Impact of secondary salinisation on freshwater ecosystems: effect of experimentally increased salinity on an intermittent floodplain wetland

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Abstract. Intermittent wetlands are particularly at risk from secondary salinisation because salts are concentrated during drawdown. We conducted a field experiment to examine the effect of adding salt at two different concentrations (to achieve nominal conductivities of $1000\,\mu S\,cm^{-1}$ (low salt) and $3000\,\mu S\,cm^{-1}$ (high salt)) on water quality, freshwater plants and epiphytic diatoms in an intermittent wetland during a 3.3-month drawdown. Conductivity increased to 3000 and $8500\,\mu S\,cm^{-1}$ in low-salt and high-salt treatments respectively. Salt was apparently lost to the sediments, causing protons to be released from the sediments and reducing water column pH from 6.9 to 5.5 in the low-salt treatment and to 4.0 in the high-salt treatments. Forty days after adding the salt, biomass, %cover and flower production in *Potamogeton cheesmanii* were significantly reduced, whereas *Amphibromus fluitans* was not significantly affected. The salt effect on *Triglochin procera* was intermediate between the other two macrophytes. Significant reductions in the density, species richness and diversity of epiphytic diatoms occurred in the high-salt, but not in the low-salt, treatments. Our work shows that increases in salinity, and thus conductivity (up to $8500\,\mu S\,cm^{-1}$), in low-alkalinity intermittent wetlands can change water quality, with significant adverse effects on some macrophyte and diatom communities.

Additional keywords: diatoms, macrophytes, mesocosms, salinity, water quality.

Introduction

An area in excess of 160 million ha of arid and semiarid land is now affected by secondary salinisation worldwide (Williams 1999). In Australia, it is now considered the most serious land and water-resource problem with more than 5.7 million hectares currently within regions 'at risk' or 'affected' by dryland salinity, and this is predicted to increase up to ∼17 million ha by 2050. Additionally, an estimated 20 000 km of riverine habitat are currently affected by increased salinity (NLWA 2001).

The environmental impacts of increasing salinisation on the biota of inland freshwater systems are poorly understood and complex as are the interactions between salinity and the physical and chemical environment of these systems (Hart *et al*. 1990, 1991; James *et al*. 2003; Nielsen *et al*. 2003; Bailey *et al*. 2006). In recent years, there has been considerable research on the effects of salinity on a range of Australian freshwater organisms, including macroinvertebrates (Zalizniak *et al*. 2006; Horrigan *et al*. 2007; Kefford *et al*. 2007), fish (Wedderburn *et al*. 2008) and wetland plants (Robinson *et al*. 2006; Nielsen *et al*. 2008; Salter *et al*. 2008).

In the present paper, we report the results of experiments designed to examine the effect of increasing salt concentration on water quality, freshwater vascular plants and epiphytic diatoms in an intermittent floodplain wetland located in Victoria,

south-eastern Australia. Hart *et al*. (1990, 1991) previously identified such intermittent or temporary wetlands as particularly vulnerable to salt impacts because of the concentrating of salt in the water column during drawdown and the likely build-up of salt in the sediment profile. The following hypotheses were tested in our field experiment: (*1*) that the concentration of salt in the treatment mesocosms will increase during drawdown in proportion to the reduction in water volume, and (*2*) that macrophyte biomass and diversity, and diversity of epiphytic diatoms, will decrease in both the low-salt and high-salt mesocosms compared with control mesocosms, and that the magnitude of the effect will be greater in the high-salt mesocosms. A subsequent hypothesis tested in two laboratory experiments was that the decrease in pH observed in the salt-added mesocosms was due to added sodium ions liberating protons from the surface sediments by ion exchange.

Materials and methods

Study site

The study site was an intermittent wetland (Raftery wetland – 36°27′S, 145°22′E, elevation 114 m) located in Raftery State Forest, on the floodplain adjacent to the Goulburn River, ∼5 km south of Shepparton, Victoria, Australia. Raftery wetland is a Y-shaped depression (∼150 ha) that typically fills after flooding

to form a shallow (<1 m) freshwater marsh (*sensu* Department of Conservation and Environment 1992). Flooding generally occurs in late winter to early spring, followed by a period of drawdown during summer, with the wetland becoming dry by February or March. This wetland also experiences extended dry periods (e.g. 1997–2004) when no flooding occurs.

Overstorey vegetation around the wetland is dominated by *Eucalyptus camaldulensis* (river redgum), whereas understorey consists mainly of small herbs and grasses. The aquatic plant community present in the wetland after flooding is typical of similar wetlands in south-eastern Australia (Sainty and Jacobs 2003; Robertson and James 2007). The submerged angiosperms *Potamogeton cheesmanii* J.H. Willis and *Triglochin procera* R.Br. are attached, rhizomatous perennials with surface-floating leaves that form a dense, homogeneous stand throughout most of the wetland. *Myriophyllum crispatum* Orch., an attached perennial with mostly submerged feathery leaves and some emergent shoots, grows mainly in shallow areas along the edge of the wetland. *Stellaria palustris* Ehrh. ex Retz., an attached submerged annual, grows in dense, scattered stands a few metres in diameter throughout the wetland. *Amphibromus fluitans* Kirk, an attached emergent perennial grass is widespread throughout the wetland whereas *Eleocharis acuta* R.Br., a rhizomatous emergent rush, grows in scattered clumps in shallow water throughout the wetland and along the edge. There are also stands of the rhizomatous perennial sedges *Carex apressa* R.Br. and *Cyperus gunni* Hook.f. scattered on the floodplain.

Experimental design and mesocosm construction

After a preliminary survey of the flooded wetland in 1990, nine square mesocosms (10-m sides) were installed across a 600-m length of the dry wetland in early May 1991. Each mesocosm contained a representative community of the dominant aquatic plants (i.e. *Potamogeton cheesmanii*, *Triglochin procera* and *Amphibromus fluitans*). Isolated patches of *Eleocharis acuta*, *Myriophyllum crispatum* and *Stellaria palustris* were also present in the mesocosms.

The sides of the mesocosms consisted of a curtain of polyethylene film ('Solargrow', Sarlon Industries Pty Ltd, Melbourne, Australia). The bottom 40 cm of the curtain was buried in a 10-cm-wide and 40-cm-deep trench leaving 50 cm of the curtain above the ground. The bottom of each mesocosm was the undisturbed bed of the wetland. The unfastened sides of the mesocosms were left folded on the ground to avoid damage as the wetland flooded. When the water level in the wetland had decreased to ∼45 cm deep, the sides of each mesocosm were fastened to metal stakes driven into the sediment and a continuous length of polyethylene flotation attached to the upper edge so that it floated just above the water surface and could accommodate changes in the water level. Each curtain partitioned off about ∼45 000 L of water in an area of 100 m^2. To identify possible mesocosm effects, three similar-sized, open-water sites (outside the mesocosms) were also monitored simultaneously and compared with control mesocosms.

On 5 November 1991, 39 days after the wetland had flooded, salt ('table grade' sea salt, Cheetham Salt Ltd, Melbourne, Australia) was dissolved in water from the wetland before being added to six randomly selected mesocosms allocated

to two treatments. The salt concentration of six mesocosms was increased, three to nominally 1000 μS cm^{-1} conductivity (low-salt treatment), and three to nominally 3000 μS cm^{-1} conductivity (high-salt treatment). The three remaining mesocosms served as controls. The mass of salt required to increase salt concentration to the required conductivity for each mesocosm was calculated from the volume and conductivity of water present in the mesocosm on Day 38 (we used a conversion factor: μS cm^{-1} (conductivity) = 0.6 mg L^{-1} (concentration of total dissolved solids (TDS)) after Williams 1987). Following weighing, salt was dissolved in 20 L of water, and 1-L aliquots of this concentrated salt solution were subsequently diluted 1 : 10 in wetland water and broadcasted over the water surface of the mesocosm. Twelve hours after the salt solution had been added, conductivity measurements (Hach meter, Model 44 600; Ames, IA, USA) were made at random locations in each mesocosm throughout the water column to determine whether there was any spatial variation within the mesocosms. These measurements showed that the salt dissipated throughout the water column and that the target conductivities were achieved. The initial salt concentrations for the treatment mesocosms were selected because they represented the minimum and maximum concentrations of 85% of the groundwater in the Shepparton Irrigation Region (Salt Force 1989). Chemical analysis of the salt showed that it was typical of sea salt and consisted of the same ionic composition as the groundwater in the region, with the dominant salt being NaCl. Samples for water quality and macrophytes were taken from the mesocosms and the wetland between October 1991 (before salt was added) and February 1992. Sampling days are related to days since flooding, which occurred on 28 September 1991.

