ECOHYDROLOGY & HYDROBIOLOGY

DOI: 10.2478/v10104-012-0003-5

Vol. 12
No. 1, 53-63
2012

Zooplankton community emerging from fresh and saline wetlands

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Abstract

Salinity is a significant factor affecting aquatic species distribution and diversity. To quantify the impact of increasing salinity on the zooplankton community structure, the emergence of zooplankton community from fresh and saline wetlands under different salinity was examined. Sediments from three wetlands from the Upper South East of South Australia were exposed to salinity levels of 300 mg dm^{-3}, 5000 mg dm^{-3} and 15 000 mg dm^{-3} for 21 days. After 21 days, more taxa had emerged from fresher wetland sediment than emerged from more saline wetlands. A reduction in the number of zooplankton species and their abundance was evident in the freshwater wetland sediment once salinity was increased from 300 mg dm^{-3} to 5000 mg dm^{-3}. Species that emerged from freshwater sediment were mainly freshwater species and their number was significantly reduced as salinity increased. Saline wetlands were colonised by more salt tolerant species such as *Brachionus plicatilis*, *Trichocerca* sp. and calanoid copepods. The results indicate that increasing salinity will potentially reduce freshwater zooplankton richness and the community will be shifted from freshwater species to more salt tolerant species.

Key words: zooplankton, eggs, salinity, wetlands.

1. Introduction

Salinity is a serious threat to freshwater ecosystems and it is becoming increasingly more serious due to environmental changes caused both by primary and secondary salinisation. In the process of primary salinisation, salt enters inland water systems through groundwater intrusion, terrestrial run-off, and salt build up on the soil due to high evaporation. Secondary or anthropogenically induced salinisation involves salt entering the system through agricultural run-off and flow regulation causing the accumulation of salt in the low land rivers and wetlands (Williams 1986). A significant number of studies have shown that many freshwater ecosystems are becoming saline particularly in coastal and semi-arid regions (Nielsen *et al.* 2007; Watt *et al.* 2007).

In Australia, freshwater ecosystems are seriously threatened by increasing salinity. Native vegetation clearing and irrigation have resulted in rising groundwater tables that significantly increase salt water intrusion into riverine and wetland systems. This phenomenon is referred as dryland salinity (Ruprecht, Schofield 1991; Halse *et al.* 2003; George *et al.* 1995). Dryland salinity is recognised to be an important cause of stream, river and wetland salinisation in Australia, in addition to natural or primary salinisation that occurred before European settlement. Dryland salinity alone has affected up to 370 000 ha of land and wetlands, and it is predicted to double by the year 2050 under current land use and groundwater trends in South Australia (Barnett 2000). According to Barnett (2000), there are more than 50 000 ha of wetlands in South Australia that have been affected by dryland salinity and this includes wetlands of national of significance listed as Ramsar Sites.

Wetlands support a variety of ecosystems and many of them supporting a wide diversity of native species. However, increasing salinity has led to deterioration of water quality and caused serious degradation of valuable habitats. There is considerable concern about the impact of increasing salinity on freshwater biota. Salinity is known as a driving factor determining the presence and dominance of aquatic organisms, for example, a study by Nielsen *et al.* (2008), showed that increasing salinity in freshwater ecosystems has caused significance reduction in the emergence of zooplankton when eggs are exposed to a high salinity environment. Similarly, reduction in freshwater zooplankton species has also been reported by Gonçalves *et al.* (2007). Salinisation in freshwater ecosystems may impair the growth and reproduction of aquatic biota (James *et al.* 2003) and lead to elimination of certain less tolerant species and, in turn, could affect ecological balance (James, Hart 2003; Bailey, James 2000).

Recently, research has shown an increased interest in studying the effect of salinity on zooplankton eggs bank and plant propagules. Zooplankton produce resting eggs during their life cycle in response to unfavourable environmental conditions and remain in the sediment until favourable conditions return. For example, cladocerans, copepods and rotifers have been found to produce diapause eggs as wetlands dry and salinity increases (Bailey *et al.* 2004).

Previous published data has shown that freshwater zooplankton will undergo growth and reproduction stress when subjected to salinity up to 1000 mg dm $^{-3}$ (Hart *et al.* 1991). Nielsen *et al.* (2008) predicted that very few biota will survive as natural wetlands salinity reaches 5000 mg dm^{-3} and suggested that natural wetland which subjected to long term salinisation will reduce the persistence of freshwater species and be replaced by more salt-tolerant taxa. The increasing salinity has resulted in a loss of wetland biodiversity such as plants, bird and microinvertebrates, including zooplankton (Nielsen *et al.* 2003b). A study on the emergence of zooplankton propagules from wetlands sediment of different areas from those that have been studied previously is needed to verify the effect of increasing salinity over a broader area.

Zooplankton propagules from sediment of various wetlands across Australia have also been used to study the response of zooplankton to different salinity regimes (Skinner *et al.* 2001; Nielsen *et al.* 2007; 2008). Even though much work has been done on the impact of increasing salinity on Australian freshwater ecosystems, the area of study only covers a small number of wetlands. Thus, the study to broaden the area of study is needed. Wetlands of the Upper South East (USE) region of South Australia is one of the areas where salinity is an increasing problem. Due to the importance of the wetlands of the USE region of South Australia, the result of the study will be useful for the Upper South East Dry Salinity & Flood Management Program. In this study, the emergence of zooplankton egg banks and how species diversity responds to induced elevated salinity is assessed for three wetlands sediments of the USE region. The main focus was to determine whether species richness and abundance, emerging from wetlands sediment propagules, are affected by increasing salinity.

