THE ROLES OF SEED BANKS AND SOIL MOISTURE IN RECRUITMENT OF SEMI-ARID FLOODPLAIN PLANTS: THE RIVER MURRAY, AUSTRALIA

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Declaration of Originality

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List of Papers

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Jensen, A.E., Walker, K.F., & Paton, D.C. (2006). The Secret Life of Tangled Lignum, *Muehlenbeckia florulenta* (Polygonaceae): little known plant of the floodplains. In *Wetlands of the Murrumbidgee River Catchment* (eds I Taylor, P. Murray & S. Taylor), 79-85. Murrumbidgee Catchment Management Authority, Leeton, NSW.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2007). Using phenology to determine environmental watering regimes for the River Murray floodplain, South Australia. In *Australian Rivers: making a difference* (Eds A.L. Wilson, R.L. Dehaan, R.J. Watts, K.J. Page, K.H. Bowmer & A. Curtis). Proceedings of 5th Australian Conference on Stream Management, 175-180. Charles Sturt University, Albury, New South Wales.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2008a). Smart Environmental Watering: getting most benefit from scant flows for floodplain trees (River Murray, South Australia). In *Proceedings of Water Down Under 2008 Conference,* (Eds) Daniell, T., Lambert, M. & Leonard, M., 15-17 April, Adelaide, 1426-1437. Engineers Australia, Melbourne, Australia.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2008b). The role of seed banks in restoration of floodplain woodlands. *River Research & Applications*, 24, 632-649 (2008). *Special Issue: Proceedings of Riverine Ecohydrology Conference,* Stirling Scotland, 2006.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2008c). The floodplain seed bank of a regulated lowland river: composition and responses to wetting treatments for the River Murray floodplain, Australia. *In prep*.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2008d). Soil Moisture for Seedling Survival: a key factor in woodland decline on the Lower Murray, Australia. *In prep*.

Statement of Authorship

For the first four published papers listed above:

Anne Elizabeth Jensen performed analysis on all samples, interpreted data, wrote the original draft manuscript and coordinated all amendments, and acted as corresponding author. Keith Forbes Walker and David Cleland Paton supervised project design and data collation, reviewed data interpretation and manuscript content, and provided editing comments on the final papers.

For the last two submitted papers listed above:

Anne Elizabeth Jensen performed analysis on all samples, interpreted data, wrote the original draft manuscript and coordinated all amendments, and will act as corresponding author. Keith Forbes Walker and David Cleland Paton supervised project design and data collation, reviewed data interpretation and manuscript content, and provided editing comments on the final papers.

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I hereby certify that the statement of contribution is accurate

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KEITH FORBES WALKER

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis

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DAVID CLELAND PATON

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Signed Date

Abstract

The decline of floodplain vegetation along the Lower River Murray, South Australia, has evoked recommendations for 'environmental flows' to restore and maintain the health of the ecosystem. To assist managers to maximize benefits from environmental flows, this thesis considers the significance of water for germination and recruitment in key floodplain plant species. Three dominant species are considered, including two trees, river red gum (*Eucalyptus camaldulensis*) and black box *(E. largiflorens*), and an understorey shrub, tangled lignum (*Muehlenbeckia florulenta*).

The soil seed bank was dominated by terrestrial annual native plants. Among 1400 seedlings, a single river red gum was found, and no black box or lignum, suggesting that these species do not contribute to the persistent soil seed bank and rely instead on aerial seed banks (*serotiny*). Sampling of the soil seed bank was continued to determine when seed fall might coincide with appropriate soil moisture conditions. Responses of the soil seed bank to varied water regimes were compared to determine requirements for seedling survival. The results indicated that species richness, rapidity of response and survival time were all promoted by sustained soil moisture.

Stands of eucalypts in various states of health (from very stressed to very healthy) were monitored to identify seasonal patterns in bud crops, flowering, fresh leaves and volumes of seed released from the aerial seed bank. Distinct seasonal phenological patterns were apparent, and suggested alternating flowering among individual trees (biennial for red gum, bi-annual for black box), producing an annual peak in summer. Peak seed rain occurred in summer (December–March) in healthy trees for both red gum and black box, with light seed rain continuing throughout the year. Seed fall from stressed trees was much reduced. Stressed trees responded after a second watering event, with much more varied and extended annual seed fall patterns.

Lignum showed a spring peak in flowering and seed production. There was a prolific response of flowering and seeding to rainfall, but few seedlings survived. Vigorous vegetative growth occurred in existing plants in response to rainfall and watering but no new cloned plants were found during the study. An investigation of chromosomes as a potential tool to appraise the balance between sexual and asexual reproduction in lignum proved inconclusive, although a previous report of octoploidy in lignum was confirmed.

Seeds from all three species and lignum cuttings were tested for their responses to varied watering regimes, based on combinations of simulated rain and flood conditions. The optimal soil moisture for continued growth and survival in all seeds and cuttings was 10-25%, with moisture values <10% causing wilting and death. The results also suggested that red gum and black box seeds which germinate in water under flooded conditions need to be stranded onto moist soil at the water's edge within 10 days, for the seedling to continue to grow. It was also concluded that germination on rain-moistened soil is a key supplementary mechanism for recruitment, particularly between irregular flood events.

For greatest benefit, the timing of environmental flows should complement any seasonal rainfall and irregular flooding that may occur. Extension of suitable soil moisture conditions (10-25%) for as long as possible after >5 mm rainfall, or after over-bank flows, would increase chances for survival of seedlings. December is the most likely month for maximal benefit from watering in the Lower Murray Valley, for germination and recruitment, based on regional rainfall and flooding patterns.

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Chapter1 Introduction

Nature of the Problem

The health of floodplain vegetation is sensitive to the flow regime of the parent river (Junk *et al.*, 1989; Stromberg, 1993), and the vegetation in turn provides habitats for other plants and animals (Naiman *et al.*, 1997; Robertson *et al.*, 1978; Ward *et al.*, 1999a). Changed regimes, associated with flow regulation and diversions, have pervasive effects on floodplain-river ecosystems (Bayley, 1991; Busch & Smith, 1995; King & Keeland, 1999; Knutson & Klaas, 1998; Micklin, 1988; Richardson & Hussain, 2006; Stromberg *et al.*, 2003; Ward *et al.*, 1999b).

In South Australia, the incidence of water stress and death in eucalypt trees along the River Murray has increased as the effects of regulation have been intensified by drought and rising saline groundwater (MDBC, 2005). There have been no significant over-bank flows since 1996, and over 70 percent of trees were dead, dying or stressed by 2004, continuing a trend first reported in 1990 (Margules & Partners *et al.*, 1990; MDBC, 2003, 2005). Since 2004, the Murray-Darling Basin Commission and state government agencies have allocated ‘environmental flows’, from a very limited reserve, to relieve the stressed trees and promote germination and seedling establishment, hence recruitment (the accrual of potentially reproductive individuals to populations). These allocations have been limited to sites where water can be delivered and retained.

The success of these allocations will depend on the health of the plants, timing of watering and the capacity of the seed bank to respond. The saplings require soil moisture to be maintained for up to 2 years, before they develop a sinker root and gain some independence from conditions at the soil surface (George *et al.*, 2004). The critical scenario, therefore, requires viable seed and sufficient moisture for germination and survival of seedlings and saplings to reproductive age.

The floodplain vegetation community is dominated by mature, deep-rooted perennial trees and shrubs which keep local groundwater levels under the floodplain low enough to prevent capillary rise of salt to the surface (Jolly *et al.* 1992). The Lower Murray floodplain is underlain by relatively shallow and highly saline groundwater, and the salt and water balance is sensitive to the influences of river regulation, water extraction and regional irrigation (Jolly *et al.*, 1992; Maheshwari *et al.*, 1995; Walker, 2006). The perennial vegetation depends on fresh water lenses overlying the saline groundwater which are periodically replenished by flooding (Mensforth *et al.*, 1994), combined with local rainfall events to replenish soil moisture (George, 2004). Regional vegetation clearance outside the Lower Murray Valley has increased regional pressure gradients which push groundwater towards the floodplain as a natural drainage sink (Allison *et al.*, 1990). In addition, excess drainage from irrigation activities on highland adjacent to the floodplain is adding to the pressure on saline groundwater under the floodplain, displacing it into the root zone of floodplain trees (AWE, 1999).

Proposed delivery of environmental flows to the Lower Murray floodplain will provide only very limited volumes of water. For ecological recovery, the ecosystem requires restoration of the small to medium floods which have been removed by river regulation (Thoms *et al.* 2000). However, such volumes cannot be delivered under present conditions, unless a major flood event occurs and overbank flooding is allowed to occur by water managers, rather than retaining water in upstream storages. While the timing of late spring-early summer flooding has been identified as a key driver in vegetation recruitment (George, 2004; Thoms *et al.*, 2000), over-bank flows can only be created in a managed environmental flow if flows > 25,000 ML d^{-1} occur in the main river channel. With reduced frequency of flow events under river regulation and the current extended regional drought, the relative value of short watering periods is being considered. While the primary aim for vegetation health and survival is to support germination of seeds or maintain germinated seedlings, other useful outcomes might be maintenance of bud crops, increased flowering, increased growth of new leaves or maintenance of fruit crops on trees, to support future seed rain.

The importance of the natural flow regime for the Lower Murray floodplain ecosystem has been elucidated over the past 30 years, with an emphasis on the mainstream and defined wetlands and individual plant and animal species, rather than the broader floodplain ecosystem (Puckridge *et al.*, 1998; Thoms *et al.*, 2000; Walker, 2006). Subsequent recommendations for environmental flows have been based on restoration or simulation of key elements of the natural regime at the river reach scale (Bunn & Arthington, 2002; Jensen *et al.*, 2000; Jones *et al.*, 2002; Thoms *et al.*, 2000). Less attention has been given to the specific needs of floodplain vegetation, with an initial assessment in the late 1980s (Margules & Partners *et al.*, 1990) followed up more than a decade later (Roberts, 2003), as vegetation health declined (MDBC, 2003, 2005).

The obvious solution to declining Lower Murray floodplain vegetation health is the provision of environmental flows to replicate the missing small to medium floods. In the short to medium term, lack of available water means that only very small volumes are available for environmental use, with single contained wetlands to be watered for periods of much shorter duration. For effective application of this water at a local scale, more detailed understanding is required of the relationship between watering events and desired responses by key plant species. While river red gum has been observed to germinate readily following floods, the volumes, timing and release mechanisms of viable seed are not known in sufficient local detail, and the requirements for sufficient moisture for germination and survival of seedlings have not been quantified. The relative roles of flood and rainfall as water sources also need further investigation, as complements to environmental flows.

Study Species

Three deep-rooted perennial species which cover large areas of the Lower Murray floodplain were chosen for investigation, including two eucalypt trees—the river red gum (Myrtaceae: *Eucalyptus camaldulensis* Dehnh.) and black box (Myrtaceae: *E. largiflorens* F. Muell.)—and an understorey shrub, tangled lignum (Polygonaceae: *Muehlenbeckia florulenta* (Meissn.) F. Muell.).

River red gum (loosely, ‘red gum’) (Figure 1.1) is the largest and one of the largest and most widespread trees in Australia, with extensive woodlands along major river channels in the Murray-Darling Basin (Cunningham *et al.*, 1981). River red gum dominates riparian zones, with its distribution dictated by its relatively high flood tolerance and high transpiration rates (Roberts & Marston, 2000).

Black box (Figure 1.2) is found on heavy clay soils in more elevated, periodically-flooded positions on the floodplain, and along dry-lake margins (Cunningham *et al.*, 1981; Jensen, 1983; Margules & Partners *et al.*, 1990). This species is found as a narrow belt of trees along the high water line (Cunningham *et al.*, 1981) or on the slopes of minor sandy rises, where favourable seed bed conditions have occurred after a flood and seedlings have survived to maturity (O'Malley & Sheldon, 1990).

Tangled lignum (hereafter ‘lignum’) (Figure 1.3) is widespread in south-eastern Australia, particularly in the Murray-Darling Basin. It is found on the nutrient-poor, grey cracking clays of the Lower Murray floodplain (Craig *et al.*, 1991). Lignum is typically found in characteristic shrublands on swamps, river-flats, gilgais (ephemerally-flooded clay pans) and other intermittently flooded areas (Cunningham *et al.*, 1981), particularly in local habitats subject to temporary ponding after rain or flooding (Roberts & Marston, 2000).

Figure 1.1 Mature river red gum at Werta Wert Lagoon, Chowilla Floodplain.

Figure 1.2 Mature black box at Banrock Station, Lower Murray Floodplain.

Figure 1.3 Tangled lignum at Racecourse Lagoon, Brenda Park, near Morgan.
Note the vertical clonal growth from root nodes surrounding the parent plant.

Objectives

This thesis concerns perceived gaps in the scientific knowledge available to support environmental flow allocations for floodplain vegetation along the River Murray. It describes the relationships between watering and recruitment in the three aforementioned key floodplain species, with field and laboratory investigations to determine the availability of viable seed and requirements for soil moisture to trigger germination and sustain seedlings. The investigations included the source and availability of viable seeds, timing of peak seed availability, critical levels of soil moisture content to support survival of seedlings, alternative recruitment strategies by lignum, the role of rainfall, and the potential disruption of recruitment by external factors such as stock grazing. Particular goals were to:

- review the literature relating to the role of seed banks and soil moisture in recruitment of semi-arid floodplain plants
- assess the composition and status of the soil seed bank with regard to germination of red gum, black box and lignum, and to investigate responses to flooding and rainfall as water sources
- investigate the phenological cycles of river red gum and black box, and to determine key elements in their reproductive cycles
- assess the viability and status of the aerial seed bank for river red gum and black box, and their patterns of seed release
- investigate the reproductive and growth strategies of lignum, and to investigate responses to flooding or rainfall as water sources
- investigate the role of soil moisture in providing appropriate conditions for germination, seedling establishment and survival (hence recruitment) for these species, and
- develop guidelines for flow management to optimise floodplain vegetation responses to environmental watering.

Hypotheses

Five hypotheses were developed from an initial literature review and preliminary field observations:

- river red gum, black box and lignum seeds are present transiently but not persistently in the soil seed bank
- there is an annual peak in volumes of seed rain in river red gum and black box, with greater volumes every alternate year
- river red gum releases seed in seasonal patterns likely to coincide with floods, and black box releases seed in seasonal patterns likely to coincide with rainfall, reflecting their respective adaptations to potential water sources and suitable recruitment conditions
- sexual reproduction (seed set) in lignum requires over-bank flows, whereas asexual reproduction (clonal growth) may be promoted by significant seasonal rainfall events
- maintenance of a minimal level of soil moisture (suggested >30%) will sustain eucalypt and lignum seedlings through the vulnerable stage of relying on soil moisture in surface layers while developing roots to access deeper soil moisture reserves.

Thesis Outline

This thesis is based on six papers published or in preparation for journal publication, as indicated below, and this has influenced the presentation of data. In particular, the phenology of the eucalypt species is presented separately from seed rain data. Some papers were published early in the project using partial data sets, so the discussion in the relevant chapters has been extended to incorporate complete data sets.

Chapter 2 contains a review of knowledge relating to recruitment of key plant species on river floodplains. The review includes consideration of comparable ecosystems and threats to ecosystem health. While wetland systems are relatively well-described in the literature, there is less information on broader floodplain habitats and recruitment processes for their plants. Key factors identified as likely to influence recruitment success include seed sources, water sources, requirements for germination and grazing impacts by stock, native animals and ants. Chapter 2 also provides information about current knowledge of river red gum, black box and lignum on the Lower Murray floodplain. Information is included on the current hydrology of the Lower Murray and how it has altered from pre-regulation flows in frequency, duration and seasonality and thus affected water sources and vegetation health. The effects of regional climate on water sources are also summarised. Other issues covered include serotiny, hydrochory, soil moisture requirements, soil and water salinity, and the coincidence of strandlines with recruitment. The scientific basis for environmental flows is also summarised and a model for recruitment developed.

Chapter 3 describes the study locations and conditions, to avoid repetition in subsequent chapters. Regional rainfall conditions for the study period are summarised, as rainfall proved to be a significant supplementary water source. Field sites were selected to take advantage of the opportunity offered by the regional experimental watering program, the varied health of sites, and varied hydrologic regime of sites.

Chapter 4[1] presents the results of an initial investigation into the status and composition of the Lower Murray Valley floodplain soil seed bank and the response of the soil seed bank to flooding. The results are based on a paper currently in preparation for publication. This investigation assessed the dominant species in the soil seed bank and the likelihood that the eucalypts and lignum demonstrate serotiny, holding seeds in aerial seed banks until suitable conditions arise for release. Experimental treatments of soil seed bank samples are described which confirm the importance of sustained soil moisture and the important role of local rainfall events as a supplementary water source.

Chapter 5[2] presents data on the phenological cycle for seed production and release in the two floodplain eucalypts from the study, tracking the cycle of bud formation, flowering and fruit production in order to establish seasonal patterns in the trees. These data have been published as a refereed paper in conference proceedings, and were analysed to determine the nature of patterns in the phenological cycles for each eucalypt, and to identify key phases which might benefit from environmental watering.

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[1] Jensen, A.E., Walker, K.F., & Paton, D.C. (2008a). The floodplain seed bank of a regulated lowland river: composition and responses to wetting treatments for the River Murray floodplain, Australia. *In prep*.

[2] Jensen, A.E., Walker, K.F., & Paton, D.C. (2007). Using phenology to determine environmental watering regimes for the River Murray floodplain, South Australia. In *Australian Rivers: making a difference* (Eds A.L. Wilson, R.L. Dehaan, R.J. Watts, K.J. Page, K.H. Bowmer & A. Curtis).

Chapter 6[3],[4] contains data from seed traps to determine timing of seed availability, to link with the phenological patterns described in Chapter 5. This part of the study examined seed availability for germination and factors affecting seedling survival in the two floodplain eucalypts, and conducted field sampling to determine the timing of peak seed fall. The results presented extend material presented in two published papers, and confirm the occurrence of serotiny. Significant quantities of available viable seed were measured, to determine volumes and seasonal patterns. Stressed trees were monitored to determine if volumes of seed released were reduced, and if temporal patterns were similar to healthy trees. Evidence was sought to support the pattern of biennial cycles in river red gums reported in Chapter 5. Additional monthly sampling of the soil seed bank was carried out to check for the presence of any river red gum, black box and lignum seeds over an annual cycle. The effect of strandlines on germination success was monitored, and compared with germination under glasshouse conditions. The impact of removal of grazing pressure by sheep was observed during the second half of the study period.

Chapter 7[5] investigates processes for germination and growth in lignum, and extends a published paper describing preliminary results. Sampling sites included inundated wetlands, moist riparian zones and dry floodplain reliant on rainfall. The responses of lignum to inundation and rainfall were monitored during the sampling period. The responses to above average rainfall in 2005 and other rainfall events were observed, and examples of seed production, seedlings and vegetative reproduction were documented.

Chapter 8[6] presents experimental results on the levels of soil moisture required for germination and seedling survival in both eucalypts and lignum, and is based on a paper in preparation for publication. Experimental treatments with four water regimes were applied for 12 weeks to red gum, black box and lignum seeds, and to lignum cuttings. The experiment sought to define more precisely the soil moisture conditions required to trigger germination and sustain seedlings, or to trigger and sustain shoot and root growth in cuttings.

In Chapter 9, the potential application of these findings to developing guidelines for environmental flows is considered, drawing in part on the paper referenced in Chapter 6. A calendar has been developed to indicate timings for application of water for different outcomes, including sustaining bud crops, promotion of flowering and soil moisture to coincide with maximum seed rain. Chapter 10 reviews the findings of these investigations, and reconsiders the hypotheses advanced in Chapter 1.

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[3] Jensen, A.E., Walker, K.F., & Paton, D.C. (2008b). The role of seed banks in restoration of floodplain woodlands. *River Research & Applications*, 24 (2008), 632-649. *Special Issue: Proceedings of Riverine Hydroecology Conference, Stirling, Scotland, 2006.*

[4] Jensen, A.E., Walker, K.F., & Paton, D.C. (2008d). Smart Environmental Watering: getting most benefit from scant flows for floodplain trees (River Murray, South Australia). In (Eds) Daniell, T., Lambert, M. & Leonard, M., *Proceedings of Water Down Under 2008 Conference*, 15-17 April, Adelaide, 1426-1437. Engineers Australia, Melbourne, Australia.

[5] Jensen, A.E., Walker, K.F., & Paton, D.C. (2006). The Secret Life of Tangled Lignum, *Muehlenbeckia florulenta* (Polygonaceae): little known plant of the floodplains. In *Wetlands of the Murrumbidgee River Catchment* (eds) Taylor, I., Murray, P. & Taylor, S., 79-85. Murrumbidgee Catchment Management Authority, Leeton, NSW.

[6] Jensen, A.E., Walker, K.F., & Paton, D.C. (2008d). Soil moisture for seedling survival: a key factor in woodland decline on the Lower Murray, Australia. *In prep*.

Chapter2 Literature Review: Factors affecting Recruitment of Semi-arid Floodplain Vegetation

Introduction

The recruitment, composition and health of floodplain vegetation are sensitive to the flow regime of the parent river (e.g. Junk *et al.*, 1989; Stromberg, 1993). In turn, the vegetation, especially trees and other perennials, determines local landscape and habitat characteristics (Naiman *et al.*, 1995; Robertson *et al.*, 1978; Ward *et al.*, 1999). Changed flow regimes due to regulation and diversions thereby have pervasive effects on floodplain communities throughout the world (Poff *et al.*, 1997; Stanford *et al.*, 1996; Tockner & Stanford, 2002, 2004). In arid and semi-arid regions, they are especially vulnerable to diversions of river flows, abstraction of groundwater and climatic changes (Busch & Smith, 1995; Nilsson *et al.*, 2005; Shafroth *et al.*, 2002; Stromberg, 1993). Changes in flood frequency, duration and intensity, combined with lowered water tables under floodplains and increased salinity, have contributed to changes in riparian vegetation for rivers in western North America (Busch & Smith, 1995). Dominant endemic species are now approaching local extinction or greatly reduced under changed water regimes.

Conceptual understanding of river and floodplain processes and the role of water regime has been evolving steadily on an international scale since the 1980s (Junk *et al.*, 1989; Junk & Wantzen, 2004; Vannote *et al.*, 1980; Ward & Stanford, 1995; Zalewski & Robarts, 2004). The hydrological connectedness of the floodplain to the main river channel has a strong influence on its productivity (Sedell *et al.*, 1989). In large rivers, reach scale ecosystem processes are more dominant than longitudinal transfers of nutrients and sediments, and interactions with floodplains are critical. The flood pulse allows for rapid recycling of organic matter and nutrients, and drives the production cycles of river-floodplain ecosystems (Junk *et al.*, 1989; Junk & Wantzen, 2004). Understanding is still incomplete of the complex nature of ecological processes and patterns in river ecosystems, and this constrains the effectiveness of management actions for conservation and restoration purposes (Ward *et al.*, 2001). Further complexity is added by the feedbacks and interactions between floodplain landforms and vegetation communities (Corenblit *et al.*, 2007).

Changes to water regime are likely to have significant effects on riverine ecosystems, including changes in inundation and life cycles, interrupted connectivity, changes in species composition, and greater likelihood of introduced species (Bunn & Arthington, 2002). The single most common cause of environmental decline reported in river ecosystems in Australia is change in water regime (Boulton & Lloyd, 1991; Boulton & Jenkins, 1997; Jensen, 1983; Jensen *et al.*, 1994; Jensen, 1998a; Jensen *et al.*, 2003; Pressey, 1990; Streever, 1997; Thoms *et al.*, 2000). It is now widely inferred at a regional scale, although not well demonstrated by research at a local scale (George, 2004), that the loss of small to medium over-bank flows during spring floods is a critical factor in the decline of biodiversity and wetland health in the Murray Valley (Jones, 2002; Thoms *et al.*, 2000; Whittington, 2002; Young, 2001). Effects from changed flow regimes observed in the Lower Murray Valley downstream of Wentworth (*see Figure 3.1*), where the river mainstream is fully regulated by a series of low-level in-stream weirs, include a serious decline in floodplain vegetation health (Jensen *et al.*, 1994; Jensen, 1998a; Jensen *et al.*, 2000; Margules & Partners *et al.*, 1990; Roberts, 2003; Thoms *et al.*, 2000; Walker, 1986, 1990).

Floodplain ecosystems in the River Murray Valley in south-eastern Australia have been characterised as flood pulse-driven, with significant lateral transformation processes occurring during interactions between the river and floodplain (Junk *et al.*, 1989; Puckridge *et al.*, 1998; Walker *et al.*, 1995). Over-bank flows created by the spring flood pulse trigger breeding and regeneration responses in key floodplain species, and sustain the local salt and water balance in wetland and floodplain hydrological regimes (Walker, 1986; Walker *et al.*, 1992; Walker *et al.*, 1994; Young, 2001).

Since concerns about the long-term effects of water deprivation on the vegetation for the Lower Murray Valley were first raised some 25 years ago (Margules & Partners *et al.*, 1990; Roberts, 2003), these effects have intensified. In the last 10 years, with diversion rates at 80% of average annual discharge, there has been a Basin-wide trend toward less rainfall and lower discharge, and a severe drought prevails. Since 2000, the incidence of dying and dead trees has increased, and three quarters or more of mature trees along 700 km of the Murray Valley were dead, dying or stressed by 2004 (MDBC, 2005). In this context of loss of mature trees, factors affecting regeneration and recruitment have not been studied or monitored until recently (George, 2004) and the broader landscape-scale wetland habitat of the floodplain in the Lower Murray has not been studied at local scales, with the exception of the Chowilla floodplain in the Riverland region (Jolly *et al.*, 1992; O'Malley & Sheldon, 1990; Sharley & Huggan, 1995).

Proposals currently being implemented to provide ‘environmental flows’ aim to arrest the decline of vegetation health, wetland health and water quality, based on the assumption that flooding of floodplain soils will lead to successful outcomes, including recruitment (Walker, 2002). Recommendations for environmental flows have been at the river reach scale (Jensen *et al.*, 2000; Thoms *et al.*, 2000). However, with drought conditions since 2000, there is no prospect of environmental flows at the river reach scale, and single wetland watering trials have been undertaken, watering sites 6-40 ha in area. Ecological responses to watering have been little studied at such localised scales, apart from limited monitoring of community wetland repair projects which reinstated wetting and drying cycles (Jensen, 2002a). Provision of environmental water is currently extremely limited by availability, feasibility and high cost of delivery. More specific scientific information on the most effective application of water can assist in achieving the best results in improved floodplain health, particularly through better understanding of vegetation recruitment processes. These results could then be up-scaled to the river reach scale when larger flow events make wider scale environmental flows possible in the future.

Successful recruitment relies on availability and viability of seed, coupled with suitable conditions for germination, including adequate soil moisture, temperature and nutrients. Factors likely to have significant influence on recruitment success in the key species on the Lower Murray floodplain include:

- decline of mature vegetation
- water sources (flood regime, quality, frequency, seasonality, duration)
- seed sources (availability, viability, volume, release patterns)
- requirements for germination (soil moisture, temperature, light, salinity)
- grazing impacts of stock, native animals and ants.

Current knowledge of these factors has been reviewed, in the context of their influence on conditions suitable to promote recovery and recruitment of vegetation on the Lower Murray floodplain, and in relation to management of environmental flows, as outlined below.

Decline of Mature Vegetation in Lower Murray Valley

The River Murray in south-eastern Australia is a meandering lowland river with extensive associated floodplains in the lower reaches (*see Figure 3.1*). The floodplain of the 830-km Lower River Murray, below the Murray-Darling junction, supports extensive woodlands of river red gum and black box (Myrtaceae: *Eucalyptus camaldulensis*, *E. largiflorens*), with a shrub understorey of tangled lignum (Polygonaceae: *Muehlenbeckia florulenta*) (Margules & Partners *et al.*, 1990). Like all dryland rivers, the Murray has an erratic 'flood pulse' (Walker *et al.*, 1995), and the floodplain vegetation is well-adapted to changeable patterns of flow.

The primary causes of ecological decline in the Lower Murray Valley have been attributed to changes in water regime (Jacobs, 1990; Maheshwari *et al.*, 1993; Young, 2001), including reduced flood frequency, duration and volume, faster flood recessions, increased permanent inundation, increased periods of drought, higher turbidity and raised groundwater levels. The natural hydrological patterns of the Murray Valley were based on highly variable, irregular floods and low flows, with the whole floodplain inundated only during very infrequent extreme high flow events. Peak flow events were usually in the spring-summer period (Jensen, 1986; Walker, 1986). The Murray-Darling river system has been intensively regulated, particularly the Lower Murray, and about 80% of the system's mean annual discharge is diverted, mainly for irrigation (Maheshwari *et al.*, 1995). Regulation is a major disturbance to the river and floodplain ecosystem because it creates a more predictable water regime (Walker & Thoms 1993). Changes in the hydrograph have been quantified, and understanding developed of the impact on various trophic levels in associated aquatic and riparian ecosystems (Walker *et al.*, 1992; Walker & Thoms, 1993; Walker *et al.*, 1994). Decline in numerous wetland species has been linked with reduced frequency, duration and volume of flooding in the regulated river system (Walker, 1986, 1990).

The primary cause of the vegetation decline noted since the 1980s is water deprivation by river regulation, which has removed smaller higher frequency floods, but salinisation and grazing also are locally significant (MDBC, 2003, 2005; Walker & Thoms, 1993; Walker, 2006). Over-bank flows in the Lower Murray have been much reduced in frequency and magnitude due to diversions and regulation (Walker, 2006). In 2007, these effects have been exacerbated by an extended severe drought and, after 11 years without flooding due to the changed water regime, red gum and black box trees are stressed and dying (MDBC, 2005). Up to 90 percent of eucalypt trees have declined or died in some locations, continuing a trend first reported in 1990 (Margules & Partners *et al.*, 1990; MDBC, 2003, 2005). Along 700 km of the Murray Valley, stressed, dying or dead river red gum and black box trees have increased from 52% in 2002 to 76% in 2004. On the severely affected Chowilla Floodplain (*see Figures 3.1, 3.2*), stressed trees rose from 54% in 2002 to 89% in 2004, with river red gums worse affected than black box trees (MDBC, 2005). A more recent assessment of tree condition in the mid-Murray Valley in the state of Victoria suggested a general trend of declining condition with distance downstream from Hume Dam, associated with an increasing decline of flood frequency due to regulation, and lower average rainfall. The study suggested that current watering regimes in the mid-Murray (including both rainfall and flooding) are insufficient to maintain river red gum communities in good condition (Cunningham *et al.*, 2007).

Water sources for Vegetation Recruitment and Maintenance

In climates with extremes of drought and flood, the water regime is a key determinant in community composition of the vegetation. On the US south-eastern coastal plain, drought cycles were shown to have the potential to drive changes in vegetation communities (Stroh *et al.*, 2008). Decreased hydro-periods led to increased invasion of terrestrial species, while longer hydro-periods resulted in expansion of aquatic species. Drought in dry seasons was found to be the limiting factor in seedling survival in seasonally flooded tropical forests (Lopez & Kursar, 2007).

The key elements of the water regime which are considered significant for maintenance of Lower Murray floodplain communities are seasonality, duration, frequency, and occurrence of serial floods

(Jensen & Nicholls, 1997). Water regime – the pattern of inundation in a wetland – is defined by the extent and depth, duration, timing, frequency and variability of flooding and drying (Rea & Ganf, 1994). Water regime is the most important factor in determining community composition in wetland ecosystems (Mitsch & Gosselink, 1993), and influencing biotic and other abiotic patterns in space and time (Brock & Crossle, 2002). Changes to water regime, making wetlands more permanently wet or dry, are likely to reduce species richness, change the vegetation and hence seed bank composition and dynamics (Brock & Casanova, 1997; van der Valk & Davis, 1978). Changed conditions to a drier cycle may favour annual or ephemeral, semi-terrestrial competitive species over aquatic species (Brock, 1998). The important role of water availability and hydrology in promoting germination and growth has been demonstrated for wetlands (Casanova & Brock, 2000), and it is expected that this would also apply to the wider floodplain habitat for terrestrial species such as eucalypts and lignum, for which flooding is the primary disturbance and dispersal agent.

The links between recruitment and water regime have been demonstrated for river red gums in the Barmah Forest, on the mid-Murray (Bren & Gibbs, 1986; Dexter, 1970). Findings from these studies, under different climatic and hydrological conditions, must be qualified for application to Lower Murray communities to account for later seasonality of floods and drier climatic conditions. Flood triggers for germination of eucalypts in the Lower Murray are 4-6 weeks later than Barmah Forest sites in the mid-Murray Valley 400 km upstream, which have a pattern of germination tied to spring floods (Dexter, 1967, 1978). Flood peaks in unregulated conditions in the Lower Murray Valley generally occurred in the period October to December (see Table 2.1 below), suggesting a later pattern of late spring-early summer germination would be required to coincide with later flood peaks.

Floods provide an opportunity for regeneration, but are only one step in the sequence for successful recruitment. Large scale regeneration historically followed large flood events in consecutive years. It is known that flooding is a primary trigger for regeneration processes in floodplain wetland communities (Bren & Gibbs, 1986; Dexter, 1967, 1978; George, 2004; Roberts *et al.*, 2001; Walker, 1990; Walker *et al.*, 1994), but the response sequence for successful recruitment is not well understood. It is often associated with high rainfall in the catchment, producing conditions potentially suitable for germination, seedling establishment and early juvenile survival (George, 2004). This underlines the importance of rainfall as a complementary water source. It is apparent that river red gums in the Lower Murray Valley have adapted to a different water regime to river red gums further upstream or in higher rainfall locations, such as the Mt Lofty Ranges in South Australia, where rainfall is the primary water source (Pudney, 1998).

Timing of the flood pulse is very important for the dispersal and establishment of riparian trees, including river red gum (Pettit & Froend, 2001). Seed fall in river red gum at a dry tropical Western Australian site is timed to coincide with falling water levels, while there is high soil moisture content, bare soil and absence of scouring (Pettit & Froend, 2001). For river red gums in the Lower Murray Valley, soil moisture content is important for survival of river red gum seedlings, scouring is not a significant factor, and bare soil is not beneficial as it lacks protection from granivorous insects and high evapo-transpiration. The applicability of features observed in other eucalypt species and at other Australian locations needs to be adjusted for the floodplain eucalypts of the Lower Murray Valley. For example, it is not known what precise sequence of floods is required for widespread recruitment of either eucalypt species in the Lower Murray Valley. There is evidence that two sequential floods are associated with surviving stands of each species (Mr J. Seekamp, Renmark, pers. comm.; George, 2004). While this gives a benchmark for optimal conditions, further work is required to understand the conditions required for production of buds and flowers, thought to occur in the previous year (Assoc Prof D. Paton, University of Adelaide, pers. comm.), which implies a requirement for a multi-year sequence of appropriate conditions, including both seasonal rainfall and flooding.

The primary source of water for river red gums in the Lower Murray comes from small to medium flood events which overflow onto the floodplain and replenish the soil moisture and fresh water lenses that buffer their root zones from saline groundwater (MDBC, 2003; Mensforth *et al.*, 1992). Successful recruitment is associated with moderate river flows of >40,000 MLd^{-1} (George, 2004). Flooding recharges shallow aquifers and maintains soil moisture (Jolly *et al.*, 1992). Local rainfall is a potential secondary water source to maintain soil moisture, with successful recruitment in both red gums and black box associated with above average rainfall > 300 mm (George, 2004). Most red gums in the Lower Murray Valley occur in zones with flooding once every 2-3 y (Jensen, 2004; MDBC, 2005), and they are stressed by more than two years of drought between floods (Roberts & Marston, 2000). Studies have shown the ability of river red gums to switch between different water sources, and to use water of differing salinities (Jolly *et al.*, 1992; MDBC, 2003; Mensforth *et al.*, 1992). Trees more than 15m away from fresh surface water are likely to be using groundwater as their primary source. River red gums become stressed when regional hydraulic pressures push saline groundwater (>20,000 mS cm^{-1}) into their root zones (Sharley & Huggan, 1995). The vigour of stands is governed by the extent and persistence of the freshwater lenses, and intrusion of the saline regional groundwater into their root zones (Walker, 1986).

Black box may endure 10 years without flooding, if soil moisture is maintained. They may subsist entirely on soil water during dry periods (Slavich *et al.*, 1999), but are stressed by saline groundwater (>40,000 mS cm^{-1}) within 2-4 m of the surface (Sharley & Huggan, 1995). Previous episodes of stress, death and partial recovery have occurred for black box at Chowilla (Mr J. Seekamp, Renmark, pers. comm.). The pre-conditions for stress are >4 years without fresh water sourced from floods or seasonal rain, where the saline groundwater is within 4 m of the surface (Jensen *et al.*, 2003; Jensen *et al.*, 1998; Jolly *et al.*, 1992). Very large floods (>100,000 ML d^{-1}) are needed to alleviate stress in black box at higher elevations. Successful recruitment is associated with moderate river flows of >80,000 ML d^{-1} (George, 2004).

Reduced Water Sources for Lower Murray floodplain vegetation

A significant change in timing of flood peaks in the River Murray below Hume Dam has occurred since river regulation began (Maheshwari *et al.*, 1995). While seasonality of flow peaks has been effectively reversed, immediately below Hume Dam, it has previously been suggested that no significant change in seasonality of flow peaks had occurred in the Lower Murray Valley, below Euston weir (Jacobs, 1990). An analysis was conducted in this study of the seasonality of annual flow peaks at Morgan in South Australia (date of peak flow) which indicates that a significant change has occurred, with seasonal peaks more variable and occurring later than under natural conditions (Figure 2.1). Under unregulated conditions (pre-1922), the greatest chance of over-bank flows (72%) coincided with the peak rainfall period in spring, but this coincidence has become less frequent with increased regulation and diversions. Under the present highly-regulated conditions, the chance of over-bank flows in spring is reduced from 72% to 52%, and there are increased chances of over-bank flows in hotter summer months (19% to 29%) and autumn (0% to 10%) (Table 2.1). The timing of flows has changed significantly, with flood peaks shifting first back into winter in the initial regulation period (1922-1975) and later, with much greater diversions since 1975, a shift to peaks later into summer. This suggests that potential germination events are deferred to hotter, drier conditions in summer, and concurs with observations that conditions on the floodplain are becoming drier (Roberts, 2003; Walker & Thoms, 1993).

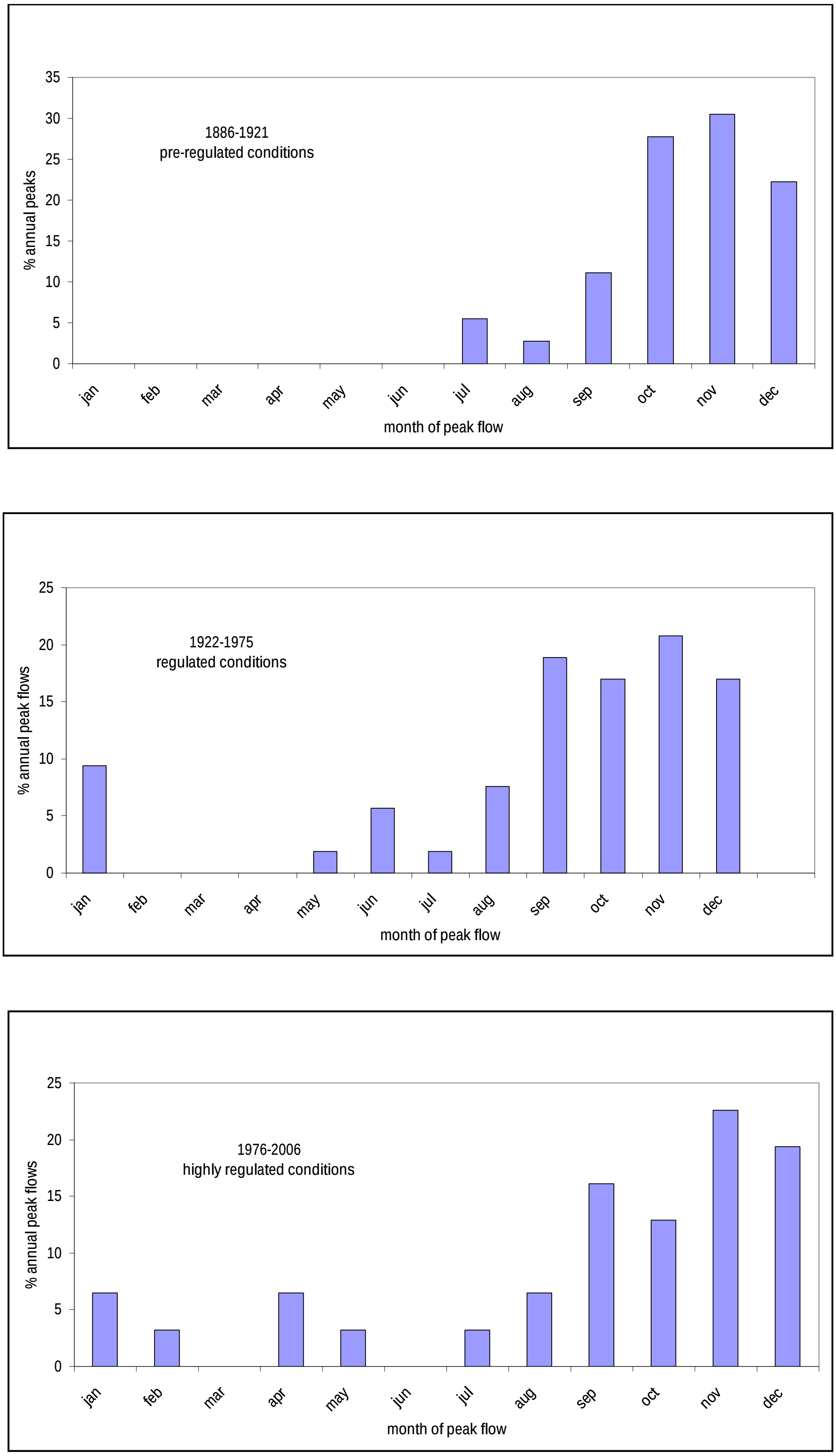

Figure 2.1 Changes in seasonal timing of flow peaks during different phases of river regulation, with frequency distribution of normalised average peak flows (Y-axis) by month (X-axis), for unregulated flows (1886-1921; 36y), regulated flows (1922-1975; 53y) and highly regulated flows (1976-2006; 31y). The strong pattern of flow peaks in October-December has been significantly reduced and occurs over a longer period (including late summer) with greater variability.
(*Sources: Ken Smith Technical Services and Dept of Water, Land & Biodiversity Conservation*).

