperm *Ruppia megacarpa* R. Mason and the charophyte species *Lamprothamnium macropogon* (A. Braun) I.L. Ophel. The two invertebrates were ostracod species *Mytilocypris henricae* (Chapman 1966) and *Australocypris* spp. Salinity treatment concentrations of 61.0 g/L or above resulted in no taxa being found in any replicates. All four of these plant and invertebrate species emerged from sediment in all replicates in the lower salinity treatments (3.4 g/L to 37.7 g/L). In contrast, in all replicates of the 61.0 g/L treatment no plant species germinated and only the invertebrate *Australocypris* spp. emerged from the sediments. No plant or invertebrate species emerged from the sediments in all replicates of the highest salinity treatments (98.7 g/L and 136.0 g/L) (Table 3.2).

Table 3.2 Presence/absence of each species of macrophyte and invertebrate, for each replicate (A,B,C,D and E) in this propagule bank study based on Lake Cullen substrate.

✓ Present ✗ Absent

		Taxon			
Treatment	**replicate**	*Ruppia megacarpa*	*Lamprothamnium macropogon*	*Mytilocypris henricae*	*Australocypris* spp.
3.4 g/L	A	✓	✓	✓	✓
	B	✓	✓	✓	✓
	C	✓	✓	✓	✓
	D	✓	✓	✓	✓
	E	✓	✓	✓	✓
5.5 g/L	A	✓	✓	✓	✓
	B	✓	✓	✓	✓
	C	✓	✓	✓	✓
	D	✓	✓	✓	✓
	E	✓	✓	✓	✓
8.9 g/L	A	✓	✓	✓	✓
	B	✓	✓	✓	✓
	C	✓	✓	✓	✓
	D	✓	✓	✓	✓
	E	✓	✓	✓	✓
14.4 g/L	A	✓	✓	✓	✓
	B	✓	✓	✓	✓
	C	✓	✓	✓	✓
	D	✓	✓	✓	✓
	E	✓	✓	✓	✓
23.3 g/L	A	✓	✓	✓	✓
	B	✓	✓	✓	✓
	C	✓	✓	✓	✓
	D	✓	✓	✓	✓
	E	✓	✓	✓	✓
37.7 g/L	A	✗	✓	✓	✓
	B	✗	✓	✓	✓
	C	✗	✓	✓	✓
	D	✗	✓	✓	✓
	E	✓	✓	✓	✓
61.0 g/L	A	✗	✗	✗	✓
	B	✗	✗	✗	✓
	C	✗	✗	✗	✓
	D	✗	✗	✗	✓
	E	✗	✗	✗	✓
98.7 g/L	A	✗	✗	✗	✗
	B	✗	✗	✗	✗
	C	✗	✗	✗	✗
	D	✗	✗	✗	✗
	E	✗	✗	✗	✗
136 g/L	A	✗	✗	✗	✗
	B	✗	✗	✗	✗
	C	✗	✗	✗	✗
	D	✗	✗	✗	✗
	E	✗	✗	✗	✗

Germination of *R. megacarpa* occurred very quickly in salinities of 3.4 g/L to 23.3 g/L with shoots being recorded in at least one replicate of each treatment in the first week of the experiment (Table 3.3). All replicates in this salinity range recorded *R. megacarpa* shoots in Week 2 of the experiment. A lag in the germination of *R. megacarpa* was observed in the 37.7 g/L treatment with only a few shoots being recorded in the second last week of the experiment.

Individuals of *L. macropogon* took longer to germinate than *R. megacarpa* at salinities ranging from 3.4 g/L to 23.3 g/L with individuals being recorded in all replicates in treatments across this range by Week 2 of the experiment. A lag in germination was again observed at a salinity of 37.7 g/L. In the 61.0 g/L treatment, *L. macropogon* appeared to have germinated faster than *R. megacarpa,* with individuals being first recorded in Weeks 4 to 6 in this treatment (Table 3.3).

Emergence of invertebrate species could not be separated into individual species, as it was impossible to identify to genus level by eye during the experiment as ostracods were too small. Invertebrate species were first observed in all replicates in treatments ranging from 3.4 g/L to 23.3 g/L by Week 6. A lag in the emergence of invertebrates was observed in the 61.0 g/L treatment with individuals being observed in all replicates of this treatment by Week 8 (Table 3.3).

Table 3.3 The week in which macrophyte and invertebrates were first observed, for each replicate (A,B,C,D and E) in this propagule bank study based on Lake Cullen substrate. Invertebrate species could not be identified at this early stage.

Treatment	Replicate	*Ruppia megacarpa*	*Lamprothamnium macropogon*	Invertebrate spp.
3.4 g/L	A	1	2	4
	B	1	2	4
	C	1	2	4
	D	1	2	5
	E	1	2	4
5.5 g/L	A	1	2	4
	B	1	2	4
	C	1	2	4
	D	2	2	4
	E	1	2	4
8.9 g/L	A	1	2	4
	B	1	2	4
	C	1	2	4
	D	2	2	5
	E	1	2	4
14.4 g/L	A	1	2	4
	B	1	2	4
	C	1	2	4
	D	2	2	4
	E	1	2	4
23.3 g/L	A	2	2	4
	B	1	2	4
	C	1	2	4
	D	2	2	4
	E	1	2	4
37.7 g/L	A		5	4
	B	13	4	4
	C		5	6
	D		6	4
	E		5	4
61.0 g/L	A			8
	B			8
	C			6
	D			8
	E			8
98.7 g/L	A			
	B			
	C			
	D			
	E			
136 g/L	A			
	B			
	C			
	D			
	E			

3.3.3 *Ruppia megacarpa* germination

Figure 3.3 shows that there was a decrease in germination success and plant growth in *R. megacarpa* as salinity increased. When salinity treatments 3.4 g/L to 37.7 g/L were included in analysis (all treatments with germination of aquatic macrophytes), a significant negative linear regression ($p < 0.001$, $R^2 = 0.915$) in the germination success of *R. megacarpa* (measured as number of shoots) was found with increasing salinity (Figure 3.3a and Appendix 8). There was a slight increase in the number of *R. megacarpa* shoots present at 5.5 g/L when compared to 3.4 g/L, however this increase was not significant. Results of a one way ANOVA indicated a significant difference in the number of shoots with increasing salinity ($p < 0.001$, $F = 31.704$ df = 5, 24). A *post hoc* Tukey's test showed that there was no significant difference between 3.4 g/L, 5.5 g/L or 8.9 g/L treatments, nor was there any significant difference between 8.9 g/L and 14.4 g/L or between 14.4 g/L and 23.3 g/L. There were significant differences however between all other treatments (Appendix 8).

A significant negative linear regression was also found between total biovolume ($p < 0.001$, $R^2 = 0.850$) and increasing salinity, and between total biomass ($p < 0.001$, $R^2 = 0.826$) and increasing salinity (Figure 3.3b and c respectively, Appendix 8). The results of a one way ANOVA indicated a significant difference between the total biovolume of *R. megacarpa* as salinity increased ($p < 0.001$, $F = 16.266$ df = 5, 24). These results show that the amount of *R. megacarpa* biovolume present in each treatment was affected by increasing salinity. A *post hoc* Tukey's test showed that there was only a significant difference in salinity treatments ranging from 3.4 g/L to 23.3 g/L and the highest salinity concentration at 37.7 g/L. But there was no significant difference found between any of the treatments ranging from 3.4 g/L and 23.3 g/L (Appendix 8). This suggests that salinity significantly affected the biovolume of *R. megacarpa* at 37.7 g/L treatment.

Results of another one way ANOVA also showed significant difference between the total dry weight biomass of *R. megacarpa* as salinity increased ($p < 0.001$, $F = 17.564$ df = 5, 24). These results indicate that the amount of *R. megacarpa* dry weight biomass present in each treatment was affected by increasing salinity. A *post hoc* Tukey's test showed very similar results to that of the effect of salinity on *R. megacarpa* biovolume, as there was only a significant difference in salinity treatments ranging from 3.4 g/L to 23.3 g/L

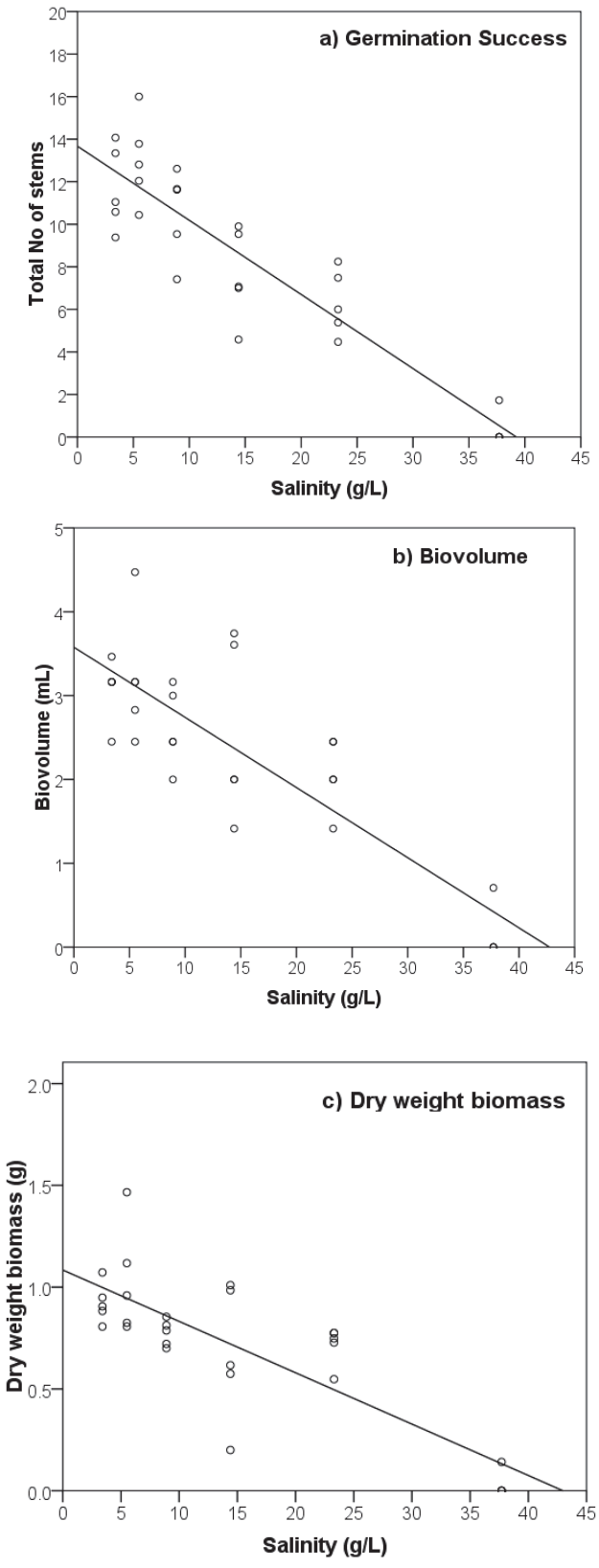


Figure 3.3 Regressions showing the effect of salinity on the **a)** germination, **b)** biovolume and **c)** dry weight biomass of *Ruppia megacarpa,* (n=5, square root transformation), trend lines only shown where regressions were found to be significant ($p < 0.05$).

and the highest salinity concentration at 37.7 g/L. There was no significant difference found between any of the treatments ranging from 3.4 g/L and 23.3 g/L (Appendix 8). This suggests that salinity significantly affects the dry weight biomass of *R. megacarpa* at 37.7 g/L treatment.

Calculated salinity concentrations for when each of the number of shoots, zero biovolume and zero dry weight biomass were calculated by solving for y = 0 in the equations developed from the regression models. These calculated values ranged from 39.3 g/L to 43.4 g/L and were supported by the germination results of this experiment as no recruitment from the propagule bank was observed at salinities ≥ 61.0 g/L.

3.3.4 *Ruppia megacarpa* - reproductive success

Reproductive structures (flowers, budding flowers, fruit and seeds) were recorded in all salinity treatments between 3.4 g/L and 23.3 g/L (Figures 3.4a and 3.4b). There were no reproductive structures recorded at 37.7 g/L salinity even though shoots were present.

When analysing treatments where flowering occurred, the time until flowers were first observed was shorter in the higher salinities. Flowering was first observed at the end of Week 4 in some replicates for treatments, ranging from 8.9 g/L to 23.3 g/L, whereas flowering was first observed at the end of Week 6 for some replicates in treatments 3.4 g/L and 5.5 g/L (Figure 3.4a). All replicates within the salinity range of 3.4 g/L to 23.3 g/L, with the exception of one replicate in the 14.4 g/L, treatment which had *R. megacarpa* flowers during the experiment.

A similar pattern was also found in the time until mature fruits were first observed for *R. megacarpa*, and again no lag or delay was found with increasing salinity. When analysing all treatments in which fruits were produced, at least one *R. megacarpa* individual with mature fruit was observed by the end of Week 7, with the exception of the lowest salinity treatment (3.4 g/L) which first mature fruits present in at least one replicate by the end of Week 8 (Figure 3.4b). All replicates within the salinity range of 3.4 g/L to 23.3 g/L had mature *R. megacarpa* fruits present during Weeks 7 to 10 in this experiment. The only exception to this was one replicate in the 14.4 g/L treatment in which no fruits were produced.

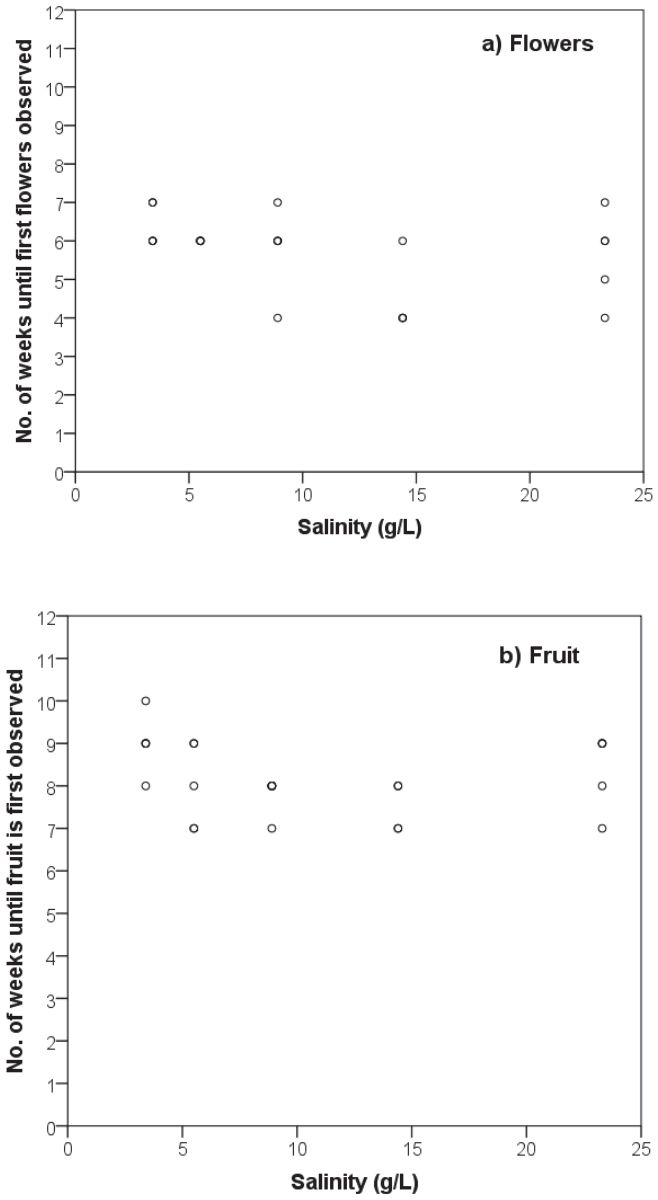


Figure 3.4 Number of weeks until *Ruppia megacarpa* reproductive structures, **a)** flowers and **b)** fruit were first observed in replicates (n=5).

3.3.5 *Lamprothamnium macropogon* germination

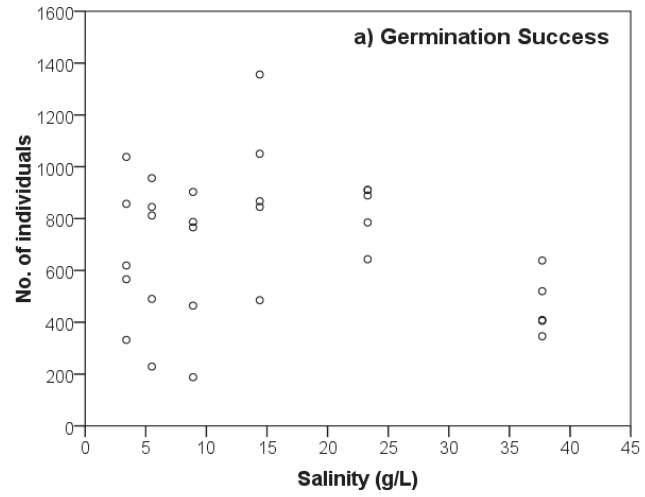
There were very large numbers of *L. macropogon* individuals recorded in this experiment, with some replicates having in excess of 1000 individuals germinating from the propagule bank. When treatments of 3.4 g/L to 37.7 g/L were included in analysis (all treatments containing germinants), no significant regression could be found between the number of individuals germinating from the propagule bank, and increasing salinity (Figure 3.5a, and Appendix 8). A one way ANOVA also indicated that there was no significant

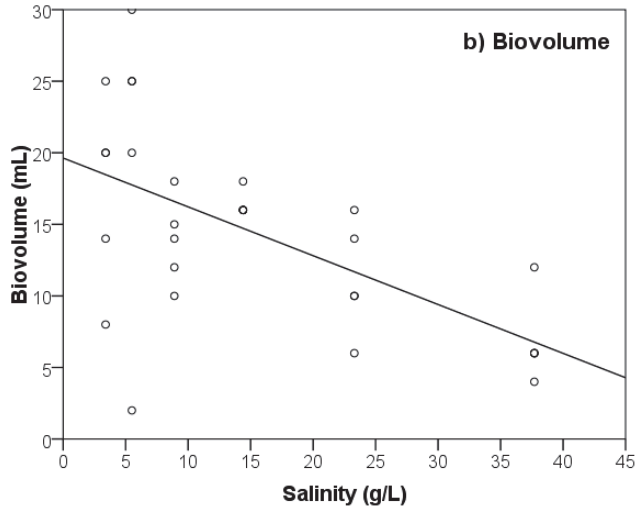
difference in the number of individuals germinating for *L. macropogon* as salinity increased ($p > 0.05$).

Figure 3.5 b and c show that with increasing salinity there is a decrease in growth for *L. macropogon*. Significant declining linear relationships were found for the biovolume ($p < 0.001$, $R^2 = 0.599$) and dry weight biomass ($p = 0.002$, $R^2 = 0.534$) of *L. macropogon* with increasing salinity. This suggests that while increased salinities below the threshold level had little affect on the number of individuals germinating, it did have an effect on the charophyte growth (Figures 3.5b and 3.5c, and Appendix 6).

A one way ANOVA indicated a significant difference in the biovolume of *L. macropogon* with increased salinity (p 0.034, F = 2.992 df = 5, 24). A *post hoc* Tukey's test showed that there was only a significant difference ($p < 0.05$) between the 5.5 g/L and 37.7 g/L treatments (Appendix 8). A one way ANOVA indicated that there was a significant difference between dry weight biomass at increasing salinities ($p = 0.007$, $F = 4.164$ df = 5, 24). A *post hoc* Tukey's test indicated a significant difference between all treatments and the 37.7 g/L treatment (Appendix 8).

Calculated salinity concentrations for the biovolume and dry weight biomass of *L. macropogon* were calculated by solving for $y = 0$ in the equations developed from the regression models. These calculated values were found to be 52.6 g/L and 57.6 g/L respectively. The maximum calculated values for biovolume was supported by the results. The maximum calculated value for dry weight biomass, however was not supported by the results of this experiment as no germination was observed at salinities ≥ 61.0 g/L.





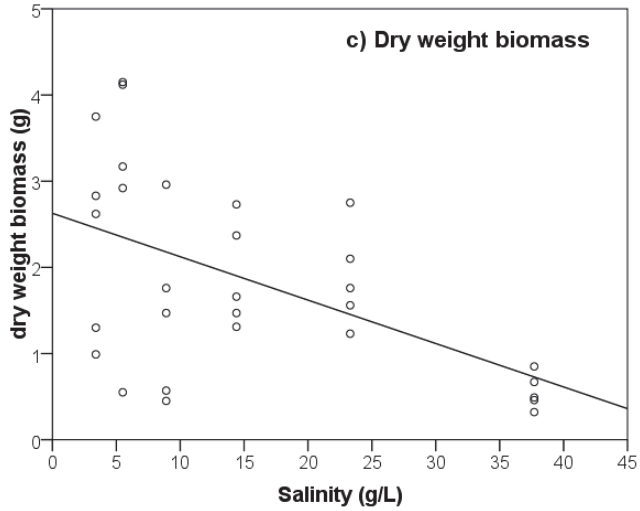


Figure 3.5 Regressions showing the effect of salinity on the **a)** germination, **b)** biovolume and **c)** dry weight biomass of *Lamprothamnium macropogon,* (n=5), trend lines only shown where regressions were significant ($p < 0.05$)

3.3.6 *Lamprothamnium macropogon* - reproductive success

Reproductive whorls (containing antheridia and oogonia) on *L. macropogon* were recorded in all salinity treatments containing germinants from 3.4 g/L to 37.7 g/L. Analysis of the different types of antheridia and oogonia present on *L. macropogon* individuals was calculated as percentage of total reproductive individuals (antheridia and oogonia) in order to take into account the varying number of individuals present in each replicate. The cumulative percentages for each replicate in most cases exceeded 100% as individuals often contained more than one type of antheridia or oogonia.

The percentage of reproductive individuals containing antheridia was very high with percentages exceeding 80% in some replicates. No significant regression was found for the percentage of reproductive individuals containing antheridia with increasing salinity (Figure 3.6a and Appendix 8). The percentage of individuals containing immature oogonia, mature oogonia and aborted oogonia were lower, (0 to 10%) in all replicates. A significant declining linear relationship ($p < 0.001$, $R^2 = 0.695$) was found for the percentage of reproductive individuals containing immature oogonia with increasing salinity (Figure 3.6b and Appendix 8). Results of a one way ANOVA indicated that there was a significant difference between the treatments ($p = 0.001$, $F = 5.835$, $df = 5, 24$). A *post hoc* Tukey's test showed that there was only a significant difference between the lower salinity treatments (3.4 g/L, 5.5 g/L and 8.9 g/L) and the 37.7 g/L treatment (Appendix 8) and also between the 8.9 g/L and 23.3 g/L treatments.

A significant linear relationship ($p = 0.003$, $R^2 = 0.442$) was also found for mature oogonia and increasing salinity, a maximum number of mature oogonia being produced in treatments 3.4 g/L to 14.4 g/L (Figure 3.6c and Appendix 8). Results of a one way ANOVA indicated that there was a significant difference between the treatments ($p = 0.015$, $F = 3.565$, $df = 5, 24$). A *post hoc* Tukey's test showed that there was only a significant difference between the 14.4 g/L and 37.7 g/L treatments (Appendix 8).

The percentage of individuals with aborted oogonia did increase with increasing salinity, and a significant linear relationship ($p = 0.001$, $R^2 = 0.576$) found between the percentage of reproductive individuals with aborted oogonia and increasing salinity (Figure 3.6d and Appendix 8). Results of a one way ANOVA indicated that there was a significant

difference between the treatments (p = 0.017, F = 3.460, df = 5, 24). A *post hoc* Tukey's test showed that there was only a significant difference between the lower salinity treatments (3.4 g/L, 5.5 g/L and 8.9 g/L) and the 37.7 g/L treatment (Appendix 8).

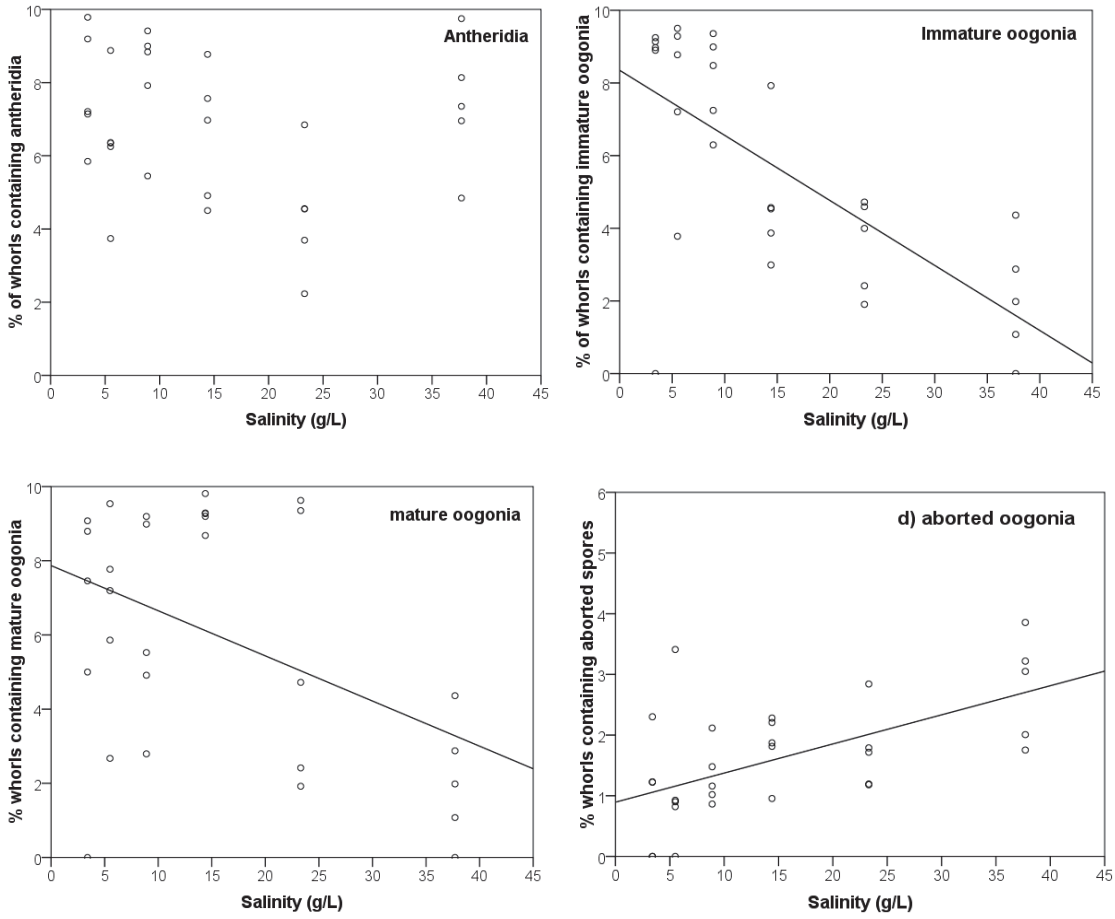


Figure 3.6 The percentage of *Lamprothamnium macropogon* individuals containing each type of reproductive structures, (n=5, square root transformation), regression trend lines only shown where regressions were significant (p <0.05)

Increased salinity concentrations in this experiment had little affect on the time until antheridia were first observed in each replicate. All treatments in which germination of *L. macropogon* occurred (salinity range 3.4 g/L to 37.7 g/L) had at least one replicate with antheridia present at the end of Week 7, with the exception of one treatment in 8.9 g/L which did not have any individuals with antheridia until Week 9 (Figure 3.7). All replicates within this salinity range (3.7 g/L to 37.7 g/L) contained antheridia for *L. macropogon* during weeks 7 to 12. When compared to the time until flowering and fruit development in *R. megacarpa* (Weeks 4 to 7 and Weeks 7 to 10 respectively), these results suggest that *L. macropogon* (Weeks 7 to 12) takes a longer than *R. megacarpa* to become reproductive (Figures 3.4a and 3.4b and 3.7).

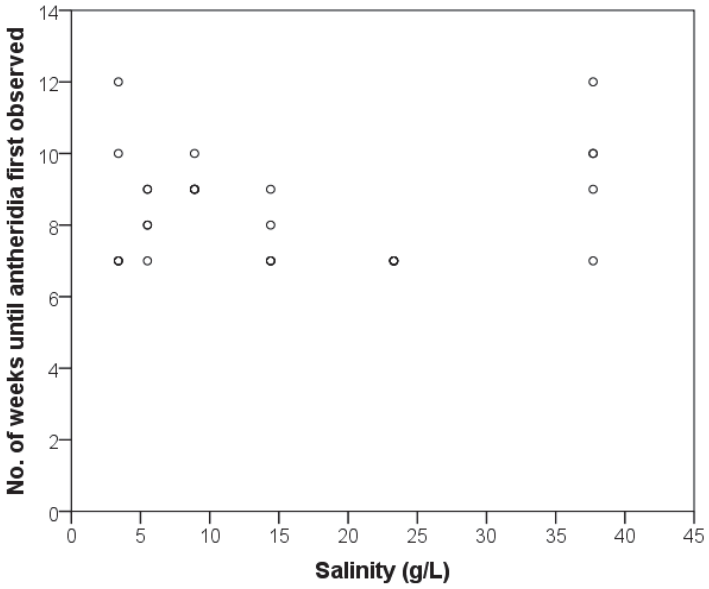


Figure 3.7 Number of weeks until *Lamprothamnium macropogon* antheridia first observed in replicates exposed to varying salinity concentrations (n=5).

3.3.7 Comparison of macrophyte species

When comparing the two aquatic macrophytes that germinated in this experiment, the number of *L. macropogon* individuals was far greater than the number of *R. megacarpa* stems produced (Figure 3.8a). The total biovolume and dry weight biomass was also higher for *L. macropogon* than *R. megacarpa* (Figures 3.8b and 3.8c).

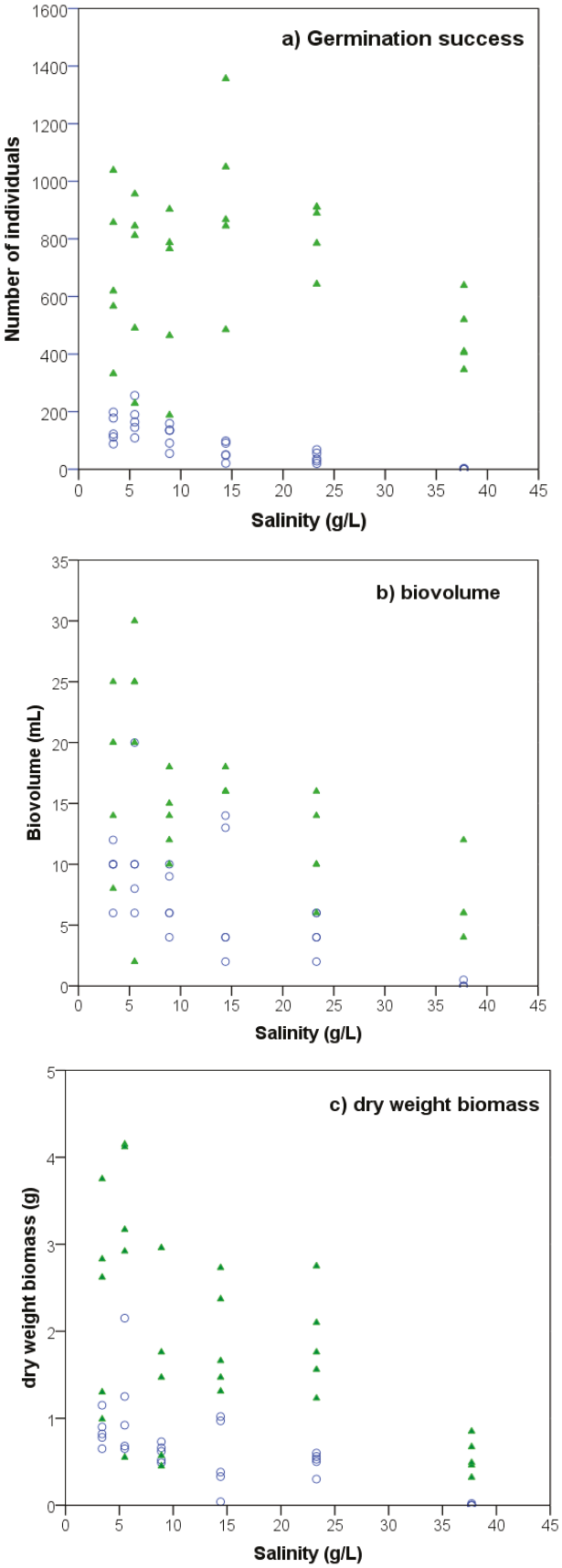


Figure 3.8 Comparison of *Ruppia megacarpa* and *Lamprothamnium macropogon* **a)** germination success, **b)** biovolume and **c)** dry weight biomass in varying salinity concentrations. Key ○ *R. megacarpa* ▲*L. macropogon*.

3.3.8 Phytoplankton blooms

Visual observations of the tubs indicated that phytoplankton blooms occurred in all replicates exposed the highest salinity treatment (136.0 g/L). These blooms formed by Week 4 for all replicates except one (which had formed in Week 3), and persisted for the duration of the experiment (Table 3.4 and Figure 3.9).

Table 3.4 Presence of phytoplankton blooms in replicates exposed to the 136.0 g/L salinity treatment for the duration of the experiment

Key ✗ absent ✓ Present

Replicate	Week												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
B	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
D	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

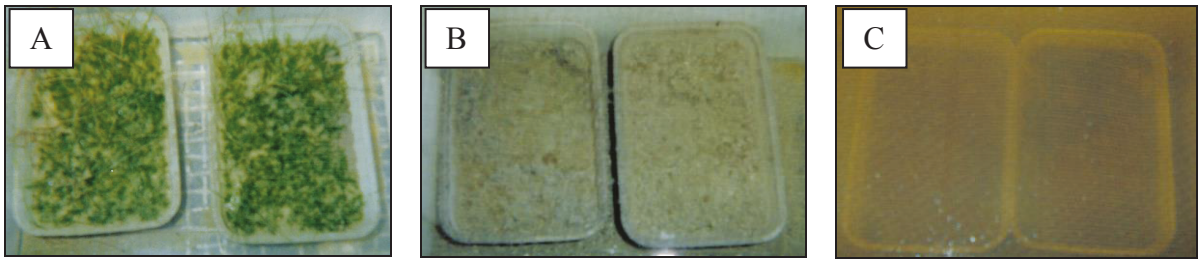


Figure 3.9 Three different replicates from various salinity treatments showing **A.** macrophyte growth (23.3 g/L treatment), **B.** no macrophyte growth (98.7 g/L treatment) and **C.** phytoplankton bloom (136 g/L treatment).

3.3.9 Invertebrates

Only two species of ostracods emerged from the substrate of Lake Cullen after four weeks. While diversity was low, the abundance of each ostracod species was very high, with over 2000 individuals recorded in many replicates and some replicates had even greater numbers (up to 8081).

Mytilocypris henricae was found in all replicates in salinity treatments (3.4 g/L to 37.7 g/L), whereas *Australocypris* spp. was present in all replicates ranging from 3.4 g/L to 61.0 g/L. This suggests that *Australocypris* spp. is the more salt tolerant of the two species. It is interesting to note that *Australocypris* spp. was able to exist in the 61.0 g/L salinity treatment even though there were no plants present (Figure 3.9 and 3.10).

The number of *M. henricae* individuals exhibited a significant declining linear regression ($p < 0.001$, $R^2 = 0.637$) with increasing salinity. In particular there were reduced population numbers in the 37.7 g/L salinity treatment in comparision to the lower salinity treatments of ≤ 23.3 g/L (Figure 3.10 and Appendix 8). Results of a one way ANOVA indicated that there was a significant difference between treatments ($p = 0.001$, $F = 6.617$, $df = 5, 24$). A *post hoc* Tukey's test showed that there was a significant difference between 37.7 g/L and all other treatments with the exception of the 14.4 g/L treatment (Appendix 8).

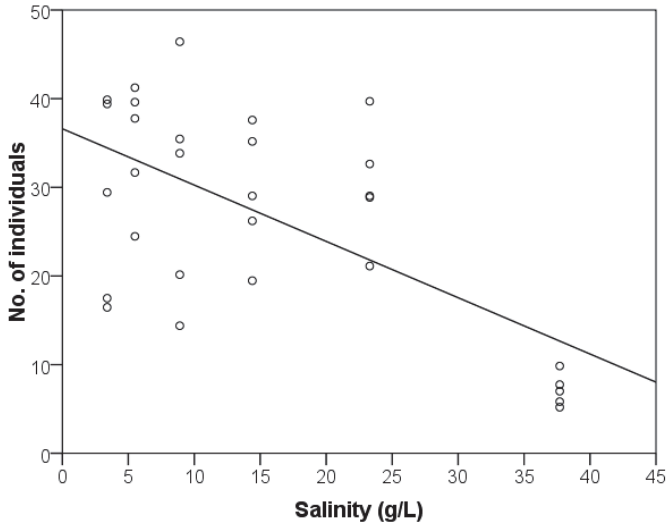


Figure 3.10 The number of *Mytilocypris henricae* individuals (n=5 square root transformation), trend line represents a significant regression ($p < 0.05$)

The total number of individuals for *Australocypris* spp. showed a significant cubic relationship ($p < 0.001$, $R^2 = 0.772$) with increasing salinity, however this relationship was not a declining one (Figure 3.11 and Appendix 8). The results show that the maximum number of individuals for this species was recorded in the mid-range salinity treatments (23.3 g/L to 61.0 g/L).

Calculated thresholds for the salinity at which the total number of individuals for each species reaches zero were calculated by solving $y = 0$ for the equations developed from the regression model equations (Appendix 8). Calculated salinity

threshold levels for *M. henricae* was calculated to be 57.6 g/L which is supported by the results obtained in this experiment as no individuals of this species emerged from the substrate exposed to ≥ 61.0 g/L salinity treatments. The calculated salinity threshold for *Australocypris* spp. was 63.9 g/L which was also supported by the results of the experiment as no individuals of this species emerged from the proopagule bank at salinity ≥ 98.7 g/L.

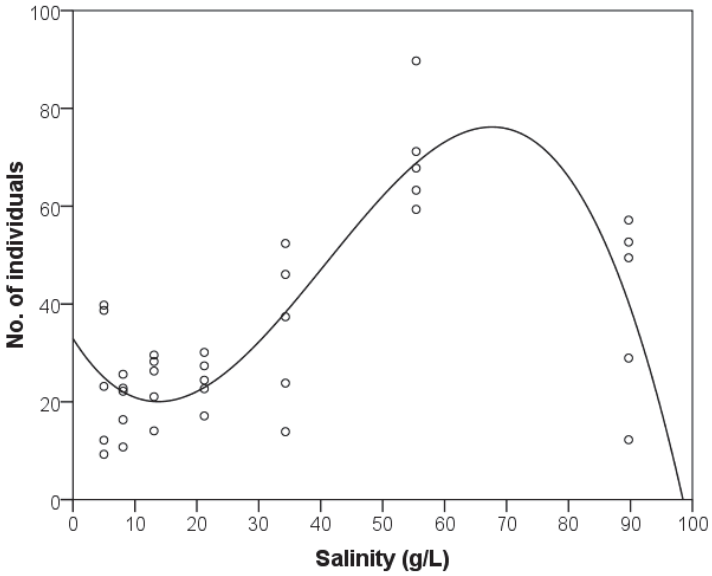


Figure 3.11 The total number of *Australocypris* spp. individuals (n=5, square root transformation), trend line represents a significant regression ($p < 0.05$).

The time until the first invertebrates were observed indicates that increased salinity concentrations may cause a lag in the time until invertebrates emerge in the 61.0 g/L treatment (Figure 3.12). It is important to note that the emergence of invertebrates was not observed for each individual invertebrate species and therefore these results represent the time until either ostracod species emerged from the sediment. Thus the lag in the emergence of invertebrates in the 61.0 g/L treatment may be due to life cycle differences between species (as *M. henricae* was not present in this treatment), rather than an effect of salinity.

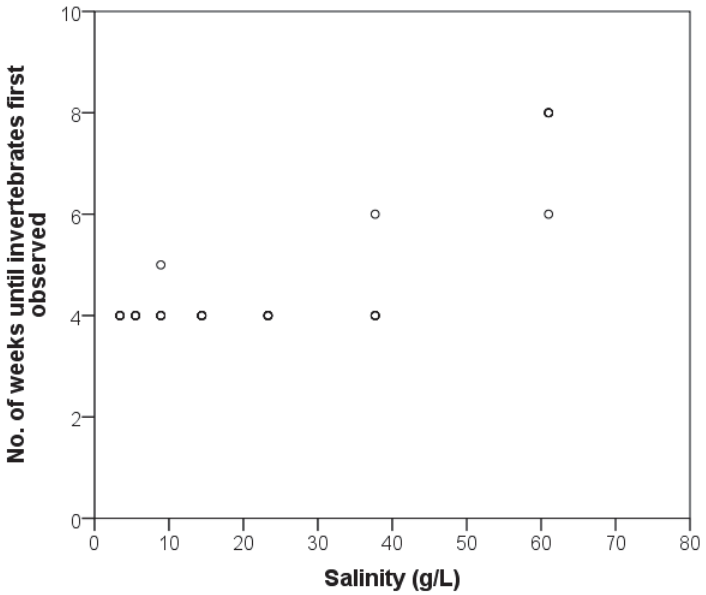


Figure 3.12 Effect of salinity on the time until invertebrates were first observed to have emerged in each replicate of substrate subjected to varying salinity concentrations (n=5).

3.3.10 Multivariate analysis

Multivariate analysis of the number of individual plants and invertebrates that emerged in each replicate during this experiment indicates four distinct clusters (Figure 3.13). Group 1 contained the lower salinity concentrations of between 3.4 g/L to 23.3 g/L and represented the treatments where all plant and invertebrate species were present, and growth and emergence was highest. Group 2 contained all of the replicates exposed to the 37.7 g/L treatment, where all plant and invertebrate species were present, but in particular the number of individuals for plant species was lower than the treatments in Group 1. Groups 3 and 4 contained all of the replicates exposed to the 61.0 g/L salinity treatment, where there were no plant species present and only *Australocypris* spp. emerged from the substrate.

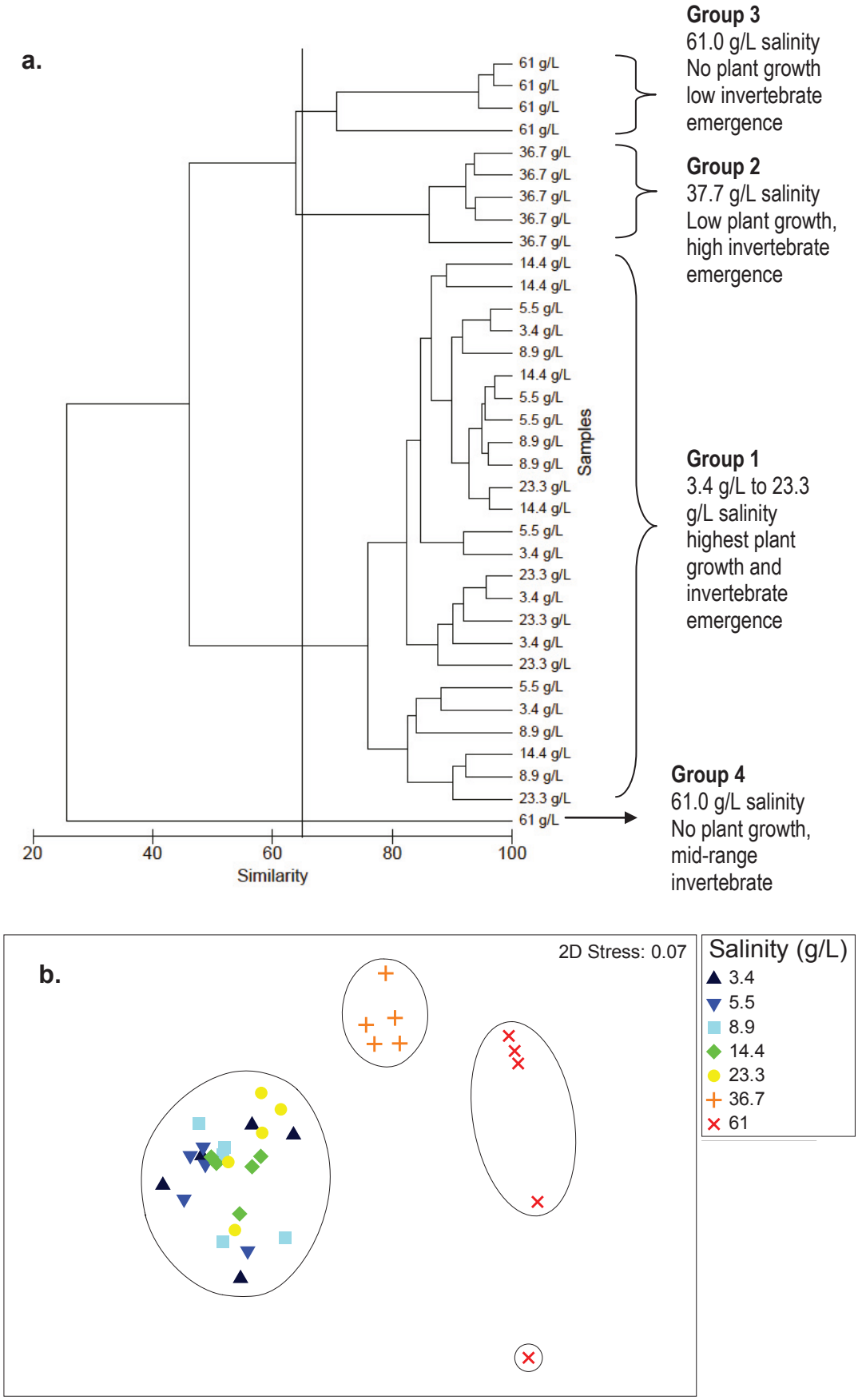


Figure 3.13 Multivariate analysis of number of plant and invertebrate individuals that emerged from the propagule bank for all salinity treatments ≤ 61 g/L. **a.** Cluster dendrogram generated from Bray-Curtis similarity matrix. Slice represents 65% similarity level **b.** MDS ordination, contours represent 65% similarity level. Stress = 0.07.

3.4 Discussion

The results of this study indicated four differing regimes or states: a submerged aquatic macrophyte regime with two aquatic macrophyte and two invertebrate species, a clear water state with no macrophytes and one invertebrate species, a clear water state with no macrophytes and no invertebrate species and an algal bloom state. No microbial mat state was observed in this study.

3.4.1 Effect of salinity on the germination of aquatic macrophytes

Both *Ruppia megacarpa* and *Lamprothamnium macropogon* are known to be perennial aquatic macrophyte species, although *R. megacarpa* can act as an annual species in ephemeral systems. It is therefore not surprising for a perennial species to be present in the propagule bank of this wetland, given its past flooding regime and management as a permanent storage lake for irrigation waters (KLAWG, 1992). The presence of these perennial species needs to be considered when determining a future watering regime for Lake Cullen. Perennial species tend to be found in permanent systems, however as evidenced by this study both species are able to survive periods of drying. Whether these species are able to persist under a more ephemeral watering regime in the long term is something that warrants investigation. A basic requirement would be that the timing and duration of inundation needs to be long enough to allow plants to reach maturity and contribute propagules to the propagule bank for these species to persist into the future.

The germination of *R. megacarpa* seeds in the experiment was quick, with shoots being recorded across all replicates in salinity treatments ranging from 3.4 g/L to 23.3 g/L within the first one or two weeks of the experiment. Other similar propagule bank studies have reported much slower germination rates of 15 days or longer in *Ruppia* species (Kahn and Durako, 2005; Sim *et al.*, 2006a). The short time observed until germination first occurred in this experiment may be attributed to environmental factors such as the high water temperatures ($\geq 30^{o}$C) experienced during the initial stages of this study (Appendix 7). Previous experiments have also found that high temperatures increase the germination success of seeds of a species from the same genus, *Ruppia maritima* (Verhoeven, 1980; Koch and Seeliger, 1988; Malea *et al.*, 2004).

A lag phase in germination of *R. megacarpa* and *L. macropogon* at a salinity of 37.7 g/L was apparent with first germinants observed at 12 weeks and 5 weeks respectively. Similar delays in germination of aquatic macrophytes at higher salinities have been recorded by Sim *et al.,* (2006a) and Kim *et al.,* (2013). Germination lags can present problems if these plant species are to persist when a wetland dries out too quickly or becomes too salty for germination to occur. These lags can also be problematic if germination occurs late in the watering period and salinity concentrations are not kept at low concentrations long enough to enable plants to reproduce. Thus over time propagule banks can become depleted and species may be lost from wetland ecosystems.

There has been little research into the longevity of saline wetland propagule banks. Recent studies on freshwater wetland species by Brock (2011) have shown that wetland seeds can persist in propagule banks and be viable after 12 years without inundation. Additionally, Brock (2011) found that the mean survival time seeds could remain dry for was 7.4 years, but the longevity of individual wetland plant species varied greatly, so further research is warranted.

Germination of both *R. megacarpa* and *L. macropogon* occurred in all salinity concentrations up to and including 37.7 g/L, with no germination recorded at or above 61.0 g/L. This concurs with the salinity threshold of 45 g/L for macrophyte dominated wetlands proposed by Sim *et al.,* (2006a) and shows a higher germination threshold that than posed by Kim *et al.,*(2013) who found that *R. megacarpa* seeds did not germinate in waters > 30 g/L. Regression analysis showed that salinity impacted the number of *R. megacarpa* shoots produced at salinities of 14.4 g/L and above, suggesting that salinity concentrations below the germination threshold level affect the number of shoots produced in this species. A gradual decline in the number of *Ruppia polycarpa* shoots was found in a similar study by Sim *et al.,* (2006a).

There was a slight increase in the number of *R. megacarpa* shoots present at 5.5 g/L when compared to 3.4 g/L, which may be due to natural variations in seed numbers across the replicates, however this pattern of reduced germination success at lower salinities has been recorded in other studies of *Ruppia* species (Sim *et al.*, 2006a) suggesting that these macrophytes may have greater germination success rate in slightly elevated salt concentrations.

A very different response was observed for the effect of salinity on the germination of *L. macropogon* with results showing that salinity concentrations below 37.7 g/L had little or no effect on the number of individuals germinating from the sediments of Lake Cullen. Sim *et al.,* (2006a) also found no relationship between increased salinity and the number of individuals of *L. macropogon* when salinity concentrations were below 37.7 g/L. High numbers of *L. macropogon* individuals have germinated in this experiment, suggesting the presence of a large number of propagules in the propagule bank.

3.4.2 Effects of salinity on macrophyte growth

Salinity negatively impacted the total biovolume and dry weight biomass of *R. megacarpa* and *L. macropogon*. *Post hoc* Tukey's test results demonstrated that increasing salinity to 37.7 g/L or higher had a significant impact on the total biovolume and dry weight biomass for *R. megacarpa*. This suggests that increased salinity concentrations may impact growth by causing stunting of these plants at higher salinities. *Post hoc* Tukey's tests for *L. macropogon* only showed significant differences between lower salinity concentrations of 5.5 g/L and 37.7 g/L for total biovolume; and between 3.4 g/L and 37.7 g/L, 5.5 g/L and 37.7 g/L for total dry weight biomass. These results indicate that salinity concentrations below 5.5 g/L are optimal for *L. macropogon* growth and salinity concentrations below 23.3 g/L are optimal for *R. megacarpa* growth. This finding supports results from Robertson and Funnell (2012) who found that there was reduced growth and cover of *R. megacarpa* in a New Zealand lagoon that was subjected to increased salinities of between 10g/L to 20 g/L.