As with previous work on the rising salinity impact on freshwater ecosystems, this study remains limited to the study area of the semi-arid region. Given that many areas of other countries and regions are subjected to rising salinity due to salt water intrusion, modified hydrological regime due to extensive agriculture practices, climate change and human intervention, this study indicates a promising area for research related to the impact of rising salinity on freshwater ecosystem in other regions. Studying the relationship between the increasing salinity and the biotic structure can be used to estimate the ecosystem's sensitivity. Therefore, proper management of vulnerable ecosystems can be applied.

2. Materials and methods

2.1. Study site

The USE region of South Australia covers over 1 million ha area and contains a number of ephemeral wetlands and according to the Upper South East Dry Salinity & Flood Management Program (USEDS&FMP) (1993 cited in Goodman *et al.* 2010), only 7% of the original wetlands remain ecologically functioning. Dryland salinity has recently been labelled as the driving factor of increasing salinity and recognised as a significant factor of loss of wetland area in the region. The majority of wetlands in the USE depend on groundwater to fill and during the drawdown of salty groundwater flow, salts are concentrated and deposited in the wetland sediment (Goodman *et al.* 2010b). Historically, flooding through surface run-off generally occurred during winter, followed by a dry period of the summer. However, prolonged dry period and flow regulation have caused the increasing salinity of the wetlands flow has been regulated (Goodman *et al.* 2010b). The wetlands in the region have been isolated from its origin source by constructed drains resulting in reduced freshwater flows to the wetland and as the result many of the remaining wetlands in the USE of South Australia are threatened by increasing salinity (USEDS&FMP 2007).

2.2. Sediment collection

Sediment samples from three wetlands, Mandina, KC-road and Willalooka, were used as the source of zooplankton eggs bank. These water bodies differ in salinity, and represent a salinity gradient. Willalooka is fresh with a salinity range from 200 to 400 mg dm^{-3}. Mandina and KC road wetlands are saline with a salinity range from 13 000 to 38 000 mg dm^{-3}) at the time of sampling. Sediment containing zooplankton eggs was collected from Willalooka wetlands in January 2010 while samples from Mandina and KC-Road wetlands were collected in March 2010. No inundation period was identified during sampling periods. Samples of sediments 15 cm^3 were collected from random locations throughout the wetlands. Three samples for each wetland were obtained and then were mixed to get one composited sample for each wetland. According to Nielsen *et al.* (2008), composited samples maximise the number of zooplankton propagules and minimise the variability within each wetland. The sediment was stored in plastic containers prior to transport to laboratory. The sediment samples were air dried on plastic trays for one week or until the sediment samples appeared dry. Air dried sediment was then gently crushed by hand to avoid damaging the egg bank and mixed thoroughly before experimental treatment (Nielsen *et al.* 2003a).

2.3. Salinity measurement

Salinity level of the wetland and experimental water was measured using an electronic conductivity (EC) meter WP-81S V.5.c U0659by TPS. The salinity of sediment samples was measured using the soil-water extract method (1:5 by weight) of Price (2006) and ANZECC (2000). The conductivity value is converted to milligram per litre using the conversion factor of 0.68 by Hart *et al.* (1991).

2.4. Experimental design

100 g dry weight (DW) of air dried sediment was added to 15 × 12 × 5 cm plastic containers and artificially flooded with 500 ml distilled water with three levels of constant salinity from fresh to saline. Salinity treatments included 300 mg dm^{-3}, 5000 mg dm^{-3} and 15 000 mg dm^{-3}. These concentration were justifie considering 300 mg dm^{-3} as the freshwater salinity level, 5000 mg dm^{-3} refers to saline to freshwater threshold based on Australian standard and 15 000 mg dm^{-3} considered as high salinity level. Salinity of 300 mg dm^{-3} was established simply by adding reverse osmosis (RO) water to the sediment. For sediment with actual salinity level above 300 mg dm^{-3}, dilution was needed to establish salinity level of 300 mg dm^{-3}. This was done by filtering the water through a 35 μm zooplankton net and replacing the water with RO water. The procedure was repeated until the desired salinity was obtained. Three replicates were assigned for each treatment using a composited sample. All samples were incubated in a temperature controlled culture room at 20°C with day and night cycle using artificial light of 12 hours day and 12 hours night for 21 days. The salinity level was measured daily for one week and weekly for the rest of incubation period. For each treatment, salinity levels were maintained either by adding synthetic sea salt (Instant Ocean by Aquarium System) to increase salinity or by adding tap water as necessary.

Zooplankton was collected on day 7, 14 and 21 from the treatment containers. On each collection day, a volume of the overlaying water from each container was decanted and filtered through a 35μm mesh net before being preserved in 70% ethanol prior for identification and counting. Filtered water was returned to the microcosm.

2.5. Zooplankton identification

A total of 5 ml from each of the 40 ml sub samples was identified to 3 major groups of zooplankton: cladoceran, copepod and rotifer. Zooplankton samples were counted and identified in a Sedgewick-Rafter counting chamber under a bright field microscope. All samples were identified to the level of genus and species where possible using the key of Shiel (1995) and Bayly (1992). Species richness was counted as the number of taxa per unit sample (100 g DW sediment) (McCune, Grace 2002) and abundance was measured as total individual per 100 g DW.

