APPLIED ISSUES

The effects of salinity on aquatic plant germination and zooplankton hatching from two wetland sediments

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SUMMARY

1. The effect of increasing salinity on the emergence of zooplankton eggs and the germination of aquatic plant seeds from the sediment of two wetlands was examined. Salinity was found to cause reductions in species richness and abundance of aquatic plants and zooplankton at salinities between 1000 and 5000 mg L\(^{-1}\)). Aquatic plants also had an associated decrease in above ground biomass.

2. Individual taxa showed different responses to salinity, and four response patterns were identified: (i) increased number of organisms emerging at 1000 mg L\(^{-1}\)); (ii) decreased number of organisms emerging above 1000 mg L\(^{-1}\)); (iii) decreased number of organisms emerging between 300 and 1000 mg L\(^{-1}\)); (iv) no difference in number of organisms emerging across the range of salinities. Response patterns (iii) and (iv) were common to both plants and zooplankton, whereas response patterns (i) and (ii) were only identified for zooplankton.

3. Results indicate that there is potential for the increasing salinity in Australian rivers and wetlands to decrease the species richness of aquatic communities resulting in loss of wetland biodiversity.

Keywords: aquatic plants, eggs, salinity, seeds, wetlands, zooplankton

Introduction

Australia has a diverse range of riverine and wetland ecosystems which vary widely in their predictability and duration of flooding (Puckridge et al., 1998), with the variability in water regime often moderating many important ecological processes (Walker, Sheldon & Puckridge, 1995). Wetlands are often traditionally grouped as either temporary or permanent, depending on natural water regime patterns. In general, as both predictability and duration of flooding decrease and variability of water regimes increase, wetlands will have greater dry to wet time ratios (Boulton & Brock, 1999). As a consequence of anthropogenic modification to water regimes many ‘permanent’ wetlands now dry during extended periods of low rainfall, whereas many ‘temporary’ wetlands are permanently inundated (Walker & Thoms, 1993; Williams, 1998; Brock, Smith & Jarman, 1999; Brock & Jarman, 2000).

Salt entering Australian aquatic ecosystems originates primarily from the atmosphere (e.g. rainfall, wind-borne), erosion of sediments (e.g. weathering, microbial activity) or saline groundwater. The relative contributions of these sources depend on factors such as distance inland, climate and geology (Williams, 1987; Baldwin, 1996; Boulton & Brock, 1999). Secondary salinisation, notably as a consequence of the
clearing of native vegetation and irrigation, has increased the amount of salt entering freshwater ecosystems (Williams, 1987; Hart et al., 1991). It has been estimated that in the future more than 15.5 million hectares of land across Australia will be affected by salinity (PMSEIC, 1998). Eventually this salt will be washed into rivers and wetlands, thereby increasing the salinity of these systems (MDBC, 1999). Predictions are that in excess of 40 000 km of streams across Australia will be affected by salinity by 2050 (ANZECC, 2001).

Under a natural water regime, periods of increased salinity generally occur in many aquatic ecosystems during periods of low flows. Biota often either have mechanisms of tolerating periods of high salinity or become locally extinct. Rainfall either results in dilution of wetland salts or a flushing of salts from wetlands and floodplains to downstream river channels. For example, regulation of the Murray-Darling river system has reduced the natural flushing of the river channel and associated wetlands (Close, 1990). While there is currently little evidence to suggest that salinity will exceed the natural tolerance of biota associated with the river channels of the Murray-Darling river system there is some evidence that salt will accumulate in wetlands as a consequence of reduced flushing and concentrations of salt will exceed critical levels for native biota (Bailey & James, 1999; Gutteridge, Haskins & Davey Pty. Ltd, 1999; Nielsen & Hillman, 2000; Clunie et al., 2002).