The effects of regulation in the Lower Murray (the tract below the Murray-Darling confluence) have been to eliminate small floods (up to 1 in 7 y frequency; 25-40,000 ML d^{-1}) and reduce the frequency and duration of medium floods (up to 1 in 20 y; 40-60,000 ML d^{-1}) (Walker & Thoms, 1993; Maheshwari *et al.*, 1995; Roberts, 2003; Walker, 2006). The last effective over-bank flows in this region were in 1996, 11 years ago. At Chowilla, a 17,770 ha complex of wetlands and woodlands near Renmark, South Australia, natural flood frequencies of 1 in 2 y have been halved (Sharley & Huggan, 1995) and up to 89% of river red gums are stressed or dying (MDBC, 2005). Once the fresh water lens is exhausted, the only water available in the soil profile is groundwater which can be as salty as seawater (55,0000 mS cm^{-1}) and may be driven by regional hydraulic pressure into the root zone of floodplain trees.

The chance of rainfall in the Lower Murray Valley is highest in winter and spring, with 58% of average annual rainfall due to the influence of weather systems driven by the westerly wind systems (Table 2.1). There is also a 20% chance of rainfall in summer, due to regional summer thunderstorms generated by heating of the semi-arid landscape. Where rainfall events occurred during summer following a flood recession, this would have the potential to temporarily reduce moisture stress for seedlings at a critical time.

The significance of rainfall in recruitment was highlighted for the Chowilla floodplain in an assessment of the decline of black box, with sudden death of mature trees in the 1980s occurring in the fourth year of rainfall drought in the absence of flood events (Jensen *et al.*, 1998). A strong correlation has been found between rainfall and subsequent seed fall events in the absence of flooding for river red gum and black box in the Riverland (George, 2004). However, no correlation was found between rainfall and flowering in that study.

Season	% total annual average rainfall	% peak flows in natural conditions	% peak flows in regulated conditions	% peak flows in highly regulated conditions
Autumn (Mar-May)	21.9	0.0	1.9	9.7
Winter (Jun-Aug)	28.0	8.3	58.5	9.7
Spring (Sep-Nov)	29.7	72.2	26.4	51.6
Summer (Dec-Feb)	20.4	19.4	15.1	29.0

Table 2.1 Average seasonal chance of rainfall for Lower Murray Valley compared with peak flows in natural (unregulated) conditions (1886-1921), peak flows in regulated conditions (1922-1975) and flows in highly regulated conditions (1976-2006), showing changing coincidence of spring peak rainfall with time of peak flows (from Figure 2.1). Rain data Bureau of Meteorology: Renmark (118 y of data).

Changed Floodplain Conditions

The shift in seasonality of flow peaks is compounded by the less obvious impact of flow regulation, which has resulted in much drier conditions on the floodplain, sufficient to trigger shifts in species distributions, and decline in vegetation health (Margules and Partners *et al.*, 1990; Roberts, 2003).

The direct impact of accumulated water stress from reduced flood frequencies is coupled with the indirect effects of exacerbated groundwater salinisation and drought. The reduction in flood frequency is 2-3 fold, and the period between inundations is at least doubled (Sharley & Huggan, 1995). Once rare dry phases are now a common occurrence, with man-induced drought occurring 1 in 2 y compared to the natural frequency of 1 in 20 y. Floods are approximately one-third of natural volumes, occur less than half as often, and last for about one-quarter as long (Close, 1990; Jensen, 1983; Thoms *et al.*, 2000; Walker, 1990). Conditions for successful recruitment of the key species therefore occur much less often, as water does not reach large sections of the floodplain, or does not persist for long enough for seedlings to establish (Jensen *et al.*, 2003; Roberts, 2003). The reduced watering effect from small to medium floods is compounded by the increasing impact of regional groundwater of very high salinity (Sharley & Huggan, 1995). Investigations on the Chowilla floodplain into groundwater processes and water sources for floodplain vegetation communities have confirmed the reduced frequency and duration of flooding as a cause of decline in vegetation health through reduction in availability of fresh water (Jolly *et al.*, 1992). The change in hydrological and salinity balances has led to an accumulation of salt in the soil profile and at the soil surface, with incidents of localised tree deaths (Jensen *et al.*, 1998; Jolly *et al.*, 1992).

Figure 2.2 Degraded river red gum and black box woodland communities at Clarks Floodplain near Berri appear as grey patches (circled). This decline is due to highly saline groundwater being displaced towards the River Murray (arrow) by irrigation on adjacent highland areas (right bottom corner), combined with reduced over-bank flows during floods to replenish fresh water lenses overlying groundwater. A groundwater interception scheme was constructed in 2005, to reduce the impact of irrigation-induced groundwater displacement, thus improving the chance of effectiveness of watering trials at this site.

A shift in species distribution would result in a significant reduction in the local range of distribution for both eucalypt species, which would be confined to those zones with minimum hydrological conditions for maintenance of adult trees, and recruitment of juveniles. No broad-scale index of floodplain condition is available (Roberts, 2003). A useful benchmark is the valley-length survey of vegetation condition in the late 1980s (Margules and Partners *et al.*, 1990). This report concluded that river red gum woodland in the Lower Murray was under threat due to the major decline of established trees with limited replacement through regeneration. Black box woodland in the same region was found to be in sub-optimal condition with low likelihood of replacement (Margules and Partners *et al.*, 1990). With changing conditions resulting in less recruitment at the regional scale, it becomes more critical to understand recruitment processes at the local scale in order to manage opportunities for triggering germination and sustaining recruitment at local sites.

Since the 1990 survey was published, no further systematic assessment was undertaken until baseline surveys were undertaken in 2002 and 2004 (MDBC, 2005). These surveys documented serious decline of 52% stressed, dying or dead eucalypts from Gunbower in Victoria to Mannum in South Australia, covering more than 700 km of river valley. When the surveys were repeated in 2004, the figure had increased to 74% in just two years. In the case of Chowilla, the figures were much worse, underlining the severity of the water stress at this site, with 54% in 2002 and 89% in 2004.

A recent assessment of river red gum forests along a 1600 km riparian corridor in Victoria recommended immediate purchase of 4,000 GL for environmental flows at least once every 5 years to maintain the river red gum forests and floodplains along the full length of the River Murray and to ensure their long term survival (VEAC, 2007). This would represent 300 GL a year on top of the 500 GL a year First Step already promised through the Living Murray Initiative, to be delivered by 2009.

No effective over-bank flows have occurred in the Lower Murray since 1996 to relieve water stress by replenishment of soil moisture reserves or fresh water lenses over saline groundwater, so conditions continue to worsen for stressed trees (Figures 2.2, 2.3). The loss of vigour raises key issues relating to recruitment and maintenance of floodplain vegetation communities, including:

- reduced reproductive performance in stressed trees
- limited ability for stressed mature trees to recover if the water regime is restored
- potential net loss of trees in the short to medium term with lack of successful recruitment.

Figure 2.3 Clarks Floodplain healthy red gum forest in 2000 (left) had seriously deteriorated by 2004 (right) with thinning canopies, dying tips and signs of significant stress, particularly to the landward edge of the floodplain (right).

Seed Sources for Vegetation Recruitment and Maintenance

In most plants, recruitment is governed, in part, by the composition and nature of the seed bank. A seed bank is a collection of ungerminated seeds with the potential to replace the vegetation community. Seeds enter the soil seed bank via seed rain, and losses from the seed bank are caused by predation, pathogens, burial and germination. Germination may be triggered by environmental change or disturbance (Leckie *et al.*, 2000; Pickett & McDonnell, 1989). Seed supply depends on source-density (density of adults), source-strength (adult fecundity) and dispersal limitations (distance and mechanisms) (Clark *et al.*, 1998). Establishment limitation depends on seed survival, germination and seedling survival. The most fundamental limitation on recruitment for trees in southern Appalachian forests was found to be absence of parent trees. Spatial variability in seed rain was high, and seeds were not always available to germinate. Low numbers of seeds shed survived to germinate. Few tree species had >5 seedlings m^{-2} surviving one year after germination.

The floodplain vegetation of the Lower Murray Valley has relatively low species diversity and is dominated by extensive woodlands of river red gum and black box, with extensive shrublands of lignum (Roberts, 2003). River red gum and black box are found in 22 vegetation communities along more than 2,000km of valley, with most of the communities in the Lower Murray Valley classified as woodlands (Roberts, 2003). River red gum tends to be distributed in zones which have ready access to fresh water at least once every two years, while the more drought and salt-tolerant black box is associated with the outer limits of larger flood events on higher elevations of the floodplain (Roberts & Marston, 2000). Lignum, the dominant shrub of the floodplain, is drought and salt tolerant, and associated with low-lying clay pans on the floodplain (Cunningham *et al.*, 1981). River red gum, black box and lignum have been selected as indicator species for this study of factors affecting vegetation recruitment on the Lower Murray floodplain, as they are the dominant species in the Murray Valley floodplain vegetation communities. Conditions suitable for the continued successful recruitment of these species are expected to support survival of associated plant communities.

Dryland riparian zones with highly variable flood regimes have been associated with persistent soil seed banks, with approximately equal proportions of hydro-riparian and xero-riparian species in south-western rivers of North America (Stromberg *et al.*, 2008). Persistent seeds were found to be smaller than transient seeds, with hydrophytes having smaller seeds than xerophytes. These features were seen as adaptations which allowed dispersal during flood periods and survival during drought periods. Research in Australia has focused on wetlands soil seed banks, and in particular, the characteristic of persistence, defined as the capability of seed to remain viable for two or more growing seasons (Baskin & Baskin, 1998), which provides a mechanism for surviving dry phases in a highly variable climate (Brock *et al.*, 2003). There is little information, however, about seed banks associated with rivers in Australia (Chong & Walker, 2005). If a floodplain might be considered as a temporary wetland, there is more scope for comparison with published data, albeit still limited (Brock *et al.*, 1994; Frears, 2001; Nicol *et al.*, 2003; Stone, 2001) (Table 2.2).

The Lower Murray Valley floodplain soil seed bank has been described as depauperate (Chong & Walker, 2005) and sparse (Siebentritt *et al.*, 2004), with low seed densities (Chong, 2002; Frears, 2001), on the basis that seed bank densities less than 1,000 m^{-2} are considered rare (Nicol *et al.*, 2003). However, the Lower Murray floodplain seed bank may not be so readily classified at the low end of the density scale, as seed densities at sites in the Lower Murray vary widely from relatively sparse densities such as 696 m^{-2} at the relatively saline Reedy Creek site near Mannum (Frears, 2001) to much higher densities of 69,905 m^{-2} at Loveday fresh water wetland in the Riverland (Stone, 2001). While the soil seed bank may have low seed densities at some sites compared to sites with higher rainfall and more fertile soils, the values for defined wetlands within the floodplain appear to be within expected ranges of seed density and diversity compared with extant vegetation for the locations and physical parameters (Table 2.2). It should also be noted that much lower seed densities occur in terrestrial forest systems, which are suggested to have densities in the range 100-1000 m^{-2} (Fenner, 1985), which may be more comparable to the floodplain woodlands of the Lower Murray Valley . For example a median density of 1218 seeds m^{-2} is reported for an average of 40 species m^{-2} in old-growth temperate deciduous forest in Quebec (Leckie *et al.*, 2000). Thus, it is likely that declining recruitment is due to factors other than seed availability.

Persistent seed banks for eucalypts, however, may not necessarily be stored in the soil, as it appears that eucalypts adopt instead the strategy of serotiny, where seeds are retained in the canopy and released when conditions are optimal for germination (O'Dowd & Gill, 1984). Both species are small seeded and prolific producers of seed (particularly river red gum) and likely to be targets for granivorous ants, so that serotiny would increase the chance of successful germination in favourable conditions (Yates *et al.*, 1996). This strategy has been noted in other eucalypt species (Yates *et al.*, 1994) and in shrubs such as banksias (Lamont & Enright, 2000), although it can also be a strategy to cope with fire rather than escape from predators. A previous study has shown that lignum does not have a persistent soil seed bank in this region (Chong & Walker, 2005).

Reduced success in episodic recruitment events is less likely to be due to seed predation and more likely to be due to unsuitable physiological conditions or reduced seed supply (Meeson *et al.*, 2002; Wellington & Noble, 1985). Reduced seed supply is likely to result from the combined effects of changed flow regime, grazing, salinisation and clearing on the floodplain (Eldridge *et al.*, 1993; Meeson *et al.*, 2002). Thus, in the context of reported vegetation decline and inadequate recruitment, the availability and seasonality of viable seed for the key species should be investigated and the likely impacts of changed flows, grazing, salinisation and clearing assessed.

Seed bank location	Wetland type	Seed density	Source
Lake Honghu, China	Fresh water lake shoreline	1227 m^{-2}	(Li *et al.*, 2008)
Mediterranean marshes	temporary marshes	≤ 800,000 m^{-2}	(Bonis *et al.*, 1995)
North American wetlands	semi-permanent prairie wetlands (submerged & shoreline sediments)	1309-9893 m^{-2}	(Poiani & Johnson, 1988)
Nanjemoy Creek, Maryland, tributary of Chesapeake Bay	riparian soil seed bank	<160 seeds m^{-2} *Vallisneria americana*	(Jarvis & Moore, 2008)
Bool Lagoon south east region of South Australia	semi-permanent fresh water wetland	22,000 -- 78,000 m^{-2}	(Nicol *et al.*, 2003)
Reedy Creek near Mannum, Lower Murray Valley	brackish semi-permanent wetland (submerged & shoreline sediments)	617-755 m^{-2}	(Frears, 2001)
Loveday Wetlands, Lower Murray Valley	temporary wetland	69,905 m^{-2}	(Stone, 2001)

Table 2.2 International range of seed density values in wetland soil seed banks, indicating high variability for different habitats and conditions.

Recruitment Processes for River Red Gum and Black Box

Successful recruitment in eucalypts occurs when a series of essential events coincide to provide optimum conditions for vigorous regeneration. Recruitment is defined here as development of new, potentially reproductive individuals. The sequence of these events needs to be understood to enhance natural regeneration in stressed eucalypt communities.

There is evidence that river red gum may follow a survival strategy of combining low-level annual maintenance recruitment with massive recruitment in boom years when conditions are highly favourable (George, 2004). This strategy has developed in response to the erratic flood pulse-driven ecosystem, with organisms evolving strategies suited to the patchiness of a 'boom-bust' environment (Walker & Thoms, 1993). Neither species has continuous recruitment (Margules & Partners *et al.*, 1990). River red gum is a free producer of seed, but it has a reputation of not flowering freely every year (Turnbull & Doran, 1987). Dense stands of young plants form after floods, and gradually thin out as they grow (Cunningham *et al.*, 1981). It has been suggested that river red gums have an annual minimum rate of seed production, with infrequent seasons of massive seed production every few years (Cremer, 1965a, 1977). Flower abundance in eucalypts increases with heavy rain at bud formation, and decreases with drought, fire and insect damage. If the previous year's crop was heavy, the current crop will be light. River red gums show a consistent pattern of heavier flower production every second year at South Australian locations with peak flowering in December (Paton *et al.*, 2004). Eucalypt seeds have been captured in seed traps throughout the year (Cremer, 1965b; George, 2004). This suggests that there is always a limited amount of seed somewhere in the population, to provide a maintenance level of recruitrment, with 'boom' years when conditions optimal for regeneration coincide with heavy seed rain (Turnbull & Doran, 1987).

Data on the process of successful seedling recruitment in river red gum is sparse and inconsistent, with vegetation surveys not including regeneration and little specific data for the Lower Murray below Wentworth (George, 2004; Kiddle, 1987). However, it is clear that soil moisture levels in the summer following germination are critical for seedling survival (Dexter, 1978; Jensen, 1983; Roberts & Marston, 2000). As a result of declining health and dry conditions, recruitment rates in these species are insufficient to maintain populations (George *et al.*, 2005).

Conditions for successful recruitment include all the processes required to initiate germination, through seedling growth and survival to reproductive age. These processes include bud, flower and viable seed production, seed release and dispersal, appropriate abiotic conditions for successful germination, development of sinker roots, avoidance of attack and damage to growing saplings, and appropriate conditions for growth to sexual maturity.

Ten stages of the regeneration process have been identified (Stages I-X; George, 2004). The parent tree supports the first four stages of flowering, seed storage in the aerial seed bank, seed fall and seed dispersal. The seedling growth stage covers the next three regeneration stages of germination, sinker root establishment and growth of seedlings. The final three stages are sapling, pole stage and mature tree. Seedlings were classified as less than 1.3m tall, and saplings as more than 1.3m in height. Trees taller than 1.3m were further subdivided into sapling stage (shorter, narrow pointed crowns), pole stage (taller, fuller rounded crowns) and mature stage (full rounded crowns). This study concentrated on the earliest stages, from bud development to germination.

The process of successful recruitment for the two eucalypt species on the Lower Murray floodplain can be summarised as a cycle which is likely to take 5-20 years (Figure 2.4). Timelines are highly variable for individual trees, which may begin to set buds as early as 5 years old, or as late as 20 years old. The steps of the recruitment process (George, 2004) are:

- bud development (9 months)
- flowering (4 months)
- pollination
- fruit set
- aerial seed bank (1-2 years)
- seed release triggered

- seed dispersal (few days)
- germination (3-8 weeks)
- seedling (sinker root) stage (2 years)
- sapling stage (3-4 years)
- bud initiation (5-20 years).

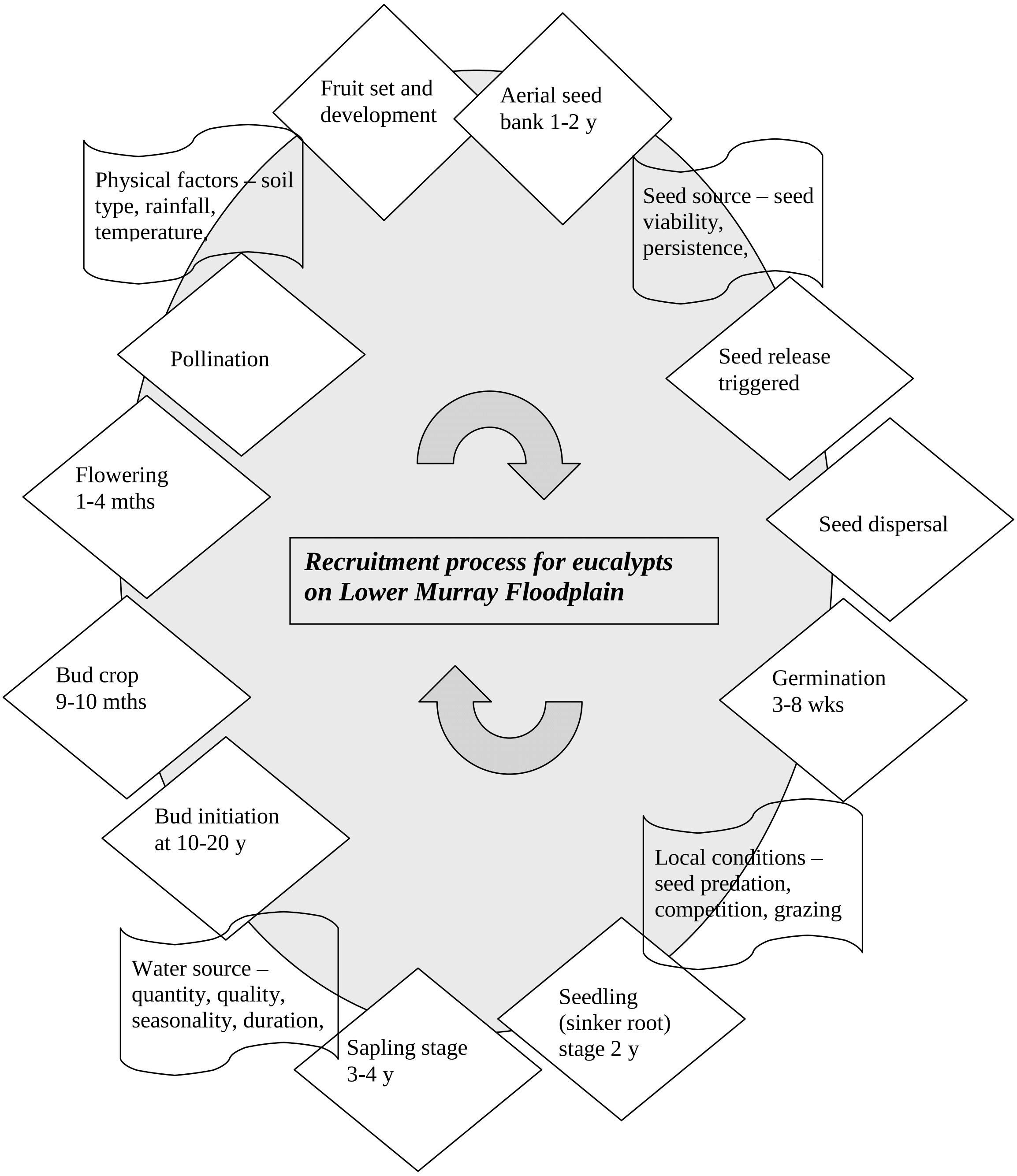

Figure 2.4 Process for successful recruitment in eucalypt species on Lower Murray floodplain, showing stages of phenological cycle and growth, from bud initiation (lower left) clockwise through flowering, fruit set and seed release to germination. Influential external factors including local conditions, water source, physical factors, and seed source are indicated in each corner of the diagram.

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Phenological Processes of Lower Murray Eucalypts

The volume of viable seed is determined by stages in the phenological cycle, including flowering, seed production, seed shed and dispersal (Page *et al.*, 2002). Limited available information about the phenology of river red gum and black box indicates significant variation in phenological processes between locations with different climatic conditions. There is evidence that peak flowering and seed fall generally occur in December-January in all areas of the Murray-Darling Basin for both species. The phenological cycle of buds, flowers, pollination, fruit set, seed fall and germination is likely to take 26-38 months for river red gum and black box, depending on how long seed is retained in the aerial seed bank (Figure 2.4).

Across its Australian range, river red gum reportedly only flowers in summer, from December to February, (Boland *et al.*, 1984), but other authors report some variations in regional locations. It is reported as flowering slightly earlier, from November to January, in the Barmah Forest (Dexter, 1967, 1978) and in the Murray-Darling Basin (Roberts & Marston, 2000). It flowers for a longer period from November to February across its South Australian range (Paton *et al.*, 2004). An even more extended period in spring and summer, from September to February, is reported in the Lower Murray Valley (George, 2004).

Less information is available on the phenology of black box, and the differences in flowering periods may reflect localised variation between populations. Black box reportedly flowers across its Australian distribution in spring and summer, from August to January (Boland *et al.*, 1984), and for a similar period in the Lower Murray Valley (George, 2004). However, flowering in the Murray-Darling Basin is reported to be significantly different, occurring through winter and into spring, from May to October (Roberts & Marston, 2000).

Flowering and Seed Production

The process of seed production in eucalypts starts from bud development (Figure 2.4). Eucalypt buds have a small cap or operculum, which falls off to reveal the circle of male stamens around the female stigma of the flower (anthesis). Pollination by insects, birds or small mammals is required for seed set (Curtis, 1985). The eucalypt flower develops into the fruit, with 3-5 locules filled with 0-2 fertile seeds and 0-10 infertile seeds (chaff) per locule (Cremer, 1965b). Viable seeds are usually in the range of 10-20% of seed and chaff by weight (Cremer, 1977), and can be less than 10% (Turnbull & Doran, 1987). The size of eucalypt seeds is highly variable. Bigger seeds tend to produce seedlings with faster initial growth and better rates of survival (Cremer, 1965b). River red gums may have higher numbers of seeds, with 7.67 seeds per fruit (range 0-23) reported from the Mt Lofty Ranges (Pudney, 1998)

Eucalypts generally produce abundant quantities of viable seed, but need at least 10 seed trees per hectare to sustain broad-scale regeneration owing to the limited dispersal on the ground (Cremer, 1965b). Mature river red gums may produce seed crops ranging from 1kg per tree in light production years, to several kilograms per tree in heavy production years (Turnbull & Doran, 1987). A single tree may produce 100-150 million seeds for one successful recruit (Jacobs, 1955), taking into account losses before germination, and low survival rates (<5%) of germinants (Fenner, 1985). Seed production in eucalypts is rarely uniform from year to year, from location to location, or within stands or on an individual tree. It has been suggested that for eucalypt species there is a relatively regular annual minimum production level, with peak production every few years (Cremer, 1965b, 1977). Mature seed in unopened capsules may persist on a tree for several years, and seed from each crop may be viable for up to two years.

Seed Fall

Very little eucalypt seed persists in the soil, and so the main seed source for eucalypt species is seed rain from the aerial seed bank (Curtis, 1985). Most eucalypt species retain seed in their canopy for 1-2 years (Cremer, 1965a). Capsules usually open on the tree and drop seed under the canopy or within 20-30 m of the canopy (Pudney, 1998; Turnbull & Doran, 1987). Most seed is shed before the capsules drop. The capsules begin to dry out, releasing the seed from the locules of the fruits, and allowing the seed to drop out under the influence of wind and gravity. Annual peaks of seed shed have been noted in eucalypts occurring after the hottest time of the year (Cremer, 1965b). Stress such as fire or drought may induce seed shed over a short period. Stress such as drought may also induce premature abscission of capsules and immature fruits, thus avoiding expenditure of energy on reproduction in unfavourable conditions (Cremer, 1965b). Seed fall is reported most likely to occur when local temperatures are high enough to trigger germination immediately. Otherwise, seed is likely to fall victim to ant predation (Yates *et al.*, 1995). Heavy continuous rains or recession of floodwaters are most likely to provide suitable conditions on the soil surface, in conjunction with relevant light and temperature conditions, for rapid germination in river red gum (Edgar, 1977).

River red gum seed fall is highly variable across locations, with seed fall across its Australian range originally suggested as late summer-autumn, in February to May (Grose, 1962), but subsequently ascribed a later autumn-winter period of March to July (Boland *et al.*, 1984). Seed fall in the Lower Murray Valley coincided with the earlier period of February to May (George, 2004), while trees in the South Australian Mt Lofty Ranges shed seed over winter, in June to September (Pudney, 1998). Trees in the Barmah Forest shed seed in spring, from August to December (Dexter, 1967, 1978). Black box seed fall is reportedly confined to late summer-autumn, from February to April, both in its Australian range and in the Lower Murray Valley (Boland *et al.*, 1984; George, 2004). These variations may correlate with the most likely water source to trigger germination in each species, i.e. flooding for river red gum and rainfall for black box. Some light seed rain may be detected at any time, triggered by stressors or localised damage to trees (Assoc Prof D. Paton, University of Adelaide, pers. comm.).

Dispersal

The majority of species in wetlands and flood-prone areas use floodwaters for dispersal and germinate when floodwaters recede, leaving moist soil to support development of seedlings (Leck & Brock, 2000; Nilsson *et al.*, 1991). Dispersal by water (*hydrochory*) can be advantageous in habitats with extremes of flood and drought, as this mechanism deposits floating seeds at higher elevations (up to 200 mm higher), where the survival rate can be better, compared to floodplain depressions (Lopez & Kursar, 2007). Greater variation in extremes of flood and drought have been found to limit diversity of species and to be a factor in creating mono-dominant communities in seasonally flooded tropical forests.

Hydrochory and flood pulsing provides a mechanism for recolonisation after flooding creates suitable conditions. In arid and semi-arid floodplains, vegetation communities include both aquatic and terrestrial species. Persistent propagules in the soil seed bank are dispersed by flood pulses, providing a temporal connection for the community and a mechanism to overcome disruptive effects of drought (Boudell & Stromberg, 2008). Smaller, lighter seeds are more suited to hydrochorous dispersal and also form longer term seed banks (Gurnell *et al.*, 2008).

The distribution of both river red gum and black box is strongly linked to flood history and elevation on the floodplain, indicating the strong influence of water as a dispersal agent for seed and determinant of receptive germination sites. Hydrochory appears to be a significant dispersal mechanism for river red gum and black box in riparian areas (George, 2004; Pettit & Froend, 2001), although the initial seed fall will be reliant on wind and wind is the main dispersal mechanism for non-riparian landscapes in higher rainfall zones (Pudney, 1998). Hydrochory is also a likely mechanism for lignum, given its lack of persistence in the soil seed bank (Capon & Brock, 2006; Chong & Walker, 2005).

Seedlings often are associated with high-water strandlines formed by twigs, leaves and other organic litter. The seeds float readily (Pettit & Froend 2001) and accumulate among debris that acts as mulch, retaining moisture and nutrients. Strandline stands of saplings and mature trees can be identified with particular flood events which created a high water line and stranded mulch and seeds in defined locations (George, 2004; Jensen, 1983; Nicol *et al.*, 2003). Dense, even-aged, restricted strandline regeneration on point bars within the main river channel is the dominant form of regeneration occurring under regulated river conditions (Kiddle, 1987). Localised floodplain recruitment of strandlines around temporary wetlands following small to medium flood events have a poor history of survival, probably due to changed groundwater conditions and lack of follow-up moisture (Jensen, 1983). No examples of broad-scale floodplain regeneration, suitable to replace senescent, large, spreading individual trees, have been located in the Lower Murray Valley for either eucalypt species (Mr J. Seekamp, Renmark, pers. comm.; Mr M. Harper, Dept of Environment & Heritage, pers. comm.; Mrs A. Jensen, University of Adelaide, pers. obs.).

Recruitment Processes for Lignum

Limited information is available on the phenology of lignum, as little systematic work has been done on its distribution and recruitment, nor any analysis of impacts of changed water regimes on this species, apart from a few student studies and recent studies on ephemeral systems of the Darling and Cooper Creek catchments (Capon, 2004; James & Capon, 2004). Lignum seeds are non-dormant, with most seeds germinating in response to moisture, and no seeds are held in the canopy (Chong & Walker, 2005). The seeds need to be on the soil surface for establishment, as buried seeds do not germinate. Seeds are spread by floodwaters and germinate in mud (Southgate, 1988).

The conditions required for germination of lignum are moisture, warm temperatures and light (Southgate, 1988). The period of the phenological cycle is relatively short. Seeds ripen and disperse within 14 days. Germination occurs within three days. Anthesis is synchronous between male and female plants, allowing pollination. Leaves are present only on young green shoots, and develop after rain or flooding (Southgate, 1988).

Lignum reproduces both vegetatively and by seed, but there is no quantitative evidence of the relative importance of each method (Lynch, 2006; Southgate, 1988). Abundant seeds are produced, but no surviving germinants were observed at a Lower Murray site (Chong & Walker, 2005). It was suggested that the lack of sexual recruitment at this site may be a result of reduced flooding due to river regulation. Asexual reproduction may be favoured by short-term increases in soil moisture (e.g. rainfall) while longer duration inundation may favour sexual reproduction. However, an investigation of the gender balance and distribution of lignum at Lower Murray sites was inconclusive, and it was suggested that long term water regime history, as well as pH and conductivity conditions, need to be included in a more comprehensive study (Lynch, 2006).

At Narran Lakes, in the upper Murray-Darling basin, where there has been a major reduction in the frequency of smaller floods, the germination, seedling survival and responses of the seed bank to significant changes in the water regime in lignum shrublands have been studied (James & Capon, 2004). There is a large persistent seed bank with heterogeneous distribution but lignum seeds were not found, even though lignum seed production has been observed throughout the year at Narran Lakes and Cooper Creek, in response to both flooding and significant rainfall (Dr S. Capon, Monash

University, pers. comm.). At Narran Lakes lignum demonstrated a rapid flowering cycle in response to flooding in January 2004, with flowering in March and prolific seed production in April but no lignum seedlings were located in the field, suggesting that vegetative reproduction is the dominant mechanism (Ms C. James, CRC Freshwater Ecology, pers. comm.). Continuing studies at Narran Lakes found that soil seed banks of lignum and chenopod communities, which have highly variable hydrological regimes, comprise suites of species which germinate in response to conditions not limited to flooding, including rainfall during dry periods (James *et al.*, 2007). The study on the Cooper Creek floodplain found that perennial species were poorly represented in soil seed bank samples and perennial plants did not appear to depend on soil seed banks for persistence (Capon & Brock, 2006). Shrub and sub-shrub species were completely absent despite their prevalence in extant vegetation. It was suggested that reproduction in many perennial plants in this region may be predominantly vegetative, including lignum.

Requirements for Lignum Germination and Recruitment

While water regime is the agent that will select which species from the seed bank will form the establishing community (Mitsch & Gosselink, 1993), climate is an important second factor (Leck & Brock, 2000). Physical conditions created by the climate of the Lower Murray Valley require specific adaptations for moisture conservation by endemic plants and recruitment strategies that maximise access to limited water sources during critical life stages and minimise the impact of high evapo-transpiration during sensitive life stages.

The lowland floodplains of the Murray-Darling Basin occur in a semi-arid climatic zone, with low rainfall and high evapo-transpiration rates. The Riverland region, covering the broad river valley from the South Australian border downstream to Overland Corner, has a semi-arid climate with an average rainfall of 250 mm/y and average annual potential evaporation of 2000 mm/y (Jolly *et al.*, 1992). It features hot, dry summers and cool, wet winters, with mean daytime temperatures in the range 9-27°C (George *et al.*, 2005). Under natural conditions, peak annual flows in the Murray with associated over-bank flows occurred in late spring and early summer (Oct-Dec) (Figure 2.1).

The primary factor for successful eucalypt recruitment on the Lower Murray floodplain is available moisture. This is determined by climatic influences (temperature, rainfall, flood, drought), and capacity for moisture retention in surface soils, as determined by structure, composition and soil transmissivity (Edgar, 1977; Grose & Zimmer, 1958; Grose, 1962). Seasonality of flood peaks is also significant as regeneration success is dependent on the flood season and flood sequence, coupled with warm temperatures post flood-recession and relatively low moisture stress during summer. Vigour, resilience and regeneration opportunities depend on the frequency and duration of floods.

The Moisture Index (measure of water available for plant growth) for the Lower Murray Valley is less than 0.2, compared to 0.7 near Albury, 1500 km upstream (Roberts 2003). This low moisture availability emphasises the necessity for a recruitment strategy to ensure access to adequate water for germination and survival of seedlings. Growing conditions for key floodplain species oscillate at irregular intervals between very dry and very wet, with plants required to survive drought and heat stress alternating with water-logging and anoxic conditions in the root zone (Roberts & Marston, 2000).

River red gum has a high sensitivity to moisture stress, with a greater effect in sand than clay, which would prevent premature or widespread germination in response to light rains, or immediately after seed fall if soil moisture was not adequate (Edgar, 1977). The optimum conditions for regeneration of river red gum in the mid-Murray Valley, 400 km upstream of the Lower Murray region, occur on the recession of floods (Dexter, 1970). Experiments to determine optimum conditions for germination of river red gum and black box for silvicultural purposes indicated that temperature and light are important factors (Bren, 1988; Dexter, 1967, 1978; Grose & Zimmer, 1958). However, although a constant temperature of 35°C is the optimum temperature for mass germination, warm temperatures are only required for 4 hours per day to obtain good germination responses (Grose & Zimmer, 1958). The minimum light requirement was found to be only 10 minutes per day (when germinants were being counted). Light becomes more important at lower temperatures (<25°C). Germination response times for both eucalypt species under laboratory conditions were in the range 3-7 days, with the longer time for germination occurring when the maximum temperature was lowered to 27°C (Grose, 1962). Sub-optimal germination still occurred at temperatures as low as 15°C.

Floodplain soils in the Lower Murray Valley are primarily alluvial grey cracking clay up to 5 m deep, with poor infiltration rates (Hollingsworth *et al.*, 1990). Sandier soil structures are found in former stream beds on the floodplain or on the edge of the actively meandering mainstream. Clay soils may retain higher levels of soil moisture than sandy soils, but sandy soils provide easier penetration for developing root systems to reach groundwater resources. Clay soils can bake hard on the surface, and can also lose structure and become non-wetting when soil salinities become sufficiently high, leading to desiccation of seedlings (Jensen, 1998b). The soil composition, texture and salinity levels are therefore likely to affect the volume of soil moisture available to seedlings.

Soil and Water Salinity

The most important factors in the decline of vegetation health on the Murray-Darling Basin floodplains are soil salinisation and declining flooding frequency (Caldwell Connell Engineers Pty Ltd, 1981; Margules and Partners *et al.*, 1990). Salinities found across the Lower Murray Valley floodplain in water bodies, soils and groundwater range from fresh to hyper-saline. Fresh water habitat is defined as <5,000 mS cm^{-1} conductivity (Nielsen & Brock, 2004), although species classified as associated with fresh water habitat may tolerate pulses of higher salinity for short periods. The median salinity of mainstream water in the Lower Murray Valley generally falls in the range 300-700 mS cm^{-1} (Close, 1990), and thus can be classified as fresh. The management target set by the Water Allocation Plan is for median salinities <1,670 mS cm^{-1} for the mainstream, wetlands and floodplain (RMCWMB, 2002). In the context of the Lower Murray Valley floodplain, salinity values consistently >5,000 mS cm^{-1} are considered to be saline in relation to impacts on floodplain vegetation health. Regional groundwater salinities are generally more than 23,000 mS cm^{-1} and frequently as high as seawater, which is 58,000 mS cm^{-1} (Evans *et al.*, 1990).

Water stress is more likely to occur in saline soils as a result of the adverse osmotic gradient away from plant roots. The combined effects of river regulation and drought lead to a prolonged lack of watering of floodplain trees, and accumulation of salt in the soil profile, with significant moisture stress (Jolly *et al.*, 1992). The reduction in frequency of flooding has also reduced the frequency of freshening effects in floodplain water bodies and lenses of freshwater overlying more saline water in the groundwater sequence. Osmotic stress is the primary cause of decline in black box in the Lower Murray region, including the Chowilla floodplain (Eldridge, 1991; Jolly *et al.*, 1992), Clarks Floodplain (Holland, 2002) and Katarapko Island (Good, 1989). In the case of black box at Disher Creek, dieback occurred after groundwater of salinities greater than 50-55,000 mS cm^{-1} rose to within 2 m of the surface (Webb & Nichols, 1997). At Chowilla floodplain, the health of floodplain vegetation declined once groundwater salinity and depth exceeded limits of 20,000 mS cm^{-1} less than 2 m from the surface for river red gum and 40,000 mS cm^{-1} within 2-4 m from the surface for black box (Sharley & Huggan, 1995).