2.6. Data analysis

Statistical analysis was carried out using SPSS (version 18). A preliminary analysis was done to test normality of the data with the Shapiro-Wilk test and data was check for homogeneity of variance. One way ANOVAs (salinity as a single treatment) were used to determine whether there were differences in the number of taxa and abundance of zooplankton emerging from each sediment sample under different salinity levels. Where significant effects were observed, means were compared with Tukey's post test to determine which treatment pairs were significantly different. Multivariate analysis of community data was performed using the PC-ORD statistical package version 5. Non-metric Multidimensional scaling, (NMS) derived from a Bray-Curtis similarity matrix was used to display the pattern of zooplankton community data within each treatment across wetlands sites. Analysis of rank of abundance was performed to determine which taxa was more abundance than others in each treatment at each sampling time.

3. Results

3.1. Sediment characteristics

The sediment from Mandina and KC-road wetlands were more saline than the sediment from Willalooka. The salinity of sediment from Mandina and KC-road wetlands were 22430±562 mg dm^{-3} and 19819±226 mg dm^{-3} respectively, while soil salinity taken from Willalooka was 6858±51 mg dm^{-3} (Table I). Average microcosm salinities were maintained at a predetermined concentration (±15%) as presented in (Table II).

Table I. Mean salinity characteristics of sampling site and sediment samples (mg dm^{-3}).

Site name	Site salinity (mg dm^{-3})	Soil salinity (mg dm^{-3})
Willalooka	400	6858±51
Mandina	26588	22430±562
KC-Road	29920	19819±226

Table II. Measured salinity level for each treatment at each sampling time.

Paratmeter	Site	Day	300 mg dm^{-3}	5000 mg dm^{-3}	15 000 mg dm^{-3}
Salinity (mg dm^{-3})	Willalooka	Day 1	350	5123	14144
		Day 2	357	4912	14575
		Day 3	320	7207	14983
		Day 4	333	5168	14484
		Day 5	319	5105	13985
		Day 6	321	5281	13691
		Day 7	304	5191	14325
		Day 14	295	5191	14371
		Day 21	290	4719	14597
	Mandina	Day 1	312	4835	14411
		Day 2	292	4851	14366
		Day 3	274	4576	13911
		Day 4	358	4576	14892
		Day 5	335	4978	14847
		Day 6	322	4871	13192
		Day 7	338	4935	13895
		Day 14	296	5281	14665
		Day 21	340	5114	15035
	KC-Road	Day 1	296	4872	14280
		Day 2	306	5127	14053
		Day 3	296	4651	13940
		Day 4	288	5012	13872
		Day 5	296	4950	14688
		Day 6	333	5055	14371
		Day 7	351	5236	14824
		Day 14	366	4658	15232
		Day 21	338	5105	15391

3.2. Communities

The zooplankton community that developed from Willalooka was significantly different from that of Mandina and KC-Road as confirmed by NMS ordination (Fig. 1). The communities that emerged from Willalooka sediment in the 300 mg dm^{-3} and 5000 mg dm^{-3} treatments were different from the community that emerged from the 15 000 mg dm^{-3}. However, the communities that developed from Mandina and KC-road sediments were consistently similar across all salinity treatments.

3.3. Species richness

After 21 days, a total of 34 species emerged from the wetland sediment. Twenty five taxa emerged from the Willalooka sediment consisting of 18 rotifer and 7 cladoceran species. Mandina sediment yielded 8 species consisting of 4 rotifers and 4 copepods; and KC-Road sediment yielded 11 species consisting of 6 Rotifers, 4 copepods and 1 cladocera species (Fig. 2). Hatching for most cladocerans and rotifers taxa was largely restricted to freshwater sediment, while hatching for Copepods (all species combined) was restricted to only saline wetland samples (Table III). Zooplankton showed a decrease in the number of taxa once salinity increased from 300 to 15 000 mg dm^{-3}. This trend was significant ($p < 0.001$) for zooplankton at Willalooka wetland but not for zooplankton that emerged from Mandina and KC-Road wetland sediments.

The number of taxa that emerged from the Willalooka sediment was significantly different across all salinity treatments ($F_{(2,6)} = 168.8$, $p < 0.0001$). Tukey's post test showed that the decreasein the number of taxa was evident once salinity was above 300 mg dm^{-3} and became apparent when sediment was exposed to salinity levels of 5000 and 15 000 mg dm^{-3}. While the number of taxa varied greatly in the low salinity treatment, only 3 taxa were present at 15 000 mg dm^{-3} treatment (Fig. 3). Rotifers were the most dominant taxa to emerge from this sediment