Sampling and analysis

Nutrients

On each sampling occasion, conductivity and pH were measured, and a 1-L water sample was taken from each mesocosm and the three open water sites. Water samples were collected from just below the water surface with polyethylene bottles (washed with phosphate-free detergent) and then temporarily stored on ice before being frozen. These water samples were used to confirm pH and conductivity measurements taken in the field, and to determine total suspended solids (TSS), alkalinity and the concentrations of the major cations (Na, K, Ca and Mg), major anions (Cl, SO$_4$), total phosphorus (TP) and total nitrogen (TN). A subsample of 60 mL was filtered in the field with a syringe filter (0.2 μm), and the filtrate was used to determine concentrations of ammonia (NH$_4$-N), nitrate (NO$_3$-N) and filterable reactive phosphorus (FRP). All chemical analyses were carried out in the Water Studies Centre, Monash University laboratory (NATA accredited), using standard methods (APHA 2007). Normal quality control/quality assurance procedures were undertaken.

Sediment interstitial water

To determine the conductivity of interstitial water and the depth of salt intrusion into the sediment, three sediment cores (5 cm diameter, 10 cm long) were taken from each mesocosm on each sampling date. In the laboratory, loose organic material was removed from the top of the core and a 1-cm-thick disc was cut from the top of each core from every collection, and also between

3–4 cm and 7–8 cm for the samples taken on Days 24, 55, 88 and 147 (i.e. final) visit. Approximately 25% of the wet disc was oven-dried to a constant weight (70°C) and the water content was calculated. The remainder of the wet disk was placed in a centrifuge tube, 15 mL of deionised water added, the tube was shaken to form a slurry, then centrifuged at 1200*g* for 10 min, and the conductivity of the supernatant measured.

Aquatic plants

Plant samples were taken from a 64-m^2 area (8 × 8 m) inside each mesocosm, leaving a 1-m-wide buffer zone adjacent to the inner side of the curtain. Five 0.25-m^2 areas in each mesocosm were sampled on each visit. Each sample area was randomly selected from the outermost 1-m-wide band of the initial 64-m^2 sampling area, and then progressively towards the centre of the sampling area on subsequent visits. In this way, we avoided trampling areas and minimised disturbance inside the mesocosm. An additional 0.5-m-wide buffer zone was left around each sample area. Pilot studies undertaken the previous year had indicated that both buffer sizes were adequate to minimise disturbance on adjacent sampling areas and the possible curtain effect.

A 0.25-m^2 quadrat was placed on the water surface at the sampling position. Emergent biomass of *Amphibromus fluitans* was harvested from within the quadrat, returned to the laboratory and oven-dried at 70°C to a constant weight. A 4-cm grid fitted to the quadrat was used to estimate %cover of green tissue of *Potamogeton cheesmanii* and *Triglochin procera* at the water surface. Once completed, an open-ended, aluminium-clad drop-box sampler (75 cm high × 50 × 50 cm) was used to sample plant biomass (and invertebrates). A period of at least 2 h was left between %cover estimates and biomass removal to minimise disturbance to the invertebrates. Once the drop box was in position above the sample area, it was rapidly pushed down through the water column into the soft sediment. Aboveground plant biomass was then removed, rinsed in the volume of water inside the drop box to dislodge animals, placed in a labelled plastic bag and put on ice. In the laboratory, plant material was sorted to species and oven-dried to a constant weight at 70°C.

Epiphytic diatom diversity

Epiphytic diatoms were analysed from samples (three per mesocosm) of randomly selected dried leaves (∼4.5 g dry weight (DW)) of *P. cheesmanii* that had been collected from each mesocosm on Days 38, 79 and 110. Samples were placed in distilled water for 2 h, boiled for 10 min and allowed to settle overnight. Extracts from each sample were individually digested, prepared and examined after Blinn and Bailey (2001). The number of cells per g DW of *P. cheesmanii* and the values of relative abundance for each taxon in each treatment mesocosm were determined. A diversity index (*H′*) was calculated for the diatom assemblage in each salt-treated and control microcosm.

Sediment ion exchange and pH

Two laboratory experiments were conducted to test the hypothesis that the decrease in pH observed in salt-treated mesocosms was due to added sodium ions liberating protons from the surface sediments by ion exchange. In the first experiment, increasing concentrations of NaCl were added to a suspension of 10 g of wet sediment in 500 mL of deionised water. The sediment was obtained from a homogenised sample of 10 cores (0–2 cm deep) collected from the wetland in March 1992. This sediment concentration corresponds to the salt solution penetrating only 0.5 mm into the sediment.

The second experiment was conducted in 1997, using sediment from Raftery wetland that had been dry for 3 years. A quantity of 7 g of air-dried wetland sediment (homogenised sample of 10 cores, 0–2 cm deep) was suspended in 100 mL of filtered (0.45 μm) Goulburn River water in 125-mL flasks. The final concentration of salt in treatment flasks was increased to 3000 mg L^{-1} (*n* = 6), 9000 mg L^{-1} (*n* = 6) and 15 000 mg L^{-1} (*n* = 6). Control flasks consisted of sediment and filtered river water (*n* = 6) or flasks were filled with 125 mL of distilled water and no sediment (*n* = 6). The flasks were incubated at 20°C at 90–95 μE on a 12/12 h light/dark cycle in a controlled environment cabinet. The pH was measured at the commencement of the experiment (6 February 1997) and at 5-day intervals during 21 days. The purpose of this experiment was to estimate, under laboratory conditions, the potential for wetland sediment that had been dry for 3 years to liberate protons and decrease the pH of the overlying water under a more extensive range of salt concentrations than were examined in the field experiment. The salt concentrations used were based on groundwater data collected for the Shepparton Irrigation district and water-column conductivities of salt-affected wetlands in northern and western Victoria.

Statistical analysis

Preliminary data screening of dependent variables involved constructing box plots to check for normality, and plots of estimated means *v.* residuals to check for homogeneity of variance. After inspection, the data were usually transformed, by 4th square root for biomass and arcsine for %cover. A nested ANOVA, with mesocosm nested within treatment, was used to examine treatment effects at each time (Quinn and Keough 2002). Mesocosm was classified as a random factor because mesocosms were randomly situated in the wetland and each allocated randomly to a particular treatment. Where significant treatment effects were observed, means were compared with Tukey's test. For the sediment ion-exchange experiment we used a repeated-measures ANOVA with time as a fixed factor.