Studies on the effects of salinity have generally focused on impacts on adult life stages, i.e. those that are potentially the most tolerant life stages (Metzeling, Doeg & O’Connor, 1995). In contrast, early life stages, such as the germinating seeds of aquatic plants and hatching eggs of aquatic animals, could be the most susceptible to increasing salinity (Choudhuri, 1968; Skinner, Sheldon & Walker, 2001). Wetland sediments contain a reservoir of dormant seeds and vegetative propagules of aquatic plants and eggs of aquatic invertebrates such as rotifers and microcrustacean, collectively termed the ‘seed bank’. The numbers of seeds and eggs in the seed bank is generally large (Leck, 1989; Finlayson, Cowie & Bailey, 1990; Brock, Theodore & O’Donnell, 1994; Brock, 1998; Brock & Rogers, 1998; Nielsen et al., 2000) and long lived (Boulton & Lloyd, 1992; Jenkins & Briggs, 1997; Brock, 1998; Leck & Brock, 2000). Hence, this seed bank provides not only refuges during dry periods but is also a means of conserving genetic, phenotypic, species and community diversity (Ellner & Hairston, 1994; Hairston, Kearns & Ellner, 1996; Leck & Brock, 2000; Brock et al., 2003).

The environmental conditions present at a particular time will determine which plants and animals emerge from the seed bank. In part, the communities of plants and animals that establish will be determined by the amount of salt present (Halse, Shiel & Williams, 1998; Euliss, Wrubleski & Mushet, 1999; Skinner et al., 2001). Knowledge of how increasing salinity influences the generation of new communities of zooplankton and plants will help in the prediction of the longer term effects of increasing salinity on biotic communities in wetlands, floodplains and rivers. The behaviour of salinity under different water regimes may also be important for these predictions and interpretations. Such information is needed in Australia as part of the scientific base on which management predictions and plans are based. This study experimentally examines how salinity and water regime influence plant germination and zooplankton emergence from sediment collected from two wetlands: a floodplain wetland associated with the River Murray and an upland wetland on the New England Tablelands of New South Wales.

Methods

Sediments containing seeds of plants and eggs of zooplankton were collected in September 2000 from two Australian wetlands: Racecourse Lagoon, an intermittent temporary wetland on the New England Tablelands, NSW (altitude 1400 m a.s.l, latitude 30°04’S, longitude 151°46’E) and Ryan’s 1 Billabong a semi-permanent wetland on the River Murray floodplain, NSW (altitude 150 m a.s.l, latitude 36°08’S, longitude 146°58’E). Random samples of sediments were collected to 5 cm depth in a defined 200 × 20 m zone at the edge of each wetland. Sediment from each wetland was lightly crushed, mixed and air dried for 8 weeks so that existing growing vegetation was killed and emergence of seeds and eggs was enhanced. Pots (17 cm diameter, 17 cm deep) were filled to within 5 cm from the top with sandy loam and then filled to the top with wetland sediment.

Three levels of salinity (<300, 1000 and 5000 mg L⁻¹) were tested (n = 4 replicates per treatment) in 1 m diameter and 1 m deep fibreglass tanks,
using a randomised split plot experimental design. Adverse effects have been predicted to occur to aquatic biota when salinity exceeds 1000 mg L\(^{-1}\). The salt used was a commercial salt ‘Aquasonic’ (by Ocean-nature) that has its major elements in similar proportions to that of sea water (NaCl dominated). Tanks were filled to a set level with tap water (Armidale town water supply).

A metal mesh frame was fixed above each tank from which pots (containing wetland sediment) were hung using chains and hooks. In November 2000, pots were placed in each of the 12 tanks and hung at ‘damp’ level, so that the soil was waterlogged but not submerged for the first 24 h. Separate pots were used for plants and zooplankton. Zooplankton pots were hung inside 35 \(\mu\)m mesh nets with catching bottles at the base. The top of each zooplankton net was suspended above the level of the tank edge. After the initial 24 h at the damp level, pots were individually raised and lowered to simulate water level fluctuations (Casanova & Brock, 2000), allowing all water regimes to be imposed within one tank.

Four water regime treatments were used to simulate flooded, damp, increasing and decreasing water levels (Table 1). For plants all water regimes were used, whereas for zooplankton only the flooded and damp water regimes were imposed. Each tank held a total of 13 pots: eight pots for plants (four water regimes \(\times\) two sediments) and four pots for zooplankton (two water regimes \(\times\) two sediments) plus one sand filled submerged control pot to test for aquatic plant propagule movement within the tank.