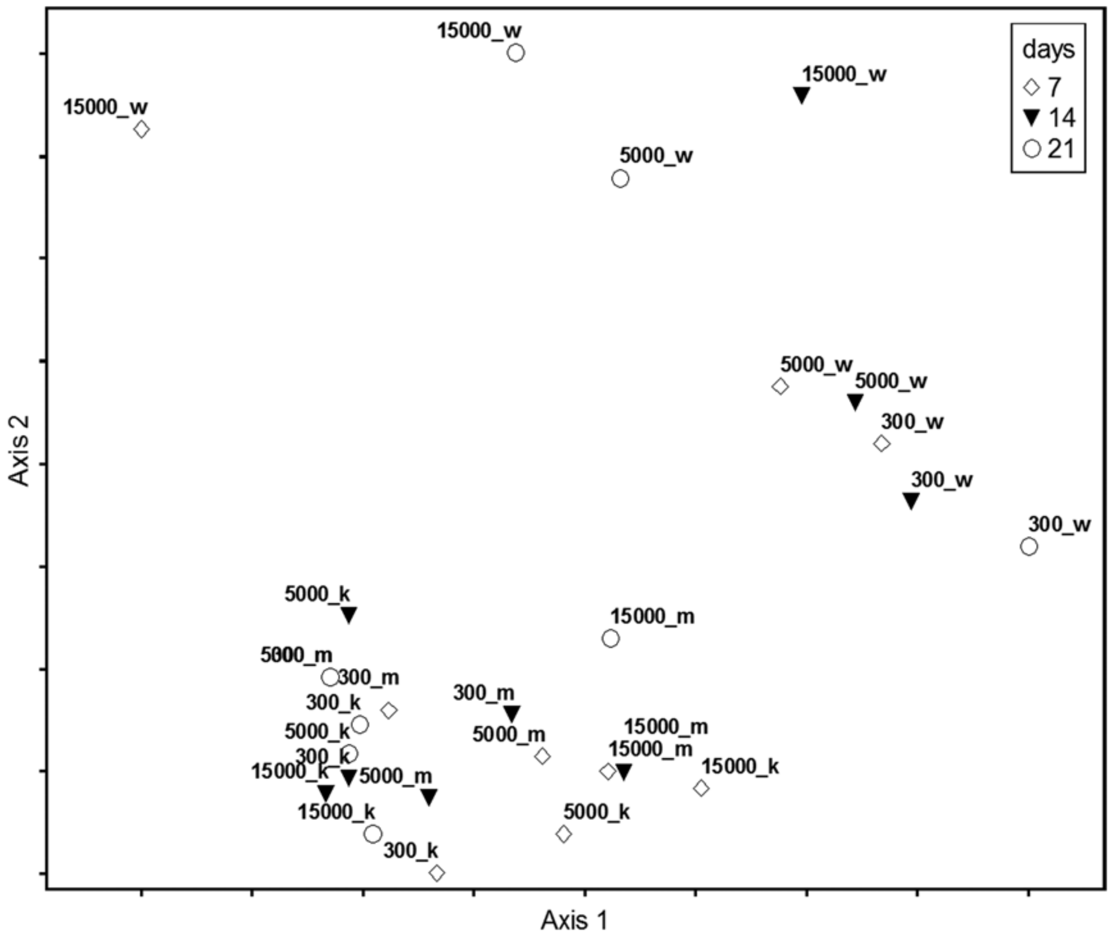

Fig. 1. Non-metric multidimensional scaling ordination of zooplankton community of all salinity treatment at each sampling location. 300, 5000 and 15 000 refers to salinity treatment in mg dm^{-3} and letters to salinity level refer to location (W = Willalooka, M = Mandina and K = KC-Road).