These results may not be a true indication of the effect of salinity on the growth of these species, given that the lag time in germination at the 37.7 g/L salinity concentration effectively reduced the length of time successful germinants had to grow, in comparison to those germinated in lower salinity treatments. In particular, this may have affected results for *R. megacarpa,* as individuals of this species did not germinate until the last week of the experiment in the 37.7 g/L salinity treatment. If the experiment had been extended for extra time, growth may not have been limited at this salinity concentrations.

3.4.3 Effects of salinity on aquatic macrophyte reproductive success

No *R. megacarpa* reproductive structures were produced at the 37.7 g/L salinity concentration, even though germinants were present in this treatment. This is possibly due to the lag in germination time occurring in this treatment, as shoots were not recorded until the last week of the experiment. Reproductive whorls for *L. macropogon* were recorded in all salinity treatments containing germinants, up to and including 37.7 g/L. In a similar study by Sim *et al.,* (2006a), *Lamprothamnium macropogon* reproductive structures were only produced in individuals up to 15 g/L. As such, this study indicates that this species is viable at salinities up to 37.7 g/L, which is higher than *R. megacarpa*.

The type of reproductive structures produced by *L. macropogon* showed differing relationships to increased salinity. The most significant results were that number of aborted oogonia increased with increasing salinity, and results of a *post hoc* Tukey's test showing that there was a significant difference between both 3.4 g/L and 37.7 g/L and 5.5 g/L and 37.7 g/L, suggesting that an increase in salinity over 5.5 g/L may significantly impact the number of aborted oogonia and therefore affect reproductive success of *L. macropogon*.

While Sim *et al.,* (2006a), have shown that an increase in salinity can cause a delay in the time it takes until plants to become reproductive, there was no lag or delay apparent in the time taken for the *R. megacarpa* to flower or until first antheridia were observed on *L. macropogon* in this experiment. It should be noted however that the aquatic macrophytes in the study by Sim *et al.,* (2006a), were monitored more regularly than that of this study and included other species of *Ruppia*. Also, whilst Sim *et al.,* (2006a) did find a significant delay in germination of *L. macropogon* individuals, the lag time was only slight, with individuals germinating after 36 days at a salinity of 30 g/L compared to 30 days at 0 g/L. Compared to the results by Sim *et al.,* (2006a) germination occurred more rapidly in this current study, with individuals germinating in all salinity treatments at or below 37.7 g/L within the first two weeks. This highlights that salinity alone may not be affecting germination success in these species, it may also be influenced by other environmental factors such as temperature or photoperiod which may be important in breaking dormancy in seeds and spores.

3.4.4 Comparison of macrophyte species

The number of *L. macropogon* individuals germinated in this experiment far outweighed the number of *R. megacarpa* shoots, and similarly there was a higher total biovolume and biomass for *L. macropogon* compared to that of *R. megacarpa*. These results can be attributed to the different colonising strategies shown by each of these species. *R. megacarpa* produced fewer stems, fewer sexual propagules and thus had fewer germinating propagules in the propagule bank. This species is also able to spread across the wetland substrate through asexual reproductive structures such as rhizomes. For the charophyte species *L. macropogon* the colonising strategy is quite different, as large numbers of individuals germinated from the propagule bank indicating that this species produces large numbers of viable spores. But unlike *R. megacarpa* these individuals are not able to colonise vast areas via asexual reproduction. Similar colonising strategies for other species of the *Lamprothamnium* and *Ruppia* species were found in a study by Sim *et al.,* (2006a) and Porter (2007).

3.4.5 Effect of salinity on the number of invertebrates in populations developing from the propagule bank

Two species of ostracods emerged from the Lake Cullen sediments namely, *M. henricae* and *Australocypris* spp. Ostracods are known to be less salt sensitive than many other invertebrates (Pinder *et al.*, 2005). The salinity tolerances of individual species within this group are variable, with some species only found in waters with salinities below 0.5 g/L, while others have been recorded in water with salt concentrations of up to 288 g/L (De Deckker, 1974; De Deckker, 1981b; De Deckker, 1981a; Morris *et al.*, 2002; Martens *et al.*, 2008).

The number of individuals of *M. henricae* emerging reduced with salinity concentrations ≥ 37.7 g/L, with a significantly lower number of individuals in the populations that developed during the experiment, at the higher salinity concentration. Results also suggest that individuals of *M. henricae* are unable to emerge at salinities ≥ 61.0 g/L. This species is known to live on halophytes such as *R. megacarpa* (De Deckker, 1981b; Williams, 1981) and has been recorded laying eggs in the hollow stems of *R. megacarpa* (Martens *et al.*, 1985). Thus even if this species was able to tolerate higher salinities than the macrophyte community, the

threshold salinity of this ostracod species will reflect that of the aquatic macrophyte community it relies on for food and refuge.

Australocypris spp. has been recorded in a number of saline lakes throughout Victoria, South Australia and Western Australia (De Deckker, 1974; De Deckker and Geddes, 1980; De Deckker, 1981b; Shelley, 2008). This species had a different response to increasing salinities, with individuals emerging from the sediments at 61.0 g/L, in the absence of aquatic macrophyte species. This suggests that this species is not reliant on the macrophyte community and species from this genera have been reported as feeding on algae (De Deckker, 1974).

3.4.6 Salinity thresholds for submerged aquatic macrophyte and invertebrate communities

Results suggest that the upper salinity threshold for a macrophyte dominated systems lies between 37.7 g/L and 61.0 g/L, and the majority of calculated threshold values determined by the regression models in this study, further support a threshold limit between these values. Sim *et al.,* (2006a), also suggested that 45 g/L is the upper salinity threshold for macrophyte dominated communities. These concentration values however need to be used cautiously, as results of this study have shown that salinity can affect the reproduction of aquatic macrophytes below this salinity concentration, and may also impact their growth.

One factor not considered in this study is the effect of time and there have been few long term studies on the effects of long term disturbances on seed and egg banks. Brock (2011) found that in a long term study of plant propagule banks that some species were still viable after 12 years without inundation, however the longevity of individual species varied greatly. Waterkeyn *et al.,* (2011) conducted a long term (3 year) study on the effects of salinity (up to 5 g/L) and watering regime on crustacean eggs in diapause present in the substrate and found that the crustacean community was able to re-establish after disturbances.

The frequency of flooding and salinity concentrtations however affected species differently. Results of this study indicated that if subjected to salinities of between 37.7 g/L and the suggested salinity threshold of 45 g/L, for a long enough period, or in temporary wetlands over sufficient wetting cycles, that species such as *R.*

megacarpa could be lost from the system due to decreased germination and reproduction rates. It was also found that number of individuals of the ostracod *M. henricae* and growth of *L. macropogon* could also be affected at these salinities. Thus maintaining a wetland below these threshold levels for the majority of the flooding period would be optimal for maintaining viable populations of macrophyte species present in the propagule bank. If the wetland reaches threshold salinity levels too early during the flooding period, or if high salinity concentrations persist for long periods of time, plants may be less able to reproduce thereby reducing their contribution to the propagule bank over time. These species may not be resilient enough to survive high salinity periods or be able to re-establish after wetlands dry out due to the scarcity of propagules present in the lake sediments.

The salinity threshold for the emergence of macrophyte and invertebrate species present in the Lake Cullen aquatic community was between 37.7 g/L and 61.0 g/L. But in order to maintain biodiversity of all species present in the propagule bank long term, salinity concentrations need to be kept below 37.7 g/L and close to 24.4 g/L, especially for aquatic macrophytes during their reproductive stages. While aquatic macrophytes will persist in salinities of ≥ 37.7 g/L, the reproductive success and growth of these species will be affected. If the wetland is maintained at high salinity concentrations for an extended period of time during the lake's wetting phase, plants will have reduced reproductive success and therefore contribute fewer propagules to the propagule bank.

Additionally, a higher salinity will decrease the amount of biomass, indirectly impacting the number of waterbirds that the wetland can support. This is particularly important for *Ruppia* species which are an important food source for waterbirds as well as the invertebrate species *M. henricae* (Martens *et al.*, 1985; Lugg *et al.*, 1989). A lower salinity concentration (24.4 g/L) would also correspond to optimal concentrations for the maximum number of individuals emerging from the wetland sediments for both invertebrate species as seen in this study.

4.0 An investigation of the effect of high salinity disturbances on the propagule bank of Lake Cullen

4.1 Introduction

This chapter covers the second part of the glasshouse experiments testing the effects of salinity on the propagule bank of Lake Cullen. Whilst the previous chapter (Chapter 3) investigated the response of the propagule bank to 9 salinity treatments, this chapter investigates the response of the propagule bank to 4 high salinity disturbances, and the effect of up to 8 recovery salinity treatments on the propagule bank of Lake Cullen. This study aims to determine not only the effect of high level disturbances, but also whether recovery salinity concentrations impact germination and emergence of species from the propagule bank.

4.1.1 Models for predicting the effect of salinity on the loss of macrophytes from wetlands

Many models have been devised to explain the loss of macrophytes from wetlands, and it has been long accepted that loss of these species from wetlands occurs in a non-linear fashion (Williams *et al.*, 1990; Williams, 1998c; Davis, 2002). An alternative stable state model has been thoroughly investigated as a possible model to explain how salinity affects wetland communities (Davis *et al.*, 2003; Strehlow *et al.*, 2005; Sim *et al.*, 2006a; Sim *et al.*, 2006b; Sim *et al.*, 2006c), as opposed to a continuous or threshold model (Figure 4.1a-c). An alternative stable states model has been used to describe other types of disturbances in various ecosystems (Scheffer and Carpenter, 2003; Folke *et al.*, 2004), particularly the eutrophication of shallow lakes (Scheffer and Carpenter, 2003), catastrophic changes in coral reefs (McClanahan *et al.*, 2002; Mumby *et al.*, 2007) and in the loss of vegetation through grazing in terrestrial ecosystems (May, 1977; Rietkerk and van de Koppel, 1997). More recently Gordon *et al.,* (2008) and Davis *et al.,* (2010) have posed an additional model based on the alternative stable states model which shows how some systems may be unable to recover after a disturbance. This model shows that the threshold level for recovery between regime ‘B” and “A” may need to be much lower than the threshold where changes originally collapsed from regime ‘A’ and ‘B’ (Figure 4.1). This type of regime is characterised by the presence of hysteresis, where after a disturbance such as high salinity, the system may only return to its original if the

recovery salinity is reduced to a much lower level than the threshold (Figure 4.1c) (Davis *et al.*, 2010). The collapse model differs from the alternative stable states model in that no matter how much the stressor is removed from the system, no recovery is possible in the wetland (Figure 4.1d) (Davis *et al.*, 2010).

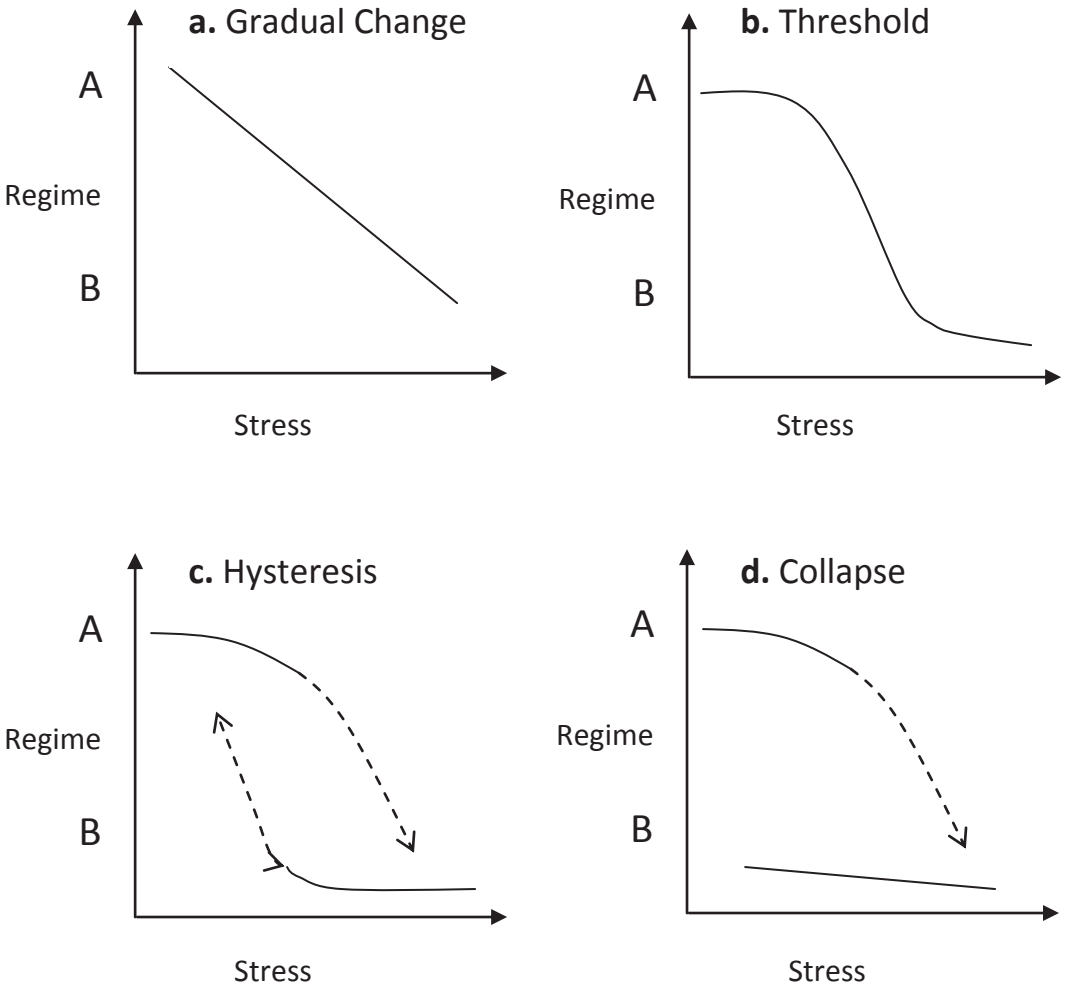


Figure 4.1 Differing models to show ways in which ecosystems can respond to external stressors such as salinity - modified from Gordon *et al.,* (2008); and Davis *et al.,* (2010). Regime A represents wetlands dominated by submerged aquatic macrophytes; regime B represents wetlands with an alternative regime (either benthic microbial mat or phytoplankton dominated wetlands).

Sim *et al.,* (2006b), and Davis *et al*., (2010), have found that salinity is not the only environmental driver of changes in wetland communities. They found that hydrology and nutrient levels also influence whether a wetland ecosystem is dominated by aquatic macrophytes, benthic microbial mats, phytoplankton, or bare sediments. They also found that drivers differ in the effects between permanent and temporary wetlands. More recently Davis *et al.,* (2010), described how acidification can also be a driver in changing regimes between those detailed above (systems dominated by

aquatic macrophytes, benthic microbial mats, phytoplankton, or bare sediments), and that it is not as simple as a single environmental factor influencing what regime a wetland shows, rather it is the interaction between watering regimes, salinity, nitrification and acidification that can drive these systems (Figure 4.2). Ultimately there are multiple stressors on any wetland system and as such relationships between one regime and the next may not be as straightforward as models represented in Figure 4.1.There may in fact be models nested within a larger and an overarching complex model.

The changes in community structure with increasing salinity found in the Lake Cullen propagule bank experiment (Chapter 3), did not follow the same patterns described in similar studies (Sim *et al.*, 2006a; Sim *et al.*, 2006b; Sim *et al.*, 2006c). Instead, a change from a macrophyte dominated community to that of a phytoplankton dominated community was found with increasing salinity. A clear water transitional stage occurred between the two "states", rather than a benthic microbial community. This provides further support for the theory that there are multiple stressors influencing wetland conditions and that wetlands do not always change from a macrophyte dominated community to a benthic microbial mat community with increased salinity (Davis *et al.*, 2010).

The presence of hysteresis and alternative stable states within an ecosystem is not easily demonstrated. Scheffer and Carpenter (2003) suggested that through controlled experiments, changes in the structure of an ecosystem can be explained by the alternative stable states model, whether it be the hysteresis model (Figure 4.1c) or the collapse model (Figure 4.1d).

The following factors are thought to exert such an influence:

- different initial states leading to differing final states
- disturbances triggering a shift to another permanent state
- presence of hysteresis in response to increases and decreases in disturbance (i.e. salinity)

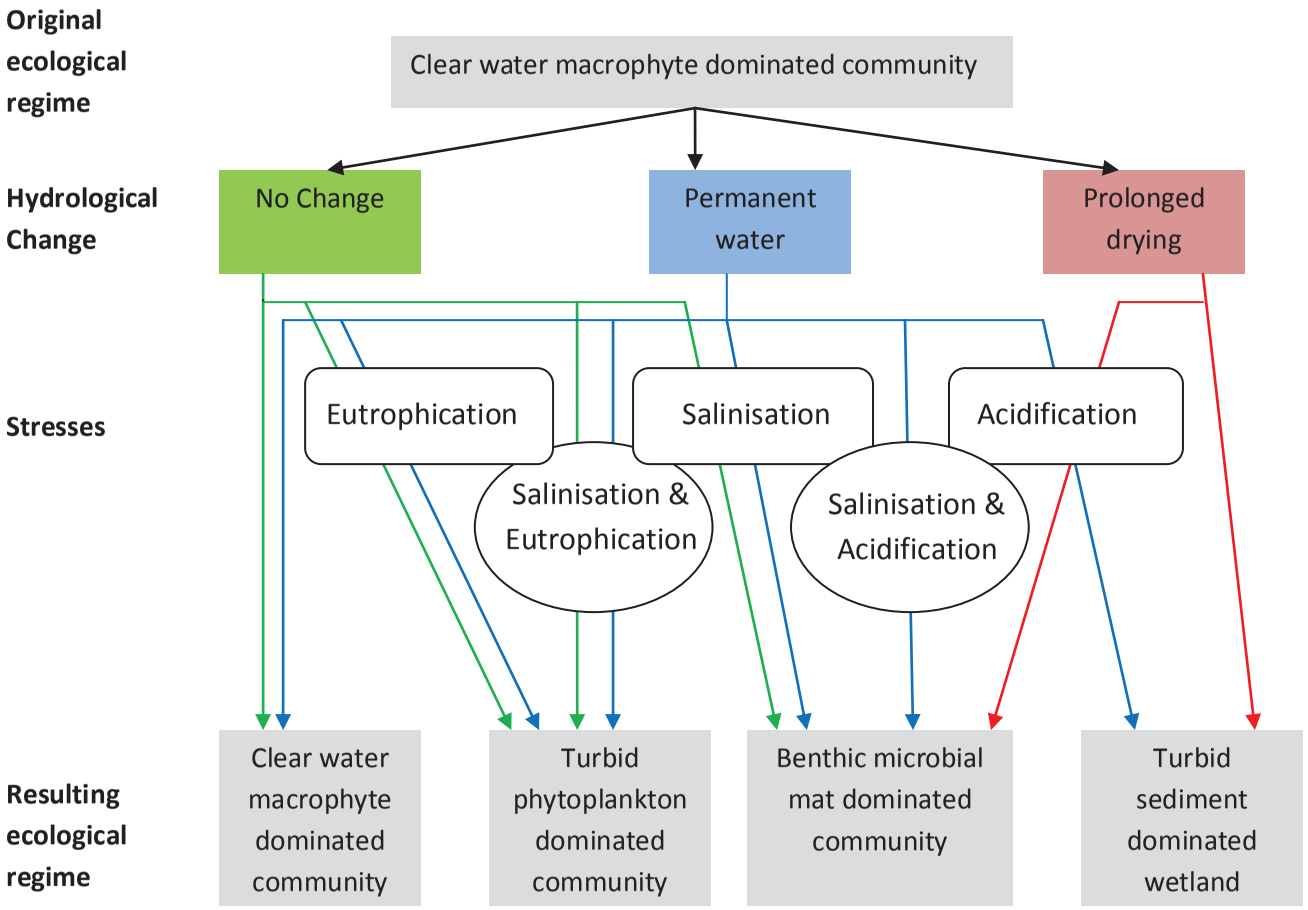


Figure 4.2: The proposed interactions between hydrological regime, salinisation, eutrophication and acidification on the state of shallow freshwater systems in southwestern Australia modified from Davis *et al.,* (2010)

Many studies of wetland propagule banks have focussed on determining the floral or invertebrate communities that emerge from sediments at various salinity concentrations after a period of drying or drought (Brock and Britton, 1995; Brock *et al.*, 2003; Nielsen *et al.*, 2003a; Nielsen *et al.*, 2003b; Brock *et al.*, 2005; Sim *et al.*, 2006a; Sim *et al.*, 2006b; Nielsen *et al.*, 2007; Nielsen *et al.*, 2008). One study by Sim *et al.,* (2006b) investigated the effect of increasing salinity concentrations on the plant germination from two saline wetlands in Western Australia and the effect of increasing salinities over a period of time on the health of *Ruppia polycarpa* (R. Mason) plants. Sim *et al.,* (2006b) not only tested the effect of increasing salinities on this macrophyte, but also the effect of the rate of increasing salinity concentrations. Results of this study showed that plant condition declined rapidly at salinities > 45 g/L and that rapid increases in salinity caused a rapid decline in plant condition.

Another study by Robinson *et al.,* (2006), tested the effect of preliminary exposure of *Melaleuca ericifolia* seeds on germination success. Seeds were exposed to a number of preliminary saline treatments up to and including 16 g/L before being returned to distilled water. Robinson *et al.,* (2006) found that seeds were able to germinate in all preliminary saline treatments. They also found that returning the seeds to distilled water greatly increased *Melaleuca ericifolia* germination success.

4.1.2 Hypotheses

Few propagule bank studies have focussed on species recovery after a period of high saline disturbance with the exception of Neilsen *et al.,* (2007) and Waterkeyn (2011). Neilsen *et al.,* (2007) investigated how 14 day high and low salinity pulses affected the plant and zooplankton from the propagule banks of three freshwater wetlands. Pulse events are short term increases in salinity, often associated with saline water disposal in rivers and wetlands (Nielsen *et al.*, 2007). Salinity pulses were found to have no effect on the emergence of plants from the propagule banks, but did have a positive effect on zooplankton emergence. All three wetlands had increased emergence of zooplankton after the low and high pulses of salinity (Nielsen *et al.*, 2007). Waterkeyn *et al.,* (2011), investigated the effect of flooding frequency and increase salinity (up to 5 g/L) on the emergence of crustaceans from the propagule

bank over a 3 year period. Waterkeyn *et al.,* (2011), found that the crustacean community was able to re-establish after disturbances, however the frequency of flooding and salinity concentrations affected species differently.

It should be noted that the high and low pulses of salinity (5 g/L and 1 g/L respectively) of the study by Neilsen *et al.,* (2007) and Waterkeyn *et al.,* (2011) were much lower than the salinities tested in both this experiment and that by Sim *et al.,* (2006b), and thus the findings may not be applicable for higher salinity disturbances.

The hypotheses were tested in this study:

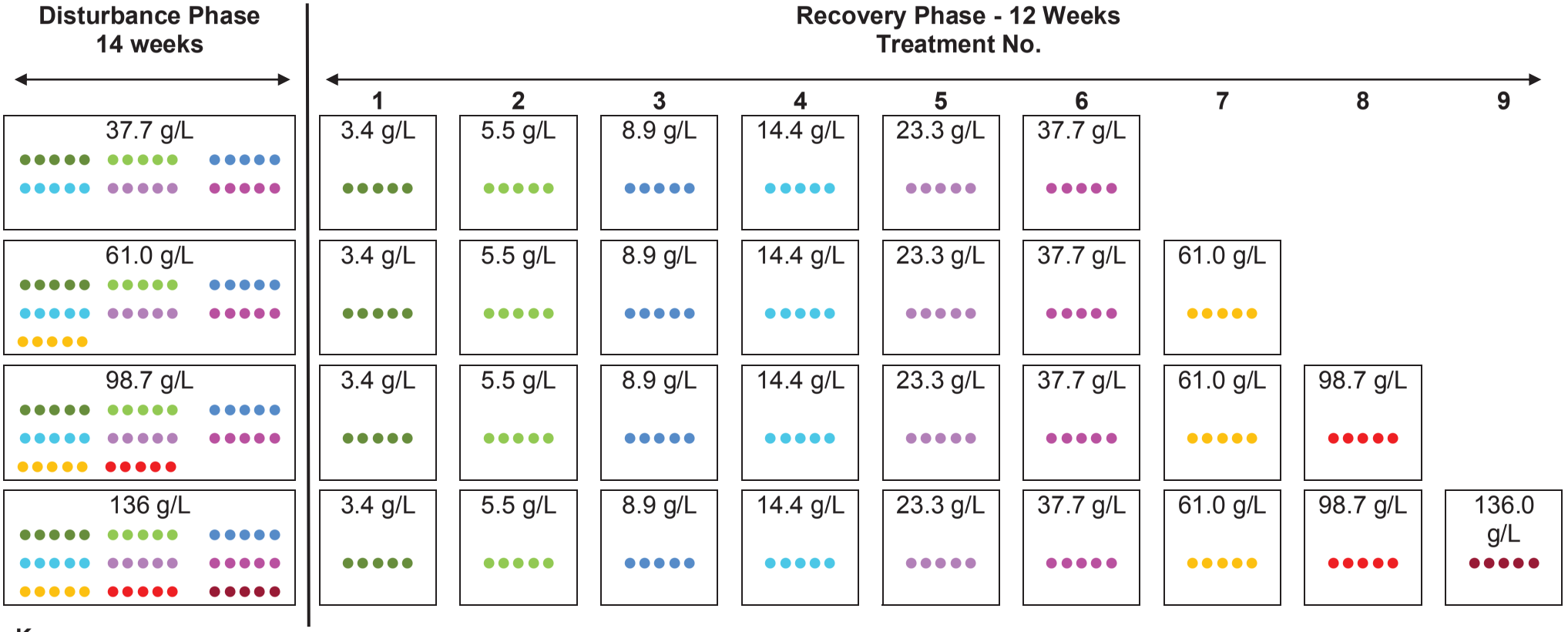
- Do high salinity disturbances affect the recovery of the dominant species present in the propagule bank of Lake Cullen and is the salinity threshold for germination the same as those propagules not subjected to a high salinity disturbance?
- What type of threshold response occurs with changing recovery salinity concentrations? Does the response follow a hysteresis, threshold response or irreversible change model?
- How do varying response salinity levels affect individual species emergence, growth or reproduction?

4.2 Methods

The effect of changing salinity regimes on the propagule bank of Lake Cullen was investigated by subjecting sediments to four high salinity treatments for 14 weeks (Disturbance Phase) before the water in these treatments was changed and the sediments were subjected to various salinity treatments which were lower or equivalent to those used in the disturbance phase for a further 12 weeks (Recovery Phase) (Figure 4.3). All treatments were determined by using a partial Fibonacci numeric sequence, which represented a natural logarithmic relationship, with the exception of the highest salinity treatment that was set at 136.0 g/L as the solution became saturated.

Sediment from Lake Cullen was collected in September 2005, when the wetland was dry and before it received an environmental water allocation. Sediments from the top 5 cm of the wetland surface were collected using a spade. Sediment collection sites were chosen from random locations 0 and 50 meters from the wetland edge. Sediment samples were transported back to the laboratory where they were stored in the dark at ± 4^{o}C for two months to prevent propagule germination. Samples were then dried thoroughly under cover in a glasshouse (to prevent contamination from airborne propagules) before being passed through a soil crusher set at a diameter of 6 mm (Jaw Crusher PEX 60 x 100). Dead vegetation was removed during this process. Crushed samples were mixed thoroughly with a spade, to minimise the influence of spatial variation in the wetland soil propagule banks.

Figure 4.3 The design of the propagule bank study showing the disturbance and recovery salinity treatments tested



Key

● – represents one replicate. ● – 3.4 g/L recovery salinity, ● - 5.5 g/L recovery salinity, ●- 8.9g/L recovery salinity, ● -14.4g/L recovery salinity, ● - 23.3 g/L recovery salinity, ● - 37.7 g/L recovery salinity, ● – 61.0 g/L recovery salinity, ● - 98.7 g/L recovery salinity, ● -136.0 g/L recovery salinity.

A total of 160, 20L clear plastic tubs were used in this experiment. There were 5 replicates for each of the 30 treatments with exception of the control treatment that consisted of 10 tubs. The tubs were arranged randomly throughout an air conditioned glasshouse. Their position was determined using a random numbers table (Appendix 9). Each tub contained a total of 1kg of sediment from Lake Cullen, distributed between two small plastic trays (120mm x 175mm x 60mm) (500 g in each). There were also 10 control tubs set up with 1 kg of sterilised sand distributed between two plastic trays, to test for contamination by airborne propagules throughout the experiment. The control tubs contained 3.4 g/L saline solution and no plants or animals were found in the tubs at the conclusion of the experiments. Tubs were filled with 15 L of solution at the required salinity and the depth of the solution was marked on the side of the tub. Salinity concentrations were checked weekly with a conductivity meter to ensure that salinity concentrations remained within the range of ± 16% for the appropriate treatment concentrations. Tubs were topped up with tap water if necessary over a 14 week period.

At the end of the Disturbance Phase (14 weeks) saline solution was drained from all replicates to a depth of 7 cm using a 25 μm mesh filter attached to a syphon to ensure that no phytoplankton, zooplankton or other invertebrates were lost from the sample. Propagules caught in the mesh were returned to the tub immediately. No plant, invertebrate or algal material was removed from the tubs at the end of the disturbance phase. Tubs were then refilled to the marked level with water to the required Recovery Phase salinity. Tubs were checked regularly and topped up with tap water if necessary over a 12 week period (Recovery Phase) to ensure that salinity concentrations remained within the desired treatment range (± 10%).

4.2.1 Air temperature and water quality monitoring

The method described in Chapter 3 was used for this study to monitor air and water quality.

4.2.2 Monitoring of aquatic macrophyte germination (seeds and asexual propagules) and invertebrate hatching from the propagule bank

The method described in Chapter 3 was used for this study to monitor aquatic macrophyte germination and invertebrate hatching from the propagule bank.

4.2.3 Invertebrate sampling

The method described in Chapter 3 was used for this study to capture and count the invertebrates that emerged during the experiment. To facilite sorting of invertebrates from detritus, the same sugar floatation method was used and the sampling efficiency (QA/QC check) of this method was conducted as described in Chapter 3.

Results of the QA/QC check of the effectiveness of the sugar flotation method showed that minimum of 95% of invertebrates for each species were successfully removed from the organic material present in the (Appendix 10). The sugar flotation method was deemed to be a successful and efficient method in sorting invertebrates from organic materials collected in the > 500 µm sieve. For the 250 µm to 500 µm and the 108 µm to 250 µm size invertebrates, no flotation method was necessary as less detritus was present in these samples.

4.2.4 Harvesting aquatic macrophytes

At the end of the 26 week study period, all emergent aquatic macrophytes were harvested and sorted according to species. For each aquatic angiosperm species the number of shoots, reproductive individuals, flowers budding, mature flowers, fruit, reproductive structures, aborted sexual reproductive structures and seeds produced by the plants were recorded. For the charophytes the number of individuals, reproductive individuals, and number of reproductive structures on whorls were recorded.

The aboveground biovolume and dry weight biomass of the measured plant material of each species was then measured using the methods and equations described in Chapter 3.

4.2.5 Data analysis

Data was analysed for linear, quadratic, and cubic regressions to determine how the species responded to increased salinity concentrations and to ascertain an upper salinity threshold. Results were also analysed using two way ANOVA tests to determine if there was any significant difference ($p < 0.05$) between salinity treatments, using the following model.

DV = constant + Disturbance Phase salinity + Recovery Phase salinity + Distrubance Phase salinity x Recovery phase salinity

Where results of the two way ANOVA's were significant, *post hoc* Tukey's tests were conducted to determine which treatments were significantly different. All statistical tests were conducted using the PASW 18 (previously known as SPSS statistics) software package. Square root transformations of the dependent variable were undertaken when the data from this experiment did not meet the assumptions of normality or homogeneity of variances for parametric tests (Levene's test).

4.3 Results

4.3.1 Air temperature and water quality monitoring

Maximum air temperature ranged between 20.1 ^{o}C and 40.5 ^{o}C, while minimum air temperatures ranged from 6.0 ^{o}C to 14.7 ^{o}C. Maximum water temperatures ranged between 15 ^{o}C and 32 ^{o}C, while minimum water temperatures ranged from 8 ^{o}C to 25 ^{o}C (Appendix 11). Monitoring results of salinity concentrations for each replicate showed that on average maximum salinity concentrations were maintained within 16% of target treatment concentration. The monitoring results for pH indicated that waters were alkaline, ranging from pH 7.35 to 9.90 (Appendix 11).

4.3.2 Emergence of aquatic macrophyte and invertebrate taxa

Three species of aquatic macrophyte germinated in this experiment; *Ruppia megacarpa, Lamprothamnium macropogon* and an unidentified *Ruppia* spp. The unidentified *Ruppia* spp. only occurred in one replicate (Disturbance Phase salinity 98.7 g/L, Recovery Phase salinity 14.4 g/L treatment) and was not possible to identify to species level as it did not flower during the experiment.

Figure 4.4, shows that the germination of aquatic macrophytes only occurred within the Disturbance Phase of this experiment (first 14 weeks) in the treatment where the salinity was ≤ 37.7 g/L. In treatments where Disturbance Phase salinity was ≤ 37.7 g/L germination did not occur at the same time for the two dominant aquatic macrophyte species. *Lamprothamnium macropogon* germinated in all replicates with a Disturbance Phase salinity concentrations of 37.7 g/L by Week 7 of the experiment. Results for *R. megacarpa* was less uniform with individuals observed to have germinated during the Disturbance Phase (salinity 37.7 g/L) of the experiment in 11 replicates, whilst other replicates were only observed to have individuals germinating much later in the Recovery Phase after salinity concentrations were reduced (Figure 4.4).

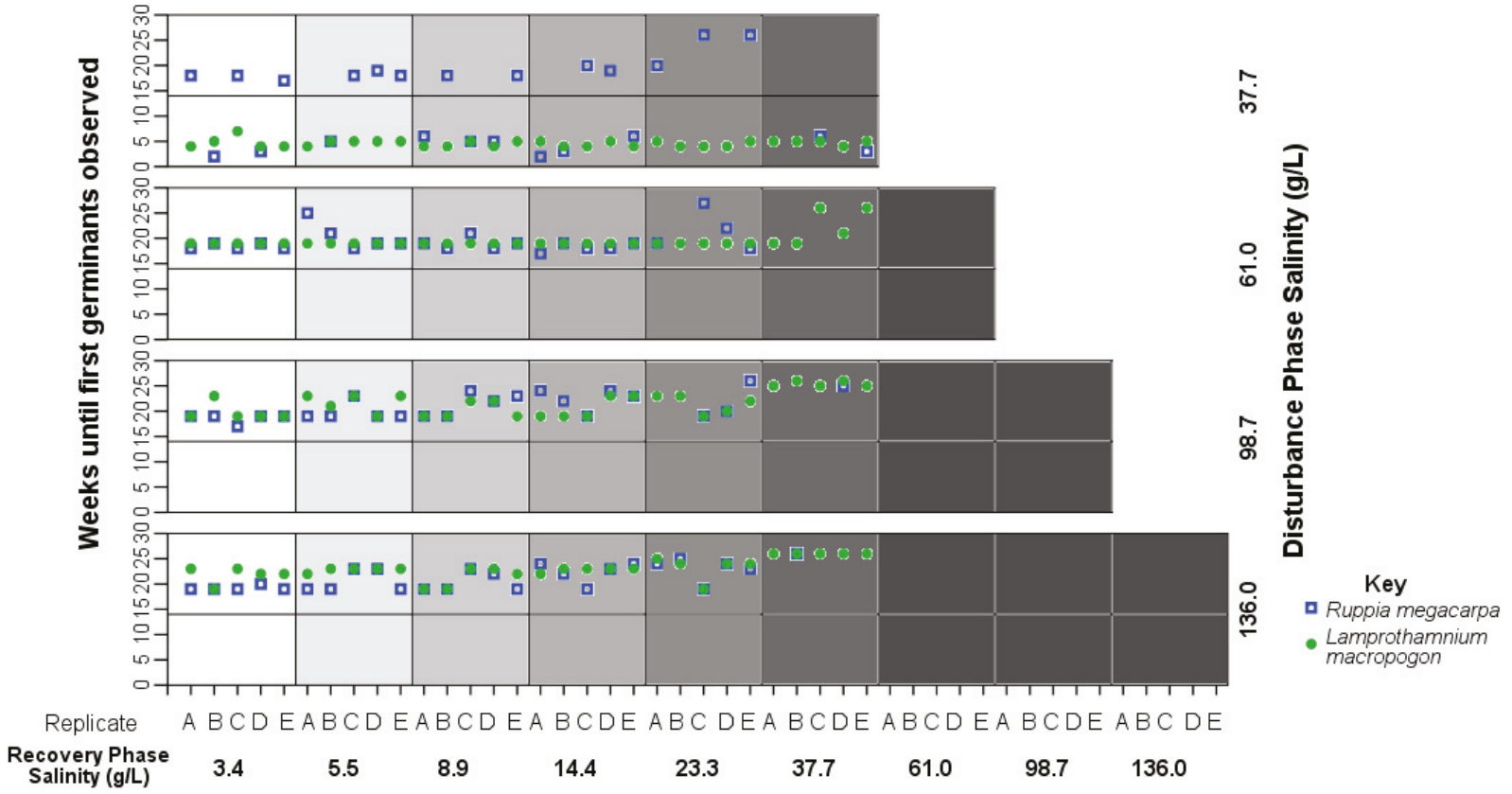


Figure 4.4 The effect of high Disturbance Phase and Recovery Phase salinity concentrations on the time until macrophyte germination.

Germination of aquatic macrophytes was observed in those salinities with a Recovery Phase salinity ≤ 37.7 g/L. Germination occurred from Week 16 until the last week of the experiment (Week 26) for both *L. macropogon* and *R. megacarpa*. Germination of *R. megacarpa* however did not occur in all replicates with a Recovery Phase salinity of 23.3 g/L or 37.7 g/L. Also, all replicates within the 61.0 g/L, 98.7 g/L and 136.0 g/L Disturbance Phases with 37.7 g/L Recovery Phase treatments, had no or low numbers of *R. megacarpa* germinants. Figure 4.4 also shows that the higher the Disturbance and Recovery salinity combination, the later the first germination of macrophytes occurred.

Six invertebrates species emerged in this experiment, the ostracods *Mytilocypris henricae* (Chapman 1966) and *Australocypris* spp., individuals from the dipterian Family Psychodidae (moth flies), flatworms from Class Turbellaria, and Collembollans from Family Sminthuridae. Only two of these species were abundant (*Mytliocypris henricae, Australocypris* spp.), with the other 4 species only present in one replicate each (Appendix 12).

Figure 4.5 shows the time until emergence for the two dominant ostracod species (*M. henricae* and *Australocypris* spp.). The data does not distinguish between individual species, because small newly emerged ostracods, are difficult to distinguish between species with confidence. Ostracods emerged in the Disturbance Phase of this study in all replicates with disturbance salinity treatments of 37.7 g/L (emergence occurred during Weeks 4 to 5) and 61.0 g/L (emergence occurred during Weeks 6 to 9). Whereas ostracods only emerged in the Disturbance Phase salinity treatments of 98.7g/L and 136.0 g/L, when the Recovery Phase salinity concentrations had been lowered ≤ 61.0 g/L during the Recovery Phase (emergence occurred between Weeks 21 to 26 for both disturbance treatments).

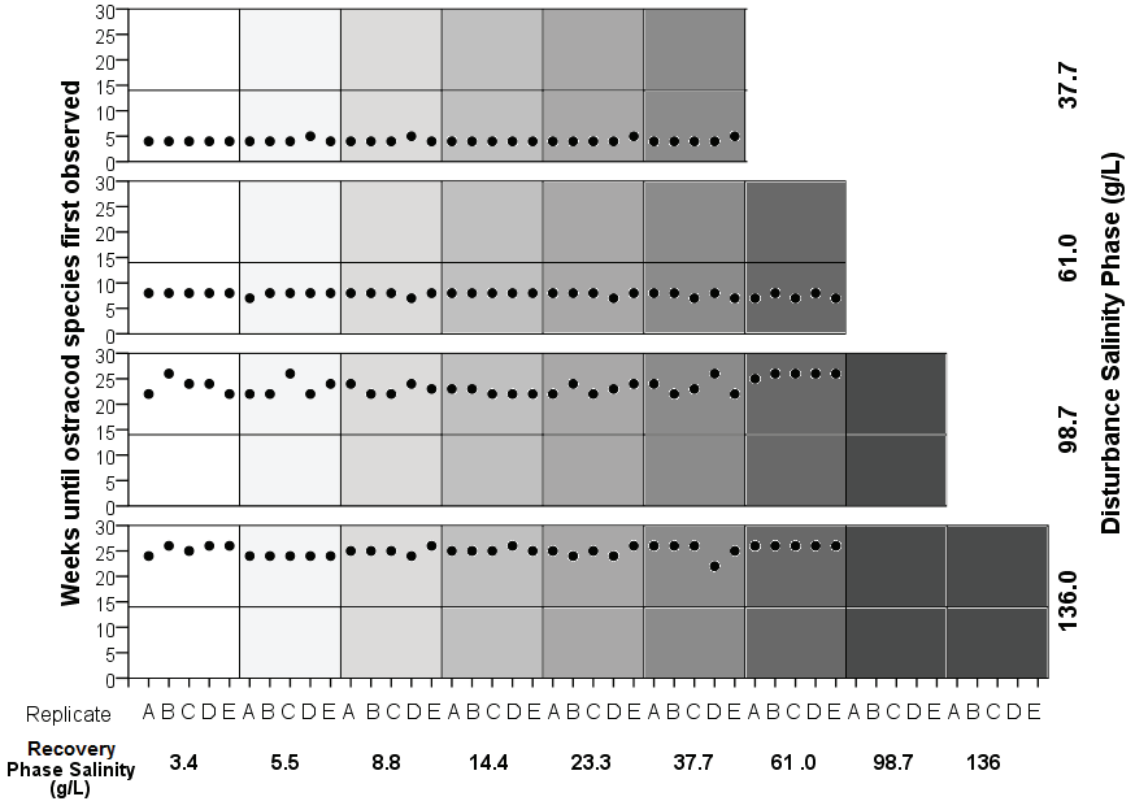


Figure 4.5 The effect of Disturbance Phase and Recovery Phase salinity concentrtaions on the time until emergence of ostracod species, *Mytilocypris henricae* and *Australocypris* spp.

4.3.3 Aquatic macrophyte germination - *Ruppia megacarpa*

In 3 out of the 4 Disturbance Phase salinity treatments, the number of *R. megacarpa* stems produced as Recovery Phase salinity increased showed significant negative linear regressions ($p < 0.05$) (Figure 4.6). Results of significant linear regressions for the 61.0 g/L ($p < 0.001$, $R^2 = 0.519$), 98.7 g/L ($p < 0.001$, $R^2 = 0.586$) and 136 g/L ($p < 0.001$, $R^2 = 0.592$) treatments were very similar indicating that whilst the Recovery Phase salinity concentrations did impact the number of *R. megacarpa* stems produced, this was minimal despite the differences in initial Disturbance Phase salinity concentrations the propagule banks were exposed to (Figure 4.6 and Appendix 13). The lowest Disturbance Phase salinity treatment (37.7 g/L) showed no significant regression, although the numbers of stems produced was similar to that of the other three Disturbance Phase salinity concentrations.

Results of a two way ANOVA showed that there was a main effect of Disturbance Phase salinity concentration on the number of *R. megacarpa* stems produced ($p = 0.046$, $F = 2.743$, $df = 3, 120$). There was also a main effect of Recovery Phase salinity concentration on number of *R. megacarpa stems* produced ($p < 0.001$, $F = 29.246$, $df = 8, 120$) and a significant interaction of Disturbance Phase salinity and Recovery Phase salinity on the number of *R. megacarpa stems* produced ($p = 0.042$, $F = 1.734$, $df = 8, 120$). This indicates that not only did both the Disturbance and Recovery salinities affect the number of *R. megacarpa* stems produced, but that the effect of the Recovery Phase salinity concentratons was different depending on the Disturbance Phase salinity treatment the seedbank was subjected to. *Post hoc* Tukey's tests indicated that there was no significant difference between any of the Disturbance phase salinity treatments. However there were significant differences found between many of the Recovery Phase salinity treatments, please refer to Appendix 13.

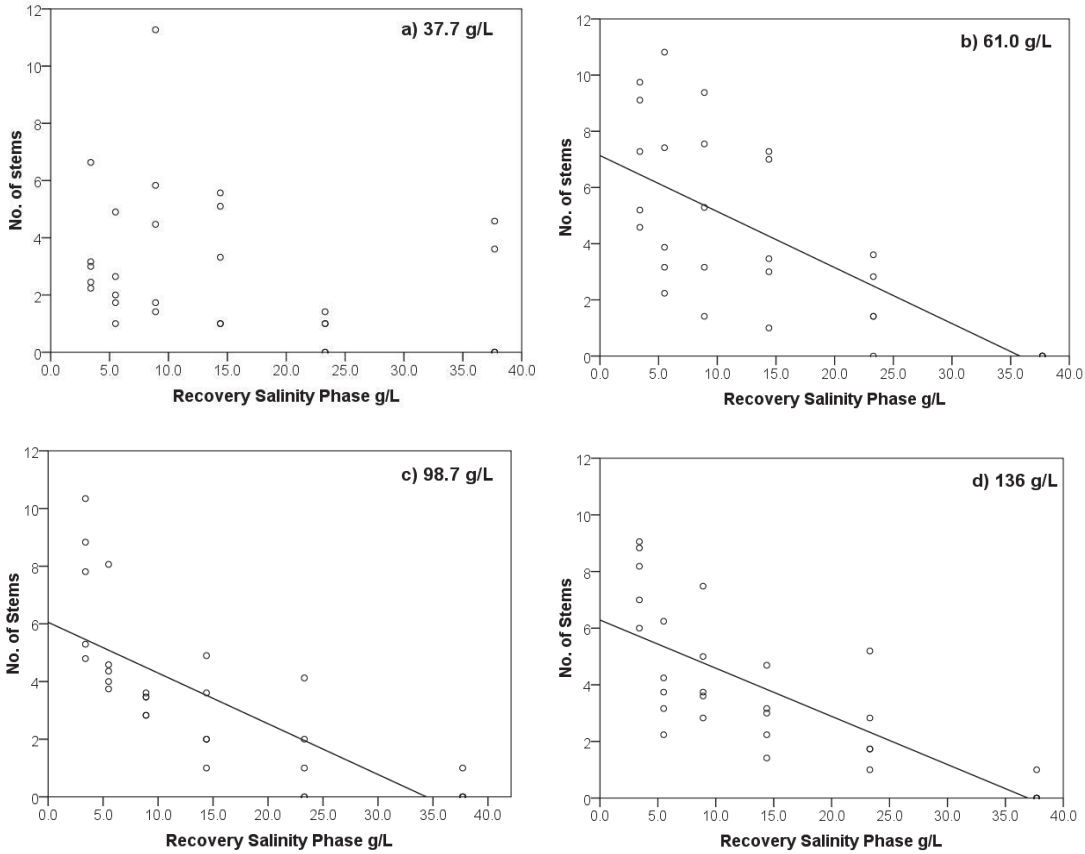


Figure 4.6 The effect of Recovery Phase salinity concentrations on the number of *Ruppia megacarpa* stems produced for each Disturbance Phase salinity treatment (n=5, square root transformation). Lines represent where regressions were significant ($p<0.05$).

Significant negative linear regressions ($p <0.05$) were found for all four disturbance salinity phase treatments being 37.7 g/L ($p = 0.022$, $R^2 = 0.174$), 61.0 g/L ($p < 0.001$, $R^2 = 0.536$), 98.7 g/L ($p < 0.001$, $R^2 = 0.666$) and 136.0 g/L ($p < 0.001$, $R^2 = 0.686$) when comparing the amount of *R. megacarpa* dry weight biomass produced as Recovery Phase salinity concentrations increased (Figure 4.7). The significant linear regressions for all Disturbance Phase salinity concentration treatments were very similar indicating that whilst the Recovery Phase salinity concentrations were impacting the amount of dry weight biomass produced. There was very little difference in biomass produced across the four Disturbance Phase salinity treatments, despite differences in initial Disturbance Phase salinity concentrations propagule banks were exposed to (Figure 4.7 and Appendix 13). It should be noted that the R^2 value for the 37.7 g/L disturbance salinity treatment regression was very low ($R^2 = 0.174$), indicating that whilst the amount of dry weight biomass produced was affected by salinity, that salinity may not be the only factor impacting on *R. megacarpa* dry weight biomass produced.

Results of a two way ANOVA showed that there was no significant main effect of Disturbance Phase salinity concentration on *R. megacarpa* dryweight biomass produced ($p = 0.803$). There was also no significant intereaction effect of Disturbance phase salinity and Recovery Phase salinity on the amount of *R. megacarpa* dryweight biomass produced ($p = 0.493$). There was however a main effect of Recovery Phase salinity concentration on on the amount of *R. megacarpa* dryweight biomass produced ($p < 0.001$, $F = 0.330$, $df = 8, 120$). This indicates that only the Recovery salinity treatments affected the amount of *R. megacarpa* dryweight biomass produced. *Post hoc* Tukey's tests indicated that there was significant differences found between many of the Recovery Phase salinity treatments, please refer to Appendix 13.

Reproductive structures (flowers and fruit) on *R. megacarpa* were recorded in a few replicates exposed to the lowest Disturbance Phase salinity treatment of 37.7 g/L and only one flower was recorded in the 61.0 g/L Disturbance Phase salinity and 5.5 g/L Recovery Phase salinity treatments. Little more can be deduced from these results and regarding the effects of Disturbance and Recovery Phase salinity concentrations on reproduction in this species, given the low concentrations of reproduction.