Grazing Impacts

Sheep Grazing

One of the major impacts of grazing by sheep on Australian ecosystems is the removal of seedlings of native species, for example loss of western myall (*Acacia papyracarpa*), sandalwood (*Myoporum platycarpum*) and saltbush (*Atriplex* spp.) seedlings in the rangelands of the arid zone (Lange, 1969; Lange & Purdie, 1976; Tiver & Andrew, 1997; Tiver & Kiermeier, 2006). The grazing impact of sheep in the rangelands has been demonstrated to be intensified at watering points (Lange, 1969). In the Lower Murray Valley, many landholdings allow sheep to graze unrestricted along water frontages of the floodplain, with numerous permanent creeks and backwaters providing ready access to water by stock. In the Murrumbidgee catchment, the tendency of grazing stock to congregate at drinking sites concentrates the negative impact on habitat in riparian zones (Jansen & Robertson, 2001). Opportunistic grazing by sheep was found to reduce seed density and species richness at a floodplain site near Wentworth in the Lower Murray region (Nicol *et al.*, 2007). Plant communities derived from the seed bank were also altered, and the cause for the reduction in the seed bank was thought to be low seed production by extant vegetation. However, emergence trials of seed banks at grazed sites did not report eucalypt seedlings in the data recorded, with herbs and native annuals dominant (Jansen & Robertson, 2001; Nicol *et al.*, 2007).

The impact of grazing by sheep has been disputed in the Murray region by local graziers, with claims that regeneration of river red gum occurs in the presence of grazing sheep, so there is no need to control grazing (Mr J Robertson, Chowilla Station, pers. comm., Mr M Stoeckel, Bunyip Reach, pers. comm.). These observations relate to massive recruitment events associated with flooding, when there are so many seedlings that a significant number survive (Yates *et al.*, 1995), or the presence of water protects them from grazing in early stages of growth. Exclosures have been erected at various sites to protect emerging seedlings from grazing following floods and watering events. In each case, there was initial success, with high seedling abundance but almost no survival within 1-2 months, indicating the need for sustained soil moisture through summer months (Mr M. Harper, Dept of Environment & Heritage, pers. comm., Dr T. Wallace, Dept of Water, Land & Biodiversity Conservation, pers. comm.; Jensen, 1983).

Ant Granivory

Ant granivory on seeds is a potential factor affecting eucalypt and lignum seedling recruitment. Ants are the most abundant and diverse component of invertebrate fauna in semi-arid and arid zones of Australia, and they have broadly omnivorous diets (Andersen, 1989, 1991; Greenslade, 1979). The dominant genus is *Iridomyrmex* which prefers high temperatures and open vegetation habitats; its dominance parallels that of eucalypts (Andersen, 1991, 2003). High rates of seed removal have been recorded in all major habitat types, but predation rates even up to 95% may not be a limiting factor in successful recruitment (Andersen, 1989; McArthur, 2003).

Under unregulated conditions, heavy ant predation of seeds may not impact on the long term viability of stable floodplain vegetation communities (Andersen, 1991, 2003; Ashton, 1979). Several instances have been recorded of dominant plant species which rely on massive irregular recruitment events, generated by major habitat disturbance such as fire, for long term maintenance of population numbers, where steady high levels of ant predation do not affect long-term population viability (Ashton, 1979; Yates *et al.*, 1995). Floods provide a similar habitat disturbance trigger for recruitment on the Lower Murray floodplain, and that the massive numbers of recruits from such episodic events could counter-balance seeds lost to seed-harvesting ants on a regular basis. Changes in conditions in the Murray Valley, creating drier conditions, may mean that the ant predation effect may be more significant in the future. *Iridomyrmex* have evolved characteristics that allow these ants to survive in dry conditions (Walters & Mackay, 2003), and thus this genus could be expected to increase on the drying Lower Murray floodplain, with a potential increase in seed predation.

Environmental Watering

The appropriate form of environmental flows for a particular river system or river reach depends on endemic ecology and functions. Internationally, a series of concepts has evolved over the past 30 years for the understanding of river ecology and functions, starting from the River Continuum Concept that biological communities along a river respond to a continuous gradient of physical conditions from the headwaters to the mouth (Vannote *et al.*, 1980). The Serial Discontinuity Concept proposed that interruptions to the river continuum effectively reset the gradients of environmental conditions (Ward & Stanford, 1983). This concept was later extended to include external interruptions, especially in lowland rivers subject to lateral exchanges with wetlands (Ward & Stanford, 1989, 1995). The Flood Pulse Concept applies better to floodplain rivers, and suggests that seasonal pulses in the discharge are the key variable governing the biota (Junk *et al.*, 1989; Junk & Wantzen, 2004). This concept recognises the distinctive nature of floodplain biota, with adaptations to tolerate periodic inundation, and has been further modified to accommodate the irregular pulses in dryland rivers (Puckridge *et al.*, 1998; Walker *et al.*, 1995). The emerging concept of ecohydrology integrates hydrological and ecological processes, and proposes using ecosystem properties for regulating biota by hydrology, and hydrology by biota (Zalewski & Robarts, 2004).

'Environmental flows', or 'water for the environment', or 'environmental water requirements', are terms which are used widely among water resource managers. In the Australian context, the National Principles for the Provision of Water for Ecosystems (ARMCANZ/ANZECC, 1966) define environmental water requirements as 'the water regime needed to maintain water-dependent ecosystems, including their processes and biological diversity, at a low level of risk'. The implicit goal in environmental flow management is to promote recruitment in populations of native flora and fauna, although frequently not stated explicitly (Walker, 2002). The primary mechanism through which this might be achieved is management of the water regime to approximate key elements of the natural hydrograph which provide appropriate habitat for germination and breeding of key ecosystem species.

The concept of environmental flows has been viewed since the early 1990s as having major potential for repairing damaged river ecosystems (Arthington *et al.*, 1991; Jensen *et al.*, 1994; Jensen & Nicholls, 1997; Jensen *et al.*, 1997; Jensen *et al.*, 2003; Pigram & Hooper, 1992). The challenge for scientists has been to understand the relationship between riverine ecosystem functions and flow regime, with a view to identification of the key elements of the hydrograph that would need to be restored for effective repair and maintenance of declining ecosystem functions in the river ecosystem (Arthington *et al.*, 1991). Major research programs are seeking to understand Australia's rivers and determine what is needed for effective environmental flows to restore river health, including populations of native flora and fauna, with particular focus on the rivers of the Murray-Darling Basin in south-eastern Australia (Jensen *et al.*, 2000; Jones, 2002; Jones *et al.*, 2002; Roberts *et al.*, 2001; Thoms *et al.*, 2000; Whittington *et al.*, 2001; Whittington, 2002; Young, 2001).

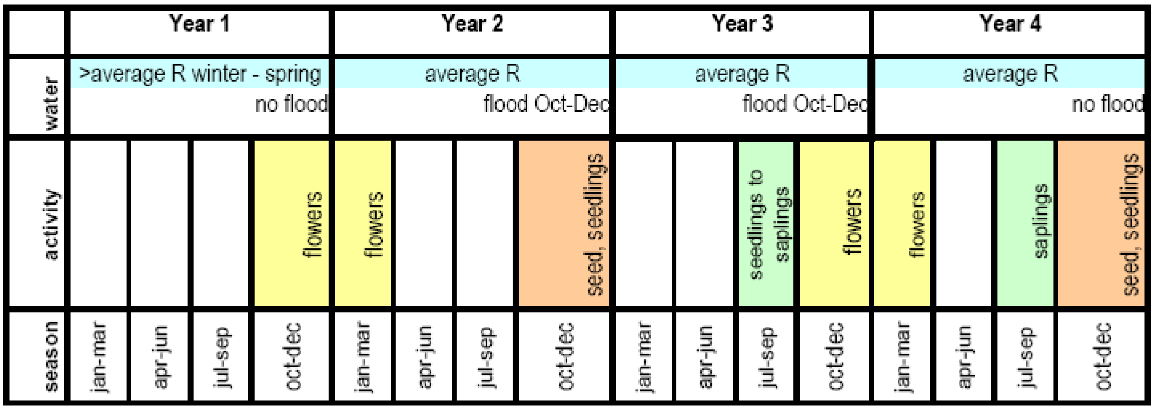
Important components of water regime for plants of rivers, wetland and floodplains are frequency of flooding, timing (ie seasonality), duration, depth and rate of change in water level. In wet/dry habitats, the periods between floods are also significant (ie length of drought) and the variability of either wet or dry conditions (Roberts & Marston, 2000; Roberts, 2003). The ideal season for drying in the Mediterranean climate of the Lower Murray Valley is late summer-autumn, when there is still a sufficiently high evapo-transpiration rate to complete the drying process before any winter rainfall cancels the effect (Jensen, 2002a, b). The timing of re-filling in the Lower Murray should coincide with the natural timing of seasonal high flow events, which occurred in October-December pre-regulation.

Flooding is expected to promote growth and germination or sprouting of propagules in a variety of flood-tolerant and flood-dependent species on the Murray Valley floodplain, with greatest responses at elevations flooded longest and deepest (Siebentritt *et al.*, 2004). A short trial flooding in 2000 generated only a temporary vegetative response which died back within 3 months, and no recruitment of new individuals. It was concluded that repeat flooding was necessary to enrich and maintain the seed bank and increase its capacity to respond to flooding (Siebentritt *et al.*, 2004). Preliminary results from the Living Murray watering trials indicate that repeat watering is required to maintain growth responses in stressed mature trees (Dr R Watts, Dept of Land, Water & Biodiversity Conservation, pers. comm.). Managed seasonal wetting and drying cycles in Lower Murray Valley wetlands (18 months inundated, 6 months dry) were successful in promoting growth of water plants, macrophytes and river red gum seedlings (Jensen, 2002a).

In the context of managed environmental flows, it is clear that water regime is a critical factor in successful recruitment of key plant species on the Lower Murray floodplain, but the details of these interactions have not been described sufficiently to prescribe effective watering at particular locations. It is also unclear how well the seed bank can adapt to the impacts of river regulation and the drier conditions now being experienced, or to respond to limited watering.

Model to Sustain Recruitment

The role of seasonal rainfall in contributing to a sequence of conditions suitable for seedling survival needs to be considered. The required sequence may be up to three years of good seasonal rainfall and seasonal floods to maintain sufficient soil moisture reserves to trigger flowering, seed fall, germination and growth of seedlings to reach a self-sufficient stage of development which is able to tap into fresh water lenses at the top of the water table. The interactive effect of rainfall with flood inundation needs to be understood to develop a watering timetable to maximise the effectiveness of limited environmental flows. It will be critical to establish the minimum level of soil moisture required. One potential sequence for seedling survival is set out in Figure 2.5.



	Year 1				Year 2				Year 3				Year 4			
water	>average R winter - spring				average R				average R				average R			
	no flood				flood Oct-Dec				flood Oct-Dec				no flood			
activity				flowers	flowers			seed, seedlings			seedlings to saplings	flowers	flowers		saplings	seed, seedlings
season	jan-mar	apr-jun	jul-sep	oct-dec	jan-mar	apr-jun	jul-sep	oct-dec	jan-mar	apr-jun	jul-sep	oct-dec	jan-mar	apr-jun	jul-sep	oct-dec

Figure 2.5 Suggested sequence of flooding and rainfall to support seedling survival requires rainfall to be above average in winter-spring of the first year and average for the next three years, coupled with floods in Years 2 and 3, to produce seedlings in spring-summer of Years 2 and 4, and saplings in Years 3 and 4. For simplicity, this scenario assumes all trees are on the same biennial cycle, flowering in Years 1 and 3.

The lack of detailed knowledge of regeneration processes for river red gum and black box at a landscape scale has also been identified as an information gap (Roberts, 2003). The mechanisms by which river red gum and black box have adapted to the irregular water regime to ensure successful recruitment need to be understood. The timing of the phenological cycle for each of the target species needs to be confirmed for regional conditions in the Murray Valley. The timing of seed fall from the aerial seed bank and the relationship to potential water sources needs to be understood in order to identify suitable conditions for germination and early juvenile survival. The role of hydrochory as a dispersal mechanism for all three key species should be taken into account, to elucidate conditions for successful germination at a floodplain scale.

The suggested combined strategy of floodplain eucalypts mixing annual low-level maintenance recruitment and infrequent massive recruitment events needs further investigation in the Lower Murray Valley. Conditions for maintenance recruitment need to be understood at the local scale in order to identify opportunities to use environmental flows to assist and extend natural processes, and to reduce recruitment failures. The boom recruitment during larger flood events also needs to be understood at the river reach scale so that appropriate post-flood conditions can be maintained to ensure recruitment success in these infrequent but critical events.

The adaptations by lignum appear to be different from those of the eucalypts, and also need further investigation. Lignum may use a combination of sexual and asexual reproduction, depending on conditions. Importantly, lignum does not have a persistent seed bank, and there are few examples of successful sexual reproduction. Further understanding is required of the extent of vegetative reproduction, the mix of reproduction strategies, and the critical conditions required for successful juvenile survival. Retention of this deep-rooted perennial shrub is likely to be a key factor in maintenance of the salt and water balance on the floodplain.

Conclusions

The major decline in vegetation health on the Lower Murray floodplain is attributed to changes in the floodplain inundation regime due to river regulation (Roberts, 2003). The dry phases have been extended and become more frequent, making growing conditions drier. The resilience of floodplain plant communities is being reduced, as they lose capacity to recover from periods of stress. If this trend continues unchecked, there could be continued decline and death in species requiring wetter conditions, a shift in species composition and wholesale habitat change (Roberts, 2003). Wetter habitats on the floodplain may shrink closer to the mainstream, leaving higher elevations with permanently changed hydrological status (Walker & Thoms, 1993).

Management options for reversal of the decline in floodplain vegetation health are based on the concept of environmental flows, where water is supplied with the aim of reversing decline and encouraging recovery in damaged floodplain vegetation (Walker, 2002). The management of environmental flows therefore requires specific knowledge of the processes for successful recruitment in the target species and vegetation communities, in order to create a local water regime which will produce the most effective outcomes in terms of vegetation recovery. Further information is required to confirm and clarify local links between changes to water regime and decline in recruitment and floodplain vegetation health. Particular attention should be given to potential options to reverse and repair damage already occurring.

The nature of the soil seed bank on the Lower Murray floodplain has not been directly investigated previously, although some wetland species and sites have been studied. It appears likely that the perennial terrestrial species which dominate the floodplain vegetation communities use aerial seed banks to retain their seed intact until release is triggered by favourable conditions, so the presence of these seeds in the soil seed bank would be only transient. Seeds which do not germinate immediately would be at high risk of ant granivory, thus very few seeds are likely to be found in samples from the soil seed bank.

The detailed responses of the seed bank to flooding, and the conditions required for successful recruitment from a wetting event remain undocumented, even though significant stands of river red gum and black box can be associated with specific larger flood events (George, 2004). More recent drying and re-wetting cycles in managed wetlands have produced local recruitment of river red gum seedlings, indicating a positive response to water regimes which aimed to mimic seasonality of natural over-bank flows (Jensen, 2001, 2002a).

Given the importance of soil moisture content as a factor in successful recruitment for both eucalypts and lignum, the conditions under which soil moisture is likely to be adequate for germination and early juvenile survival need more precise definition. As a measure of the combined impacts of water regime and evapo-transpiration, this parameter may prove to be a useful indicator of favourable conditions for early juvenile survival. It could be used to measure the need for additional environmental water allocations at critical times to maintain soil moisture and thus recruits.

It is anticipated that climatic, soil salinity and soil moisture conditions in the Murray Valley region could be suitable for germination of river red gum and black box at any time of the year, with germination taking longer in the cooler months. Ideal temperature conditions would occur from mid-spring through the summer months into autumn, subject to soil moisture levels being adequate to trigger germination of seedlings. Survival of seedlings would then depend on sufficient soil moisture to sustain the seedlings through summer and autumn, assuming normal winter and spring rainfall occurs.

There are thus gaps needing investigation to establish the composition of the floodplain seed bank in the Lower Murray Valley, the patterns of seed storage in the key floodplain species, the nature of the response of the soil and aerial seed banks to flooding, germination requirements, conditions for early juvenile survival, and critical steps in the recruitment process. From this literature review, the primary questions identified for further study are as follows:

- Are river red gum, black box and lignum seeds present in the Murray Valley floodplain soil seed bank?
- What is the seasonal pattern of seed rain for river red gum and black box?
- How do the patterns of seed rain for river red gum and black box coincide with potential water sources provided by flood and rainfall?
- When does lignum reproduce sexually or asexually, and is there a link to the alternative water sources of flood and rainfall?
- What is the level of soil moisture required for germination and early juvenile survival for river red gum, black box and lignum?

For effective application of environmental flows to Lower Murray floodplain vegetation, more detailed understanding is required of the relationship between watering events and desired responses by key plant species. The volumes, timing and release mechanisms of viable seed are not known in sufficient detail, and the requirements for sufficient moisture for germination and survival of seedlings have not been quantified. The relative roles of flood and rainfall as water sources also need further investigation, as a scientific basis for identifying the most effective complementary use of environmental flows. The Living Murray watering trials on the Chowilla floodplain, commenced in 2004, offered an opportunity to investigate these questions in the Lower Murray Valley, especially for the key floodplain perennial species river red gum, black box and lignum.

Chapter3 Lower Murray Floodplain Study Sites and Conditions

Introduction

The Murray-Darling Basin in south-eastern Australia has a catchment of more than 1,000,000 km^2, with the slow-flowing, turbid River Murray flowing through more than 2000 km of semi-arid lowlands to the Murray Mouth in South Australia. The region of interest in this study is the Lower Murray Valley, for approximately 680 km downstream of the South Australian border with Victoria (Figure 3.1). The river meanders through a broad valley up to 10 km wide over 400 km downstream to Banrock Station, and then is contained within a narrow gorge 1-2 km wide (the Murray Trench) for 280 km to Wellington, before discharging into Lake Alexandrina and eventually reaching the sea through a series of five barrages between barrier islands (Figure 3.1). This section of the river is fully regulated by a series of weirs.

The Lower Murray Valley presents a specific set of climatic and hydrological conditions which influence the survival and regeneration of floodplain vegetation communities. The locations and conditions during the period of this study are described in this chapter, to provide a context for the research described in following chapters.

Location of Study Sites

Eight sites were monitored on the Lower River Murray floodplain in South Australia (Figure 3.1). Five sites (Pilby Creek Floodplain, Pipeclay Lagoon, Twin Creeks, Monoman Island Horseshoe Billabong, Werta Wert Lagoon) were located on the Chowilla floodplain (Figure 3.2), a 17,700 ha complex of anabranches, creeks, wetlands and billabongs near Renmark (584 km from the Murray Mouth). The other sites (Figure 3.1) were at Clarks Floodplain (500 ha), near Weir No 4 (at 511 km), Banrock Station (700 ha), near Weir No 3 (at 427 km) and Brenda Park (550 ha) near Morgan (at 312 km).

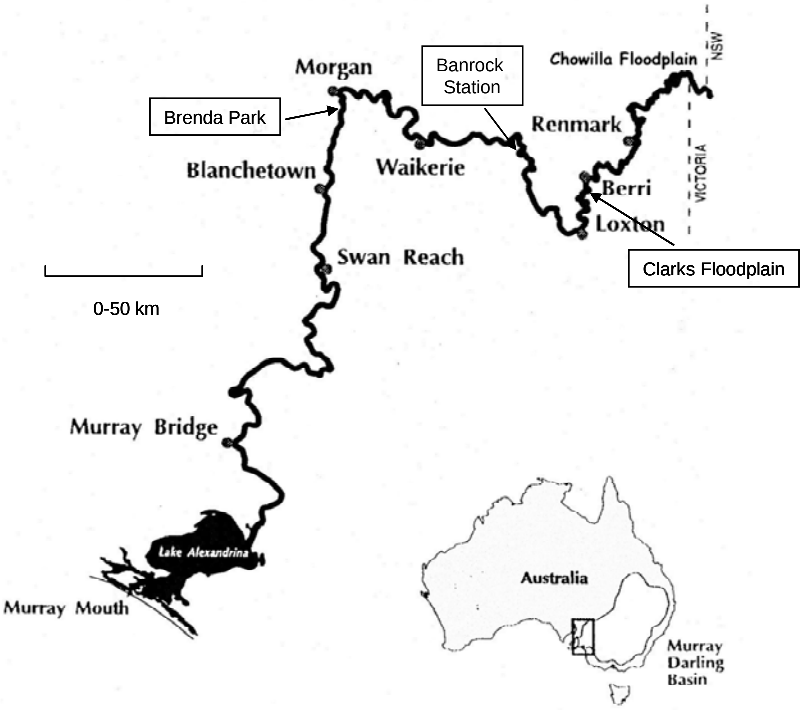


Figure 3.1 Sampling sites on the Lower River Murray Floodplain, South Australia.

The principal site, Chowilla, is part of a former sheep pastoral lease (Figure 3.2). Grazing was discontinued in September 2005, and the area now is a reserve managed by the SA National Parks and Wildlife Service. It is also a Wetland of International Importance under the Ramsar convention (signed by Australia at its inauguration in 1971), having qualified for listing as internationally important on the grounds of habitat diversity, several rare species and >1% of the world breeding population of several species of waterfowl. The vegetation includes red gum forest (748 ha) and woodland (1030 ha) and black box woodland (3458 ha) (Roberts, 2003; Sharley & Huggan, 1995). The distinction between 'forest' and 'woodland' is in height and density, with woodland generally < 8 m tall and < 30% canopy cover (Specht & Wood, 1972).

NOTE: This figure is included on page 3-2 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.2 Chowilla Floodplain, with Chowilla Creek left centre joining the mainstream, Werta Wert Lagoon top centre (arrow), and River Murray mainstream in foreground flowing right to left. (*source: Google Earth*).

Field Site Selection

The sampling sites were selected to represent various soil salinities and flooding histories. Monitoring at the selected sites was carried out during a period when experimental watering trials were being conducted under *The Living Murray Program*. Further descriptions of field sites, habitat types and hydrological regimes are given in Figure 3.3. The project was primarily designed around extensive collection of field data for statistical analysis, using ordination methods to elucidate associations and patterns related to the phenological cycles and process for recruitment in the three key species. Data collected at the selected field sites was used in investigations including the status of the soil seed bank (*see Chapter 4*), the phonological cycle of the eucalypts (see Chapter 5), the seed rain patterns of the eucalypts (*see Chapter 6*), and the growth mechanisms for lignum (*see Chapter 7*).

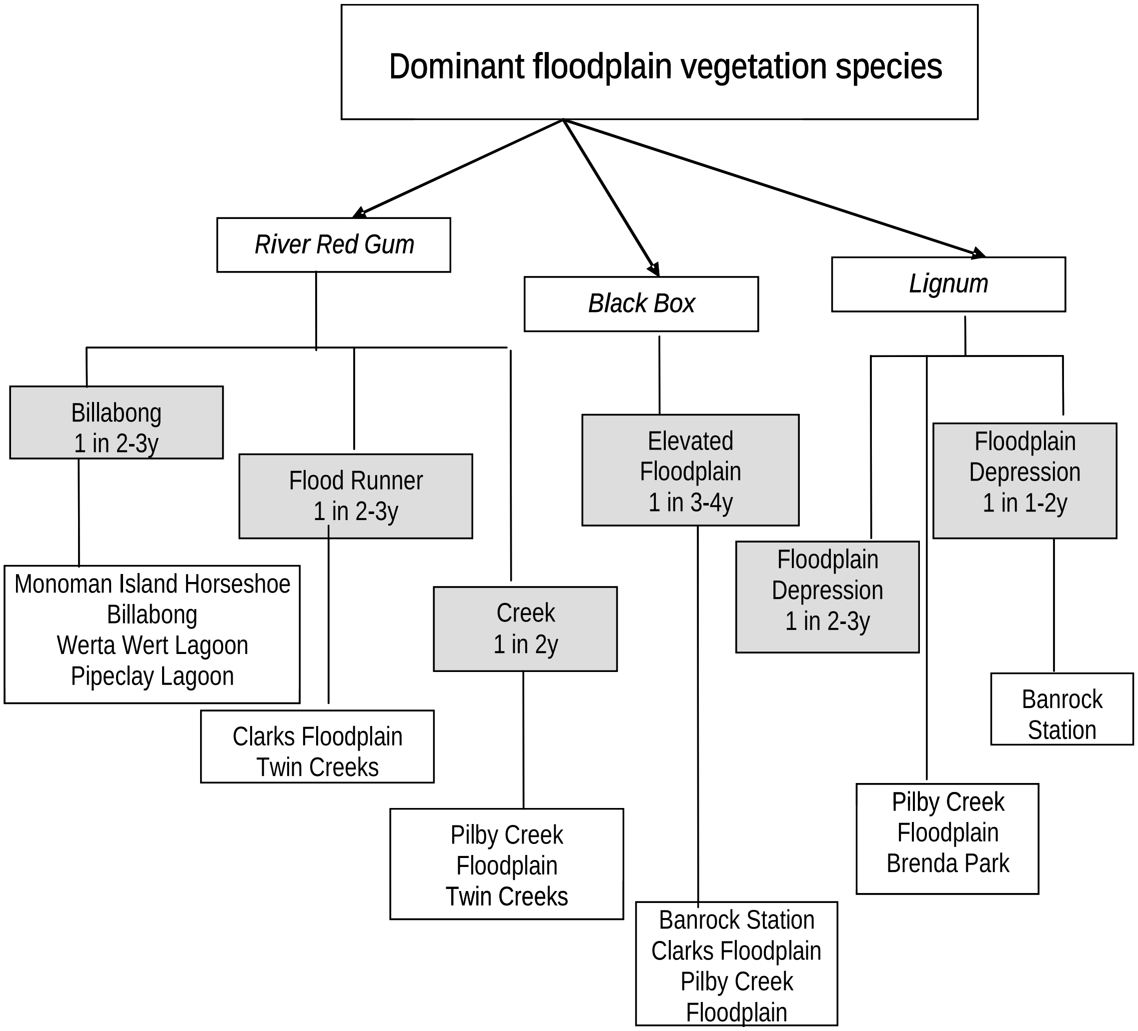


Figure 3.3 Classification of field sampling sites, showing dominant vegetation species, habitat type and natural flood frequencies for locations

Altered Water Regime

Due to river regulation, water extractions and the current extended regional drought, the time of last watering of the study sites by natural overbank flooding was 1996, except for elevated sites with black box at Banrock Station, which were last inundated in the 1973-75 floods. Thus all sites are significantly water-stressed.

As discussed in Chapter 2, river regulation has had significant impacts on the flow regime of the River Murray, including reduced frequency and duration of floods, and changed seasonality (*see Figure 2.1, Table 2.1*). In the Lower Murray Valley, the water regime is regulated both by upstream storage dams, and by local effects from Weirs 1–7, along the mainstream of the River Murray, which in effect have turned the river into a series of weirs pools (Close, 1990; Walker, 2006). As a consequence of regulation, small floods

(<25,000 ML d-1) no longer occur and medium floods (25-50,000 ML d-1) are less frequent and of shorter duration. Flood frequencies in the low-lying areas populated by red gums on the Chowilla Floodplain have decreased from about 1 in 1.2 y under natural conditions to 1 in 3.3 y (Sharley & Huggan, 1995) (Table 3.1). A flood of 62,000 ML d-1 is required to water the entire area of red gum, and 85,000 ML d-1 is needed to water black box communities at Chowilla. Flows of this magnitude occurred 1 in 1.7 y under natural conditions, but now occur 1 in 4.8 y. Flood durations also are much reduced (Table 3.1).

Similar reductions in the flood regime apply to all study sites. Clarks Floodplain, downstream of Weir No 4, has significantly reduced flooding. Prior to regulation, floods at this site occurred once in 2-3 y, but the frequency now is once in 4-5 y (Jensen *et al.*, 2002). Similar topography at Banrock Station suggests an equivalent change in flood regime. Racecourse Lagoon at Brenda Park is located within the Murray Trench, where the much narrower valley results in higher frequencies of flooding. However, this site has also had its flood regime reduced by blockages in distributary channels due to access roads, with a similar depressive effect on water regime as experienced at the other field sites (Jensen *et al.*, 1999).

NOTE: This table is included on page 3-4 of the print copy of the thesis held in the University of Adelaide Library.

Table 3.1 Reductions in flood frequency and duration for the Chowilla floodplain due to river regulation impacts.
Source: (Sharley & Huggan, 1995).

Climatic Conditions for Study

The field program covered three summer seasons from October 2004 to March 2007 with widely varying rainfall conditions. Monitoring covered a dry season (total rainfall 190 mm, 2004), a wet season (301 mm, 2005), a very dry season (119.4 mm, 2006) and a very dry start to 2007 (Bureau of Meteorology data for Renmark: average 260 mm). Climate data from Renmark was representative for all study sites. Figure 3.4 shows the annual rainfall patterns, with 73%, 116% and 46% of annual average rainfall in those years, as well as minimum and maximum temperatures. Generally, rainfall coincides with lower temperatures, although there was a significant rainfall peak in early summer 2004-05, when temperatures were higher (Figure 3.5).

Local rainfall is an important water source. A fall of ≥5 mm was deemed sufficient to germinate seeds, as it caused floodplain tracks to be water-logged. In 2004, rainfall was below average with 11 events ≥5 mm and

4 events ≥10 mm, in 2005 above average rainfall provided 18 events ≥5 mm and 10 events ≥10 mm) while in 2006 with well below average rainfall only 5 events ≥5 mm and 2 events ≥10 mm occurred.

NOTE: This figure is included on page 3-5 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.4 Daily rainfall recorded at Renmark including the study period, showing a relatively dry year in 2004, above average falls in 2005 drought conditions in 2006 and very dry conditions in early 2007; Y-axis 0-25mm d-1.
(*Source: Bureau of Meteorology*).

Cumulative rainfall and average temperatures were calculated for each 5-day period prior to sampling (0-5, 6-10, 11-15, etc days). Days since last rainfall, and days ≥30ºC in the previous month were determined, as regional evaporation rates far exceed rainfall, and high summer temperatures cause a moisture deficit. Also, red gum seeds germinate optimally at high temperatures (>30ºC), although they may germinate from 15ºC (Grose & Zimmer, 1958). These data were pertinent to determine seasonal conditions and their potential impact on germination and seedling survival.

NOTE: This figure is included on page 3-6 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.5 Maximum (black bars) and minimum temperatures (white bars) compared to rainfall (line) during the study period from October 2004 to March 2007, showing higher rainfall in 2005 coincided with cooler winter and spring temperatures; Y-axes 0-40ºC temperature (left axis) and 0-80 mm rainfall (right axis).
(*Source: Bureau of Meteorology*).

Key Species for Study

River Red Gum

The largest trees on the Murray floodplain are river red gum. The largest river red gum forests occur in the mid-Murray valley between Yarrawonga and Swan Hill, where the braided floodplains flood frequently and provide ideal growth habitat for tall, dense forest communities such as Barmah-Millewa Forests, where red gums have been intensively studied (Bren, 1988a, b; Dexter, 1978). Further downstream, the Lower Murray floodplain supports more open, shorter woodland river red gum communities such as on the Chowilla floodplain (Roberts, 2003).

River red gums are most vulnerable to drought, as they require frequent watering and so in this habitat favour areas on the floodplain where flood frequencies are approximately 1 in 2 y (Roberts & Marston, 2000). Red gums can produce floating, adventitious rootlets in water-logged soil, to cope with anoxia during prolonged flooding (Heinrich, 1990). Successful recruitment in the Lower Murray is associated with medium river flow events >40,000 ML d-1 (George, 2004). The trees are stressed by saline groundwater (>20,000 µS cm-1) within 2 m of the surface (Sharley & Huggan, 1995).

Black Box

In the southern and western floodplains of the Murray-Darling Basin, black box is on higher elevations, with its distribution dictated by its relatively low flood tolerance, high drought tolerance and low transpiration rates. It usually forms open woodland stands, where canopy cover is less than 30% (Roberts & Marston, 2000). Black box is largely dependent on flooding for natural regeneration (Treloar, 1959), although local rainfall becomes critical between floods (Jensen *et al.*, 1998). Black box is less affected by the loss of small floods than red gum (Cunningham *et al.*, 1981), but its long term survival could be affected by reduced duration of larger floods (Roberts, 2003).

Black box relies on soil water during prolonged inter-flood dry periods (Slavich *et al.*, 1999). It frequently demarcates the outer limits of larger floods and the extreme edge of the floodplain (Jensen, 1983; Slavich *et al.*, 1999). Seedlings are generally successful as a narrow band along the high-water line of the flood of origin (Cunningham *et al.*, 1981). Black box are more drought-tolerant, but they too are showing signs of water stress, although they are reputed to endure 10 years without flooding (George, 2004; George *et al.*, 2005; MDBC, 2003). Recruitment is associated with river flows >80,000 ML d^{-1} (George, 2004). Black box trees can subsist on soil water during dry periods (Slavich *et al.*, 1999), but are stressed by saline groundwater (>40,000 $\mu S\ cm^{-1}$) within 2-4 m of the surface (Sharley & Huggan, 1995). Unlike red gums, black box cannot cope with anoxia during prolonged flooding (Heinrich, 1990), and the distributions of seedlings and mature trees often indicate the limits of the biggest floods (Slavich *et al.*, 1999).

The extremely slow growth rate of black box meant that it was not of commercial interest, and consequently the species has not been studied with the same intensity as the commercially significant river red gum, so less is known about its phenology and recruitment process (Roberts & Marston, 2000).

Lignum

Lignum is a long-lived, deep-rooted perennial shrub that can attain 3 m height (Roberts & Marston, 2000). Lignum grows in characteristic shrublands on floodplains of the western Murray-Darling Basin, often in shallow clay pans. It grows best in local habitats subject to temporary ponding of water, after rain or flooding (Roberts & Marston, 2000). Lignum is typically found on swamps, river-flats, gilgais and other intermittently flooded areas. It is particularly common in the Murray-Darling Basin, including on grey cracking clays on the River Murray floodplain in South Australia, in zones with flood frequencies of approximately 3 in 10 y (Craig *et al.*, 1991). The habit is distinctive, with multiple tangled woody stems (hence the common name), which remain leafless except when new growth occurs in response to significant local rains or river flooding (Roberts & Marston, 2000). The small leaves are shed again after flowering. Lignum appears to be opportunistic, ready to respond rapidly to either significant rains or floods (Southgate, 1988). Lignum reproduces sexually and vegetatively, with new plants striking from nodes on roots or on branches contacting the soil. The effects of reduced flooding and drought on lignum communities have been given little attention.

Soils of Lower Murray Study Sites

Soils of the sampling sites were dominated by grey cracking clays, in various mixes with river sand, thus clays, sandy clays and more rarely, clayey sands. Sites with clay soils tended to wet slowly but retained moisture at deeper levels while drying hard on the surface or developing deep cracks. Sites with more sand tended to wet quickly but also drain quickly. Surface soil salinity, pH and organic carbon content of field soil samples were determined in the pilot study (see Chapter 4) by standard methods (Klute, 1986; Walkley & Black, 1934), with results indicating very low field soil moistures (2.4-7.0%) in dry seasons, relatively low surface soil salinities at low elevations on the floodplain (294-1230 $\mu S\ cm^{-1}$) and slightly acidic soil pH (5.4-6.0) (Jensen *et al.*, 2007). These values are unlikely to inhibit germination of the subject species (Tucker, 2003).

Experimental Watering Trials

The five sites at Chowilla are part of the Murray-Darling Basin Commission's *The Living Murray Program* watering trials, which began in March 2004 (http://www.mdbc.gov.au) (Figure 3.6). Clarks Floodplain was added to this program in June 2005. Banrock Station is a Ramsar-listed wetland, with water control structures that has been subjected to a managed, near-natural wetting and drying regime since 1994. The Brenda Park site had a control structure installed in 2000, to operate a wetting and drying regime.

All study sites were last flooded for three weeks in December 2000 (too short for long term effect on soil moisture reserves), and for eight weeks in 1996, thus by the end of 2007 there had been 11 years since effective over-bank flows. This reduced frequency and duration of flooding, combined with severe regional drought, has created a situation of water stress at all locations, reflected in rapidly declining health of river red gums and black box, with seriously escalating decline between surveys in 2002 and 2004 (MDBC, 2005).

NOTE: This figure is included on page 3-8 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.6 Five field sites at Chowilla, adjacent to Living Murray watering trials. (Map source: Sharley & Huggan, 1995)

Key 1 = Pilby Creek Floodplain, 2 = Monoman Island Horseshoe Billabong,
3 = Pipeclay Lagoon, 4 = Twin Creeks, 5 = Werta Wert Lagoon

The study sites were watered at least twice during the field study period from October 2004 to March 2007 (Table 3.2). Watering trials were timed to simulate natural wetting cycles, in late spring-early summer, but in March additional water was delivered to highly-stressed trees. Four of the five sites at Chowilla had been dry for four years when watering began in 2004, but Monoman Island Horseshoe Billabong had been dry for 10 years. Clarks Floodplain had been dry for five years when watered in 2005. The sites at Twin Creeks and Clarks Floodplain are on sandy soil and retained water for only a few weeks, but Werta Wert Lagoon, Monoman Island Horseshoe Billabong, Pilby Creek Floodplain and Pipeclay Lagoon are on clay and remained wet for several months.

At Banrock Station Lagoon, water levels have been raised artificially in late spring each year since 1994. Higher levels occurred in November 2005 when (for purposes of environmental management) the No 3 Weir pool on the River Murray adjacent to Banrock Station was raised to augment a small flood river red gum and lignum communities were flooded for 2-3 weeks. The stressed black box trees on Banrock Station floodplain are at a higher elevation and have not been flooded since 1975. They did not benefit from the watering trials or weir-pool raising. The Racecourse Lagoon in the Brenda Park wetlands complex was filled in December 2004 with the intention of managing near-natural wetting and drying cycles. However, the wetland was unable to be dried out due to a leaky control structure and it remained wet throughout the study period until finally drying in March 2007, at the end of field sampling.

Chowilla Sites	Volume (ML)	Area (ha)	Date filled	Period dry (y)	Duration of fill (weeks)	Water retained (weeks)	Regime
Monoman Island Horseshoe	140	6	Mar 2004	10	12	36	water stressed trees
			Dec 2005		8	28	
Werta Wert	680	37	Aug 2004	4	12	28	≤ 120 d
			Nov 2005		10		83 in 100 y
Pipeclay Lagoon	40	6	Aug 2004	4	12	20	≤ 120 d
			Sep 2005		12	20	8 in 10 y
Twin Creeks	75	6	Nov 2004	4	2	6	water stressed trees
			May 2005		4	10	
			Mar 2006		4	8	
Pilby Junction	300	24	Sep 2004	4	12	24	≤ 120 d
			Oct 2005		12	28	6 in 10 y
			Sep 2006		12	24	

Table 3.2 Summary of watering trials at selected field sites on Chowilla Floodplain, indicating timing and duration of watering events and management objectives.

Vegetation Health

Tree health (H) was rated on a 7-point scale, adapted from Holland (2002) and used by the SA Department of Water, Land and Biodiversity Conservation for Chowilla Floodplain to assess responses by stressed trees to watering trials (Table 3.3). Healthy trees (H = 4-6) have >50% of their original canopy and < 10% epicormic growth, but may have dead branchlets (H = 4). Stressed trees (H = 1 3) have <50% of their canopy and show increasing degrees of foliage loss, dead branches and epicormic growth.

NOTE: This table is included on page 3-10 of the print copy of the thesis held in the University of Adelaide Library.

Table 3.3 Scale of tree health as adapted by the SA Department of Water, Land and Biodiversity Conservation from Holland's scale developed for Clarks Floodplain (Holland, 2002).

The field sites covered a range of vegetation condition, including relatively healthy (low stress), medium stress, and highly stressed (Table 3.4). Most sites exhibited varying degrees of stress, and the issue was highlighted on the site selected for a pilot study of the soil seed bank at Clarks Floodplain (*see Chapter 4*). This site had dramatically deteriorated in just four years, with mature trees previously in excellent health showing thin canopies, loss of leaf colour and loss of vigour. The site at Monoman Island Horseshoe Billabong was also seriously stressed, with many dead trees. Figure 3.7 gives examples of a healthy red gum and a very stressed black box tree from the 36 trees sampled.

Site	Low stress	Medium stress	Highly stressed
Banrock Station	RRG BB (ref)	Lignum	BB
Racecourse Lagoon	RRG	Lignum	
Brenda Park	Lignum		
Clarks Floodplain (healthy)	RRG		
Clarks Floodplain (stressed)			RRG
Pilby Creek Floodplain	BB	RRG, Lignum	RRG (ref)
Pipeclay Lagoon	RRG	Lignum	
Monoman Island		BB (ref)	BB
Horseshoe Billabong		RRG (ref)	RRG
Twin Creeks		RRG	RRG (ref)
Werta Wert Lagoon		RRG	

Table 3.4 Field sites covered a range of combinations of vegetation condition and types (RRG = river red gum, BB= black box).