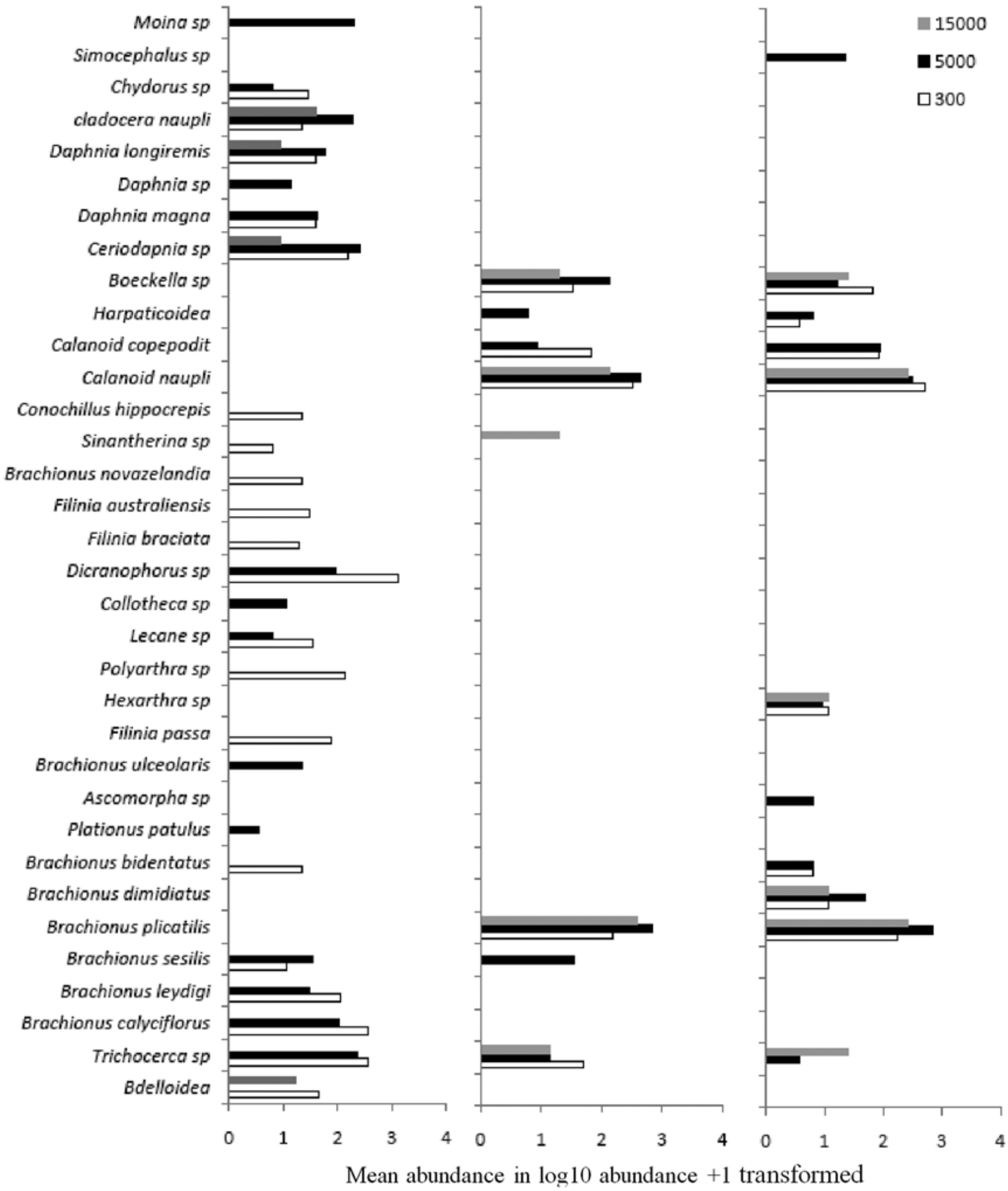

Fig. 2. Zooplankton composition and abundance emerged from Willalooka, Mandina and KC-Road wetlands, respectively, after 21 days of inundation period subjected to experimental increases in salinity. 300, 5000, 15 000 refer to salinity level in mg dm^{-3}.

Table III. Mean number of taxa of rotifers, cladocerans and copepods after 21 days of incubation across all salinity treatments.

Taxa	Willalooka			Mandina			KC-road		
	300 mg dm^{-3}	5000 mg dm^{-3}	15 000 mg dm^{-3}	300 mg dm^{-3}	5000 mg dm^{-3}	15 000 mg dm^{-3}	300 mg dm^{-3}	5000 mg dm^{-3}	15 000 mg dm^{-3}
Rotifers	15	9	1	3	3	3	4	5	4
Copepods	0	0	0	3	4	3	3	4	3
Cladocerans	5	7	2	0	0	0	0	1	0

compared to the number of cladocera. A significant decrease in the number of rotifers and cladocerans was evident once salinity increased ($F_{(2,6)}$ = 147.1, $p < 0.0001$; $F_{(2,6)}$ = 97, $p < 0.0001$) (Table IV). On each sampling occasion,(day 7, 14 and 21) the taxa that emerged from Willalooka sediments in the 300 mg dm^{-3} and 5000 mg dm^{-3} treatments were consistently higher than that in the 15 000 mg dm^{-3} treatment. More taxa emerged by day 7 and emergence remained constant up to day 21 at the lower salinity level, while no zooplankton emerged in the 15 000 mg dm^{-3} treatment by day 7 and only 2 taxa had emerged by day 14 and 21 (Table V).

The effect of salinity after three weeks of exposure on species richness was also significant for the KC-road sediment ($F_{(2,6)}$ = 14.71, $p = 0.0049$) (Fig. 3). However, Tukey'spost test showed the difference was significant only for treatment pairs 300 mg dm^{-3} vs 5000 mg dm^{-3} and 5000 *vs* 15 000 mg dm^{-3} ($p = 0.014$ and $p = 0.05$ respectively) but not for 300 *vs* 15 000 mg L-1 ($F_{(2,6)}$ = 14.71, $p = 0.645$) treatment pairs. Zooplankton speciesthat emerged from KC-Road sediments were similar in all treatments and sampling days combined; the highest number emerged from the 5000 mg dm^{-3} treatment by day 14 (n = 7) (Table V).

There was no significant effect on the number of taxa that emerged from Mandina sediment after three weeks exposure to the three salinity levels ($F_{(2,6)}$ = 2.4, $p = 0.1715$) (Fig. 3). Taxa that emerged from Mandina wetlands in all treatments and sampling days were consistently similar (Table V).

3.4. Taxa abundance

At the lower salinity level in all sediment samples, mean zooplankton abundance increased after day 7 and continued to increase until day 21. In the 5000mg dm^{-3} treatment for Willalooka and KC-Road microcosms, the abundance peaked at day 14 and slightly decreased there after (Fig. 4). The composition of zooplankton of all treatments on each sampling occasion based on rank of abundance is presented in Table V.

After three weeks of inundation, a significant decrease in total zooplankton abundance with increased salinity was observed in Wilallooka sediment ($F_{(2,6)}$ = 152.9, $p < 0.0001$). This trend was significant for all salinity treatment pairs (Fig. 4a). Rotifers were the most abundant taxa; however rotifer abundance significantly decreased once salinity increased above 300 mg dm^{-3} ($F_{(2,6)}$ = 218.33, $p < 0.0001$). A significant decrease in abundance of cladocerans was observed once salinity was above 5000 mg dm^{-3} ($F_{(2,6)}$ = 218.33, $p = 0.001$).