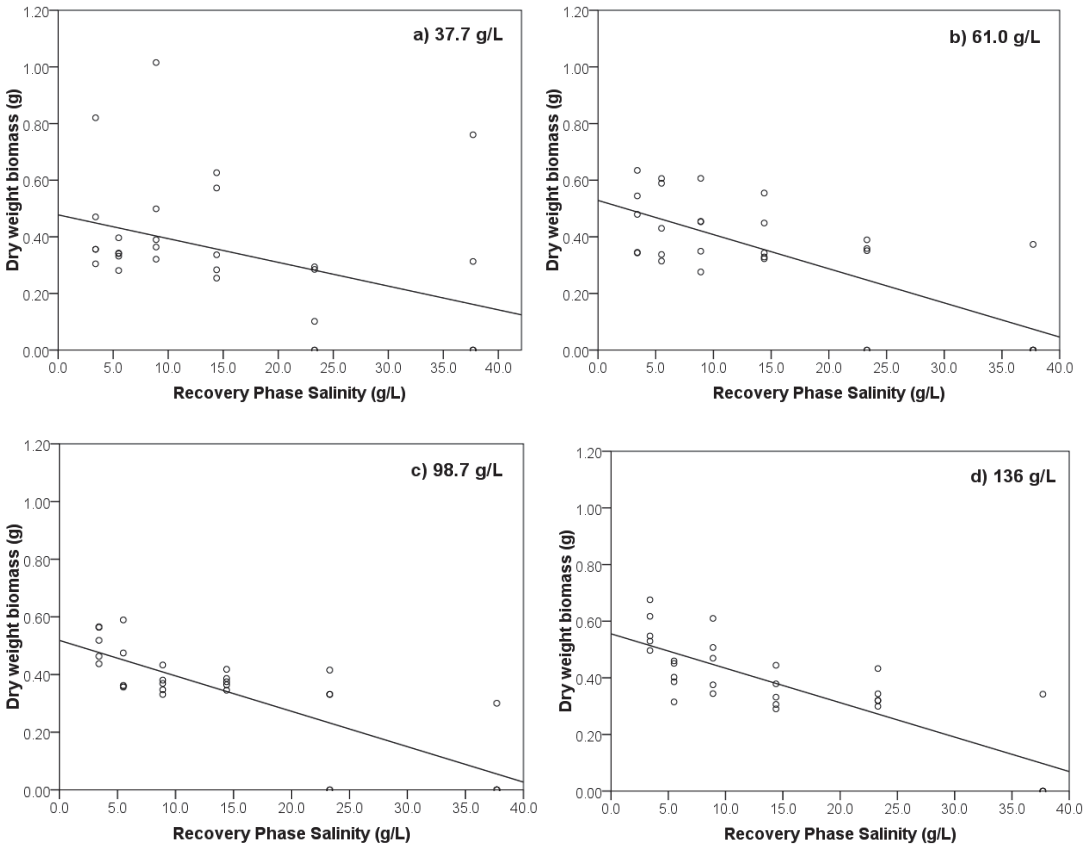


Figure 4.7 The effect of Recovery Phase salinity concentrations on the amount of *Ruppia megacarpa* dry weight biomass produced for each Disturbance Phase salinity treatment (n=5, square root transformation), Lines represent where regressions were significant ($p<0.05$).

4.3.4 Aquatic macrophyte germination – *Lamprothamnium macropogon*

In all Disturbance Phase salinity treatments, the number of *L. macropogon* individuals produced as Recovery Phase salinity concentrations increased showed significant negative linear regressions ($p < 0.05$) (Figure 4.8). Significant linear regressions for the 61.0 g/L, 98.7 g/L and 136 g/L treatments were very similar indicating that whilst the recovery salinity concentrations were impacting the number of *L. macropogon* germinants, there was very little variation in the number produced when comparing the different initial salinity concentrations the propagule banks were exposed to (Figure 4.8 and Appendix 13). The lowest Disturbance Phase salinity treatment (37.7 g/L) showed the same negative trend, with increasing Recovery Phase salinity. The number of germinants was higher in each of the Recovery Phase salinity treatments, when compared to the same Recovery Phase concentrations in each of the three higher Disturbance Phase treatments. This indicates that having a lower Disturbance Phase salinity concentration of ≤ 37.7 g/L may increase germination in this species. It should be noted that for the Disturbance Phase salinity

treatments of 37.7 g/L and 61.0 g/L that the R^2 values were low (<0.3) indicating that the Reovery Phase salinity may not be the only factor affecting results. All other regressions were much stronger with R^2 values of > 0.6.

Results of a two way ANOVA showed that there was a main effect of Disturbance Phase salinity concentration on the number of *L.macropogon* germinants ($p = <0.001$, $F = 260.486$, $df = 3, 120$). There was also a main effect of Recovery Phase salinity concentration on number of *L.macropogon* germinants ($p < 0.001$, $F = 113.037$, $df = 8, 120$) and a significant interaction of Disturbance Phase salinity and Recovery Phase salinity on the number of *L.macropogon* germinants ($p = 0.005$, $F = 2.246$, $df = 18, 120$). This indicates that not only did both the Disturbance and Recovery salinities affect the number of *L.macropogon* germinants, but that the effect of the Recovery Phase salinity concentratons was different depending on the Disturbance Phase salinity treatment the seedbank was subjected to. *Post hoc* Tukey's tests indicated that there was a significant difference between all of the Disturbance phase salinity treatments. There were also significant differences found between many of the Recovery Phase salinity treatments, please refer to Appendix 13.

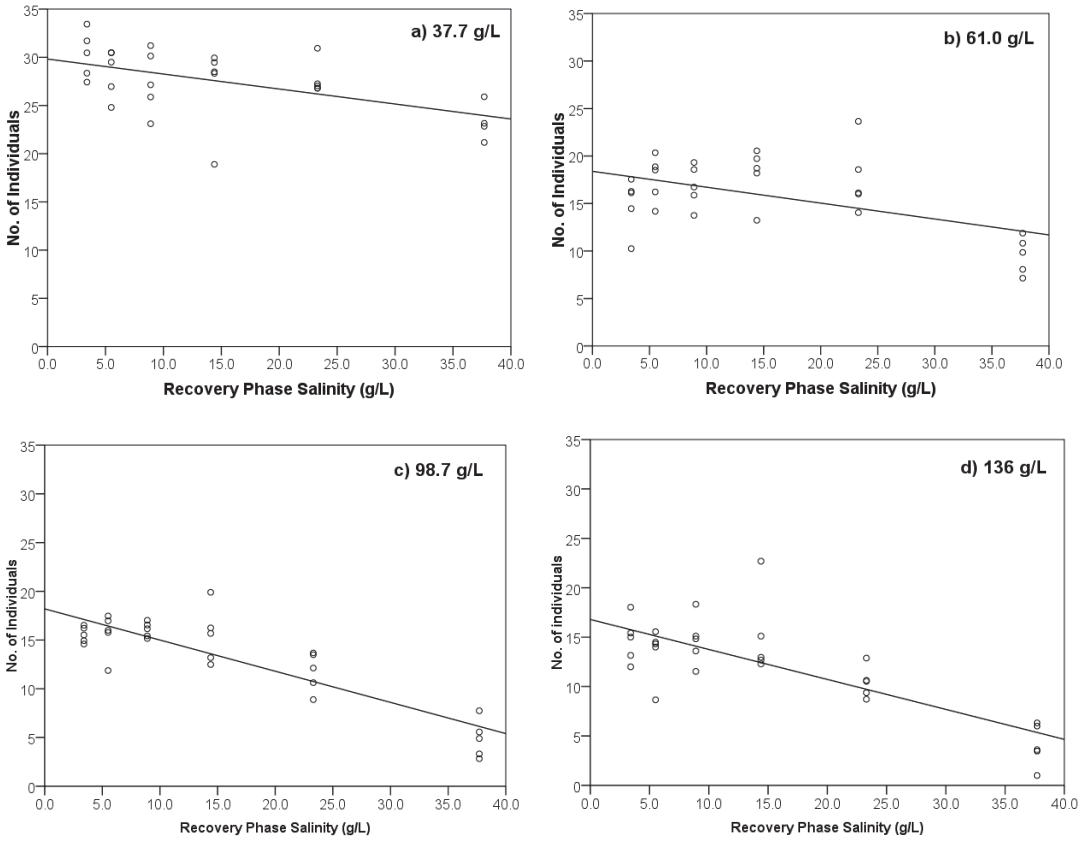


Figure 4.8 The effect of Recovery Phase salinity concentrations on the number of *Lamprothamnium macropogon* germinants in each Disturbance Phase salinity treatment (n=5, square root transformation). Lines represent where regressions were significant ($p < 0.05$).

Significant negative linear regressions ($p < 0.05$) were found, for all four Disturbance Phase salinity treatments, when comparing *L. macropogon* dry weight biomass produced as Recovery Phase salinity concentrations increased (Figure 4.9). The significant linear regressions for all disturbance treatments were very similar indicating that whilst the recovery salinity concentrations were impacting *L. macropogon* biomass, there was very little effect produced by the Disturbance Phase salinity concentrations that the propagule banks were exposed to (Figure 4.9 and Appendix 13). It should be noted that for all Disturbance Phase salinity treatments, the R^2 values were low (<0.5) indicating that whilst recovery salinity concentrations did effect the germination of *L. macropogon*, that this may not be the only factor impacting on the results.

Results of a two way ANOVA showed that there was a main effect of Disturbance Phase salinity concentration on the amount of *L.macropogon* dry weight biomass

(p = <0.001, F = 25.608, df = 3, 120). There was also a main effect of Recovery Phase salinity concentration on the amount of *L.macropogon* dry weight biomass (p < 0.001, F = 51.148, df = 8, 120) and no significant interaction of Disturbance Phase salinity and Recovery Phase salinity on the amount of *L.macropogon* dry weight biomass (p = 0.063). This indicates that did both the Disturbance and Recovery salinities affected the amount of *L.macropogon* dry weight biomass. *Post hoc* Tukey's tests indicated that there was a significant difference between all of the Disturbance phase salinity treatments. There were also significant differences found between many of the Recovery Phase salinity treatments, please refer to Appendix 13.

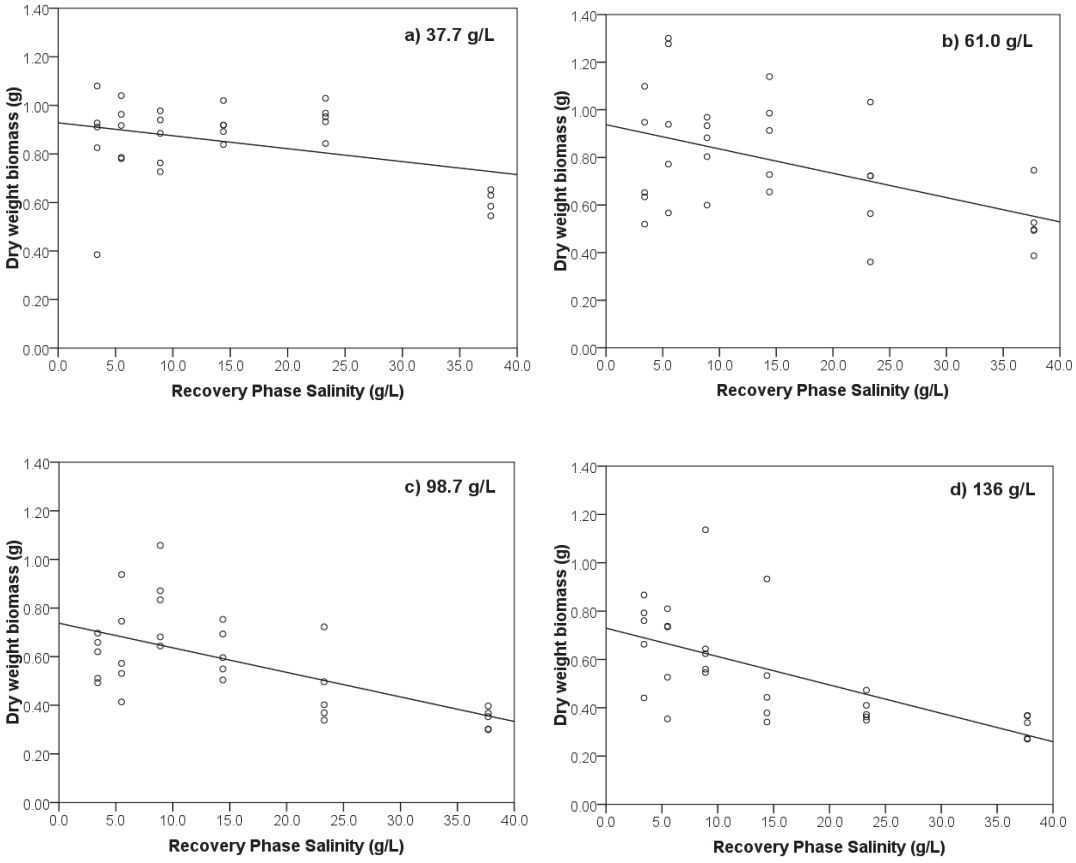


Figure 4.9 The effect of Recovery Phase salinity concentrations on the amount of *Lamprothamnium macropogon* dry weight biomass in each Disturbance Phase salinity treatment (n=5 square root transformation). Lines represent where regressions were significant (p <0.05).

Lamprothamnium macropogon individuals only contained antheridia in Disturbance Phase salinities of 37.7 g/L and 61.0 g/L, with the exception of one replicate in each of the higher Disturbance Phase salinity concentrations, which had individuals with antheridia (98.7 g/L Disturbance Phase and 13.3 g/L Recovery Phase salinity

treatment and 136 g/L Disturbance Phase and 24.4 g/L Recovery Phase salinity treatment). A higher percentage of individuals had antheridia in the Disturbance Phase salinity of 37.7 g/L when compared to the Disturbance Phase salinity of 61.0 g/L (Appendix 13). There were no oogonia observed to be produced on any *L. macropogon* individuals in this experiment.

4.3.5 Phytoplankton Blooms

Visual observations of the tubs indicated that phytoplankton blooms occurred in all replicates in the highest disturbance salinity treatment (136.0 g/L). These blooms formed by Week 4 and persisted for the duration of the disturbance phase of the experiment (Table 4.1). In the recovery phase of the experiment, phytoplankton blooms were only present in the treatment with Recovery Phase salinity of 136.0 g/L, however these algal blooms occurred in every replicate with this salinity.

Table 4.1 Presence of phytoplankton blooms in replicates in the 136.0 g/L Disturbance Phase salinity treatment for the duration of the experiment. Numbers (up to 5) indicate the number of replicates were algal blooms were present.

Key ✗ absent ✓ present

Replicate		Week																								
		Disturbance Phase														Recovery Phase										
Disturbance Phase g/L	Recovery Phase g/L	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
136.0	3.4	0	0	0	5	5	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
136.0	5.5	0	0	0	5	5	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
136.0	8.9	0	0	0	5	5	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
136.0	14.4	0	0	0	5	5	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
136.0	23.3	0	0	0	5	5	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
136.0	37.7	0	0	0	5	5	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
136.0	61.0	0	0	0	5	5	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
136.0	98.7	0	0	0	5	5	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
136.0	136.0	0	0	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

4.3.6 Invertebrate emergence - *Mytilocypris henricae*

Significant quadratic regressions were found between the number of *M. henricae* individuals and Recovery Phase salinity concentrations for each the Disturbance Phase salinity concentrations with the exception of the 37.7 g/L Disturbance Phase salinity (Figure 4.10, Appendix 13). The significant quadratic relationships indicate that higher numbers of *M. henricae* were found in populations that developed from the propagule bank in the mid-range Recovery Phase salinity treatments (8.9 g/L to 24.4 g/L). The highest number of individuals was recorded in the 37.7g/L Disturbance Phase treatment which can be attributed to fact that the salinity was low enough for this ostracod species to emerge and thus allowed further time for this population to grow. It should be noted that for all Disturbance Phase salinity treatments, the R^2 values were low (<0.6) indicating that whilst recovery salinity concentrations did effect the population numbers of *M. henricae* that developed from the propagule bank, that there may be other factors effecting these resutls.

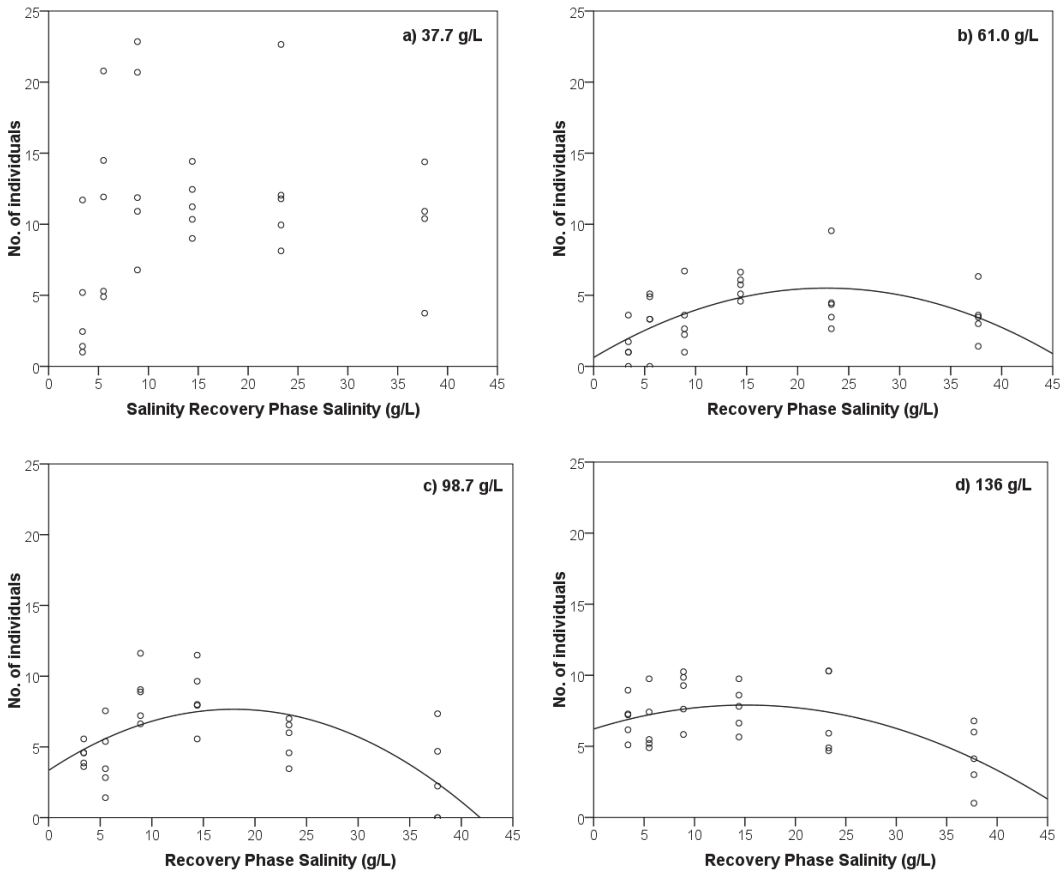


Figure 4.10 The effect of Recovery Phase salinity concentrations on the number of *Mytilocypris henricae* individuals in populations that developed from the sediments in each Disturbance Phase salinity treatment (n=5, square root transformation). Lines indicate where regressions were significant (p<0.05).

Results of a two way ANOVA showed that there was a main effect of Disturbance Phase salinity concentration on the number of *M. henricae* individuals ($p = <0.001$, $F = 33.157$, $df = 3, 120$). There was also a main effect of Recovery Phase salinity concentration on number of *M. henricae* individuals ($p < 0.001$, $F = 16.961$, $df = 8, 120$) and a significant interaction of Disturbance Phase salinity and Recovery Phase salinity on the number of *M. henricae* individuals ($p = 0.024$, $F = 1.872$, $df = 18, 120$). This indicates that not only did both the Disturbance and Recovery salinities affect the number of *M. henricae* individuals, but that the effect of the Recovery Phase salinity concentratons was different depending on the Disturbance Phase salinity treatment the propagule bank was subjected to. *Post hoc* Tukey's tests indicated that there was only a significant difference between the higher Disturbance Phase salinity treatments (61 g/L, 98.7 g/L and 136 g/L) and the lowest Disturbance Phase salinity treatment (37.7 g/L). There were also significant differences found between many of the Recovery Phase salinity treatments, please refer to Appendix 13.

4.3.7 Invertebrate emergence - *Australocypris* species

Significant quadratic regressions were found with Recovery Phase salinities for the Disturbance Phase salinity concentrations of 61.0 g/L and 98.7 g/L (Figure 4.11, Appendix 13). No significant regressions were found for Disturbance Phase salinity treatments of 37.7 g/L or 136 g/L. It should also be noted that the R^2 value for all quadratic regressions were quite low (< 0.5). The results show that higher numbers of *Australocypris* spp. individuals in populations that had developed from the sediments in the 37.7g/L and 61.0 g/L Disturbance Phase salinity concentrations compared with the two higher Disturbance Phase salinity treatments. This may be attributed to this species being able to emerge in these salinity concentrations during the Disturbance Phase of the experiment, thus allowing extra time for these populations to grow. The results also show that the Recovery Phase salinity treatments had little effect on the population numbers of *Australocypris* spp. as there was little difference in the number of individuals in the populations that developed from the propagule bank across the different recovery salinity concentrations within a Disturbance Phase treatment.

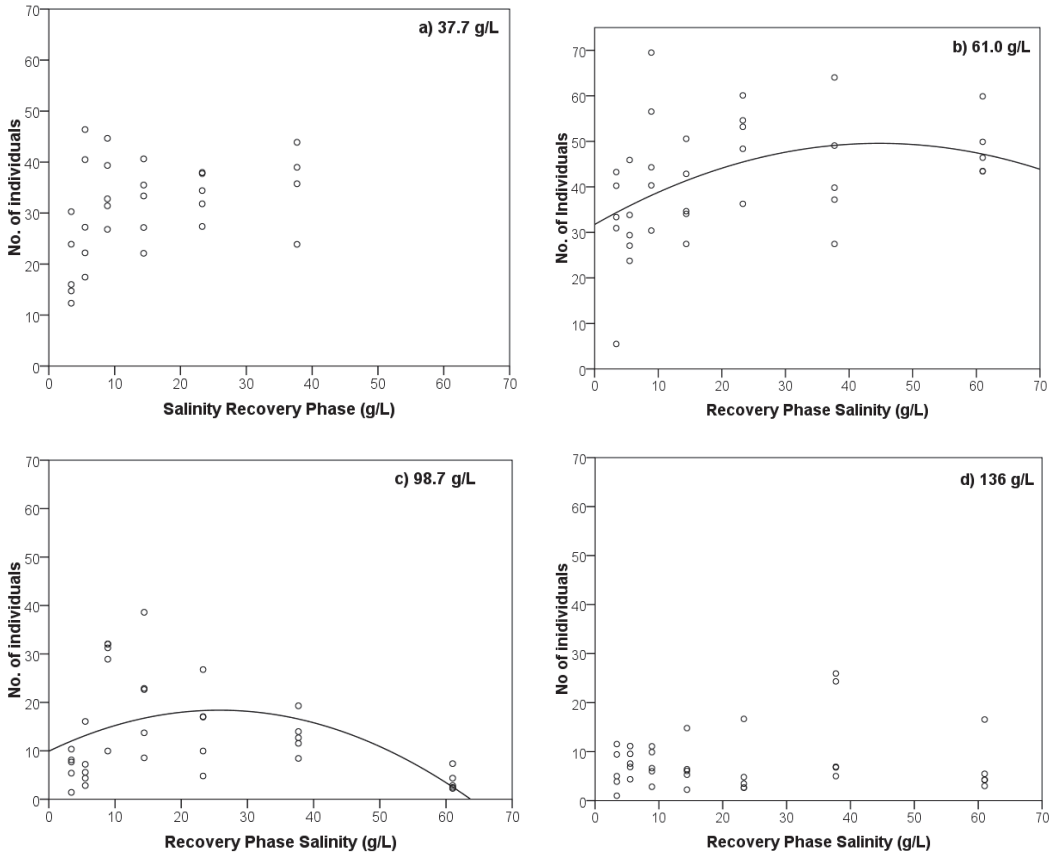


Figure 4.11 The effect of Recovery Phase salinity concentrations on the number of *Australocypris* spp. individuals that emerged from the sediments in each Disturbance Phase salinity treatment (n=5, square root transformation). Lines indicate where regressions were significant ($p < 0.05$).

Results of a two way ANOVA showed that there was a main effect of Disturbance Phase salinity concentration on the number of *Australocypris* spp. individuals ($p = <0.001$, $F = 132.585$, $df = 3, 120$). There was also a main effect of Recovery Phase salinity concentration on number of *Australocypris* spp.individuals ($p < 0.001$, $F = 7.532$, $df = 8, 120$) and a significant interaction of Disturbance Phase salinity and Recovery Phase salinity on the number of *Australocypris* spp.individuals ($p = 0.003$, $F = 2.364$, $df = 18, 120$). This indicates that not only did both the Disturbance and Recovery salinities affect the number of *Australocypris* spp.individuals, but that the effect of the Recovery Phase salinity concentratons was different depending on the Disturbance Phase salinity treatment the propagule bank was subjected to. *Post hoc* Tukey's tests indicated that there was a significant difference between all Disturbance Phase salinity treatments. There were also significant differences found between many of the Recovery Phase salinity treatments, please refer to Appendix 13.

4.4 Discussion

The abundant macrophytes, *Ruppia megacarpa* (Large Fruit Tassel), and *L. macropogon* (Stonewort) and two abundant invertebrate species *Mytilocypris henricae* (Chapman 1966) and *Australocypris* spp. (both ostracods) were present in the majority of replicates in the Recovery Phase salinity treatments ≤ 37.7 (threshold between 37.7 g/L and 61.0 g/L), regardless of the Disturbance Phase salinity concentrations these replicates were subjected to. This suggests that emergence of these species is not affected by high salinity disturbances experienced over a short period of time. In particular, *L. macropogon* and *M. henricae* were found in all replicates with a Recovery Phase salinity concentration ≤ 37.7 g/L and *Australocypris* spp. which was present in all replicates with a Recovery Phase salinity ≤ 61.0 g/L. These results correspond to the salinity threshold levels found for all three of these species in Chapter 3. The only species with a less uniform pattern of emergence (refer to Chapter 3), was the angiosperm *R. megacarpa* which was present in all replicates with Recovery Phase salinity treatments ≤ 14.4 g/L but only some replicates with a Recovery Phase salinity concentration of 23.3 g/L and 37.7 g/L (Figure 4.5).

4.4.1 Evidence for models predicting the effect of increased salinity concentrations on community change in the propagule bnak biota

Results of species presence or absence for each treatment indicated that the aquatic macrophyte and invertebrate communities have recovered in way that reflects the salinity threshold levels determined in Chapter 3 (salinity threshold of levels from 37.7 g/L to 61.0 g/L for *R. megacarpa, L. macropogon* and *M. henricae* and a salinity threshold of between 61.0 g/L and 98.7 g/L for *Australocypris* spp.). For all abundant species, with the exception of *R. megacarpa*, varying Disturbance Phase salinity concentrations had little effect on their germination in the Recovery Phase. The similarity in threshold levels shown in this experiment and those of Chapter 3 indicates that this aquatic community recovery followed a threshold model, rather than an alternative stable states model, a linear model, or a collapse model (Figure 4.1).

Results of other experimental studies have found that hysteresis is apparent in field data from Western Australia, where macrophyte dominated wetlands and benthic microbial mat dominated wetlands occur in the same salinities (Sim *et al.*, 2006c). Hysteresis is where systems show both alternative states at the same salinity level. For example, in this

current experiment some replicates had macrophyte dominated communities whilst others exposed to the same salinity had predominately phytoplankton communities (Scheffer and Carpenter, 2003). In this study however, hysteresis was not evident as all replicated exposed to the same salinity showed the same community, e.g. all treatments with a recovery concentration ≤ 37.7 g/L showed a macrophyte community (conisisting of *R. megacarpa* and *L. macropogon*) and contained two ostracod species (*M. henricae* and *Australocypris* spp).

Sim *et al.,* (2006c), suggested that other factors apart from salinity may be adding to the conflicting results reported in different studies. In particular, Sim *et al.,* (2006c) found that the way in which wetlands fill with water, the permanence of the watering regime and the nutrient load are important in determining the biota that establishes within a wetland. Sim *et al.,* (2006c) and Strelow *et al.,* (2005) also reported four differing 'regimes/states' being present in wetlands in south western, Western Australia (namely aquatic macrophyte dominated, clear water dominated, benthic microbial mat dominated and phytoplankton dominated), further complicating the model, if an alternative stable states system actually exists.

Scheffer and Carpenter (2003) state that the following factors are all experimental evidence for the existence of alternative stable states:

- different initial states leading to different final states
- disturbances triggering a shift to another permanent state
- or presence of hysteresis in response to changes back and forth between differing states

Taking the points above into account, the results of this current study show little evidence supporting the presence of hysteresis or alternative stable states. The higher salinity treatments did affect the number or growth of some species when recovery salinity concentrations were below 37.7 g/L. Communities in these treatments however still exhibited the characteristics of a submerged aquatic community and thus the clear water or algal bloom "state" did not persist in any of the recovery treatments of ≤ 37.7 g/L. Also, the differing initial (disturbance) salinity concentrations tested did not result in differing final states with all Recovery treatments at ≤ 37.7 g/L resulting in a macrophytes dominated system, and Recovery treatments set at ≥ 61.0 g/L resulted in a clear water system or algal bloom.

Whilst alternative stable states were suggested as a possible model describing how communities change from macrophyte dominated to benthic microbial mat dominated wetlands in Western Australia (Davis, 2002; Strehlow *et al.*, 2005; Sim *et al.*, 2006a; Sim *et al.*, 2006b; Sim *et al.*, 2006c), studies have stressed that there may be multiple influences from a number of stressors including salinity, watering regime and eutrophication affecting wetlands and thus simple models may not be very useful for predicting community change (Davis *et al.*, 2010). The results of this study and those of the previous chapter are in support of this multiple stresses model. This is particularly evident given that the shift between "states" in this current experiment and those described in the previous chapter are different to those reported from Western Australia (Davis, 2002; Strehlow *et al.*, 2005; Sim *et al.*, 2006a; Sim *et al.*, 2006b; Sim *et al.*, 2006c). Studies in Western Australia have noted the change from a macrophyte dominated wetland to a benthic microbial mat community with increased salinity (Davis, 2002; Strehlow *et al.*, 2005; Sim *et al.*, 2006a; Sim *et al.*, 2006b; Sim *et al.*, 2006c), in contrast to this study where a change from a macrophyte dominated to a phytoplankton dominated community was observed.

Whilst in terms of presence or absence of plant and invertebrate species responses to each treatment, the overall aquatic community was very similar to the results of Chapter 3. But individual species responses to Recovery salinity concentrations in terms of number of individuals, biomass, and production of reproductive structures were different. Number of individuals, biomass, and the production of reproductive structures were much lower in this study compared to results reported in Chapter 3.

4.4.2 Effect of disturbance and recovery salinities on germination and growth of individual aquatic macrophyte species

The various Disturbance Phase salinity treatments had a similar effect on the number of shoots or dry weight biomass for *R. megacarpa,* or the number of individuals or dry weight biomass of *L. macropogon*. It was only the Recovery Phase salinity treatments that appeared to affect the number of shoots and dry weight biomass for *R. megacarpa* and the number of individuals or dry weight biomass of *L. macropogon*.

These results indicate that both species are able to recover from hypersaline disturbance (e.g. 136 g/L) in a similar way to intermediate salinity disturbances of 37.7 g/L. This is despite the fact that germination had occurred in the Disturbance Phase of the experiment

in the 37.7 g/L Disturbance Phase salinity treatment, whereas no germination occurred in the Disturbance Phase salinity concentrations ≥ 61.0 g/L. This indicates that germination was suppressed by the high salinity but also demonstrates the resilience of both plant species as the propagules were not killed by the high salinity disturbance. This form of resilience by suppression of germination is common in wetland plants and not only allows some species to tolerate short periods of increased salinity by also other disturbances such as drying (Brock and Casanova, 1991: Brock and Britton, 1995; Brock and Rogers, 1998; Casanova and Brock, 1999; Leck and Brock, 2000; Skinner *et al.,* 2001; Brock *et al.,* 2003; Nicol *et al.,* 2003; Nielson *et al.,* 2003a; Capon and Brock 2006; Sim *et al.,* 2006a; 2006b; Nielson *et al.,* 2007; Porter, 2007; Roberton and James, 2007; Goodman *et al.,* 20011).

Goodman *et al.,* (2010), also conducted an experiment on four common wetland plants *Triglochin procerum* R. Brown (Water ribbon), *Myriophyllum simulans* Orchard (Milfoil), *Cotula coronopifolia* L. (Water buttons) and *Baumea arthrophylla* (Nees) Boeckeler (Fine twig sedge) investigating the effect of 3 and 6 week duration salinity disturbances (4 g/L and 8 g/L) on the survivorship and growth of adult plants. Results of Goodman *et al.,* (2010) study were also consistent with those presented here as all four species were able to survive short term exposure to high salinities. They also reported that these salinity disturbances could impact on growth and thus recovery of submerged plant species. Whilst lower dry weight biomass results were reported in this current study when compared to that of Chapter 3, it is hard to ascertain whether the lower biomass is a result of the high salinity concentrations or as result of lower ambient temperatures, as the experiments reported in the previous chapter were undertaken in summer whilst the Recovery Phase of this experiment occurred during the cooler autumn and winter months (Appendix 11). There have been a number of studies suggesting seasonality (and thus day length, temperature and rainfall) can affect not only plant growth but also influence the species that emerge from propagule banks (Britton and Brock, 1994; Casanova and Brock, 2000; Warwick and Brock, 2003). As such the effect of season on growth, as well as high salinity disturbances warrant further investigation.

4.4.3 Effect of disturbance and recovery salinities on aquatic macrophyte reproductive structures

It was difficult to determine the effects of Disturbance and Recovery Phase salinity treatments on the reproductive output of *R. megacarpa* in this experiment, as flowering in this species and many other aquatic angiosperms seems to be affected by other environmental factors such as water temperature. During this study there was a difference in the temperature and photoperiod that replicates were exposed to in Disturbance Phase when compared to the environmental conditions in Recovery Phase of the experiment, as shown by the minimum and maximum temperatures recorded throughout the experiment (Disturbance Phase air temperatures = 8.7 ^{o}C to 40.5 ^{o}C, Recovery Phase air temperatures = 5.3 ^{o}C to 35.1^{o}C, see Appendix 11). Therefore the result of flowering only occurring in the Disturbance Phase salinity treatment of 37.7 g/L needs to be treated with caution as flowering may have occurred in response to warmer temperatures. If the response shown here is due to an indirect effect of salinity in a lag in flowering rather than a direct result of salinity in reduced concentrations of flowering, this still has implications for management. Wetlands would need to be maintained at lower salinites during the warmer months to allow for maximum flowering for this species (*R. megacarpa*).

Increased salinity has been reported to cause delayed and reduced flowering in a number of plant species. Van Zant (2002) reported that increases in salinity concentrations above 4 g/L delayed flowering in *Iris hexagona* Walt. (Dixie iris) and that this effect of delayed flowering continues even after salinity concentrations are lowered. Sim *et al.,* (2006a); James *et al.,* (2003) and James *et al.,* (2009) also reported that plants exposed to treatments of ≤ 6 g/L flowered later than those in 0 g/L salinity treatments for *R. polycarpa*. In order for species to persist long term in wetlands they must be able to complete their life cycle and contribute viable propagules to the propagule bank. Thus any delay in germination or flowering can impact the long term viability of wetland species over the long term. Further investigation into the effect of Disturbance Phase salinities and time on the production of *R. megacarpa* flowers is needed.

Lamprothamnium macropogon individuals containing reproductive structures (antheridia) were only found in the lower Disturbance Phase salinity treatments of 37.7 g/L and 61.0 g/L with the exception of one replicate in 98.7 g/L, and one replicate in the 136 g/L Disturbance Phase salinity treatment which produced oogonia. Similar to the number of reproductive structures recorded for *R. megacarpa*, these results need to be

treated with caution as germination of this species in some replicates occurred late in the experiment (Figure 4.1). This is particularly relevant in the higher Disturbance Phase salinity concentrations of 98.7 g/L and 136 g/L. Therefore the lack of individuals containing reproductive spores in these treatments may not be due to Disturbance Phase salinity treatments, but because there was insufficient time for these individuals to mature and produce reproductive structures. Again if the response shown here is a result of an indirect effect of salinity as a lag in production of antheridia and oogonia rather than a direct effect of salinity in a reduced production of spores, this still has implications for management. Wetlands would need to be maintained at lower salinites during the warmer months to allow for maximum production of reproductive antheridia and oogonia for this species (*R. megacarpa*).

4.4.4 Effect of disturbance and recovery salinities on invertebrate emergence from the propagule bank

The two dominant invertebrate species exhibited complex responses to varying Disturbance and Recovery Phase salinity treatments in this experiment. The ostracod *M. henricae* had peak emergence recorded in the Disturbance Phase 37.7 g/L salinity treatment, which was lower than the threshold (between 37.7 g/L and 61.0 g/L) reported for this species (Chapter 3). The results also showed that fewer individuals were observed in the 61.0 g/L Disturbance Phase salinity treatment, with Disturbance Phase salinity concentrations of 98.7 g/L and 136 g/L recording more individuals emerging from the propagule bank after the Recovery Phase was complete. This may be due to competition between *M. henricae* and *Australocypris* spp., as *Australocypris* spp. emerged in the first phase of the experiment (Disturbance Phase salinity of 61.0 g/L), whereas *M. henricae* only emerged in the second phase of the experiment after the salinity concentrations had been lowered to 37.7 g/L. The number of individuals for *Australocypris* spp. was much higher in the 37.7 g/L and 61.0 g/L Disturbance Phase salinity treatments, which is probably because salinity concentrations were lower than the reported threshold (Chapter 3) (≥ 61.0 g/L) for this species thus allowing emergence to occur in the first phase of the experiment.

These results indicate that Disturbance Phase salinity concentrations did impact the recovery of these invertebrate species. The increased numbers in the lower concentrations of the Disturbance Phase (37.7 g/L and 61.0 g/L) however may be due to the length of

time available for species to emerge from the propagule bank rather than salinity. If the lag in emergence is due to elevated salinity concentrations, this may impact the species indirectly over time as individuals would have less time to reach reproductive maturity and may also be affected by a lack of available food caused by increased salinity concentrations (James *et al.*, 2003).

Many studies on the effects of salinity disturbances focus on freshwater communities; many field studies have indicated that salinity increases negatively impact the wetland biota (Brock and Lane, 1983; Brock and Sheil, 1983; Hart *et al.*, 1990; Hart *et al.*, 1991; Clunie *et al.*, 2002; James *et al.*, 2003; Nielsen *et al.*, 2003b). In contrast, studies on the effects of salinity pulses on the emergence of invertebrates from the propagule bank have found the reverse, i.e. salinity pulses increase the invertebrates abundance (Nielsen *et al.*, 2007). Neilsen *et al.,* (2007) suggested that these field studies may be including invertebrate species that drift into wetlands or streams, whereas the propagule bank studies only measure those invertebrates that emerge from the sediments. Further study is also required in this area.

Overall, results of this study show that the plant and invertebrate communities of Lake Cullen recover from high salinity disturbances in a very similar way to that described in Chapter 3. Threshold concentrations established for each individual species in the previous chapter were consistent to those of this study, with *R. megacarpa, L. macropogon,* and *M. henricae* all germinating or emerging from the propagule bank in salinities of ≤ 37.7 g/L and *Australocypris* spp. emerging in salinities of ≤ 61.0 g/L.

5.0 The effects of environmental conditions on the germination of *Ruppia megacarpa* seeds

5.1 Introduction

The germination requirements of seeds vary considerably depending on the species, and different environmental factors that can inhibit or promote germination. Factors known to affect germination of wetland plants include: physiological responses e.g. seed coat breakage or age of the seed, and environmental factors such as water temperature, salinity, as well as the influence of photoperiod at the time of germination, or between seed maturity and germination (Crowther and Hynes, 1977; Baskin and Baskin, 1998; Casanova and Brock, 2000; Taton *et al.*, 2006)

Field studies of wetland plant germination from the propagule bank have focused on large propagule bank studies where environmental factors such as temperature and photoperiod are difficult to control. Even when experiments are conducted in glasshouses often temperature and photoperiod will vary throughout the experiment especially in long term studies such as those described in Chapters 3 and 4. Optimal temperature or photoperiod conditions for the germination of individual plant species are hard to determine from these studies. There have been few highly controlled germination studies conducted to investigate the impact of environmental factors on the germination of *Ruppia* species. The most notable studies are those conducted by Brock (1982a) and Vollenberg and Congdon (1986).

Brock (1982a) conducted a series of experiments to investigate the effects of cold pre-treatment, salinity, seed coat breakage and photoperiod on the germination of *Ruppia megacarpa* R. Mason (Large-Fruit Widgeon Grass) and *Ruppia tuberosa* J.S. Davis and Toml. (Tuberous Tassel). This study found that cold pre-treatment had no effect on the germination of either species. Additionally it found that mechanical seed coat breakage had no affect on the germination of *R. megacarpa*. Germination of *R. tuberosa* however was positively affected by scarification of the seed coat and exposure to wetting and drying events. A higher percentage of seeds germinated when they had undergone seed coat scarification as well as being exposed to wetting and drying events when compared to scarification of seed coat alone (Brock, 1982a). These results suggest that in the field this species will respond with a higher germination rate when flooding and drying events occur in ephemeral aquatic habitats, and seeds are subjected to scarification.

Brock (1982a) found that changes in salinity had different effects on germination in *R. megacarpa* and *R. tuberosa*. Increased germination rates for *R. megacarpa* were found in the lowest salinity treatment (0 g/L), whereas increased germination rates for *R. tuberosa* were found in the highest salinity treatment (32 g/L). This corresponds to the environments that these two species are found, as *R. megacarpa* is often distributed in fresher conditions than *R. tuberosa*. Additionally Brock (1982a) found that photoperiod had little affect on the germination of *R. tuberosa*, but a shorter photoperiod of 8 hours light/16 hours dark was optimal for the germination of *R. megacarpa* when compared to the other photoperiod treatments (16 hours light/ 8 hours dark; 24 hours light/0 hours dark and 0 hours light/24 hours dark).

An experiment investigating the effect of an after ripening time period on the germination of *R. megacarpa, R. tuberosa* and *Ruppia polycarpa* R. Mason (Many-Fruit Widgeon Grass) was also conducted by Brock (1982a). Mature seeds were collected in the field to ensure the seeds were all the same age. These seeds were placed in four different treatments: distilled water (0 g/L), saline solution (19 g/L), saline solution with lake substrate (19 g/L), and ephemeral conditions. Seeds were stored in lake water and maintained at 18^{o}C or kept outdoors under normal diurnal temperature and light conditions. Seed germination was then monitored over an 18 month period. Results of this study found that germination was recorded in the first growing season for all species. In the second growing season a larger number of *R. megacarpa* seeds germinated than in the initial growing season suggesting that an after ripening period had a positive effect on seed germination of this species. The opposite was found for *R. tuberosa* and *R. polycarpa* with maximum number of seeds germinating in the first growing season (Brock, 1982a).

Seeds without substrate germinated in distilled water but not in the saline solution (Brock, 1982a). In the second growing season, seeds with substrate under saline conditions did germinate but only after seasonal rains diluted the overlying water suggesting that *R. megacarpa* can germinate with or without substrate, but only when salinity concentrations are reduced.

Another germination study by Vollenbergh and Congdon (1986) investigated the effects of salinity and temperature on the germination of macrophytes including *R. polycarpa*

Results of this study found that *R. polycarpa* had increased germination rates at lower salinities (0 to 35 g/L) when compared to the higher salinity concentrations (78.8 g/L and 157.5 g/L). Vollenbergh and Congdon (1986) also found that soaking *R. polycarpa* seeds in a 157.5 g/L salt concentration for 6 days before returning the seeds to freshwater had little effect on the germination success. Temperatures from 10^{o}C to 30^{o}C had little affect on the number of *R. polycarpa* seeds germinating.

5.1.1 Hypotheses

Previous studies show that favourable environmental conditions for successful germination can vary within a genus, and therefore are species specific (Crowther and Hynes, 1977; Brock, 1982b; Brock, 1982a; Vollebergh and Congdon, 1986; Casanova and Brock, 2000; Taton *et al.*, 2006). These studies also indicated that environmental conditions can impact the germination success of a species. The purpose of the following investigation is to build on the findings of Brock (1982a) and to test the following hypotheses:

- What effects do changes environmental conditions such as the presence of substrate, temperature and photoperiod have on the germination success of *R. megacarpa*?
- Do *R. megacarpa* seeds from different locations have varying germination success?

5.2 Methods

5.2.1 The effect of environmental variables on the germination of *Ruppia megacarpa* seeds.

The germination of *R. megacarpa* seeds was tested over a four week period to determine if the location of the seed source or the presence or absence of lake substrate affected germination success. Two different salinities were tested to determine which was optimal for *R. megacarpa* seed germination as results from Chapter 3 and Sim *et al.*, (2006a) suggest that *Ruppia spp.* may require slightly elevated saline conditions for optimal germination rates. Seeds were collected from the dry bed of Lake Cullen, Golf Course Lake and Lake Wandella in November 2006. Lake substrate, with seeds was collected and stored in 20 L dry plastic containers, transported back to the laboratory where the seeds were removed from the sediment. Seeds were removed from the sediment by hand as they are easy to see. Average *R. megacarpa* seed size is 3mm (Brock, 1982). Twelve treatments were set up with the following as variables: location (Lakes Cullen, Golf Course or Wandella), substrate (present or absent) and salinity (1.4 g/L or 3.4 g/L), (Table 5.1).

Table 5.1 Treatments testing the effect of locality, substrate presence and salinity on germination success of *Ruppia megacarpa.*

Treatment No.	Location	Substrate	Salinity (g/L)
1	Lake Cullen	Present	1.4
2	Lake Cullen	Absent	1.4
3	Lake Cullen	Present	3.4
4	Lake Cullen	Absent	3.4
5	Golf Course Lake	Present	1.4
6	Golf Course Lake	Absent	1.4
7	Golf Course Lake	Present	3.4
8	Golf Course Lake	Absent	3.4
9	Lake Wandella	Present	1.4
10	Lake Wandella	Absent	1.4
11	Lake Wandella	Present	3.4
12	Lake Wandella	Absent	3.4

Sixty 500 mL plastic sample jars were used, with five replicates for each of the 12 treatments. A total of 150 *R. megacarpa* seeds were used for each treatment so each replicate contained 30 *R. megacarpa* seeds. For treatments without substrate the seeds were placed directly in the jar. For treatments with substrate, 2 cm of sediment (with all seeds removed) was placed in the bottom of the jar and seeds were placed on the substrate surface. Tap water was used to make up the required salinity concentration by adding

"Ocean Nature" sea salt and checking the salinity (g/L) using a salinity meter (Orion Multimeter model 1230). A solution of the appropriate salinity concentration was added to the sample jars to a level of 7 cm (Figure 5.1). Salinity was checked again after 24 hours to determine if salt already present in the substrate had dissolved. If necessary the solution in the sample jars was diluted to the required salinity and surplus solution was syphoned off to maintain a depth of 7 cm.

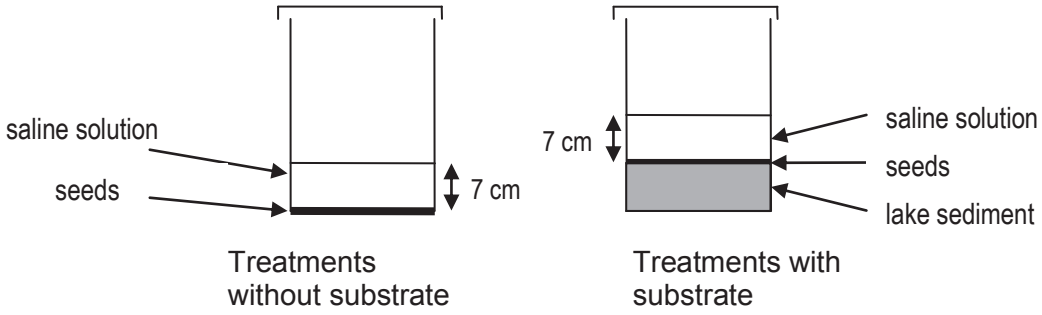


Figure 5.1 Set up of sample jars in pilot germination experiments

Sample jars were placed randomly in a germination cabinet (Appendix 14), with photoperiod set to 14 hours light/10 hours darkness and an ambient temperature of 30^{o} C. The sample jars were checked at the same time each day over a 4 week period. The number of germinants were recorded and then removed.

5.2.2 The effect of photoperiod on the germination of *Ruppia megacarpa* seeds

A second study investigated the effect of photoperiod on *R. megacarpa* seed collected from the dry bed of Lake Wandella, in November 2006. Seeds were collected with lake substrate, stored dry in plastic containers and transported back to the laboratory where all seeds were removed from the sediment. Three photoperiod treatments were tested: 14 hours light/10 hours dark, 12 hours light/12 hours dark, and 10 hours light/14 hours dark. These photoperiods approximate the seasonal changes in photoperiod recorded in the Kerang region of northern Victoria which is the nearest location to the seed source for these experiments (Geoscience Australia, 2013).

Fifteen 500 mL plastic sample jars were used, with five replicates for each of the 3 treatments. A total of 150 *R. megacarpa* seeds were used for each treatment and each sample jar contained 30 *R. megacarpa* seeds. Each sample jar was set up with lake

substrate, seeds and a saline solution (3.4 g/L concentration), as described in Methods 5.2.1.

Each treatment was carried out one at a time; the order of which the treatments were conducted was determined using random numbers (Appendix 15). The position of the jars within each germination cabinet was also determined using random numbers (Appendix 16). The temperature of the germination cabinet was 30^{o}C for all three treatments. Sample jars were left in the cabinets for four weeks and were checked at the same time each day for this period. The number of germinants was recorded and then the germinants were removed.

5.2.3 The affect of temperature on the germination of *Ruppia megacarpa* seeds

A third study tested the effect of temperature on germination of *R. megacarpa* seeds collected from the dry bed of Lake Wandella, in November 2006. Seeds were collected with the lake substrate, stored dry in plastic containers and transported back to the laboratory where the seeds were removed from the lake sediment. Two temperature treatments (25^{o}C and 30^{o}C) were applied which are typical ambient daytime air temperatures recorded in the Kerang region (Bailey *et al.*, 2006).

Ten 500 mL plastic sample jars were used, with five jars for each of the two treatments. A total of 150 *R. megacarpa* seeds were used for each treatment with each replicate containing 30 *R. megacarpa* seeds. Each sample jar was set up with lake substrate, seeds and a saline solution (3.4 g/L), as described in Methods 5.2.1.

Each treatment was carried out one at a time; the order that the treatments were conducted was determined using random numbers (Appendix 15). The position of the jars within each germination cabinet was also determined using random numbers (Appendix 17). The photoperiod in the germination cabinet was 14 hours light and 10 hours dark for both treatments. Samples were checked at the same time each day, for a four week period with the number of germinants was recorded and then the germinants were removed.

5.2.4 Data Analysis

Results were analysed using either one way ANOVA or where there were only two treatments, independent T-tests, to determine if there was any significant difference ($p < 0.05$) between treatments. The ANOVA model tested was as follows

DV = constant + treatment (either presence of substrate, location of seed source, temperature or photoperiod)

Where results of the one way ANOVA's were significant, *post hoc* Tukey's tests were conducted to determine which treatments were significantly different. All statistical tests were conducted using the PASW 18 (previously known as SPSS statistics) software package. Square root transformations of the dependent variable were undertaken when the data from this experiment did not meet the assumptions of normality or homogeneity of variances for parametric tests (Levene's test).