Zooplankton samples were collected on days 2, 4, 7, 11, 14 and 18 by raising the pots above the water surface, removing the catching net and washing the residue into a catching jar. Samples were preserved in 70\% ethanol. Samples were counted under dark-field microscopy and identified to the level of genus using the keys of Shiel (1995). Counts were pooled to give the total number emerging under each salinity–water regime combination.

Plants were identified after week 16 and taxon richness, abundance and biomass recorded. The above ground biomass of the three most abundant species *Myriophyllum varifolium* Hook f., *Eleocharis acuta* (R.Br) and *Lythrum hyssopifolia* L was harvested and weighed. The remaining plants were grouped and weighed together. Plants were dried at 80 °C to constant weight and weighed to ±0.01 g. Identification and nomenclature of angiosperms and ferns follows Harden (1990–93), liverworts Sainty & Jacobs (1981) and charophytes (M. Casanova, pers. comm.).

**Water quality**

Electrical conductivity (EC) was measured using an Orion salinity meter (Model 135A; Orion Research Inc., Beverley, MA, U.S.A.). Conductivity values were converted to milligram per litre using a conversion factor of 0.68 (Hart *et al.*, 1991). Salinity values of each tank were adjusted to stay within the predetermined range of values for each level by either the addition of tap water or salt as necessary.

**Data analysis**

Analyses were based on a split-plot ANOVA design with salinity and water regime as the two treatments. The model used was: Model DV = Constant + Salinity + Water regime + (Salinity \(\times\) Water regime) + Tank(Salinity) + [Water regime \(\times\) Tank(Salinity)]. Each source of variation was then tested against the appropriate error term. When a significant difference between salinities was detected Bonferroni pairwise corrections were carried out to determine which treatment means were significantly different. All data were square root transformed to remove heterogeneity of variances (Underwood, 1997), and this was

<table>
<thead>
<tr>
<th>Water regime</th>
<th>Samples collected</th>
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<tbody>
<tr>
<td>Flooded</td>
<td>Plants, zooplankton</td>
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<tr>
<td>Damp</td>
<td>Plants, zooplankton</td>
</tr>
<tr>
<td>Increasing depth</td>
<td>Plants</td>
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<tr>
<td>Decreasing depth</td>
<td>Plants</td>
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confirmed by testing for the removal of the linear relationship between mean values and variances.

Multivariate analysis of the community data was performed using PC-ORD (version 4.10), (McClune & Mefford, 1999). Non-metric multidimensional scaling (NMS) derived from a Bray–Curtis similarity matrix was used to display community data. Multi-Response Permutation Procedures (MRPP) was used to determine if communities developing under the different salinity regimes were different. All data were square-root transformed prior to analysis.

Results

Communities

Zooplankton and plants showed a decrease in the number of taxa, abundance and biomass of organisms with increasing salinity (Fig. 1, Table 2). This general trend was significant for zooplankton at both wetlands, but for plants only at Ryan’s Billabong.

Zooplankton communities showed a decrease in number of animals and number of taxa emerging at 5000 mg L\(^{-1}\) when compared with the two lower salinities (Fig. 1). More zooplankton emerged from the Racecourse Lagoon sediment exposed to <300 mg L\(^{-1}\) when only shallowly flooded (damp), but this pattern did not occur across all three salinities. Although the zooplankton community was dominated by rotifers, different communities emerged from the three salinities (\(P < 0.05\)). In contrast to salinity, water regime did not modify zooplankton communities (Fig. 2).

For the aquatic plants, there was a significant decrease in the number of taxa germinating from Ryan’s Billabong sediment exposed to 5000 mg L\(^{-1}\) but no significant differences in the number of plants or taxa germinating from the Racecourse Lagoon sediments were found (Fig. 1, Table 2). The aquatic plant communities that developed from both sediments did not differ between <300 and 1000 mg L\(^{-1}\) or between 1000 and 5000 mg L\(^{-1}\) salinities (Fig. 2). Differences were, however, noted between <300 and 5000 mg L\(^{-1}\) salinities (\(P < 0.05\)). The imposed water regime was a stronger determinant of aquatic plant community structure than salinity, with the communities developing under damp conditions being different from those under flooded conditions (Fig. 2).