Results

Hydrology

The wetland flooded to a maximum depth of ∼1.5 m during the time the Goulburn River was in flood, with the flood peak estimated to have occurred on 28 September 1991. The first water-depth measurements in the wetland were taken 16 days (i.e. Day 16) after this date, when the wetland was full. The mean water depth in the nine mesocosms at this time was 56 ± 3 cm (mean ± s.d.) (Fig. 1). The water depth dropped rapidly between Days 16 and 29 as the last of the floodwater drained back to the Goulburn River. During the period from 11 November 1991 to 13 January 1992, the mean water depth in the nine mesocosms on each sampling occasion was very similar (Fig. 1). The decrease in water depth followed the linear relationship: depth (cm) = 62 − 0.53 time (days); r^2 = 0.99 (Fig. 1). The water loss
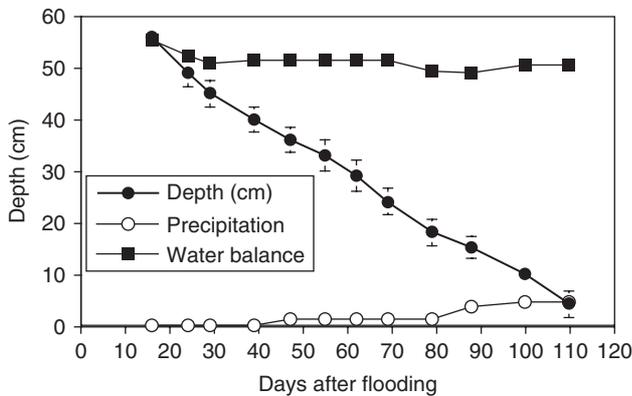
Fig. 1. Water depth, rainfall and water balance at Raftery wetland, Shepparton, Victoria, between September 1991 and February 1992. Water balance = depth + evaporation − rainfall. Flooding occurred on 28 September 1991. Figures provided as mean ± s.d.

occurred primarily because of evaporation where, apart from the period between Days 16 and 24, the sum of water depth and cumulative evaporation less cumulative precipitation was constant (Fig. 1). There was a drop to 1/10 (from ∼42 m^3 to 4 m^3) of water from the mesocosms during the study period.

Conductivity and pH

In the period before salt was added, conductivity in the nine mesocosms and three wetland sites was $100 \pm 24\,\mu S\,cm^{-1}$ (mean ± s.d., $n = 17$). As expected, this was similar to the conductivity in the Goulburn River at high flow ($86\,\mu S\,cm^{-1}$). The conductivity in the wetland and the control mesocosms increased as water depth decreased from Day 24 to Day 110 (Fig. 2a). Mean conductivity in the wetland increased from 100 to $485\,\mu S\,cm^{-1}$ and in the three control mesocosms from 100 to $195\,\mu S\,cm^{-1}$. After the addition of salt, there was a 5-fold increase in the mean conductivity in the low-salt and high-salt mesocosms, with the final conductivities rising to ∼$3000\,\mu S\,cm^{-1}$ and ∼$8500\,\mu S\,cm^{-1}$ respectively (Fig. 2a).

The pH in the control mesocosms and the wetland decreased from 6.9 to 6.4 during the study period (Fig. 2b). In contrast, the pH decrease in the salt-treated mesocosms was considerably greater, with mean pH decreasing by 1.3 and 2.9 units to a final pH of 5.6 in the low-salt mesocosm and 4.0 in the high-salt mesocosm (Fig. 2b). The pH change in the high-salt mesocosms was most rapid in the 40-day period immediately after the salt was added (i.e. between Days 39 and 79).

Water-column nutrient concentrations

The concentration of TP in the wetland approximately doubled in the first part of the study (from Day 16 to Day 39) and then remained relatively constant at ∼$200\,\mu g\,L^{-1}$, until the last 2 weeks of the study when a very rapid increase in the concentration was noted (Fig. 2c). The variations in the TP concentration in the control mesocosms broadly mirrored those in the wetland. Very low concentrations of FRP were recorded in both the wetland and the control mesocosms on most sampling occasions. In the wetland, the concentration of FRP ranged between <2 and $5\,\mu g\,L^{-1}$, except for Days 39 and 55 (Fig. 2d). Equally, the

concentration of FRP in the control mesocosms was very low (<2–3 $\mu g\,L^{-1}$) on most sampling occasions.

The concentration of TP generally decreased in the salt-dosed mesocosms (certainly compared with that in the control mesocosms) between Days 39 and 88, after which it increased dramatically in all mesocosms. The concentrations of FRP in the salt-dosed mesocosms were very low, except for two spuriously high readings in one of the high-dose mesocosms, and for higher values generally on Day 39.

The trend in the concentration of TN was broadly similar in the wetland, control mesocosms and salt-dosed mesocosms throughout the study period, with a gradual increase observed between Days 24 and 100, and then a dramatic increase during the last 10 days (Fig. 2e). In the control and salt-dosed mesocosms, it is possible that there was a slight decrease in the concentration of TN between Days 69 and 88.

The NO$_3$-N concentration was very low during the study period, being below the analytical detection limit of $5\,\mu g\,L^{-1}$. The one exception was on Day 55 when the three samples taken from the wetland had elevated NO$_3$-N concentrations (6, 14 and $82\,\mu g\,L^{-1}$), probably owing to the presence of cattle in the wetland at that time. The concentrations of FRP and NH$_4$-N were also elevated in the wetland on this day. The concentration of NH$_4$-N showed a similar trend in the wetland (excluding three very high values that are assumed to be due to cattle) and the control mesocosms, with a gradual increase from ∼$6\,\mu g\,L^{-1}$ to ∼$35\,\mu g\,L^{-1}$ by Day 80, and then a more rapid increase to the end of the study (∼$150\,\mu g\,L^{-1}$) (Fig. 2f). The trend was similar in the salt-dosed mesocosms, although the increase in the concentration towards the end of the study was not as great as in the controls (Fig. 2f).

Sediment interstitial water

Immediately before salt addition, the conductivity of the sediment interstitial water was the same in all treatment mesocosms ($F_{2,26} = 1.023$, $P = 0.3735$). However, after salt addition a consistent pattern developed where the concentration of salt was higher in the upper sediment profile than deeper down (Table 1). By Day 79 (i.e. 40 days after salt addition), the salt concentration of the interstitial water was significantly higher in the high-salt mesocosms than in the low-salt mesocosms and both were higher than that in the control mesocosms ($F_{3,35} = 9.118$, $P = 0.0023$). However, the salt concentration at the three different depths was the same (Table 1). A similar pattern existed 1 month later. However, on this occasion the concentration of salt in the upper sediment profile was higher than that found both at 2–5-cm and 6–10-cm depth (Table 1). The high variance may reflect subtle microscale differences in the distribution of clay or sediment type in the cores.