5.3 Results

5.3.1 The effect of environmental variables on the germination of *Ruppia megacarpa* seeds

There was little difference in the germination success across the three different seed sources (Figure 5.2, Table 5.2). Results of a one-way ANOVA showed that the only treatment to show any significant difference ($p < 0.05$) in mean number of germinated seeds for the different seed sources was the substrate present, 3.4 g/L treatment (Table 5.2). Results of a *post hoc* Tukey's test showed that there was only a significant difference between the mean number of *R. megacarpa* seeds germinated between Lake Cullen and Lake Wandella in the 3.4 g/L with substrate present treatment (Table 5.3).

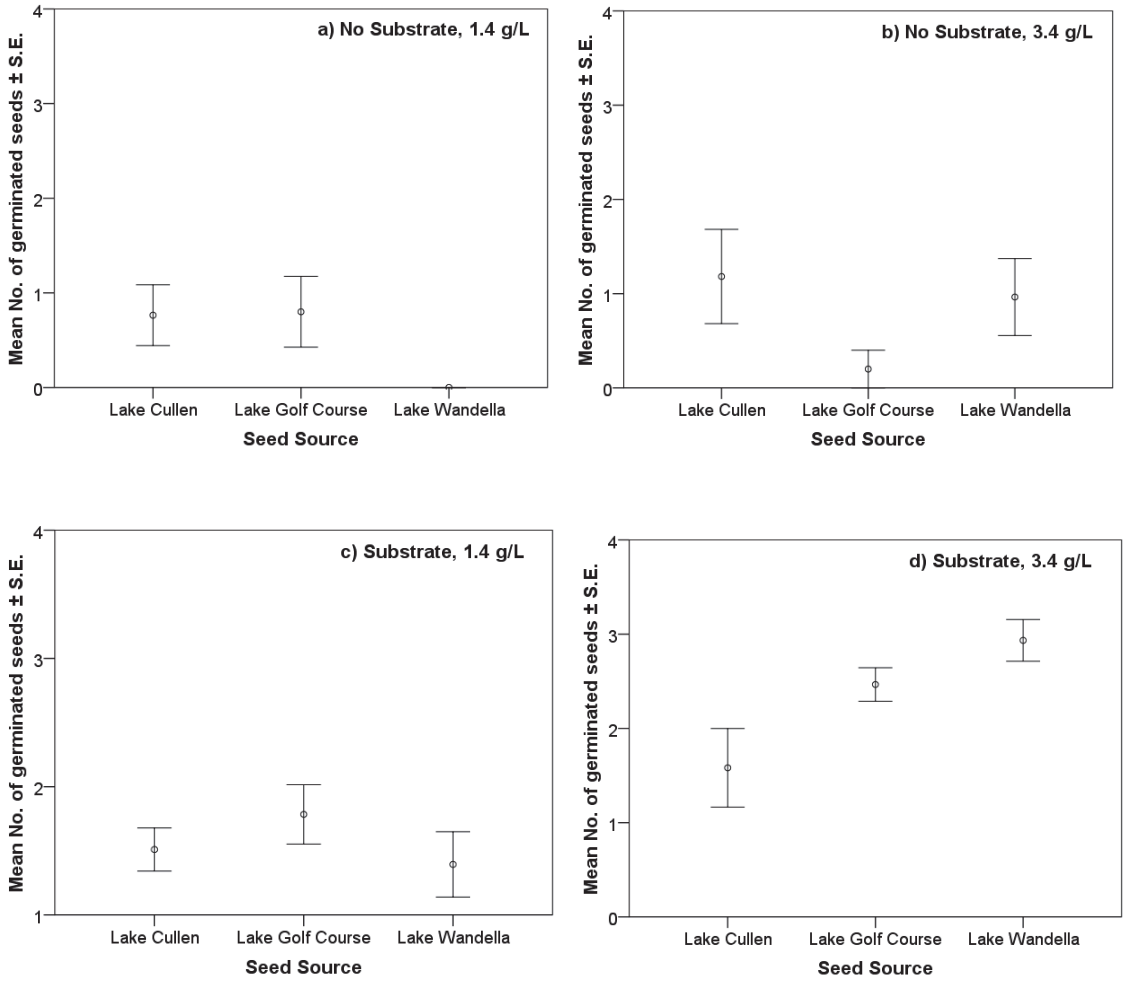


Figure 5.2 The effect of different seed sources on the germination rate for *Ruppia megacarpa* seeds in **a)** no substrate 1.4 g/L, **b)** no substrate 3.4 g/L, **c)** substrate 1.4 g/L, **d)** substrate 3.4 g/L treatments (n=5, square root transformed)

Table 5.2 Results of One Way ANOVA on the effect of seed source on the germination rate of *R. megacarpa* seeds. * Indicates significance p < 0.05 level

Treatment				
Substrate	**Salinity (g/L)**	**F**	**df**	**P**
Absent	1.4	2.522	2,12	0.122
Absent	3.4	1.747	2,12	0.216
Present	1.4	0.820	2,12	0.464
Present	3.4	5.557	2,12	**0.020***

Table 5.3 Results of *post hoc* Tukey's tests for the substrate 3.4 g/L salinity treatment. * Indicates significance p <0.05 level

	Lake Cullen	**Lake Golf Course**	**Lake Wandella**
Lake Cullen			
Lake Golf Course	0.122		
Lake Wandella	**0.017***	0.511	

Germination success for *R. megacarpa* seeds varied considerably between individual treatments tested, the lowest mean germination success was 0 (± 0 S.E), a no substrate with 1.4 g/L salinity treatment from Lake Wandella, while the highest mean germination success 8.8 (± 1.4 S.E).from Lake Wandella with substrate with a salinity of 3.4 g/L. There was greater germination success of *R. megacarpa* seeds in the treatments with substrate present. Similarly, the treatments with higher salinity (3.4 g/L) had more seeds germinate than those at a salinity of 1.4 g/L. The exception was the no substrate with a salinity of 3.4 g/L treatment from Lake Golf Course with mean germination success of 0.2 (± 0.2 S.E) seeds compared the no substrate in 1.4 g/L salinity with mean 1.2 (± 0.7 S.E) seeds (Figure 5.3).

Results of a two-way ANOVA show that there is a significant main effect of salinity ($p = 0.011$, $F = 6.936$, df 1, 56) and a significant main effect of the presence of substrate on the germination of *R. megacarpa* seeds ($p < 0.001$, $F = 44.144$, df 1, 56). There was no significant intereaction effect of salinity and presence of substrate ($p = 0.201$). Results of independent t-tests showed that the presence of sediment significantly increased ($p < 0.05$) the mean number of *R. megacarpa* seeds germinating in the 1.4 g/L and 3.4 g/L salinity treatments from Lake Wandella, and in the 3.4 g/L treatments of seeds collected from Lake Golf Course. Further independent t-tests showed that the presence of substrate significantly increased the mean number of *R. megacarpa* seeds germinated ($p < 0.10$) in the 1.4 g/L salinity treatments in from Lake Cullen and Lake Golf Course. The only treatment that did not show a significant increase in germination with the presence of

substrate ($p > 0.10$) was the 3.4 g/L salinity treatment from Lake Cullen (Tables 5.4a, 5.5a and 5.6a). Results of independent t-tests showed that an increase in salinity from 1.4 g/L to 3.4 g/L significantly increased ($p < 0.05$) the mean number of *R. megacarpa* seeds germinated in the substrate and no substrate treatments from Lake Wandella, and the substrate treatment from Lake Golf Course (Tables 5.4b, 5.5b, and 5.6b).

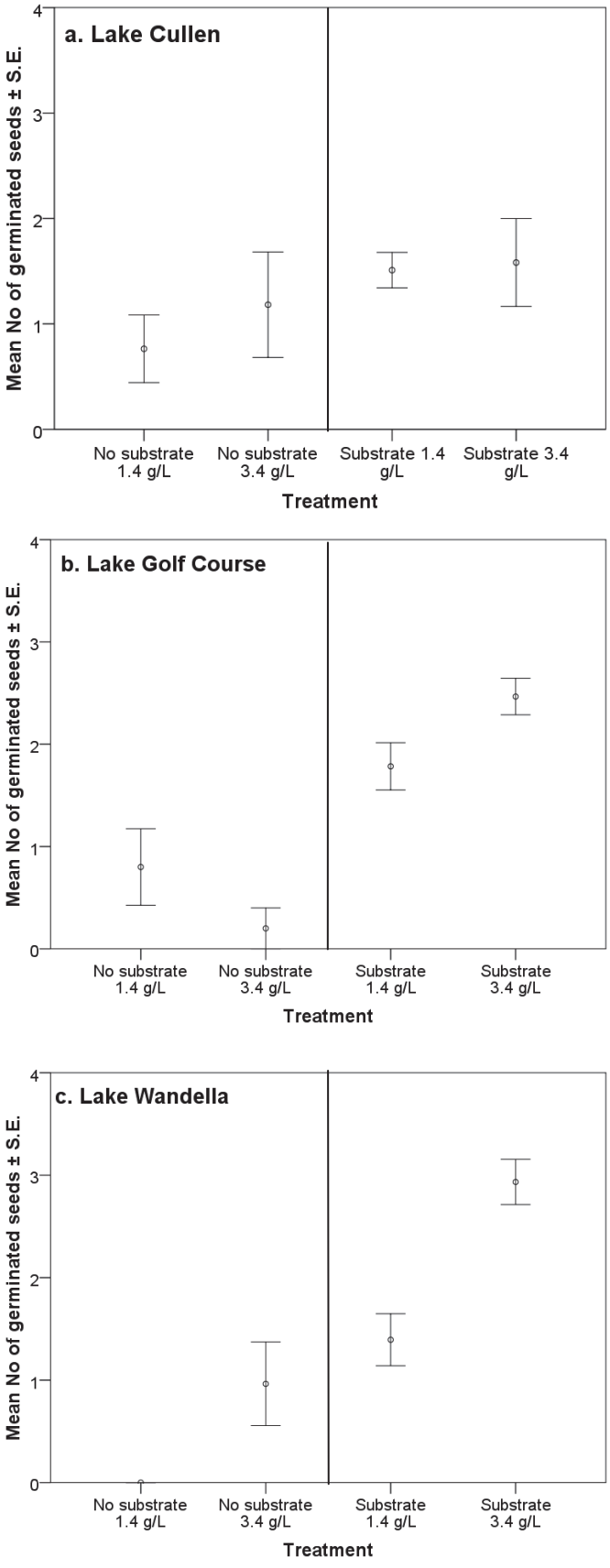


Figure 5.3 Effect of the presence of lake substrate and salinity on the germination of *Ruppia megacarpa* seeds from **a.** Lake Cullen, **b.** Lake Golf Course and **c.** Lake Wandella (n=5, square root transformed)

Table 5.4 t-test results for Lake Cullen comparing **a.** effect of substrate and **b.** effect of salinity on the rate of germination in *Ruppia megacarpa* seeds. * Indicates significance p < 0.10.

a. Effect of substrate

Substrate	Present 1.4 g/L	Present 3.4 g/L
Absent 1.4 g/L	**0.074***	
Absent 3.4 g/L		0.556

b. Effect of salinity

Substrate	Absent 3.4 g/L	Present 3.4 g/L
Absent 1.4 g/L	0.502	
Present 1.4 g/L		0.877

Table 5.5 t-test results for Lake Golf Course comparing **a.** effect of substrate and **b.** effect of salinity on the rate of germination in *Ruppia megacarpa* seeds * Indicates significance to the p < 0.10. ** Indicates significance p <0.05.

a. Effect of substrate

Substrate	Present 1.4 g/L	Present 3.4 g/L
Absent 1.4 g/L	**0.056***	
Absent 3.4 g/L		**0.048****

b. Effect of salinity

Substrate	Absent 3.4 g/L	Present 3.4 g/L
Absent 1.4 g/L	0.195	
Present 1.4 g/L		**>0.001****

Table 5.6 t-test results for Lake Wandella comparing **a.** effect of substrate and **b.** effect of salinity on the rate of germination in *Ruppia megacarpa* seeds. ** Indicates significance p < 0.05.

a. Effect of substrate

Substrate	Present 1.4 g/L	Present 3.4 g/L
Absent 1.4 g/L	**0.001****	
Absent 3.4 g/L		**0.003****

b. Effect of salinity

Substrate	Absent 3.4 g/L	Present 3.4 g/L
Absent 1.4 g/L	**0.046****	
Present 1.4 g/L		**0.002****

5.3.2 The effect of photoperiod on *Ruppia megacarpa* seed germination

The results indicate that germination success between the 14 hour light/10 hour dark and 12 hour light/12 hour dark photoperiod treatments were very similar, with mean of 8.8 (± 1.4 S.E.) and 8.0 (± 1.7 S.E) seeds germinating in these treatments respectively. The third photoperiod treatment of 14 hours light/ 10 hours dark had lower germination success with mean 4.0 (± 1.6 S.E) seeds germinating over the four week period (Table 5.7).

Table 5.7 The total, percentage and mean number of *Ruppia megacarpa* seeds germinated for each photoperiod treatment

Seed source	Photoperiod	No of seeds germinated	% of seeds germinated	Mean number of germinated seeds (± S.E)
Lake Wandella	14 hours light/10 hours dark	44	29.3%	8.8 (± 1.4)
	12 hours light/12 hours dark	40	26.7%	8.0 (± 1.7)
	10 hours light/14hours dark	20	13.3%	4.0 (± 1.6)

Results of a one-way ANOVA found no significant difference ($p < 0.05$) between the results, even though there was a reduction in the number of germinants in the 10 light/14 hours dark treatment.

5.3.3 The effect of temperature on *Ruppia megacarpa* seed germination

The germination of *R. megacarpa* was very different in the two temperature treatments with mean 3.11 (± 1.4 S.E) seeds germinating at 30°C and a mean of 0.55 (± 0.2 S.E) seeds germinating at 25°C (Table 5.8).

Table 5.8 The total, percentage and mean number of germinated *Ruppia megacarpa* seeds from a total of 150 seeds, at two different temperatures

Seed Source	Temperature (°C)	Number of germinated seeds	% of germinated seeds	Mean number of germinated seeds (± S.E)
Lake Wandella	30	44	29.3	3.11 (± 1.4)
	25	13	8.7	0.55 (± 0.2)

Results of an independent t-test showed that there was a significant difference ($p < 0.05$) between the two temperatures, indicating that germination of *R. megacarpa* is significantly reduced at 25°C.

5.4 Discussion

Results show that there was a low germination of *R. megacarpa* seeds across all treatments with maximum germination success of less than 30%. Brock (1982a) also reported germination rates in both *R. megacarpa* and *R. tuberosa* of less than 30%. Brock (1982a) found that *R. megacarpa* seeds required an after ripening period because not all seeds germinated within one growth cycle or wetting phase period. This could explain the lower germination success found in other *Ruppia* studies (Brock, 1982a; Vollebergh and Congdon, 1986). Low germination success could also be due to non-viable seeds within the propagule bank.

Results from *R. megacarpa* germination studies demonstrate that differing environmental conditions can affect germination. The findings of this study show that:

1. Seed source location had little effect on the germination success of *Ruppia megacarpa* seeds

Overall there was no significant difference in seed viability regardless of the seed source location in this study. Results of this study indicated that generally there was no significant difference in the germination success between seeds sourced from different locations. The exception was one treatment (3.4 g/L salinity with substrate present) with a significant difference between Lake Wandella and Golf Course Lake ($p < 0.05$).

2. The presence of substrate increases the germination success of *Ruppia megacarpa* seeds

In most seed source locality and salinity treatments the presence of substrate increased *R. megacarpa* seed germination success ($p < 0.10$). One exception was the Lake Cullen 3.4 g/L salinity treatment where no significant difference was found between number of germinants from the substrate and no substrate treatments. Whilst these results were not significant there was a trend to suggest that there was an increase in the number of seeds germinating in the presence of substrate.

A possible reasons why the germination success of *R. megacarpa* seeds is increased with the presence of substrate is that lake substrates may promote the germination of seeds through a mycorrhizal association, similar to that seen in orchids (Stewart and Kane,

2007). Whilst this relationship is not common in wetland plants, mycorrhizal associations have been found in some submerged and emergent aquatic macrophyte species (Khan and Belik, 1995; Cooke and Lefor, 1998; Ingham and Wilson, 1999). Zhongqiang *et al.,* (2005) found that there was a significant increase in the number of seeds germinated in three species of *Vallisneria* (Eelgrass) in the presence of freshwater microalgae. The association between the presence of substrate and the increase in germination of *R. megacarpa* seeds needs further investigation.

3. Photoperiod had little effect on the germination success of *Ruppia megacarpa* seeds

No significant difference was found between the germination success of *R. megacarpa* seeds exposed to three different photoperiod regimes. There was however a lower germination recorded in the 10 hours light/14 hours dark treatment (mean 4 ± 1.6 S.E) compared to the 12 hour light/12 hour dark and 14 hour light and/10 hour dark treatments (mean 8.8 ± 1.4 S.E and 8.0 ± 1.7 S.E. respectively). Results suggest that more seeds germinate when exposed to photoperiods with a longer light phase. Brock (1982a), reported a different pattern, with higher germination success between 24% and 30% at a photoperiod of 8 hours light/16 hours dark compared to germination success between 18% and 20 % in the 16 hours light/8 hours dark photoperiod treatment. It should be noted that whilst an increased number of seeds germinating was found in the 8 hours light/16 hours dark photoperiod treatment, no significant difference was found between the two treatments. This present study indicates that *R. megacarpa* seed germination increased in longer light photoperiods, but these results need to be treated with caution as neither study demonstrates conclusively that photoperiod has an effect on germination success. Photoperiod could be a less important factor as day length not only varies from year to year, but also day to day depending on cloud cover. Also aquatic vegetation can experience shadowing from riparian vegetation or other aquatic plants. Brock (1982a) also reported that a number of seeds remain viable in the propagule bank and do not germinate until the following year. These seeds would experience a range of photoperiods throughout the year. Further study is warranted to determine the effects of varying photoperiods on the germination success of *R. megacarpa* with confidence.

4. Increased temperature, increased the germination success of *Ruppia megacarpa* seeds

There was a significant difference in the number of *R. megacarpa* germinants observed in the two different temperature treatments with increased germination success at 30^{o}C. There have been no previous studies on the effect of water temperature on the germination of *R. megacarpa* seeds. This higher temperature however, does correspond with mean maximum summer air temperature (31.8^{o}C) in the Kerang region as it is likely that high water tmepreatures would occur in these wetlands over summer (Bailey *et al.*, 2006).

Studies on other species of submerged macrophytes have demonstrated the effect of temperature on germination success. Temperatures of above 15^{o}C resulted in increased germination success in *Myriophyllum spicatum* L. (Water Milfoil) seeds and temperatures of above 20^{o}C resulted in increased germination success in *Potamogeton malaianus* Miq. (Pondweed) seeds (Xiao *et al.*, 2010). Other species studied include *Hydrilla verticillata* (L.f) Royle (Water Thyme) with optimal germination success at 28^{o}C (Lal and Gopal, 1993), *Vallisneria americana* Michx. (American Eelgrass) with an optimal germination success at 22^{o}C (Jarvis and Moore, 2008) and *Vallisneria natins* (Lou) H. Hara with optimal germination at 25^{o}C (Ke and Li, 2006). It is thought that temperature triggers germination and may enable plants to begin growth under conditions supporting optimal growth (Xiao *et al.*, 2010).

Given the low rates of *R. megacarpa* seed germination it is important that further studies investigating the effects of salinity are conducted in optimal environmental conditions. The results of this study indicate that seed source location has no effect on germination success in *R. megacarpa*. This suggests that seeds are fairly homogenous across the different wetlands in the region. Photoperiod was also found to have no significant effect on the germination success of *R. megacarpa*. The presence of substrate and a temperature of 30^{o}C did significantly affect germination success *in R. megacarpa*.

6.0 The effect of salinity and desiccation disturbances on germination success of *Ruppia megacarpa*

6.1 Introduction

The germination requirements of seeds is species specific and environmental factors can either inhibit or promote germination in wetland plants (Brock, 1982a; Britton and Brock, 1994; Baskin and Baskin, 1998; Casanova and Brock, 2000; Kahn and Durako, 2005; Taton *et al.*, 2006; Cho and Sanders, 2009; Goodman *et al.*, 2010). Salinity and drying periods are two such environmental factors that can affect a wetland plant's germination success (Cho and Sanders, 2009). Many studies have shown that flood frequency and timing, depth, and duration can strongly influence species composition in wetland plant communities (Casanova and Brock, 2000; Nicol *et al.*, 2003; Porter *et al.*, 2007). Drying periods after floods can break dormancy and thus increase germination in some species growing in ephemeral habitats during the next wetting phase (Bonis *et al.*, 1995; Casanova and Brock, 1996). In some species however this is not the case and macrophytes that grow in these temporary habitats must at least have seeds or propagules that are able to survive periods of desiccation between flooding events. This survival trait is essiential for macrophyte establishment in wetlands in northwestern Victoria as water availability is becoming an increasingly important issue due to drought and climate change. These factors have resulted in a number of permanently inundated wetlands drying out and becoming temporary habitats (as discussed in Chapter 2).

Many studies of temporary wetlands have investigated how propagule banks contribute to the plant and invertebrate communities that become established after dry substrates are rehydrated by floods (Brock and Lane, 1983; Casanova and Brock, 1990; Brock and Casanova, 1991; Brock and Britton, 1995; Brock, 1997; Casanova and Brock, 2000; Nielsen *et al.*, 2002; Brock *et al.*, 2003; Sim *et al.*, 2006a). Some of these studies, together with past germination studies and investigations discussed in Chapter 4 have shown that *Ruppia megacarpa* R. Mason (Large-Fruit Widgeon Grass), and many other species of *Ruppia,* are able to tolerate periods of desiccation (Brock, 1982a; Brock, 1982b; Sim *et al.*, 2006a). But the effect of desiccation on propagule bank viability and germination success is not as well understood. In some cases researchers are unsure whether drying enhances or inhibits germination success in *Ruppia* species. Koch and Seeliger (1988) found that drying enhanced the germination of *Ruppia* seeds collected

from an ephemeral habitat. In contrast, seed viability tests (Tetrezolium tests) conducted by Cho and Sanders (2009) found that drying greatly reduced seed viability when comparing *Ruppia maritima* L. (Widgeon Grass) seeds dried over a ten month period with seeds that had just been deposited.

Many wetland propagule bank and germination studies have also investigated the effect of salinity on various macrophytes species (Galinato and Van Der Valk, 1986; Salter *et al.*, 2010; Goodman *et al.*, 2011). Results from the propagule bank studies discussed in Chapters 3 and 4 indicated that *R. megacarpa* seeds are able to germinate in salinities of ≥ 37.7 g/L. These findings concur with propagule bank studies conducted by Sim *et al.,* (2006a) who reported that *Ruppia polycarpa* R. Mason (Many-fruit widgeon grass) was able to germinate in salinities ≤ 45 g/L and that seeds of *R. megacarpa* germinated in salinities ≤ 30 g/L. Vollenbergh and Congdon (1986) found that the optimal salinity range for the germination of *R. polycarpa* seeds was 0 g/L to 35 g/L.

It is important to note that different species within the same genus can have varied responses to salinity. Brock (1982a) found that the germination success of *R. megacarpa* seeds increased in freshwater (0 g/L) whereas for *Ruppia tuberosa* J.S. Davis and Toml. (Tuberous Tassle) germination success was greater in higher salinity concentrations (42 g/L). This reflects the environments that these two species are distributed in, as *R. megacarpa* is often found under fresher conditions that *R. tuberosa* (Brock, 1982a).

Few controlled studies have focused on the effect of decreasing salinities after a period of high salinity disturbance, on the germination success of wetland plants. Results from the propagule bank studies discussed in Chapter 4 suggest that higher salinity concentrations had little affect on the germination success of *R. megacarpa* seeds, once they had been returned to the recovery phase. Vollenberg and Congdon (1986) reported similar results for
R. polycarpa seeds soaked in high salt solution (up to 157.5 g/L) for 6 days before returning the seeds to freshwater (0 g/L).

6.1.1 Hypotheses

The purpose of this study is to further build on the findings of Chapters 3, 4 and 5 and to look specifically test the following hypotheses

- Does a drying period affect the germination success of *R. megacarpa*?
- Does the combination of a drying period and decreasing salinity, post exposure to high salinity affect the germination success of *R. megacarpa*?

6.2 Methods

The effect of drying and lowering of salinity concentrations on *Ruppia megacarpa* seed germination was tested over a 50 day period. Seeds were collected from the dry bed of Lake Wandella in November 2006, stored dry in plastic containers and transported back to the laboratory where they were removed from the substrate. Eleven treatments were selected to test the effects of the following parameters on *R. megacarpa* germination success (Table 6.1) namely the effects of:

- desiccation
- reducing salinity concentrations
- desiccation as well as reducing salinity concentrations

This 50 day period was divided into two phases (Phase 1 and 2), each of 25 days in length, with an extra two days (dry phase) between each phase for treatments subjected to desiccation.

Table 6.1 Description of treatments used to test germination success of *Ruppia megacarpa* seeds collected from Lake Wandella

Treatment Type	Phase 1 (25 days) Salinity (g/L)	Dry Phase (2 days)	Phase 2 (25 days) Salinity (g/L)
Controls, no change in salinity, no desiccation period	10	N/A	10
	30	N/A	30
	50	N/A	50
No change in salinity, desiccation period	10	Dry	10
	30	Dry	30
	50	Dry	50
Reducing salinity, no desiccation	30	N/A	10
	50	N/A	10
Reducing salinity and desiccation period	30	Dry	10
	50	Dry	10
	50	Dry	30

A total of fifty-five 500mL plastic sample jars were used, with five jars for each treatment (11 treatments). A total of 150 *R. megacarpa* seeds were used, with each sample jar containing 30 *R. megacarpa* seeds. Each sample jar contained 2 cm of substrate (with all seeds removed) and *R. megacarpa* seeds were placed on the surface of this substrate. Saline solutions were made up to the required concentration by adding Ocean Nature Sea Salt to tap water and checking the salinity with a salinity meter (Orion Multimeter Model No. 1230). Solutions of the required salinity (g/L) were added to the sample jars to a depth of 7 cm. The salinity of the solution was checked after 24 hours to determine if any

salt present in the substrate had dissolved. If necessary the solution in the sample jars was diluted to the desired salinity concentration and any surplice water was syphoned off so that the solution depth remained at 7 cm (see Figure 5.1 in chapter 5).

Sample jars were arranged in a germination cabinet according to a random number generated pattern (Appendix 18). Photoperiod of the germination cabinet was 14 hours light/10 hours dark and the ambient temperature was 30^{o}C, as these were the optimal conditions found for *R. megacarpa* in the experiments discussed in Chapter 5. Sample jars were checked, at the same time each day over 25 days, the germinants were counted and removed.

At the end of the first phase (25 days), the solution was syphoned off from the "control" and "effect of reducing salinity, no desiccation" treatments taking care not to disturb the *R. megacarpa* seeds. A solution was made up to the required Phase 2 salinity concentration by adding Ocean Nature Sea Salt to tap water, using a salinity meter (Orion Multimeter Model No. 1230) to check salinity concentrations (g/L) and then added to the sample jars to a depth of 7 cm. The solution concentration was checked after 24 hours to determine if salt present in the substrate had dissolved. If necessary the solution in the sample jars was diluted to the required salinity and any surplus solution syphoned off so that the depth remained at 7 cm. Sample jars were returned to the germination cabinet with the same photoperiod and temperature conditions as applied in Phase 1, for a further 25 days. Sample jars were checked, at the same time each day over this period and germinants were counted and removed.

At the end of the first phase (25 days), the solution was also syphoned off from the "effect of desiccation, no change in salinity" and "effect of reducing salinity and desiccation" treatments. These jars were placed in an oven at 40^{o}C for 2 days (48 hours) to allow the substrate to dry out. After this drying period a solution was made up to the required Phase 2 salinity concentration and checked as previously described, prior to being added to the sample jars to a depth of 7 cm. The salinity of the solution was checked after 24 hours to determine if salt present in the substrate had dissolved. If necessary the solution was diluted to the required salinity and surplus water was syphoned off so that the depth of the solution remained at 7 cm. Sample jars were returned to the germination cabinet with the same photoperiod and temperature conditions as applied in Phase 1 of the experiment, for

a further 25 days. Sample jars were checked, at the same time each day over this period and the germinants were counted and removed.

6.2.1 Data Analysis

Results were analysed using three way mixed ANOVA to determine if there was any significant difference ($p < 0.05$) between salinity treatments, using the following model.

DV = constant + Phase 1 salinity + Phase 2 salinity + Presence of desiccation + Phase 1 salinity x Phase 2 salinity + Phase 1 salinity x Presence of desiccation + Phase 2 salinity x Presence of desiccation + Phase 1 salinity x Phase 2 salinity x Presence of desiccation

Where results of the three way ANOVA were significant, *post hoc* Tukey's tests were conducted to determine which treatments were significantly different. All statistical tests were conducted using the PASW 18 (previously known as SPSS statistics) software package. Square root transformations of the dependent variable were undertaken when the data from this experiment did not meet the assumptions of normality or homogeneity of variances for parametric tests (Levene's test).

6.3 Results

Ruppia megacarpa seeds germinated in the two lower salinity treatments of 10 g/L and 30 g/L and no germinants were recorded when seeds were subjected to the highest salinity concentration of 50 g/L (Table 6.2). Overall germination success was below 30%. Germination was higher in treatments where *R. megacarpa* seeds were subjected to the lowest salinity (10 g/L) (Table 6.2). The greatest germination success was recorded in the effect of reducing salinity and drying treatment of 30 g/L, Phase 1 salinity and 10 g/L Phase 2 salinity where 26% (39) seeds germinated. A lag in the time before germination of up to four days was also observed in the 30 g/L treatments, compared to the lower salinity treatment of 10 g/L (Appendix 19).

Table 6.2 Overall germination rates of *Ruppia megacarpa* seeds collected from Lake Wandella in each treatment at the completion of the experiment, highlighted treatments (bold) represent the highest and lowest germination rates.

Treatment Type	Phase 1 (25 days) Salinity (g/L)	Dry Phase (2 days)	Phase 2 (25 days) Salinity (g/L)	Total no. of seeds germinated at end of phase 1	Total no. of seeds germinated at end of phase 2	% of seeds germinated	Mean no. of seeds germinated per jar	Standard error of the mean no. seeds germinated per jar (±)
Controls no change in salinity, no drying period	10	N/A	10	29	32	21.3	5.3	2.4
	30	N/A	30	8	16	10.7	3.2	1.8
	50	**N/A**	**50**	**0**	**0**	**0**	**0**	**0**
Effect of drying, no change in salinity	10	Dry	10	19	29	19.3	5.8	1.8
	30	Dry	30	19	38	25.3	8	2
	50	**Dry**	**50**	**0**	**0**	**0**	**0**	**0**
Effect of lowering salinity, no drying	30	N/A	10	13	19	12.7	3.8	1.2
	50	N/A	10	0	21	14.0	4.2	1.2
Effect of lowering salinity and drying	**30**	**Dry**	**10**	**14**	**39**	**26.0**	**7.8**	**1.4**
	50	Dry	10	0	20	13.3	4	1.4
	50	Dry	30	0	10	6.7	2	0.9

Results of the within treatment effects in the mixed three-way ANOVA showed that only Phase 2 salinity levels had a significant ($p < 0.05$) main effect on the number of *R. megacarpa* germinants (Table 6.4). There were no other significant ($p < 0.05$) main or significant ($p < 0.05$) interaction effects found between the different treatments (Table 6.3). Table 6.2 further supports these findings as the lower the Phase 2 salinity concentration the greater the number of *R. megacarpa* germinants were recorded overall.

Table 6.3 Results of a mixed three-way ANOVA testing the effect of Phase 1 salinity, Phase 2 salinity and presence of desiccation period on the number of *Ruppia megacarpa* germinants. ** Indicates significance to the $p < 0.05$ level

Factors	***df***	***F***	***p***
Phase 1 Salinity	2,45	2.727	0.076
Phase 2 Salinity	2,45	3.709	**0.006****
Desication	1,45	0.021	0.887
Phase 1 salinity x Phase 2 salinity	1,45	0.291	0.592
Phase 1 salinity x Desication	1,45	0.047	0.830
Phase 2 salinity x Desication	1,45	<0.01	1.000
Phase 1 salinity x Phase 2 salinity x Desication	1,45	<0.01	1.000

Results of *post hoc* Tukey's tests indicated a significant difference ($p < 0.05$) between the lowest salinity treatments of 10 g/L and the highest salinity treatment of 50 g/L (Table 6.4).

Table 6.4 Results of *post hoc* Tukey's test comparing Phase 2 salinity treatments on number of *Ruppia megacarpa* germinants. ** Indicates significance $p < 0.05$ level

Salinity (g/L)	10	30	50
10			
30	0.101		
50	**< 0.001****	0.064	

6.4 Discussion

Results of this study further build on the results of Chapter 5 by investigating how other environmental conditions such as lowering salinity concentrations and drying can affect the germination of *Ruppia megacarpa* seeds. The results of this study indicate that:

1. The presence of a drying period had no effect on *Ruppia megacarpa* seed germination

A 48 hour drying period had no significant effect on the number of *R. megacarpa* seeds germinating in this experiment. But this result must be treated with caution as the seeds were collected from the dry bed of Lake Wandella and thus had already been exposed to a much longer drying period. Additionally, the age of these seeds was not known. Cho and Sanders (2009) used Tetrazolium tests to revel that exposing *R. maritima* seeds to a 10 month drying period reduced seed viability by 35.7%. In a germination study, Cho and Sanders (2009) observed a 90% to 95% germination success rate in newly formed *R. maritima* seeds compared to a 18% to 30% germination rate in *R. maritima* seeds that had been desiccated for 10 months. This is in contrast to the findings of Salter *et al.,* (2010) who found that a drying period increased the germination success of *Vallisneria australis* seeds.

Germination success of *R. megacarpa* seeds in this study were also low, with less than 30% of seeds germinating in all treatments. This suggests that the conditions seeds were exposed to prior to this experiment may have affected their viability. Similar low germination rates in *R. megacarpa* seeds were reported in Chapter 5, as well as in studies by Brock (1982a).

Brock (1982a), conducted an after ripening trial on three *Ruppia* species to determine if all seeds germinated in the first growing season. Results indicated that the greatest germination success in *R. megacarpa* was in the second growing season, after either permanent wet, or dry, and rewetting regimes. This suggests that *R. megacarpa* seeds need an after ripening period to optimise germination. The findings of this current study did not concur with those of Brock (1982a), with only one treatment showing a significant effect of phase and no treatments demonstrating a significant effect in response to a drying period. But this current study was conducted over 50 days at a controlled temperature and photoperiod environment with a short desiccation event, whereas the

study by Brock (1982a) was conducted over 15 months in the field. As such, the environmental conditions that the seeds were exposed to differed greatly.

Many other wetland plants have shown increased germination success after periods of drying and after ripening. Brock (2011) reported that seeds can be long lived and that some wetland species were able to germinate after a dry period of 12 years. Brock (2011) also found that some species germinated from wetland sediments that had been subjected to several wetting and drying regimes over a 7 year period, even when new plants were prevented from contributing new propagules to the seed bank. Carta *et al.,* (2013) found that an after ripening period increased germination success in wetland species from the Mediterranean Basin, whilst Jensen (2004) found similar patters in fen grassland species from Northern Germany. Thus the mechanism of after ripening in *R. megacarpa* seeds and the possible effects of other environmental conditions between growing seasons requires further investigation.

2. Lower salinity concentrations and freshening salinity concentrations had a positive effect on the germination of *Ruppia megacarpa* seeds

Both Phase 1 and Phase 2 salinity concentrations had a significant effect on the germination of *R. megacarpa* seeds. Results indicated that the lower the salinity concentration, the higher the germination success in *R. megacarpa* seeds. Significant differences were found between all salinity treatments, with the exception of the Phase 1 salinity treatments of 10 g/L and 30 g/L. No germination occurred in the 50 g/L salinity treatment, which also supports the findings of propagule bank studies previously discussed (Chapters 3 and 4) where the highest salinity in which germination occurred was 37.7 g/L. This also concurred with the propagule bank studies conducted by Sim *et al.,* (2006a) where the germination of *R. megacarpa* was observed in salinity treatments of up to 30 g/L. Maximum germination was found in the lowest salinity treatment of 10 g/L, which is also supported by the findings of Brock (1982a) who reported that the lowest salinity concentration promoted the highest germination success in *R. megacarpa* seeds, when compared to the higher salinity treatments of 19 g/L and 42 g/L.

Few studies have investigated the effect of briefly reducing salinity concentrations on the germination success of wetland plants. In this study, every instance where salinity concentrations were reduced resulted in increased *R. megacarpa* seed germination, especially when salinity concentrations were dropped to the lowest salinity concentration

(10 g/L). The initial high salinity concentrations had little impact on the overall germination of *R. megacarpa* when salinities were later reduced, and the germination success in these treatments was similar to those of the controls after Phase 1. Similar observations are discussed in Chapter 4, where initial high salinity variations were found to have little impact in the overall germination of *R. megacarpa* during a Recovery Phase.

Although some germination occurred in 30 g/L salinity, the germination success was significantly lower and there was a short lag in time until germinants appeared. Delays in the germination of seeds can be problematic for a species survival in wetlands. If germination occurs late in the wetting cycle, there may not be enough time before the wetland dries for plants to reach maturity and reproduce. As a result, propagule banks can become depleted over time. Lag times in the germination of *R. megacarpa* at intermediate salinity concentrations are discussed in the propagule bank experiments described in Chapters 3 (see section 3.4.1), and also in the propagule bank studies conducted by Sim *et al.,* (2006a).

Brock (1982a) and van Vierssen *et al.,* (1982) concluded that *R. megacarpa* was a perennial species found in permanently watered, relatively stable environments. Brock (1982a) also suggested that *R. megacarpa* seeds remain dormant within the propagule bank and that high germination success of this species only occurs in the field when salinities are reduced by heavy rains. This may have implications for how wetlands with *R. megacarpa* present are managed, if this species is considered important in maintaining ecosystem function. Through this study and those previously discussed (Chapters 3, 4 and 5) it is clear that *R. megacarpa* seeds can survive periods of desiccation. But, the long term effect on the viability, germination and colonisation success of *R. megacarpa* is unknown and will need to be monitored into the future when managing these wetlands as ephemeral habitats.

Many plant and animal species can survive not only periods of high salinity, but also periods of drying by producing seeds or eggs with the ability to persist in the substrate until conditions become more favourable. *Ruppia megacarpa* seeds avoid increased salinity by remaining dormant until fresher conditions predominate, indicating that this species can be resilient not only to higher salinity concentrations but also to periods of desiccation (Skinner *et al.*, 2001; James *et al.*, 2003). Managers however need to be careful when applying this knowledge to management regimes and watering plans for

wetlands. An important factor that needs to be considered, is the maximum length of time these propagules are able to remain viable in the propagule bank. As already discussed there is evidence to suggest that *R. megacarpa* seeds have reduced viability if dried for extended periods of time (10 months) before germination occurs (Cho and Sanders, 2009). Further long-term studies into this area are required to determine the effect of drying and salinity concentrations on the viability of these propagules.

Overall this study found that increased salinity concentrations negatively impact the germination success of *R. megacarpa* seeds, and that no germination occurred in the highest salinity treatment of 50 g/L. Reducing salinity concentrations was found to positively impact germination success, particularly when levels were lowered to a salinity of 10 g/L. The presence of a short drying period however, did not have any significant impact on the germination of *R. megacarpa* seeds. Further studies are needed to understand the effect of both after ripening and drying on the seeds of this species, given the low germination success observed, and the findings of Cho and Sanders (2009) regarding the negative impacts of drying in *R. maritima* seeds.

7.0 Implications for management of wetlands of intermediate salinity in northwest Victoria

Wetlands of intermediate salinity are often characterized as areas of low flora and fauna diversity, yet high productivity, leading to systems that support numerous water bird and fish populations (Brock and Lane, 1983; Brock, 1986; Timms, 1993; Kingsford and Porter, 1994). These wetlands are of high ecological value especially in comparison to hypersaline lakes which are often less complex systems (Bauld, 1981; Davis, 2002; Davis *et al.*, 2003; Strehlow *et al.*, 2005; Sim *et al.*, 2006a; 2006b; 2006c; Davis *et al.*, 2010). Management of these wetlands in the past focused on providing adequate water flows in order to maintain salinity concentrations low enough to support plant and animal communities. The negative impacts of increased salinity and reduced water flows on wetlands of intermediate salinity in northwestern Victoria will only be exacerbated in the future as the effects of climate change and alterations in weather patterns occur (Herbst and Blinn, 1998; James, 2005; Nielsen and Brock, 2009). There are many policies and agencies responsible for the management saline lakes in northwestern Victoria and as a result coordinated efforts will be required between these agencies to effectively manage these systems. Results of this study not only provide further evidence of how productive saline wetlands can be, but additionally they have demonstrated how careful management of these wetlands is crucial for maintaining viable plant and animal communities into the future. Many of the wetlands in this study are subjected to a number of environmental stressors that may impact on their future ability to support a macrophyte dominated community that provides viable habitat for vertebrate and invertebrate species.

Currently there are a number of state and national Acts governing the management of wetlands in northwestern Victoria and their implementation Acts falls to a number of agencies and organizations (Table 7.1). Consequently the management of these wetlands is often a cooperative partnership between differing agencies and organisations (Table 7.1).

Table 7.1 Australian and Victorian Acts, and the agencies and organisations responsible for the management of wetlands in northwestern Victoria

Australian and Victorian Acts relating to the management of wetlands	Agencies and organisations responsible for the management of wetlands in northwestern Victoria.
Environmental Protection and Biodiversity Act (1999 - National)	Department of Environment and Primary Industries
Flora and Fauna Guarantee Act (1988 - Victoria)	Environmental Protection agency
Planning and Environment Act (1987 - Victorian)	Local Catchment Management Authorities (Mallee CMA and North Central CMA)
Catchment and Land Management Act (1994 - Victorian)	Victorian Catchment Management Council
Environment Protection Act (1970 - Victorian)	Goulburn Murray Water
Water Act (1989 – Victorian), Water Act (2007 – National)	Department of Planning and Community Development

The most important aspect of the management of these wetlands in terms of their salinity is the watering regime. The watering regimes of wetlands considered in this study included Lake Elizabeth, Lake Cullen, Lake Wandella, Long Lake, Golf Course Lake, Round Lake, Woorinen North Lake. These watering regimes of wetlands in northwestern Victoria and the allocations of environmental flows is decided based on three factors, firstly the presence of any endangered species, secondly connectivity to the irrigation channels, and thirdly the amount of water available, which varies from year to year(Seabloom *et al.*, 1998).

7.1 The distribution of biota in wetlands of intermediate salinity in northwestern Victoria

Historically wetlands of northwestern Victoria have been a mosaic of fresh, hyposaline, saline and hypersaline lakes across the landscape, with varied flooding regimes. The development of farms, irrigation schemes, towns and levee banks over time has resulted in dramatic changes in salinity concentrations and reduced permanence of wetlands in this area. In the Kerang to Swan Hill region many wetlands have become increasingly saline or hypersaline, and some have become completely dry (KLAWG, 1992). This has affected the distribution of biota in the region over time, in particular *Craterocephalus*

fluviatilis (Murray Hardyhead) which is now restricted to just a few wetlands in Victoria, and is now considered to be endangered (Ebner and Raadik, 2001).

Wetlands investigated in Chapter 2, (Lake Elizabeth, Round Lake, Woorinen North Lake and Lake Hawthorn) were all found to be wetlands of intermediate salinity (mean salinity range of 8.22 g/L to 29.28 g/L), with low turbidity (mean < 30 NTU) and tended to be alkaline (mean range of pH 8.15 to 8.80). Although the biotic diversity of these saline wetlands is low, their productivity is generally very high which is a common finding in this type of system and concurs with the work of Timms (1997). Wetlands investigated in Chapter 2 were highly productive and supported the growth of the submerged aquatic macrophyte *Ruppia megacarpa* R. Mason (large-fruit widgeon grass) and most supported the charophyte *Lamprothamnium macropogon* (A. Braun) L.Ophel (Stonewort). Whilst productivity was high in all wetlands investigated, the distribution of these macrophyte species was heterogeneous across wetlands, with some areas supporting extensive macrophyte beds and other areas of the wetlands being bare substrate with little or no plant cover.

All wetlands studied, (except Lake Elizabeth, where no fish were caught) supported communities of *C. fluviatilis* and *Gambusia holbrooki* (Eastern Mosquito fish), in addition to these species Woorinen North Lake also supported populations of *Philypnodon grandiceps* (Flat Headed Gudgeon). These wetlands are able to support productive invertebrate, fish and water bird populations, providing salinity concentrations remain low enough (< 16 g/L), and where possible should be managed to support such communities.

7.1.1 The distribution of *Craterocephalus fluviatilis* (Murray Hardyhead) in wetlands of northwest Victoria

Craterocephalus fluviatilis has a restricted distribution within Victoria and is mainly found within lakes of intermediate salinity. The distribution of this species was thought to be restricted to the Cardross Lakes (Mildura), Lake Hawthorn (Mildura), Lake Woorinen North (Swan Hill), Round Lake (near Lake Boga) and Lake Elizabeth (Kerang) (Flemming, 1990; Allen *et al.*, 2002; Lyon *et al.*, 2002; Ebner *et al.*, 2003). Previous studies also suggested that this species was reliant on *Ruppia* spp. for survival, and that *Ruppia* spp. may be used as an indicator species for the presence of *C. fluviatilis* in

wetlands (Lyon *et al.*, 2002). Results of this study (Chapter 2) indicate that *C. fluviatilis* populations no longer exist in Lake Elizabeth but populations were found in Lake Hawthorn, Lake Woorinen North and Round Lake. Populations of *C. fluviatilis*, are associated with *Ruppia megacarpa* stands in Victorian wetlands, but this fish does not co-occur with *R. megacarpa* in South Australia (Wedderburn *et al.*, 2007; Wedderburn *et al.*, 2008). In South Australia populations are associated with *Myriophyllum* spp., which suggests that *C. fluviatilis* is not reliant on *Ruppia* spp. *per se*, but is actually reliant on the presence of submerged macrophyte cover for egg laying (Ellis, 2005a). Thus the existence of this type of macrophyte in healthy stands in important for the survival of *C. fluviatilis* populations in saline wetlands in Victoria.

The loss of the *C. fluviatilis* population from Lake Elizabeth and surrounding areas has been attributed to a number of possible environmental stressors including increased salinity, acid sulphate events, drying of wetlands, introduced fish species, as well as loss of connectivity to the flood plain (Backhouse *et al.*, 2006). Lake Elizabeth is no longer connected to the flood plain and only receives flood waters under extreme floods due to a number of anthropogenic changes in the area including the implementation of irrigation schemes, elevated roads and railway lines, and levee banks (KLAWG, 1992). Whilst many of these factors have occurred, the suggestion that acid sulphate soils are responsible for the loss of this species from Lake Elizabeth seems unlikely given the extensive growth of macrophytes and presence of water bird and invertebrate species in this lake (Chapter 2). Increased salinity concentrations are the most probable reason for the loss of this population, as salinity concentrations were high in past (up to 35 g/L) and may have been elevated above the threshold that this species can tolerate (Kelly, 1996). Given *C. fluviatilis* is an annual species (Ellis, 2006); populations may have been lost quickly from Lake Elizabeth as a result of extreme salinity concentrations preventing reproduction.

Populations of *C. fluviatilis* in South Australian wetlands, occur in less saline environments than those in Victoria (Wedderburn *et al.*, 2008). Results of the case study discussed in Chapter 2 indicate that historically *C. fluviatilis* was distributed in fresher waters in areas around Kerang and Swan Hill. One factor that may have contributed to the loss *C. fluviatilis* in fresher systems of the Kerang Lakes region is the reduction in submerged macrophyte cover. Many of the lakes within the Kerang Lakes area that are fresh in nature have been used for irrigation water storage and water transport. This

anthropogenic use has resulted in increased turbidity and subsequent loss of macrophyte cover (KLAWG, 1992). Which may be the reason that *C. fluviatilis* populations have been lost from wetlands such as Lake Boga (which is used to store irrigation and flood waters), and others along the irrigation system. Loss of *C. fluviatilis* populations from many of the other lakes in the district is probably due to increased salinity (e.g. Lake Tutchewop and Lake Elizabeth), or wetlands completely drying out (e.g. Lake Golf Course, Long Lake, Lake Wandella and Lake Cullen) (Ebner and Raadik, 2001; Backhouse *et al.*, 2006; Wedderburn *et al.*, 2008).

The most important factors affecting the distribution of *C. fluviatilis* in wetlands in Victoria are salinity concentrations, presence of submerged macrophytes and a permanent watering regime. There are hundreds of wetlands in the Kerang Lakes area that range in salinity from fresh to hypersaline. Some of these wetlands may be suitable for reintroduction of *C. fluviatilis*. Wetlands in this region should be screened for appropriate salinity concentrations (approximately < 22 g/L), presence of submerged macrophytes and low turbidity, and have a permanent water supply.

For the 2013/2014 watering season Round Lake and Lake Elizabeth were identified as priority wetlands due to receive environmental flows even under drought conditions. Round Lake was identified as a priority wetland as it currently supports a population of *C. fluviatilis* which rely on freshwater flows for survival, whereas Lake Elizabeth was identified as a potential site for the reintroduction of *C. fluviatilis* from captive breeding programs and thus would requires an environmental flow to maintain salinity at a concentration suitable for their release.

Other sites where *C. fluviatilis* populations occur, or where reintroductions of *C. fluviatilis* have occurred (e.g. Cardross Lakes, Lake Koorlong and Brickworks Lagoon, all lakes not included in this study) will receive environmental flows under dry, average or wet conditions (Seabloom *et al.*, 1998). Woorinen North Lake is managed as a permanent lake and has secured flows under the Woorinen pipeline scheme, and Middle Reedy Lake (not included in this study) is an irrigation lake and is therefore also managed as a permanent lake as a part of the Torrumbarry scheme (KLAWG, 1992; Lyon *et al.*, 2002).

The remaining wetlands of intermediate salinity that do not have *C. fluviatilis* populations, are managed either as temporary wetlands with set flooding regimes (e.g. Lake Cullen is currently managed to flood one in every four years), flood storage areas in times of high flows (i.e. Lake Golf Course), or if they are disconnected from water supplies there is no management of the watering regime (i.e. Lake Wandella) (KLAWG, 1992; Department of Sustainability and Environment, 2004). Given the existing knowledge and management of these wetlands and the findings of this study, managers of intermediate saline wetlands in northwestern Victoria should consider the following management options:

In all wetlands of intermediate salinity in northwestern Victoria

- Wetlands without *C. fluviatilis* populations can be managed as semi-permanent systems and may be allowed to dry. Watering regimes should include a flooding period over the summer months to allow for maximum macrophyte germination, growth and reproduction.
- Salinities should be maintained below 45 g/L for as long as possible during the flooding period to allow for the establishment of a clear water, macrophyte dominated wetlands.
- Monitoring of water quality parameters (especially salinity, dissolved oxygen and turbidity) and extensive surveys of biota (macrophyte, fish and invertebrate species) should be conducted within wetlands during the flooding period. Monitoring of fish populations should include methods for catching smaller bodied species such as *C. fluviatilis*, so that any migration of this species to new sites can be monitored.