Conclusions

The study sites provided a wide sample of floodplain conditions in proximity to watered sites, where the impacts of the managed water regime could be evaluated. The climatic conditions during the study ranged from 73% of average annual rainfall in 2004, through 116% in 2005 and 43% in 2006, providing contrasting seasons. The effect of grazing was observed in before and after comparisons, once sheep were removed in September 2005.

Figure 3.7 A healthy red gum (left) and a very stressed black box tree with epicormic growth (right) on Banrock Station floodplain, as examples of the trees sampled. The healthy red gum is at a sheltered, well-watered site, while the stressed black box is at an exposed, saline site at a higher elevation. The trees are at approximately the same scale, showing that red gums are usually approximately twice the height of black box. The red gum was within the zone of influence of watering in 2004 and 2005, while the black box is likely to have benefited from effect the 1991 flood on groundwater, and was last inundated in the 1973-75 floods.

Chapter4 Status and composition of the Lower Murray floodplain soil seed bank[7]

Introduction

In most plants, recruitment is governed by the composition and nature of the seed bank, which contains ungerminated seeds of the vegetation community, and acts as a reservoir of species. Germination is triggered by change or disturbance, such as temperature, changes in day length, fire, or floods (Leckie *et al.*, 2000; Pickett & McDonnell, 1989). There is little information, however, about seed banks associated with river floodplains in Australia (Chong & Walker, 2005), with most focus being on wetland seed banks, where seeds may lie dormant for extended periods between floods. If a floodplain might be considered as a temporary wetland, there is more scope for comparison with published data, albeit still limited (Brock *et al.*, 1994; Frears, 2001; Nicol *et al.*, 2003; Stone, 2001). Work on wetland seed banks in Australia has focused on soil seed banks, and in particular, the characteristic of persistence, defined as the capability of seed to remain viable for two or more growing seasons (Baskin & Baskin, 1998), which provides a mechanism for surviving dry phases in a highly variable climate (Brock *et al.*, 2003). Such a mechanism might be expected in the Murray floodplain seed bank, but the persistence of eucalypts and lignum in the soil seed bank has not been tested.

Other mechanisms such as aerial seed banks may be important, as seen in other eucalypt species (Yates *et al.*, 1994). The plants of the River Murray floodplain have perhaps more in common with terrestrial systems, where long-lived woody perennials rely on a strategy of annual production of short-lived seeds, as seen in floodplains of ephemeral rivers of south-western USA (Stromberg, Boudell & Hazelton 2008). The persistent seed bank is more likely to be dominated by short-lived species such as annuals and herbaceous perennials.

As a first step to understanding successful recruitment in eucalypts and lignum on the Lower Murray floodplain, the status and composition of the soil seed bank was explored, with reference to river red gum and black box, and the common shrub, tangled lignum. Owing to the frequent observation of river red gum germination in disturbed soil, such as along new tracks on the floodplain, or after floods, it is a common assumption among natural resource managers that the seeds are present in the soil and just require the addition of water, although lignum seed was not found in the soil in a previous study (Chong & Walker, 2005). The process for seed distribution and contact with soil moisture needed clarification in order to target watering events. This information had potential to be significant for plans to rehabilitate selected floodplain sites by artificial watering (‘environmental flows’) to relieve stressed plants and promote seed germination. With this in mind, the responses of the seed bank to experimental watering regimes were also tested.

Study Site and Methods

Samples were taken at Clarks Floodplain, on the River Murray near Berri, South Australia (*see Figure 3.1*). The site is a 500 ha complex of point bars, terraces, meander scrolls and flood runners (distributary channels), in an area where meanders have created sandy peninsulas supporting dense vegetation (Figure 4.1). It is downstream of Weir & Lock No 4, constructed in 1929. Prior to regulation, floods at this site occurred once in 2-3 y, but the frequency now is once in 4-5 y, so the lower floodplain has lacked effective freshwater inundation for 11 years (*see Chapter 3*).

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[7] Jensen, A.E., Walker, K.F., & Paton, D.C. (2008a). The floodplain seed bank of a regulated lowland river: composition and responses to wetting treatments for the River Murray floodplain, Australia. In prep.

The greatest impact at this site has been on river red gums, which require inundation every 2-3 y. Stressed trees on the higher floodplain were first noticed in 1997 (PPK, 1998), linked to displacement of highly saline groundwater by nearby irrigation, with associated salinisation, ponding and scalding (Jensen *et al.*, 2002) (Figure 4.2). In 1997, grazing stock were removed and recreational access was controlled, allowing successful regeneration of black box in localised areas. River red gum regeneration has been confined to river sand bars, with no regeneration found under the mature woodland and forest stands. Some grazing damage still occurs due to feral hares (*Lepus capensis*).

Figure 4.1 Clarks Floodplain, downstream of Weir & Lock No 4 (top centre across river mainstream), includes three sandy peninsulas with river red gum forest, river red gum woodland, black box open woodland and lignum shrubland communities. Denser, healthier communities, including tall forest, are concentrated on inner bends where river water refreshes the shallow water table through sandy soils.
(*Source: Department of Lands aerial photography 1988*).

At the study site, the state of health varied between vegetation layers, with the more salt-tolerant groundcover and small shrub species growing vigorously, flowering and fruiting, in contrast to the stressed and dying river red gums. Species in good health included prostrate chenopods (*Rhagodia* spp., *Atriplex* spp.), native boxthorn (*Lycium australe*) and ruby saltbush (*Enchylaena tomentosa*). Black box trees on higher elevations adjacent to the study site were in good health, but stands close to the outer river bend 2-300 m downstream (Figure 4.2) were under medium to severe stress (H = 2-3) (Holland, 2002) (*see Table 3.3*). The mature stand of river red gums at the higher edge of the flood runner (Figure 4.3) were showing severe stress and death (H = 0-2). These large mature trees had lost all leaves, were losing fine twigs and shedding large quantities of bark. Most of the larger trees, likely to be more than 500 years old, appeared to be beyond recovery (Figure 4.3). River red gums at lower elevations (i.e. higher flood frequencies) showed medium stress (H = 3 4).

Samples were taken in April 2004, during a dry period. Local annual rainfall was near average (264 mm) in 2003, but negligible in 2004 prior to sampling (12 mm to 31 March) (Bureau of Meteorology data for Loxton). A 100 m transect was selected, starting in an area of river red gum saplings on the bed of a major flood runner and leading to a zone of dead mature trees on more elevated flats with approximately 2.5 m increase in elevation (Figure 4.3). The transect covered a zone where river red gum and lignum seeds were likely to be found but black box was less likely, based on distance to potential source plants. Five 1200 g samples were taken at 20 m intervals along the transect. Each was transferred to a 2 L plastic container, with topsoil uppermost, transported to a greenhouse and subdivided into four equal parts, in 1 L containers, for experimentation.

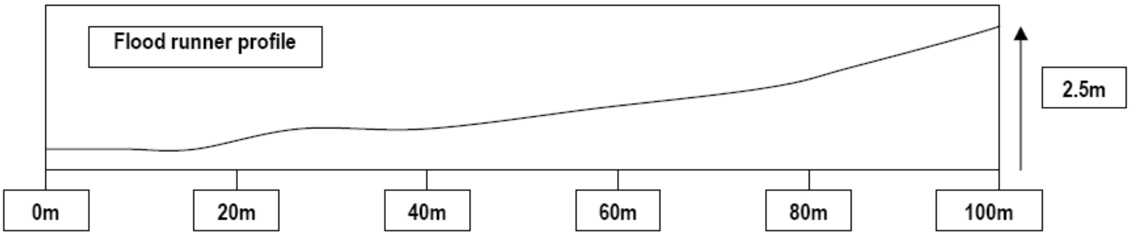
Figure 4.2 Aerial view of Clarks Floodplain, with highland irrigation (lower right) and a pool of saline seepage on the adjacent lower-lying floodplain. The River Murray mainstream flows from right to left, with sandbars on each inner bend (centre left). Vegetation on the outer bend (study site) is declining and dying as saline groundwater is displaced by irrigation drainage under the floodplain towards the river. Vegetation on the sandy peninsula (top centre) is very healthy, with the river on three sides replenishing the water table. The flood runner allows water to short-cut across the peninsula in small floods >50,000 ML d^{-1}.

Three watering treatments were devised to simulate a short flood, a longer duration flood and rainfall, respectively. These were (1) short wetting (inundated to 10 mm for 7 days, dry for 49 days), (2) long wetting (10 mm for 28 days, dry for 35 days) and (3) two rainfall events (5 mm on day 1, 5 mm on day 29). Rain treatments consisted of a single application of 50 mL, which equated to a rainfall event of 5 mm in 100 mm x 100 mm pots. A control was added with sustained moisture (10-20% soil moisture for 63 days). To simulate floodwater salinities, sea salt (Lotus™) was added to yield an initial salinity of 670 µS cm^{-1}; this was applied for one week, and thereafter 500 µS cm-1 was applied. In the River Murray locally the median salinity is 600 µS $cm\text{-}^{1}$, tending to lower values during spring freshes (Mackay & Eastburn, 1990). Controls received water of 500 µS cm^{-1}. Rain simulations used laboratory Reverse Osmosis water only, with 50 mL (equivalent of 5 mm rainfall) sufficient to wet the samples for up to 7 days.

Samples were maintained in a glasshouse at temperatures in the range 10-33°C (exceeding 30°C on several days) and conducive for germination (Grose & Zimmer, 1958). Emergent seedlings were counted and monitored weekly for 63 days. They were left to grow in situ so that taxa could be identified. The numbers of individual plants (plant density) and species per sample (species richness) were recorded. Introduced grasses were abundant in some samples, and were recorded separately to preserve the option of isolating them in later data analyses.

Soil moisture was measured by weighing sealed samples, drying for 72 hours at 65°C and weighing again to determine water loss (Klute, 1986). Soil salinity was measured using a 1:5 soil water extract, mixing 8 g dry soil and 40 mL distilled water and shaking for 2 hours (Klute, 1986). The salinity of the solution then was measured using a handheld probe.

Figure 4.3 Site of field transect (L to R) from base of flood runner to upper edge in zone of dead mature red gums at Clarks Floodplain. Stressed red gums can be seen at lower elevations in the flood runner (left middle distance). Approximate elevation is 2.5 m from left to right. The dead trees appeared healthy in 2000, with no obvious signs of stress. The picture shows the transect from 40 m (left) to 90 m (right) and the profile below indicates the position of sample points relative to elevation.



At the study site, the state of health varied between vegetation layers, with the more salt-tolerant groundcover and small shrub species growing vigorously, flowering and fruiting, in contrast to the stressed and dying river red gums. Species in good health included prostrate chenopods (*Rhagodia* spp., *Atriplex* spp.), native boxthorn (*Lycium australe*) and ruby saltbush (*Enchylaena tomentosa*). Black box trees on higher elevations adjacent to the study site were in good health, but stands close to the outer river bend 2-300 m downstream (Figure 4.2) were under medium to severe stress (H = 2-3) (Holland, 2002). The mature stand of river red gums at the higher edge of the flood runner (Figure 4.3) were showing severe stress and death (H = 0-2). These large mature trees had lost all leaves, were losing fine twigs and shedding large quantities of bark. Most of the larger trees, likely to be more than 500 years old, appeared to be beyond recovery (Figure 4.3). River red gums at lower elevations (i.e. higher flood frequencies) showed medium stress (H = 3 4).

Spatial and temporal sampling of soil seed bank

Initially soil seed bank samples to determine composition were taken initially in dry conditions prior to watering at several other sites, including Pilby Creek Floodplain, Pipeclay Lagoon and Werta Wert Lagoon, along transects that intersected riparian zones and wetlands, and near piezometer lines associated with watering trials (*see Figure 3.1*). Samples (0.01 m^2 × 60 mm deep) were collected in 1 L plastic ice-cream containers, returned to the glasshouse, watered and monitored over two months for emergent eucalypt seedlings. All non-eucalypt seedlings then were removed, and the samples were monitored for a further two months. To monitor seasonal patterns, ongoing monthly intact soil samples (15 × 0.01 m^2 × 60 mm deep) were taken for 17 months from November 2005 to March 2007 during watering trials at Pipeclay Lagoon (healthy site) and Clarks Floodplain (stressed site).

Results

Composition of soil seed bank

Plants grown out from the soil seed bank samples were 18 species of mainly native terrestrial annuals, predominantly Chenopodiaceae, including four saltbushes (*Atriplex* spp., *Rhagodia* spp.) and ruby saltbush (*Enchylaena tomentosa*) (Table 4.2). Sneezeweed (Asteraceae: *Centipeda cunninghamii*) was the single most abundant species. It flowered and set seed in the controls, completing its life cycle in eight weeks, as did other ground-cover and herb species.

Other plants from wetter habitats were less well-represented, but included riparian sedges and rushes (*Cyperus gymnocaulos*, *Eleocharis* spp.) and rats tail couch (*Sporobolus mitchellii*), marginal wetland plants like common joyweed (*Alternanthera nodiflora*), native licorice (*Glycyrrhiza acanthocarpa*), slender knotweed (*Persicaria decipiens*) and creeping monkey flower (*Mimulus repens*).

The total density across all samples (n = 100) was 1496 seedlings m^{-2}. The densities and species richness of emergent seedlings were highly variable within and between locations on the sample transect (cf. flood frequencies). Only one red gum seedling was found. No black box seedlings were found, but this could be because mature black box trees were more than 50 m from the sampling sites. No lignum seedlings were found, although several lignum bushes were within 20 m.

The single river red gum seedling was recovered from a sample taken directly under dead trees; there was no living overhead canopy and the nearest likely seed source was more than 100 m away (Figure 4.3). Along the transect there was more than 50% ground cover from small prostrate shrubs, bark and leaf litter, providing a mulching effect for seedlings. The river red gum seedling emerged in the control, and was first observed 28 days after watering commenced.

common name	species	Family
lesser joyweed	*Alternanthera denticulata*	Aramanthaceae
daisy	*Aster sublatus*	Asteraceae
prostrate saltbush	*Atriplex sp.*	Chenopodiaceae
Saltbush 1	*Atriplex sp.*	Chenopodiaceae
saltbush 2	*Atriplex sp.*	Chenopodiaceae
saltbush 3	*Atriplex sp.*	Chenopodiaceae
common sneezeweed	*Centipeda cunninghamii*	Asteraceae
chenopod	*Chenopodium pumilis*	Chenopodiaceae
Australian stone crop	*Crassula sieberana*	Crassulaceae
rush	*Cyperus gymnocaulis*	Cyperaceae
native carrot	*Daucus brachiatus*	Apiaceae
ruby saltbush	*Enchylaena tomentosa*	Chenopodiaceae
river red gum	*Eucalyptus camaldulensis*	Myrtaceae
creeping monkey-flower	*Mimulus repens*	Scrophulariaceae
prostrate rhagodia	*Rhagodia sp.*	Chenopodiaceae
seed grass	*Senapsis alba*	Brassicaceae
unknown groundsel	*Senecio sp.*	Asteraceae
unknown dicot	*Trachymene sp.*	Apiaceaea

Table 4.1 Species germinated as seedlings in soil seed bank samples from flood runner at Clarks Floodplain.

Soil moistures in the topmost 100 mm of soil samples were low (2.4-7.6%, n = 25), reflecting the prevailing dry season (Table 4.3). Surface soil salinities (294-1940 µS cm^{-1}, or 490-1160 ppm; n = 25) were variable at low elevations and higher at the most elevated margin of the flood runner. Soil pH was slightly acidic (5.4 6.0, n = 25), with no apparent spatial pattern.

Transect	n	pH	Salinity (EC)
0-20 m	5	5.57 (0.09)	590 (45.83)
20-40 m	5	5.42 (0.05)	634 (149.49)
40-60 m	5	5.91 (0.06)	294 (39.82)
60-80 m	5	6.01 (0.11)	420 (84.56)
80-100 m	5	5.85 (0.17)	1940 (489.49)

Table 4.2 Mean pH and salinity (standard errors) of soil seed bank samples along 100 m transect traversing flood runner over approximately 2.5m elevation change, with 0 m being at the lowest elevation (i.e. higher flood frequency), and 100 m at the highest elevation (i.e. lower flood frequency).

Seasonal Germination from Soil Seed Bank

Plants grown out from soil seed bank samples from floodplain terrace, wetland bed and riparian sites were mainly annual native terrestrial species, particularly chenopods (*see Chapter 4*). A total of 14,464 native plant seedlings were grown out from an aggregate 6.55 m^2 of soil taken from 4 sites, including a sequence of 17 monthly samples at a stressed site (Clarks Floodplain) and at a healthy site (Pipeclay Lagoon) (Table 6.3). Of the emergent seedlings, only 76 were red gum seedlings, equivalent to 11.6 m^{-2}

seedlings. No black box seedlings were found, but this was not unexpected as mature black box trees were more than 100 m from the sampling sites. No lignum seedlings were found, even though lignum bushes were present close to both sampling sites.

The healthy site in the riparian zone of Pipeclay Lagoon, which was watered each spring during the study period, had germination rate of 25 red gum seedlings m^{-2} (Table 6.3). Much lower numbers were recovered from Clarks Floodplain, where seed fall from stressed trees was significantly reduced (Figure 6.4), with 1.18 seedlings m^{-2} on the bed of the flood runner where seedlings emerged following experimental watering.

A single freshly-fallen fruit with spilled seed captured in a sample from Pipeclay Lagoon in September 2006 yielded 22 seedlings, but these seedlings were excluded from the count as they did not derive from seed rain. If these seeds had been included, the total from the Pipeclay riparian zone over 16 months would have been 91 seedlings, or 33 seedlings m^{-2}.

Site	Period	*n* samples (0.2 m^3)	Total area sampled (m^2)	Total native sdlgs	Red gum sdlgs	Density (sdlgs m^{-2})	Potential red gum sdlgs ha^{-1}
Clarks Floodplain	Jul-04, Nov-05--Mar-07	280	3.4	5087	4	1.18	11,800
Pipeclay Lagoon	Jul-04, Nov-05--Mar-07	280	2.75	9062	69	25.09	250,909
Pilby Creek Floodplain	Jul-04	10	0.2	235	2	10.00	100,000
Werta Wert Lagoon	Jul-04	10	0.2	80	1	5.00	50,000
TOTAL		580	6.55	14,464	76	11.6	116,000

Table 4.3 Total native seedlings (excluding introduced grass and non-native weeds) and river red gum seedlings germinated from soil seed bank samples on the Lower Murray floodplain. The larger sample at Clarks Floodplain includes initial sampling of the soil seed bank .

Effects of Water Regime on Germination

Different watering treatments produced distinctive responses (Figure 4.4). The short wetting treatment (flooded for 7 days) evoked rapid germination, peaking over 21-28 days, and a higher plant density than other treatments, but all seedlings died after 49 days without additional moisture. In the long wetting treatment (flooded for 28 days), germination was slower (starting underwater), with lower densities of seedlings, and seedlings survived for 63 days without additional moisture.

The control attained maximum density by 12 days and maintained similar species density throughout the experiment (Figure 4.4). This result indicated the maximum species richness which could be germinated under these conditions from the soil seed bank. Density of seedlings germinated declined along the sample transect from the more frequently inundated bed to the less frequently inundated higher edge.

The simulated ‘rain’ events produced rapid, short-lived responses of relatively low plant densities, and seedlings within 14 days (Figure 4.4). New seedlings were produced on the two separate wetting events from the same seed bank sample, with similar plant densities but higher species richness for the second event.

In all treatments, there was a higher seedling density in the lower elevation samples on the flood runner bed where inundation would be more frequent, compared with higher elevation samples which would have lower flood frequencies.

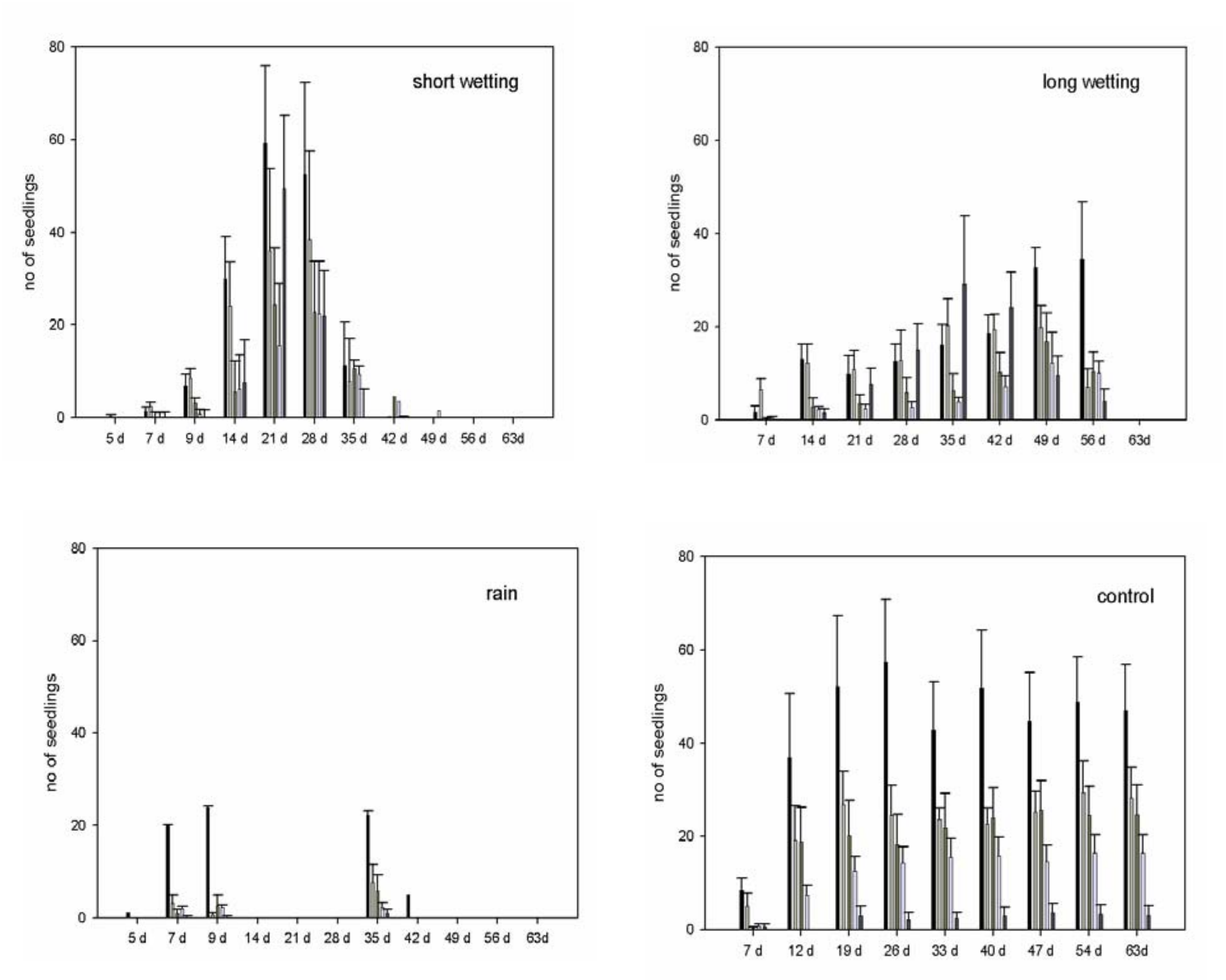


Figure 4.4 The number of seedlings grown from soil seed bank samples over 9 weeks to four different water regimes were plotted as combined replicates for each 20 m interval along the transect (left column in group at lowest elevation), with standard error bars. The response to the short wetting treatment (inundated for 7 days) showed rapid germination and highest density of plants within 14 days, declining as the soil dried out. The response to the long wetting treatment (inundated for 28 days) showed a slower germination of lower density, but longer survival. The response to two simulated rain events (R = 5 mm) with a dry period between showed two separate germinations from the same seed bank sample. All seedlings died after 14 days with no additional moisture. The greatest response was from the low elevation samples. The response to constant 10-20% soil moisture showed a steady rate of germination (no significant difference in values over time). Plant densities were consistently lower at higher elevations across the flood runner.

A comparison of the relative seedling densities of seed bank responses to the wetting treatments shows the higher density, more rapid response to the short wetting, compared to the slower, lower density response to the long wetting (Figure 4.5). Each ‘rain’ event produced a similarly low density of seedlings, while the control indicates the maximum potential germination of seedlings produced by these soil seed bank samples under these experimental conditions.

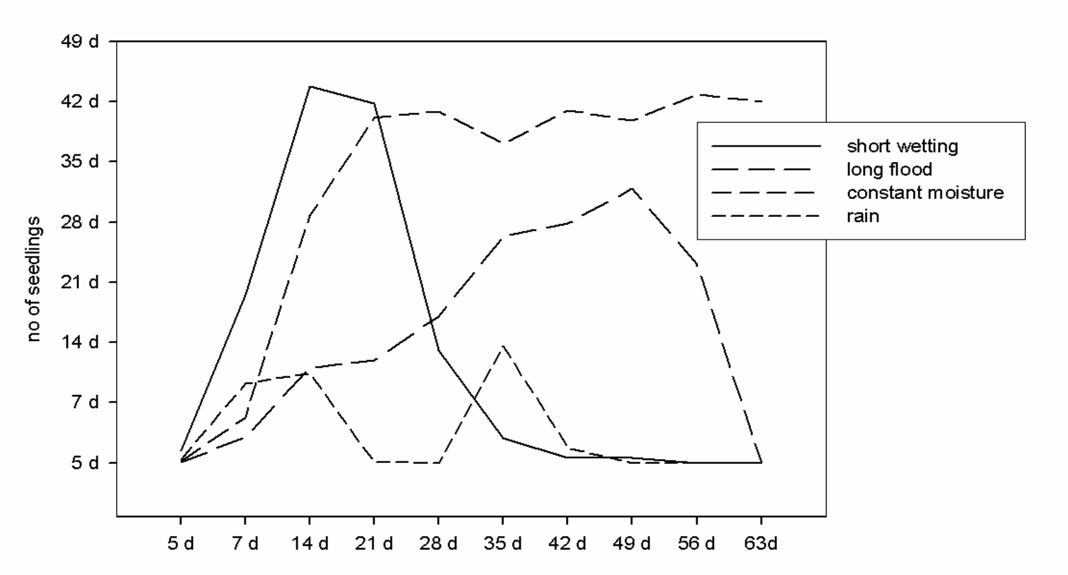


Figure 4.5 Comparison of responses in seedling densities to wetting treatments (shown individually in Figure 4.4) showing the rapid response of native seedlings to the short wetting (7 d), compared to the slower response to the long wetting (28 d). Two small peaks show the response to individual ‘rain’ events, while constant moisture conditions in the control indicate the maximum potential response from the seed bank.

The highest density of seedlings occurred in the bed of the flood runner (20 m) for all treatments, associated with highest flood frequencies (Figure 4.6). Most samples yielded their highest plant density under constant moisture. The exception was in the samples from the high edge of the flood runner (100 m), which peaked under the long wetting treatment, due to the high density of small cotyledons. These did not survive as soil moisture declined.

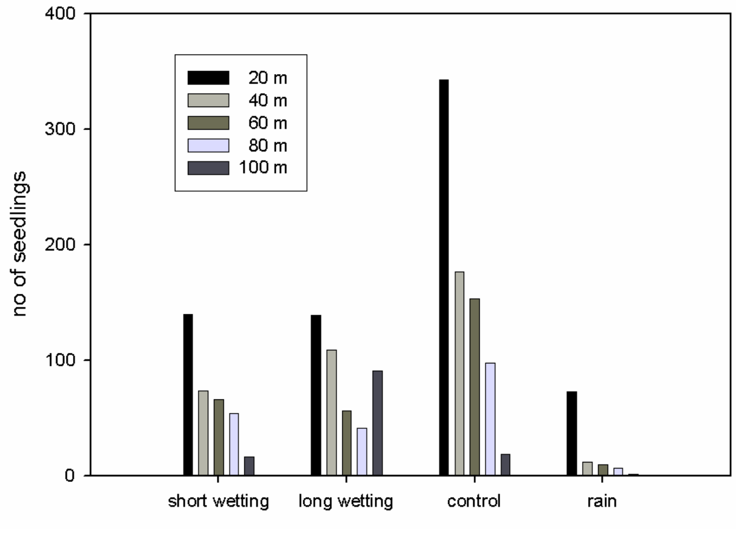


Figure 4.6 The average density of seedlings germinated under each treatment is shown for samples taken from the bed of the floodrunner (20 m), with a general decline in germination as the elevation increases up the edge of the floodrunner to higher levels of the floodplain (100 m), ie with reducing flood frequency. The highest densities of seedlings for each transect location were generally produced in the control, with the exception of the 100m samples which had highest richness in the long wetting treatment.

Species richness in weekly samples increased with sustained soil moisture (Figure 4.8). It was higher for lower elevations in the flood runner, corresponding with higher frequencies of inundation. Species richness in the different treatments was similar for the control and long wetting while moist, but was significantly lower for the short wetting, and even lower for the 'rain' treatments. The spatial pattern in species richness showed highest numbers of species growing in the interval 0-40 m along the sample transect, associated with higher frequencies and 25-40,000 ML d^{-1} flows (Figure 4.9).

Figure 4.7 Soil seed bank sample in 1 L pot showing seedling growth in response to constant moisture.

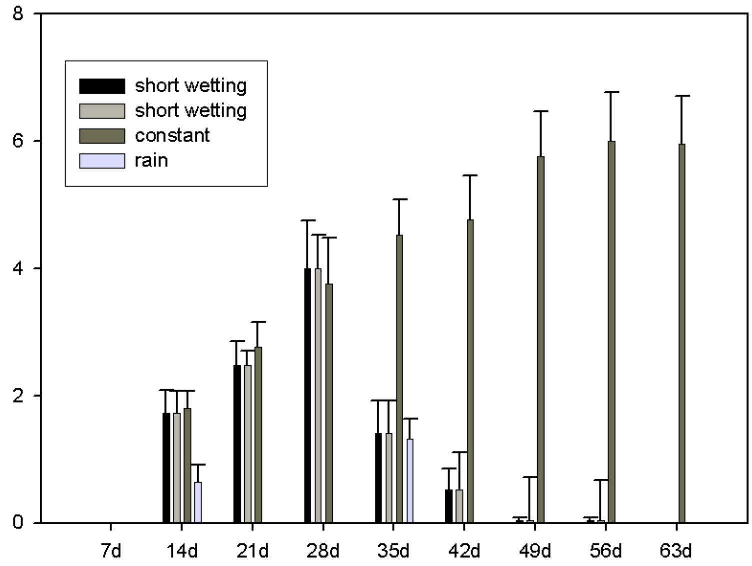


Figure 4.8 Maximum mean species richness in weekly samples was associated with sustained soil moisture. The total number of species in the long wetting was 16, in the control 14, with 9 in the short wetting and 7 in the second 'rain' treatment. The species which comprised the minimum totals in samples were common to all sites.

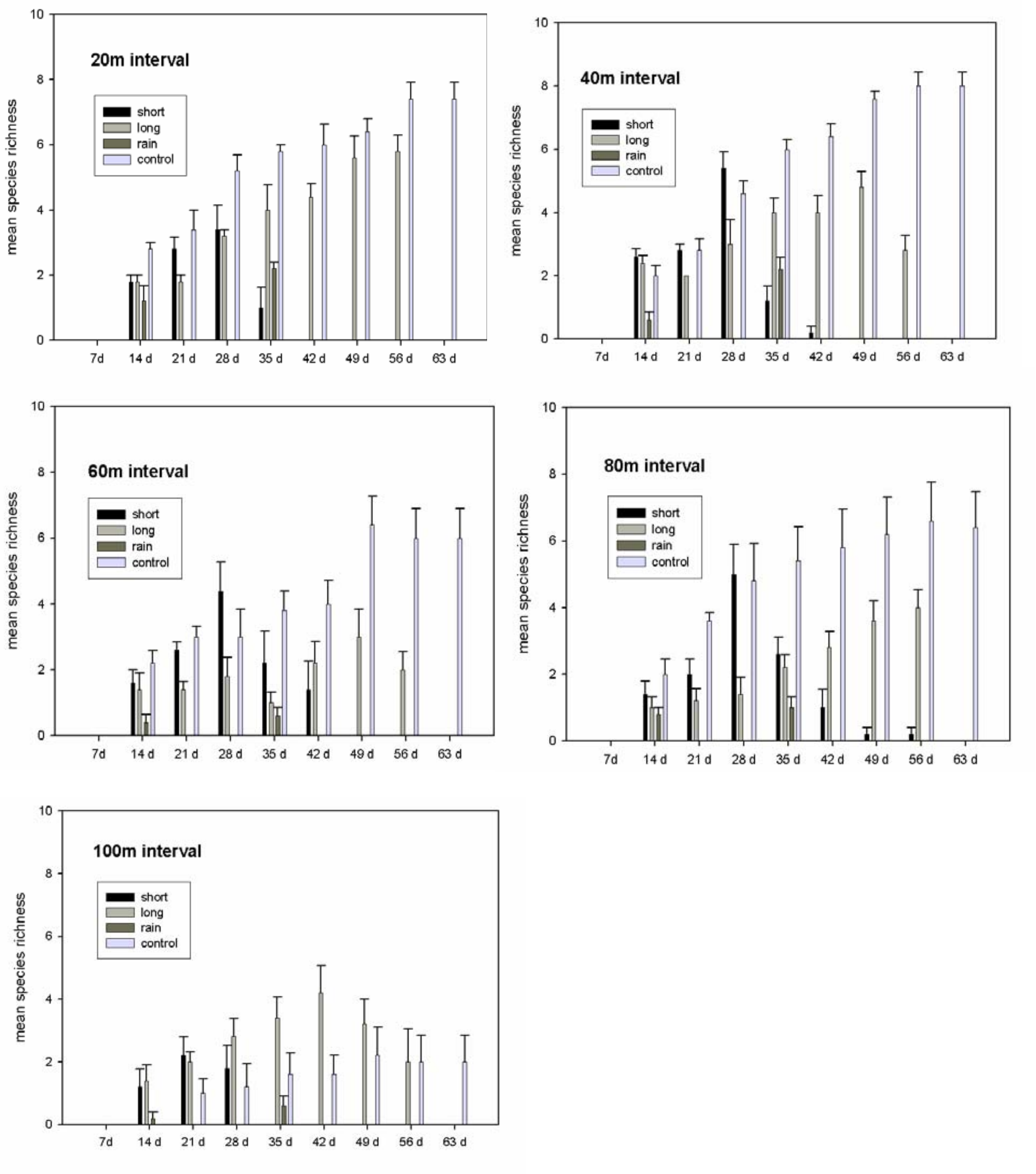


Figure 4.9 Maximum mean weekly species richness was associated with locations subject to higher flood frequencies. Data are presented at five transect locations across the flood runner from the lowest point (0 m) to the higher runner edge (100 m), with elevation increasing by approximately 2.5 m (see transect profile in Figure 4.3). Most species germinated in the 0-40m interval, i.e. at lower elevations and higher flood frequencies.

Discussion

Composition of soil seed bank

The floodplain soil seed bank was found to be dominated by native annual species, primarily ground cover and small shrub species typical of regional terrestrial communities not necessarily confined to floodplain habitats. Small numbers of riparian and semi-aquatic species were also found. Many demonstrated short life cycles which could be completed within one flooding cycle, presumably an adaptation for opportunistic responses to water availability in wetland species (Brock & Rogers, 1998).

The dominant tree species on the floodplain, river red gum, had only a sparse and patchy presence in the soil seed bank. This was unexpected, given repeated observations of significant numbers of trees regenerated in strandlines associated with flood events (Bren & Gibbs, 1986; George *et al.*, 2005), and the high volume of seed produced by this species, perhaps 600,000 seeds per tree (Jacobs, 1955). Samples in this study were taken in April, the month recommended for seed harvesting in both river red gum and black box (Boland *et al.*, 1984; Gunn, 2001). River red gum releases its seeds from capsules while still on the tree (Dexter, 1967; Pudney, 1998). Seed fall in the Lower Murray Valley has been reported as highest during March-May (George, 2004), and seeds might therefore be expected in the soil seed bank at this time. The sample transect was near surviving saplings, which would indicate that seeds should be found in the vicinity.

The single germinant found suggests that river red gum seeds have a short lifespan once in the soil seed bank. Their absence at this site could be caused by a high loss of seeds from the soil surface after seed fall (e.g. granivory by ants), or reduced seed fall, or a local variation in timing of seed fall. At this location, the highly stressed mature trees may be producing less seed.

A single red gum seedling out of 1496 seedlings germinated from 1 m^2 sampled was not significant statistically relative to the density of all seedlings grown from soil seed bank samples. Nevertheless, this potential density of one seedling per square metre may be sufficient for maintenance recruitment to replace the local population of scattered trees over the lifetime of the current mature trees (150-1,000 y), even with the normal low 5% survival rate (Fenner, 1985). However, the soil seed bank apparently does not contain high enough numbers of seeds to be the primary source of seed for flood-triggered germination of dense strandline stands of eucalypts, which form the bulk of the population structure (George *et al.*, 2005). Further testing should be done for the presence of river red gum in the soil seed bank across all seasons (*see Chapter 6*).

Black box seeds were not found in this initial study, but this species generally grows at more elevated locations on the floodplain than the sample site. Most eucalypt seeds rarely travel more than three times tree height from the trunk on seed fall (Pudney, 1998), and the nearest mature black box trees were <10 m high and more than 50 m from the sampling transect. Thus, further testing should be done at more locations and in different seasons to determine whether black box seeds are present in the soil seed bank (*see Chapter 6*).

Seeds from the dominant perennial shrub lignum were also not found in the soil seed bank, even though mature shrubs were close to the transect. Nor were they found in samples taken from under lignum bushes, confirming a previous report that lignum does not have a persistent soil seed bank (Chong & Walker, 2005). Lignum also has the capability of vegetative reproduction, but the relative roles of sexual and asexual reproduction are little known. Sexual reproduction may be linked to protracted watering (e.g. flooding), while asexual reproduction may be triggered by short-term watering events (e.g. local rainfall) (Lynch, 2006). This question requires further investigation, to determine whether lingum is present in the soil seed bank or utilises alternative reproductive strategies (*see Chapter 7*).

The dominant perennial species (eucalypts and lignum) thus appear to practise serotiny, holding the majority of their seeds in an aerial seed bank, rather than in the soil seed bank. It is thought that the majority of species in wetlands and flood-prone areas use floodwaters for dispersal (*hydrochory*) and germinate when floodwaters recede, leaving moist soil to support development of seedlings (Leck & Brock, 2000). The coincidence of peak seed fall with flooding would therefore provide a mechanism for germinating large, even-aged stands of saplings in a boom recruitment event (Dexter 1978). Ongoing light seed rain would also be dispersed by hydrochory during experimental watering events at any season (see Chapter 6). However, the presence of seeds in the soil seed bank appears to be very transitory, possibly due to ant granivory.

Thus, the seeds of river red gum, black box and lignum do not appear to achieve persistence in the soil seed bank, in that they do not persist for more than two growing seasons. Instead these eucalypts adopt the strategy of serotiny, where seeds are retained in the canopy and released when conditions are optimal for germination. This strategy has been noted in other eucalypt species (Yates *et al.*, 1994) and in shrubs such as banksias (Lamont & Enright, 2000).

The density of seedlings germinated from the soil seed bank at this site of 1496 m^{-2} is relatively low. The Lower Murray seed bank has previously been described as depauperate (Chong & Walker, 2005) and sparse (Siebentritt *et al.*, 2004), as indicated by low seed densities (Chong, 2002; Frears, 2001). Wetland seed bank densities less than 1000 m^{-2} are generally considered rare (Nicol *et al.*, 2003). Regional data on wetland seed banks includes widely varying values, from relatively sparse densities such as 696 m^{-2} at Reedy Creek near Mannum, in a brackish semi-permanent wetland (Frears, 2001) to much higher densities of 69,905 m^{-2} at a temporary wetland at Loveday in the Riverland (Stone, 2001). Data from North American semi-permanent prairie wetlands are in the range 1309-9893 m^{-2} (Poiani & Johnson, 1988). However, these studies have focused on wetland sites, and the broader Lower Murray floodplain seed bank as tested in this study appears to be dominated by terrestrial rather than wetland species. The lack of seeds contributed from the three dominant floodplain species would be a factor in the low density.