**Fig. 1** Abundance (number of individuals) and taxon richness of zooplankton emerging (A and B) and aquatic plants germinating (C and D) and biomass (g) of aquatic plants (E) from sediments from Ryan’s Billabong (solid bars) and Racecourse Lagoon (open bars). Lower case letters denote significant difference between treatments.

Zooplankton taxa

Thirty-two taxa of zooplankton were identified, consisting of 22 rotifer and 10 microcrustacean taxa. The responses of individual zooplankton taxa to salinity could be broadly classified into four response types (Fig. 3):

Response 1: increased number of organisms emerging at 1000 mg L\(^{-1}\) (e.g. Bdelloids, Cephalodella spp.).

Response 2: decreased number of organisms emerging at 5000 mg L\(^{-1}\) (e.g. Eosphora sp., Lecane spp., Lepadella spp.).

Response 3: decreased number of organisms emerging between 300 and 1000 mg L\(^{-1}\) (e.g. Euchlanis spp., Trichocerca spp.).

Response 4: no difference in number of organisms emerging across the range of salinities (e.g. Ostracods).
For those taxa (Lecane spp., Lepadella spp. and Trichocerca spp.) that had significant salinity and water regime interactions the effect of salt was stronger at the greater depth.

Aquatic plant taxa

Thirty-five plant taxa were identified, comprising submerged, amphibious and terrestrial plant groups. Two of the four response types were shown by aquatic plants (Types 3 and 4, Fig. 3).

Response 3: decreased number of organisms germinating between 300 and 1000 mg L\(^{-1}\) (e.g. Nitella spp.).

Response 4: no difference in number of organisms germinating across the range of salinities (e.g. Juncus spp.).

The water regime imposed within each salinity treatment also had a role in modifying plant communities. Species that have preferences for specific water regimes only germinated under those regimes. For example, L. hyssopifolia (an amphibious species that tolerates water level fluctuations) only germinated in the damp sediment and emergence decreased with increasing salinity (Fig. 4). In contrast, Nitella spp. (a submerged species) did not germinate in the damp treatments but germinated from all other treatments with a reduction in germination as salinity increased (Fig. 4).

Discussion

Community response

Increasing salinity significantly affected both community composition and individual taxa in terms of germination of aquatic plants and the emergence of zooplankton. In the case of aquatic plants there was also an interaction with water regime. Hence, our findings show that both water regime and salinity are important, with water regime imposing the stronger pressure, particularly between edge communities with damp water regimes and communities developing under flooded conditions (Brock & Casanova, 1997; Casanova & Brock, 2000).
Communities germinating within each water regime were similar under each salinity treatment. Primarily, this appears to be due to the preference of individual plants to specific habitats. Examples from this work include the amphibious plant *Lythrum hyssopifolia* and the submerged plants of *Nitella* spp. *Lythrum hyssopifolia* is found as part of the edge community in many wetlands, capable of tolerating fluctuating water levels (Brock & Casanova, 1997). *Nitella* spp. on the other hand, are species of charophytes also common in many wetlands which require flooded conditions (Brock & Casanova, 1997). Such habitat preferences of plants result in differences in communities under different water regimes. Both of these species germinated under all salinities in their preferred habitat, but germination and establishment declined as salinity increased. Our results indicate that there is a gradient of response of aquatic plant communities with increasing stress as salinity increases. This increased stress results in a loss of aquatic plant taxa and an associated reduction in abundance and above ground biomass.

**Fig. 2** Multi-dimensional plots of zooplankton and aquatic plant communities emerging from sediments from Ryan’s Billabong and Racecourse Lagoon. Symbols: open = <300 mg L⁻¹; grey = 1000 mg L⁻¹; black = 5000 mg L⁻¹. ⊗ = damp; □ = flooded, △ = decreasing depth; ◇ = increasing depth.

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The zooplankton communities were not influenced as strongly by water regime as the aquatic plant communities. However, differences between communities based on salinity are supported by MRPP analysis. Salinity was a strong determinant of communities, with changes found to occur between 300 and 1000 mg L$^{-1}$ and at salinities above 1000 mg L$^{-1}$ a clear effect on community composition was shown.