In wetlands where Craterocephalus fluviatilis *(Murray Hardyhead) populations are present:*

- Wetlands should be managed to ensure the presence of permanent water at intermediate salinity concentrations, as this species is not able to survive desiccation or hypersaline conditions.
- Salinities should be kept low. For Round Lake, populations proved to be tolerant of salinities ≤ 22 g/L. It would be advisable to maintain lower salinity concentrations

(<16 g/L) at Lake Hawthorn and Woorinen North Lake. This would also support *P. grandiceps* (Flat Headed Gudgeon) populations in Woorinen North Lake that prefer lower salinity concentrations.

- Monitoring of *C. fluviatilis* populations should be conducted every 6 – 12 months to assess their health and abundance in these wetlands. Monitoring of other biota (especially submerged macrophytes) should also occur, but could be less frequent. Monitoring of water quality parameters (especially salinity, dissolved oxygen and turbidity), should occur regularly throughout the year to ensure salinity concentrations are maintained within acceptable limits.

7.2. The effect of salinity on aquatic macrophyte dominated communities

Results discussed in Chapters 3 and 4, have supported previous studies by Sim *et al.,* (2006a) that macrophyte dominated wetlands cease to exist in salinities above 45 g/L. In propagule bank studies of sediments from Western Australian wetlands, Sim *et al.,* (2006a; 2006b; 2006c) found that microbial mat communities developed in salinity treatments above 45 g/L. As shown in this current study (Chapters 3 and 4) however, microbial mat communities did not develop. However Sim et al., (2006b: 2006c) did find that the establishment of benthic microbial mats not only driven by salinity but also by water regimes. Thus the absence of microbial mat dominated communities may be due to the ephemeral watering regime of the Lake Cullen study site, as microbial mats tend to develop in wetlands that remain inundated (Sim *et al.*, 2006b). Phytoplankton blooms may have also developed in response to elevated nutrient concentrations in the wetland substrate (Sim *et al.*, 2006c). Four different communities were identified including:

- Macrophyte and invertebrate dominated (3.4 g/L to 37.7 g/L)
- Clear water, invertebrate (developed in 61.0 g/L salinity)
- Clear water, no macrophyte or invertebrate emergence (developed in 98.7 g/L salinity)
- Phytoplankton bloom (developed in 136 g/L salinity)

Only two species of aquatic macrophytes germinated (*R. megacarpa* and *L. macropogon*) and two species of ostracod emerged namely *Mytilocypris henricae* (Chapman 1966) and *Australocypris* spp. from the propagule bank of Lake Cullen in the propagule bank

investigation. The upper salinity threshold for most of these macrophyte and invertebrate species was between 37.7 g/L and 61.0 g/L, with *Australocypris* spp. having a greater salinity tolerance of between 61.0 g/L and 89.7 g/L.

This study also investigated the sub lethal effects of salinity on the aquatic macrophytes *R. megacarpa* and *L. macropogon* (Chapter 3). Whilst both of these species were able to germinate in salinities ≤ 37.7 g/L, a number of adverse effects where identified at concentrations below this threshold including:

- Lag in germination of *R. megacarpa* (12 weeks) and *L. macropogon* (5 weeks) in salinities of 37.7 g/L
- Reduced number of *R. megacarpa* shoots in salinities > 14.4 g/L
- Reduced growth of *R. megacarpa* (in salinities > 5.5 g/L) and *L. macropogon* (in salinities > 23.3 g/L)
- No *R. megacarpa* flowers produced in salinities of 37.7 g/L, and increased number of aborted spores in *L. macropogon* with increased salinity

Whilst this suggests that elevated salinities, below the threshold concentration may impact growth and reproduction in aquatic macrophytes, these results need to be interpreted with caution. The lag in germination of these species observed in the 37.7 g/L salinity treatment meant that germinants had less time to grow before harvesting occurred and thus reduced dry weight biomass and biovolumes may be due to this delay in germination, rather than a direct effect on growth. However in wetlands that are managed as ephemeral systems, the salinity continues to increase as the wetlands dry. Thus lags in germination will have a significant impact on the macrophyte community as plants would be unable to grow as well as those germinating under lower salinity.

The results recorded in Chapters 3 and 4 indicated that the propagule bank of Lake Cullen is resilient to high salinity disturbances and that macrophyte dominated communities can return once salinity concentrations are established below threshold concentrations. These results indicate that these plant species may also be resilient to the possible impacts of climate change on wetlands in Victoria. Recent studies have predicted that wetlands in northwestern Victoria will probably experience longer periods of drought, reduced water flows and pulsed flooding regimes as a result of climate change (Swinton *et al.*, 2000; James, 2005; Nielsen and Brock, 2009). The macrophyte and invertebrate species that emerged from the Lake Cullen propagule bank in this experiment could be resilient to the

effects of climate change as they had the ability to reestablish after periods of drying and do not need a long watering cycle to complete flowering and propagule production. Further investigations on the effects of prolonged drought, short pulsed flooding and the overall longevity of these propagule banks is required to provide additional evidence for resilience of these species to climate change.

7.3 The existence of alternative stable state and the response of the Lake Cullen propagule bank to high salinity disturbance

The adverse effects of increased salinity on the distribution of biota in Australian wetlands and other freshwater systems has been researched extensively over many years through surveys of biota and propagule bank analyses (Brock and Lane, 1983; Brock and Sheil, 1983; Hart *et al.*, 1990; Hart *et al.*, 1991; Clunie *et al.*, 2002; James *et al.*, 2003; Nielsen *et al.*, 2003b). Studies have focused on the relationship between salinity and changes in community structure, with Sim *et al.,* (2006b; 2006c) and Davis *et al.,* (2003) reporting that the relationship between increased salinity and community structure could be best explained by an alternative stable states model, and more recently Davis *et al.,* (2010) proposed a multiple stressor model.

The study discussed in Chapter 4 further tested the multiple stressor model on the propagule bank of Lake Cullen by subjecting sediments to four high salinity disturbances and then up to 9 recovery phase salinity treatments. Results from this study found little evidence to suggest that alternative stable states existed. The alternative stable states described by Sim *et al.,* (2006c), were not the same as the communities produced during the propagule bank experiments conducted on Lake Cullen (Chapters 3 and 4). During these experiments, four distinct communities developed at differing salinity concentrations. In the fresher treatments (3.4 g/L to 37.7 g/L), macrophyte dominated community was found in all replicates. This community consisted of the aquatic macrophytes *R. megacarpa* and *L. macropogon* and the invertebrates *M. henricae* and *Australocypris* spp. The second community that developed in all replicates in the 61.0 g/L salinity treatment was a clear water community with no macrophytes and only a single ostracod species *Australocypris* spp. A clear water, no macrophyte or invertebrate regime developed in the 98.7 g/L salinity concentrations. The final community was one dominated by phytoplankton blooms that were observed in all replicates of the 136 g/L salinity treatments.

Another key aspect of an alternative stable states model is the concept of hysteresis, where more than one regime may be present at a particular environmental level (Davis *et al.*, 2010). Thus if the model of alternative stable states was correct in describing the relationship between change in community structure and increased salinity in wetlands, then the presence of two alternative states should be present around threshold salinity levels. This however was not the case as discussed in results of the popagule bank studies in Chapters 3 and 4. The response of the Lake Cullen sediments to a high salinity disturbance, followed by a recovery phase, (Chapter 4) showed the same pattern in terms of species emerging from the popagule bank, as that of the first propagule bank experiment where sediments were not subjected to high salinity disturbances (Chapter 3). There was also no evidence supporting the need for a lower salinity threshold to initiate the transition back to a macrophyte dominated community.

Results discussed in Chapter 4 support the multiple stressors model described by Davis *et al*., (2010). This model suggests that wetland community structure is affected by a range of stressors (including nutrient concentrations, acidification, salinity and watering regime) and that these stressors may act independently or synergistically to affect community structure. Davis *et al.,* (2010) identified that linear, threshold, alternative stable state and collapse models are oversimplified and that a combination of these models nested within a more complex model was required to explain how wetlands react to not only to increased salinity but other stressors as well. Regression analysis of propagule bank experiment results, investigating the recovery of the Lake Cullen propagule bank exposed to high salinity disturbances indicated that the macrophyte community response was similar to a threshold model. This was in direct contrast to similar experiments conducted by Sim *et al.,* (2006b) who proposed an alternative stable states model. One plausible reason for the difference in the results is that the wetlands of south west Western Australia are seasonally drying wetlands were as Lake Cullen in Victoria undergoes a more prolonged wetting and drying cycle. Many studies have identified that salinity is not the only driver for change in wetlands and that watering regime in particular is an important factor in influencing wetland community structure (Sim *et al.*, 2006c; Davis *et al.*, 2010; Raulings *et al.*, 2011). Davis *et al.,* (2010) and specifically identified that seasonally drying wetlands appeared to follow simple threshold models whereas wetlands with a permanent watering regime were more likely to show hysteresis following an alternative stable states model.

Results of Chapters 3 and 4 indicated that there was dramatically higher plant growth in the propagule bank study conducted over the summer months (Chapter 3), in comparison to those conducted over winter months (Chapter 4). Thus seasonality may affect the emergence and productivity of the propagule bank, and consequently salinity concentrations should be kept as low as possible during the summer months to promote plant germination and growth. Salinity is also known to effect sensitive life stages in organisms such as flowering for angiosperms (Van Zandt and Mopper, 2002) or survival of juvenile vertebrates (James *et al.*, 2003). Results of this current study indicated that salinity may have impacted flowering. Results presented in Chapter 3 demonstrated that no flowering occurred at a salinity concentration of 37.7 g/L, despite the fact that plants had germinated in this high salinity treatment. The lag in germination observed in this treatment may have contributed to the absence of flowers, as flowering was observed in the second propagule bank experiment (Chapter 4) at a salinity concentration of 37.7 g/L. Lags in germination or flowering can still impact on wetland community structure as flooding of wetlands needs to be long enough to allow flowering and seed production to occur. Results presented in Chapter 4 also indicated that flowering only occurred in the high salinity disturbance treatment of 37.7 g/L. This result may be attributed to not only the lower disturbance phase salinity concentration, but also that plants germinated earlier in these treatments during the disturbance phase. Thus allowing more time for flower development, and also these plants were exposed to a higher ambient temperature. The development of flowers has been linked to increased temperatures (Seabloom *et al.*, 1998), which further supports the management option of keeping salinity concentrations lower over the summer months to promote flowering and seed production.

7.4 Effect of environmental factors on germination of *Ruppia megacarpa* seeds

Germination experiments described in Chapters 5 and 6 identified a number of environmental factors that affected germination of *R. megacarpa* seeds. The presence of substrate, a temperature of 30 ^{o}C, and reduced salinity of 10 g/L were found to optimize *R. megacarpa* germination. Photoperiod, seed source location and desiccation period were found to have no affect on germination. These results indicated that germination of this species is likely to occur in the warmer months, when salinity concentrations are lower due to environmental flows being released into wetlands. Therefore wetlands where watering occurs during summer and salinity concentrations are kept low (>10 g/L) are likely to promote the germination of *R. megacarpa*. Warwick and Brock (2003), reported

similar findings in other submerged macrophyte species, in their study of watering regimes and seasonality and their effects on propagule banks. They found that a higher number of species germinated, higher biomass was produced and more species were reproducing in the treatments inundated in summer in comparison those inundated in winter.

7.5 Further Research

Results of this study have addressed a number of knowledge gaps about the resilience of wetlands of intermediate salinity. More specifically:

- Identified the distribution of wetlands of intermediate salinity in selected regions of northwest Victoria and documented some their plant and fish biota
- Identified optimum salinity thresholds suitable for submerged macrophyte and invertebrate communities in wetlands of intermediate salinity (including the identification of salinity thresholds for individual plant and invertebrate species)
- Investigated possible sub-lethal and indirect effects of salinity on individual plant and invertebrate species
- Identified ideal and practical watering regimes designed to promote the germination of plants and emergence of invertebrates from propagule banks of intermediate saline wetlands.

There are however aspects of the ecology and management of intermediate saline wetlands that warrant further investigation.

7.5.1 Further investigations into the effect of salinity on, and the management of *Craterocephalus fluviatilis* populations

This study and surveys conducted by Arthur Rylah Institute for Environmental Research, have identified that *C. fluviatilis* populations no longer exist in Lake Elizabeth (Dixon, 2007). Populations that were there, were known to survive periods of salinity of up to 35 g/L (Kelly, 1996; Dixon, 2007). One plausible reason for this is that whilst individual adults were able to tolerate the high salinity concentrations, they may not have been able to breed. Thus the effect of salinity on the breeding success of *C. fluviatilis* and salinity tolerances of egg, fry, juvenile and adults of this species warrants further investigation. Studies by Dixon (2007) reported that individual populations of *C. fluviatilis* may have

varying salinity tolerances so an investigation into the acclimation and possible differences in salinity tolerances of different populations of *C. fluviatilis* is also required.

As already discussed, *C. fluviatilis* was once thought to rely on *Ruppia* spp. for breeding as this species was only found in wetlands with *Ruppia* spp. present. Populations of *C. fluviatilis* however have been found associated with *Myriophyllum* spp. in South Australia. *Craterocephalus fluviatilis* are known to rely on submerged macrophytes to lay their eggs on. Thus further investigation of the relationship between breeding success of *C. fluviatilis* and the presence of other species submerged macrophytes is warranted as it could identify other wetlands where the successful reintroduction of *C. fluviatilis* is possible.

Since this study was completed the Kerang Lakes area was extensively flooded (Seabloom *et al.*, 1998; Darvas, 2007). Recent investigations of irrigation lakes in the Kerang area have found populations of *C. fluviatilis* in Middle Reedy lake, a site that previously did not support this species (Williams, 1966). A captive breeding program for *C. fluviatilis* was also conducted during the drought by the Murray Darling Freshwater Research Institute and reintroductions of *C. fluviatilis* have taken place at sites around Victoria (Brock, 2011). Further investigation could identify other sites for the reintroductions of *C. fluviatilis*, particularly in the Kerang Lakes area. Possible sites could include Lake Golf Course, Long Lake, Lake Cullen and Lake Wandella, which all supported *C. fluviatilis* populations in the past. Suitable wetlands would need to be able to maintain a permanent watering regime, salinities < 22 g/L, and the presence of submerged macrophyte species.

7.5.2 Further investigations into the effect of salinity on propagule banks of wetlands of intermediate salinity

This study investigated the effects of salinity on the vegetation and invertebrate community emerging from the propagule bank of Lake Cullen. Further investigations using the same methodology on other wetlands of intermediate salinity are warranted to determine if the same response is apparent in other locations, and by the same or different macrophyte and invertebrate species. In particular studies focusing on the types of stressors that drive community changes in wetlands would be useful in determining which

model is able to explain how wetlands change, and what stressors drive community changes.

Results from this study were able to establish that the propagule bank of Lake Cullen was resilient to short term, high salinity disturbances. Longevity of the propagule bank however is yet to be determined and thus long term studies into the effect of high salinity concentrations on propagule bank viability would be useful in identifying the impact of increased time and disturbance on the recovery of communities subjected to hypersaline conditions. Given the potential effects of climate change, it is projected that wetlands in Victoria will be subjected to extreme weather events, and that overall, there will be less run off to wetlands (James, 2005; Nielsen and Brock, 2009). Thus understanding propagule bank longevity will be important to ensure macrophyte communities can continue to exist under these altered climatic conditions.

Findings of the propagule bank studies (Chapter 3 and 4), indicated that reproductive success of the macrophytes may have been affected by increased salinity. Further investigations to determine if reduced reproductive success and growth of *R. megacarpa* and *L. macropogon* is due to increased salinity, or lag times in germination of these species is warranted and will inform the management of intermediate saline wetlands.

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Appendix 1 Table of random numbers to indicate sites for water quality monitoring

Table A1.1 Table of Random numbers to indicate sites for water quality montioring

Lake Hawthorn	Woorinen North Lake	Round Lake	Lake Elizabeth
7	11	8	12
1	6	3	4
9	9	10	6
3	4	4	7

Appendix 2. Selection of quadrat size for belt transects

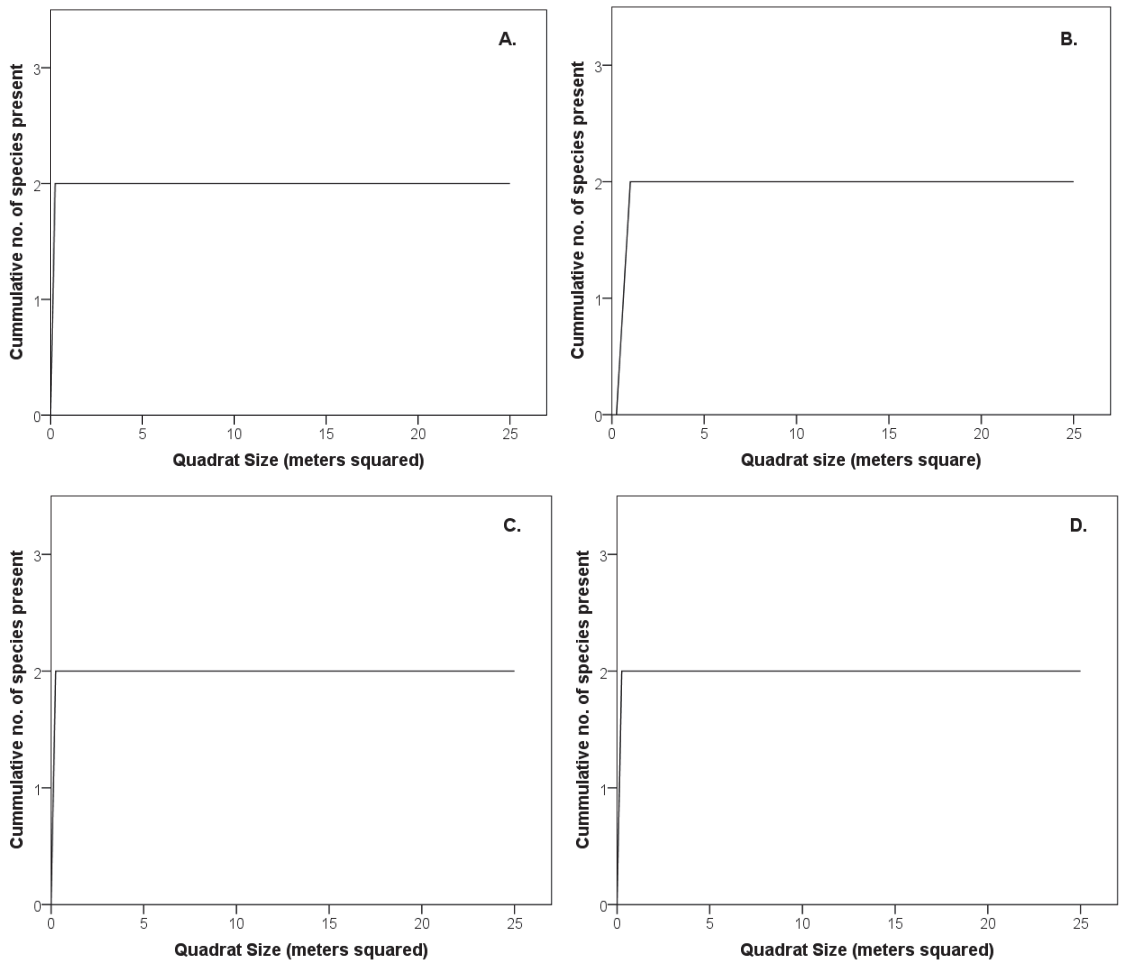


Figure A2.1 Species area curves for four sites (A-D) at Lake Elizabeth

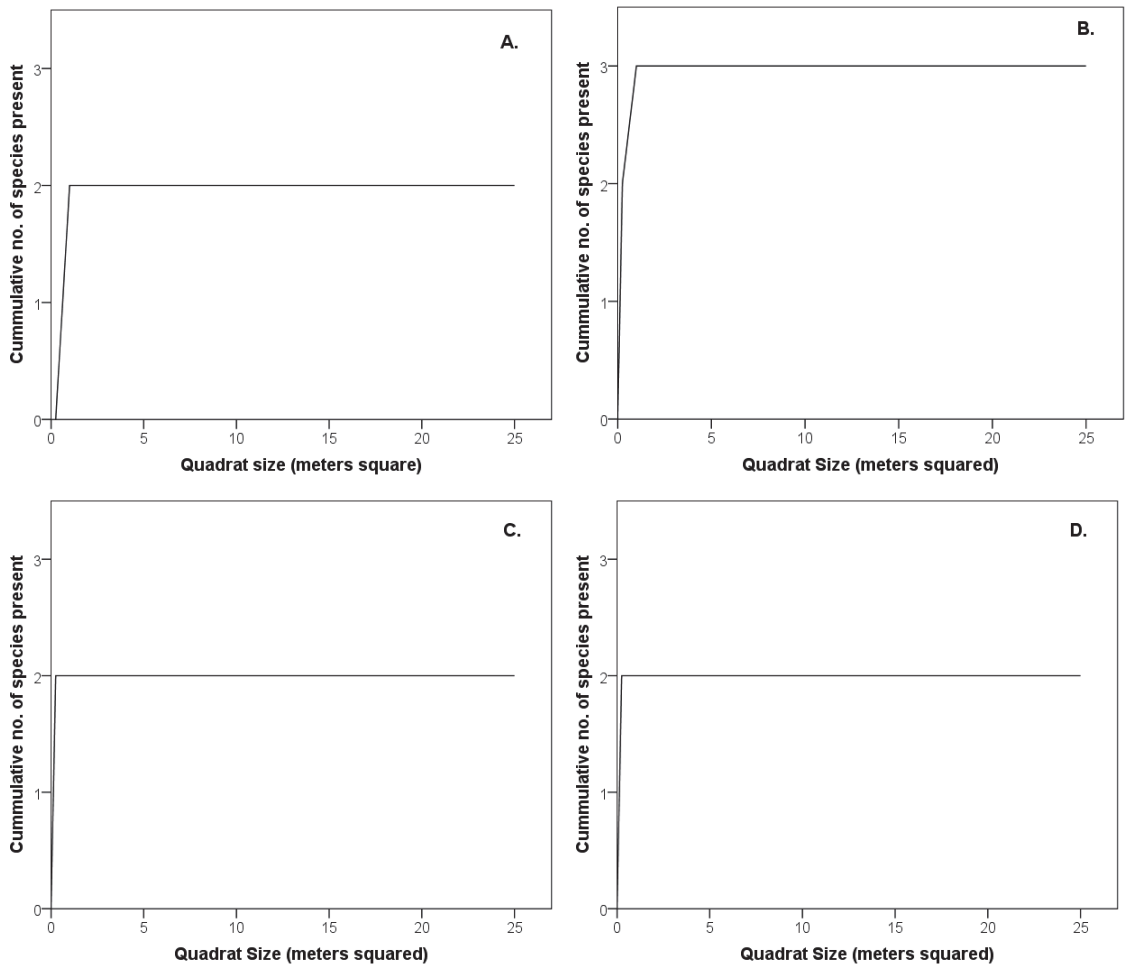


Figure A2.2 Species area curves for four sites (A-D) at Round Lake

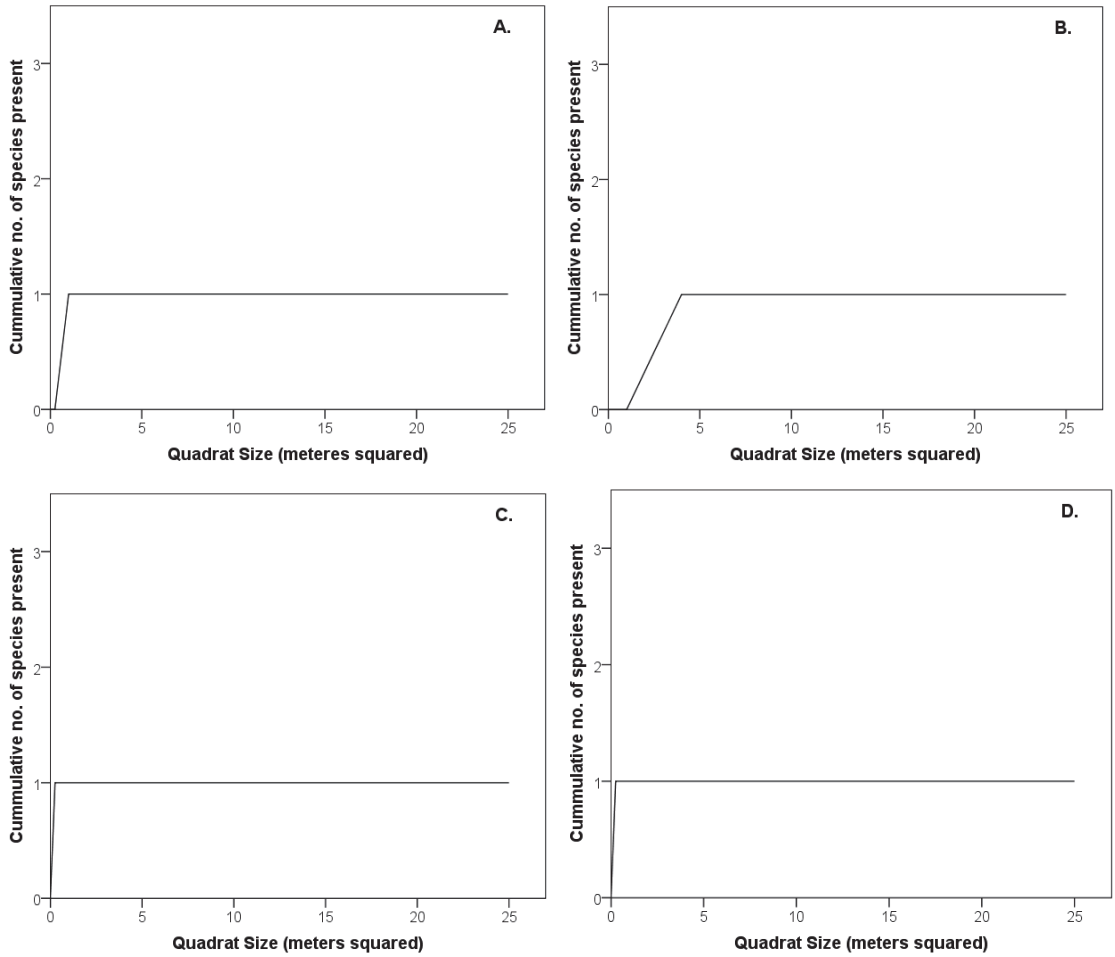


Figure A2.3 Species area curves for four sites (A-D) at Lake Woorinen North

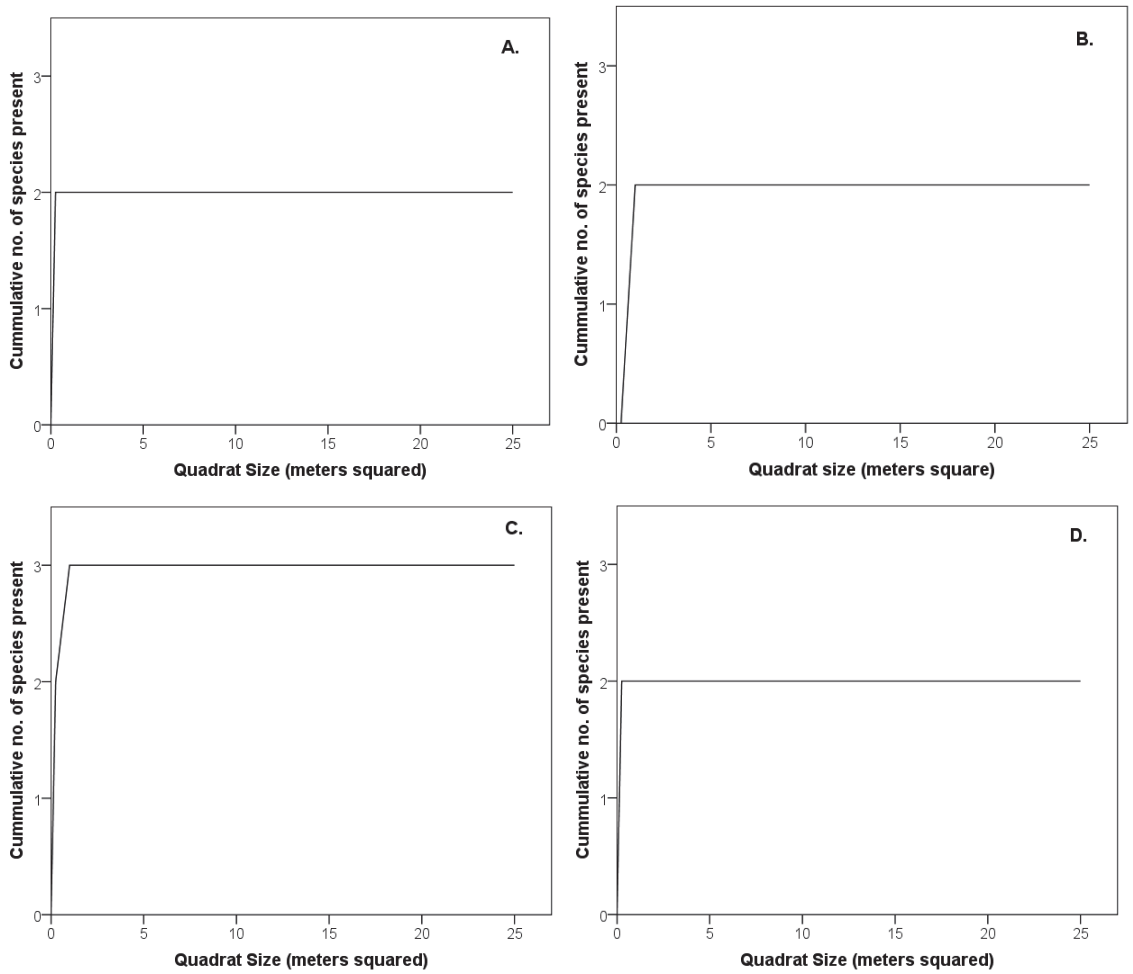


Figure A2.4 Species area curves for four sites (A-D) at Lake Hawthorn

Appendix 3 Belt transect results (depth, percentage vegetation cover, dry weight biomass and biovolume)

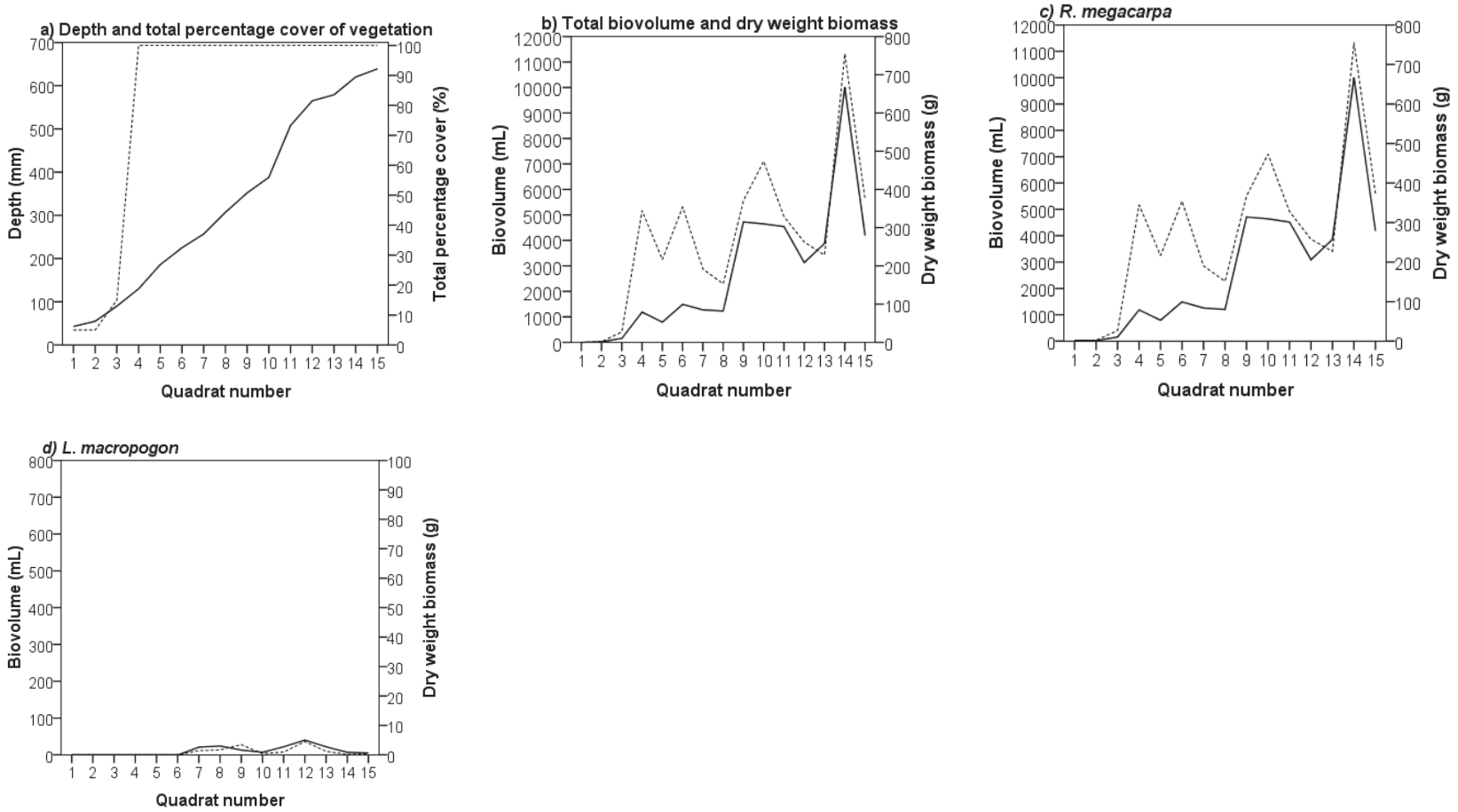


Figure A3.1 Lake Elizabeth Transect A. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c) and d) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa,* and *L. macropogon.*

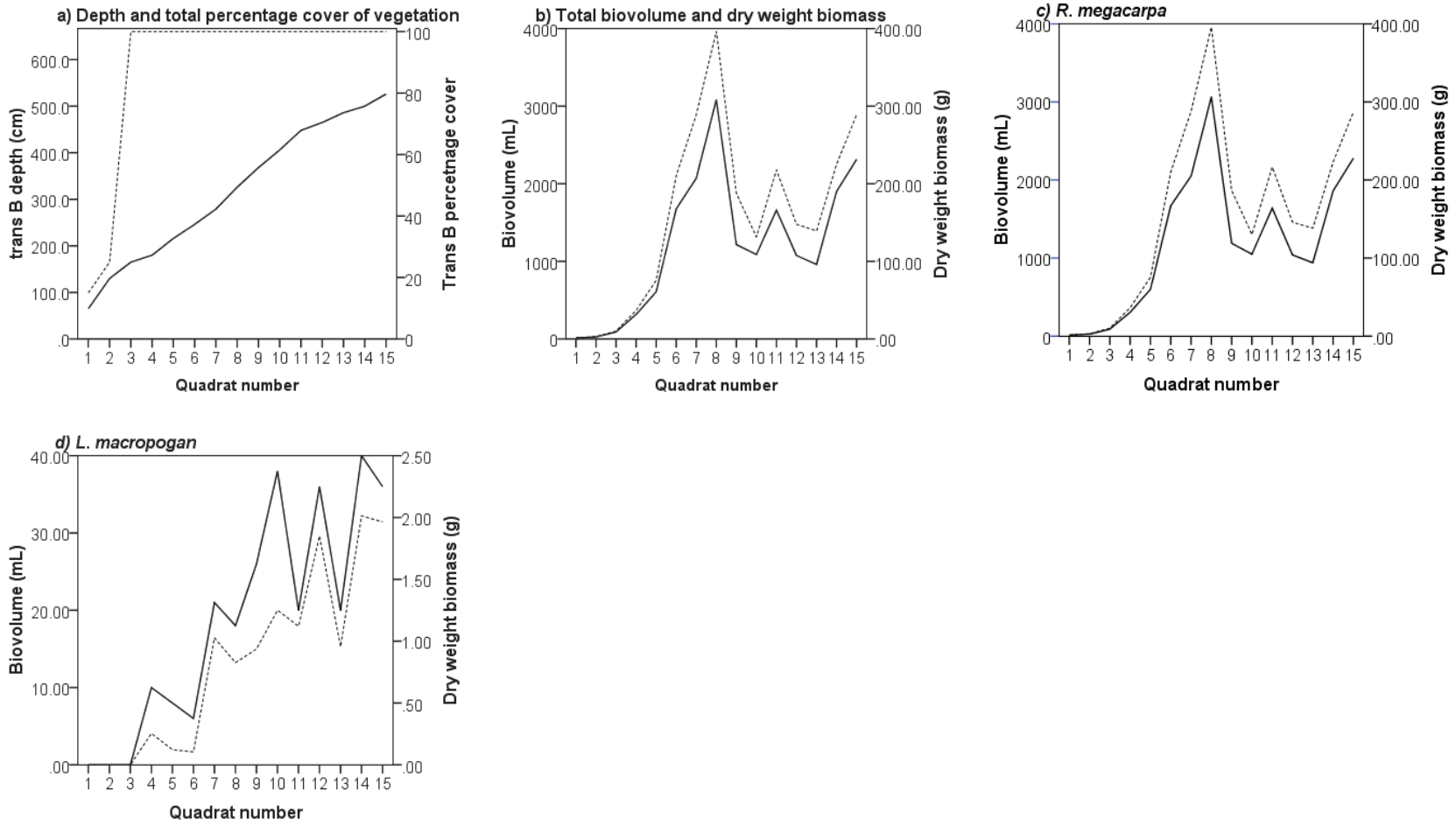


Figure A3.2 Lake Elizabeth Transect B. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c) and d) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa,* and *L. macropogon.*

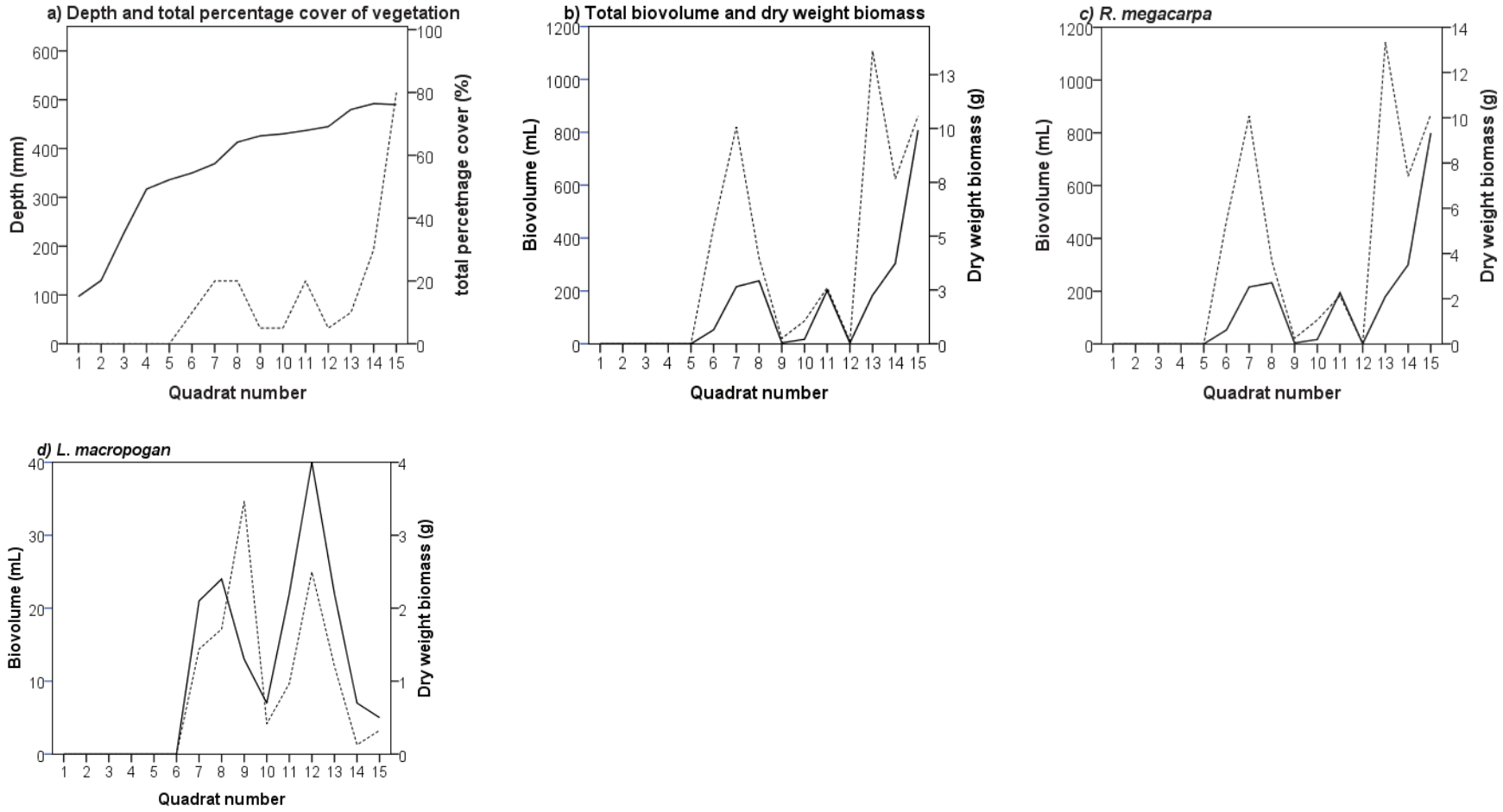


Figure A3.3 Lake Elizabeth Transect C. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c) and d) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa,* and *L. macropogon.*

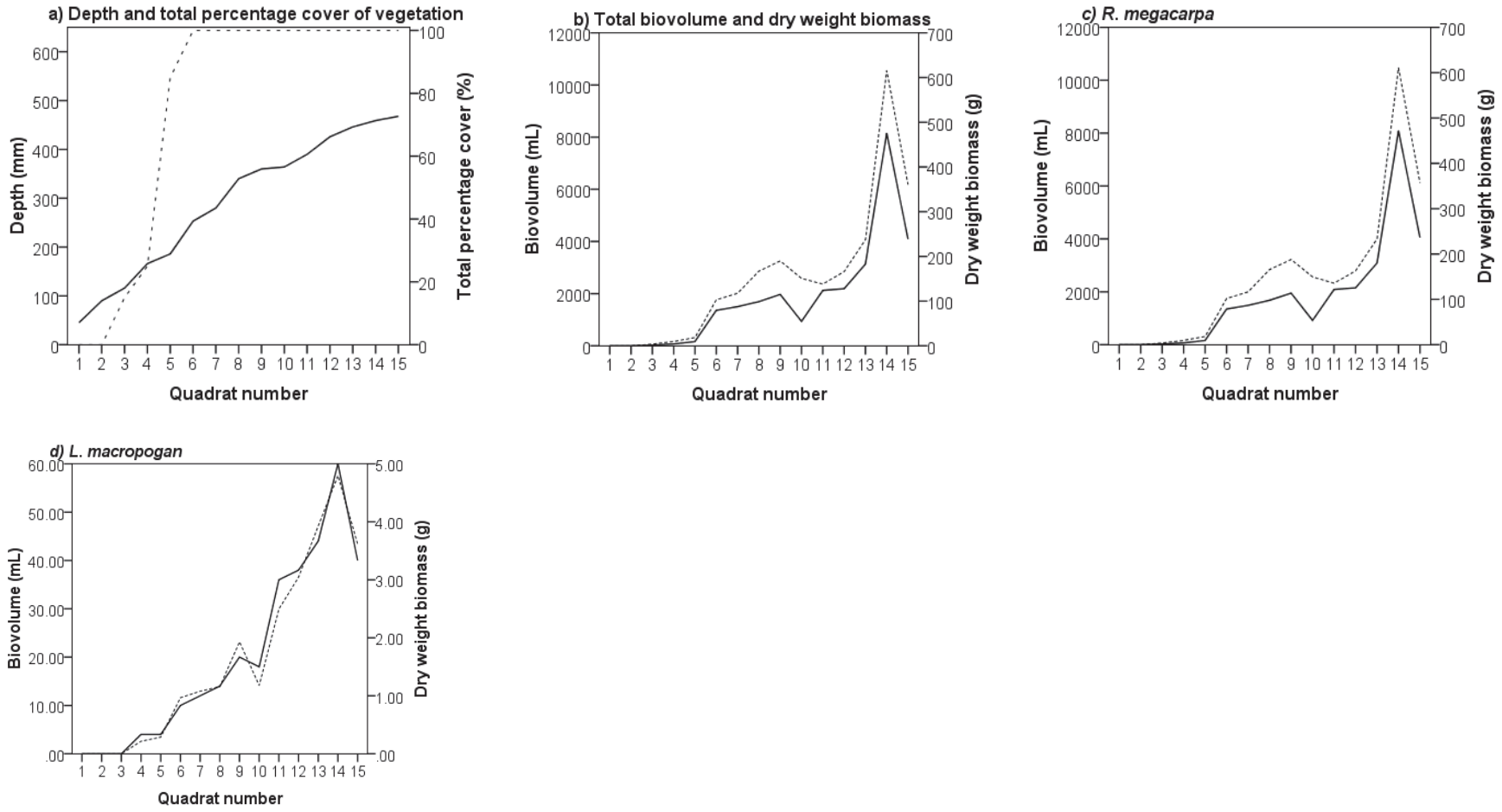


Figure A3.4 Lake Elizabeth Transect D. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c) and d) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa,* and *L. macropogon.*

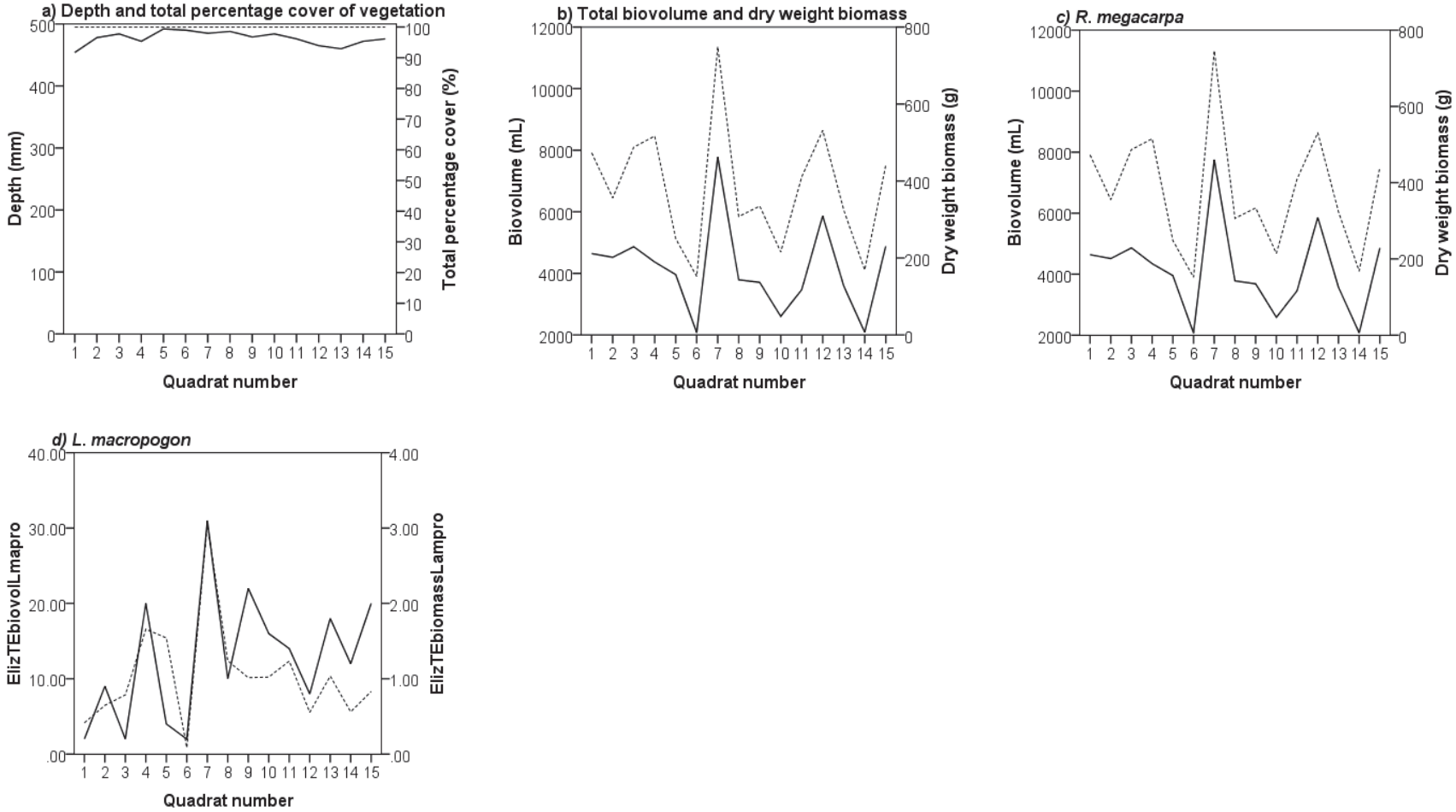


Figure A3.5 Lake Elizabeth Transect E. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c) and d) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa,* and *L. macropogon.*

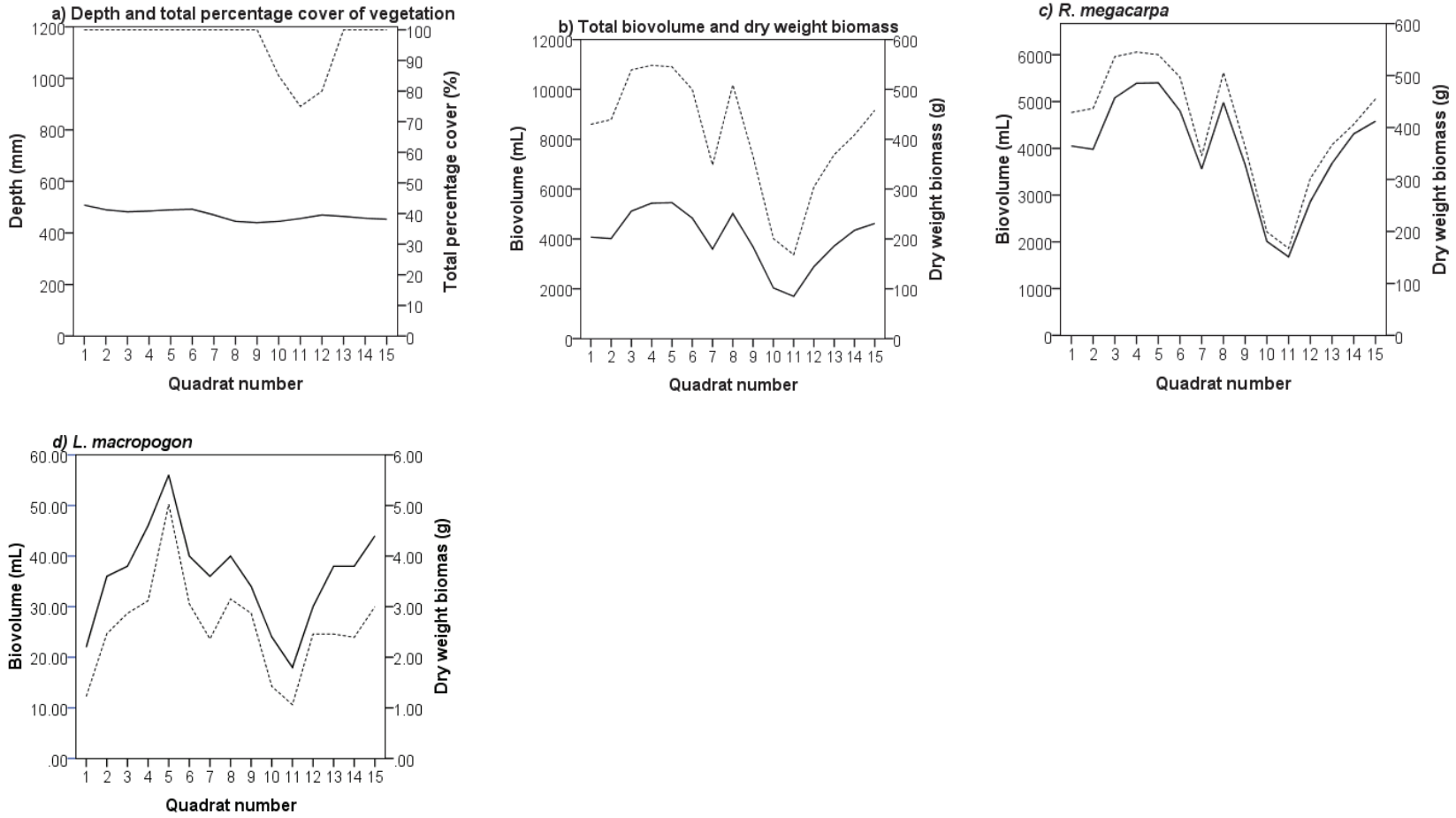


Figure A3.6 Lake Elizabeth Transect F. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c) and d) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa,* and *L. macropogon.*