The effect of increasing salinity on total zooplankton abundance was also significant for zooplankton emerged from the Mandina wetland sediment ($F_{(2,6)}$ = 12.44, $p = 0.007$). Total zooplankton abundance significantly increased at salinity levels of 5000 mg dm^{-3} ($F_{(2,6)}$ = 12.44, $p = 0.14$) and then decreased significantly in the 15 000 mg dm^{-3} ($p = 0.010$) treatment (Fig. 4b). *B. plicatilis* and *Trichocerca* sp. were the two most abundant rotifers and calanoid copepode was the most abundant

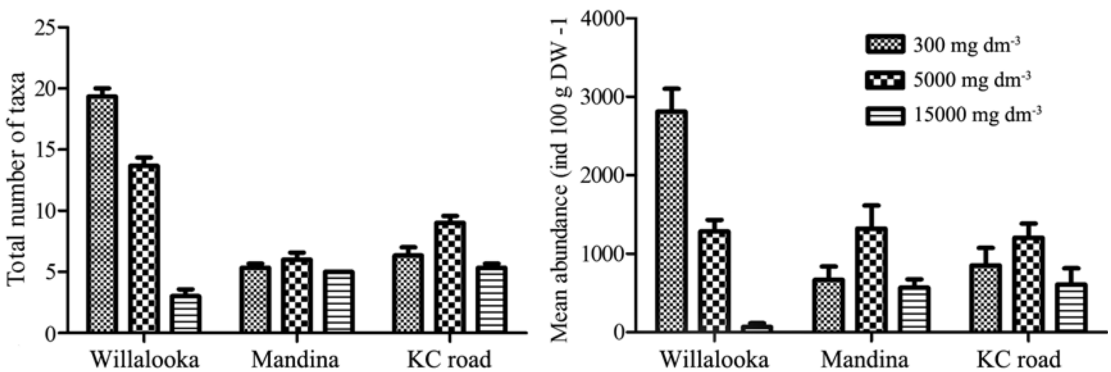

Fig. 3. Total number of taxa and mean abundance that emerged after 21 days of incubation and subjected to different salinity. Value represents mean ± standard error based on three replicates.

Table IV. Mean abundance of rotifers, cladocerans and copepods after 21 days of incubation across all salinity treatments (ind. in 100 g DW).

Taxa	Willalooka			Mandina			KC-road		
	300 mg dm^{-3}	5000 mg dm^{-3}	15 000 mg dm^{-3}	300 mg dm^{-3}	5000 mg dm^{-3}	15 000 mg dm^{-3}	300 mg dm^{-3}	5000 mg dm^{-3}	15 000 mg dm^{-3}
Rotifers	2520	531	16	200	739	419	200	757	315
Calanoids	0	0	0	413	584	149	600	427	293
Cladocerans	275	755	53	0	0	0	0	21	0

copepod across all salinity treatments and sampling times. A significant increase in rotifers abundance was observed once salinity rose above 300 mg dm^{-3} ($F_{(2,6)}$ = 18.57, p = 0.002) but as salinity rose above 5000 mg dm^{-3}, abundance decreased significantly (p=0.026). The abundance of copepods also decreased as salinity exceeded 5000 mg dm^{-3} ($F_{(2,6)}$ = 9.53, p = 0.014) .

The impact of increasing salinity on zooplankton abundance in the KC-road sediment was only significant when salinity exceeded 5000 mg dm^{-3} ($F_{(2,6)}$ = 6.5, p = 0.27) (Fig. 4c). Same as in Mandina wetland, Rotifers, *B. plicatilis*, and calanoid copepod were the two most abundance taxa across all treatments and sampling days (Table V). The abundance of rotifers and calanoid copepods was low at salinity levels above 5000 mg dm^{-3} ($F_{(2,6)}$ = 23.85, p = 0.001; $F_{(2,6)}$ = 6.59, p = 0.031).

4. Discussion

Relatively few taxa emerged from the sediment of Mandina and KC-road, the two more saline wetlands, compared with the freshwater sediment of Willalooka. This suggests that salinity affects the composition of zooplankton assemblage that

Table V. Community composition of each sampling occasion at each treatment based on individual species rank of abundance. Legend: *Asco – Ascomorpha* sp.; Bde – Bdelloidea; *Boeck – Boeckella* sp.; *B. bid – Brachionus bidentatus*; *B.cal – B. calicyflorus*; *B.dim – B. dimidiatus*; *B. ley – B. leydigi*; *B. nov – B.novazealansis*; *B. pli – B. plicatilis*; *B. ses – B. sesilis*; *B.ulc – B. ulceolaris*; *Cer – Ceriodaphnia* sp.; *Chy – Chydorus* sp.; Cla.naup – Cladoceran nauplii; *Collo – Collotheca* sp.; *Cono – Conochilus* sp.; Cop.naup – Copepod nauplii; Cop – Copepodit stage; *Daph.sp – Dapnhia* sp.; *D.long – Daphnia longiremis*; *D.mag – D. magna*; *Dic – Dicranophorus* sp.; *F.aus – Filinia australis*; *F.bra – F. braciata*; *F.pas – F. passa*; Harp – Harpaticoidea; *Hexa – Hexarthra* sp.; *Lec – Lecane* sp.; *Moi – Moina* sp.; *Pla – Plationus patulus*; *Poly – Polyarthra* sp.; *Simo – Simochephalus* sp.; *Sin – Sinantherina* sp.; *Tri – Trichocerca* sp.