The ongoing monthly assessment of the soil seed bank for 17 months recorded an overall average 11.6 m^{-2} eucalypts across all sites, suggesting potential germination of 116,000 seedlings ha^{-1} in suitable habitats. Monitoring at the stressed Clarks Floodplain site indicated 1.18 m^{-2} seedlings in response to watering (potentially 11,800 ha^{-1} even at stressed sites). The persistence of six surviving seedlings out of 359 germinants along 120 m^2 on the Monoman Billabong strandline over 18 months (including drought) suggested a potential 500 seedlings ha^{-1} in suitable strandline habitats.

Most red gum seedlings were found in the riparian zone of a wetland with a fringe of healthy mature trees (Pipeclay Lagoon at Chowilla). The monthly numbers of seedlings found in the soil seed bank of the riparian zone at Pipeclay Lagoon increased in proportion to the monthly fluctuations in seed rain from trees overhanging the sampling site. There were sufficient red gum seedlings to suggest ongoing potential for low-level recruitment of individual trees from rain-triggered germination, as seen at Monoman Island Horseshoe Billabong, Chowilla and Clarks Floodplain (Jensen *et al.*, 2008a).

Effects of surface water regime on germination

The effects of different water treatments suggest the importance of sustained soil moisture in successful recruitment. Moist soil is required to sustain germinating seedlings through the establishment stage, beyond initial germination. This indicates that more than one soil-wetting event is required for survival, especially to sustain seedlings through desiccating conditions if germination is triggered in early-mid summer on a floodplain which is becoming drier. The single red gum seedling recovered in the control was not present at 21 days but was first observed after 28 days, which suggests a minimum requirement of at least 22 days of 10-20% soil moisture prior to germination.

The responses to the 'rain' treatments were quick and short-lived, which suggests that additional sources of soil moisture are required after rain for seedling survival. Seedlings only survived for 14 days after a simulated rain event of 5 mm rain. Two separate germinations in response to two separate rainfall events occurred from the same soil sample, with similar densities of plants but higher species richness in the second germination. Thus, not all seeds germinate on the first wetting event, providing a mechanism for further germination when conditions are favourable, rather than exhausting the seed bank in the first germination, as also observed in wetland seed banks of aquatic species (Brock & Crossle, 2002).

There was a rapid germination and longer-lived response to the short wetting treatment (10 mm inundation for 7 days), but the seedlings did not survive beyond 42 days without additional soil moisture. The germination response to the long wetting (10 mm inundation for 28 days) was slower, and lower in plant density, with some species starting germination underwater. The seedlings were sustained for longer, but eventually all had died by 63 days without additional moisture.

Species richness was similar in the long wetting treatment and the control, but significantly lower in the short wetting treatment, and very low in the 'rain' treatment. There was spatial variation in germination responses to watering, with reduced plant density and species richness from relatively higher elevations in the flood runner, where flooding frequencies are lower.

Surface soil salinities across most of the flood runner were relatively low (<650 µS cm^{-1}), although there was a significant increase at the higher edge of the flood runner (1940 µS cm^{-1}), under the dead mature trees (Table 4.2). While local conditions during flooding may elevate water salinity levels to as much as 1000 µS cm^{-1}, the salinity levels applied in experimental conditions (500-640 µS cm^{-1}) are considered unlikely to inhibit a germination response from the seed bank samples (Nielsen & Brock, 2004).

Natural sources of soil moisture

Soil moisture content was critical for seedling survival following germination. The regeneration response and survival of seedlings were shown to be highly sensitive to continued soil moisture availability. Rain is thus a key factor in the sequence of water sources to maintain soil moisture levels and contribute to seedling survival, as well as flood inundation events. Sunchrony between seed fall and hydrological flood regime has been observed in red gum forests further upstream (Dexter 1978), and the change in timing of flood peaks to a much more variable pattern in the Lower Murray Valley would reduce the chances of seed falling onto soil with sufficient moisture to support germination and survival of seedlings (*see Chapter 2*)

Residual soil moisture levels between wetting events were very low (<6%). While seasonal rainfall is sufficient to support life cycles of native shrubs and groundcover species, it has not been sufficient to counteract the combined effects of lack of flood inundation for an extended period, plus rising saline groundwater intruding into the root zones of mature river red gums (McEwan *et al*., 2003). Black box trees within the zone of invading groundwater are also showing significant signs of stress, in spite of their greater tolerance of salinity.

It is thus a key priority to understand in more detail how minimum soil moisture levels for seedling survival are created and sustained in the context of altered hydrology and drier habitat conditions on the Lower Murray floodplain (*see Chapter 8*). This has implications for the timing of artificial watering events in environmental flow programs, particularly sites such as Clarks Floodplain where the health of mature trees has been adversely affected by reduced flood frequency and rising saline groundwater.

Effects of Saline Groundwater on Tree Health

The rapid decline in vegetation health at this site since 1997 led to groundwater investigations which concluded that the decline was linked to the intrusion of saline groundwater into the root zones (AWE, 1999). Groundwater investigations confirmed that the Bookpurnong groundwater mound fed by local irrigation is displacing groundwater directly towards the major river bend adjacent to the study site (Figure 4.2) (AWE, 1999) (R Doble, CSIRO, pers. comm.). This measured groundwater movement coincides with the observed pattern of decline in vegetation health. River red gums in the Lower Murray region have been observed to be adversely affected when groundwater of salinity exceeding 20,000 µS cm^{-1} rises to <2 m below the surface (Sharley & Huggan, 1995). Local investigations recorded groundwater salinities in the range 55,800-58,400 µS cm^{-1} at <3.6 m below the surface in 2001 (Holland, 2002) and bank seepage indicated that levels had risen to within 2 m of the surface under the dying river red gums by 2004 (Jensen, pers. obs.). In contrast, groundwater salinities from nearby stands of healthy river red gums were relatively fresh at 720 µS cm^{-1} with depth to groundwater of 3.52 m (McEwan *et al.*, 2003).

Since 1996, with no effective over-bank flows, it is likely that fresh water lenses overlying the groundwater have been exhausted under the affected trees. The nearest source of fresh water for the river red gums is the river mainstream, more than 100 m away. River red gums do not access surface water more than 15 m away from the trunk (MDBC, 2003; Mensforth *et al.*, 1994), so the stressed trees are expected to be drawing on groundwater directly below them. The trees on the inland edge of the flood runner, which have been most badly affected, would have to exert greater transpiration pressures to pull river water back into their root zones against the increasing flux of groundwater (A Telfer, Australian Water Environments, pers comm.). Poor black box health on the Clarks Floodplain site coincided with shallow groundwater of salinity >50,000 µS cm^{-1} (≤2.5m depth) (Holland, 2002).

In response to the salinisation of the floodplain, proposals have been implemented since the completion of this pilot study to intercept the groundwater which is being displaced towards the river by irrigation drainage (AWE 1999a,b). Fresh river water is also being pumped into the flood runner. These measures are designed to increase the ‘air space’ above the water table, and to replenish fresh water lenses which can provide water for the stressed trees. Recent studies have confirmed that the distribution of shallow groundwater of high salinity coincides with the pattern of tree decline and death at the Clarks Floodplain site (Berens et al).

Further Investigations

The responses of the soil seed bank to various water regimes indicate that more than one soil-wetting event is required for seedling survival, especially to sustain seedlings through desiccating conditions if germination is triggered in early-mid summer. Appropriate serial wetting regimes should be tested, incorporating the contribution of local rainfall events to soil moisture. The watering trial which has since pumped fresh river water into the flood runner in 2005 and 2006 provided an opportunity for further field study of the effects of artificial watering events on eucalypt and lignum recruitment. Further work has been undertaken by government agencies at Clarks Floodplain to investigate groundwater responses to surface flooding and interception of groundwater, confirming that watering trials have been locally effective in reducing the impact of rising saline groundwater and contributing to the responses measured in sample trees (*see Chapter 6*).

Chapter5 Investigating the Phenological Cycle of Lower Murray Floodplain Eucalypts[8]

Introduction

Phenology is the study of times of seasonally recurring biological functions such as flowering, fruiting and seed fall (Curtis, 1985). In both species, the phenology of flowering and seed fall varies geographically. River red gums flower from September to February (George, 2004; Roberts & Marston, 2000) along the Murray, but in December and January elsewhere (Boland *et al.*, 1984; Paton *et al.*, 2004). Seed fall peaks in autumn and spring in the Lower Murray (George, 2004), in winter in the nearby Mount Lofty Ranges (Pudney, 1998), and in spring in the Barmah Forest, Victoria (Dexter, 1970). Black box trees flower from August to January in the Lower Murray (George, 2004), and from May to October in most of the Murray-Darling Basin (Roberts & Marston, 2000). Seed fall is from February to April (Boland *et al.*, 1984; George, 2004).

The plant communities of the Lower Murray floodplain, characterised by the distinctive river red gum and black box (Myrtaceae: *Eucalyptus camaldulensis*, *E. largiflorens*), rely on serotiny, releasing seed from aerial seed banks. A pilot study found that river red gum seeds were not present in the persistent soil seed bank in statistically significant numbers (see Chapter 4). Black box seeds were not found at the sites sampled initially, but these sites were at lower elevations of the floodplain and distant from mature black box trees. Subsequent sampling of soil beneath the canopies of healthy black box trees yielded no seedlings in emergence trials.

This part of the study aimed to investigate the phenological patterns of the two eucalypts, river red gum and black box, and to assess the factors affecting various stages of the phenological cycle, particularly timing of various stages which are likely to interact with available water sources. It may be that seed fall in the eucalypts is timed to be synchronous with two separate moisture sources, as seed fall in autumn and winter could coincide with significant rainfall, and seed fall in spring and summer could coincide with flooding near river red gums or black box with mature seed. The study included some trees included in a previous study of eucalypt regeneration and its relationship to hydrological regime at Banrock Station (George, 2004) (*see Figure 3.1*), and extended sampling over a wider section of the Lower Murray Valley.

River Red Gum

Most river red gum communities are found in floodplain zones with a natural flood frequency of at least 1 in 3 y (George, 2004; Jensen, 2004; MDBC, 2003; Sharley & Huggan, 1995). River red gums in the Murray-Darling Basin require relatively frequent flooding, with not more than 2 years of drought between wet periods, but that they do not tolerate more than 2 years of water-logging (Roberts & Marston, 2000). Germination is encouraged by flooding (Walker, 1986).

River red gum is found on heavy grey clay soils and sandier soils, typically forming ribbon stands along riverbanks and watercourses, and on floodplains and river flats subject to frequent or periodic flooding (Margules & Partners *et al.*, 1990). Limits of recent big floods (eg 1973-74) are marked by copses of young trees (Walker, 1986).

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[8] Jensen, A.E., Walker, K.F., & Paton, D.C. (2007). Using phenology to determine environmental watering regimes for the River Murray floodplain, South Australia. In Australian Rivers: making a difference (Eds A.L. Wilson, R.L. Dehaan, R.J. Watts, K.J. Page, K.H. Bowmer & A. Curtis). Proceedings of 5th Australian Conference on Stream Management, 175-180. Charles Sturt University, Albury, New South Wales.

River red gum is a medium to large sized tree, 25-40 m high when mature (Cunningham *et al.*, 1981). Basal girth can be 3-4 m and trees may live for hundreds of years (Walker, 1986). The cream flowers occur in clusters of 5-10, and have beaked caps. The fruit is 7-8 mm wide, with the valves protruding (Cunningham *et al.*, 1981).

Black Box

Across its Australian distribution, black box is a medium sized tree 10-20 m high, with a large spreading crown and drooping branches (Cunningham *et al.*, 1981). The off-white flowers occur in clusters of 3-7 as part of a large panicle. The fruits are approximately 5 mm, with the valves enclosed. The species normally flowers in spring-summer, but some winter flowering has been observed (Mrs A Jensen, University of Adelaide, pers obs).

In the Murray-Darling Basin, this small to medium tree has an open form, with low-level branching and often exhibiting a twisted trunk habit (Roberts & Marston, 2000). Height of trees varies with flood frequency, and erectness and vigour are dependent on water availability (Roberts & Marston, 2000). Heights on the Chowilla floodplain have been measured in the range 3-21 m, with the tallest trees associated with well-watered sites (Roberts & Marston, 2000).

Materials and Methods

Observations were recorded at seven sites in South Australia, between Morgan and the New South Wales border (*see Figure 3.1*). These were Racecourse Lagoon at Brenda Park, near Morgan, Banrock Station floodplain, near Barmera and Monoman Island Horseshoe Billabong, Pipeclay Lagoon, Pilby Creek Floodplain, Twin Creeks and Werta Wert Lagoon on the Chowilla floodplain near Renmark (see Figures 3.2, 3.5). All sites were linked to controlled watering events, and those at Chowilla are part of *The Living Murray Program* developed by the Murray-Darling Basin Commission (*see Chapter 3*).

Monitoring from October 2004 to March 2007 covered a dry season (total rainfall 190 mm, 2004), a wet season (301 mm, 2005) and a very dry season (119.4 mm, 2006) (Bureau of Meteorology data for Renmark: average 260 mm) (*see Figures 3.3, 3.4*).

Phenological data were recorded monthly for 3 selected branches (marked with coloured flagging tape) on each of nine river red gums and nine black box trees across the seven sites, to complement data from 12 seed traps at each location (Table 5.1). A rank scale (0-4) was used to indicate the relative volumes of buds, flowers, immature fruit, mature fruit with closed valves and fresh leaves. Scores were: 0 = absent, 1= low volume (<25% of influorescences), 2= medium volume (25-50%), 3=high volume (50-75%) and 4=very high volume (>75%). These data were plotted to reveal seasonal and inter-annual patterns. Other variables such as local rainfall, soil moisture and insect herbivory were monitored as part of the larger study; these were included in later analyses of the complete data set (*see Chapter 6*).

Species	watered	water delayed	not watered	healthy	low stress (H=4-5)	medium stress (H=3)	high stress (H=1-2)	Total
River red gum	7	0	1	4	2	2	0	8
Black box	0	1	8	4	1	2	2	9

Table 5.1 Trees sampled for phenological patterns allocated by water regime category and health status.

Results

River red gum bud crops followed an annual cycle, maintaining relative volumes to May-July and declining toward December or January as buds were shed prior to flowering (Figure 5.1). Buds were set in January-February, and surviving mature buds were held on the trees until flowering in November. Flowering peaked in December in each year, although most individual trees flowered predominantly at two-year intervals (either odd or even years). Immature fruits occurred from October 2004 until April 2006, then were not recorded for 7 months (presumably these were aborted due to dry conditions from autumn 2006). Mature fruits persisted throughout the sampling period and increased in volume in 2006, when immature fruit from 2005 matured. There was a substantially larger fruit crop in 2006 than in 2005, and even larger numbers in early 2007. Fresh leaf growth peaked in December and continued through autumn, but with progressively smaller volumes from 2004 to 2006.

In overview, individual river red gums peaked in bud production in different years (for example, five trees produced higher bud crops in 2005, one in 2005 and four in 2006). The flowering patterns showed that trees either peaked in both December 2004 and December 2006, or only in December 2005. Abundances of closed mature fruit were higher on six trees in 2006 and on two trees in 2005 (two trees had equivalent numbers of fruit in both years and so were not counted as having a single peak). At Twin Creeks there was an additional low-intensity winter flowering event in June 2006, possibly due to a-seasonal watering in May-July 2005 and again in March-April 2006 (natural flow peaks normally occur in October-December).

Black box bud crops also showed an annual cycle, maintaining bud numbers until May-June (Figure 5.1). Very small bud crops were produced in January 2005 and December 2005. As with river red gums, there was a larger bud crop in 2006 than in 2005. Buds were set in January-February, and mature buds were held on the tree until flowering. Flowering occurred in October 2004-January 2005, July 2005-December 2005 and April 2006-December 2006. The only months when flowering was not recorded in this study were February and March. Winter flowering (May-October) occurred mainly at Chowilla sites in 2006 (Monoman Island, Pilby Creek Floodplain), while Banrock Station trees flowered mainly in summer(November-January 2004 and 2006). Immature fruit occurred throughout the monitoring period, but abundances were low in September 2005 and April 2006, and generally lower in 2006 than in 2005. Numbers of immature fruit increased again in December 2006. Mature fruits with closed valves persisted at relatively consistent volumes throughout the entire study period. Fresh leaf growth peaked each summer, but continued through most of 2005 when good rainfall occurred. No new leaves were recorded in dry conditions from April 2006 to November 2006, and a relatively smaller volume was produced in summer 2006.

In general, the abundances of buds on individual black box trees peaked in different years (for example, one tree peaked in 2004, none in 2005 and eight in 2006). Numbers of flowers produced were highest for three trees in 2004, no trees in 2005 and three in 2006. Numbers of closed mature fruit were highest for four trees in 2004, one in 2005 and two in 2006. Table 5.2 compares the phenology of river red gums and black box trees at the study sites in 2004-2007. These observations show that the summer months (Dec-Feb) are more critical for production in river red gum, with a short flowering season, while black box trees have more protracted stages, but also with peak volumes in summer. Both species retain bud crops (before flowering) and mature closed fruit (before shedding seed) in their canopies for extended periods, although the crop may be shed in response to poor conditions post-bud initiation and pre-flowering (Figure 5.1).

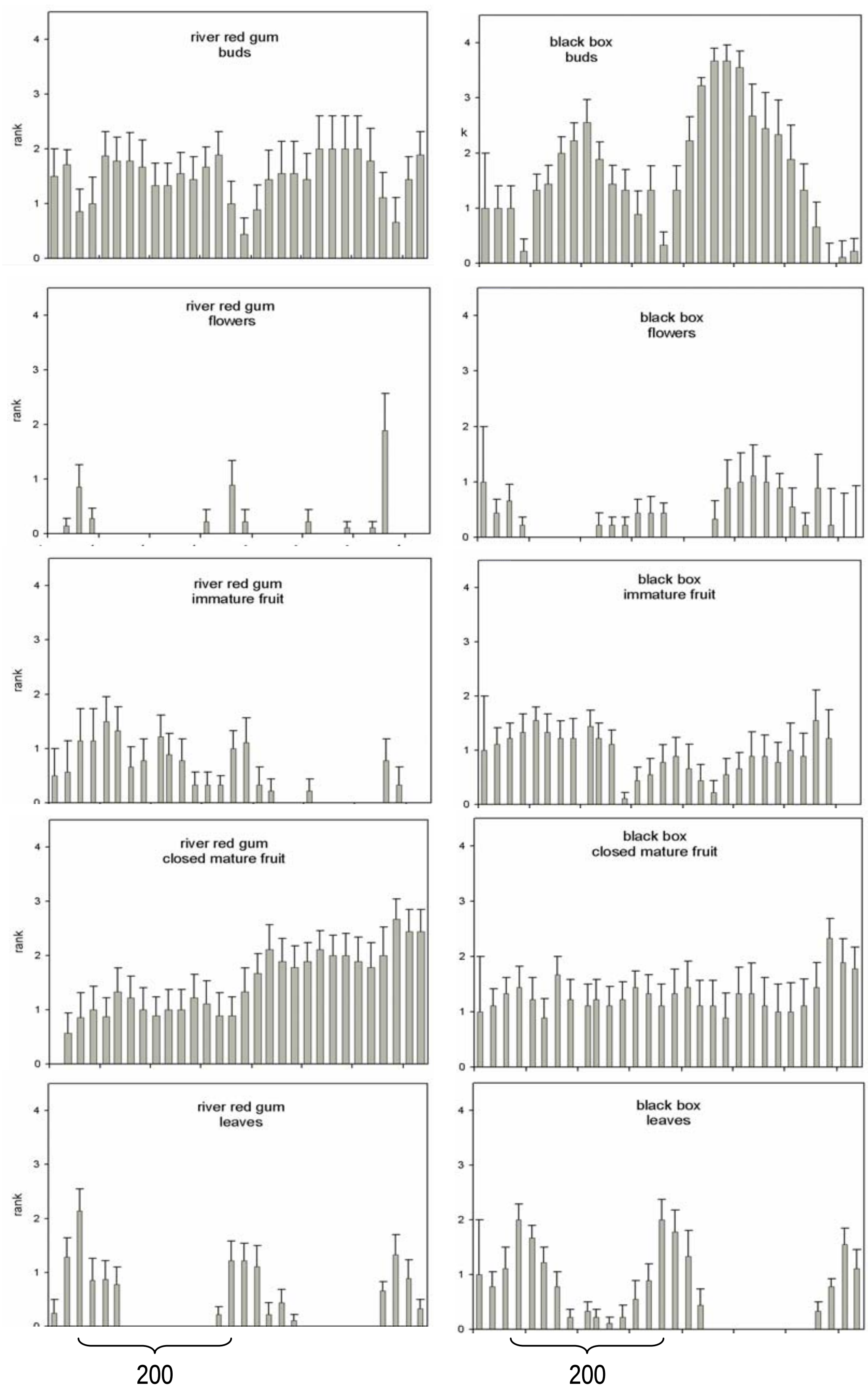

Figure 5.1 Phenology of nine river red gum (left) and nine black box trees (right) during October 2004 to December 2006 (2004, 2006 indicated by shading), showing relative volumes of production (average rank 0-4) for buds, flowers, immature fruit, closed mature fruit and fresh leaves. Data are shown as means with standard error.

Phenological Stage	River red gum	Black box
Bud crops	Surviving mature buds retained for up to 12 months before flowering. Crop reduced from Jun-Nov before flowering. Similar crops in wet and dry years	Surviving mature buds retained for up to 12 months before flowering. Crop reduced in response to drought. Larger bud crop (170%) in 2006 after wet season in 2005. Reduced bud crop likely 2007 after drought
Flowering	Mainly Dec. Most (78%) with biennial cycle; minority with annual cycle; higher production in alternate years Approx. half on opposite cycle	All months except Feb, Mar. Most with annual cycle and similar production each year. Trees peak in summer or winter. Minority with biennial cycle
Leaf production	Mainly Nov-Jan. Lower numbers of new leaves in dry conditions in 2006; some trees (45%) ceased production	Year-round, peaking in Nov-Feb. Numbers of new leaves lower in 2006; most trees (55%) ceased production in dry conditions
Immature fruit	Varied annual cycle: longer in 2004 (Dec-Sep), shorter in 2005 (Dec-Jan). Only 30% of sample producing in Dec 2006, suggests smaller crop in 2007	Annual cycle peaks in Jan-Feb, declining to Sep. Reduced crop in 2006 compared to 2005
Mature fruit	Retained on trees (closed valves) for up to 24 months before shedding seed. Increase in 2006 (173%) thought to be product of high numbers of immature fruit in 2005	Retained on trees (closed valves) for up to 24 months before shedding seed. Similar numbers in 2005, 2006

Table 5.2 Comparative phenological activity of trees at seven sites on the Lower Murray, 2004-2007. (*Note that this information has been expanded to include seed data in Table 6.2*)

Discussion

It appears that in both species the flower crop is determined by conditions occurring 12 months prior to flowering and not by conditions in the current season. A similar time lag was apparent for the mature fruit crop, which peaked in 2006, 12 months after a wet season of 2005. Flowering cycles in individual trees may operate alternately, so that within a stand there are always likely to be some trees flowering in a given year. Trees at Pipeclay Lagoon, Werta Wert Lagoon and Twin Creeks at Chowilla flowered in the same year as those at Morgan (2005), while trees at Banrock Station and Pilby Creek Floodplain (Chowilla) flowered in alternate years (2004, 2006).

River red gum and black box exhibit serotiny, that is, they retain most of their seeds in the canopy and release them as light, continuous seed rain, or they may produce heavier falls after seasonal rain (Lamont & Enright, 2000). Seed release on the Lower Murray peaks in summer for river red gum

(Dec-Feb) and over a longer period for black box (Oct-Mar) (Jensen *et al.*, 2007). Seeds are held in the aerial seed bank for at least two years.

The study was conducted during a period when potential moisture sources were limited, with no effective overbank flows to inundate the floodplain since 1996. In 2004, rainfall was below average (190 mm, 73%); while in 2005 it was above average (301 mm, 116%), and in 2006 it was far below average (119 mm,46%) (*see Figure 3.4*). Rainfall conditions in 2005 matched the conditions found to be a pre-requisite for successful recruitment, when coupled with a medium flood (George 2004). River red gum seedlings emerged in the field in response to rainfall in 2005 (Jensen *et al.*, 2007), and in response to watering in 2006 (Mrs A Jensen, per sobs). There appears to have been a time-lag from rainfall in 2005 until production in 2006 of flowers and mature fruit in river red gum and buds and flowers in black box (Figure 5.1). In 2006, it is suggested that drought caused abortion of immature fruit in river red gum and suppressed leaf growth in black box (Figure 5.1).

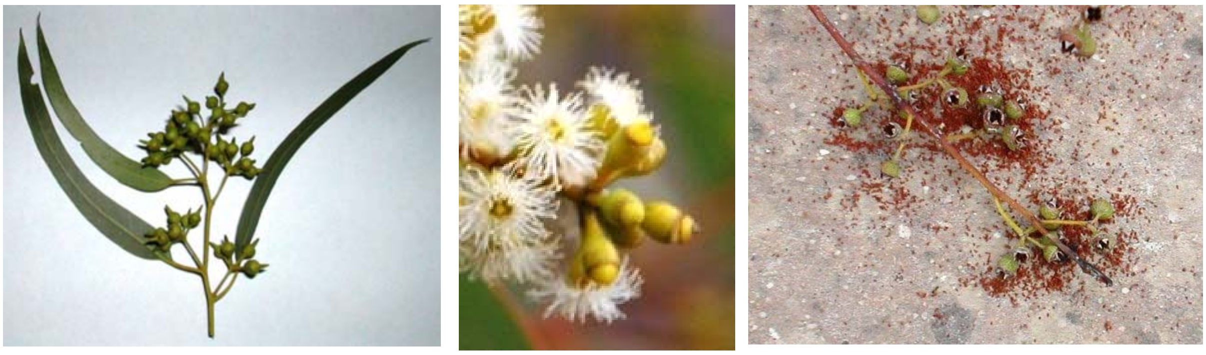

Figure 5.2 Ripe river red gum buds (left), black box buds and flowers (centre), and river red gum seed spilled from open mature fruit (right).

Conclusions

Healthy eucalypts are showing distinctive phenological patterns, with seasonal patterns of bud, flower and fruit production which appear to be adapted to natural (pre-regulation) cycles of rainfall and flood peaks for the Lower Murray floodplain. Flowering peaks around December for river red gum on the Lower Murray floodplain, and appears to be timed to coincide with natural flood peaks in October-December. While black box also showed a strong peak in summer, some trees peaked in autumn-winter, indicating an alternative strategy.

While only a small sample of trees was monitored, they appeared to be representative of other trees at those locations. Approximately 50% of river red gums at each site were on opposite biennial cycles, thus about half of the trees would flower in a given year (December is the peak month). Conversely, most black box trees were on annual cycles, which peaked either in summer or in winter (May-June, or December). These strategies would ensure that some flowers are available each summer in river red gum, and in either summer or winter in black box. The winter flowering in black box would coincide with reasonable chance of rain, and summer flowering in red gum and black box would coincide with the natural pattern of flooding.

The size of the flower crop in eucalypts appeared to be set by soil moisture levels 12 months previously, with the phenological cycle of bud development, maturation, retention, flowering, fruit set and maturation of seed taking two years. In years of increased moisture stress, trees shed buds and immature fruit, resulting in significantly reduced crop volumes, and this was observed during drought conditions in 2006.

This study, although limited in sample size, demonstrated the usefulness of phenological data, which is critical in identifying local patterns of flowering and fruit production, to supplement data on seed rain from seed samples (*see Chapter 6*). It is also easier data for environmental managers to collect and analyse, and provides very useful information in relation to timing of environmental flows to support maintenance of bud and fruit crops on the trees. Further investigation is required on a longer seasonal data set to confirm the annual and biennial cycles suggested here, and to evaluate the effects of drought and variable rainfall conditions on phenological patterns.

Chapter6 Aerial seed banks and seed sources for floodplain eucalypts[9,10]

Introduction

Floodplain plant communities are prone to moisture stress, especially in dry climates, and the frequency and intensity of stress are increased by river flow regulation. The plants generally respond with slower growth, dieback or mortality (Mahoney and Rood 1992, Naiman *et al.* 1995, Shafroth *et al.* 2000, Stromberg *et al.* 2003). They may continue to produce seed under stress, and germination may occur opportunistically in response to local rainfall, but the seedlings are insecure until they have developed 'sinker' roots to free them of dependence on near-surface soil moisture (Cooper *et al.* 1999, George 2004). Maintenance of soil moisture therefore is a prerequisite for conservation and rehabilitation of woodlands, and requires active intervention (Beauchamp 2001) or restoration of natural flows (Stanford *et al.* 1996, Poff *et al.* 1997).

The vegetation of the River Murray floodplain in South Australia is an exemplar. Eucalypt trees (Myrtaceae: river red gum, *Eucalyptus camaldulensis*; black box, *E. largiflorens*) on the Murray are affected by flow regulation, rising saline groundwater and land clearance and grazing, and regeneration is sparse or patchy (Margules and Partners *et al.* 1990, Roberts 2003). In some areas, 50 95% of trees have been pronounced 'dead or dying' from water stress (MDBC 2003), and recruitment clearly is insufficient for population maintenance (MDBC 2003, Roberts 2003, George 2004, George *et al.* 2005b). Water sources for germination and seedling survival have been significantly reduced, with frequency of flood events reduced and more likely to occur during dry climatic conditions (*see Chapter 3*). Artificial watering has promoted survival and leaf production on mature river red gums, and encouraged germination at some sites (Dr T. Wallace, SA Dept Water, Land and Biodiversity Conservation, pers. comm.). Further 'environmental flow' allocations are planned, although watering alone does not guarantee an ecologically-useful response. In particular, the presumption that target species are represented in the soil seed bank is unsupported by empirical data.

Plant communities on the Lower Murray floodplain, South Australia, are degraded by flow regulation, salinisation, land clearance and grazing, and in some areas up to 95% of eucalypt trees are dead or dying from water stress. The soil seed bank included mainly introduced grasses and annual native species, particularly chenopods, with no significant numbers of eucalypt or lignum seeds (*see Chapter 4*), whereas the dominant trees, river red gum and black box retained most of their seeds in a canopy seed bank for up to 2 years (*serotiny*) (*see Chapter 5*).

This chapter continues investigation of the floodplain seed bank and its capacity to respond to floods or managed 'environmental flows', and considers the volume and timing of seed fall from the aerial seed bank to create patterns of 'seed rain', and the influence of alternate moisture sources, tree health, grazing and other factors on seed release.

Materials and Methods

Observations were recorded at eight sites in South Australia, between Morgan and the New South Wales border (*see Figure 3.1*). These were Racecourse Lagoon at Brenda Park, near Morgan, Banrock

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[9] Jensen, A.E., Walker, K.F., & Paton, D.C. (2008b). The role of seed banks in restoration of floodplain woodlands. *River Research & Applications*, 24, 632-649 (2008). *Special Issue: Proceedings of Riverine Hydroecology Conference, Stirling Scotland, 2006.*

[10] Jensen, A.E., Walker, K.F., & Paton, D.C. (2008a). Smart Environmental Watering: getting most benefit from scant flows for floodplain trees (River Murray, South Australia). In *Proceedings of Water Down Under 2008 Conference*, 15-17 April, Adelaide (Eds) Daniell, T., Lambert, M. & Leonard, M., 1426-1437. Engineers Australia, Melbourne, Australia.

Station floodplain, near Barmera, Clarks Floodplain near Berri, and Monoman Island Horseshoe Billabong, Pipeclay Lagoon, Pilby Creek Floodplain, Twin Creeks and Werta Wert Lagoon on the Chowilla floodplain near Renmark (see *Figures 3.1, 3.2, 3.7*). All sites were linked to controlled watering events, and those at Chowilla are part of The Living Murray Program developed by the Murray-Darling Basin Commission (*see Chapter 3 for site descriptions*).

Monitoring from October 2004 to March 2007 covered a dry season (total rainfall 190 mm, 2004), a wet season (301 mm, 2005) and a very dry season (119.4 mm, 2006) (Bureau of Meteorology data for Renmark: average 260 mm) (*see Figure 3.3*). Sheep were present at the Chowilla sites during experimental watering from March 2004 to September 2005.

Thirty one red gums at seven sites were monitored to determine levels of stress and responses to watering and the roles of aerial (canopy) and soil seed banks. Incidental observations were made of four black box trees at Banrock Station and one at Pilby Creek Floodplain, but these were at higher elevations and unlikely to benefit from the watering trials. Tree health (H) was rated on a 7-point scale, adapted from Holland (2002) and used at Chowilla by the SA Department of Water, Land and Biodiversity Conservation (*see Table 4.1*). Healthy trees (H = 4-6) have >50% of their original canopy and <10% epicormic growth, and may have dead branchlets (H = 4). Stressed trees (H = 1 3) have <50% of their canopy and increased foliage loss, dead branches and epicormic growth. Trees sampled presented a varied combination of health (healthy, low, medium and high stress) and watering regimes (watered, water delayed, not watered) (Table 6.1, *see Figure 3.6*).

	Watered	water delayed	not watered	Health (H=5-6)	Low stress (H=4)	Medium stress (H=3)	High stress (H=1-2)	Total
River red gum	22	4	5	13	5	8	5	31
Black box	0	1	4	2	0	1	2	5

Table 6.1 Trees sampled for seed release by water regime category and health status. Very few black box trees grew within the monitored zone around watering sites.

Individual trees varied significantly in horizontally-projected area covered by the canopy, from a minimum of 20 m^2 in a highly stressed black box to a maximum 774 m^2 in an extremely healthy river red gum. The average canopy cover area was 241 m^2 (calculated from the radius of the canopy from the trunk), with a mode of 85 m^2 and median of 174 m^2. Seed traps were set under canopies, close to the outer edge.

Aerial seed bank

Seasonal seed rain was monitored for 36 trees (three traps per tree) at seven sites (thus, 108 traps). At each site, a sample of three trees in similar conditions was selected, plus one ‘reference tree’, likely to be unaffected by watering. Reference trees were either in better condition than the sample group (‘positive’ reference) or in worse condition (‘negative’ reference). Traps were located at the canopy edge, leeward of the prevailing south-westerly wind, to maximise annual seed capture. Examples were observed in the field confirming that seedlings grew predominantly to the north-east of mature trees. The traps were built following a modified design developed by George (2004): each had a 0.2 m^2 circular opening over a 500 mm deep cone of porous 100 µm mesh UV-stabilised Rally Mulch Mat™ to catch seeds >200 µm. Traps were weighted and suspended 1 m above the ground on a ring of plastic pipe, supported by three 1200 mm wooden stakes. On each sampling occasion, the trap contents were transferred to paper bags, stored at 4ºC and later sieved (2, 1 mm; 500, 200 µm mesh). Items >1 mm were counted, including leaves, fruit, buds, and opercula (flower caps). Material <1 mm was again sieved to separate >500 µm and >200 µm fractions of seeds, chaff and micro-debris. Fractions were weighed and stored in glass tubes at 4ºC for subsequent counting of viable seeds.

All samples were fully counted, as sub-sampling was too variable, and weight was not correlated to viable seed due to fine debris contamination of samples. Seeds were counted using germination or flotation, depending on quantity. In the former method (Gunn, 2001), high-density samples were divided into 2 mg portions of sifted material to enable rapid counting. These were transferred to 90 mm Petri dishes and spread on Whatman No. 1 filter paper over 30 mg vermiculite saturated with 30 mL distilled water. Samples were kept for five days at 32ºC from 0800–1800 h and 15°C from 1800-0600 h, when a first count of germinants was made (cf. Grose & Zimmer, 1958). The seedlings remained attached to their seed coats for 5 days, red gum seedlings having golden coats and black box having larger, striated, dark brown coats. Once the coats were shed there was no perceptible difference between seedlings over the next 14 days (the duration of counts). Subsequent germinants were counted, and viable seeds that failed to germinate also were counted. The squash test for viability (Gunn, 2001) was unsuccessful, but fully-imbibed seeds would produce white endospermic material when squeezed with forceps, and some had partly-developed cotyledons, confirming that they were viable. Flotation was effective for low-density samples. Red gum seeds were soaked for 24 h in 50 mL distilled water; fully-imbibed seeds then would float, leaving debris and chaff. For black box, counting was completed within 4 h, before the seeds began to sink.

Sieved materials >1 mm from seed trap samples were counted to supplement seed counts and compared with field observations of phenological status of trees which had been analysed earlier (*see Chapter 5*). The parameters measured included leaves shed, open mature fruits (indicating seeds shed), immature fruits (fruiting aborted), mature buds (bud crop reduced), immature buds (bud crop aborted), opercula (flowering), immature opercula (flowering aborted), and stamens (flowering completed).

Viability, germination and survival

Seed viability and germination rates were assessed in floodplain soil samples or in pots with seeds added and kept in a glasshouse. Additional samples were germinated under constant 10-20% soil moisture to determine maximum viability and germination rates. The number of seeds per fruit was tested from opportunistic field samples of mature un-opened fruit which were grown out in laboratory conditions. In October 2005, river red gum seedlings on the margins of Monoman Billabong were staked for monitoring, but most were drowned by a water-level rise in January 2006. Thirty seedlings at higher elevations were staked, and height, survival and condition were assessed monthly from March 2006. At Clarks Floodplain, 15 seedlings were monitored from June 2006 until drowned by a subsequent watering event in October 2006.

In the laboratory, a sample of 6 large river red gum seeds were spread on 90 mm Whatman No. 1 filter paper over 30 mg vermiculite saturated with 30 mL distilled water, in a covered Petri dish. The sample was placed in a covered box at normal room temperature, with permanent lighting. A time-lapse film was taken of the germination of red gum seeds to show the process of root and shoot emergence, and loss of the seed coat. Photographs were taken every 10 minutes over 8 days using the AMcap video capture and preview application by Noël Danjou, modified from Microsoft freeware (http://www.noeld.com/programs.asp?cat=video). The series of images was merged into a video film of about 10 seconds by Scott Mills, a postgraduate at the University of Adelaide, using ImageJ, image processing software to combine Java images into AV1 format (http://rsb.info.nih.gov/ij/).

Strandlines

Seedlings often are associated with high-water strandlines formed by twigs, leaves and other organic litter. The seeds float readily (‘hydrochory’: cf. Pettit & Froend 2001) and accumulate among debris that acts as mulch, retaining moisture and nutrients. Accordingly, strandline seedlings were monitored with regard for soil moisture and the effects of grazing. At Chowilla, five strandlines were monitored (two from July 2004, three after subsequent water rises). At Werta Wert Lagoon, an artificial strandline was created by sowing red gum seeds mixed with sand in 20 pots (0.01 m^2 × 60 mm deep; 15-20 seeds per pot). Ten pots were buried to surface level on site, adjacent to two monitored natural strandlines, and for comparison 10 duplicate pots were kept in a glasshouse, consistently moist and safe from grazers. At Pilby Creek Floodplain, a vigorous strandline formed after a second watering in October 2005, coinciding with the removal of grazing sheep.

Ordination Analyses

Data collected from the seed traps were analysed to detect phenological patterns in bud crops, flowering and leaf drop, and any associations with abiotic factors such as rainfall and temperature. Phenological material retrieved from the seed traps (leaves, fruit, buds, etc) was examined by ordination analysis (non-metric multi-dimensional scaling) with PC-ORD (v. 4.41, MJM Software, Oregon, USA), using the Relative Euclidean distance metric and all default options. Seed data from seedling counts were analysed by Non-metric Multi-dimensional Scaling, using the Relative Sorensen distance metric and all default options (PC-ORD, v. 5.0: MJM Software, Oregon, USA)

Results

Seed Release from River Red Gums

Seed rain volumes and timing were highly variable for river red gums within and between sites, with significant reductions in seed volumes and less defined seasonal patterns in stressed trees (Figure 6.1, 6.2). There was evidence indicating a summer seasonal release pattern for healthy river red gum and black box trees. The results are presented across 30 months, from October 2004 to March 2007, thus three summer seasons.

Healthy river red gums were monitored at three sites: Clarks Floodplain, Pipeclay Lagoon and Banrock Station. At Clarks Floodplain, a sample of three healthy trees (H = 4-5) outside the watered zone produced an individual peak of 47,500 seeds m^{-2} in March 2005 (late summer), with minor production in October 2005 (235 seeds m^{-2}) and extremely low seed rain in summer 2006 (<70 seeds m^{-2}) (*see Table 3.3 for health scale*).

At Pipeclay Lagoon, Chowilla, three healthy trees (H = 5-6) on the lagoon edge were monitored, with a positive reference tree (H = 5) 50 m distant from the watered zone. The lagoon, with a healthy fringing woodland and red gum saplings on its bed, was filled September-January in 2004, 2005 and 2006. The responses of trees on the lagoon edge were much less than the reference tree, which had the highest monthly individual seed rain for the study period at 17,562 seeds m^{-2} in December 2004 but a much lower volume of 2,755 seeds m^{-2} in December 2006. The trees at the water’s edge shed an average of 1068 seeds m^{-2} in December 2004 and 4558 seeds m^{-2} in December 2006.