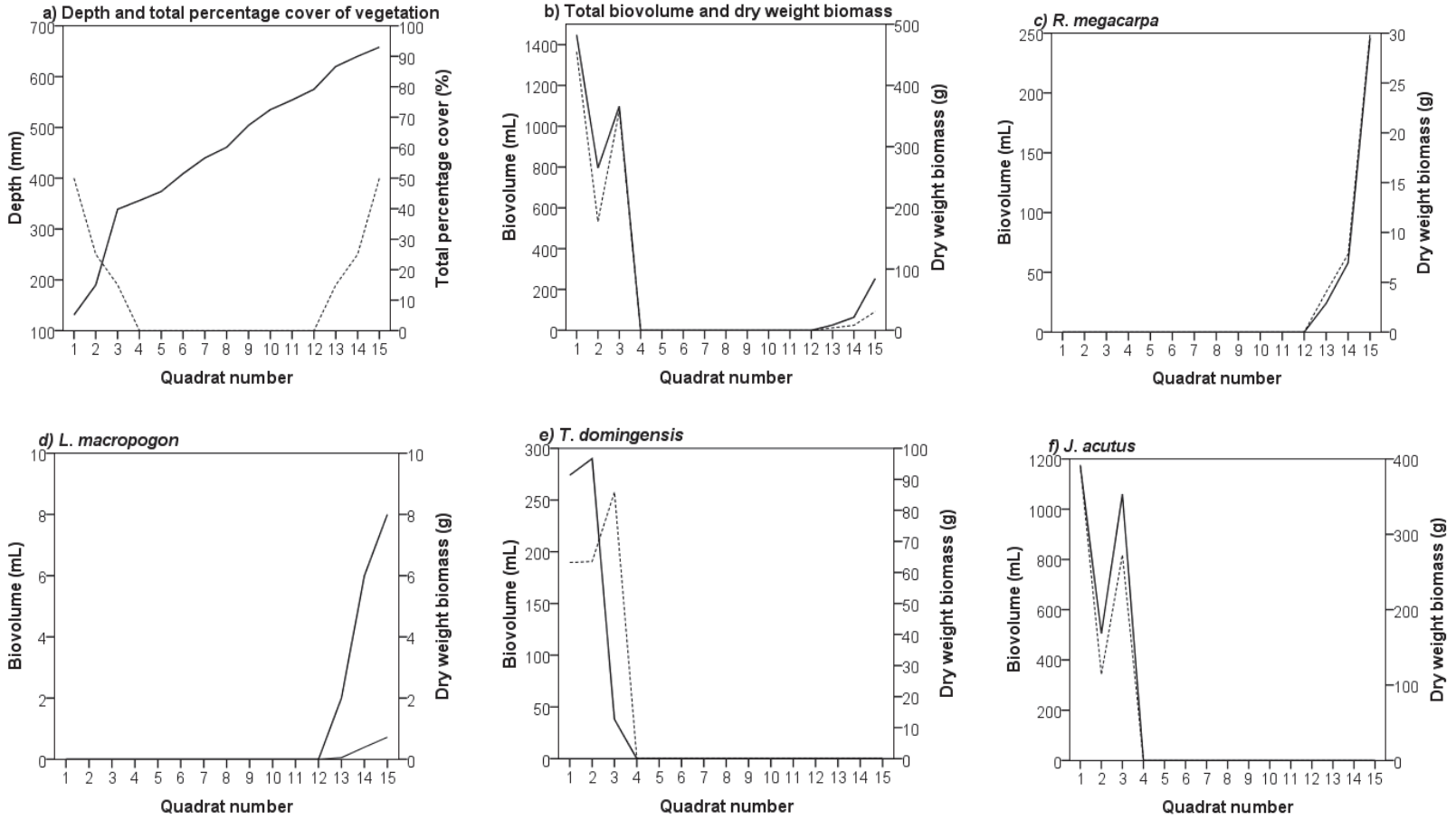


Figure A3.7 Round Lake Transect A. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), d), e) and f) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa, L. macropogon, J. acutus* and *T. domingensis.*

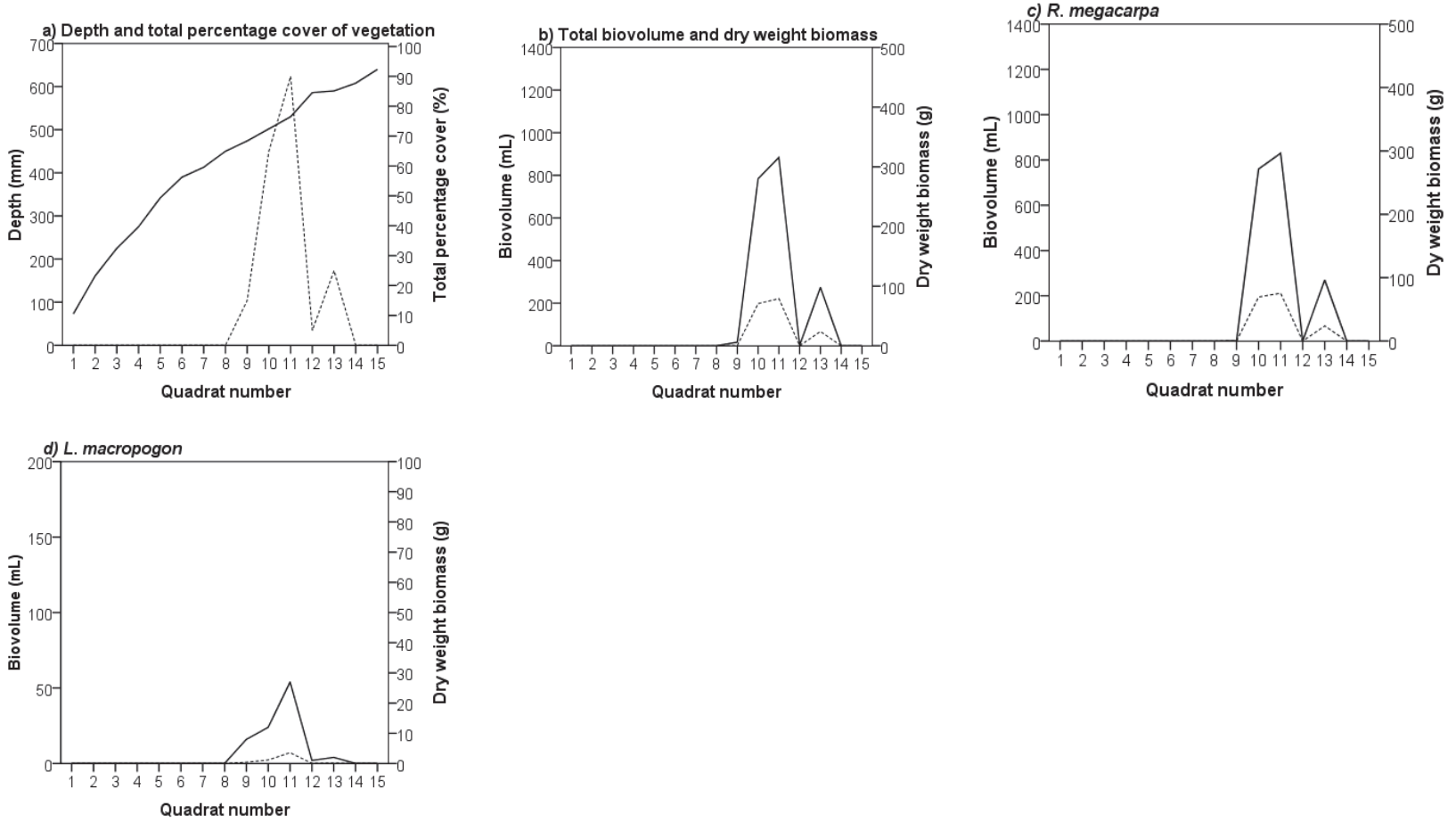


Figure A3.8 Round Lake Transect B. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon.*

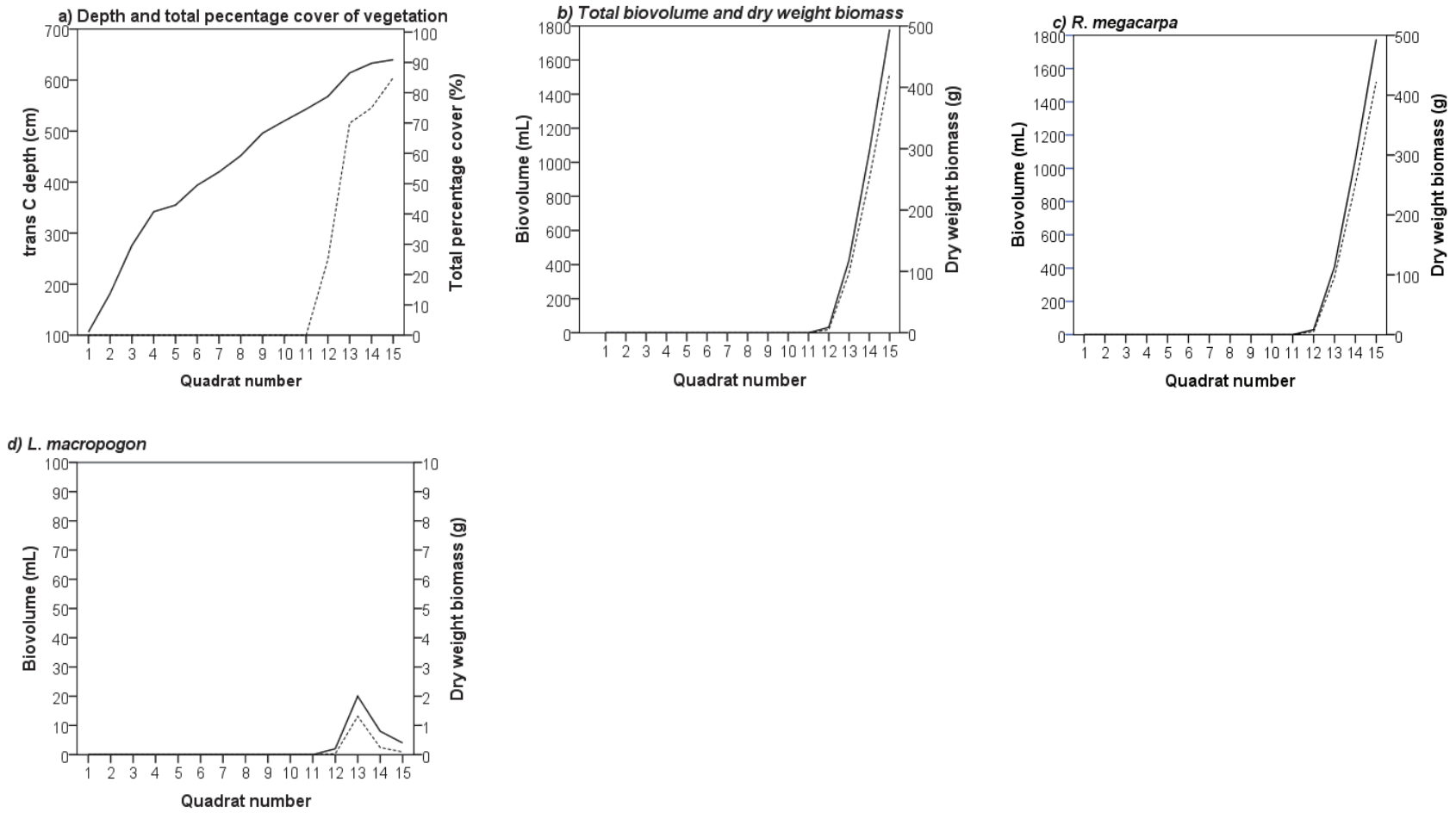


Figure A3.9 Round Lake Transect C. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon.*

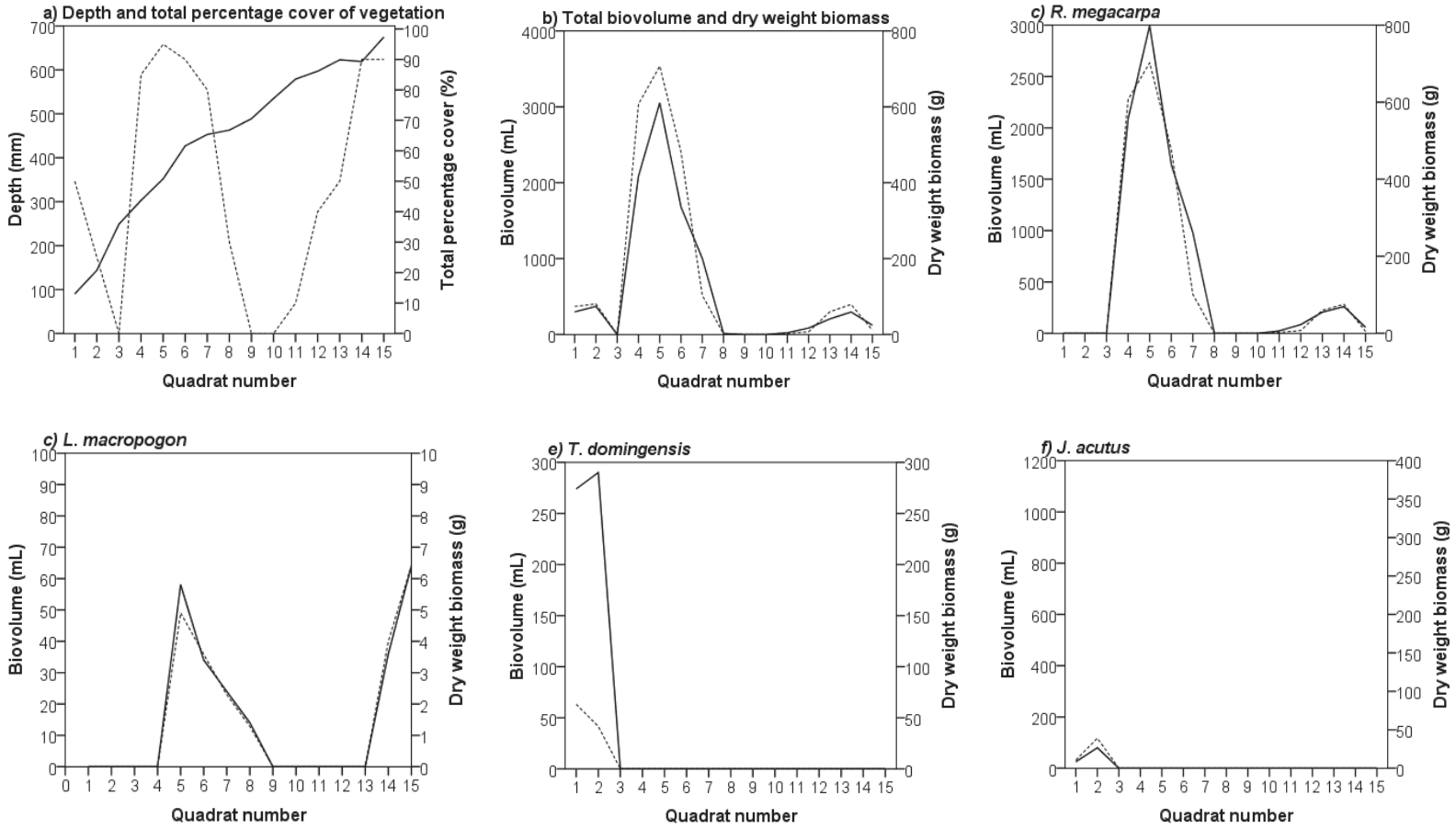


Figure A3.10 Round Lake Transect D. A. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), d), e) and f) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa, L. macropogon, J. acutus* and *T. domingensis.*

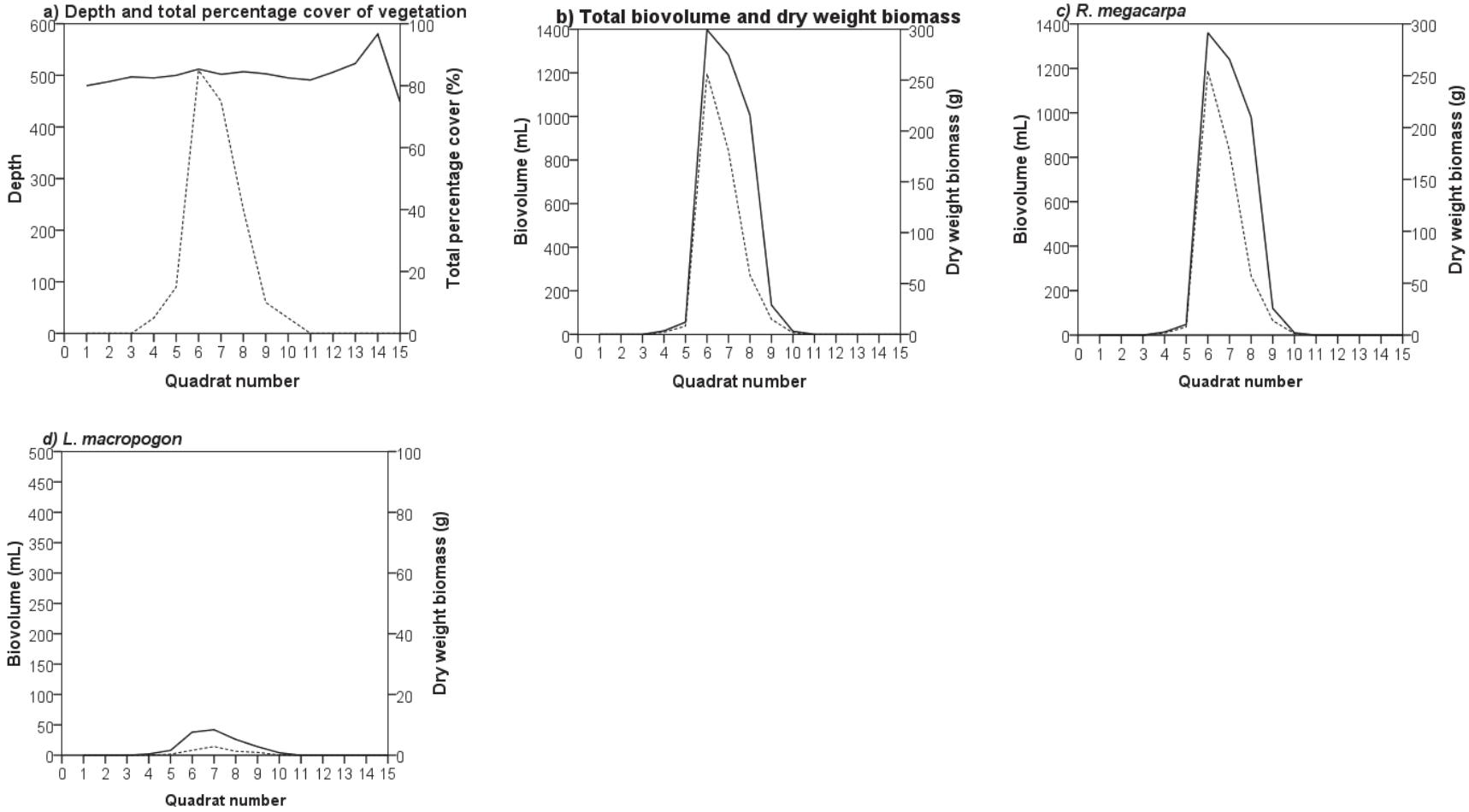


Figure A3.11 Round Lake Transect E. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon.*

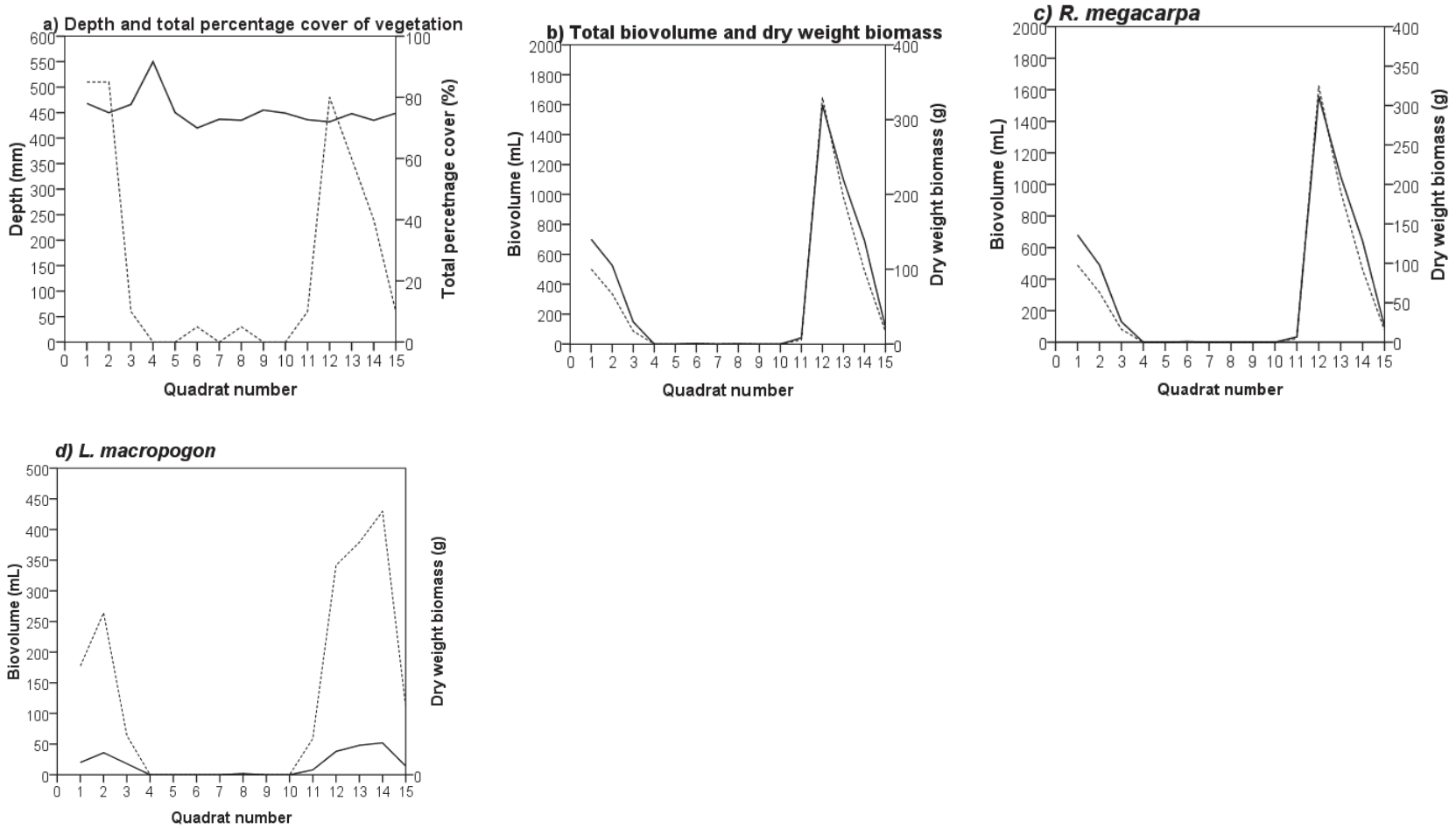


Figure A3.12 Round Lake Transect F. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon*

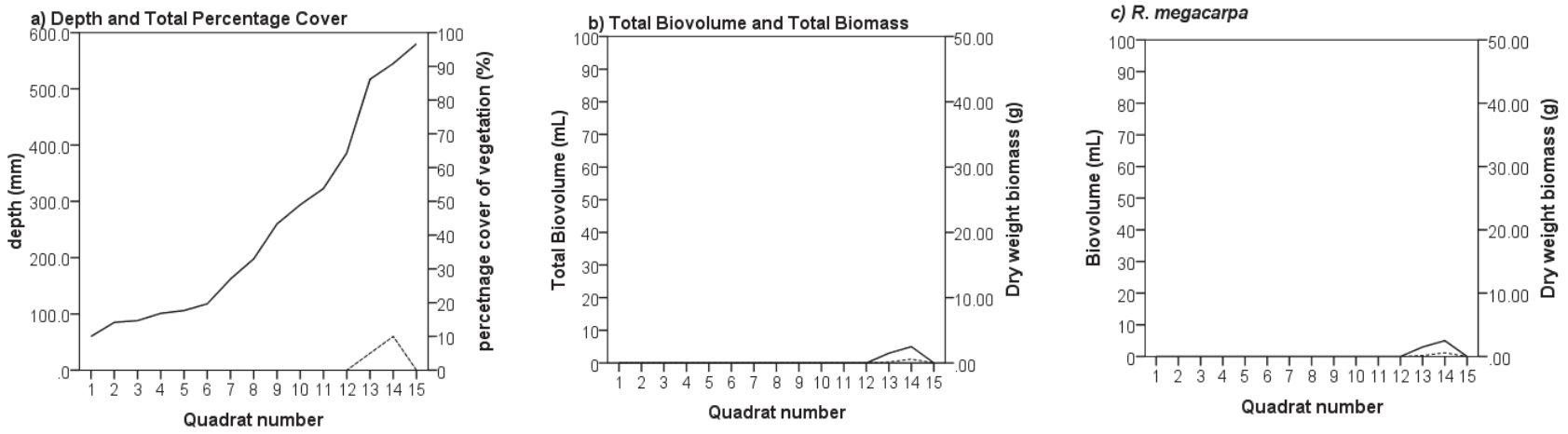


Figure A3.13 Woorinen North Transect A. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa*.

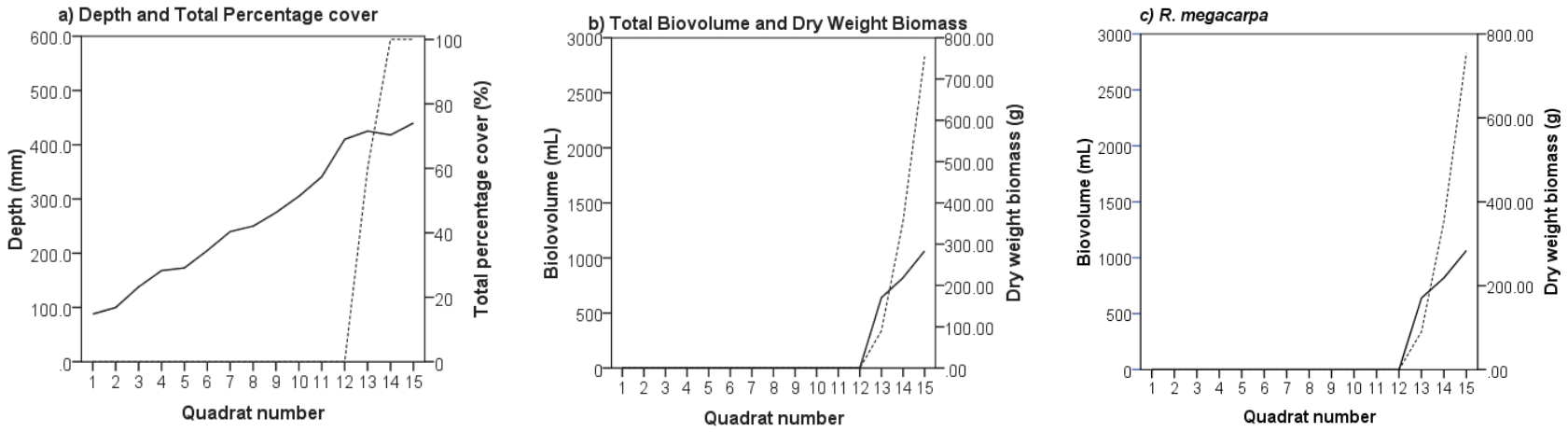


Figure A3.14 Woorinen North Transect B a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa*.

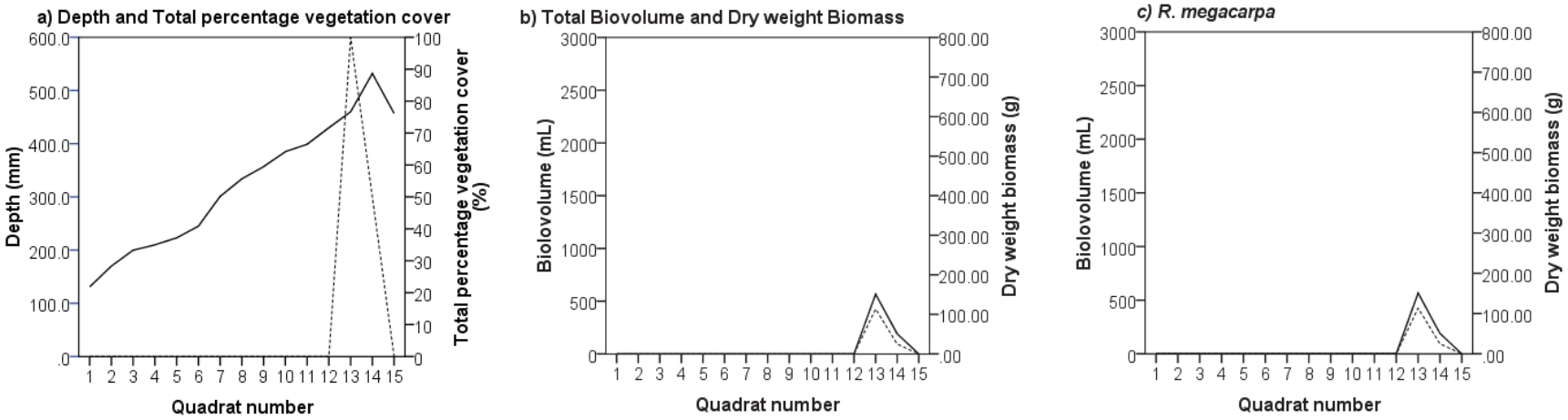


Figure A3.15 Woorinen North Transect C. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa*.

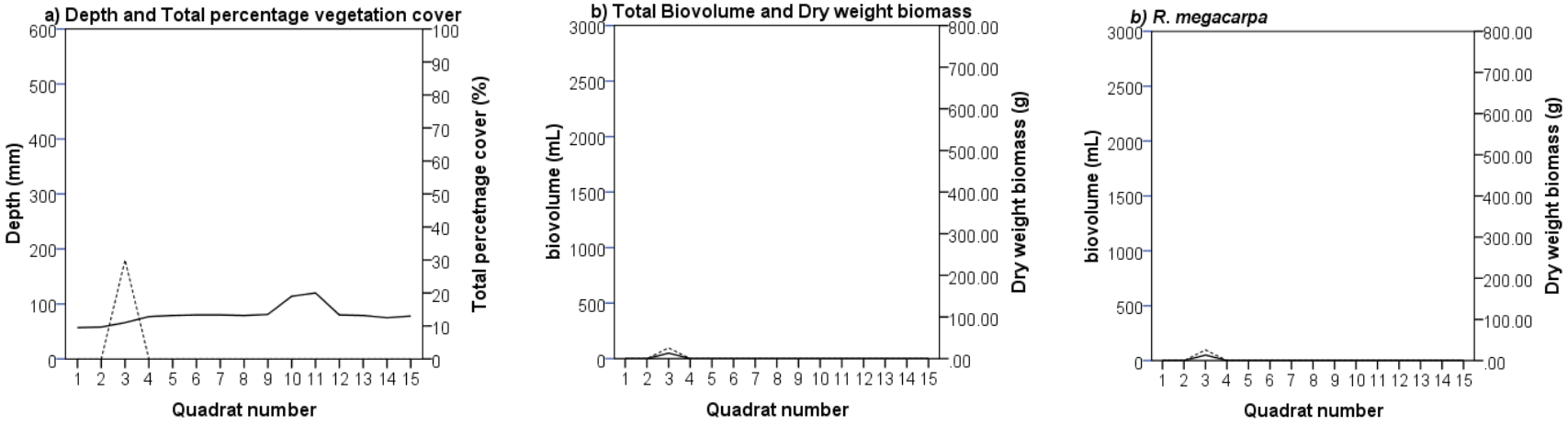


Figure A3.16 Woorinen North Transect D. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa*.

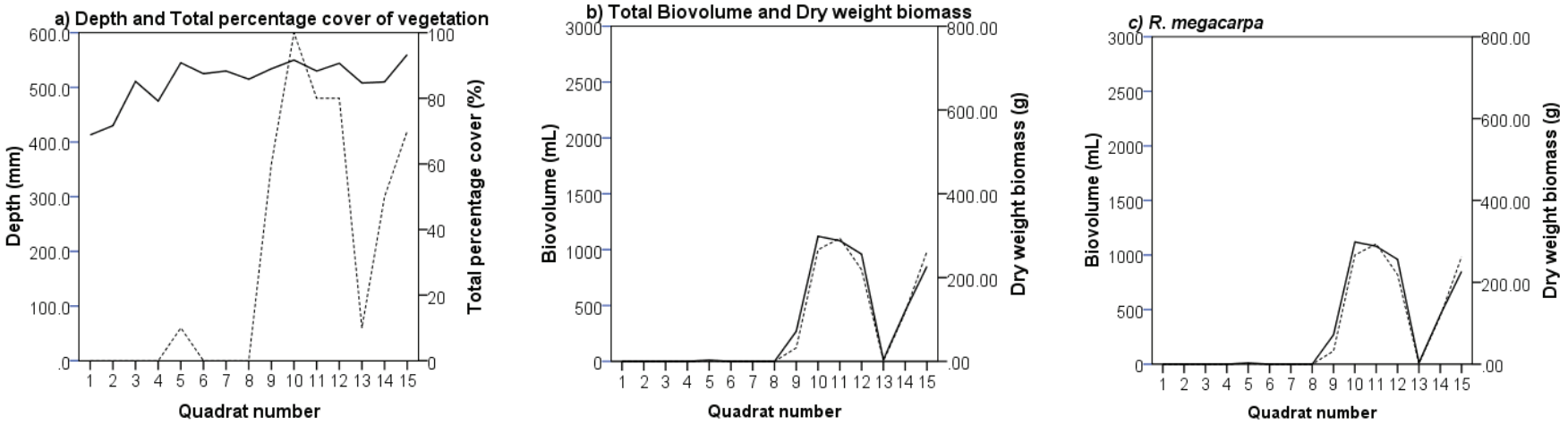


Figure A3.17 Woorinen North Transect E. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa*.

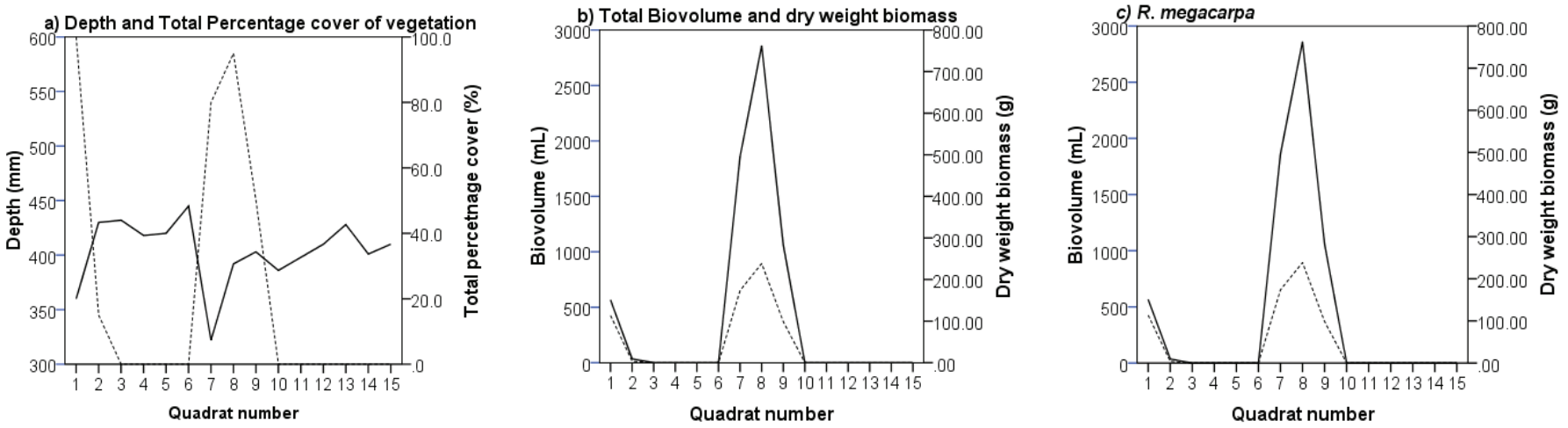


Figure A3.18 Woorinen North Transect F. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa*

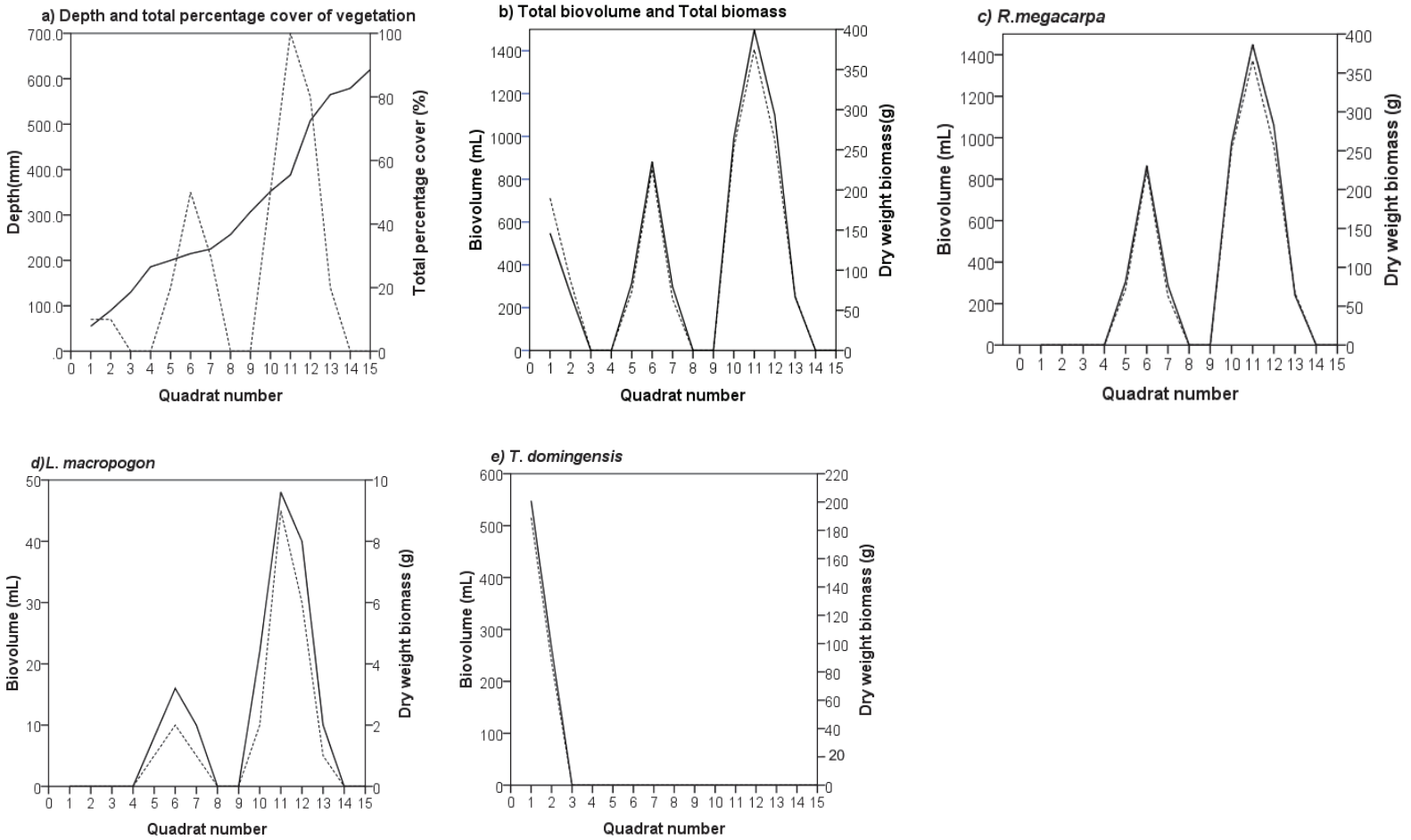


Figure A3.19 Lake Hawthorn Transect Aa) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), d), e) and f) shows biovolume (solid line) and biomass (dashed line) for *R. megacarpa, L. macropogon, J. acutus* and *T. domingensis.*

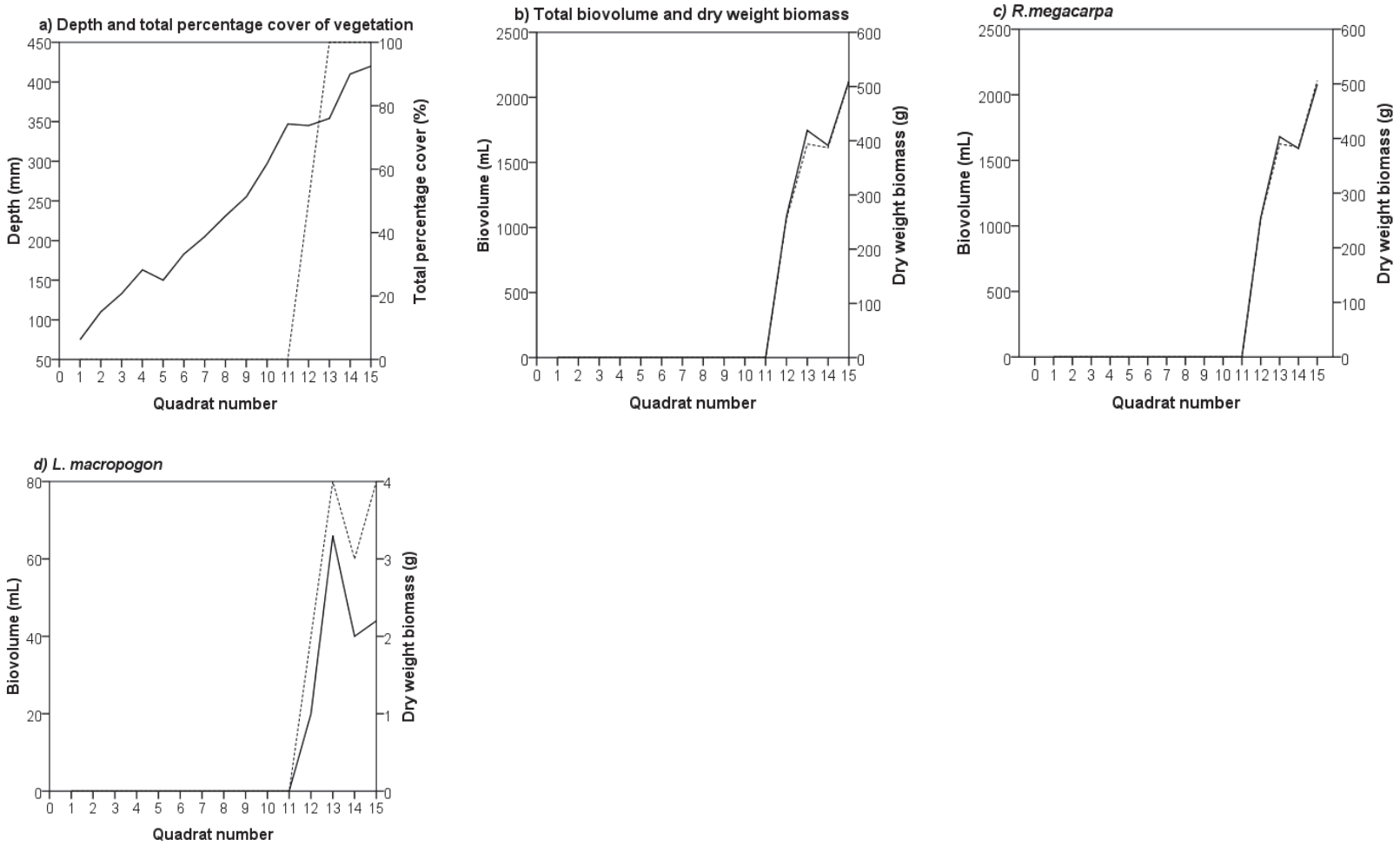


Figure A3.20 Lake Hawthorn Transect B. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon.*

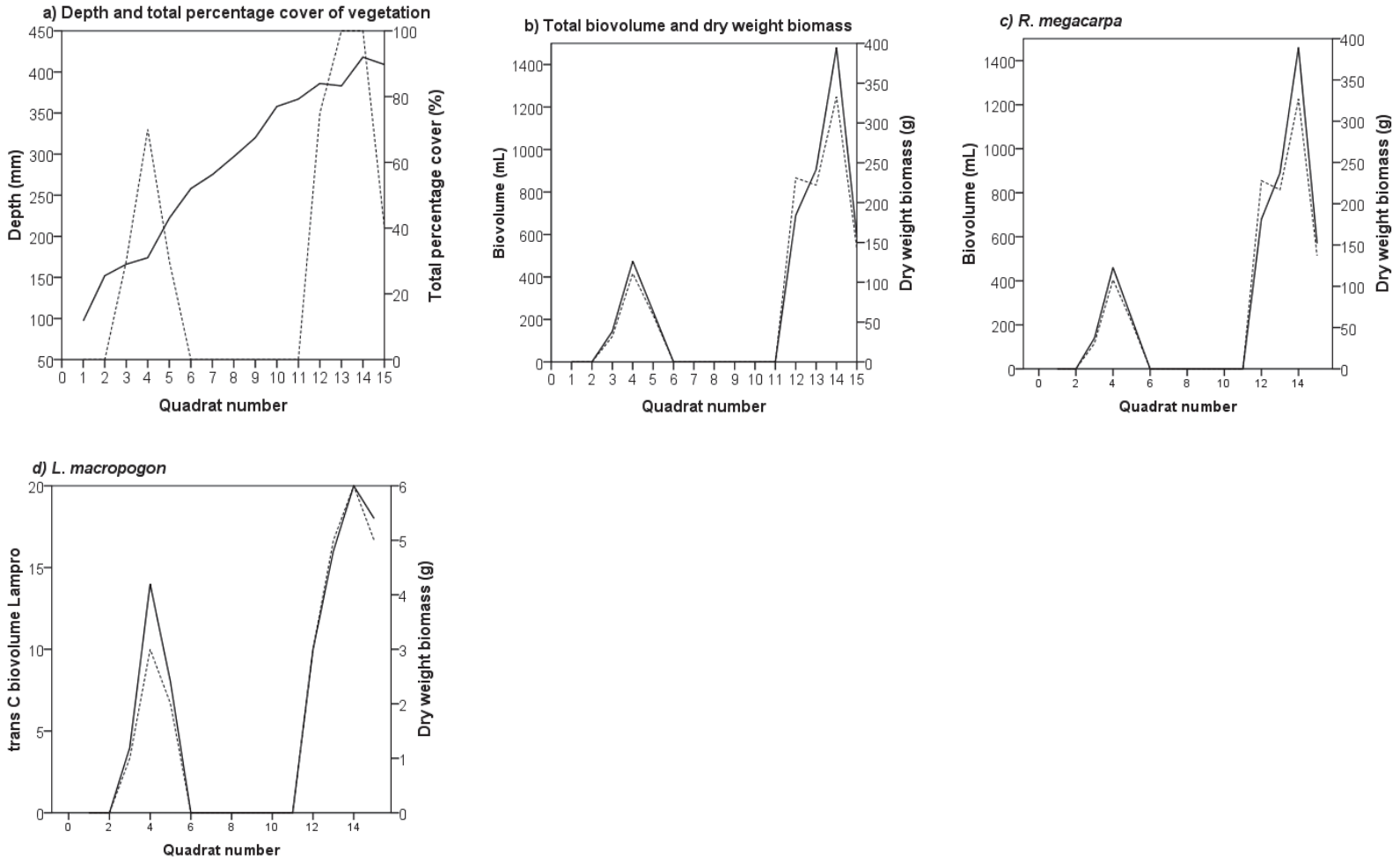


Figure A3.21 Lake Hawthorn Transect c. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon.*