Sediment ion exchange and pH

In Experiment 1, the addition of only $500\,mg\,L^{-1}$ of NaCl to fresh wetland sediment collected at the time of the field experiment in 1992 resulted in the pH of the solution decreasing by almost 2 units, from 6.7 to 4.9. The solution pH then only decreased a further 0.2 units after the addition of a further $3500\,mg\,L^{-1}$ of NaCl.
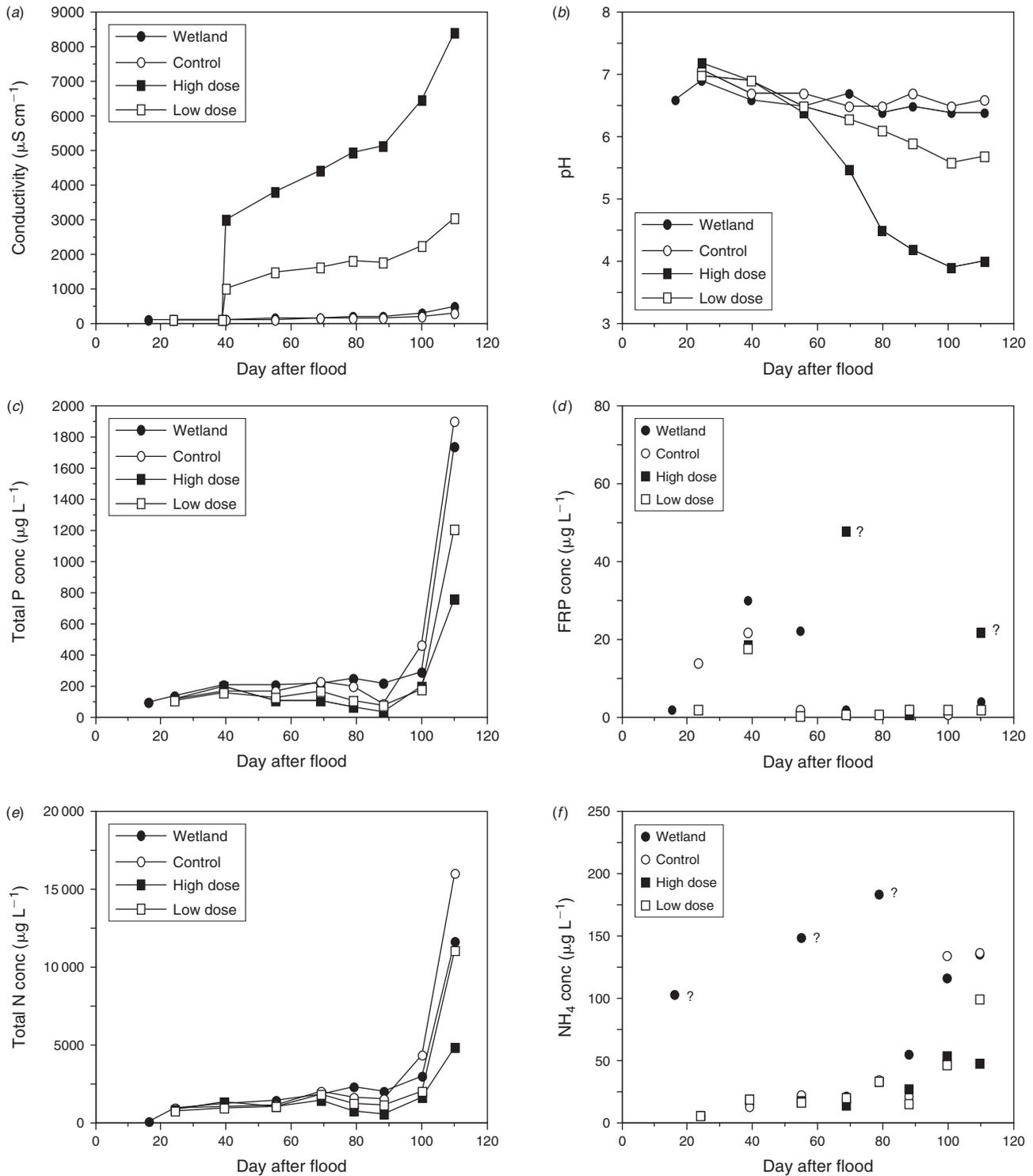
Fig. 2. Changes in water-quality indicators at Raftery wetland, Shepparton, Victoria, from October 1991 (16 days after flooding) to February 1992. (*a*) Conductivity, (*b*) pH, (*c*) total P, (*d*) FRP, (*e*) total N and (*f*) NH_4.

In Experiment 2, the pH in flasks containing sediment and filtered water from the Goulburn River was 6.3 ± 0.2 (mean $\pm$ s.d.) before the addition of salt. After the salt addition, the pH in the treatment flasks fell on average 1.2 units compared with the control solutions ($F_{3,19} = 21.9$, $P = 0.0012$, Table 2). However, after 21 days there was no difference between the mean pH for each salt treatment (5.5 ± 0.1 for $3000\,mg\,L^{-1}$, 5.4 ± 0.1 for $9000\,mg\,L^{-1}$ and 5.3 ± 0.1 for $15\,000\,mg\,L^{-1}$, Table 2).

Table 1. Conductivity of interstitial water at various depth profiles in sediment from Raftery wetland following salt application at Day 39

Values show mean $\pm$ s.d. ($n = 3$) in control, low-salt and high-salt mesocosms and at open wetland (WL) sites. NA = not applicable; SL = sample lost

Days since flooding (days since salt added)	Sediment depth (cm)	Conductivity ($\mu S\,cm^{-1}$)			
		Control	Low	High	WL
38 (NA)	Surface	2050 ± 750	3390 ± 2260	2670 ± 1740	SL
	2–5	2270 ± 710	2110 ± 770	2960 ± 1760	SL
	6–10	1660 ± 530	1610 ± 490	2730 ± 2360	SL
55 (16)	Surface	1920 ± 1510	2140 ± 1020	3850 ± 2180	900 ± 540
	2–5	1920 ± 920	2100 ± 960	2950 ± 1030	920 ± 750
	6–10	1270 ± 560	1730 ± 800	2960 ± 1250	1210 ± 170
78 (40)	Surface	990 ± 500	1800 ± 210	4580 ± 1740	1200 ± 470
	2–5	830 ± 270	1820 ± 410	4670 ± 1760	1000 ± 650
	6–10	720 ± 250	1620 ± 320	4810 ± 2360	670 ± 340
110 (72)	Surface	650 ± 240	3640 ± 1620	$12\,500 \pm 1740$	87 ± 490
	2–5	480 ± 100	2930 ± 650	7310 ± 1760	690 ± 360
	6–10	490 ± 85	3000 ± 609	$66\,700 \pm 2360$	620 ± 1

Table 2. Change in the water-column pH following the flooding of dry wetland sediment with water containing TDS at 3000, 9000 or 15 000 mg L^{-1}, in the laboratory experiments (mean $\pm$ s.e.m.)

Sea salt was dissolved in filtered (0.45 μm) Goulburn River water. Controls consisted of sediment and filtered river water only

Days since flooding	Salt concentration (mg L^{-1})			
	Control	3000	9000	15 000
0	6.3 ± 0.2	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1
7	6.7 ± 0.1	5.9 ± 0.1	5.6 ± 0.1	5.5 ± 0.1
11	6.6 ± 0.2	5.6 ± 0.1	5.5 ± 0.1	5.3 ± 0.1
21	6.5 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.3 ± 0.1

Response of macrophytes

Diversity

Twenty-one days after the flood, the plant community was well established with the apical leaves of *A. fluitans* floating on the surface of the water, although with very few emergent shoots. The tips of the leaves of *T. procera* and the stems of *S. palustris* were just at the water surface. Apical floating leaves of *P. cheesmanii* were just below the surface of the water. In most samples, aboveground parts of *E. acuta* (found in one sample) and *M. crispatum* (found in three samples) were not detected. *A. fluitans*, *P. cheesmanii* and *T. procera* were the most common species in the wetland, occurring in 85–100% of quadrats throughout the study. In contrast, *M. crispatum* and *S. palustris* occurred in 36 and 33% of quadrats, respectively, and *E. acuta* was rare (18% of quadrats). Increasing salinity did not change the plant species present or the order of frequency of occurrence of the six species relative to each other.

Biomass

Immediately before the addition of salt on Day 39, the total macrophyte biomass (DW) in the mesocosms was $134 \pm 7\,g\,m^{-2}$ (mean $\pm$ s.d.) (Fig. 3*a*), with *A. fluitans* and *P. cheesmanii* accounting for ~84% ($113 \pm 7\,g\,m^{-2}$) and 11% ($14.3 \pm 1.7\,g\,m^{-2}$), respectively, of the biomass, with minor contributions from *T. procera* ($6.5 \pm 1.9\,g\,m^{-2}$) and *S. palustris* ($1.9 \pm 1.7\,g\,m^{-2}$). Maximum biomass in the control mesocosms was reached on Day 100 ($929 \pm 35\,g\,m^{-2}$ DW), the DW biomass declining to $826 \pm 30\,g\,m^{-2}$ on the last sampling occasion (Day 147, Fig. 3*a*). The relative contribution from each plant species remained essentially the same as that established on Day 39 throughout the sampling period.