Monitoring at Banrock Station floodplain included a sample of three healthy red gums (H = 5) in the watered zone and one healthy reference tree (H = 6) at a higher elevation more than 30 m distant from the highest water level. The sample group received indirect watering from an adjacent creek replenishing water tables in spring 2004, and direct surface watering in spring 2005. The seed rain data were dominated by Tree 3 in the watered zone, which had individual peaks in February 2005 (9455 seeds m^{-2}, cf. average 3578 seeds m^{-2}), August 2005 (6572 seeds m^{-2}, cf. 2236) and September 2006 (9,260 seeds m^{-2}, cf. 3126). The other sample trees and the reference tree also peaked in February

2005 (<2300 seeds m^{-2}), but the reference tree had no significant seed rain for the remainder of the study.

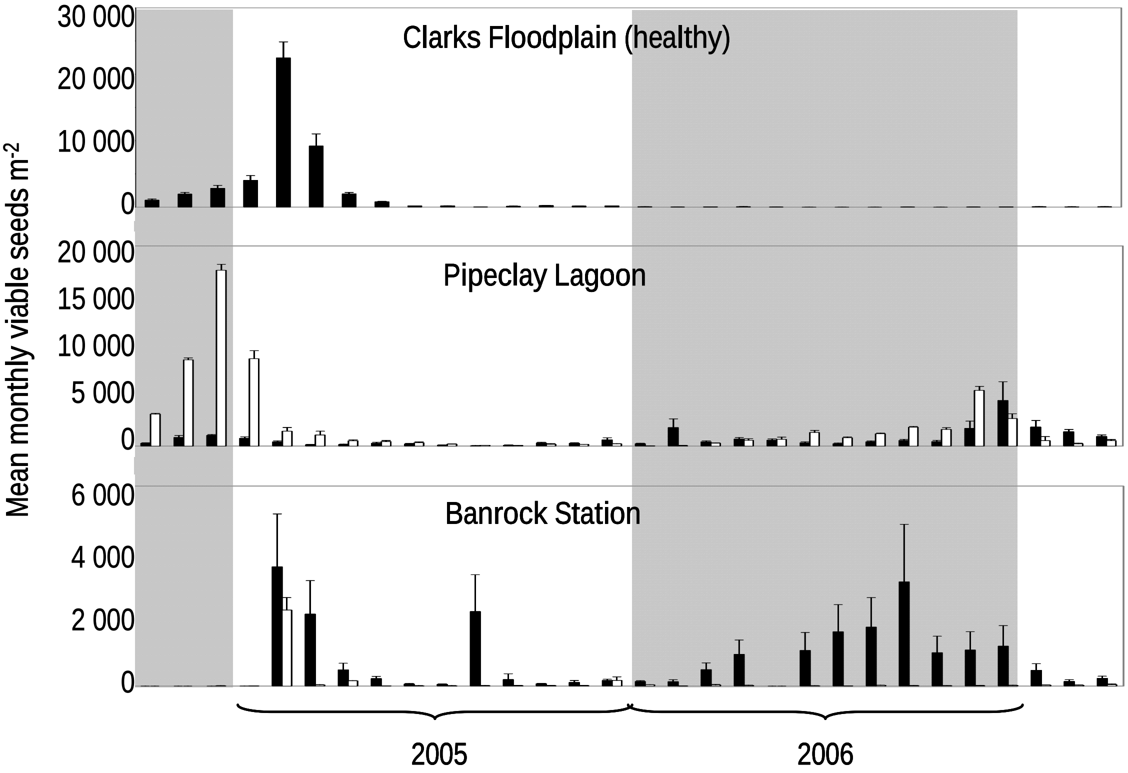

Figure 6.1 Mean (standard error) monthly measurements from October 2004 to March 2007 of viable seed in seed rain for sample river red gum groups (black bars) and reference trees (white bars) at healthy sites. Note that seed rain tends to peak in summer months of alternate years, but a single tree at Banrock Station also shed copious seed In August 2005 andhealthy trees at Clarks Floodplain had negligible seed rain in summer 2006. Sites are shown in descending order of seed rain volumes (note that vertical scales are not equivalent, owing to the wide divergence in values).

Stressed river red gums were monitored at four sites on Chowilla Floodplain, and at Clarks Floodplain. At Monoman Island Horseshoe Billabong, Chowilla, a sample group of three stressed trees (H = 1-3) was compared with a positive reference tree (H = 4) 50 m outside the watering zone. This was the first site watered, commencing in March 2004.The site was watered again from December 2005, with water persisting in the billabong for approximately 6 months due to the low transmissivity of the clay bed. All trees at this site responded off-cycle, with seed rain occurring April-September 2005, two years after the initial watering. The most stressed trees (H = 1-2) showed only a minor seed rain response, with a pattern similar to the reference tree. Tree 3 (H= 3) responded most positively, with peak seed rain of 2657 seeds m^{-2} in April 2006.

At Twin Creeks, Chowilla, three stressed trees (H = 3) on the creek bank were monitored, with a negative reference tree (H = 1) 50 m distant from the watered zone. This site was watered Nov-Dec 2004, and again in May-Jul 2005 and Apr-Jun 2006. Water was not retained, as the site has sandy, highly transmissive soils. The growth response of the trees was much faster than at clay sites, with green leaves replacing stressed brown leaves within two weeks of the first watering. The timing of the second and third watering events was constrained by pumping costs, so watering did not coincide with natural flooding patterns. The reference tree showed a delayed growth response after six weeks, and its health improved (H = 2). Two of the creek-side trees shed seeds (3350-4220 seeds m^{-2}) in November 2006, but had very low volumes of seed rain compared to healthy trees at other sites.

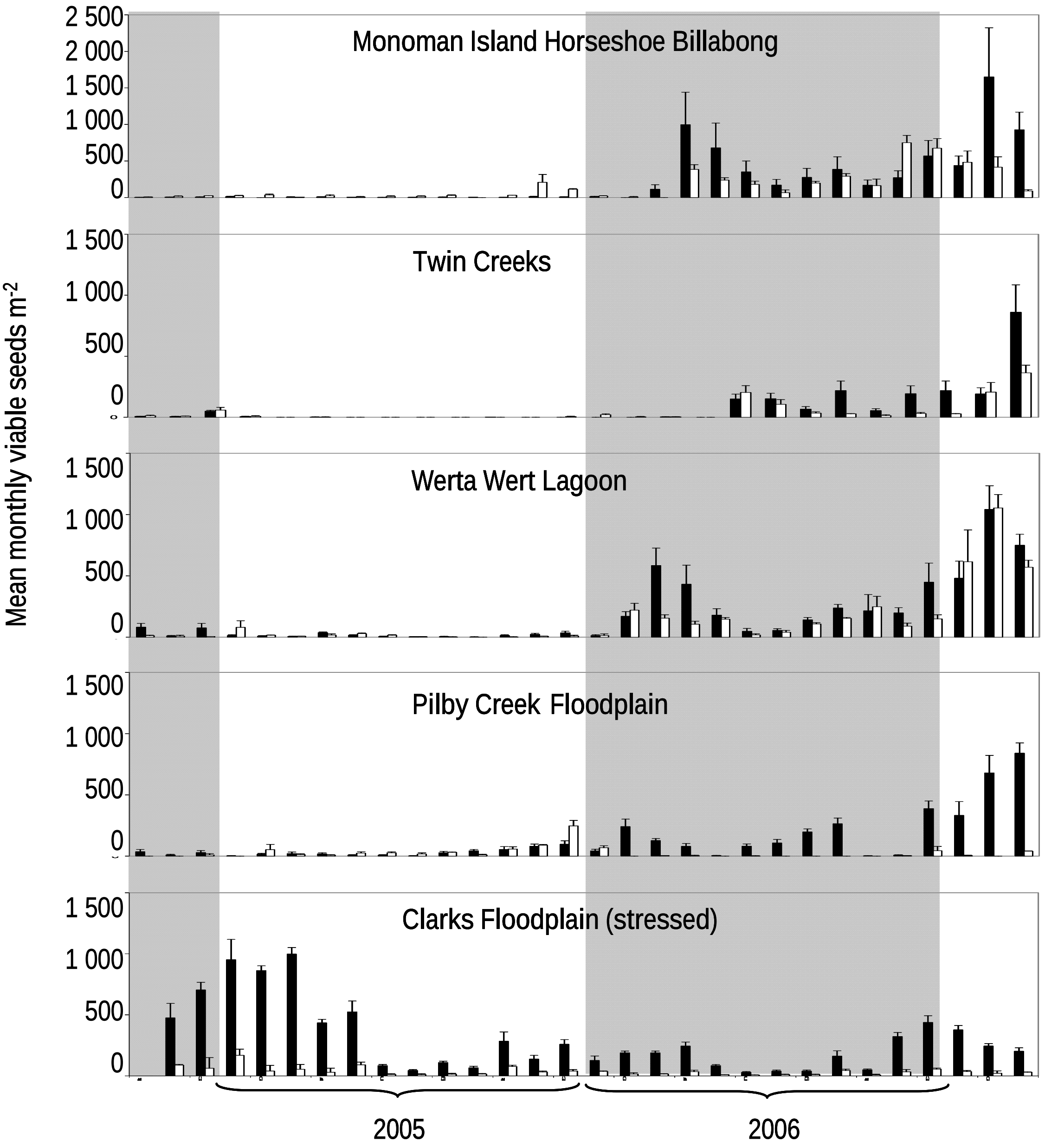

Figure 6.2 Mean (standard error) monthly measurements from October 2004 to March 2007 of viable seed in seed rain for sample river red gum groups (black bars) and reference trees (white bars) at stressed sites. Note that seed rain is reduced 20 times in stressed trees at Clarks Floodplain, compared to healthy trees. Sites are shown in descending order of seed rain volumes (note that vertical scales are not equivalent, owing to the wide divergence in values).

At Werta Wert Lagoon on Chowilla floodplain, a group of three healthy trees (H = 4-5) and a relatively healthy reference tree (H = 4) were monitored. They were all located in the narrow band of river red gums fringing this deflation basin lagoon, with the lunette forming a sloping bank which confines the species to a narrow elevation of suitable flooding regimes. Thus the reference tree was only marginally further from the wetland edge, and showed a delayed but similar growth response to watering. The lagoon was filled in September 2004 and again in December 2005. The volume of seed shed from these healthy trees was significantly less than at other sites, with an average peak of 585 seeds m^{-2} in April 2006 and a higher peak of 1042 seeds m^{-2} in February 2007.

At Pilby Creek Floodplain on Chowilla floodplain, three sample trees on the creek bank were monitored, with a negative reference tree 50 m distant from the watered zone. Pilby Creek was filled in Sept-Dec 2004, Sept-Dec 2005 and Sept 2006-Jan 2007. The reference tree was also inundated four weeks after the creek-side trees, from overflows further downstream. The creek-side trees ranged from medium stress (H = 3) to healthy (H = 4-5), while the reference tree began as very stressed (H = 1) and improved to medium stress (H = 3). The creek-side trees had limited seed rain, with individuals starting to respond in February 2006 (average 241 seeds m^{-2}) and a stronger response in late summer (December-February 2006-07) (average 840 seeds m^{-2}). The stressed reference tree responded earlier to watering, with 247 seeds m^{-2} in December 2005 but very few seeds for the remainder of the study period. The volumes of seed rain for all trees remained very low, compared to healthy trees.

At Clarks Floodplain, a sample group of three stressed trees (H = 3) was 500 m from the healthy group of trees, and the negative reference tree was even more stressed (H = 1). These trees shed nearly 20 times less seeds (<1000 seeds m^{-2}) in a more variable extended pattern compared to seed rain from the nearby healthy trees. Watering at this site occurred in July-September 2005 and October-December 2006. An unexpected result was the negligible seed rain shed by healthy trees in summer 2006, when a biennial peak was predicted, based on the phenological pattern observed (*see Chapter 5*).

Seed Release from Black Box

Limited data were obtained for seed release from black box, with data collected from the stressed sample trees at Banrock Station with a healthy reference tree, and a single healthy black box on the fringe of the watered zone at Pilby Creek floodplain (Figures 6.3, 6.4). However, the decline in seed release from stressed trees was highly significant, and seasonal patterns of summer seed release with varying annual volumes were confirmed in the healthy trees. At Banrock Station, stressed black box trees (H = 2-3) had maximum releases of only 5-60 seeds m^{-2} per month.

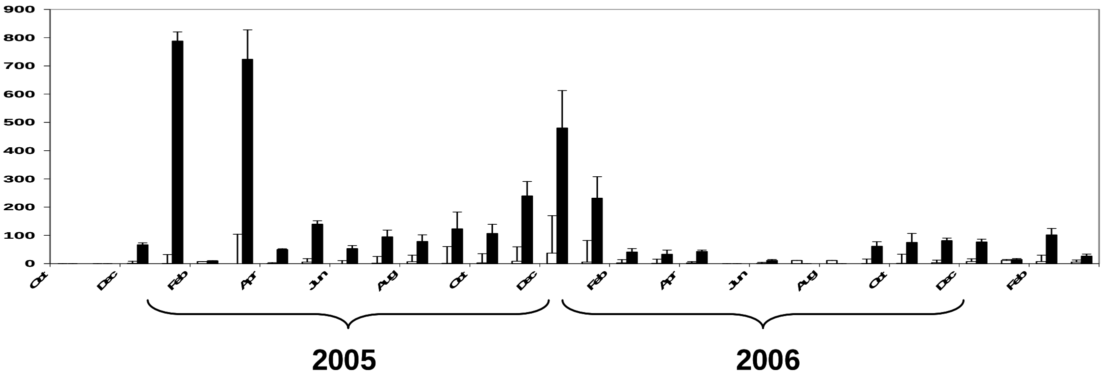

Figure 6.3 Mean (standard error) monthly measurements of viable seed in seed rain (0-900 seeds m^{-2} Y-axis) for stressed black box trees (white bars) and healthy reference tree (black bars) at Banrock Station. Note that seed rain is reduced 10-20 times in stressed trees, compared to the healthy reference tree.

Healthy black box trees (H = 5) released a maximum of 788 and 2670 m^{-2} seeds per month at Banrock Station in summer (January-March 2005) and Pilby Creek Floodplain in spring-summer (October-January 2004-05), respectively. Lower volumes of seed rain were noted in summer 2006, thought to be due to drought conditions.

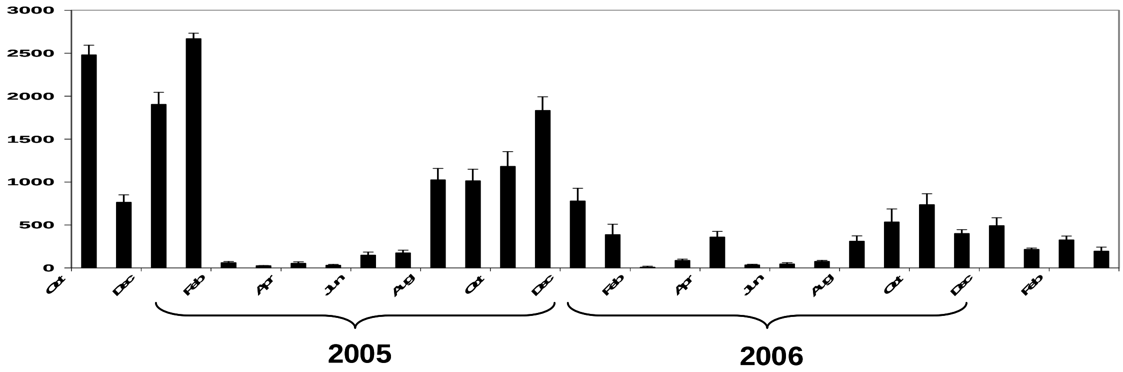

Figure 6.4 Mean (standard error) monthly measurements of viable seed in seed rain (0-3000 seeds m^{-2} Y-axis) for a healthy black box tree at Pilby Creek Floodplain. Note that seed rain peaks annually in summer months for this tree.

Effect of Stress on Seed Release

Stressed trees released less seed. Initial results from the first summer were analysed and indicated that healthy river red gums (H = 5-6) released 605-17,562 seeds m^{-2} (trees = 12, *n* = 58), generally in summer (November-March). By comparison, trees with medium stress (H = 3) released 25-4305 m^{-2} (trees = 9, *n* = 51), and those with high stress (H = 1-2) released 5-915 seeds m^{-2} (trees = 5, *n* = 29). At Clarks Floodplain, stressed trees (H = 2-3) released up to 9 times less seed than healthy trees (H = 4-5) at the same site. However, trees with low stress (H = 4) also had very low maximum monthly seed rain in the range 85-240 m^{-2} (trees = 5, *n* = 30), suggesting that this was a seasonal response rather than stress-related.

Across the full monitoring period of 30 months, it became apparent that this reduction of seed rain volume was very severe, with most stressed trees producing average peak seed volumes <1000 seeds m^{-2} throughout the study. A direct comparison of the stressed and healthy trees at Clarks Floodplain shows the major reduction of seed numbers, and the changed pattern of seed rain with higher variability. Individual healthy river red gums had peak seed rain in summer 2004, no significant seed rain in 2005 (expected pattern), but no significant summer seed rain occurred in the drought year of 2006 (<1,000 seeds m^{-2}), when the next biennial peak in seed rain would be expected in suitable conditions (Figure 6.5). Individual stressed river red gums at the same site (shown at the same scale as the healthy trees 500 m away) had 10 times lower volumes of seed rain in late summer 2004 (Figure 6.6). Stressed trees showed a much more varied pattern of low level seed rain. Individual stressed river red gums had small varied amounts of seed rain in summer 2005 (following local watering), with another peak of summer seed rain in 2006 after a second watering event (Figure 6.7).

Seed rain at stressed sites responding to watering occurred over a more extended seasonal period and was annual rather than biennial. The natural pattern is suggested by the Clarks Floodplain healthy trees, with an average peak of approximately 22,000 seeds m^{-2} in summer 2004-05, followed with only minor production in October 2005 (235 seeds m^{-2}). However, the expected peak of seed rain in summer 2006-07 did not occur, indicating that a smaller crop of seeds occurred in healthy trees, probably due to drought conditions.

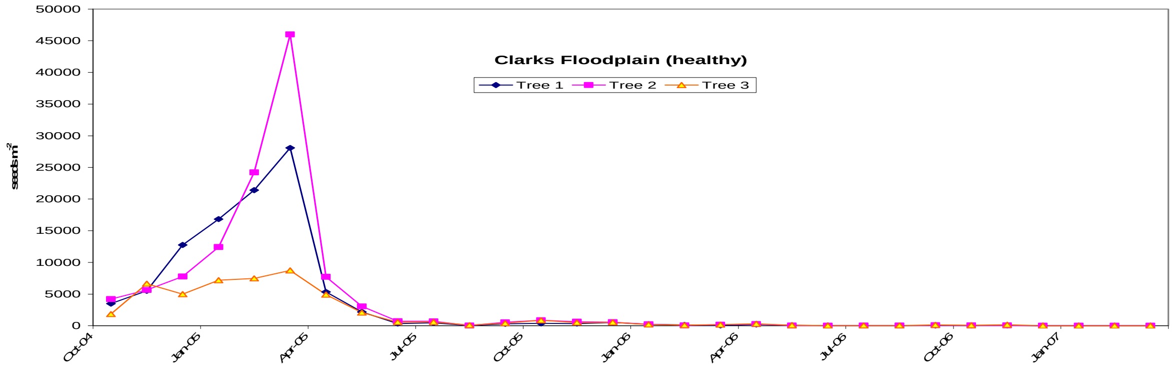

Figure 6.5 Monthly seed rain measured at healthy individual trees at Clarks Floodplain from Oct 2004 to March 2007, showing no significant seed rain in summer 2006.

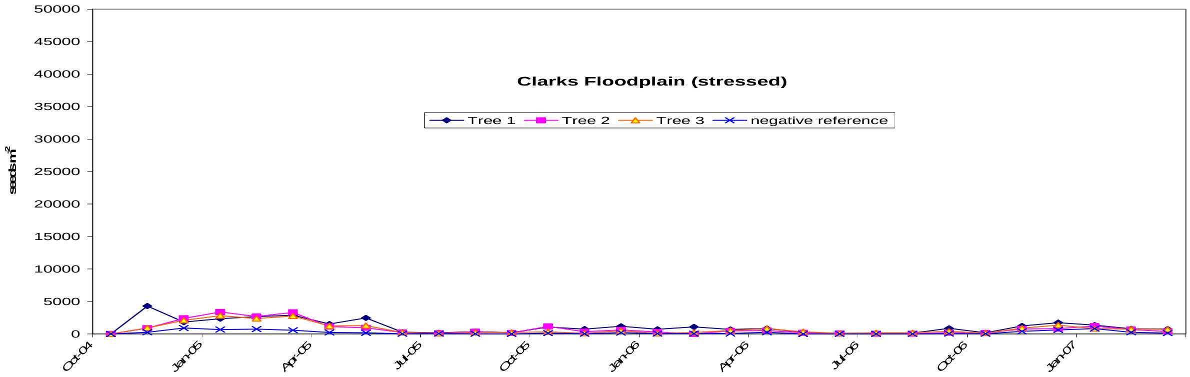

Figure 6.6 Monthly seed rain measured at stressed individual trees at Clarks Floodplain from Oct 2004 to March 2007, showing significantly reduced seed rain compared to healthy trees (Figure 6.5).

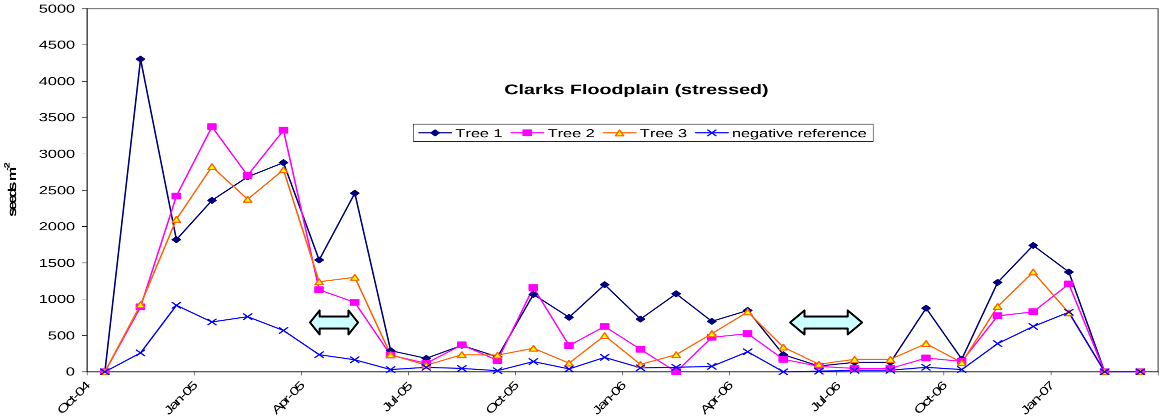

Figure 6.7 Monthly seed rain measured at stressed individual trees at Clarks Floodplain from Oct 2004 to March 2007, drawn at a larger scale to show highly variable and extended seed rain pattern, compared to healthy trees (Figure 6.5)

Phenological patterns

In the ordination analysis of the phenological data, the primary (biological) matrix included leaves, fruit (open valves), buds, opercula (indicating flowering), and immature fruit (closed valves) and opercula and buds (indicating aborted flower, bud and fruit production). The secondary (environmental) matrix included temperature, rainfall and distance to surface water. The ordination (Figure 6.8) yielded a 3D solution with stress 12% (r^2 = 0.87) (Axis 1, 23.5% of total variance; Axis 2, 23.7%; Axis 3, 40.7%). The separation of samples was most influenced by immature opercula (indicating aborted buds), mature dehisced fruit and leaf drop. When data were grouped in 'tri-mesters' (thus: spring, Aug-Nov; summer, Dec-Mar; autumn-winter, Apr-Jul), key factors distinguishing samples were immature fruit (seed not developed) and mature opercula (indicating flowering), mature dehisced fruit and immature opercula (aborted buds) (Figure 6.9). Strong associations are evident between bud formation, flowering (opercula shed) and fruit shed (after seed rain) in the spring trimester, coinciding with peak rainfall.

Table 6.2 compares the phenology of sample trees over three summer seasons in 2004-2007, updating an earlier summary (*see Chapter 5*, Jensen *et al.*, 2007) and incorporating data on seedlings grown from monthly samples of the soil seed bank (*see Chapter 4*). Summer (Dec-Feb) is more critical for production in red gum, which has a short flowering season, while black box trees have more protracted stages, but also peak seed rain in summer. Both species retain bud crops (before flowering) and mature closed fruit (before shedding seed) in their canopies for extended periods, although some of the crop may be aborted in response to poor conditions post-bud initiation and pre-flowering (*see Chapter 5*, Jensen *et al.*, 2007).

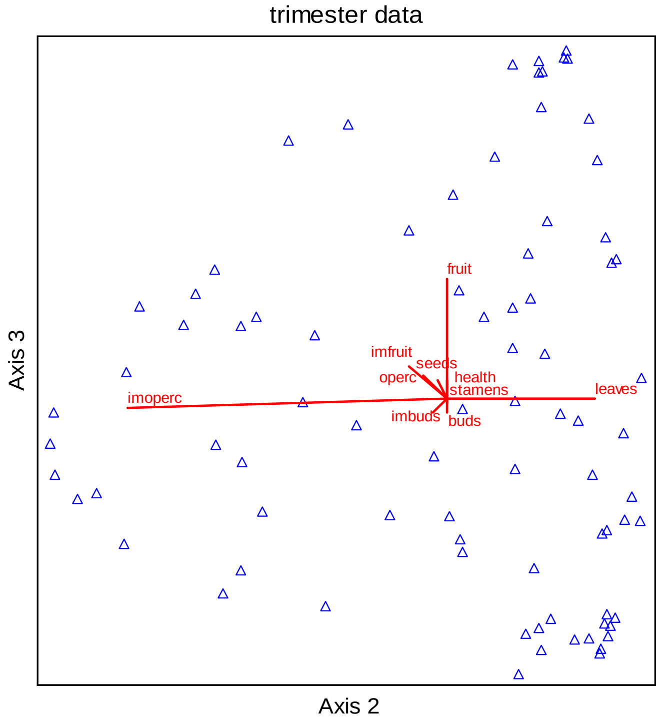

Figure 6.8 Ordination showing that immature operculae (aborted flowering), immature fruit and mature fruit are the key factors discriminating between samples (r2= 0.87 Axis 1 = 23.5%, Axis 2 = 23.7%, Axis 3 = 40.7%).

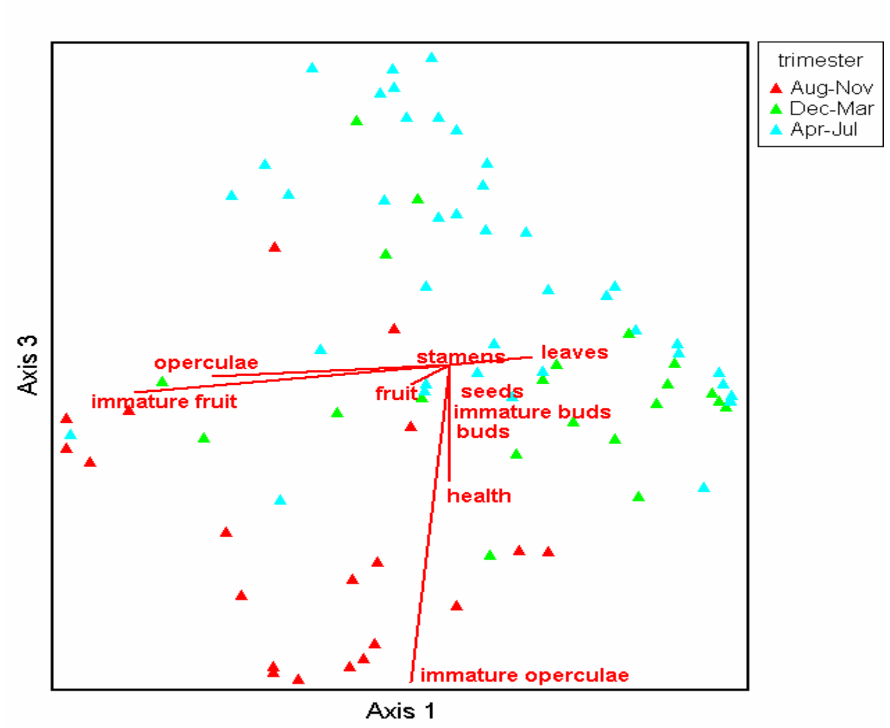

Figure 6.9 Ordination data partitioned as spring, summer and autumn-winter trimesters. Data for the summer and autumn-winter trimesters are negatively correlated with most variables, as most reproductive activity occurs in spring (r^2=0.85, Axis 1 = 28.3%, Axis 2 = 25.4%, Axis 3 = 31.6%).

Plant material in seed traps for the Pipeclay Lagoon trees from the initial summer period suggested that flowering and seed set peaked in summer (Figure 6.10). Phenological patterns for Pipeclay Lagoon sample trees (T1-T3 along riparian zone of wetland, T ref 50 m from water body at slightly higher elevation) show clear seasonal patterns, although volumes vary significantly among trees. Opercula (flower caps) dropped in December, with stamens in January indicating flowering. Leaf drop also peaks in summer, with a sharp reduction in March at the beginning of autumn. Under-developed buds (also under-developed opercula) are shed in February, and well-developed buds are shed in March and April as the trees reduce their crop. Mature (dehisced) fruit are shed in January, following maximum seed rain in December. Evidently, these red gums were adjusting during autumn the quantity of flowers they could display in the following summer. At Clarks Floodplain, fall of dehisced fruit also followed one month after seed release but this pattern peaked in March-April, three months later than the trees at Pipeclay Lagoon.

Stage of cycle	River red gum	Black box
Bud crops	Surviving mature buds retained for up to 12 months before flowering. Crop reduced from Jun-Nov before flowering. Similar crops in wet and dry years	Surviving mature buds retained for up to 12 months before flowering. Crop reduced in response to drought. Larger bud crop (170%) in 2006 after wet season in 2005. Reduced bud crop likely 2007 after drought
Flowering	Mainly Dec. Most (78%) with biennial cycle; minority with annual cycle; higher production in alternate years Approx. half on opposite cycle	All months except Feb, Mar. Most with annual cycle and similar production each year. Trees peak in summer or winter. Minority with biennial cycle
Leaf production	Mainly Nov-Jan. Lower numbers of new leaves in dry conditions in 2006; some trees (45%) ceased production	Year-round, peaking in Nov-Feb. Numbers of new leaves lower in 2006; most trees (55%) ceased production in dry conditions
Immature fruit	Varied annual cycle: longer in 2004 (Dec-Sep), shorter in 2005 (Dec-Jan). Only 30% of sample producing in Dec 2006, suggests smaller crop in 2007	Annual cycle peaks in Jan-Feb, declining to Sep. Reduced crop in 2006 compared to 2005
Mature fruit	Retained on trees (closed valves) for up to 24 months before shedding seed. Increase in 2006 (173%) thought to be product of high numbers of immature fruit in 2005	Retained on trees (closed valves) for up to 24 months before shedding seed. Similar numbers in 2005, 2006
Seed rain	Peak release Dec-Feb. Max. monthly peak 17,562 seeds m^{-2}. Seed numbers 2006 less than 2004	Peak release Oct-Mar. Max. monthly peak 7,445 seeds m^{-2}. Seed numbers 2006 less than 2004
Germination from soil seed bank	Average germination of red gum from all soil samples 4.4 m^{-2}. Germination for Pipeclay Lagoon 25.09 m^{-2} (healthy), for Clarks Floodplain 1.18 m^{-2} (stressed)	No seedlings found in field or germinated from soil samples

Table 6.2 Summary of phenology patterns in river red gum and black box trees at six sites on the Lower Murray Floodplain, 2004-2007. (*Note that phenology data is included from Table 5.2 and seedling data from Table 4.3*).

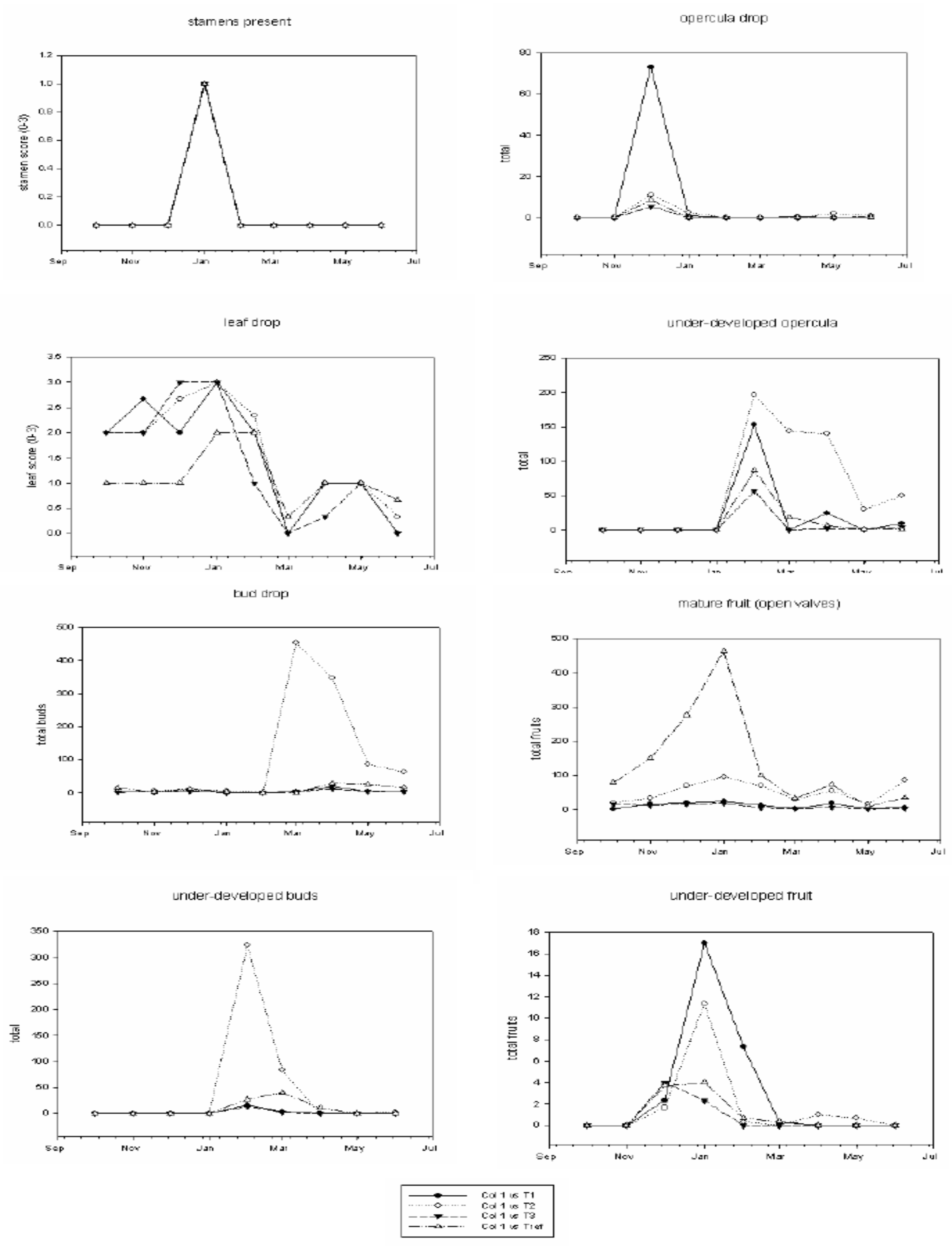

Figure 6.10 Phenological patterns for material collected in seed traps from Pipeclay Lagoon sample trees (T1-T3 along riparian zone of wetland, T ref 50 m from water body at slightly higher elevation) showing clear seasonal patterns.

In the ordination analysis of the seed trap data, the primary (biological) matrix included counts of leaves, fruit (open valves), mature buds, and opercula (indicating flowering), and immature fruit (closed valves), opercula and buds (indicating aborted flowers, buds and fruits) (Figures 6.11, 6.12). The secondary (environmental) matrix included temperature, rainfall and distance to surface water. Data for Banrock Station (Figure 6.11) yielded a 3D ordination with stress 16% (r^2 = 0.860) (Axis 1, 29% of total variance; Axis 2, 36%; Axis 3, 21%). The separation of samples was most influenced by mature dehisced fruit, mature buds and leaves, all associated with healthier trees. An earlier analysis of part of this data set suggested that immature opercula rather than mature buds were influential, but the influence of mature fruit and leaves was consistent (Jensen *et al.*, 2007a). At Clarks Floodplain, a 3D solution with stress 13% (r^2 = 0.850) (Axis 1, 28% of total variance; Axis 2, 38%; Axis 3, 19%) attributed 71% of the separation of samples to rain in the previous 21-25 days in summer, and in the previous 6-10 days in cooler months (Figure 6.12).

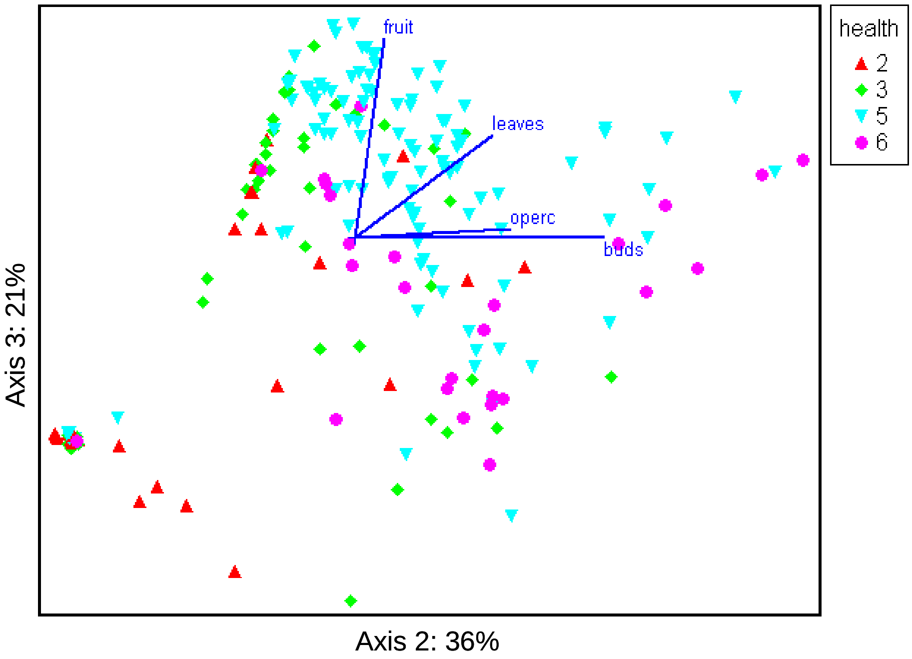

Figure 6.11 Ordination of Banrock Station sieved materials shows that numbers of mature open fruit (seed already shed), leaves and mature buds explain most of the distribution of samples, in association with tree health (health scale: H=6 is excellent, H=2 is poor).

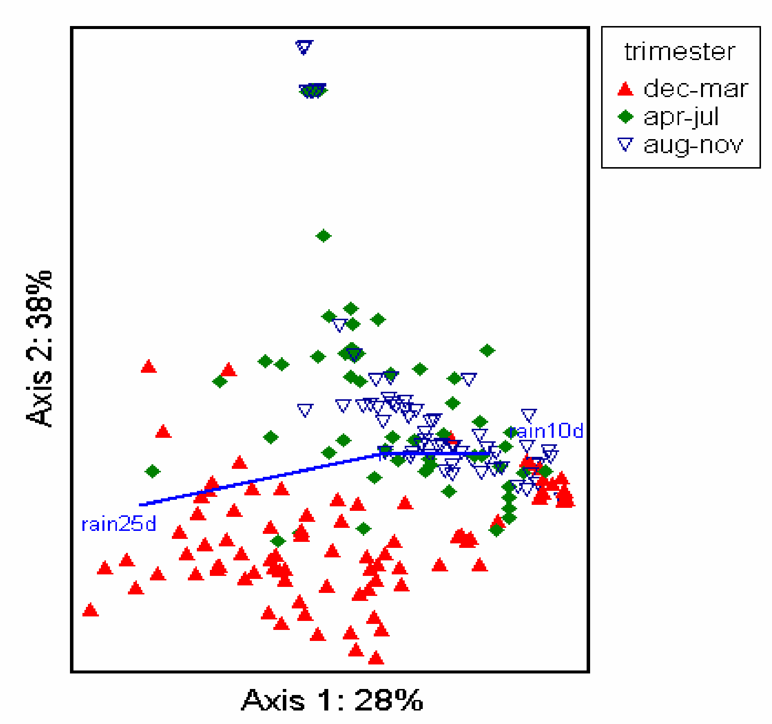

Figure 6.12 At Clarks Floodplain, samples separate according to seasonal trimesters, with rain 21-25 days prior to sampling most influential in the hotter summer months, and rain 6-10 days prior to sampling more influential in cooler autumn-spring months.