For both aquatic plants and zooplankton, increased salinity resulted in increased stress on the seed bank and a decrease in species richness and abundance. At salinities between 1000 and 5000 mg L$^{-1}$ the number and abundance of taxa decreased.

**Individual taxon response**

The responses of various taxa can be described by four patterns of response to salinity (Fig. 3). For most zooplankton and aquatic plant taxa emerging or germinating from the sediment the upper range of tolerance was between 1000 and 5000 mg L$^{-1}$. Very few animals or aquatic plants were capable of emerging when the sediment was exposed to a salinity of 5000 mg L$^{-1}$. Several zooplankton taxa (e.g. *Cephalodella* spp. and the Bdelloid group) showed a preference to some salt (response type 1). For these rotifers, emergence from eggs in the sediment was stronger at salinities of 1000 mg L$^{-1}$ than at salinities of <300 or 5000 mg L$^{-1}$. Response type two taxa indicate the ability to cope with salinity up to at least 1000 but not 5000 mg L$^{-1}$ (e.g. *Eosphora* sp.). Neither type one nor type two responses were recorded for aquatic plant taxa during this experiment whereas both zooplankton and aquatic plant had taxa demonstrating the other two patterns. Taxa with reduced emergence levels at salinities of 1000 mg L$^{-1}$ (response type three), included the rotifer, *Trichocerca*....
spp. and the charophytes, *Nitella* spp. Ostracods and the aquatic plant *Juncus* spp. showed a type four response (i.e. salinity tolerance) and were capable of emerging or germinating in salinities up to 5000 mg L\(^{-1}\).

There is little information on the effects of naturally high salinity levels on rotifers and microcrustaceans. In general, as a group they are not tolerant of increasing salinity, and rotifers and most microcrustaceans are generally considered to be of non-marine origin (De Deckker, 1983; Hammer, 1986). However, members of the genus *Trichocerca* have been recorded in the saline lakes of Victoria (Timms, 1981) and Western Australia (Brock & Shiel, 1983). Field studies have suggested that there is a decrease in the number of rotifer species occurring in lakes at salinities above 2000 mg L\(^{-1}\) (Green & Mengestou, 1991). This decrease has been linked to specific physiological tolerance to increasing salinity as opposed to food availability (Green & Mengestou, 1991). The relative proportions of the main cations (Na, Ca, Mg) and anions (Cl and HCO\(_3\)) does modify the way biota respond to high salinities (Bayly, 1969; Bailey & James, 1999). The proportions of the various ions in the dissolved salts varies between localities and influences the ability of some microcrustaceans to colonise across a broad range of saline water (Bayly, 1969; Hammer, 1986). For example, *Boekella triarticulata* (Thomson) is a freshwater species that has been shown to penetrate into highly saline waters (Bayly, 1969). This implies that differences in ionic composition between dissolved salts are important in determining distributions and abundance of zooplankton in wetlands.

Increasing salinity could influence the emergence of aquatic plants from seeds and zooplankton from eggs in three possible ways. Increasing salinity may: (i) reduce the viability of the eggs and seeds, thus reducing seed bank potential, (ii) block the cues that trigger emergence leaving propagules alive but dormant in the seed bank or (iii) not affect emergence but death follows quickly. High salinity has been linked to blocking emergence of the rotifer *Brachionus plicatilis* (Müller) (Pourriot & Snell, 1983) and the microcrustacean, *Daphniopsis pusilla* (Serventy) (Geddes, 1976). Similarly, high salinity is usually inhibitory or toxic to seed germination (Ungar, 1962; Williams & Ungar, 1972; Baskin & Baskin, 1998).

**Conclusion**

Increasing salinity appears to reduce the abundance and diversity of aquatic plants and zooplankton taxa emerging from wetland sediments. Hence, increased salinity of Australian rivers and wetlands may result in loss of wetland biodiversity. Repeated wetting with, or long-term exposure to, increased salinity may reduce the size of the seed and egg bank and reduce the ability of communities to respond when an inundation of fresh water occurs. The information from this study is currently being tested across a much broader range of wetlands to verify the trend of a decrease in abundance and diversity with increased salinity.

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**References**


Bayly I.A.E. (1969) The occurrence of calanoid copepods in athalassic saline waters in relation to salinity and


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