	300mg dm^{-3}			5000 mg dm^{-3}			15 000 mg dm^{-3}		
	Day 7	**Day 14**	**Day 21**	**Day 7**	**Day 14**	**Day 21**	**Day 7**	**Day 14**	**Day 21**
Willalooka	B.cal	Tri	Dic	B.cal	Tri	Cer	-	Bde	Cer
	Tri	Poly	Cer	Cer	Cer	Cla.naup		Cla.naup	D.long
	Dic	Dic	B.ley	Cla.naup	Moi	Moi			
	Cer	B.cal	Tri	Tri	Dic	D.long			
	D.mag	B.ley	B.nov	B.ley	Cla.naup	B.cal			
	Bde	D.long	Poly	D.mag	B.ses	Tri			
	Chy	F.pas	F.aus	B.ses	D.mag	B.ley			
	F.pas	Cer	F.pas	B.ulc	B.cal	Dic			
	Cla.naup	Lec	Bde	Plat	B.ulc				
	Cono	F.bra	B.bid		D.long				
	B.Ley	F.aus	Cono		Daph.sp				
	B.Ses	Sin	Lec		Collo				
	B.bid	Cono			Lec				
	Lec	B.bid			Chy				
	Poly	B.nov							
Taxa present	15	15	12	9	14	8	0	2	2
Mandina	Tri	B.Pli	Cop.naup	Tri	B.pli	Cop.naup	Tri	B.pli	Tri
	Cop.naup	Cop.naup	Tri	Cop.naup	Boeck	B.pli	B.pli	Tri	Cop.naup
	Cop.	Tri		Cop	Cop.naup	B.ses	Cop.naup	Cop.naup	Sin
	Boeck	Cop		Harp	Tri	Boeck	Boeck	Boeck	B.pli
		Boeck			Cop	Harp			
Taxa present	4	5	2	4	5	5	4	4	4
KC-Road	B.pli	Cop.naup	Cop.naup	B.dim	B.pli	B.pli	Boeck	B.pli	B.pli
	Cop.naup	B.pli	Cop	Cop.naup	Cop.naup	Cop.naup	Tri	Hexa	Cop.naup
	B.dim	Boeck	B.pli	B.pli	Simo	Cop	B.pli	Cop.naup	
		Hexa	Boeck	Asco	B.bid	Boeck	B.dim		
		Harp	B.bid	Harp		Simo			
				Tri		Hexa			
						B.bid			
Taxa present	3	5	5	6	4	7	4	3	2

emerged from wetlands sediment. This is further strengthened by the results of the elevated salinity experiment where fewer species emerged from Willalooka wetlands sediment as salinity increased from 300 to 5000 and 15 000 mg dm^{-3}. In other studies of species richness-salinity relationships, it has been suggested that zooplankton diversity will be affected once salinity reaches 1000 mg dm^{-3} (Hart *et al.* 1991; 2003; Nielsen *et al.* 2003b). In this study, a significant decreased in taxa number and abundance were observed as salinity increased from 300 to 5000 mg dm^{-3} and continued to decrease once salinity rose above 5000 mg dm^{-3}, suggesting a salinity threshold of between 300 and 5000 mg dm^{-3}. The salinity threshold range between 300 and 5000 mg dm^{-3} was too wide, suggesting that more works is needed to narrow down the range.

Zooplankton that emerged from Mandina and KC-Road were predominantly salt tolerant species such as rotifer, *Brachinous plicatilis*, *Trichocerca* sp. and calanoid copepods. Even though rotifers are generally of freshwater origin (De Deckker 1983), Brachionus plicatilis is known as a salt tolerant species and has been found to successfully exist at salinity levels up to 49 g dm^{-3} (Walker 1981; Dodson Frey, 2001). This study also recorded Trichocerca sp. that emerged from saline wetlands and was still recorded in freshwater samples at salinity above 5000 mg dm^{-3}, indicating that genus Trichocerca sp. is also a salt tolerant group. Trichocerca sp. has also been previously recorded from several saline lakes in Australia (Brock, Shiel 1983; Timms 1981; Timms 1998). Calanoid copepods are of marine system origin and largely salt tolerant in saline inland waters (Sarma *et al.* 2006). Research on internationally recognised, Ramsar listed saline wetlands of the Coorong Lakes system, South Australia, showed that almost all species found in 25 salt lakes studied were halophilic crustaceans (De Deckker, Geddes 1980). Our data showed that even though the experimentally increased salinity levels (300, 5000 and 15 000 mg dm^{-3}) were in fact lower than the current salinity recorded at both Mandina and KC-road wetlands, it did not necessarily replenish the freshwater community. A decrease in taxon richness associated with salinity increases was not significant for sediment taken from the Mandina wetland, suggesting that long term salinisation in this wetland had affected the longevity of egg banks which further resulted in very few zooplankton emerging from the artificially flooded sediment, even at the lowest salinity. We suggested that salinisation at Mandina and KC-Road may have contributed to the low number of taxa emerging rather than the effect of short term artificially elevated salinity. It has been suggested that the ability of a particular salinised wetland to replenish a freshwater community will not only depend on restoring the wetland to freshwater condition but also depend on the capacity of the wetland to preserve freshwater species (Nielsen *et al.* 2008). Long term salinisation impacts on zooplankton communities. It has been reported that there was a declineof faunal diversity following the increase of salinity from about 10 g dm^{-3} to 30 g dm^{-3} within 40 years (Aladin 1991).