By Day 79 (i.e. 40 days after the addition of salt), a pattern had been established where the total biomass was highest in the control mesocosms, intermediate in the low-salt mesocosms and lowest in the high-salt mesocosms (Fig. 3*a*). On Days 88, 100 and 110, an ANOVA detected a significant treatment effect ($F_{2,6} = 13.921$, $P = 0.0056$; $F_{2,6} = 8.647$, $P = 0.0171$; $F_{2,6} = 16.789$, $P = 0.0035$ respectively) and Tukey's *post hoc* tests showed that on Days 88 and 100, biomass (g m^{-2} DW) was significantly ($P < 0.05$) higher in the control mesocosms than in the low-salt and high-salt mesocosms. However, on Day 110 the biomass in the low-salt mesocosm was significantly different from that in the high-salt mesocosm (Fig. 3*a*).

However, not all plant species were as adversely affected. For example, with *A. fluitans* the total biomass, and the biomass of emergent and submerged components showed no significant reduction, although the biomass was consistently lower in the low-salt and high-salt mesocosms (Fig. 3*b*, *d*, *f*). In contrast, biomass of *P. cheesmanii* showed some decrease with increasing salinity on Day 55 (16 days after salt addition), with the effect becoming significant by Day 79 and on each sampling occasion thereafter (Day 79: $F_{2,6} = 8.536$, $P = 0.0184$; Day 88: $F_{2,6} = 15.891$, $P = 0.004$; Day 100: $F_{2,6} = 39.913$, $P = 0.0003$; Day 110: $F_{2,6} = 61.347$, $P < 0.001$; Day 142: $F_{2,6} = 56.240$, $P < 0.001$) (Fig. 3*c*). Moreover, on each of these sampling occasions, Tukey's *post hoc* tests showed that the biomass in the high-salt mesocosms was significantly lower than that in the low-salt mesocosms, with both of these lower than that in the control
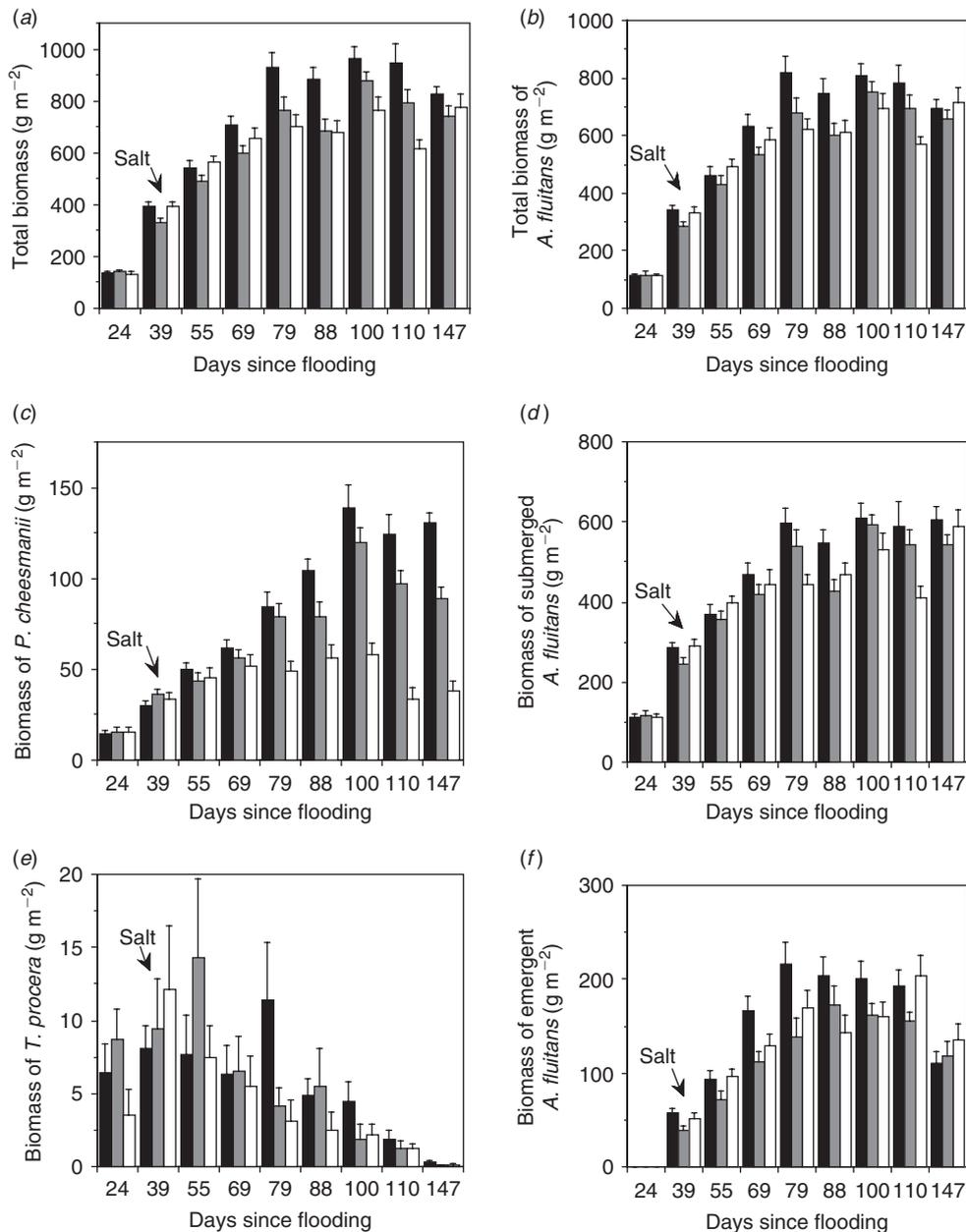
Fig. 3. Changes in macrophyte dry-weight biomass (g m^{-2}) in Raftery wetland, Shepparton, Victoria, between September 1991 and February 1992, following salt addition at Day 39. (*a*) Total plant biomass; (*b*) total biomass of *Amphibromus fluitans*; (*c*) biomass of *Potamogeton cheesmanii*; (*d*) biomass of submerged *A. fluitans*; (*e*) biomass of *Triglochin procera*; (*f*) biomass of emergent *A. fluitans* in the control (black), low-salt (shaded) and high-salt (white) mesocosms. Bars show mean ± s.e. (*n* = 15).

mesocosms ($P < 0.001$) (Fig. 3*c*). It was not possible to confidently discern an obvious salt effect on *T. procera* biomass, since the biomass decreased linearly to close to zero during the study period in all mesocosms (Fig. 3*e*).

Percentage cover of P. cheesmanii *and* T. procera

Percentage cover of green tissue in *P. cheesmanii* increased from 0.3 ± 0.3% on Day 24 to a maximum of 36 ± 3% on Day 88, and then declined gradually as the wetland dried