New Germination

Of 30 rain-germinated red gum seedlings on the outer bank of Monoman Billabong, 87% remained after 11 months. Of these survivors, 50% had their tips grazed, and 30% had varying degrees of insect attack. Heights were 100-1304 mm, with grazed seedlings tending to develop multiple shoots and less height. At Pipeclay Lagoon, a total of 103 seedlings was found in February 2005, as the wetland dried, but only 19 (18%) survived until November 2005, when they were drowned by a second watering. Only one new seedling was found after the second watering receded in February 2006, although mature fruit with open valves were found at the monitoring site (up to 750 fruit m^2), and nearby traps (T1-3) contained abundant seed. Existing seedlings and saplings grew strongly; the tallest increased after two watering events from 0.5-1 m in July 2004 to 3-4 m by June 2006. Five new seedlings (50-100 mm) were recorded on the wetland margins (10 × 2 m; 0.25 seedlings m^{-2}), in the same habitat where seedlings had germinated prior to the watering trials. Post-watering monitoring at the stressed Clarks Floodplain site indicated 57 new seedlings over 450 m^2 (0.13 m^{-2}).

Germination rates

Germination rates of river red gum seeds from seed traps in October 2004–April 2005 were 55-3,856 seedlings g^{-1} (including chaff and debris), with an average 869.3 seedlings g^{-1} in the peak months of January and February 2005. Seed production per red gum fruit was highly variable. Five samples of 25 mature, unopened fruit from healthy trees were taken as available, from Pipeclay Lagoon in November 2005, Banrock Station in March 2005, Brenda Park in February 2006, Clarks Floodplain in June 2006 and Werta Wert in November 2006. These samples yielded 0-57 seedlings per fruit, with an average of 12.03 seedlings per fruit (*n* = 125). The mode was 3 and the median was 9 seedlings per fruit, with an average of 300 seedlings from 25 fruit from five sites.

Two distinct size classes of river red gum seeds were identified in sieving material from seed traps, viz seeds >500 µm and seeds >200 µm. In germination counts in the laboratory, the smaller seeds constituted approximately 15-20% of seedlings germinated, but these smaller, less vigorous seedlings germinated more slowly than seedlings from the larger seeds. In glasshouse trials for soil moisture conditions (*see Chapter 8*), only one seedling grew from smaller red gum seeds, out of 20 pots with sufficient seed and moisture for at least 30 seedlings to germinate in each pot. All six of the large river red gum seeds monitored in the time-lapse film germinated immediately. Within 8 days, these seedlings grew roots (5-10 mm long) grew a pair of kidney-shaped cotyledons on stalks 5-10 mm high. The translucent golden seed coat was shed in 5- 8 days.

Seed volumes from black box seeds were 0-4 seeds per fruit, with an average of 1.48 seeds per fruit. The mode was 2 and the median was 2 seeds per fruit, with a total of 37 seeds from 25 fruit, sampled from Pilby Creek Floodplain in November 2006. This limited data indicates that black box trees, with much smaller fruit and similar-sized seeds to river red gum, have a maximum of 4 seeds per fruit due to size limits, compared to an average 12 seeds per fruit in red gum, with a maximum of 57 seeds in one fruit. However, seed trap material indicated that black box trees have significantly more individual fruit than river red gum, so that seed rain volumes increase with the volume of fruit.

Soil moisture and characteristics

From June 2005 to June 2006, soil moisture along the Monoman Billabong strandline attained 38% in July 2005, and declined through spring to a mere 1.9% in summer (Figure 6.13). Measurements at the very dry end of the scale (<1.5%) were inaccurate, as summer penetrometer readings in hard soils frequently were off-scale (>4.5 kg cm^{-2}) and low soil moisture volumes sometimes did not register on the Theta probe (<0.1%). Surface soil salinities were 250-1200 µS cm^{-1} (Electrical Conductivity at 25°C; mean 776, n = 25), generally below the threshold of 3,120 µS cm^{-1} associated with stress in red gums (Tucker 2003). The clay floodplain soils were slightly acidic with pH 5.3 6.4 (mean 5.8, *n* = 25). Organic carbon content of soil samples was low (0.18-2.92%; mean 1.41, *n* = 41).

Strandline germination

On the inner-bend strandline at Monoman Billabong, monitored from July 2004, 359 seedlings were counted along a transect (60 × 2 m) to sample a strandline formed by stranded ferns (*Azolla pinnata*) and woody debris. The numbers declined relatively rapidly due to grazing by sheep and drying, but 20 additional seedlings emerged after rainfall in November 2004 (Figure 6.13). By March 2005, numbers had declined to six survivors, and by July 2005 virtually no mulch remained. The survivors were stunted (<50 mm) but a spurt of growth (to >100 mm) occurred after above average rainfall in 2005. This growth occurred after removal of sheep from the site (see below). A new strandline formed at a lower elevation at the same site in January 2006, and five new seedlings emerged after rain between March and May 2006, with all 11 seedlings surviving to March 2007. Average soil hardness (unconfined compressive strength (kg m^{-2})) was generally inverse to soil moisture, ie low hardness values reflect high soil moisture.

At Werta Wert Lagoon, two natural strandlines yielded up 10 seedlings in January 2005, but none survived grazing by sheep or goats. On the artificial strandline of seeded pots, two seedlings germinated and survived for only two months. In contrast, 35 seedlings germinated over five months in the 10 duplicate pots (0.1 m^2) in the glasshouse, under constant moisture. Seedling densities in these pots were in the range 0-9 seedlings (mode = 3).

Grazing

Sheep grazed at Monoman Billabong and several other Chowilla sites until September 2005. Following watering in 2004, hundreds of seedlings appeared in strandlines and patches in the watered zone at Monoman Billabong, but these disappeared within a few weeks. Grazed seedlings as small as 10-

20 mm were observed, and few reached 150-200 mm before being grazed. Following significant rainfall in June-October 2005, and the removal of sheep, vigorous 150-200 mm seedlings re-appeared on a grazed former strandline in November, indicating original strandline seedlings regenerated from rootstock, and further watering in December 2005 encouraged them to reach 300-500 mm. Numerous seedlings <10 mm were observed at Pilby Creek Floodplain in 2004-2005, adjacent to healthy red gums (T2), but none survived beyond 20-30 mm, due to grazing by sheep. A strandline 10 × 2 m formed along the creek's edge at T1 in October 2005, after removal of sheep, and all 80 seedlings were still alive in June 2006, reaching 50-100 mm, but with 20% lightly grazed at the tip by kangaroos. At Pipeclay Lagoon in 2004-2005, there was grazing by sheep and kangaroos and there were signs of grazing on both seedlings and ground-cover plants.

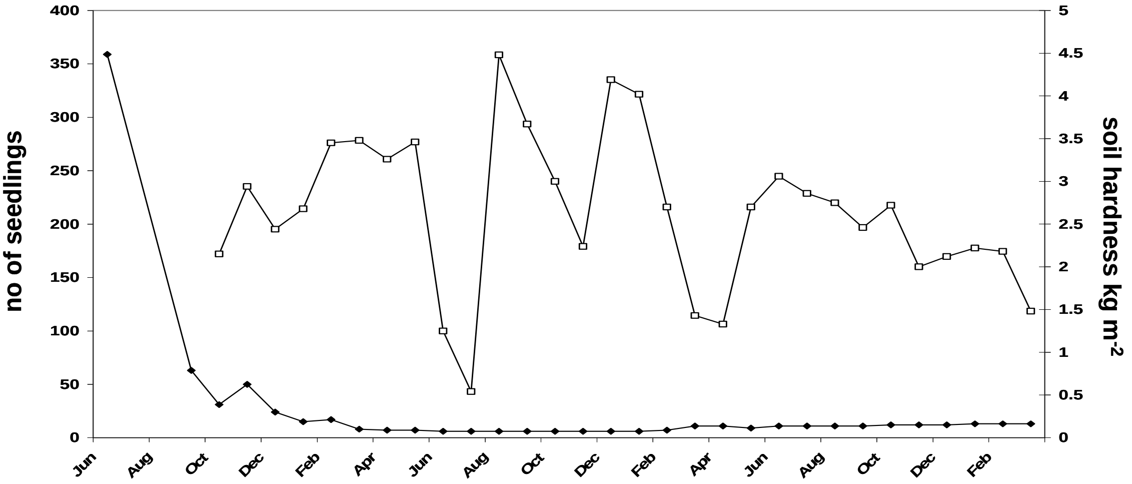

Figure 6.13 Initial germination of 360 river red gum seedlings (lower line) along the Monoman Billabong strandline rapidly declined from July 2004 due to grazing by sheep and desiccation. Average soil hardness (unconfined compressive strength (kg m^{-2}); top line) was generally inverse to soil moisture.

Discussion

Seed Release Patterns

These results provide useful information on the behaviour and responses of the aerial seed bank of the River Murray floodplain, particularly in relation to the dominant eucalypt, river red gum, based on a 30-month data series from seed-trap samples. They indicate patterns in the availability of seeds and the potential response to water sources, leading to germination, survival and maturation of seedlings. There are also useful observations on the impacts of local rainfall, tree health and grazing.

For red gum in the Lower Murray Valley, the results suggest a pattern of summer peak seed rain in alternate years in healthy trees, with approximately 50% of trees sampled in the same community being on opposite cycles, ensuring some flowers and shed seed in each year (*see Chapter 5*, Jensen *et al.*, 2007). Black box trees have longer periods of flowering and seed fall, peaking annually in summer or winter.

Previous results in this study indicated that red gum and black box seeds are held in aerial seed banks and do not persist in the soil seed bank (*see Chapter 5*, Jensen *et al.*, 2008a). This was supported by the observations from seed traps that mature closed fruit were maintained for at least 10-12 months on sample trees. Field observations indicated that the bud crop was set by environmental conditions in the preceding year, and that buds persisted for up to 12 months, as observed in other regions (Paton *et al.*, 2004). Trees gradually shed their bud or fruit crops as the seasons progressed, and there was a substantial reduction in immature fruit during the course of 2006, when rainfall was only 43% of the long-term annual average (Jensen *et al.*, 2007). The expected biennial peak of seed fall in summer 2006 was much reduced or nearly zero in healthy trees, as expected from the reduction in the immature-fruit crop observed during drought in 2006.

The pattern of seed rain for stressed trees appeared to be more varied, lower in volume and extended over a longer period, when compared with healthy trees. A lag of about 12 months was observed in the seed rain responses of healthy and stressed trees following watering. The data for stressed trees confirm reductions in quantities of viable seed by up to one order of magnitude. Ordinations showed that all growth factors increased with increased tree health, including buds, flowers and fruit. Clearly, new leaf production in healthy river red gums is highly seasonal, peaking around December, which suggests that leaf production is not an appropriate parameter to measure growth responses to watering.

At the more stressed site (Clarks Floodplain), very few red gum seedlings occurred, although there were surviving older saplings and new seedlings were generated during the study in response to watering. The lower density at Clarks Floodplain suggests a regenerative capacity of up to 11,765 red gums ha^{-1} under stressed conditions, with about 580 trees ha^{-1} likely to survive.

The importance of rainfall events was confirmed by the growth responses to higher than average rain in 2005. In particular, ordination of trap samples showed the influence of rain in the 21-25 days prior to sampling was a determining factor in the dry summer trimester (Dec-Mar), and rain in the previous 6-10 days was influential in the cooler months (Apr-Nov).

Small patches of red gum seedlings germinated at watered sites, both in strandlines at high watermark and across gently sloping banks and beds of wetlands. The likely seed source for these germination events was ongoing light seed rain onto the water surface, with seeds then being transported by wind and water (*hydrochory*) to strandlines at suitable sites for germination. Red gum seeds can float for up to 10 days before seeds begin to germinate (Jensen *et al.*, 2007a). Large recruitment events stimulated by flooding are likely to occur when flood water is pooled during the period of maximal seed rain from healthy trees. A supplementary mechanism for moderate recruitment appears to be rain-triggered germination from the soil seed bank plus light seed rain during above average rainfall in spring, followed by a flood to maintain soil moisture for the seedlings. River red gum seed is transient rather than persistent in the soil seed bank, but can survive for some months in the soil, germinating on a second watering event (*see Chapters 4 & 8*).

Seed sources

Both river red gum and black box rely on aerial (canopy) seed banks, and the soil seed bank contains few seeds regardless of the health of the parent trees. Seed release peaked in summer (Nov-Feb for river red gum, Oct-Mar for black box), with small volumes of seed rain in other months. The aerial seed bank released substantially higher volumes of seed rain from healthy trees than from stressed trees, with reductions of up to two orders of magnitude in stressed trees.

Healthy river red gum trees demonstrate serotiny, a typical feature of terrestrial woodland communities whereby seed is retained in the canopy until release is triggered by favourable conditions (Lamont and Enright 2000, M. Holland, Univ. Mississippi, pers. comm.). Many eucalypts exhibit serotiny as an apparent strategy to protect seed from predation and maximise the range of dispersal (Cremer 1965, Yates *et al.* 1994, Yates *et al.* 1995, Yates *et al.* 1996, Pudney 1998, George 2004). It may also boost the available seed in the aerial seed bank when release is triggered by fire (Lamont and Enright 2000), flood or other disturbances.

Volumes of seed rain for four healthy trees at Banrock Station measured in this study indicate higher yields over 7 months in 2004-2005 (915-17,526 seeds m^{-2} per tree, canopy areas 49-110 m^2) compared to those recorded over a 2 year dry period 2002-2003 for six healthy trees at Banrock Station (total 28,000 viable seeds) (George *et al.* 2005a), including three trees sampled both studies. The 'normal' volume suggested for river red gums by Jacobs (1955) is 600,000 seeds per tree per annum, but the highest seed volumes recorded in the present study were well above this figure (eg for the reference tree at Pipeclay Lagoon, with 24,413 seeds m^{-2} annual average seed fall over the study period and a canopy area of 163 m^2, this suggests at least 994,816 seeds per tree in annual production, assuming that most seeds fall in the north-east quadrant and calculated using 25% of the canopy area) (Figure 6.14). At Banrock Station, healthy black box had high summer releases of seed rain (maximum monthly peak 7,445 m^{-2}) and stressed trees had reduced volumes (maximum monthly peak 60 m^{-2}) (George 2004).

There was high seasonal variability in patterns of seed rain among trees within and between habitats. In river red gums, the healthy trees had maximum seed release in December, February and March, while stressed trees with lower volumes of seed had more varied patterns, some with multiple peaks. This may reflect opportunistic responses to changing conditions, with some trees releasing seed to coincide with flooding and others responding to rainfall. It may also reflect marked differences in the quantity of seeds held in the canopies of different trees at any one time, which are determined by variability in flowering (Paton *et al.* 2004).

Continual light seed rain may be due partly to external factors causing early abscission of fruits and release of seed. Stress may cause small numbers of fruit to dry out and shed seed, or parrots feeding on the fruit may spill seed, or the death of twigs or branches may cause fruit to open. Fruit damaged by parrots peaked in trap samples at Pipeclay Lagoon, Clarks Floodplain and Monoman Billabong in late summer (Jan-Feb), but the number of damaged fruit was not significant overall (n=27).

The floodplain soil seed bank for river red gum and black box resembles that of a terrestrial woodland rather than a wetland. Similar communities occur, for example, along rivers in Western Australia (Pettit & Froend 2001) and in the south-western United States (Stromberg 1993, Cooper *et al.* 1999). The soil seed bank was dominated by annual terrestrial species, with fewer wetland and riparian species. Seedlings of the perennial tree species were either few (river red gum) or absent (black box). These findings are comparable to observations on the Mississippi floodplain (M. Holland, Univ. Mississippi, pers. comm.), where species in seed banks are mainly early successional species rather than forest trees. Similar patterns are reported for a semi-permanent wetland in the Murray Valley (Robertson & James 2004) and ephemeral wetlands of the Cooper Creek floodplain, central Australia (Capon 2004).

Potential for Recruitment

Estimated potential regeneration densities from red gum seed in the aerial and soil seed banks appear to be sufficient to maintain communities, given normal rates of seed and seedling loss. Although sampling of the soil seed bank returned low numbers of red gum seedlings compared to other native species, and no black box, there may be sufficient seedlings germinating to maintain long term populations. It has been suggested that eucalypts generally need 10 seed trees ha^{-1} to sustain broadscale regeneration (Cremer, 1965), so it appears that there may still be sufficient potential regenerative capacity for this community.

The density of red gum seedlings in the soil seed bank at Pipeclay Lagoon suggests the potential for regeneration could be up to 250,900 red gums ha^{-1} in a healthy ecosystem where topography, soil and moisture conditions are suitable (*see results in Chapter 4*). The expected survival rate of 5% (Fenner, 1985) suggests a potential recruitment of 12,500 river red gums ha^{-1} in suitable habitats with healthy mature trees. However, significant reduction of seed fall in stressed trees could reduce potential recruitment by an order of magnitude (Jensen, Walker & Paton, 2008b).

Potential germination per hectare shown here is used for comparison only, given that actual regeneration will be less due to competition, predation, desiccation, grazing and other factors. Although these estimates may appear to be adequate when compared to theoretical numbers in the literature, there is some on-site evidence that recruitment rates are too low for replacement of existing communities. Recruitment of eucalypts at Banrock Station is insufficient to maintain present populations (George *et al.* 2005b, a), with missing cohorts reflecting reduced flood frequencies and recruitment rates below the rate of mature tree loss. In the late 1980s, mean regeneration densities in Lower Murray floodplain communities ranged from 475 saplings ha^{-1} for river red gum forest to 160 ha^{-1} for red gum woodland and 62 ha^{-1} for black box woodland, at a time when the communities were in poor condition and regeneration was patchy (Margules & Partners *et al.* 1990). To achieve these densities, a higher than normal proportion of the seedlings and seeds recorded in this study would need to survive (cf Fenner 1985). In general, low recruitment of red gum in the Lower Murray region is attributable to reduced seedling survival, due to dry conditions that create less frequent, more patchy opportunities for germination and survival. The rapid decline in health of mature trees will increase the risk of recruitment failures.

Recruitment Mechanisms

Red gums employ complementary recruitment strategies of intermittent mass recruitment and low-level annual recruitment (Florence 1996). Local rainfall is a trigger for germination, and flood events provide the mechanism for hydrochory, producing relatively dense stands of seedlings. Low density 'maintenance' recruitment associated with local rainfall produces seedlings in suitable patchily-distributed habitats. High-level 'boom' recruitment occurs during medium floods (40,000 ML d^{-1} for river red gums: George 2004), producing strandline stands and dense copses of seedlings. The most conspicuous form of recruitment in the Lower Murray since regulation intensified from about 1950 is dense strandline stands on inner sandy bends of the main channel (Maheshwari *et al*., 1995). These occur at lower elevations where flood frequencies are adequate to sustain seedlings and saplings. Recruitment on higher terraces is virtually non-existent.

The paucity of eucalypt seeds in the soil seed bank indicates that seed for germination of dense stands after floods comes from ongoing seed rain, transported by water to form strandlines. Laboratory tests showed that river red gum seeds will float for at least 10 days when immersed in water, sinking and germinating underwater from day 9. The period of flotation is similar to that found for Western Australian river red gums (Pettit & Froend, 2001).

Most river red gum seeds germinate within 10 days of release and landing on moist soil, but a small proportion persists to enable responses to subsequent favourable conditions. In the glasshouse, some seeds maintained viability in soil for several months prior to germination. Smaller seeds (200-500 µm) found in trap samples produced smaller, slower-growing seedlings in temperature controlled conditions, compared to seedlings germinating from larger seeds (>500 µm). The rate of germination of the smaller seeds in glasshouse conditions was insignificant, compared to larger seeds.

Persistence of seeds

‘Persistent’ seeds remain viable in the soil for two or more germination seasons following dispersal (Baskin & Baskin 1998). Persistence thereby is a strategy for seeds to endure dry periods in temporary wetlands, and the eucalypts of the Murray floodplain might be expected to use this strategy, but river red gums and black box are scarcely represented in the soil seed bank. For these eucalypts the floodplain soil seed bank is better described as a Type III seedbank (*sensu* Thompson & Grime 1979), in which most seeds germinate immediately after dispersal, and a small proportion is incorporated into the persistent seed bank. Serotiny provides an alternative strategy to persistence (Dr M Casanova, pers comm.), as seeds held in the canopy may remain viable for up to 2 years (Cremer 1965, 1977), ready for opportunistic release given favourable conditions. Glasshouse observations in this study suggest that not all seeds released from a tree germinate with the first onset of suitable conditions. Germination of eucalypt seeds could occur more than once in an annual season, determined by watering events.

Germination, Maturation and Survival

River red gum seedlings may take two years to develop sinker roots (George, 2004), which have been observed up to 100 mm long in wetter conditions of the Barmah Forest (Bren, 1988). This suggests that more than one soil-wetting event would be required to sustain seedlings through desiccating conditions, if germination occurs in early-mid summer. New seedlings appeared at study sites in response to rainfall, and most have survived for 6 months (so far) with additional rainfall. Even with summer surface soil moisture values as low as 2%, six of the seedlings in the Monoman strandline have survived for at least 27 months, indicating that the roots have found adequate moisture. Advance growth (where seedlings wait for favourable conditions) has been observed previously for red gums following local rainfall (Florence, 1996).

Along localised strandlines, desiccation effects may be offset by the mulching effect of organic litter, especially on soils with very low organic carbon content. Germination did not always recur where strandlines formed in the field, suggesting that other factors such as variability in seed release and local climatic conditions also affect germination. Rain-triggered germination had higher survival rates than strandlines generated at the high water mark by watering trials during the period monitored.

Surface soil salinities are low and are unlikely to inhibit germination and early growth if soil moisture is replenished by rainfall or flooding. Intrusions of highly saline groundwater into mature tree root zones at these field sites are causing death of mature trees. This process presents a future risk for long-term survival of saplings, and increases the need for ongoing managed watering events and floods to maintain freshwater lenses overlying the groundwater layer (Jolly *et al.,* 1992).

Water sources

Potential sources of water in the Lower Murray are floods that replenish soil water and local rainfall sufficient for germination and early seedling growth. A combination of medium floods (>40,000 ML d^{-1}) with higher than average rainfall (300 mm) is suggested as a pre-requisite for successful recruitment in river red gums, and similar rainfall linked with larger floods (>80,000 ML d^{-1}) for black box (George 2004). In the Lower Murray, rainfall was below average in 2004 (190 mm) and wetter than average in 2005 (301 mm). There were no overbank flows during this period, with river flows confined to the main channel due to drought in the catchment.

The patchy emergence of river red gum seedlings at study sites following the wetter than average spring in 2005, independent of a flood event or watering, indicated rain-triggered germination. Trap samples indicated that bud formation, flowering and fruit development on river red gums were mainly in spring (Aug-Nov), when rainfall peaked. Germination closely followed rainfall at two sites (Monoman Island, Clarks Floodplain), where seedlings germinated in spring 2005 (Aug-Sep), and attained 300-1000 mm height by June 2006. This demonstrated that local rainfall can trigger germination. Rainfall may be a trigger for germination during spring (Aug-Nov), with floods in early-mid summer (Nov-Dec) providing extra soil moisture to sustain germinants.

Effect of reduced flood regime

The timing and extent of adequate soil moisture conditions are critical for germination and recruitment, as the Lower Murray floodplain is deprived of water by regulation and diversion. If the changed regime persists, the floodplain may contract to 30% of its original area (Walker & Thoms, 1993, Roberts 2003) (*see Chapter 8*). An analysis of the seasonality of annual flow peaks at Morgan, South Australia indicated a significant change in timing since river regulation began (Maheshwari *et al,.* 1995), with seasonal peaks spread over a larger period of the year and occurring more often in hotter months than under natural conditions, so that potential germination events are deferred to hotter, drier conditions in summer or watering is less likely to coincide with peak seed fall (*see Figure 2.1, Table 2.1*). Current drought conditions in the Murray-Darling Basin indicate that no overbank flows are likely in the next several years, as priority is given to filling storages (Murray-Darling Basin Commission, 2006). These dry conditions have continued beyond the primary inflow season for 2008, extending the drought on the floodplain.

Response to Watering Events

The low volumes of seed rain immediately after watering events point to a lag time to allow seed crops to set and mature in response to watering events. Crops are determined by conditions that affect bud development up to a year before flowering (Paton *et al.* 2004). Low seed rain may reflect sub-optimal climatic conditions for seed development in the previous season, and an enhanced seed response may appear in the next season, provided that watering coincided with the reproductive cycles of the trees. Thus, a succession of flooding or rainfall events over several years may be required to maximise seed production and recruitment. Single, short-lived watering events clearly are not sufficient to sustain germinants through dry periods. Soil moisture availability is critical for plant recruitment in other semi-arid zones (Cooper *et al.,* 1999).

Managed watering offers some flexibility in timing but limited duration and volumes. Volumes of water available under regulation are much less than under natural floods, and there are constraints on delivery of water to higher elevations on the floodplain. The effectiveness of ‘environmental flows’ for recruitment of eucalypts could be increased by linking the timing of late spring-early summer watering with local rainfall, to promote seedling survival and retention of bud crops in autumn, hence increased seed production in the following summer.

Strandlines

Examples were found in the field during watering trials where seeds germinated readily along high-water strandlines where a mulch of organic litter retained moisture and gave initial protection from grazing. However, longer term survival was limited by desiccation and grazing. The emergence of strandline germination suggests that seed rain onto the water surface has been transported by hydrochory to the water’s edge. Survival rates were higher for rain-triggered germinants among dense ground-cover adjacent to watered sites, although these seedlings were vulnerable to later flooding by successive watering trials.

Grazing and Seed Predation

The impact of sheep grazing was demonstrated by comparisons of river red gum germination with sheep present in 2004-2005, and after their removal in September 2005. The emergence of dense ground cover in the absence of sheep provided protection for seedlings, as well as reducing evaporative losses. At Pilby Creek Floodplain and Monoman Island Horseshoe, red gum seedlings survived for 16 months. It was clear that livestock reduced the survival of seedlings, with seedlings grazed to ground level and most seedlings lost. By comparison, kangaroo grazing had low impact. At Pipeclay Lagoon, ground-cover species were significantly more abundant in summer 2005-2006 than the previous year, indicating that the impact of sheep grazing is much higher than kangaroo grazing. At Pilby Creek Floodplain, after removal of sheep, 100% of seedlings in a strandline continued to survive after 16 months, with ~20% mildly affected by kangaroo grazing of the tips.

Disparities between seed rain and numbers of germinants suggested high rates of seed predation, probably by ants. Peak monthly seed rain of 17,562 m^{-2} suggests volumes in the seed fall zone of 176 ×

106 red gum seeds ha^{-1}, but soil seed bank volumes were only 1-2000 seedlings ha^{-1}, indicating high losses of seeds at the soil surface. Seed-harvesting ants are likely predators (Yates *et al.* 1994). Parrots harvested seeds from mature fruits in the canopy at several sites, but the numbers of fruits lost were insignificant.

Conclusions

This section of the study examined conditions for germination, early seedling survival and successful recruitment in river red gums and black box. The eucalypts exhibit serotiny, retaining seeds in the canopy and releasing them in a continuous light seed rain, peaking in summer. This timing for seed release coincides with the likelihood of floodplain inundation from seasonal flood events, although ongoing light seed rain allows germination whenever suitable conditions are present. Rain-triggered germination was also demonstrated to be an important factor in ongoing low-level seasonal recruitment. This strategy supplements flood-triggered germination, where hydrochory is an important mechanism producing strandlines of seedlings. However, early seedling survival is dependent on adequate soil moisture reserves until a tap root is formed to reach subsoil moisture, thought to take about 2 years. Soil moisture is supplied by a combination of rainfall and flooding. The required conditions of soil moisture are further investigated in Chapter 8.

While seed rain from healthy trees does not appear to limit recruitment, seed volumes released by unhealthy, water-stressed trees are much reduced. Thus, reduced seed rain and reduced flooding are significant factors contributing to observed reduction in recruitment of river red gum and black box. Grazing by sheep was observed to have major impact, with almost total loss of seedlings, while grazing by kangaroos was restricted to light pruning of seedlings without any losses. Granivory of eucalypt seeds from the soil surface by ants is suggested to be significant, and is a topic for further study. The effects on successful recruitment of river red gum and black box of reduced flood frequency and duration, drier floodplain conditions, and grazing, are paramount.

Figure 6.14 Set of three eed traps in north-east quadrant of canopy under sample tree T1 at Werta Wert Lagoon. The red colour is a small plant of the samphire group growing on the moist wetland bed as it dried after watering. A small amount of water remains in the centre of the lagoon.

Chapter7 Germination and Growth in Lignum[11]

Introduction

Alongside the dominant eucalypts river red gum and black box, the dominant perennial shrub of the Lower Murray floodplain is tangled lignum *Muehlenbeckia florulenta* (Meissn.) F. Muell.: Polygonaceae), hereafter 'lignum'. It appears brown and lifeless in dry conditions, and is often overlooked, but its abundance and ubiquity on riverine floodplains throughout central and eastern Australia make it influential in the salt and water balance of the groundwater underlying the floodplains. The plants attain 1-3 m height, and have a persistent rootstock at least 2-3 m deep (Craig *et al.* 1991). Lignum covers significant areas of clay depressions, as well as occurring in mixed communities with both river red gum and black box, and thus has a significant influence on the evaporative balance and groundwater levels of floodplains. This study aimed to investigate the reproductive and growth strategies of lignum, and to investigate its preferred water sources.

Lignum is typical of swamps, river-flats, gilgais and other flood-prone areas (Cunningham *et al.* 1981). It is particularly common in the Murray-Darling Basin, including the nutrient-poor, grey cracking clays of the River Murray floodplain in South Australia. Flooded lignum is a nesting habitat for water birds like ibis (*Plegadis flacinellus*, *Threskiornis spinicollis*, *T. aethiopica*) and freckled duck (*Stictonetta naevosa*) (Lowe 1983; Jensen 1983; Briggs & Maher 1985; Boulton & Lloyd 1991). It also shelters Murray cod (*Maccullochella peelii peelii*) and golden perch (*Macquaria ambigua*), and the young stages of many other fish species. Associated terrestrial species include small birds like fairy wrens (*Malurus* spp.) and mammals and reptiles (e.g. Cunningham *et al.* 1981). Dense stands protect eucalypt seedlings from grazing animals, especially introduced rabbits (*Oryctolagus cuniculus*) (Jensen 1983).

The many tangled, woody stems often remain bare until the soil is wetted by rainfall or flooding, when they rapidly develop green shoots, leaves and flowers. Lignum reproduces both sexually (by seeds) and asexually (by vegetative means, through root-suckering or branch-layering) (Southgate 1988; Craig *et al.* 1991; Roberts & Marston 2000; Chong and Walker 2005). Lignum is dioecious, with small, yellow-green flowers of five petals clustered in interrupted racemes (Southgate 1988; Chong & Walker 2005). The female flowers are smallest, with a tri-branched style and eight barren filaments, and are held close to the branch; the male flowers, with eight fertile stamens and a residual stigma, are more obvious (Figure 7.1). Flowering may occur throughout the year (Cunningham *et al.* 1981), but particularly from August to December in the Lower Murray region (Southgate 1988). The shiny, brown seeds are contained within achenes (Chong & Walker 2005). Germination is cued by soil moisture and temperature, and although seedlings are difficult to find, they have been observed after summer-autumn floods (Roberts & Marston 2000) and winter rains (Chong & Walker 2005). They are vulnerable to variations in temperature and soil moisture, and to grazing animals.

Stands of lignum are tallest and most vigorous where there is persistent surface or subsurface soil moisture (Roberts & Marston 2000), in soils of relatively low pH and salinity (Craig *et al.* 1991). The soil-moisture requirement increases where salinity is high, and decreases where salinity is low. On the Lower Murray floodplain, lignum occurs in areas where flooding (to less than 60-cm depth) occurs for 45-115 days per year (Blanch *et al.* 1999). In other parts of New South Wales and South Australia, it occurs in areas flooded for 6-12 months between once in two years to once in 10 years (Goodrick 1984; Craig *et al.* 1991; King *et al.* 1995).

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[11] Jensen, A.E., Walker, K.F., & Paton, D.C. (2006). The Secret Life of Tangled Lignum, *Muehlenbeckia florulenta* (Polygonaceae): little known plant of the floodplains. In Wetlands of the Murrumbidgee River Catchment (Eds I. Taylor, P. Murray & S. Taylor), 79-85. Murrumbidgee Catchment Management Authority, Leeton, NSW.

Figure 7.1 Male (left) and female (right) flowers of lignum, showing the distinctive extruding anthers of the male flower and star-shaped, low-set female flower (automontage images).

Previous studies of lignum had led to observations that lignum reproduction at some sites appeared to be associated with historic flood events (Assoc Prof K. Walker, University of Adelaide, pers comm.).This study undertook investigations to test the hypothesis that sexual reproduction (seed set) in lignum requires over-bank flows, whereas asexual reproduction (clonal growth) may be promoted by significant seasonal rainfall events.

Methods

Field observations were recorded monthly from November 2004 to March 2007. Lignum field sites included Brenda Park, Banrock Station and Chowilla Floodplain (see Figure 3.1). At Brenda Park, using the relative elevations created by historic contour banking around a wetland, 24 lignum plants were sampled from more elevated dry riparian areas (6 plants), from moist riparian areas (10 plants) and from a wetland that was inundated from December 2004 to February 2007 (8 plants) (Figure 7.2).

The edge of a large lignum stand at Banrock Station was monitored to observe responses to watering (15 plants) (Figure 7.3). The stand received spring-summer flooding in 2004 and 2005, but not in 2006. The plants being monitored were not directly watered, but were within 20 m of the inundated area. In November 2005 a wider than normal area, including the outer edges of the stand and the adjacent open floodplain, was flooded for 3 weeks to 250-mm depth, thus directly watering the plants being monitored. At Chowilla Floodplain and Clarks Floodplain, lignum bushes were monitored in moist riparian areas adjacent to watered sites (9 plants). These plants were within 20 m of the inundated areas but were not directly watered.

Plants were selected to reflect different water regimes, generally indicated by elevation above the river. An even mix of male and female plants was sought but not always achieved where the gender could not be confirmed (from flower anatomy) until after flooding. Biological variables monitored included height, width, percent greenness, percent leaf cover, relative abundance of flowers and Southgate's (1988) health index. Environmental parameters included soil hardness, litter cover under the canopy, rainfall and distance to surface water. For the first 18 months, a Soiltest CL-700 Pocket Penetrometer was used to estimate soil moisture via unconfined compressive strength; from April 2006 complementary measurements were made using a Delta-T ML2 Theta Probe. Surface soil salinity, pH and organic carbon content were determined by standard methods (Klute, 1986; Walkley & Black, 1934).

Supplementary rainfall and temperature data were obtained from the Bureau of Meteorology (*see Figure 3.3*). Cumulative rainfall and average temperatures were calculated for each 5-day period prior to sampling (1-5, 6-10, 11-15, 16-20, 21-25, 26-30 days). The numbers of days since last rainfall, and the numbers of days greater than 30 ºC in the previous month, were also determined. These observations are pertinent because seasonal conditions during the study included one dry summer, a wetter summer following above-average spring rainfall, and a very dry summer (*see Figures 3.3, 3.4*).

Growth responses to watering and rainfall were explored by ordination (Non-metric Multi-dimensional Scaling, NMS) of biological and environmental data recorded over 27 months at Brenda Park, Banrock Station and Chowilla Floodplain, using PC-ORD software (v. 4.41, MjM Software, Oregon, USA) with the Relative Euclidean distance metric and all default options. The primary (biological) matrix included measures of lignum health, growth and vigour, and the secondary (environmental) matrix included external factors expected to influence plant responses.

NOTE: This figure is included on page 7-3 of the print copy of the thesis held in the University of Adelaide Library.

Figure 7.2 Aerial view of Racecourse Lagoon looking south, with main Brenda Park lagoon to right and River Murray to left of picture. Lines of river red gums parallel the south-east bank of Racecourse Lagoon, where they have grown on old irrigation contour banks. Sampling at this site included lignum bushes in the wetland, bushes along the eastern riparian zone, and bushes on a higher contour east of the lagoon (arrow).
(*Photo supplied by Elise Byrne of Overland Vineyards*).

Seeds were harvested from female plants and stored in paper bags at 4ºC. In the laboratory, seeds were spread on 90 mm Whatman No. 1 filter paper over 30 mg vermiculite saturated with 30 mL distilled water, in covered Petri dishes. Samples were kept on a well-lit window sill at normal room temperature (Figure 7.3). A time-lapse film was taken of germination of lignum seeds to show the process of root and shoot emergence, and loss of the seed coat. Photographs were taken every 10 minutes over 8 days using the AMcap video capture and preview application by Noël Danjou, modified from Microsoft freeware (http://www.noeld.com/programs.asp?cat=video). The series of images was merged into a video film of about 10 seconds by Scott Mills, a postgraduate at the University of Adelaide, using ImageJ, image processing software to combine Java images into AV1 format (http://rsb.info.nih.gov/ij/).

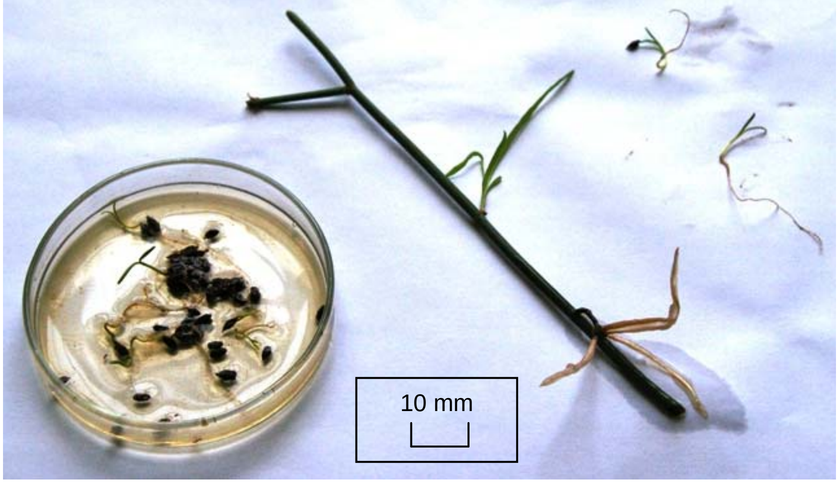

Figure 7.3 Lignum seeds germinating in a petri dish (left), alongside a lignum cutting which has sprouted roots from the first node and shoots from the second node. Two single seedlings appear top right, with the upper seedling still retaining the seed coat on the left shoot.

Lignum Ploidy

As previously described, lignum reproduces by both asexual and sexual methods. As well as production, dispersal and germination of seed, this species can produce new individuals by layering of branches, or new shoots from root nodes. The asexual mode of reproduction may offer an advantage in the fluctuating water regimes of the floodplain habitat where lignum is a dominant perennial shrub. The balance between sexual and clonal reproduction is likely to vary in response to limiting ecological factors, with sufficient soil moisture critical to survival for both forms of recruitment.

Instances have been found in the field where one gender dominates a local population (Mrs A. Jensen, University of Adelaide, pers obs). This suggests that the distribution of male and female lignum plants may be reflected in the ploidy of individual plants and local populations, and an assessment of the ploidy status of lignum could determine if plants have reproduced by sexual or clonal means.

Most *Muehlenbeckia* species are diploid (2*n*=20 chromosomes), so the finding of octoploidy (2*n*=80) by Dr John Conran (Chong & Walker, 2005) was unusual. This study sought to confirm the finding of octoploidy, and to investigate the relationship between ploidy and distribution of male and female plants, working in conjunction with a concurrent study of the gender balance and distribution of lignum in the same region (Lynch, 2006).

The methodology used is described in Appendix I. These tests failed to produce satisfactory samples of chromosomes, from over 50 attempts, using two preparation methods and three staining methods. Material from cuttings grown in RO water also failed to produce successful chromosome samples

(Lynch, 2006). The slide preparation technique was checked by an expert, who also failed initially to obtain chromosome samples from this material but finally obtained a single example, which confirmed octoploidy but the process to preserve the slide for photography using fixing gel was unsuccessful (Dr J Conran, University of Adelaide, pers comm.), and the attempt to isolate samples of lignum chromosomes was discontinued.