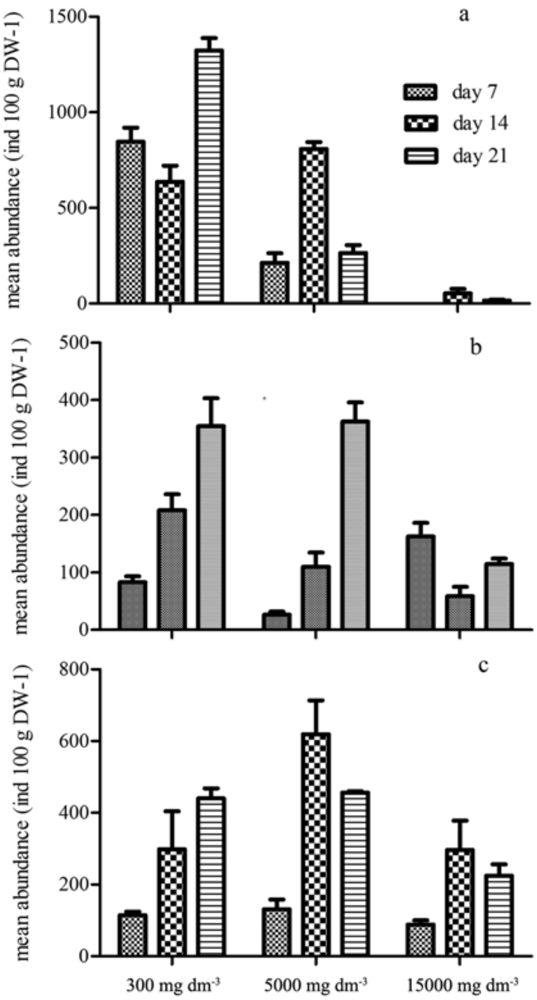

Fig. 4. Mean abundance of zooplankton that emerged after 7, 14 and 21 days of incubation at different salinity levels from (a) Willalooka sediment, (b) Mandina sediment and (c) KC-road sediment. Value represents mean ± standard error based on three replicates.

While copepods are known as salt tolerant taxa, most rotifers and cladocerans are largely restricted to freshwater systems (De Deckker 1983; Sarma *et al.* 2006). This is further confirmed by relatively more rotifers and cladocerans emerging from the Willalooka wetland sediment which was relatively fresher than that of Mandina and KC-Road wetlands. Willalooka wetlands is one of the wetland in the USE region which still fresh and of high conservation value. Experimentally increased salinity from 300 to 5000 and 15 000 mg dm^{-3} also showed a significant reduction in both taxa richness and abundance of rotifers and cladocerans emerging from the Willalooka sediment. Thus, salinity increase in a freshwater system is a influencing factor for the emergence and abundance of rotifers and cladocerans. This study produced results supporting previous studies which link increasing salinity with increasing stress on freshwater biota (Nielsen *et al.* 2003a; Kefford *et al.* 2007), resulting in more salt tolerant species persisting in the community (James *et al.* 2003). Very few zooplankton were able to persist once salinity exceed 10000 mg dm^{-3} (Pinder *et al.* 2005) and this was further confirmed in our study where only three species emerged in the 15 000 mg dm^{-3} from the freshwater sediment. The low number of zooplankton recorded in high salinity treatments and in sediment that had been subjected to long term salinisation in-situ, may also be related to the impact of salinity on emergence and survival. Early life stages of eggs and juveniles are known to be more susceptible to increasing salinity (Hart *et al.* 1991). Previous work has shown that a reduction in egg viability is related to increasing salinity which further reduces the longevity of eggs banks (De Stasio 2007). Increasing salinity may also block the cues for the eggs to emerge resulting in dormant eggs in the sediment (De Stasio 2007; Nielsen *et al.* 2007) and even if young emerge, the newly hatched may die quickly (Nielsen *et al.* 2007).

In relation to wetland salinisation in the USE region, salinity recordings conducted in September and October 2009 by Goodman (2010, unpublished work) showed the majority of remaining wetlands in this region have salinity above 5000 mg dm^{-3} to 120 000 mg dm^{-3} and only a few wetlands with salinity below 3000 mg dm^{-3}. The present study shows that within the salinised wetlands, very low numbers of zooplankton species emerged and only salt tolerant taxa persisted. On the other hand, the wetland that is still fresh was colonised by freshwater species, but, as salinity increased salt sensitive species could not tolerate it and disappeared. Hence, increasing salinity in the USE region may result in a change in community composition, with the loss of freshwater species to more salt tolerant species.

Conclusion

Salinity is a significant factor affecting the diversity and abundance of zooplankton emerging from wetland sediment. Elevated salinity reduce the diversity and abundance of zooplankton emerge from a wetlands propagules. Returning the saline to fresh condition has the potential to encourage sensitive species to return. However long term salinisation of a wetland may reduce the longevity of propagules and change the composition of egg bank over time, resulting in a reduction of the viability of propagules following a return to fresh conditions. The major implication of salinity on zooplankton community structure is the loss of sensitive species which potentially affect the wetland biodiversity if salinisation continues. Knowing the relationship between the increasing salinity and the zooplankton can be used to estimate the ecosystem's sensitivity. Therefore, proper management of vulnerable wetlands can be applied.

Acknowledgement

I gratefully acknowledge the assistance and advice of Associate Professor George G. Ganf, especially for his supervision, and valuable comments on earlier drafts, and Dr. Russel Shiel for his advice and valuable help during the project. I would also like to acknowledge Adam Bennet and Dae Heui Kim for assisting with fieldwork and Abigail Goodman for her advice in data analysis.

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