(Fig. 4*a*). Sixteen days after the addition of salt (i.e. Day 55), a pattern had been established where %cover of *P. cheesmanii* reflected the trend for the biomass, being highest in the control mesocosms, intermediate in the low-salt mesocosms and lowest in the high-salt mesocosms (Fig. 4*a*). This effect became more pronounced with time and an ANOVA showed a significant effect on each sampling occasion from Day 69 (Day 69: $F_{2,6} = 18.718$, $P = 0.0026$; Day 79: $F_{2,6} = 27.334$, $P = 0.001$; Day 88: $F_{2,6} = 51.996$, $P = 0.002$; Day 100: $F_{2,6} = 69.547$,
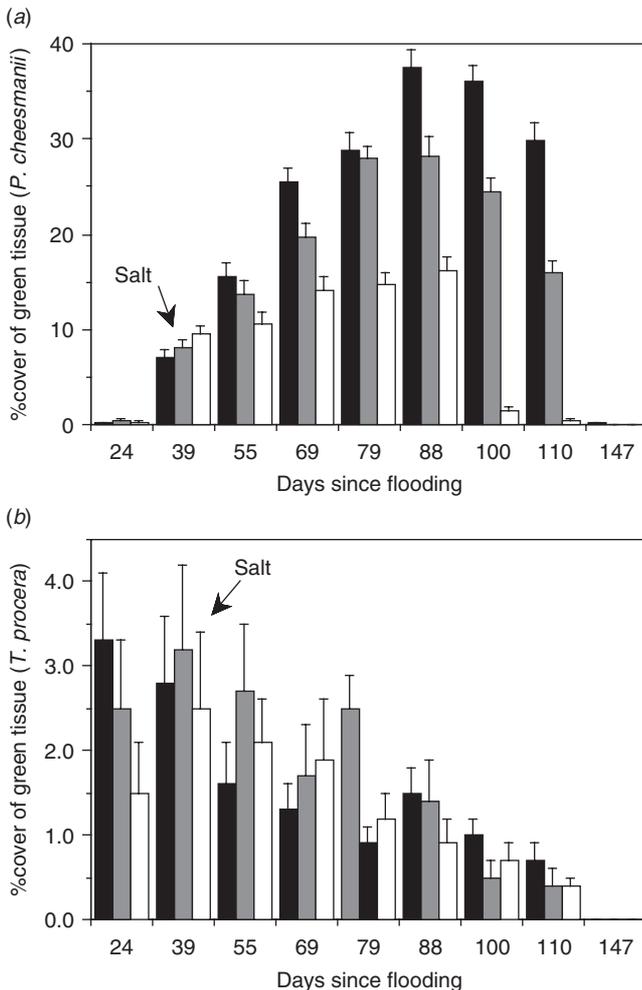
(a)

(b)

Fig. 4. Change in %cover of macrophyte green tissue in (a) *Potamogeton cheesmanii* and (b) *Triglochin procera* in the control (black), low-salt (shaded) and high-salt (white) mesocosms, following salt application at Raftery wetland, Shepparton, Victoria. Bars show mean $\pm$ s.e. ($n = 15$).

$P < 0.0001$; Day 110: $F_{2,6} = 88.391$, $P < 0.0001$). Tukey's *post hoc* tests showed that on Days 69 and 79, %cover of green leaf material in the high-salt mesocosms was significantly ($P < 0.05$) lower than that both in the control and low-salt mesocosms, which were not significantly different from each other. By Days 88, 100 and 110, the mean %covers for all treatments were significantly ($P < 0.001$) different from each other. By Day 147, there was no green leaf material in any of the six salt-treated mesocosms and very little in the control mesocosms, coinciding with minimal surface water following drawdown (Fig. 4*a*).

Twenty-four days after the flood, the %cover of green tissue in *T. procera* was 2.4 ± 0.8%, and this decreased to 0.6 ± 0.2% by Day 110. There was no difference between the salt-treated and control mesocosms (Fig. 4*b*).

Inflorescence production

When salt was added on Day 39, *A. fluitans*, *P. cheesmanii* and *T. procera* were all flowering. However, whereas inflorescence

production had only just commenced in *A. fluitans* (number of inflorescences $4 \pm 2\,\mathrm{m}^{-2}$) and *P. cheesmanii* (number of inflorescences $2 \pm 1\,\mathrm{m}^{-2}$), and reached a maximum by Day 55 (number of inflorescences $23 \pm 12\,\mathrm{m}^{-2}$, and $15 \pm 3\,\mathrm{m}^{-2}$ respectively), in *T. procera* it had apparently already peaked (number of inflorescences $5 \pm 2\,\mathrm{m}^{-2}$) and the number declined on subsequent sampling occasions.

Inflorescence production by *P. cheesmanii* was strongly suppressed in the high-salt mesocosms. For example, by Day 79 there were no inflorescences present in any of these mesocosms. Although the number of inflorescences produced in the low-salt and control mesocosms was about the same ($11 \pm 1\,\mathrm{m}^{-2}$ and $13 \pm 3\,\mathrm{m}^{-2}$ respectively), the inflorescences produced in the low-salt mesocosms were smaller than those in the control mesocosms, and usually rotted. None of the flowers produced in the salt-treated mesocosms was observed to set seed, although seed was freely produced and shed in the control mesocosms.

In contrast, salt did not appear to affect flowering in *A. fluitans* as severely as in *P. cheesmanii*. *A. fluitans* was flowering abundantly in all mesocosms by Day 55, and at Days 79 and 88 seed had matured and was being shed; at Day 100, virtually all the seed had been shed. There was no evidence of any difference between the treatments in the quality or quantity of seed produced.

Because most inflorescence production of *T. procera* had occurred before salt was added, we are unable to speculate on possible negative effects of salt. Certainly by Day 79, the number of inflorescences was similar across all treatment mesocosms, ranging from $2 \pm 1\,\mathrm{m}^{-2}$ in the control mesocosms to $1 \pm 0\,\mathrm{m}^{-2}$ in the high-salt mesocosms.

Epiphytic diatom assemblages on
Potamogeton cheesmanii

Forty-six diatom taxa were associated with the epiphytic assemblage of *P. cheesmanii*. Diatom densities were significantly (ANOVA, $F_{2,9} = 5.5$; $P = 0.0275$) reduced after 60 days in the high-salt mesocosms, but not in the control or low-salt mesocosms (*post hoc* test). Densities were not significantly different 30 days after salt application, but were reduced by 17% after a further 30 days. Densities averaged $336\,000 \pm 31\,000$, $290\,000 \pm 39\,000$ and $222\,000 \pm 112\,000$ frustules per gram of dry-weight *P. cheesmanii* for control, low-salt and high-salt treatments respectively.

Prior to salt additions, the diatom taxa *Planothidium lanceolatum* (Breb.) Round & Bukhtiyarova, *Eunotia bilunaris* (Ehr.) Mills, *Fragilaria capucina* Desm., *Fragilaria ulna* (Nitz.) Lange-Bertalot, *Gomphonema parvulum* Kütz., *Hantzschia amphioxys* (Ehr.) Grun., *Nitzschia* sp., and *Nitzschia palea* (Kütz.) W.Sm. made up >80% of the diatom assemblage in the control, 70% in the low-salt and 66% in the high-salt mesocosms. Sixty days later, these same taxa made up 51% of the assemblage in the control, 53% in the low-salt and 71% in the high-salt mesocosms. *E. bilunaris* and *G. parvulum* showed the greatest increase in density in the high-salt mesocosms after 60 days, whereas *Achnanthidium minutissimum* (Kütz.) Czar., *Epithemia sorex* Kütz., *Navicula cryptocephala* Kütz, *Nitzschia acicularis* W.Sm., *Nitzschia amphibia* Grun., *Pinnularia brevicostata* Cl., and *P. divergens* W.Sm disappeared. H' diversity was ~4.0

before salt additions and up to 60 days after treatment, except for the high-salt mesocosms, where H' was 3.2. Species richness averaged ~31 species throughout the 60-day period after treatment for all collections except for the high-salt mesocosms, which averaged only 15 taxa.

Discussion

There have been very few studies that have examined salinisation processes experimentally with *in situ* mesocosms (Herbst and Blinn 1998; Marshall and Bailey 2004). In the experiments reported here, we have demonstrated that significant changes in selected water-quality parameters and plant communities occurred after addition of salt to mesocosms located in Raftery wetland.

Water quality

There have been several studies of water-quality changes in Australian natural wetlands; however, these are of limited relevance to the present study. For example, Qiu and McComb (1994, 1996) and Mitchell and Baldwin (1998) investigated the influence of wetting and drying on the release of phosphorus from wetland sediments, whereas Briggs *et al.* (1993) related the dissolved and particulate organic matter concentrations in two wetlands to the high rates of macrophyte production and leaf input from river red gums. In our study, the conductivity in all mesocosms increased ~5-fold during the 5 months, which was considerably less than the 10-fold increase expected on the basis of the decrease in the water volume. To balance this, salt must have been lost from the water column, most likely to the sediments or incorporated into plant biomass. The amounts lost were 2 kg, 29 kg and 75 kg from the control, low-salt and high-salt treatments respectively. Similarly, the conductivity increase in the wetland was almost 5-fold (from $106\,\mu S\,cm^{-1}$ to $485\,\mu S\,cm^{-1}$).