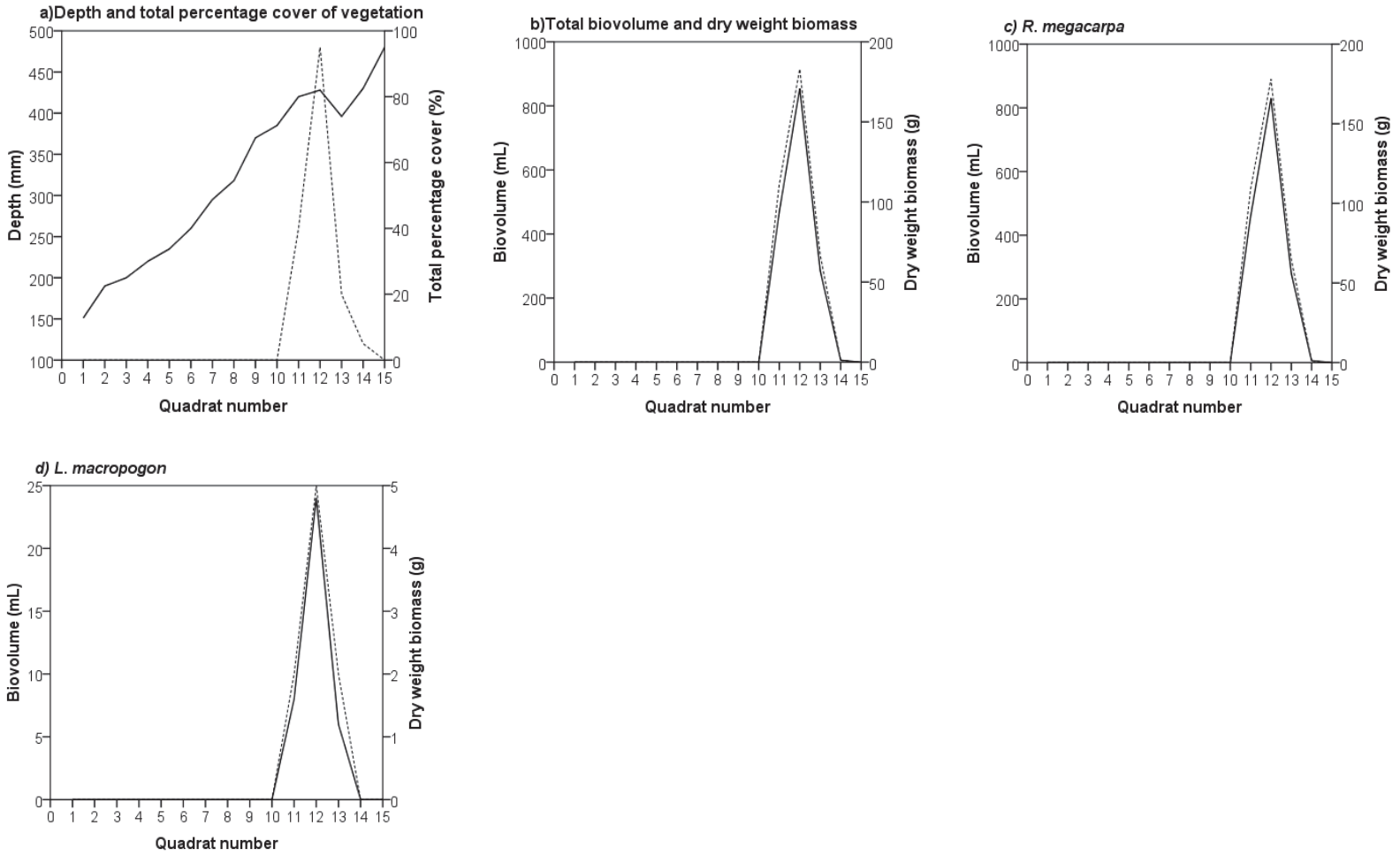


Figure A3.22 Lake Hawthorn Transect D. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon.*

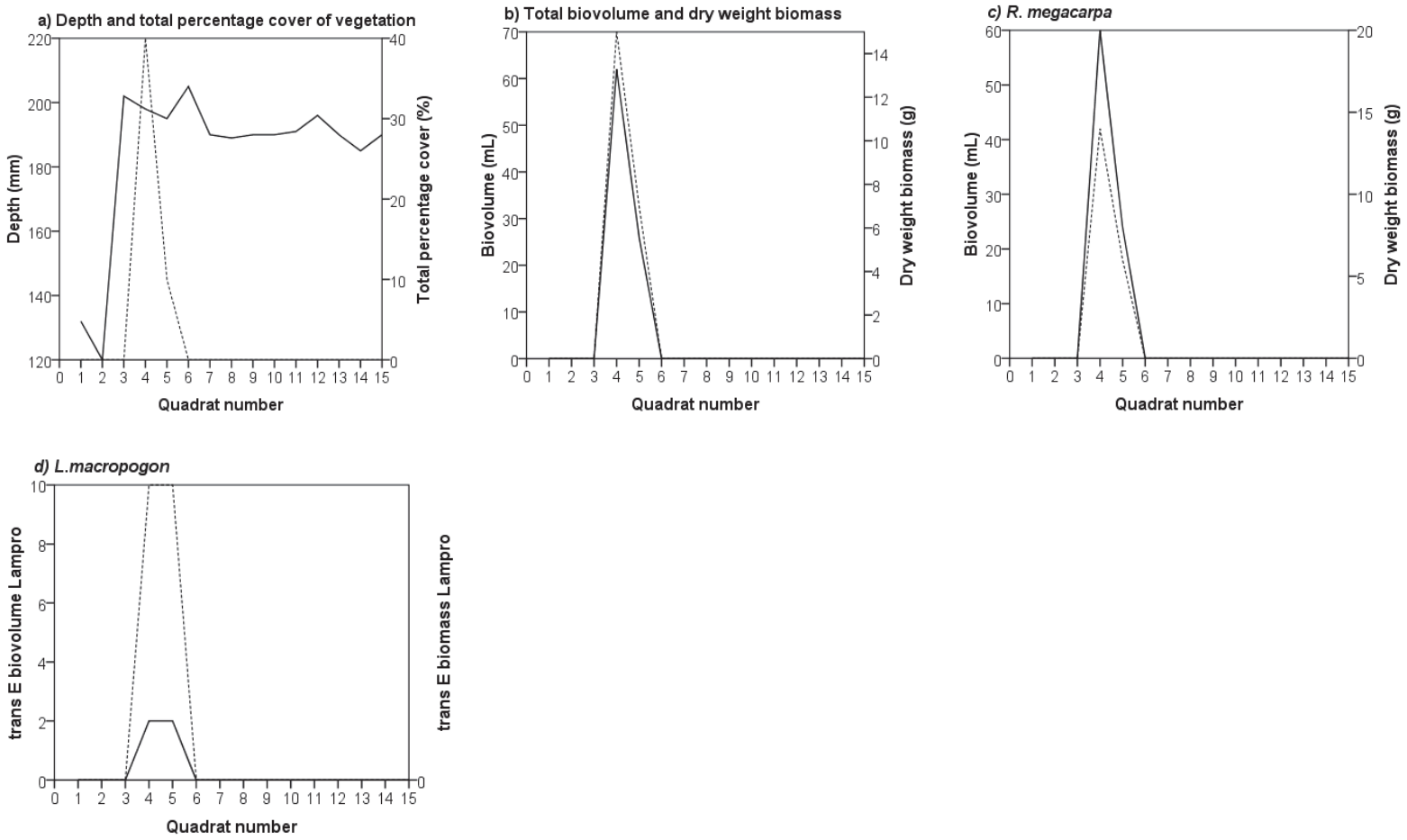


Figure A3.23 Lake Hawthorn Transect E. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon.*

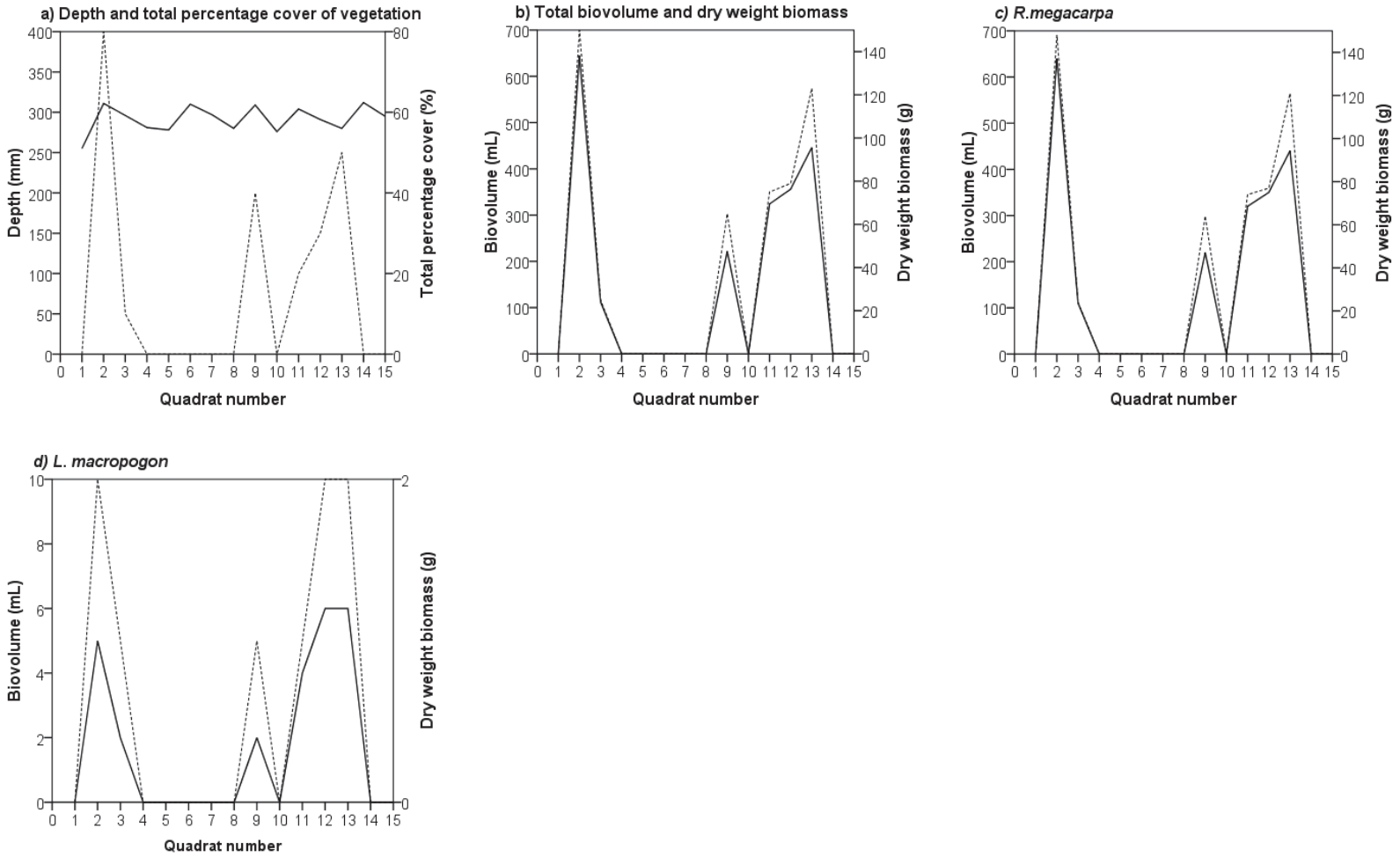


Figure A3.24 Lake Hawthorn Transect F. a) Depth of transect (solid line) and total percentage cover of vegetation (dashed line), b) Total biovolume (solid line), and total biomass (dashed line); c), and d) shows biovolume (solid line) and biomass (dashed line) *R. megacarpa,* and *L. macropogon.*

Appendix 4 Results of fish survey in wetlands of intermediate salinity in northwest Victoria – detailed results

Table A4.1 Fish measurements Round Lake

Murray Hardyhead *Craterocephalus fluviatilis*		**Eastern Mosquito fish** *Gambusia holbrooki*	
Length (mm)	**No. of fish**	**Length** (mm)	**No. of fish**
12		19	
13		20	
14		21	
15		22	
16		23	
17	3	24	
18	5	25	
19	4	26	1
20	3	27	
21	7	28	
22	6	29	
23	6	30	
24	12	31	
25	3	32	
26	5	33	
27	10	34	
28	9	35	
29	4	36	
30	3	37	
31	3	38	
32		39	
33	2	40	
34	2	41	
35	5	42	
36	1	43	
37	1	44	
38	2	45	
39	2	46	
40	1	47	
41		48	
42		49	
43		50	
44		51	
45		52	
46		53	
47		54	
48	1	55	
49		56	
		57	
		58	

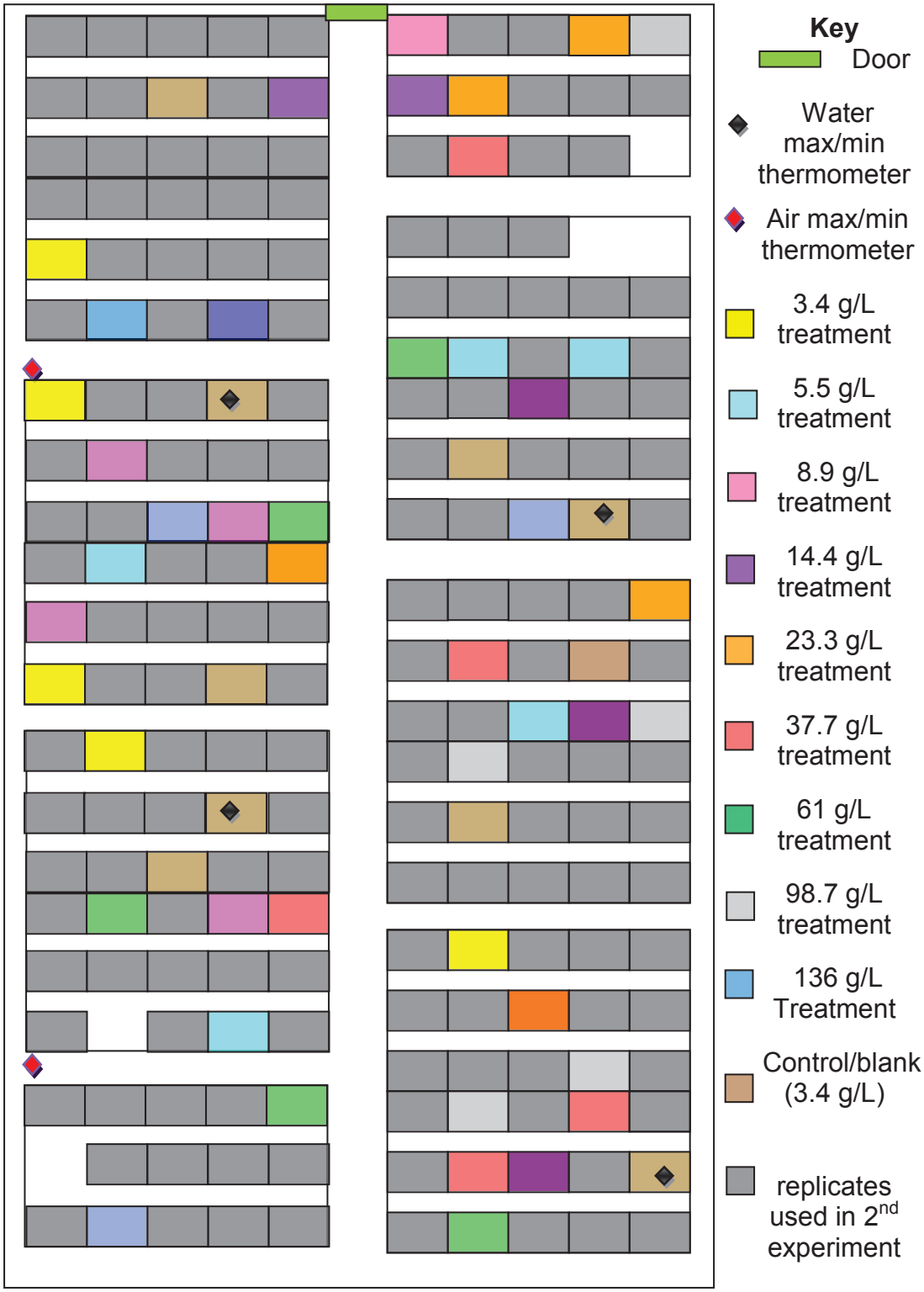
Table A4.2 Fish measurements Lake Woorinen North

Murray Hardyhead *Craterocephalus fluviatilis*		**Eastern Mosquito fish** *Gambusia holbrooki*		**Flat Headed Gudgeon** *Philypnodon grandiceps*	
Length (mm)	**No. of fish**	**Length** (mm)	**No. of fish**	**Length** (mm)	**No. of fish**
12		19		29	2
13		20		30	
14		21		31	
15		22		32	
16		23		33	
17		24		34	1
18		25	1	35	
19		26		36	
20		27		37	
21		28		38	
22		29		39	
23		30		40	
24		31		41	
25		32		42	
26		33		43	
27		34		44	
28		35		45	
29		36		46	
30		37		47	
31		38		48	
32	1	39		49	
33		40		50	
34		41		51	
35	1	42		52	
36		43		53	
37	1	44		54	
38		45		55	
39	1	46		56	
40		47		57	
41	2	48		58	
42	2	49		59	
43		50		60	
44		51		61	
45		52		62	
46		53		63	
47		54		64	
48		55		65	
49		56		66	1
		57			
		58			

Table A4.3 Fish measurements Lake Hawthorn

Murray Hardyhead *Craterocephalus fluviatilis*		**Eastern Mosquito fish** *Gambusia holbrooki*	
Length (mm)	**No. of fish**	**Length** (mm)	**No. of fish**
12	1	19	1
13	2	20	
14	9	21	
15	10	22	
16	8	23	1
17	15	24	2
18	16	25	2
19	11	26	
20	9	27	
21	8	28	1
22	4	29	
23	2	30	
24	1	31	
25		32	1
26	1	33	
27		34	
28		35	2
29		36	
30		37	
31		38	
32		39	
33		40	
34		41	
35		42	
36		43	
37		44	
38		45	
39		46	
40		47	
41		48	
42		49	
43		50	
44	1	51	
45		52	
46		53	
47		54	
48	1	55	
49	1	56	
		57	
		58	1

Appendix 5 Random location of replicates for the experiment investigating the effect of salinity on the egg and propagule bank of Lake Cullen



Appendix 6 QA/QC checks of the effectiveness of sugar flotation methods for separating invertebrates from the organic material (Chapter 3 experiments).

Table A9.1 QA/QC results of the effectiveness of the sugar flotation method on separating invertebrates from organic materials in >500µm size class for seedbank experiment 1.

Replicate	Invertebrate species	Number collected using sugar flotation method	Total number of individuals in sample	Percentage of individuals sorted by sugar floatation method
3.4 g/L A	*Mytilocypris henricae*	1532	1553	99%
	Australocypris spp.	62	62	100%
3.4 g/L D	*Mytilocypris henricae*	1574	1590	99%
	Australocypris spp.	94	100	94%
8.9 g/L C	*Mytilocypris henricae*	2152	2155	100%
	Australocypris spp.	538	538	100%
13.4 g/L D	*Mytilocypris henricae*	1159	1237	94%
	Australocypris spp.	53	53	100%
24.4 g/L D	*Mytilocypris henricae*	833	833	99%
	Australocypris spp.	216	216	100%

Appendix 7 Air temperature and water quality monitoring for experiment investigating the effect of salinity on the egg and propagule bank of Lake Cullen

Table A6.1 Maximum/minimum air temperature monitoring in the glasshouse

Date	Thermometer 1		Thermometer 2	
	Min (°C)	Max (°C)	Min (°C)	Max (°C)
23/11/05	9.9	31.4	8.7	33.1
30/11/05	11.5	34.5	9.9	36.2
7/12/05	13.3	34.5	11.6	40.5
14/12/05	12.3	31.1	10.5	33.5
21/12/05	12.7	28.5	11.1	32.1
28/12/05	13.1	31.3	11.3	32.9
3/1/06	14.7	32.1	13.6	36.3
11/1/06	12.3	28.5	9.9	36.3
18/1/06	13.5	28.8	11.5	33.0
25/1/06	13.3	34.3	11.7	38.3
1/2/06	12.9	31.3	11.8	32.0
8/2/06	13.1	27.5	11.9	31.1
15/2/06	12.3	33.3	10.7	39.3
22/2/06	12.2	30.7	10.9	33.5

Table A6.2 Maximum/minimum water temperature monitoring in the glasshouse

Date	Thermometer 1		Thermometer 2		Thermometer 3		Thermometer 4	
	Min (°C)	Max (°C)	Min (°C)	Max (°C)	Min (°C)	Max (°C)	Min (°C)	Max (°C)
23/11/05	13	26	15	23	13	26	15	23
30/11/05	15	28	17	26	15	23	14	29
7/12/05	11	29	18	29	16	22	15	32
14/12/05	15	27	17	24	16	21	16	25
21/12/05	16	26	18	21	16	22	14	26
28/12/05	15	27	18	25	16	25	15	26
3/1/06	16	30	18	28	18	26	17	29
11/1/06	15	27	18	26	15	23	15	28
18/1/06	16	26	18	24	18	25	16	25
25/1/06	15	32	19	29	17	26	16	31
1/2/06	17	30	17	23	18	29	17	30
8/2/06	18	27	20	25	19	26	16	27
15/2/06	16	29	17	20	18	23	15	24
22/2/06	17	27	16	25	16	24	17	28

Table A7.3 Salinity monitoring of replicates for the experiment investigating the effect of salinity on the egg and propagule bank of Lake Cullen, for al treatments (all measurements are in g/L)

Treatment	3.4 g/L					5.5 g/L					8.9 g/L				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
1	3.4	3.4	3.4	3.4	3.4	5.5	6.0	5.5	5.5	5.5	8.9	8.9	8.9	8.9	8.9
3	4.0	4.0	3.9	4.2	4.1	6.2	6.1	6.1	6.3	6.3	9.7	9.6	9.5	9.5	9.8
5	4.4	4.3	4.2	4.5	4.4	6.6	6.5	6.6	6.7	6.8	10.3	10.3	10.1	10.3	10.3
7	4.4	4.4	4.3	4.7	4.4	6.5	6.4	6.6	6.7	6.6	10.2	10.1	10.0	10.0	10.2
9	4.7	4.4	4.2	4.5	4.4	6.6	6.4	6.6	6.7	6.6	10.0	10.0	9.9	9.8	10.0
11	4.4	4.4	4.3	4.7	4.4	6.5	6.2	6.5	6.7	6.5	9.9	9.9	9.8	9.9	10.0
13	4.6	4.6	4.5	4.8	4.6	6.6	6.6	6.7	7.0	6.7	10.4	10.1	10.0	9.8	10.2
Mean	4.3	4.2	4.1	4.4	4.2	6.4	6.3	6.4	6.5	6.4	9.9	9.8	9.7	9.7	9.9
Standard deviation	± 0.4	± 0.4	± 0.3	± 0.4	± 0.4	± 0.4	± 0.2	± 0.4	± 0.5	± 0.4	± 0.5	± 0.4	± 0.4	± 0.4	± 0.4

Treatment	14.4 g/L					23.3 g/L					37.7 g/L				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
1	14.4	14.4	14.4	14.4	14.4	23.3	23.3	23.3	23.3	23.3	37.7	37.7	37.7	37.7	37.7
3	15.0	14.9	15.3	15.1	15.0	23.7	23.9	24.0	23.1	24.4	38.8	37.2	38.2	38.4	38.9
5	15.8	15.6	16.9	16.3	15.7	25.4	25.8	25.2	25.1	25.8	41.6	38.7	40.1	40.0	40.9
7	15.5	15.2	15.4	15.4	15.2	23.9	24.2	23.9	23.9	24.7	39.2	38.8	38.7	38.5	39.6
9	15.2	14.8	15.2	15.4	15.3	23.7	23.9	23.7	23.1	23.9	38.4	37.9	38.8	38.5	37.9
11	15.2	15.0	15.4	15.2	15.0	23.9	23.9	23.5	23.6	24.2	38.6	36.7	37.9	38.3	38.6
13	15.2	14.8	15.5	15.5	15.1	23.7	24.5	23.7	23.3	24.3	38.9	38.1	37.7	39.9	38.2
Mean	15.2	15.0	15.4	15.3	15.1	23.9	24.2	23.9	23.6	24.4	39.0	37.9	38.4	38.8	38.8
Standard deviation	± 0.4	± 0.3	± 0.7	± 0.5	± 0.4	± 0.6	± 0.7	± 0.6	± 0.7	± 0.7	± 1.1	± 0.7	± 0.8	± 0.8	± 1.0

Treatment	61 g/L					89.7 g/L					136 g/L				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
1	61.0	61.0	61.0	61.0	61.0	89.7	89.7	89.7	89.7	89.7	136.0	136.0	136.0	136.0	136.0
3	61.2	62.6	60.9	62.6	61.7	100.2	99.9	100.6	100.1	99.8	140.1	138.0	137.4	140.1	138.0
5	64.6	65.8	64.8	65.7	63.9	98.6	103.9	104.2	105.0	104.7	146.2	145.5	144.2	146.9	143.5
7	62.6	63.4	61.6	63.2	61.6	99.3	100.5	100.2	100.7	99.2	141.4	138.0	136.7	143.5	140.8
9	60.9	61.1	60.8	61.8	59.4	98.2	97.9	98.5	97.6	99.1	135.9	134.6	135.5	138.7	136.7
11	61.5	62.2	60.8	60.5	60.2	98.5	98.9	97.9	99.6	99.6	139.4	136.0	134.5	140.1	136.7
13	62.2	61.8	61.7	64.1	60.7	98.3	99.9	99.1	100.2	100.0	140.8	136.7	138.0	140.8	136.0
Mean	62.0	62.6	61.7	62.7	61.2	97.5	98.7	98.6	99.0	98.9	140.0	137.8	137.5	140.9	138.2
Standard deviation	± 1.2	± 1.5	± 1.3	± 1.7	± 1.3	± 3.3	± 4.1	± 4.1	± 4.3	± 4.2	± 3.3	± 3.3	± 3.0	± 3.2	± 2.6

Treatment	Control									
Week	1	2	3	4	5	6	7	8	9	10
1	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
3	3.5	3.5	3.5	3.5	3.6	3.5	3.6	3.5	3.5	3.5
5	3.7	3.7	3.7	3.6	3.6	3.7	3.8	3.6	3.8	3.8
7	3.6	3.6	3.7	3.5	3.6	3.5	3.6	3.5	3.5	3.6
9	3.5	3.5	3.5	3.5	3.4	3.5	3.5	3.4	3.5	3.6
11	3.6	3.5	3.5	3.5	3.6	3.5	3.5	3.6	3.5	4.2
13	3.7	3.5	3.6	3.6	3.8	3.6	3.6	3.4	3.5	3.7
Mean	3.6	3.5	3.6	3.5	3.6	3.5	3.6	3.5	3.5	3.7
Standard deviation	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.2

Appendix 8 Detailed regression analysis and ANOVA results for experiment investigating the effect of salinity on the egg and propagule bank of Lake Cullen

Table A7.1 Regression results for *Ruppia megacarpa*

Variable	Transformation	Model	Equation	R^2	df	F	P
Number of stems per replicate	√x	Linear	y = 13.67- 0.348x	0.915	1,28	143.27	**<0.001**
Biovolume	√x	Linear	y = 3.577- 0.084x	0.850	1,28	72.999	**<0.001**
Dry weight Biomass	√x	Linear	y = 1.084 – 0.025x	0.826	1,28	59.916	**<0.001**

Table A7.2 Tukey's test for number of *Ruppia megacarpa* stems

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L
3.4 g/L						
5.5 g/L	0.856					
8.9 g/L	0.925	.0316				
14.4 g/L	**0.020**	**0.001**	0.15			
23.3 g/L	**0.001**	**< 0.001**	**0.014**	0.867		
37.7 g/L	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	

Table A7.3 Tukey's test for *Ruppia megacarpa* biovolume

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L
3.4 g/L						
5.5 g/L	0.999					
8.9 g/L	0.839	0.650				
14.4 g/L	0.761	0.557	1.000			
23.3 g/L	0.141	0.071	0.730	0.812		
37.7 g/L	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	

Table A7.4 Tukey's test for total *Ruppia megacarpa* dry weight biomass

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L
3.4 g/L						
5.5 g/L	0.932					
8.9 g/L	0.809	0.279				
14.4 g/L	0.334	0.059	0.960			
23.3 g/L	0.443	0.090	0.999	1.000		
37.7 g/L	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	

Table A8.5 Regression results for *Lamprothamnium macropogan*

Variable	Transformation	Model	Equation	R^2	df	F	p
Biovolume	√x	Linear	y = 19.632-0.341x	0.599	1,28	15.693	**<0.001**
Dry weight Biomass	√x	Linear	y = 2.627-0.05x	0.534	1,28	11.171	**0.002**

Table A8.6 Tukey's test for biovolume of *Lamprothamnium macropogan*

	3400 mg/L	5500 mg/L	8900 mg/L	14400 mg/L	23300 mg/L	37700 mg/L
3400 mg/L						
5500 mg/L	0.999					
8900 mg/L	0.941	0.882				
14400 mg/L	1.000	0.998	0.984			
23300 mg/L	0.663	0.468	0.974	0.724		
37700 mg/L	0.074	**0.037**	0.302	0.092	0.729	

Table A8.9 Tukey's test for dry weight biomass of *Lamprothamnium macropogan*

	3400 mg/L	5500 mg/L	8900 mg/L	14400 mg/L	23300 mg/L	37700 mg/L
3400 mg/L						
5500 mg/L	0.932					
8900 mg/L	0.809	0.279				
14400 mg/L	0.334	0.059	0.960			
23300 mg/L	0.443	0.050	0.998	1.000		
37700 mg/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	

Table A8.10 Regression results of reproductive structures for *Lamprothamnium macropogon*

Variable	Transformation	Model	Equation	R^2	df	F	p
Percentage of total reproductive whorls containing immature oogonia	√x	Linear	y= 8.848 - 0.179x	0.695	1,28	26.100	**<0.001**
Percentage of total reproductive whorls containing mature oogonia	√x	Linear	y= 7.869 - 1.22x	0.442	1,28	6.995	**0.003**
Percentage of total reproductive whorls containing aborted spores	√x	Linear	y= 0.945 + 0.94x	0.576	1,28	13.901	**<0.001**

Table A8.11 Tukey's test for % of total reproductive whorls containing immature oogonia

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L
3.4 g/L						
5.5 g/L	1.000					
8.9 g/L	0.992	1.000				
14.4 g/L	0.543	0.362	0.245			
23.3 g/L	0.146	0.078	**0.046**	0.452		
37.7 g/L	**0.017**	**0.003**	**0.004**	0.443	0.910	

Table A8.12 Tukey's test for % of total reproductive whorls containing mature oogonia

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L
3.4 g/L						
5.5 g/L	1.000					
8.9 g/L	1.000	1.000				
14.4 g/L	0.461	0.657	0.536			
23.3 g/L	1.000	0.992	0.999	0.318		
37.7 g/L	0.227	0.129	0.182	**0.004**	0.345	

Table A8.13 Tukey's test for % of total reproductive whorls containing aborted oogonia

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L
3.4 g/L						
5.5 g/L	1.000					
8.9 g/L	1.000	1.000				
14.4 g/L	0.714	0766	0.845			
23.3 g/L	0.828	0.870	0.932	1.000		
37.7 g/L	**0.022**	**0.027**	**0.039**	0.346	0.348	

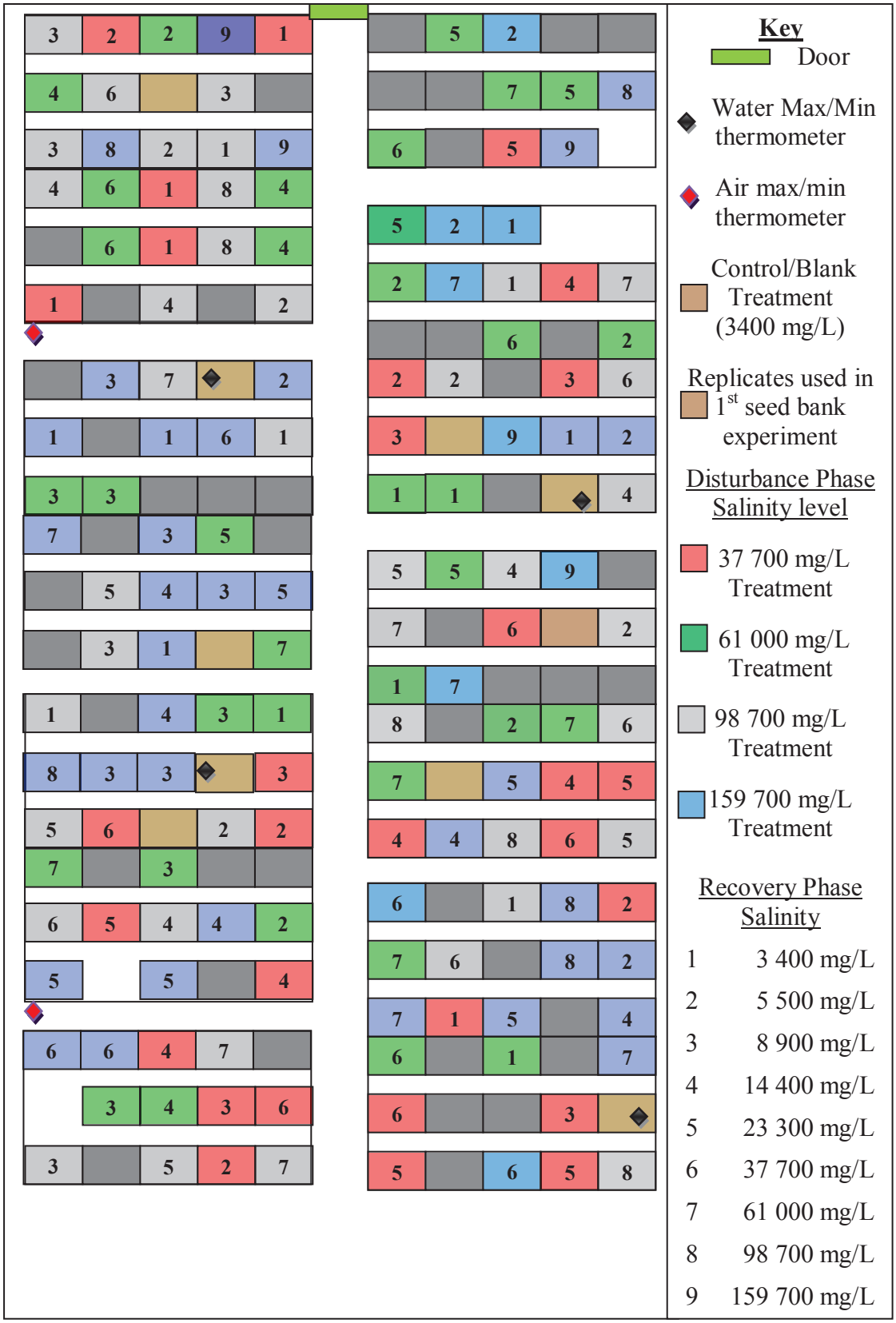
Table A8.14 Regression results for Invertebrate Species

Variable	Transformation	Model	Equation	R^2	df	F	p
Mytilocypris henricae	√x	linear	y=36.604-0.635x	0.637	1,28	19.149	**<0.001**
Australocypris spp.	√x	Cubic	$y=32.941-2.009x+0.088x^2-0.001x^3$	0.772	3,31	15.247	**<0.001**

Table A8.15 Tukey's test for total number of *Mytilocypris henricae*

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L
3.4 g/L						
5.5 g/L	0.840					
8.9 g/L	1.000	0.942				
14.4 g/L	1.000	0.911	1.000			
23.3 g/L	0.999	0.952	1.000	1.000		
37.7 g/L	**0.007**	**< 0.001**	**0.04**	**0.05**	**0.003**	

Appendix 9 Random location of replicates experiment investigating the effect of high salinity disturbances on the propagule bank of Lake Cullen



Appendix 10 QA/QC checks of the effectiveness of sugar flotation methods for separating invertebrates from the organic material (Chapter 4 experiments).

Replicate	Invertebrate species	Number collected using sugar flotation method	Total number of individuals in sample	Percentage of individuals sorted by sugar floatation method
89.7 g/L disturbance, 3.4 g /L recovery A	*Mytilocypris henricae*	27	27	100%
	Australocypris spp.	2	2	100%
136 g/L disturbance, 8.9 g /L recovery D	*Mytilocypris henricae*	99	103	96%
	Australocypris spp.	5	5	100%
89.7 g/L disturbance, 24.4 g /L recovery C	*Mytilocypris henricae*	90	93	97%
	Australocypris spp.	382	398	96%
136 g/L disturbance, 14.4 g /L recovery D	*Mytilocypris henricae*	90	93	97%
	Australocypris spp.	22	22	100%
136 g/L disturbance, 37.7 g /L recovery E	*Mytilocypris henricae*	46	46	100%
	Australocypris spp.	523	545	96%
61 g/L disturbance, 37.7 g /L recovery A	*Mytilocypris henricae*	9	9	100%
	Australocypris spp.	148	154	96%
37.7 g/L disturbance, 24.4 g /L recovery B	*Mytilocypris henricae*	488	513	95%
	Australocypris spp.	11	11	100%
61 g/L disturbance, 61 g /L recovery B	*Mytilocypris henricae*	0	0	N/A
	Australocypris spp.	1935	2016	96%
61 g/L disturbance, 3.4 g /L recovery E	*Mytilocypris henricae*	129	130	99%
	Australocypris spp.	17	18	95%
61 g/L disturbance, 5.5 g /L recovery A	*Mytilocypris henricae*	11	11	100%
	Australocypris spp.	308	324	95%

Appendix 11 Air temperature and water quality monitoring for experiment investigating the effects of high salinity disturbance on the propagule bank of Lake Cullen

Table A11.1 Maximum/minimum air temperature monitoring in the glasshouse

Date	Thermometer 1		Thermometer 2	
	Min (°C)	Max (°C)	Min (°C)	Max (°C)
Disturbance Phase				
23/11/05	9.9	31.4	8.7	33.1
30/11/05	11.5	34.5	9.9	36.2
7/12/05	13.3	34.5	11.6	40.5
14/12/05	12.3	31.1	10.5	33.5
21/12/05	12.7	28.5	11.1	32.1
28/12/05	13.1	31.3	11.3	32.9
3/1/06	14.7	32.1	13.6	36.3
11/1/06	12.3	28.5	9.9	36.3
18/1/06	13.5	28.8	11.5	33.0
25/1/06	13.3	34.3	11.7	38.3
1/2/06	12.9	31.3	11.8	32.0
8/2/06	13.1	27.5	11.9	31.1
15/2/06	12.3	33.3	10.7	39.3
22/2/06	12.2	30.7	10.9	33.5
Recovery Phase				
1/3/06	12.9	28.5	11.3	30.1
8/3/06	13.2	27.3	11.5	29.2
15/3/06	12.1	27.7	10.9	30.8
22/3/06	12.5	32.2	11.3	35.1
29/3/06	11.9	28.1	10.3	29.8
5/4/06	10.4	26.4	8.6	29.1
19/4/06	7.3	27.5	5.7	28.9
26/4/06	7.1	22.9	6.0	27.8
3/5/06	8.9	24.7	7.7	28.3
10/5/06	6.7	20.1	5.3	22.7
17/5/06	8.0	22.6	6.9	27.2

Table A11.2 Maximum/minimum water temperature monitoring in the glasshouse

Date	Thermometer 1		Thermometer 2		Thermometer 3		Thermometer 4	
	Min (°C)	Max (°C)	Min (°C)	Max (°C)	Min (°C)	Max (°C)	Min (°C)	Max (°C)
Disturbance Phase								
23/11/05	13	26	15	23	13	26	15	23
30/11/05	15	28	17	26	15	23	14	29
7/12/05	11	29	18	29	16	22	15	32
14/12/05	15	27	17	24	16	21	16	25
21/12/05	16	26	18	21	16	22	14	26
28/12/05	15	27	18	25	16	25	15	26
3/1/06	16	30	18	28	18	26	17	29
11/1/06	15	27	18	26	15	23	15	28
18/1/06	16	26	18	24	18	25	16	25
25/1/06	15	32	19	29	17	26	16	31
1/2/06	17	30	17	23	18	29	17	30
8/2/06	18	27	20	25	19	26	16	27
15/2/06	16	29	17	20	18	23	15	24
22/2/06	17	27	16	25	16	24	17	28
Recovery Phase								
1/3/06	25	26	25	27	20	24	21	27
8/3/06	18	27	18	25	16	23	16	31
15/3/06	12	25	11	23	11	24	12	23
22/3/06	14	24	13	23	15	24	13	23
29/3/06	10	23	10	22	10	21	9	23
5/4/06	13	24	13	24	12	20	11	27
19/4/06	10	24	8	24	11	24	9	28
26/4/06	10	23	11	23	10	20	8	25
3/5/06	11	24	14	21	12	22	11	22
10/5/06	8	19	10	15	9	16	8	20
17/5/06	11	22	10	21	9	22	7	25

Table A11.3 Salinity monitoring of replicates for the experiment investigating the effect of high salinity disturbances on the propagule bank of Lake Cullen, selected treatments (all measurements are in g/L)

Treatment	37.7g/L Disturbance/ 3.4 g/L recovery					37.7g/L Disturbance/ 5.5 g/L recovery					37.7g/L Disturbance/ 8.9 g/L recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7
3	37.6	40.1	38.9	39.2	38.9	37.9	38.4	38.6	39.0	38.8	38.2	38.4	38.4	38.4	38.4
5	40.1	38.8	40.5	40.7	40.2	40.5	40.5	41.0	40.1	40.9	39.8	41.0	41.3	40.5	41.3
7	38.1	39.2	38.8	39.3	38.8	39.6	39.3	38.9	39.3	39.4	39.0	38.7	39.8	39.7	39.2
9	37.4	38.9	40.7	38.5	37.1	39.6	37.6	38.4	38.4	38.6	38.5	38.0	38.3	38.2	38.1
11	37.1	38.8	38.3	38.1	37.8	38.4	38.3	38.3	37.7	38.2	37.2	37.9	38.1	38.8	37.9
13	37.7	38.1	38.3	38.7	38.3	37.9	37.8	39.5	37.8	37.8	38.1	38.5	37.9	38.1	39.2
Recovery Phase															
15	3.8	3.8	3.9	3.6	3.9	5.5	5.6	5.7	6.3	6.0	8.7	9.2	8.7	8.9	8.6
17	4.5	4.1	4.1	4.1	4.2	5.7	5.8	6.1	5.9	6.1	9.3	9.3	9.3	9.3	8.8
19	4.1	3.9	3.9	4.0	3.7	5.7	5.7	5.9	5.9	5.7	9.0	9.2	8.8	9.0	8.9
21	3.9	3.8	4.0	4.0	3.8	5.6	5.7	5.8	5.8	5.8	8.6	9.2	8.4	9.1	8.9
23	4.3	3.9	4.2	4.1	4.2	5.7	5.8	6.2	5.9	6.2	8.6	9.4	9.1	9.4	9.1
25	3.8	3.8	3.9	3.6	3.9	5.5	5.6	5.7	6.3	6.0	8.7	9.2	8.7	8.9	8.6
Mean disturbance phase	38.0	38.8	39.0	38.9	38.4	38.8	38.5	38.9	38.6	38.8	38.4	38.6	38.8	38.8	38.8
Mean recovery phase	4.1	3.9	4.0	3.9	4.0	5.6	5.7	5.9	6.0	6.0	8.8	9.3	8.8	9.1	8.8
Standard deviation disturbance phase	± 1.0	± 0.8	± 1.1	± 1.0	± 1.0	± 1.1	± 1.1	±1.1	± 0.9	± 1.1	± 0.9	±1.1	± 1.3	± 1.0	± 1.2
Standard deviation disturbance phase	± 0.3	± 0.1	± 0.1	± 0.2	± 0.2	± 0.1	± 0.1	±0.2	± 0.2	± 0.2	± 0.3	±0.1	± 0.3	± 0.2	± 0.2

Treatment	37.7g/L Disturbance/ 14.4 g/L recovery					37.7g/L Disturbance/ 23.3 g/L recovery					37.7g/L Disturbance/ 37.7 g/L recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.7
3	38.2	38.4	39.0	38.5	38.3	38.4	38.3	37.8	37.9	38.4	38.0	38.4	37.7	38.3	38.4
5	39.9	40.7	41.6	40.6	36.7	39.8	40.7	41.6	40.6	36.7	40.5	40.6	39.8	40.7	40.7
7	38.8	38.4	40.0	39.0	38.3	39.4	39.1	39.2	39.0	38.8	39.1	39.4	38.2	38.9	38.8
9	37.8	37.6	38.8	38.4	37.6	38.4	38.0	38.4	38.2	37.5	38.2	38.4	37.4	38.1	39.0
11	38.1	37.3	38.6	37.6	38.3	38.2	38.1	38.4	37.5	38.1	38.4	37.9	37.3	38.2	38.1
13	38.5	38.5	39.8	37.8	37.9	39.7	38.6	38.6	38.3	38.8	38.1	38.6	38.0	37.9	38.5
Recovery Phase															
15	14.5	15.4	14.1	15.3	16.2	24.0	22.6	23.3	23.6	23.3	36.7	37.0	37.1	36.7	34.5
17	14.3	15.1	14.8	16.6	16.6	23.1	22.4	23.3	23.0	21.8	37.1	36.7	35.5	35.8	34.8
19	14.6	15.7	14.8	16.4	16.9	23.3	22.6	23.5	23.4	22.2	37.0	36.7	36.6	36.3	34.8
21	14.3	16.3	15.0	16.3	16.5	23.3	22.6	23.3	23.3	22.5	37.1	37.0	36.4	36.2	35.1
23	14.3	15.5	14.8	16.5	16.7	23.5	22.3	23.4	23.2	22.3	36.9	36.6	36.7	36.0	34.9
25	14.5	15.4	14.1	15.3	16.2	24.0	22.6	23.3	23.6	23.3	36.7	37.0	37.1	36.7	34.5
Mean disturbance phase	38.4	38.4	39.4	38.5	37.8	38.8	38.6	38.8	38.5	38.0	38.6	38.7	38.0	38.5	38.7
Mean recovery phase	14.4	15.6	14.6	16.1	16.5	23.5	22.5	23.4	23.4	22.6	36.9	36.8	36.6	36.3	34.8
Standard deviation disturbance phase	±0.8	±1.1	±1.3	±1.0	±0.6	±0.8	±1.0	±1.3	±1.1	±0.8	±1.0	±1.0	±0.8	±1.0	±1.0
Standard deviation disturbance phase	±0.1	±0.4	±0.4	±0.6	±0.3	±0.4	±0.1	±0.1	±0.2	±0.6	±0.2	±0.2	±0.6	±0.4	±0.2

Treatment	61 g/L Disturbance/ 3.4 g/L recovery					61 g/L Disturbance/ 5.5 g/L recovery					61 g/L Disturbance/ 8.9 g/L recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0
3	62.8	61.8	62.4	62.3	62.5	61.8	61.5	61.5	62.5	61.3	62.8	62.6	61.9	63.2	61.6
5	66.7	63.9	64.0	65.3	65.8	64.6	64.6	60.9	66.8	64.2	66.8	65.1	66.4	66.7	64.0
7	62.6	63.4	61.6	63.2	61.6	63.6	61.7	61.3	63.3	63.1	61.8	60.7	59.7	63.1	60.6
9	61.9	63.0	60.8	61.8	59.4	61.6	61.1	61.2	61.5	60.6	60.8	60.2	62.5	61.5	60.5
11	62.7	60.6	60.7	61.7	61.3	60.5	60.5	58.1	61.5	59.8	61.7	60.6	60.9	61.9	59.6
13	62.2	60.3	60.9	60.4	60.7	61.3	61.3	58.1	64.5	60.9	63.7	63.0	62.9	62.3	61.2
Recovery Phase															
15	3.9	4.3	5.8	3.7	3.8	5.9	5.6	5.7	5.6	5.8	9.6	8.8	9.0	8.4	8.8
17	4.4	3.9	4.3	4.3	4.1	5.8	5.7	5.9	6.1	6.0	10.0	9.3	9.7	9.3	9.3
19	4.2	4.0	4.0	4.2	4.3	5.7	5.6	5.8	5.7	5.9	9.4	9.0	9.6	9.2	9.0
21	3.8	3.9	4.0	4.0	4.1	5.9	5.7	5.9	5.7	5.8	9.2	8.9	9.1	8.9	8.9
23	4.4	4.0	4.4	4.5	4.4	6.3	6.2	6.2	6.0	6.2	10.1	9.4	9.7	9.4	9.3
25	3.9	4.3	5.8	3.7	3.8	5.9	5.6	5.7	5.6	5.8	9.6	8.8	9.0	8.4	8.8
Mean disturbance phase	62.8	62.0	61.6	62.2	61.8	62.1	61.7	60.3	63.0	61.6	62.7	61.9	62.2	62.8	61.2
Mean recovery phase	4.1	4.1	4.7	4.1	4.1	5.9	5.7	5.9	5.8	5.9	9.7	9.0	9.4	8.9	9.0
Standard deviation disturbance phase	±1.8	±1.4	±1.2	±1.6	±2.0	±1.5	±1.3	±1.5	±2.1	±1.5	±2.1	±1.8	±2.1	±1.9	±1.4
Standard deviation disturbance phase	±0.3	±0.2	±0.9	±0.3	±0.2	±0.2	±0.2	±0.2	±0.2	±0.2	±0.3	±0.3	±0.4	±0.4	±0.2

Treatment	61 g/L Disturbance/ 14.4 g/L recovery					61 g/L Disturbance/ 23.3 g/L recovery					61 g/L Disturbance/ 37.7 g/L Recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0
3	62.8	61.8	62.4	62.3	62.5	61.8	61.5	61.5	62.5	61.3	62.8	62.6	61.9	63.2	61.6
5	66.7	63.9	64.0	65.3	65.8	64.6	64.6	60.9	66.8	64.2	66.8	65.1	66.4	66.7	64.0
7	62.6	63.4	61.6	63.2	61.6	63.6	61.7	61.3	63.3	63.1	61.8	60.7	59.7	63.1	60.6
9	61.9	63.0	60.8	61.8	59.4	61.6	61.1	61.2	61.5	60.6	60.8	60.2	62.5	61.5	60.5
11	62.7	60.6	60.7	61.7	61.3	60.5	60.5	58.1	61.5	59.8	61.7	60.6	60.9	61.9	59.6
13	62.2	60.3	60.9	6.4	60.7	61.3	61.3	58.1	64.5	60.9	63.7	63.0	62.9	62.3	61.2
Recovery Phase															
15	14.6	14.5	15.0	14.3	15.1	22.6	23.7	23.5	2.5	23.4	37.1	36.3	36.0	37.1	37.7
17	15.2	15.0	15.2	14.8	15.2	22.0	24.5	24.4	22.6	20.4	37.9	35.8	35.6	37.9	38.5
19	15.2	14.9	15.3	15.0	15.3	22.6	23.3	24.5	23.0	20.5	36.4	35.8	35.8	38.4	37.9
21	15.4	15.1	15.2	14.7	15.3	23.0	23.9	24.4	23.2	21.3	36.8	35.8	35.6	38.1	38.0
23	15.3	15.0	15.4	15.0	15.4	22.4	24.6	24.5	23.3	20.4	38.1	36.2	35.8	37.9	38.6
25	14.6	14.5	15.0	14.3	15.1	22.6	23.7	23.5	2.5	23.4	37.1	36.3	36.0	37.1	37.7
Mean disturbance phase	62.8	62.0	61.6	54.5	61.8	62.1	61.7	60.3	63.0	61.6	62.7	61.9	62.2	62.8	61.2
Mean recovery phase	15.1	14.8	15.2	14.7	15.2	22.5	24.0	24.1	16.2	21.6	37.2	36.0	35.8	37.8	38.1
Standard deviation disturbance phase	±1.8	±1.4	±1.2	±21.3	±2.0	±1.5	±1.3	±1.5	±2.1	±1.5	±2.1	±1.8	±2.1	±1.9	±1.4
Standard deviation recovery phase	±0.4	±0.3	±0.2	±0.3	±0.1	±0.3	±0.5	±0.5	±10.6	±1.5	±0.7	±0.3	±0.2	±0.5	±0.4

Treatment	61 g/L Disturbance/ 61 g/L recovery					89.7 g/L Disturbance/ 3.4 g/L recovery					89.7 g/L Disturbance/ 5.5 g/L recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0
3	62.8	61.9	61.5	62.8	63.0	60.6	61.5	62.6	61.9	61.9	61.9	61.5	62.2	61.9	61.3
5	65.1	64.9	65.8	65.3	64.3	63.4	66.4	66.1	64.9	63.2	65.8	63.0	65.5	64.9	65.2
7	63.6	62.5	62.6	64.2	61.1	62.9	62.8	61.1	62.4	61.7	61.3	62.4	62.2	62.6	61.1
9	61.4	60.7	61.2	62.5	59.6	62.4	61.5	60.3	60.4	60.5	60.3	61.2	61.0	60.8	60.1
11	61.3	60.5	61.1	60.4	59.7	60.5	61.2	60.9	61.1	58.8	61.6	59.2	61.2	61.0	61.7
13	62.5	62.3	63.6	60.1	66.5	61.3	62.6	61.3	61.3	60.2	60.2	59.6	61.5	60.6	62.0
Recovery Phase															
15	61.5	61.7	60.9	60.2	60.5	3.3	3.3	3.5	3.7	3.8	6.0	5.7	5.4	5.6	5.6
17	60.3	60.0	59.3	59.0	58.5	4.4	4.3	4.3	4.4	4.1	6.7	5.9	5.8	6.0	6.0
19	59.4	60.9	59.4	59.6	59.0	4.0	3.9	4.1	4.4	4.1	6.1	5.8	5.5	5.9	5.8
21	60.8	59.6	59.6	59.9	58.4	3.9	3.9	4.0	4.1	4.1	5.9	5.6	5.8	5.6	5.7
23	60.9	61.6	59.9	60.5	59.7	4.6	4.2	4.3	4.5	4.6	6.3	6.0	6.3	6.1	6.4
25	61.5	61.7	60.9	60.2	60.5	3.3	3.3	3.5	3.7	3.8	6.0	5.7	5.4	5.6	5.6
Mean disturbance phase	62.5	62.0	62.4	62.3	62.2	61.7	62.4	61.9	61.9	61.0	61.7	61.1	62.1	61.8	61.8
Mean recovery phase	60.7	60.9	60.0	59.9	59.4	3.9	3.8	4.0	4.1	4.1	6.2	5.8	5.7	5.8	5.9
Standard deviation disturbance phase	±1.5	±1.5	±1.8	±2.0	±2.6	±1.2	±1.9	±2.0	±1.5	±1.4	±1.9	±1.4	±1.6	±1.5	±1.6
Standard deviation recovery phase	±0.8	±0.9	±0.7	±0.5	±0.9	±0.5	±0.4	±0.4	±0.4	±0.3	±0.3	±0.1	±0.3	±0.2	±0.3

Treatment	89.7 g/L Disturbance/ 8.9 g/L recovery					89.7 g/L Disturbance/ 14.4 g/L recovery					89.7 g/L Disturbance/ 23.3 g/L Recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	61.0	61.0	61.0	61.0	61.0	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7
3	62.2	62.3	61.4	61.9	61.8	100.7	101.0	100.9	101.7	102.8	100.6	101.4	99.6	101.8	100.7
5	65.8	66.6	63.9	66.1	64.5	107.7	105.7	104.7	102.4	107.5	106.2	103.2	105.5	105.8	103.6
7	63.4	60.5	62.1	61.7	60.9	99.3	99.9	100.6	101.0	103.8	99.5	101.0	101.0	101.7	99.3
9	61.5	59.7	61.3	60.5	60.9	98.2	97.9	98.5	97.6	99.1	99.3	98.9	97.9	98.9	97.7
11	61.3	61.0	60.9	61.1	60.6	99.3	98.9	97.9	97.9	100.6	98.5	97.9	97.8	99.8	98.0
13	62.0	62.8	61.2	61.1	61.2	100.1	100.2	100.9	97.9	100.4	100.4	98.8	99.5	101.9	99.3
Recovery Phase															
15	9.2	8.7	9.1	9.5	9.4	16.2	16.0	14.9	15.1	15.0	23.0	22.8	22.8	23.5	22.7
17	9.7	9.5	9.6	9.5	10.1	16.7	16.3	14.9	15.2	15.9	23.7	23.4	22.0	23.9	23.6
19	9.1	9.0	9.2	9.0	9.5	16.4	16.4	15.0	14.9	15.8	23.9	23.1	22.2	23.9	23.8
21	8.7	9.0	8.9	9.1	9.1	16.5	16.6	14.8	15.0	15.8	23.7	23.1	22.0	23.9	23.7
23	10.2	9.5	9.9	10.2	10.4	16.5	16.5	15.0	15.3	16.0	23.8	23.3	20.8	23.9	23.5
25	9.2	8.7	9.1	9.5	9.4	16.2	16.0	14.9	15.1	15.0	23.0	22.8	22.8	23.5	22.7
Mean disturbance phase	62.5	62.0	61.7	61.9	61.6	100.6	100.3	100.3	99.6	101.8	100.5	100.0	100.0	101.2	99.6
Mean recovery phase	9.4	9.1	9.3	9.5	9.7	16.4	16.3	14.9	15.1	15.6	23.5	23.1	22.1	23.8	23.3
Standard deviation disturbance phase	±1.7	±2.3	±1.1	±1.9	±1.4	±3.3	±2.6	±2.3	±2.0	±3.1	±2.7	±1.9	±2.7	±2.4	±2.0
Standard deviation recovery phase	±0.5	±0.4	±0.4	±0.4	±0.5	±0.2	±0.3	±0.1	±0.1	±0.5	±0.4	±0.2	±0.7	±0.2	±0.5

Treatment	89.7 g/L Disturbance/ 37.7 g/L recovery					89.7 g/L Disturbance/ 61 g/L recovery					89.7 g/L Disturbance/ 89.7 g/L recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7
3	104.1	100.6	102.4	100.8	101.4	99.7	99.8	100.6	103.6	101.0	99.6	101.6	100.8	100.7	100.0
5	104.7	105.9	103.6	105.0	102.7	107.0	104.7	107.1	106.8	103.6	105.5	106.8	104.4	105.8	101.9
7	101.6	100.3	100.0	100.2	100.8	99.6	99.0	101.6	97.7	99.4	100.0	102.5	100.8	101.0	97.8
9	99.6	97.9	98.3	100.5	97.9	98.5	98.7	98.8	99.6	97.6	98.1	98.9	97.8	96.4	98.9
11	97.4	97.8	97.4	100.6	97.4	98.2	98.5	99.3	98.9	98.4	99.5	99.1	97.2	99.5	97.4
13	97.8	100.4	97.5	97.1	99.0	98.0	101.3	98.6	100.0	97.5	99.1	98.0	100.2	99.1	97.5
Recovery Phase															
15	36.9	37.5	38.6	36.2	36.7	59.9	61.8	60.9	61.5	60.6	99.3	97.6	99.1	98.6	98.7
17	37.0	37.7	36.7	35.4	37.0	61.0	59.2	59.9	59.2	60.8	97.0	97.0	95.7	94.2	97.3
19	37.3	38.2	36.5	35.7	37.5	61.7	59.8	60.1	59.4	60.7	97.5	96.8	96.2	94.9	97.9
21	37.2	37.7	36.9	35.5	37.5	60.9	60.2	60.1	59.9	60.9	97.4	97.2	96.2	95.2	98.1
23	37.5	37.7	36.8	36.0	37.5	61.7	59.8	60.0	59.7	61.1	97.7	97.6	96.6	95.7	98.3
25	36.9	37.5	38.6	36.2	36.7	59.9	61.8	60.9	61.5	60.6	99.3	97.6	99.1	98.6	98.7
Mean disturbance phase	100.6	100.2	99.7	100.4	99.7	100.0	100.1	100.7	100.8	99.5	100.1	100.8	100.0	100.2	98.9
Mean recovery phase	37.1	37.7	37.4	35.8	37.2	60.9	60.4	60.3	60.2	60.8	98.0	97.3	97.2	96.2	98.2
Standard deviation disturbance phase	±3.0	±2.8	±2.4	±2.4	±2.0	±3.2	±2.3	±3.0	±3.3	±2.2	±2.5	±3.1	±2.4	±2.9	±1.6
Standard deviation recovery phase	±0.2	±0.3	±1.0	±0.4	±0.4	±0.8	±1.1	±0.5	±1.0	±0.2	±1.0	±0.4	±1.5	±1.9	±0.5

Treatment	136 g/L Disturbance/ 3.4 g/L recovery					136 g/L Disturbance/ 5.5 g/L recovery					136 g/L Disturbance/ 8.9 g/L Recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7
3	99.8	99.6	99.8	100.3	101.7	104.1	98.9	101.0	100.2	101.4	100.5	102.5	96.8	103.2	100.6
5	104.2	102.4	105.6	107.3	106.8	108.4	101.9	106.1	105.3	107.2	104.2	104.4	100.1	104.4	105.2
7	99.4	98.3	101.9	99.1	101.3	101.9	97.8	100.0	101.9	102.2	100.1	101.0	103.5	101.3	99.5
9	96.7	96.3	99.0	96.4	98.0	100.0	97.0	98.2	99.1	98.9	99.6	98.8	95.5	99.3	98.5
11	97.9	96.8	98.3	97.5	99.6	99.1	96.2	97.9	100.0	99.6	98.6	99.1	94.5	99.0	97.4
13	98.9	96.2	96.1	99.5	98.0	100.4	97.0	97.3	100.3	98.9	99.4	100.6	95.7	100.8	99.1
Recovery Phase															
15	3.4	3.7	3.8	3.6	3.8	6.0	5.4	5.7	5.5	5.7	9.4	8.7	9.0	8.9	9.1
17	4.4	4.1	4.0	4.1	4.6	5.9	6.0	5.7	6.0	5.7	9.8	10.7	9.5	10.5	10.2
19	4.3	4.0	3.8	4.0	4.2	5.5	5.8	5.7	5.6	5.6	9.2	10.8	9.1	9.8	10.0
21	3.9	3.9	3.7	3.6	4.1	5.9	5.8	5.7	5.5	5.6	9.2	9.4	8.8	9.5	9.0
23	4.5	4.6	3.9	4.1	4.3	6.3	6.0	6.5	5.9	5.6	9.2	9.9	10.1	10.1	9.9
25	3.4	3.7	3.8	3.6	3.8	6.0	5.4	5.7	5.5	5.7	9.4	8.7	9.0	8.9	9.1
Mean disturbance phase	99.4	98.3	99.9	99.8	100.6	101.8	98.2	99.9	100.8	101.0	100.2	100.7	97.8	101.0	99.9
Mean recovery phase	4.0	4.0	3.8	3.8	4.1	5.9	5.7	5.8	5.7	5.7	9.4	9.7	9.3	9.6	9.6
Standard deviation disturbance phase	±2.4	±2.2	±3.0	±3.5	±3.1	±3.4	±1.9	±3.0	±2.2	±3.1	±1.9	±2.1	±3.2	±2.2	±2.6
Standard deviation recovery phase	±0.5	±0.3	±0.1	±0.3	±0.3	±0.3	±0.3	±0.3	±0.2	±0.1	±0.2	±0.9	±0.5	±0.6	±0.5

Treatment	136 g/L Disturbance/ 14.4 g/L recovery					136 g/L Disturbance/ 23.3 g/L recovery					136 g/L Disturbance/ 37.7 g/L Recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0
3	138.7	135.3	140.8	139.4	138.7	128.6	138.0	130.7	150.6	136.0	138.0	138.0	150.0	138.0	136.0
5	144.2	143.5	147.6	146.2	146.9	128.2	144.2	136.7	143.5	144.2	146.2	145.5	146.9	144.8	144.8
7	141.4	138.0	136.7	143.5	140.8	140.8	136.7	141.4	139.4	138.0	129.4	138.0	142.1	139.4	135.3
9	135.7	133.4	140.1	135.2	135.9	126.9	134.8	127.2	136.0	133.8	135.1	136.0	134.6	136.0	134.9
11	136.0	134.6	140.1	136.0	137.4	127.2	137.4	136.0	135.9	133.1	136.0	136.0	135.4	137.4	135.3
13	136.7	140.1	137.4	138.0	136.0	136.0	140.1	135.7	135.0	134.2	138.0	140.8	142.8	139.4	138.0
Recovery Phase															
15	17.3	15.7	15.6	15.2	15.1	22.9	23.1	22.4	23.7	23.2	36.4	37.1	37.2	37.6	37.3
17	16.9	16.6	16.6	16.5	15.8	22.2	23.7	23.8	23.9	24.3	37.1	36.5	37.4	37.6	38.4
19	17.3	15.5	16.7	16.3	16.3	22.6	23.9	23.7	24.0	24.2	37.1	36.9	36.9	37.7	38.4
21	16.7	15.3	16.5	16.4	16.3	22.6	23.8	23.8	24.1	24.1	37.5	37.4	38.3	37.8	37.0
23	17.7	16.6	16.7	16.4	16.4	22.6	23.8	21.4	23.9	23.7	37.3	36.7	35.6	38.0	38.7
25	17.3	15.7	15.6	15.2	15.1	22.9	23.1	22.4	23.7	23.2	36.4	37.1	37.2	37.6	37.3
Mean disturbance phase	138.4	137.3	139.8	139.2	138.8	132.0	138.2	134.8	139.5	136.5	137.0	138.6	141.1	138.7	137.2
Mean recovery phase	17.2	15.9	16.3	16.0	15.8	22.6	23.6	22.9	23.9	23.8	37.0	37.0	37.1	37.7	37.9
Standard deviation disturbance phase	±3.3	±3.5	±3.9	±4.2	±4.0	±5.5	±3.1	±4.6	±5.7	±3.8	±5.0	±3.5	±6.0	±3.0	±3.5
Standard deviation recovery phase	±0.4	±0.6	±0.5	±0.6	±0.6	±0.3	±0.4	±1.0	±0.2	±0.5	±0.5	±0.3	±0.9	±0.2	±0.7

Treatment	136 g/L Disturbance/ 61 g/L recovery					136 g/L Disturbance/ 89.7 g/L recovery					136 g/L Disturbance/ 136 g/L Recovery				
Week	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Disturbance Phase															
1	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0	136.0
3	137.4	134.0	137.4	137.4	137.4	134.7	135.0	130.6	138.7	138.0	136.7	137.4	136.0	138.0	138.0
5	144.8	140.8	145.5	144.8	142.1	144.2	142.8	147.6	146.2	146.9	144.2	146.2	136.7	144.2	144.2
7	136.0	139.4	138.7	139.4	138.0	139.4	135.0	137.4	140.1	138.0	137.4	138.0	138.0	140.8	140.8
9	135.5	132.5	134.6	136.0	135.5	133.8	133.8	137.4	136.0	136.7	133.8	135.7	135.0	135.1	135.5
11	136.0	132.6	135.5	137.4	136.7	133.6	134.5	139.4	139.4	138.0	136.0	136.0	136.0	136.0	137.4
13	142.1	135.7	140.1	140.8	135.5	142.1	140.1	143.5	137.4	137.4	135.0	137.4	135.3	135.1	137.4
Recovery Phase															
15	57.3	61.0	60.4	61.2	60.5	98.7	99.0	98.7	95.5	99.8	134.6	135.0	136.7	135.8	136.7
17	60.9	63.0	60.7	59.9	58.2	97.3	97.4	98.9	97.9	98.7	135.4	135.2	134.6	132.7	133.8
19	61.4	60.6	61.3	60.0	58.7	97.5	98.1	98.3	99.2	98.7	134.8	134.5	135.5	133.6	134.6
21	61.1	61.6	61.1	60.2	59.4	97.9	97.3	99.7	98.8	99.3	136.7	135.0	135.8	133.5	134.6
23	61.2	63.0	61.3	59.5	59.5	99.0	96.6	99.6	99.7	99.5	137.4	135.9	135.7	134.4	134.8
25	57.3	61.0	60.4	61.2	60.5	98.7	99.0	98.7	95.5	99.8	134.6	135.0	136.7	135.8	136.7
Mean disturbance phase	138.3	135.9	138.3	138.8	137.3	137.7	136.7	138.8	139.1	138.7	137.0	138.1	136.1	137.9	138.5
Mean recovery phase	59.9	61.7	60.9	60.3	59.5	98.2	97.9	99.0	97.8	99.3	135.6	135.1	135.8	134.3	135.2
Standard deviation disturbance phase	±3.7	±3.2	±3.7	±3.2	±2.3	±4.3	±3.4	±5.5	±3.5	±3.7	±3.4	±3.7	±1.0	±3.4	±3.0
Standard deviation recovery phase	±2.0	±1.1	±0.4	±0.7	±0.9	±0.7	±1.0	±0.6	±1.9	±0.5	±1.2	±0.5	±0.8	±1.3	±1.2

Treatment	Controls									
Week	1	2	3	4	5	6	7	8	9	10
Disturbance Phase										
1	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
3	3.5	3.5	3.5	3.5	3.6	3.5	3.6	3.5	3.5	3.5
5	3.7	3.7	3.7	3.6	3.6	3.7	3.8	3.6	3.8	3.8
7	3.6	3.6	3.7	3.5	3.6	3.5	3.6	3.5	3.5	3.6
9	3.5	3.5	3.5	3.5	3.4	3.5	3.5	3.4	3.5	3.6
11	3.6	3.5	3.5	3.5	3.6	3.5	3.5	3.6	3.5	4.2
13	3.7	3.5	3.6	3.6	3.8	3.6	3.6	3.4	3.5	3.7
Recovery Phase										
15	3.5	3.5	3.5	3.5	3.5	3.4	3.5	3.8	3.5	3.8
17	3.6	3.6	3.6	3.7	3.8	3.7	3.6	3.8	3.6	3.6
19	3.5	3.5	3.4	3.6	3.7	3.6	3.5	3.9	3.6	3.6
21	3.4	3.6	3.4	3.7	3.7	3.6	3.6	3.6	3.6	3.7
23	3.6	3.6	3.6	3.7	3.8	3.6	3.6	3.9	3.6	3.4
25	3.5	3.5	3.5	3.5	3.5	3.4	3.5	3.8	3.5	3.8
Mean disturbance phase	3.6	3.6	3.6	3.5	3.6	3.6	3.6	3.5	3.6	3.7
Mean recovery phase	3.5	3.6	3.5	3.6	3.7	3.6	3.6	3.8	3.6	3.6
Standard deviation disturbance phase	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.3
Standard deviation recovery phase	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±<0.1	±0.1

Appendix 12 The abundance of minor invertebrate species for the experiment investigating the effect of high salinity disturbances on the propagule bank of Lake Cullen

Table A12.1 The presence of the 4 minor invertebrate species.

Species	Treatment where individuals were observed to emerge from sediments	Number of individuals
Class Insecta *Order Diptera,* *Family Psychodidae*	61.0 g/L disturbance phase salinity/ 5.5 g/L recovery phase salinity	4
Class Turbellaria	136.0 g/L disturbance phase salinity/ 5.5 g/L recovery phase salinity	1
Class Insecta *Order Collembola* *Sminthuridae spp.*	136.0 g/L disturbance phase salinity/ 5.5 g/L recovery phase salinity	1

Appendix 13 Detailed regression analysis and ANOVA results for experiment investigating the effect of high salinity disturbances on the propagule bank of Lake Cullen

Table A13.1 Regression results for the effect of Recovery Phase salinity levels on the number of *Ruppia megacarpa* stems produced for each disturbance phase salinity treatment.

Disturbance Salinity level	Transformation	Equation	R^2	df	F	p
37.7 g/L	√x	N/A	0.124	1,28	3.967	0.056
61.0 g/L	√x	y = 7.138 – 1.99x	0.519	1,28	29.903	**<0.001**
98.7 g/L	√x	y = 6.055 – 0.176x	0.586	1,28	39.685	**<0.001**
136.0 g/L	√x	y = 6.287 – 0.170x	0.592	1,28	40.662	**<0.001**

Table A13.2 *Post hoc* Tukey test for Disturbance Phase salinity effects on the number of *Ruppia megacarpa* stems produced

	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
37.7 g/L				
61.0 g/L	0.309			
98.7 g/L	0.941	0.091		
136 g/L	0.886	0.054	0.999	

Table A13.3 *Post hoc* Tukey test for Recovery Phase salinity effects on the number of *Ruppia megacarpa* stems produced

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
3.4 g/L									
5.5 g/L	**0.003**								
8.9 g/L	**0.020**	1.000							
14.4 g/L	**<0.001**	0.784	0.422						
23.3 g/L	**<0.001**	**<0.001**	**<0.001**	0.083					
37.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.573				
61.0 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.176	0.995			
98.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.327	0.998	1.000		
136 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.674	1.000	1.000	1.000	

Table A13.4 Regression results for the effect of Recovery Phase salinity levels on the amount of *Ruppia megacarpa* biomass produced for each disturbance phase salinity treatment ($p<0.05$).

Disturbance Salinity level	Transformation	Equation	R^2	df	F	p
37.7 g/L	$y = \sqrt{x}$	$y = 0.477 – 0.008x$	0.174	1, 28	5,910	**0.022**
61.0 g/L	$y = \sqrt{x}$	$y = 0.528 – 0.012x$	0.536	1, 28	32.365	**<0.001**
98.7 g/L	$y = \sqrt{x}$	$y = 0.518 – 0.012x$	0.666	1, 28	55.833	**<0.001**
136.0 g/L	$y = \sqrt{x}$	$Y = 0.555 – 0.012x$	0.686	1, 28	60.465	**<0.001**

Table A13.5 *Post hoc* Tukey test for Recovery Phase salinity effects on the amount of *Ruppia megacarpa* biomass produced

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
3.4 g/L									
5.5 g/L	0.361								
8.9 g/L	0.905	0.992							
14.4 g/L	0.135	1.000	0.899						
23.3 g/L	**<0.001**	**0.002**	**<0.001**	**0.010**					
37.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.095				
61.0 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.366			
98.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	0.543	1.000		
136 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.025**	0.828	1.000	1.000	

Table A13.6 Regression results for the effect of Recovery Phase salinity levels on the number of *Lamprothamnium macropogon* individuals germinated for each disturbance phase salinity treatment ($p<0.05$).

Disturbance Salinity level	Transformation	Equation	R^2	df	F	p
37.7 g/L	√x	y = 29.816 – 0.155x	0.284	1, 28	10.700	**0.003**
61.0 g/L	√x	y = 18.383 – 0.167x	0.267	1, 28	10.186	**0.003**
98.7 g/L	√x	y = 18.199 – 0.320x	0.755	1, 28	86.511	**<0.001**
136.0 g/L	√x	y = 16.788 – 0.303x	0.609	1, 28	43.533	**<0.001**

Table A13.7 Post hoc Tukey test for Disturbance Phase salinity effects on the number of *Lamprothamnium macropogon* individuals germinated

	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
37.7 g/L				
61.0 g/L	**<0.001**			
98.7 g/L	**<0.001**	**<0.001**		
136 g/L	**<0.001**	**<0.001**	**0.002**	

Table A13.8 Post hoc Tukey test for Recovery Phase salinity effects on the number of *Lamprothamnium macropogon* individuals germinated

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
3.4 g/L									
5.5 g/L	1.000								
8.9 g/L	1.000	1.000							
14.4 g/L	1.000	1.000	1.000						
23.3 g/L	0.180	0.237	0.238	0.147					
37.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**				
61.0 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**			
98.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1.000		
136 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1.000	1.000	

Table A13.9 Regression results for the effect of Recovery Phase salinity levels on the amount of *Lamprothamnium macropogon* biomass produced in each disturbance phase salinity treatment ($p<0.05$).

Disturbance Salinity level	Transformation	Equation	R^2	df	F	p
37.7 g/L	√x	y = 0.928 – 0.005x	0.141	1, 28	4.450	**0.044**
61.0 g/L	√x	y = 0.937 – 0.010x	0.243	1, 28	9.003	**0.006**
98.7 g/L	√x	y = 0.737 – 0.010x	0.389	1, 28	17.846	**<0.001**
136.0 g/L	√x	y = 0.729 – 0.012x	0.419	1, 28	20.158	**<0.001**

Table A13.10 Post hoc Tukey test for Disturbance Phase salinity effects on the amount of *Lamprothamnium macropogon* dry weight biomass

	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
37.7 g/L				
61.0 g/L	**<0.001**			
98.7 g/L	**<0.001**	**<0.001**		
136 g/L	**<0.001**	**<0.001**	0.153	

Table A13.11 Post hoc Tukey test for Recovery Phase salinity effects on the amount of *Lamprothamnium macropogon* dry weight biomass

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
3.4 g/L									
5.5 g/L	0.942								
8.9 g/L	0.782	1.000							
14.4 g/L	1.000	0.985	0.903						
23.3 g/L	0.413	**0.026**	**0.008**	0.305					
37.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.029**				
61.0 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**			
98.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1.000		
136 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1.000	1.000	

Table A13.12 Regression results for the effect of Recovery Phase salinity levels on the number of *Mytilocypris henricae* individuals in the population developed from the propagule bank in each disturbance phase salinity treatment ($p<0.05$).

Disturbance Salinity level	Transformation	Equation	R^2	Df	F	P
61.0 g/L	√x	$y = 0.621 + 0.428x - 0.009x^2$	0.557	2, 27	6.071	**0.007**
98.7 g/L	√x	$y = 3.34 + 0.482x - 0.013x^2$	0.595	2, 27	7.401	**0.003**
136.0 g/L	√x	$y = 6.216 + 0.223x - 0.007x^2$	0.551	2, 27	5.887	**0.008**

Table A13.13 Post hoc Tukey test for Disturbance Phase salinity effects on the number of *Mytilocypris henricae* individuals in the population developed from the propagule bank

	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
37.7 g/L				
61.0 g/L	**<0.001**			
98.7 g/L	**<0.001**	0.324		
136 g/L	**<0.001**	0.121	0.960	

Table A13.14 Post hoc Tukey test for Recovery Phase salinity effects on the number of *Mytilocypris henricae* individuals in the population developed from the propagule bank

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
3.4 g/L									
5.5 g/L	0.333								
8.9 g/L	**<0.001**	0.155							
14.4 g/L	**<0.001**	0.405	1.000						
23.3 g/L	**0.008**	0.805	0.934	0.997					
37.7 g/L	0.990	0.903	**0.002**	**0.014**	0.124				
61.0 g/L	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**			
98.7 g/L	**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1.000		
136 g/L	0.065	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.011**	1.000	1.000	

Table A13.15 Regression results for the effect of Recovery Phase salinity levels on the number of *Australocypris* spp. individuals in the population that developed from the propagule bank in each disturbance phase salinity treatment ($p<0.05$).

Disturbance Salinity level	Transformation	Equation	R^2	df	F	P
61.0 g/L	√x	$y = 31.731 + 0.797x - 0.009x^2$	0.419	2, 32	3.416	**0.045**
98.7 g/L	√x	$y = 9.922 + 0.657x - 0.13x^2$	0.488	2, 32	5.002	**0.013**

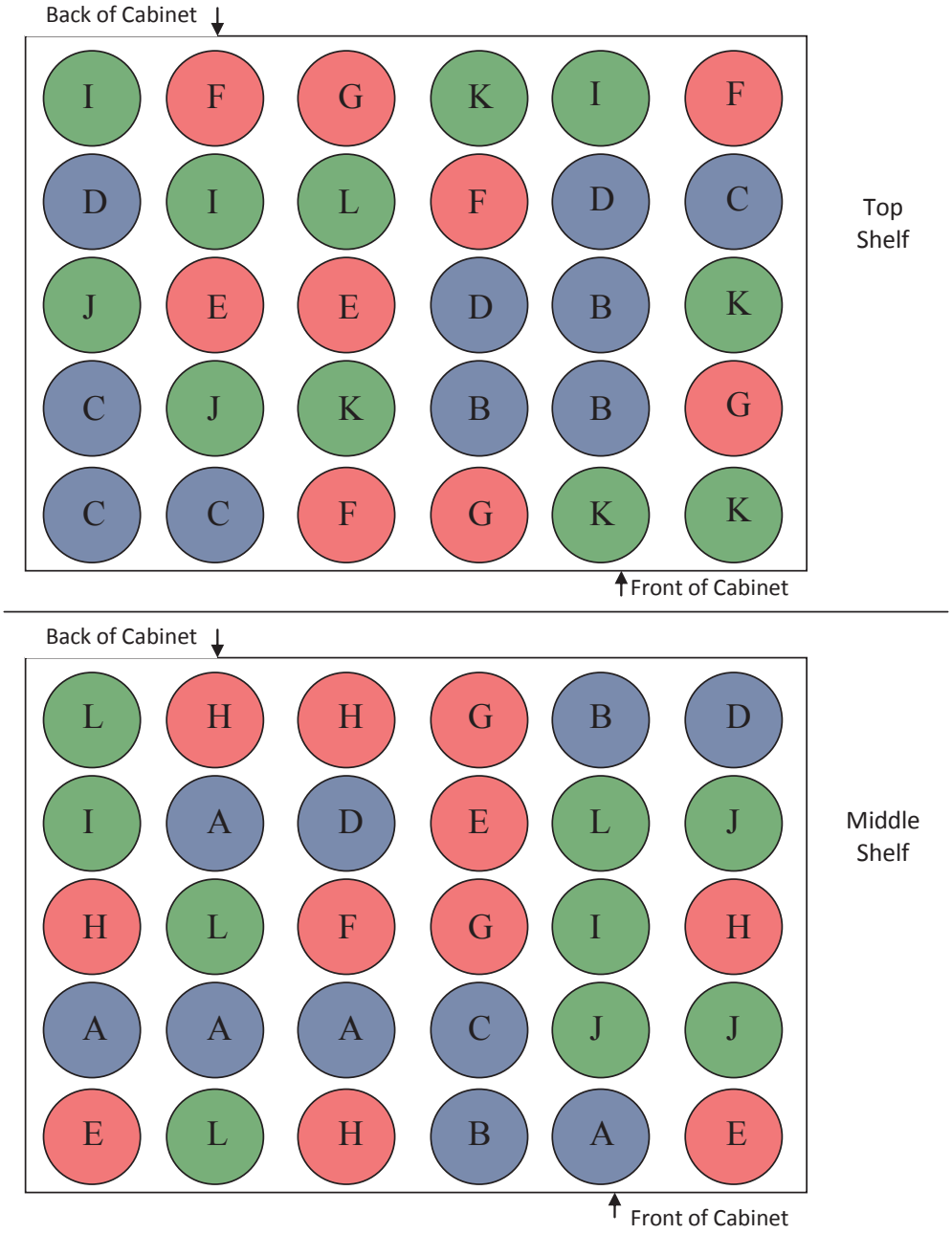
Table A13.16 Post hoc Tukey test for Disturbance Phase salinity effects on the number of *Australocypris* spp. individuals in the population developed from the propagule bank

	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
37.7 g/L				
61.0 g/L	**<0.001**			
98.7 g/L	**<0.001**	**<0.001**		
136 g/L	**<0.001**	**<0.001**	**0.007**	

Table A13.17 Post hoc Tukey test for Recovery Phase salinity effects on the number of *Australocypris* spp. individuals in the population developed from the propagule bank

	3.4 g/L	5.5 g/L	8.9 g/L	14.4 g/L	23.3 g/L	37.7 g/L	61.0 g/L	98.7 g/L	136 g/L
3.4 g/L									
5.5 g/L	0.858								
8.9 g/L	**<0.001**	**0.004**							
14.4 g/L	**0.019**	0.546	0.591						
23.3 g/L	**0.001**	0.139	0.960	0.998					
37.7 g/L	**0.001**	0.138	0.960	0.998	1.000				
61.0 g/L	0.864	1.000	**0.016**	0.711	0.264	0.263			
98.7 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		
136 g/L	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1.000	

Appendix 14 Random location of replicates in germination cabinet for the experiment investigating the effect of location of seed source and presence of substrate on the germination of *Ruppia megacarpa* seeds at two differing salinities.



Key

	Location	Substrate	Salinity mg/L		Location	Substrate	Salinity mg/L
A	Lake Cullen	No	1 360	G	Lake Golf Course	Yes	1 360
B	Lake Cullen	No	3 400	H	Lake Golf Course	Yes	3 400
C	Lake Cullen	Yes	1 360	I	Lake Wandella	No	1 360
D	Lake Cullen	Yes	3 400	J	Lake Wandella	No	3 400
E	Lake Golf Course	No	1 360	K	Lake Wandella	Yes	1 360
F	Lake Golf Course	No	3 400	L	Lake Wandella	Yes	3 400

Appendix 15 Table of random numbers to determine order of experiments testing the effect of photoperiod and temperature on the germination of *Ruppia megacarpa* seeds.

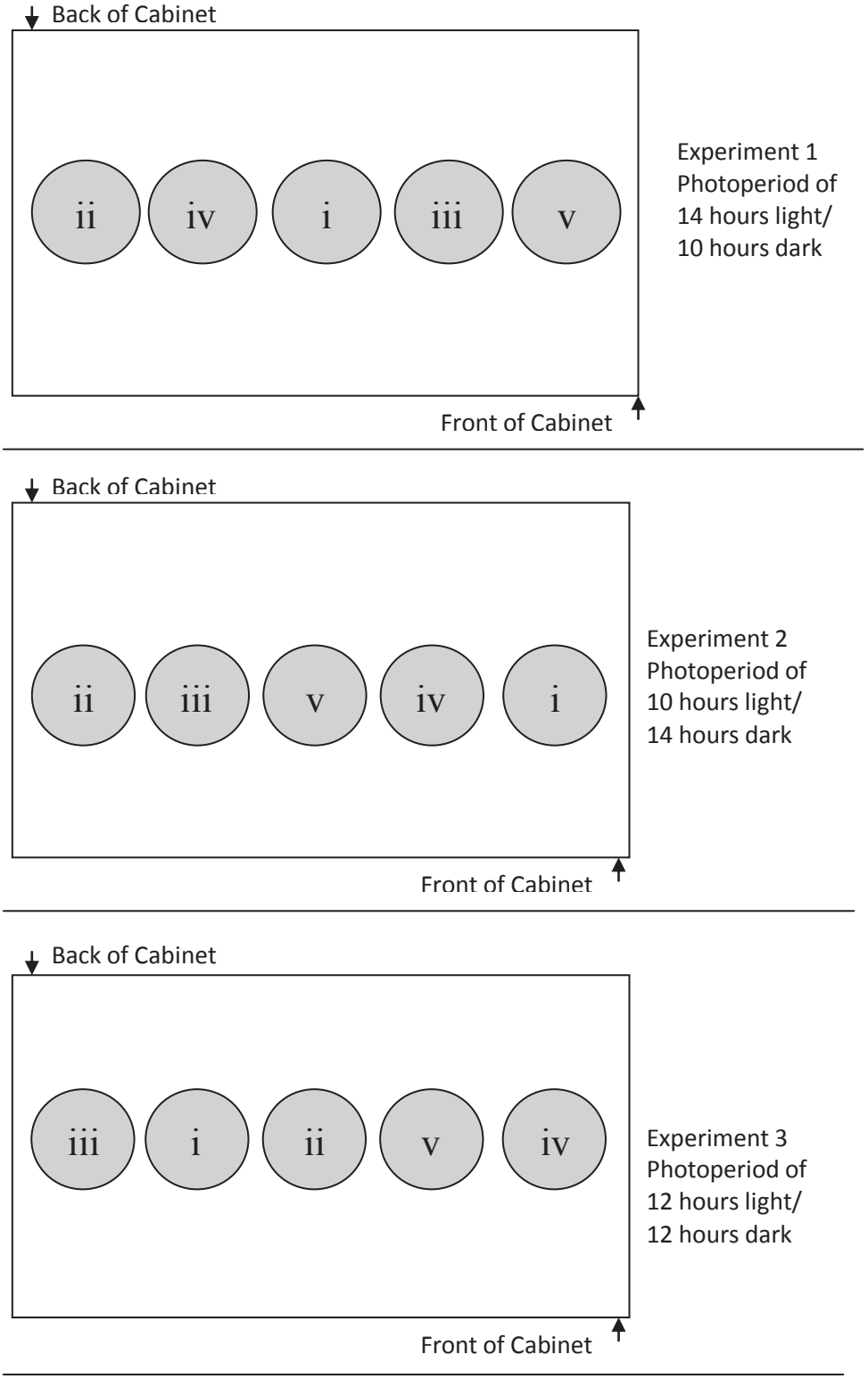
Table A15.1 Order in which treatments were conducted for photoperiod studies

Treatment	Order Treatment conducted
14 hours light, 10 hours dark	1
12 hours light, 12 hours dark	3
10 hours light, 14 hours dark	2

Table 15.2 Order in which treatments were conducted for temperature studies

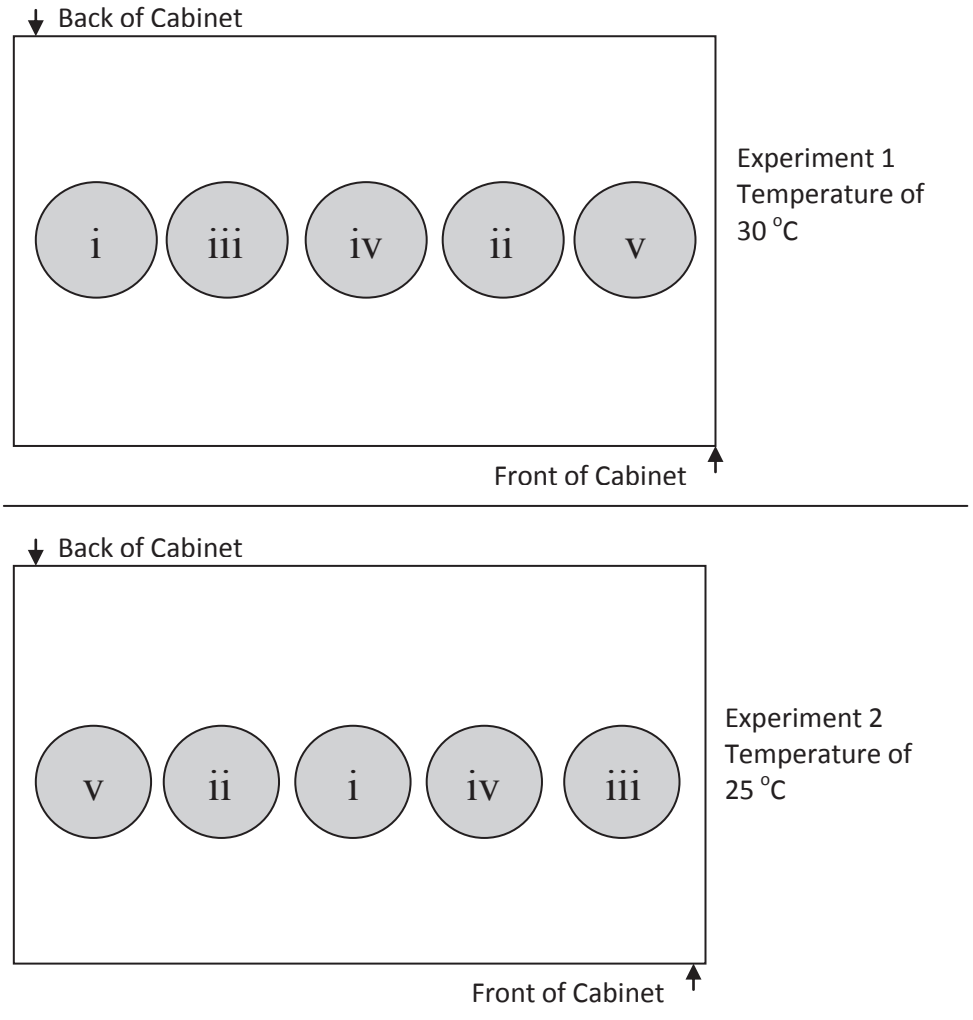
Treatment	Order Treatment conducted
25^{o}C	2
30^{o}C	1

Appendix 16 Random location of replicates in germination cabinet for the experiment investigating the effect of photoperiod on the germination of *Ruppia megacarpa* seeds.



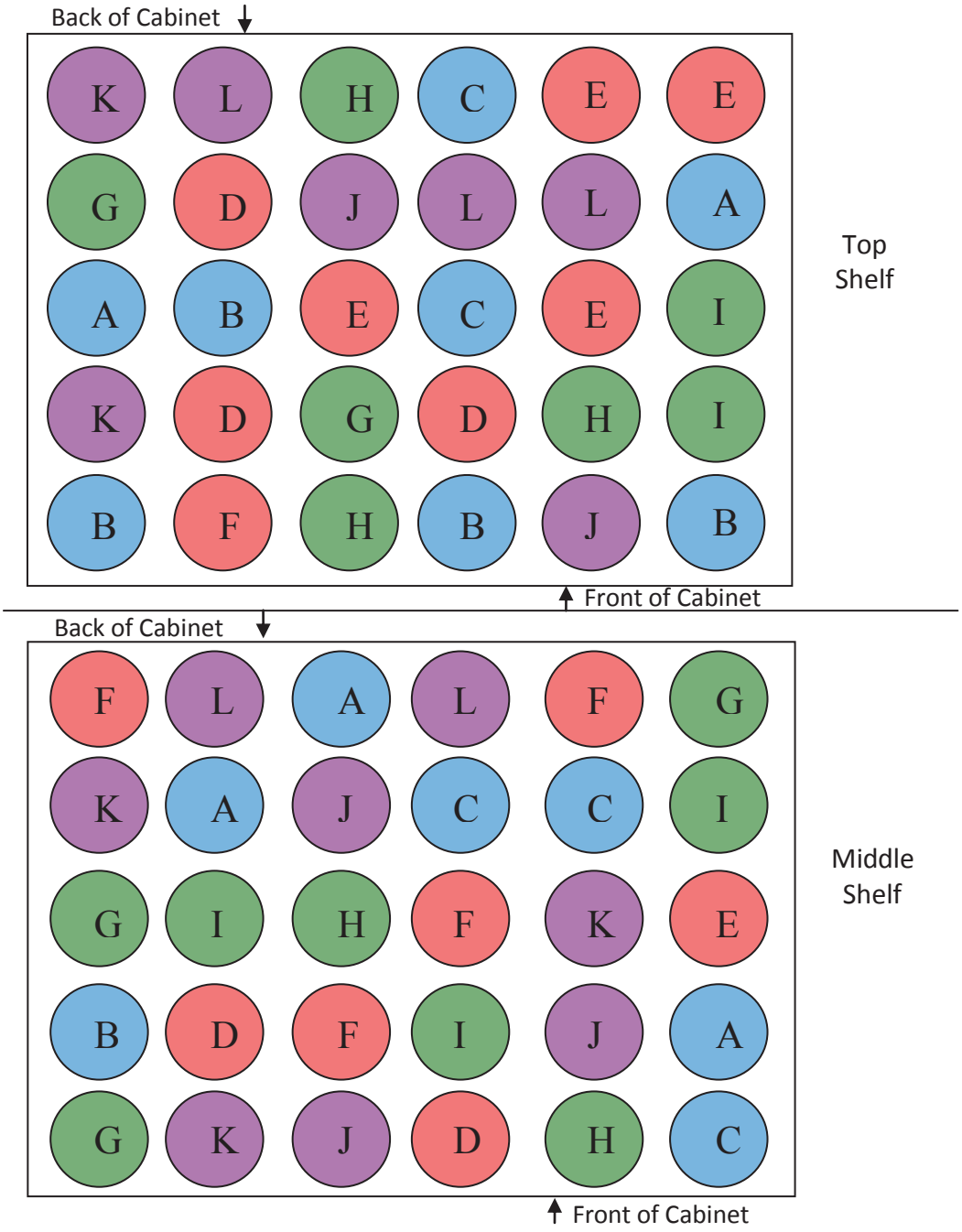
Note
Roman Numerals i – v indicate replicate number for each treatment

Appendix 17 Random location of replicates in germination cabinet for the experiment investigating the effect of temperature on the germination of *Ruppia megacarpa* seeds.



Note
Roman Numerals i – v indicate replicate number for each treatment

Appendix 18 Random location of replicates in germination cabinet for the experiment investigating the effect of temperature on the germination of *Ruppia megacarpa* seeds.



Key

	Phase 1 mg/L	Phase 2 mg/L	Phase 3 mg/L		Phase 1 mg/L	Phase 2 mg/L	Phase 3 mg/L
A	10 000	10 000	10 000	**G**	30 000	10 000	
B	30 000	30 000	30 000	**H**	50 000	30 000	
C	50 000	50 000	50 000	**I**	50 000	10 000	
D	10 000	Dry	10 000	**J**	30 000	Dry	10 000
E	30 000	Dry	30 000	**K**	50 000	Dry	30 000
F	50 000	Dry	50 000	**L**	50 000	Dry	10 000

Appendix 19 Day to Day Results – The effect of various drying periods and salinity treatments on the germination of *Ruppia megacarpa* seeds

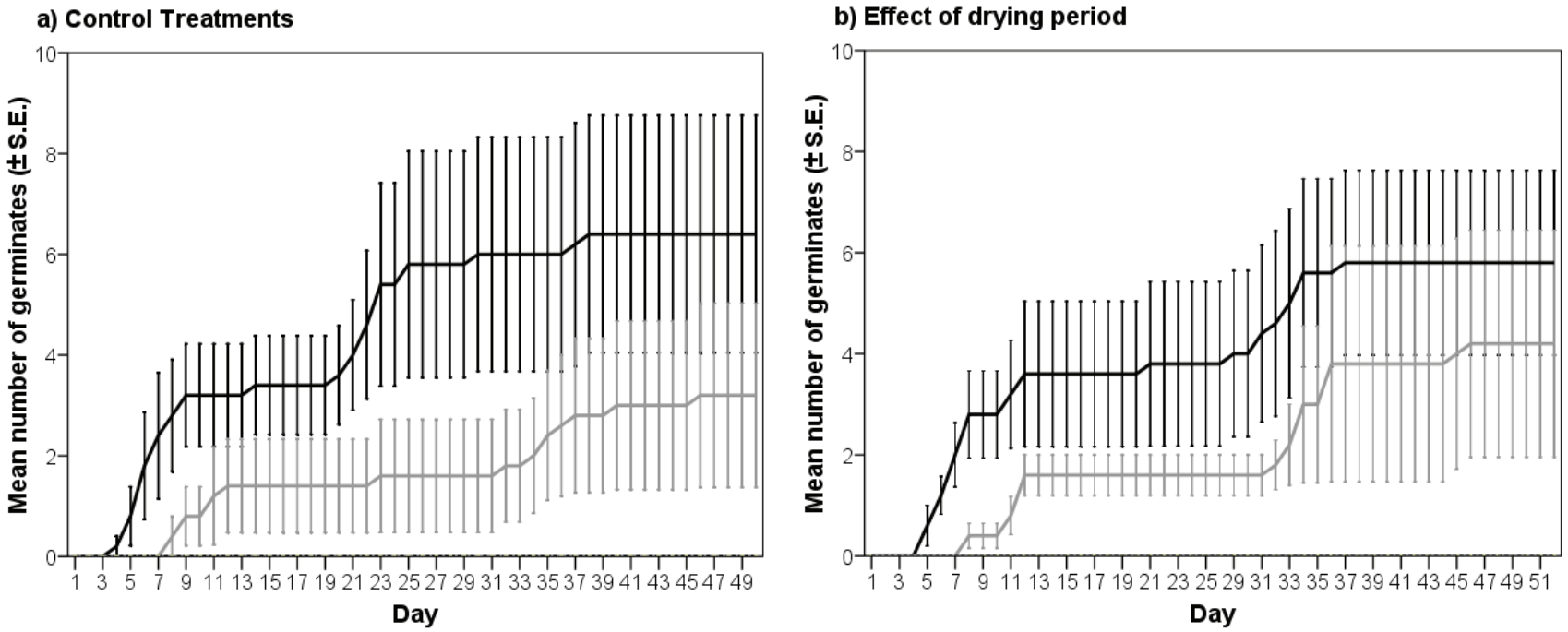


Figure A19.1 Day to Day Results – The effect of various drying periods and salinity treatments on the germination of *R. megacarpa* seeds a) Control treatments, b) effect of desiccation

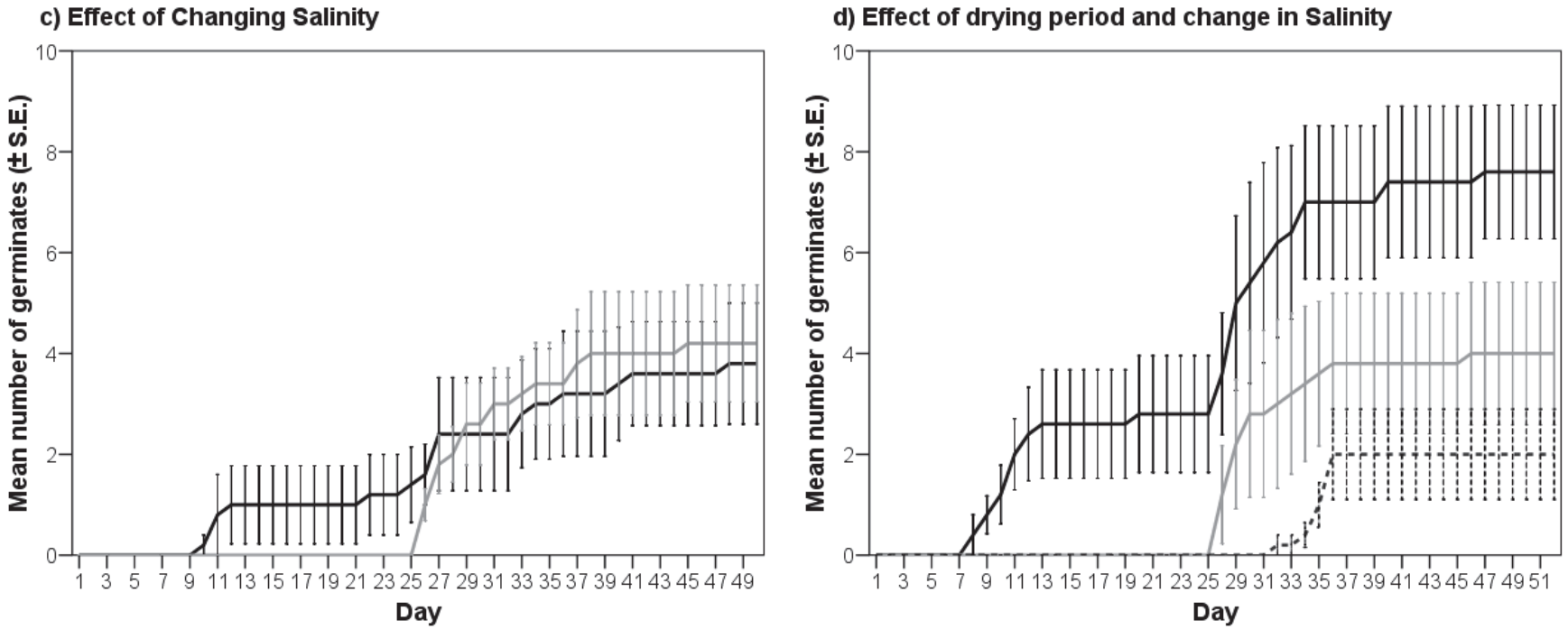


Figure A19.2 Day to Day Results – The effect of various drying periods and salinity treatments on the germination of *R. megacarpa* seeds c) changing salinity, b) effect of desiccation and change in salinity