Addition of salt to the mesocosms resulted in significant decreases in the pH of the overlying water. The effect was most noticeable in the high-salt mesocosms, where pH decreased from 6.9 to 4.0. The effect was smaller in the low-salt mesocosms, although it was still considerable, with a reduction of 1.3 units to a final pH of 5.6. We have been unable to find any other studies of freshwater wetlands where such a change in pH has been observed. However, there are several possible reasons for the observed pH decrease in the salt-dosed mesocosms. The first is that the dead plants in the salt-dosed mesocosms liberated a high concentration of organic acids. However, if this had occurred, the dissolved organic carbon concentration would have been expected to increase with time, and this was not the case. A more likely reason is that the added sodium ions liberated protons from the surface sediments by ion exchange. Indeed, both laboratory experiments supported this hypothesis. In the first sediment experiment, the observed pH change corresponded to $\sim1.2 \times 10^{-5}\,mol\,L^{-1}$ of protons liberated from the sediments, and suggested that the protons are bound to the sediment ~1500 times more strongly than Na^+ ions, assuming that Na^+ ions bind only to proton sites in the sediment. The results from the second sediment experiment further supported this hypothesis, although the magnitude of the effect was smaller. However, these results showed that the proton-exchange process was still viable for sediments that had been dried for 3 years.

It is interesting to speculate on whether this liberation of protons would occur once only on the addition of salt, or whether protons could be liberated each year if salt were added. Obviously, the process can occur only if exchangeable protons exist in the sediments, and this would occur only if the added Na^+ ions were in some way removed from the sediment back into the water column. This could occur during the early part of each 'fill' cycle when low-conductivity water ($\sim100\,\mu S\,cm^{-1}$) from the Goulburn River is added to the wetland. If this water remained in the wetland for a short time, it would be possible for Na^+ ions to be exchanged from the sediments back into the water column. The exchanged Na^+ ions would then be flushed from the wetland system. Even if this did not occur each year, it would still be possible for the sediments to retain exchangeable protons. Certainly, the high variation associated with the conductivity of interstitial water at different depths suggests that the sediment processes are not evenly distributed within the sediment profile.

The implications of this experimentally induced pH reduction to 'real' wetlands will depend on both the initial conductivity of the overlying water and on whether the wetland is regularly 'flushed' with flood waters. On the basis of the above hypothesis, we predict that in brackish wetlands that are not regularly flushed there would be little pH change because all the exchangeable protons would have been removed from the sediments. We also predict that in wetlands, such as Raftery wetland, where the initial conductivity is low and the system is regularly flushed, pH change during the drying period would be minimal because the relative concentration of Na^+ ions would be insufficient to cause release of sediment-bound protons (see Fig. 2*b*).

Changes in the concentration of five nutrients (TP, FRP, TN, NH_4-N, NO_3-N) and dissolved oxygen were measured. The behaviour of the individual nutrients differed across the study period, although in general the addition of salt did not cause any striking differences among them. The concentration of TP in the wetland and control mesocosms approximately doubled across the main part of the study to $\sim200\,\mu g\,L^{-1}$, and then increased very rapidly in the last 2 weeks of the study. Except for a few spurious high values, very low FRP concentrations (<2–$5\,\mu g\,L^{-1}$) were recorded in both the wetland and the control mesocosms across the study period. The trends in the concentrations of TP and FRP in the salt-dosed mesocosms were little different from those in the wetland and controls, with perhaps the exception being that concentrations of TP in the salt-dosed mesocosms generally decreased across most of the study period.

The rapid increase in the concentration of TP observed at the end of the study period was possibly due to a combination of the large amount of decomposing plant material releasing organic P, and the difficulties in obtaining a 'clean' sample when the water depth was only ~4 cm. Interestingly, the concentrations of FRP were not noticeably elevated at the end of the study, despite the large amount of decomposing plant material present at this time, and the concentration effect owing to evaporation. Additionally, if the sediments were anaerobic as suggested below, phosphorus would have been released from the sediments (Jordan *et al.* 2008). Thus, the very low water-column FRP concentration measured at all sites on most occasions, suggests that phosphorus is readily taken up by the attached epiphytes and the microbial

community in this wetland system, and may actually limit the growth of these biota (Harris 1999). The macrophytes would be expected to obtain most of their nutrient requirements from the sediments.

The trend in the concentration of TN was broadly similar in the wetland, control mesocosms and salt-dosed mesocosms across the study period. In the control and salt-dosed mesocosms, it is possible that there was a slight decrease in the concentration of TN between Days 69 and 88. The rapid increase in the concentration of TN observed at the end of the study period was possibly due to the large amount of decomposing plant material present in the system at this time, which would have released organic N to the water column. Additionally, there was very little water present at this time and this made it difficult to obtain a 'clean' water sample.

The concentration of NO_3-N was very low ($<5\,\mu g\,L^{-1}$) in all systems during the study period, with the exception of three samples from the wetland, where elevated nitrate concentrations were observed probably due to the presence of cattle in the wetland at that time. There are three possible reasons for the low NO_3-N concentrations in this system. The most obvious is that little nitrate is actually being formed, which would be the case if the wetland was maintained in a largely anaerobic state. The relatively high concentrations of NH_4-N measured in the system throughout the study period (Fig. 2*d*) suggest that this was the case. Equally, the nitrate concentration could be maintained at a low level if nitrate was rapidly taken up by the macrophytes, epiphytes or the microbial community, or was being lost by denitrification (Bowden 1987). Unfortunately, it is not possible to distinguish between these three possibilities with the available data.

The concentration of NH_4-N was surprisingly high (6– $150\,\mu g\,L^{-1}$) and showed a similar trend in the wetland and all mesocosms throughout the study period, although the increase in the concentration towards the end of the study was not as great in the salt-dosed mesocosms as it was in the controls. The general increase in the concentration of NH_4-N across the study period probably reflects the increased decomposition of plant material as the water level decreased. These relatively high concentrations of NH_4-N suggest that reducing conditions existed for most of the time, because if oxidising conditions had existed, the ammonia would have been rapidly oxidised to nitrate (Bowden 1987).

Aquatic plants

Several studies by Brock *et al.* (1981, 1998) have found that a salt concentration of $\sim$4000 mg L^{-1} is the upper limit tolerated by widespread freshwater macrophytes with an affinity for fresh waters. This finding has been confirmed in other studies of freshwater plants by James and Hart (1993) and Warwick and Bailey (1997, 1998). In our experiment, when the conductivity was increased to $\sim$3000 $\mu S\,cm^{-1}$ in the low-salt mesocosms and to $\sim$8500 $\mu S\,cm^{-1}$ in the high-salt mesocosms, there was no change in the species composition of aquatic plants because of the death of species or because the entry of new species was encouraged. James and Hart (1993) demonstrated a reduction in plant growth rate and size for six species of macrophytes at salt concentrations $>$1000 mg L^{-1} across a similar period

of time ($\sim$4 months) as in the present experiment. However, they found that death occurred only in two species, *P. tricarinatus* (syn. *P. cheesmanii*) and *M. crispatum*, after $\sim$1.5–2.5 months at salt concentrations of 5000 mg L^{-1} or higher. Because the rate of colonisation of a wetland by new species is dependant on the proximity of a seed source (Brock 1985), it is not surprising that colonisation by salt-tolerant species did not occur within the time frame of our investigation as there are no sources of seed of salt-tolerant species in the vicinity of Raftery wetland.

Amphibromus fluitans was not found to be sensitive to the increased salt concentration and reduced pH observed in these experiments. In contrast, *P. cheesmanii* was very sensitive. James and Hart (1993) showed in glasshouse trials that $\sim$4% of *P. tricarinatus* (syn. *P. cheesmanii*) plants were killed when exposed for 72 days to a TDS concentration of 5000 mg L^{-1}. In the present study, although the biomass of *P. cheesmanii* plants was measurably affected, death of the plants and a resultant drop in the frequency of occurrence would not be expected during the 71 days of salt exposure caused by increasing salinity. It is possible that the decrease in pH proved to be more harmful than changes in the concentration of Na^+ or Cl^- ions.

The effect of increasing salt concentration was more pronounced in the submerged biomass of *A. fluitans*, which may indicate that the effect is greatest on the tissues in contact with the saline water, rather than the emergent parts. James and Hart (1993) reported that submerged parts of *M. crispatum* lost their submerged leaves and failed to produce roots and shoots at submerged nodes when the salt concentration of the surrounding water was 2000 mg L^{-1} or more.

Increased salinity generally results in a reduction in plant vigour, which may be reflected in reduced dry-weight biomass depending on the morphology of the plant; fleshy plants will show a greater change in biomass. Macek and Rejmánková (2007) also reported reductions in plant height, shoot number, leaf length and root biomass in three emergent macrophytes (*Eleocharis cellulosa*, *Cladium jamaicense*, *Typha domingensis*) at increased salinity.

The %cover of green leaf material was a more sensitive indicator than biomass of the salt effect on *P. cheesmanii*. Thirty days after salt addition, there was a significant reduction of %cover of green leaf material in the high-salt mesocosms, whereas a significant reduction in biomass was not observed until 10 days later. We contend that biomass values alone do not illustrate the full extent of the effect of elevated salinity on *P. cheesmanii*, with %cover of green tissue probably being a more useful indicator.

There was an obvious negative effect of increased salt concentration on sexual reproduction for two of the three dominant plant species observed in the present study. The reduction in seed production observed for *T. procera*, and the blocking of seed production in *P. cheesmanii*, in the salt-treated mesocosms has important implications for the seedbank in this wetland. *P. cheesmanii* and *T. procera* have both sexual and asexual reproductive strategies (Sainty and Jacobs 2003), enabling these species to persist under a range of environmental conditions (Brock 1991; Titus and Hoover 1991). However, the long-term persistence of an aquatic plant species within a community would be threatened if sexual and/or vegetative reproductive

success were impaired by increased salinity and if replenishment of the seedbank were reduced. The size and number of *P. cheesmanii* tubers were found to be significantly reduced by increased salinity, with tuber numbers declining by ~70% and individual tuber biomass declining by 50% (K. James, P. Bailey and N. Warwick, unpubl. data).

These differential impacts on the plants and their reproductive capacity represent a reduction in the ecological resilience of the macrophyte community of Raftery wetland. This reduction may lead to the type of shift in ecological regime from a macrophyte-dominated to an alternative regime as postulated for salinising wetlands of south-western Australia (Davis *et al.* 2003; Strehlow *et al.* 2005; Sim *et al.* 2006).

Diatom communities have been shown to be sensitive to human-induced changes in the conductivity of low-salinity waters (Blinn and Bailey 2001; Blinn *et al.* 2004). The density, species richness and diversity of epiphytic diatoms were all reduced in the salt-dosed mesocosm experiment reported here, which suggests that these species would be adversely affected by increasing salinities in intermittent wetlands such as Raftery wetland. Sixty days after the salt addition, diatom densities dropped to approximately 1/6, species richness dropped >50%, and diversity dropped 20% in the high-salt mesocosms (these had a 5-fold increase in salinity). One implication of this decrease in abundance and species richness is that the number of linkages in scraper-based food webs may be reduced, and ultimately so would the carbon transfer within these wetlands (Bailey *et al.* 2006).

Increases in salinity may become exponential through time, with the continual accumulation of salts in wetland sediments. As salinity increases, new inoculations of diatoms by waterfowl from saline aquatic ecosystems will occur (Kristiansen 1996; Figuerola and Green 2002). Presently, the resting spores of diatom taxa in the dried Raftery wetland sediments represent those that are tolerant to increased salinities. However, the extreme environments of high salinity and low pH are rare on the Australian continent and it may become more difficult to inoculate diatom populations if these extreme conditions become more pronounced (Blinn *et al.* 2004).

The increase in the numerical importance of diatom species, such as *E. bilunaris*, in the high-salt mesocosms suggests that pH may override the effect of increased salinity for some taxa. This species typically prefers habitats with pH values near or below circumneutral (<7.5) and conductivities <500 μS cm^{-1} (Sonneman *et al.* 1999). The loss of taxa in the high-salt (>8000 μS cm^{-1}) and low-pH (<6.5) mesocosms suggested that there are subtle interactions between salinity and pH in diatom assemblages. For example, *A. minutissimum*, *E. sorex*, *N. cryptocephala* and *N. acicularis* all tolerate low pH although generally prefer conductivities of <2000 μS cm^{-1}, whereas *C. placentula* occurs in conductivites >5000 μS cm^{-1}, but is sensitive to low pH. In contrast, species with wide ecological tolerances such as *E. bilunaris*, *F. ulna*, *G. parvulum*, *H. amphioxys* and *N. palea* are numerically important taxa in the Raftery wetland today and are expected to be good indicators of increasing salinities in the future (Sonneman *et al.* 1999; Blinn and Bailey 2001). These taxa prefer habitats with pH values between 6.5 and 9.0 and conductivities up to 5000 μS cm^{-1} (Sonneman *et al.* 1999; Blinn and Bailey 2001).

Conclusions

The present study was designed to address three hypotheses. The first, that the concentration of salt in the treatment mesocosms would increase during drawdown in proportion to the reduction in water volume, was shown not to be true. Certainly, the salt concentration did increase with drawdown; however, it was considerably less than the 10-fold reduction in the volume. We speculate that the salt lost from the water column was incorporated into the surface layers of the sediments.

A second and related hypothesis was that the decrease in pH observed in the mesocosms with added salt was due to the added sodium ions liberating protons from the surface sediments by ion exchange. Two sets of laboratory experiments provided evidence supporting this hypothesis. However, the large pH changes (decrease of 1.4 and 2.9 pH units in the low-salt and high-salt treatments respectively) observed in these mesocosm experiments were also the result of the very low alkalinity of water in Raftery wetland, and would be expected to be considerably smaller in wetlands where the water had a greater buffering capacity. We predict that this experimentally induced pH reduction is unlikely to occur in 'real' wetlands, such as Raftery wetland, where the initial conductivity is low and the system is regularly flushed, because the increased concentration of Na$^+$ ions during the drying period would be insufficient to cause release of sediment-bound protons.

The third hypothesis was related to the effect of salt on the biomass and diversity of macrophytes, and the diversity of epiphytic diatoms. We hypothesised that these measures would decrease in both the low-salt and high-salt mesocosms compared with control mesocosms, and that the magnitude of the effect would be greatest in the high-salt mesocosms. The behaviour of *P. cheesmanii* during the first 40 days after adding the salt supported this hypothesis. However, *Amphibromus fluitans* was not significantly affected in either salt treatment, and the effects on *Triglochin procera* were intermediate between the other two macrophytes. The density, species richness and diversity of epiphytic diatoms were all significantly reduced in the high-salt mesocosms, but not in the low-salt mesocosms.

In conclusion, the present work has shown that large increases in conductivity (and thus salinity) in intermittent wetlands, up to ~8500 μS cm^{-1} (~5000 mg L^{-1}), can result in changes to water quality and significant adverse effects on some freshwater macrophyte and diatom communities.

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