Results

This investigation aimed to understand the growth and reproductive responses of lignum to water availability, in the context of controlled watering events. All field sites were influenced by watering events during the period of the study, as well as receiving significant local rainfall, particularly in 2005 (*see Chapter 3*). Soil moisture values from laboratory analysis of field samples were very low in the dry zones (1.1 - 6.3%, *n* = 9). Surface soil salinities were also relatively low (200 – 700 EC units, *n* = 9) (*see Chapter 4*). The soil pH was slightly acidic (5.4 - 6.3, *n* = 25). Soil moisture for samples from moist riparian sites was in the range 14.5 - 32.2% (*n* = 9), indicating the key role of repeated rain or flood events to sustain soil moisture to promote growth and reproduction (*see Chapter 8*). Field measurements of % soil moisture volume using the Theta probe sampler varied significantly across samples at any one location on any one occasion, and between visits and between sites. Readings were limited to dry and moist sites, with the highest reading of 38.6% recorded in May 2005 in the moist riparian zone at Brenda Park. Dry readings <3% were not regarded as completely reliable as the crumbly dry soil could not provide sufficient contact with the five probes for an accurate reading. However, values in this low range were sufficient to show that surface soil moisture was below desirable levels for sustaining germination and growth of seedlings, or continued growth of mature plants.

Growth Patterns

Plants were relatively dormant for the initial 12 month period, with patchy periodic light flowering. An intense flowering episode followed above-average rains in winter-spring of 2005, with dense blossoms on most plants at all sites in October 2005. Rainfall at Morgan was above average between December 2004 - November 2005 (322.8 mm, 125% of mean annual rainfall: Bureau of Meteorology records), with peaks in late June-July followed by falls of more than 15 mm in September and October 2005. Similar rainfall patterns were recorded at Overland Corner (for Banrock Station) and at Renmark (for Chowilla Floodplain). Lesser flowering responses occurred after heavy rain in December 2004, October 2006 and May 2007 (*see Chapter 3*), when scattered plants responded with no more than moderate levels of flower production and patchy seed production approximately four weeks after rain events.

Vigorous growth and seasonal flowering but only light seed production were maintained by the plants in the wetland at Brenda Park, inundated from December 2004 to February 2007. Plants in the more elevated dry riparian zone also responded to the rain with flowers, leaves and new stems, but to a lesser degree than those in the wet and moist riparian zones at this site. Some individual plants had moderate seed production but no seedlings were found and no new vegetative clones were established. Similarly, vigorous growth was noted at the Chowilla sites in response to both rain and watering (Figure 7.4) but no seedlings or clones. However, the Banrock Station site only showed a significant growth response to direct watering in November 2005, with a period of approximately 6 weeks of growth (stems, flowers and leaves) before reverting to a dormant state of no leaves or flowers (with improved ratings of greenness of stems) (Figure 7.5). Otherwise, the plants remained dormant throughout the study, with no new seedlings or vegetative clones.

Figure 7.4 Lignum in blossom in response to spring rains on Chowilla Floodplain.

Although past clonal vertical growth from root nodes and layering from branches were evident in plants at all sites (*see Figure 3.1*), few instances of new vegetative growth by layering or shoots from root nodes were found. At moist sites, the pattern was for vigorous vertical shoots to arch under their own weight, eventually touching the ground and sometimes developing roots at branch nodes. Root formation did not occur in dry conditions. At the flooded Brenda Park site, flooding appeared to promote the development of roots on branch nodes once these contacted the water, but while the plants remained flooded they could not set roots into the soil.

Figure 7.5 Lignum at Banrock Station remained relatively dormant throughout the study period, apart from a growth response to inundation at the base of the plants in November 2005. The layering extending from this plant (arrow) did not develop further during the monitored period.

Seed production continued at all sites for over two months following flowering in October 2005 (cf. Chong & Walker 2005), but lignum seeds were not found in the soil seed bank in soil samples from Clarks Floodplain or Chowilla (*see Chapters 4 & 6*). Seeds collected from the field germinated readily in the laboratory when placed on filter paper over vermiculite in Petri dishes and left on a windowsill in natural light. It was critical they were covered only 50% of the time (to balance saturation by condensation leading to development of mould on the seeds, against drying out too quickly). Germination was rapid (2-5 d) and approximately 50% of seeds germinated in each dish.

Soil Moisture

Relative soil moisture, as indicated by hardness measurements, clearly demonstrated the importance of local rainfall events, with short periods of elevated soil moisture (usually in winter months) interspersed with periods of very low moisture levels (particularly in summer months). At the Banrock Station site, the record of soil moisture was more variable than expected (Figure 7.6). The effect on soil moisture of the flooding event in November 2005 had disappeared by January 2006, even with the added effect of the highest monthly rainfall for the study period.

NOTE: This figure is included on page 7-7 of the print copy of the thesis held in the University of Adelaide Library.

Figure 7.6 Soil hardness at Banrock Station lignum site from January 2005 to March 2007 indicates elevated soil moisture levels in July and very low levels in February, but local rainfall events can increase soil moisture at any time (eg March 2005, November 2006). The rainfall trace (maximum 80mm) indicates monthly rainfall recorded at Renmark, 40 km east of Banrock Station (data from Bureau of Meteorology).

Grazing Impacts

Viable seeds were recovered from female plants still encased in achenes, but seeds recovered from the soil surface had their achenes removed and the kernels of all seeds had been removed by insects, probably ants (Figure 7.7). Only a small patch of 10 seedlings was found at Chowilla through the entire 30 months of field sampling, and none were found at Brenda Park, Clarks Floodplain or Banrock Station. The sole record of new seedlings was at Monoman Island on the Chowilla Floodplain, where 10 seedlings were monitored for height and survival from February 2006 to March 2007. While sheep had been removed from the site in September 2005, grazing kangaroos (Macropus gigantaea) were abundant, and appeared to graze the lignum seedlings heavily. Plants only attained 300 mm in height and all new shoots were grazed to ≤10 mm length. All 10 seedlings survived with stunted habits to March 2007 and continued to produce new shoots, with as many as 20 grazed shoots per plant. Based on their position relative to water levels and micro-topography, and lack of nearby seed sources, these seedlings probably originated from seeds transported by water (hydrochory) when the wetland was filled in December 2005 (see Chapter 8).

Figure 7.7 Lignum achenes collected from the soil surface beneath lignum bushes at Banrock Station had been ripped open by insect predators (probably ants) and the seeds removed.

Analysis of growth responses to water

A preliminary analysis of data from Brenda Park over the first 9 months showed an interesting pattern separating male and female plants. The ordination (Figure 7.8) yielded a 2D solution (stress 5%, total explained variance 98%; Axis 1: 20% of total variance, Axis 2: 70%). The superimposed vectors indicated correlations between environmental factors and ordination axis scores. Key factors distinguishing samples on Axis 1 (abscissa) were days with no rain and rain in the previous 16-20 days. The factors influencing separation of sample points on Axis 2 (ordinate) were distance to water and percentage cover on ground under canopy (note that the vertical axis explains 3.5 times more variance than the horizontal axis). There was a striking, consistent separation between males and females in the ordination, suggesting that response of the plants to environmental variables is gender-based. This separation is most strongly inversely correlated with distance from the wet zone, and suggests that reproduction from either seed or vegetative cloning may be influenced by water source.

Analyses of the full dataset from Brenda Park over 24 months showed a less definite pattern separating male and female plants. The ordination for gender (Figure 7.9) yielded a 3D solution with 1% stress. The factors on Axis 1 (abscissa) were dominant, with water depth and soil hardness having opposite effects on the distribution of samples. Samples from female plants were most closely associated with increasing soil hardness and lower water depth.

The ordination for lignum habitats at Brenda Park (Figure 7.10) showed that the separation of plants across the three habitat zones was relatively clear, although it was surprising that the inundated and dry samples were partially inter-mixed while the riparian samples were clearly separate. The separation of samples is again associated with increasing soil hardness and decreasing depth of water, with female plants tending to drier conditions, contrary to earlier findings.

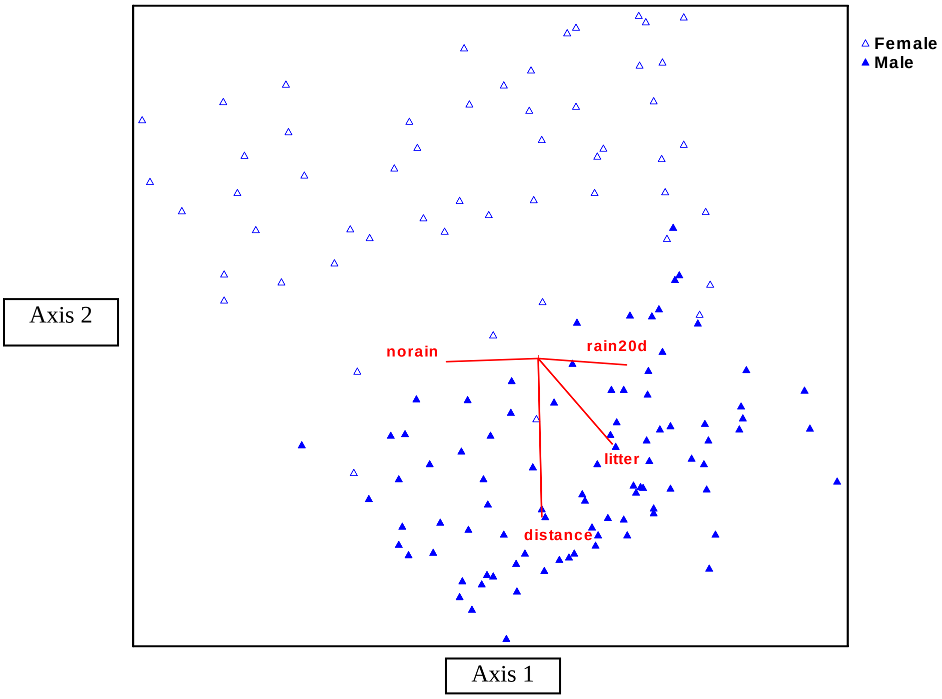

Figure 7.8 Initial ordination results for the influence of environmental factors on the distribution of sample points for lignum growth and environmental parameters at the Brenda Park site indicate that distance from the wetland (inundated throughout the period of monitoring) and amount of cover on the soil surface under lignum canopies were the most significant environmental parameters, with days with no rain and rainfall 16-20 days prior of lower significance (r^2= 0.98, Axis 1 = 20%, Axis 2 = 70%). The responses of male and female plants are clearly separated. Monthly data were recorded from February 2005 to October 2005.

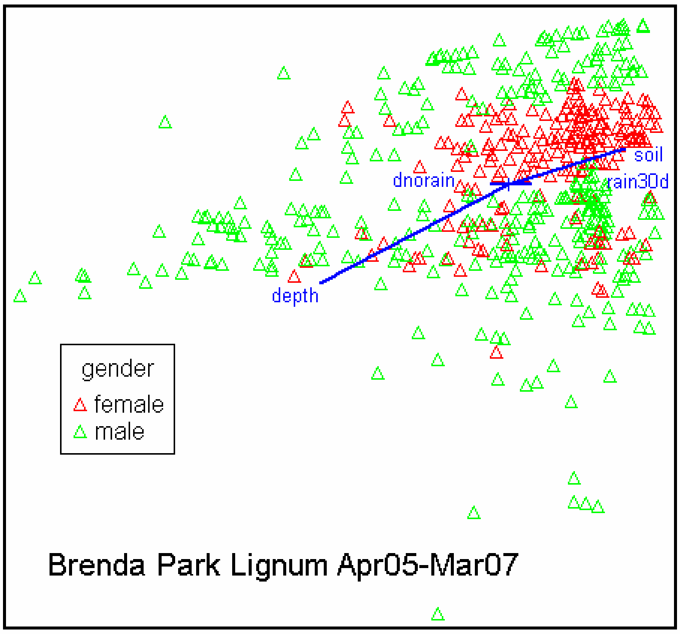

Figure 7.9 NMS ordination results for 21 lignum plants over 24 months at Brenda Park, covering wet, riparian and dry zones, gave a 3D solution with 1% stress (1-2 axes). The separation of male and female plants was less clear than in the initial analysis (Figure 7.8), although the female plants are still relatively closely clustered. The separation of samples is associated with increasing soil hardness and decreasing depth of water.

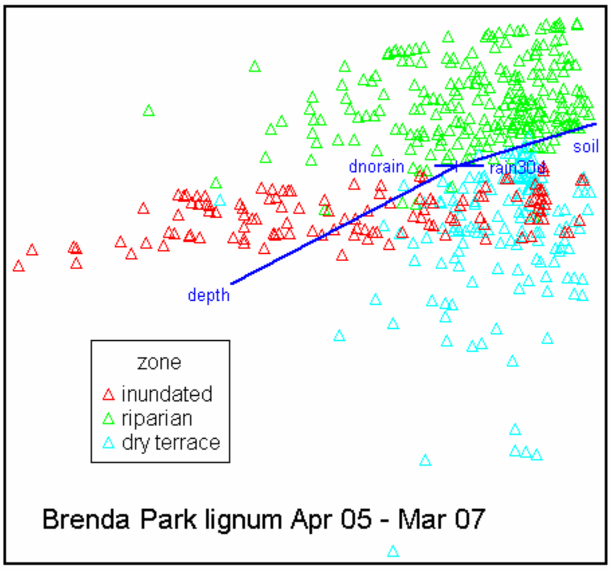

Figure 7.10 NMS ordination results for 21 lignum plants over 24 months at Brenda Park, covering inundated, riparian and dry terrace zones, gave a 3D solution with 1% stress (1-2 axes). The separation of plants across the three habitat zones was relatively clear. The separation of samples is again associated with increasing soil hardness and decreasing depth of water.

The ordination results for the Banrock Station site were unsurprising, showing a clear response to local watering in increased height and width of plants during the inundation and post-inundation period (higher X-values, lower Y-values), with a gradual return to a dormant state (lower X-values, higher Y-values) (Figure 7.11).

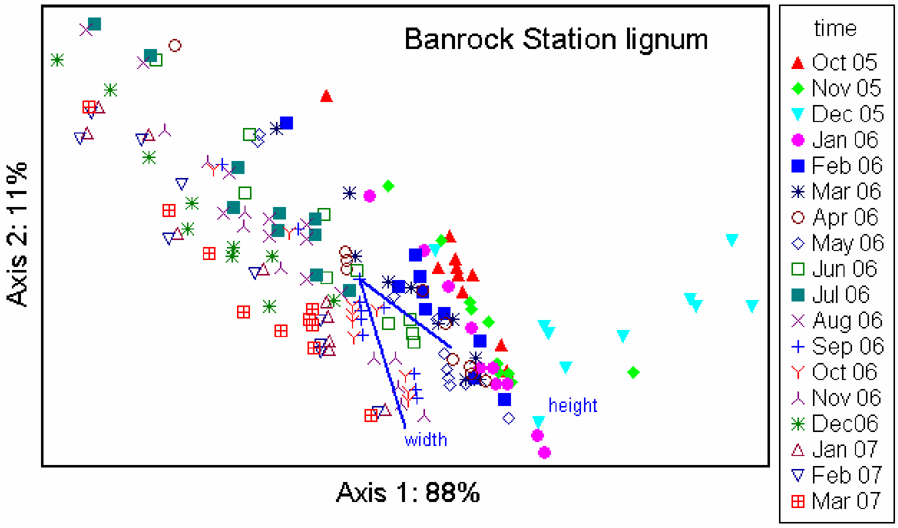

Figure 7.11 NMS ordination results for 10 lignum plants over 18 months at Banrock Station, including inundation in November 2005 and residual moist conditions in December 2005, gave a 2D solution with 3% stress which explained 99% of the pattern. The separation of samples was closely associated with the increased soil moisture generated by the local inundation event. The separation of samples is associated with increasing width and height measurements of bushes.

The lignum results for the Chowilla Floodplain also indicated a separation of sites with more frequent inundation (Figure 7.12). Lignum plants as little as 5 m outside the watered zone showed a reduced response, and the ordination clearly separated plants in drier habitats from those inundated more frequently. The separation of samples reflects the different elevations on the floodplain, with the Pilby Creek Floodplain at a lower elevation and being inundated twice during the study period, while the Pipeclay Lagoon plants were >5 m from the highest water level and thus benefited only from rain. Plants 29 and 30 at Pilby Creek Floodplain were at the highest elevation of that group of plants, and were not directly inundated, while plants 25 and 26 were inundated in all three waterings.

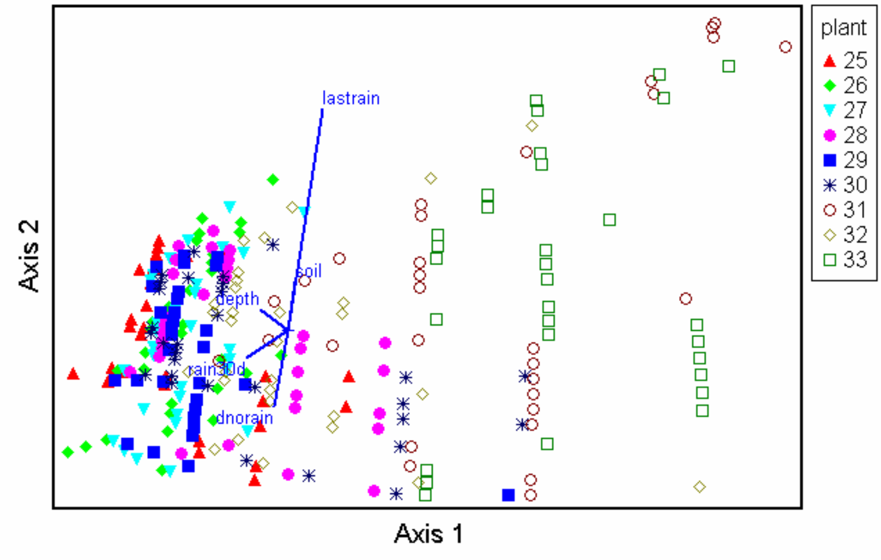

Figure 7.12 NMS ordination results for 9 lignum plants over 28 months at Chowilla Floodplain, covering inundated, riparian and dry terrace zones, gave a 2D solution with 1% stress. The separation of plants between sites (25-30 = Pilby Creek Floodplain, 31-33 = Pipeclay Lagoon) was relatively clear across the abscissa, with drier sites further to the right side of the plot..

Discussion

Lignum plants at all sites were observed to respond rapidly (within 2-3 weeks) to direct watering, whether from watering or from rainfall, with new green leaves, flowers and vigorous shoots. These strongly similar responses dominated statistical analysis of data, which highlights patterns based on differences rather than similarities between plants. An initial analysis was undertaken with only 9 months of data, and this demonstrated a strong separation of male and female plants which appeared to be related to distance from the wetland and the percentage of soil surface covered under bush canopies. However, further examination of the complete data set did not show such a clear division between genders, and increasing soil hardness and decreasing depth of water had become the most influential factors.

At Brenda Park the lignum plants continued to thrive and flower for over 24 months standing in water, although unable to recruit either vegetatively or sexually. In contrast, dry lignum at Banrock Station did not benefit from repeated seasonal watering only 20 m away from the base of plants on heavy clay soils. These plants showed no significant change in height, seed production or expansion by vegetative growth during the study period, and essentially remained dormant. This site was selected on the edge of a lignum community with existing small lignum plants in adjacent open areas in anticipation that the lignum community would colonise into the open areas, but this did not occur.

A small managed over-bank flow at Banrock Station in November 2005 demonstrated that watering benefits for lignum were limited locally, with direct inundation of lignum plants having the greatest impact. At this site, the response of the monitored lignum to a short period of 2-3 weeks of flooding directly at the base of the plants was restricted to green shoots, few leaves and few flowers over 4 weeks, followed by a return to a dormant (but greener) state. Annual wetting of a larger lignum stand within 20 m of the monitored site had no apparent effect on the sampled plants, while the wetted lignum responded vigorously with strong new growth. No new seedlings were found on the monitored site, even where older seedlings had germinated previously, and no new cloned plants established, even though clones existed within the monitored transect. It appeared that the very low transmissivity of the heavy clay soils prevented moisture moving 20-30 m by lateral migration from the watered zone to reach the root systems of the plants being monitored.

No successful vegetative regeneration was noted at any field sites, although vigorous layering was noted in response to watering. At the Brenda Park wetland site, roots developed on branch nodes once they arched underwater, but these branches remained underwater for several months (the wetland remained inundated for 24 months due to a faulty water control structure), and there was no opportunity for the branches to land on moist soil and strike the roots to create a new plant. Arching branches touching dry soil did not generate roots or strike into the soil.

Light seed production was noted throughout the study and there was very dense seed production in October-November 2005 following significant rainfall. Glasshouse experiments indicated that lignum germinated more frequently in flooded conditions, rather than in response to rain events, with germinated seedlings floating until they could establish in moist soil (*see Chapter 8*). No seedlings were found following the heavy seeding in October-November 2005. Only one case of very limited sexual reproduction was found across all field sites, and this appeared to be associated with hydrochory depositing seed during a watering trial. The limited success in germination of lignum seeds appears to be due to insect granivory and soil moisture requirements (*see Chapter 8*). Temperature is also likely to be a factor, with the development of mould on seeds and seedlings in cooler conditions.

Lignum seeds appeared to have a high level of viability, with at least 50% of seeds germinating readily in petri dishes, provided that lids were removed and replaced regularly to reduce condensation to ensure that excessive mould did not develop while maintaining sufficient moisture. A time-lapse film of lignum germination was produced which showed that, within 8 days, seedlings grew roots first (5-10 mm long) before commencing growth of a pair of elongated cotyledons (10 mm long) (Figure 7.3). The seed coat was shed in most cases within 8 days. The figure of 50% germination may be an under-estimate, as seeds which did not germinate quickly became mouldy. However, it was noted in one case of field germination in winter that seedlings become covered in mould after germination and died (Chong, 2002).

The importance of local rainfall in increasing soil moisture and triggering flowering was highlighted, and future management of flow regimes to benefit lignum can be timed to value-add on this effect (*see Figure 9.2*).

The ploidy of lignum was investigated as a possible tool to separate sexual reproduction from vegetation reproduction in individual plants and thus to identify the mode of reproduction in local populations.Polyploidy is considered to provide adaptive advantages, which might assist lignum in its highly variable habitat (Lynch, 2006). A previous study had indicated that lignum was octoploid, which is unusual for the genus *Muehlenbeckia* (Chong & Walker, 2005). A single slide of lignum chromosomes was produced which confirmed that lignum is octoploid, but could not be replicated. The methodology for producing slides with chromosomes sufficiently visible for counting proved extremely difficult and time-consuming. It may be possible to use ploidy as a tool to investigate the relative distribution of sexual and asexual germinants and further to examine links to flooding or rain as water sources. However, it would require concentration and training for an investigator to become proficient enough to produce consistent slides for examination. Useful initial work has been carried out by Lynch (2006).

Further research

Questions requiring further investigation include examination of the impact of insect granivory on seed survival and thus sexual reproduction of lignum, and the relative impact of kangaroo grazing on the survival of lignum seedlings post-germination.

Another area requiring investigation is the relative importance of evapo-transpiration by lignum on local salt and water balances for the Murray Valley floodplain and its groundwater system. This perennial, deep-rooted large shrub grows predominantly on extensive clay flats at medium elevations on the floodplain, thus preventing evaporative losses from bare soil surfaces, and associated capillary rise of salt from highly saline groundwater which can be within 2 m of the surface. Thus any reduction in active growth phases, die-back or reduced recruitment would have potential for increased accumulation of salt stored in the floodplain, and increased accession of salinity to the river mainstream in subsequent floods. The status of lignum populations also needs examination, to determine if recruitment is being reduced by changed water regimes on the floodplain and drier conditions.

The potential use of lignum ploidy to separate cloned individuals and heterosexual individuals should be explored further, with a view to using this tool to identify the mode of reproduction in different populations. If successful analysis of the level of ploidy in lignum can be achieved,, the distribution of each reproductive strategy could be mapped in order to determine what conditions are conducive to each form, and environmental watering strategies might be developed to promote regeneration. The impact of changed water regimes on the relative success of each strategy should also be explored, particularly the role of flooding in promoting root growth prior to striking of vegetative clones from arched branches.

Further investigations are needed to understand the relative roles of sexual and asexual reproduction, and the conditions required to support successful recruitment. It will then be necessary to test and develop guidelines to manage inundation (flood or environmental watering) through its timing, extent, depth and frequency to trigger lignum seed production or to support lignum seedling survival. This may require serial watering to provide sufficient soil moisture to enable lignum seedlings to reach a self-supporting state, or to promote active vegetative cloning to produce new individuals. It is also important to analyse historic distributions of lignum populations, to determine whether there has been a retraction in these distributions and, if so, whether that could be attributed to changed water regimes and drier conditions on the floodplain.

Conclusions

Within the context of changed (reduced) hydrological regimes and drier floodplain conditions, the germination process for lignum is still occurring, with healthy lignum shrubs producing large quantities of seed in seasonal patterns which appear to be adapted to natural (pre-regulation) cycles of rainfall and flood peaks for the Lower Murray floodplain. Peak lignum seed fall in November would coincide with natural flood peaks in November-December (*see Figure 9.2*). However, few surviving seedlings were found during the study period, indicating that sexual reproduction is less successful than vegetative cloning through arching (layering) of branches or shoots developing from root nodes.

While many examples of past vegetative reproduction were found, no active examples were observed in this study. Many existing clones were included in monitoring, but no new clones were observed and little change was seen in existing ones. New growth on lignum plants took the form of strong vertical branches which eventually arched under their own weight, and potentially could strike into the ground adjacent to the parent bush to create a new individual. However, successful growth of a new plant was not observed in any non-inundated areas. In inundated areas, arching branches struck roots at nodes which dipped underwater after approximately 3-4 weeks. This indicated a mechanism where these branches would be lowered on falling water levels to reach moist soil as the flood receded, and then strike roots to create a new plant. This suggests that inundation is required to trigger the process of vegetative reproduction from arching branches.

Several examples of root cloning (multiple shoots from underground root nodes) were found, with a ring of individual vertical shoots emerging from the soil surrounding the parent plant. However, it was not clear what triggers this specific growth response. Root cloning was limited to a few individual plants in any given location, so this response could not be attributed to an external event such as flooding or rainfall which would have affected all plants in that location.

Thus the evidence was inconclusive on the link between water source and mode of reproduction. The results indicated that flooding may be a necessary condition for both sexual and asexual reproduction, triggering root development at nodes on arching branches prior to striking in moist soil to establish clones, providing the medium for hydrochory to distribute seeds, and providing necessary soil moisture for both forms of reproduction.

Chapter8 Soil Moisture for Seedling Survival: a key factor in woodland decline on the Lower Murray, Australia

Introduction

Floodplain plant communities are governed by the water regime imposed by the parent river (Poff *et al.*, 1997; Tockner & Stanford, 2002). Especially in arid and semi-arid regions, they are highly vulnerable to diversions of river flows, abstraction of groundwater and climatic changes (e.g. Stromberg, 1993; (Busch & Smith, 1995; Nilsson *et al.*, 2005; Shafroth *et al.*, 2002; Stromberg, 1993).

The floodplain of the River Murray in south-eastern Australia is dominated by woodland communities of eucalypt trees (Myrtaceae), notably river red gum (*Eucalyptus camaldulensis*) and black box (*E. largiflorens*), with a shrub understorey of tangled lignum (Polygonaceae: *Muehlenbeckia florulenta*). Like all dryland rivers, the Murray has an erratic ‘flood pulse’ (Walker *et al.*, 1995), and the floodplain flora is well-adapted to changeable patterns of flow. The Murray-Darling river system is intensively regulated (Maheshwari *et al.*, 1995), and about 80% of the system’s mean annual discharge is diverted, mainly for irrigation. Ongoing concerns about the long-term effects of water deprivation on the vegetation were documented in a detailed study some 25 years ago (Margules & Partners *et al.*, 1990; Roberts, 2003). In the last 5-10 years, the effects have intensified: there has been a Basin-wide trend toward less rainfall and lower discharge, and a severe drought prevails. Since 2000, the incidence of dying and dead trees has increased, and three quarters or more of mature trees along 700 km of the Murray Valley now are lost or nearly so (MDBC, 2005).

Flooding is the main source of water to recharge shallow aquifers and maintain soil moisture (Jolly *et al.*, 1992). The effects of regulation in the Lower Murray (the tract below the Murray-Darling confluence) have been to eliminate small floods (up to 1 in 7 y frequency; 25-40,000 ML d^{-1}) and reduce the frequency and duration of medium floods (up to 1 in 20 y; 40-60,000 ML d^{-1}) (Walker & Thoms, 1993; Maheshwari *et al.*, 1995; Roberts, 2003; Walker, 2006). The last effective overbank flows in this region were in 1996, 11 years ago. Local rainfall is a secondary water source. Rain may fall throughout the year, but most is in winter (28%) and spring (30%) (*see Table 2.1*). Rainfall was below average in 2004 (73% of annual average), above average in 2005 (116%) and well below average in 2006 (43%) (Bureau of Meteorology data for Renmark: annual average 260 mm).

River red gums are vulnerable to drought and reduced flooding, as they require frequent watering and so favour areas where flood frequencies are 1 in 2 y (Roberts & Marston, 2000). Red gums produce floating, adventitious rootlets in water-logged soil, to cope with anoxia during prolonged flooding (Heinrich, 1990). Successful recruitment is associated with river flows >40,000 ML d^{-1}, with associated over-bank flows onto the floodplain (George, 2004). The trees are stressed by saline groundwater (>20,000 µS cm^{-1}) within 2 m of the surface (Sharley & Huggan, 1995).

Black box are more tolerant of drought, and occur at higher elevations on the floodplain, but they too are showing signs of water stress, although they are reputed to endure 10 years without flooding (George, 2004; George *et al.*, 2005; MDBC, 2003). Recruitment is associated with larger river flows >80,000 ML d^{-1} (George, 2004). Black box trees can subsist on soil water during dry periods (Slavich *et al.*, 1999), but are stressed by saline groundwater (>40,000 µS cm^{-1}) within 2-4 m of the surface (Sharley & Huggan, 1995). Black box cannot cope with anoxia during prolonged flooding, and the distributions of seedlings and mature trees often indicate the outer limits of larger floods (Slavich *et al.*, 1999).

Tangled lignum (hereafter ‘lignum’) is a long-lived, deep-rooted perennial shrub that attains 3 m height (Jensen *et al.*, 2006). The effects of reduced flooding and drought are little studied, but the plants become dormant and recover rapidly when water becomes available (Jensen *et al.*, 2006) (*see Chapter 7*). Lignum reproduces sexually and vegetatively, with new plants striking from nodes on roots or on branches contacting the soil. Episodes of flowering and seed production have been recorded (Chong, 2002; Lynch, 2006; Southgate, 1988).

Successful recruitment in Lower Murray floodplain communities relies on the accrual of mature, potentially reproductive individuals (*see Figure 2.5*), in which germination and seedling survival are critical stages. While some information is available about the germination biology of each species (Chong & Walker, 2005; Dexter, 1970), little information was found about the processes of germination and seedling survival applicable to the increasingly dry Lower Murray floodplain. At the population scale, recruitment rates in river red gum and black box have been shown to be insufficient to maintain current populations, which are suffering higher adult mortality due to changed floodplain conditions (George *et al.*, 2005). While local stands of eucalypt seedlings and saplings can be associated with known over-bank flows (George, 2004), few studies were found where examples of eucalypt or lignum germination were recorded in the field (Chong, 2002; Jensen, 1998; Jensen, 1983) and seedling counts were not included in emergence trials for seed bank investigations (Jansen & Robertson, 2001; Nicol *et al.*, 2007) (*see Chapter 2*).

The importance of sustained soil moisture was demonstrated following a flood in 1981, when hundreds of river red gum and black box seedlings grew to 100-150 mm in grazing exclosures but did not survive heatwave conditions (> 40°C) in mid-summer (Jensen, 1983). Similar events have been reported in relation to Living Murray watering trials, with strong germination responses in grazing exclosures but 100% mortality of seedlings within 1-2 months (Dr T. Wallace, Dept of Land, Water & Biodiversity Conservation, pers comm.). Experimental data from this project suggest that seed production from healthy trees and germination rates do not appear to be limiting in recruitment processes, although seed fall is significantly reduced in stressed trees and grazing by sheep removes most seedlings < 100 mm (Jensen *et al.*, 2008b, c) (*see Chapters 5 & 6*). Earlier results indicated that seedling survival is closely linked to soil moisture levels, with minimum soil moisture of 10-20% required for seedling survival (Jensen *et al.*, 2008) (*see Chapter 4*). This is a critical factor for germination and early survival of seedlings, as well as maintenance of mature plants. Recent research indicates that river red gums need up to 2 years to develop sinker roots into sub-surface moisture sources (George, 2004) and thus are reliant on near-surface soil moisture reserves to survive this stage, indicating that soil moisture is a critical factor in the early establishment period.

Under the currently over-allocated conditions of the catchment, it appears unlikely that seedlings would be able to obtain sufficient moisture to survive their first summer after germination, once the now shorter, , less frequent floods recede, given the extremely high regional rates of evapo-transpiration which rapidly dry out surface soil. If the soil moisture reserves are insufficient to support seedlings, the only other source of water would be local rainfall, such as summer thunder-storms, which are a feature of regional weather patterns in the Lower Murray valley (*see Chapter 3*).

Therefore, the experiment described here was undertaken to elucidate critical levels of soil moisture for germination and survival of seedlings, and to test the response of lignum cuttings to various water regimes. It was anticipated that seeds would germinate and cuttings would develop roots and/or shoots in response to a minimum amount of moisture, and that seedlings and shoots would not survive if soil moisture was not maintained above a minimum level. The water regimes applied were designed to test the effects of a combination of rainfall events and floods/watering events.

Methods

Source Material

Soil, seeds and lignum cuttings were collected from sites on the Lower Murray floodplain in South Australia, between Morgan and the New South Wales border (*see Figure 3.1*). These were Brenda Park, near Morgan, Banrock Station, near Barmera, and Monoman Island Horseshoe Billabong, Pipeclay Lagoon, Pilby Floodplain, Twin Creeks and Werta Wert Lagoon on the Chowilla floodplain near Renmark. All sites were linked to controlled watering events, and those at Chowilla are part of The Living Murray Program developed by the Murray-Darling Basin Commission (http://www.mdbc.gov.au). Seeds were collected as mature closed fruits from the eucalypts and as achenes from fruiting female lignum bushes and stored at 4°C in paper bags until use. The eucalypt fruits shed their seed within a few days. The lignum cuttings were taken from the mid-sections of new stems, cut with a minimum of two nodes.

Soil samples from Clarks Floodplain and Chowilla Floodplain were sieved (5 mm) and mixed for experimental use. The floodplain soils are mainly grey cracking clays mixed with river sand and silt (Hollingsworth *et al.*, 1990). They have low transmissivity, and are likely to become very hard on dry, elevated areas and develop deep cracks in dry flood-prone areas. They resist wetting, but retain moisture for longer than sandy soils. Previous soil analyses (Jensen *et al.*, 2007a) indicate some acidity (pH 5.4-6.0), low surface salinity at low elevations (294-1230 μS cm^{-1}) and very little moisture (2.4-7.0%) in dry seasons. The pH and salinity conditions are unlikely to inhibit germination of the eucalypt species (Tucker, 2003).

The source material included seeds of river red gum and black box, and seeds and cuttings from lignum. Two distinct sizes of red gum seeds (>200, >500 μm) were found in seed traps. The smaller seeds were about 20% of the total seed volume; they were less viable than the larger seeds, slower to germinate and producing smaller seedlings (Jensen, unpubl.). They were separated in the experiment to compare their responses to soil moisture. Lignum cuttings were kept in Reverse Osmosis (RO) water in the laboratory for 14 d, until roots and/or shoots developed at the nodes.

Sample Preparation

Seed from field sites was sorted into river red gum (>200, >500 μm) and black box seeds (>500 m). Samples were weighed using a Powder & Bullet scale (Redding® Model #2: 1 grain = 65 mg), according to a visual estimate of the number of seeds expected to germinate, thus 65 mg red gum >200 μm (c. 20 seedlings expected) and >500 μm (30 seedlings), 32.5 mg black box >500 μm (30 seedlings) and 97.5 mg lignum seeds (30 seedlings). The weight of black box samples was halved because chaff of this species could be excluded by sieving, but red gum samples included chaff of similar size and weight to the seeds. Seeds were mixed with 10 g coarse washed sand, dispensed over the soil surface of experimental pots (1 L plastic pots containing c. 360 g soil) using a 1 mm tea strainer. For the lignum cuttings, two pre-struck cuttings were laid on the surface of each experimental pot.

Experimental Design

The experiment was designed to determine the ranges of soil moisture needed to germinate and maintain eucalypt and lignum seeds and seedlings, and to encourage the growth of cuttings from lignum. It compared the responses of five types of source material to four watering treat¬ments, replicated five times. A 'constant soil moisture' control (10-25% moisture) was included to indicate the potential germination rates of viable seeds, and a 'dry' control was added to see whether seedlings would germinate without added water. A further three samples without seeds or cuttings were subjected to constant moisture to confirm that the soil had no remnant eucalypt or lignum seeds. Pots were grouped in a glasshouse, according to source, and treatments were then randomly assigned (Figure 8.1).

The experiment lasted for 12 weeks. Four watering treatments were devised to simulate rainfall, flood, rainfall followed later by flooding, and flooding followed later by rain. Rain treatments consisted of a single application of 50 mL, which equated to a rainfall event of 5 mm in 100 mm x 100 mm pots. Flood treatment consisted of 10 mm depth of inundation maintained for 4 weeks. The sample pots were allowed to dry out after a rain or flood treatment before any further treatment. The four treatment scenarios were (1) rain (50 mL added beginning weeks 1, 5 and 9), (2) flood (10 mm inundation maintained weeks 1-4, then allowed to dry weeks 5-12), (3) rain plus flood (50 mL added beginning week 1, allowed to dry, flooded weeks 5-8, then allowed to dry weeks 9-12) and (4) flood plus rain (flooded weeks 1-4, allowed to dry weeks 5-8, 50 mL added beginning week 9, allowed to dry weeks 9-12). In the River Murray locally the long-term median salinity is 600 µS cm^{-1}, tending to lower values during spring freshes (Mackay & Eastburn, 1990). To simulate typical floodwater salinities such as are likely to be experienced from over-bank flows, sea salt (Lotus™) was added to yield a salinity of 500 µS cm^{-1}. Controls also received water of 500 µS cm^{-1}. Rain simulations used laboratory RO water.

Fixed volumes of water added (50 mL) proved not to have equal effects on soil moisture in the constant moisture treatment, and it was necessary to add more water to pots that were visibly drying out and stressing seedlings. Soil moisture levels in the moist control therefore were more variable than anticipated, apparently due to the presence in some pots of small (<5 mm) lumps of clay. Soil moisture (% volume) was logged every four days using a Delta-T ML2 Theta Probe. A visual assessment was also made to establish a relationship between visible changes in moisture status with measured moisture content (0 = dry, 1 = drying, 3 = moist, 4 = very wet, 5 = flooded). It was too difficult visually to distinguish an intermediate stage between drying and moist, so no category 2 was scored.

Figure 8.1 Soil samples at commencement of experiment, showing dry soil samples spread with 200 µm river red gum seeds prior to application of water (left) and lignum seed samples after watering, with colour coded stickers to indicate randomised application of water regimes (right).

Experimental conditions

The experiment commenced on 1 May 2006 (thus, autumn-winter), in a glasshouse on The University of Adelaide campus, without shadecloth and at a temperature of 30°C (5-10°C above local daily maximum temperatures in winter, and similar to those in the Murray Valley). A control malfunction allowed temperatures to exceed 30°C several times. Temperatures and relative humidity were monitored with a wet-dry bulb hygrometer over a 20 s interval at 1100 h on alternate days, and the data were converted to relative humidity calculating vapour pressure from conversion tables (Pearcy *et al.*, 1989). Relative humidity was in the range 51-84%, which would have mitigated the drying effect of elevated temperatures (Figure 8.2). For all eucalypt and lignum seed samples, the number of germinating seedlings was recorded every four days. For lignum cuttings (50% male and 50% female), the number of roots and shoots, the length of shoots, the greenness of the cuttings (green, yellow, dead) and number of flowers were recorded, also every four days.

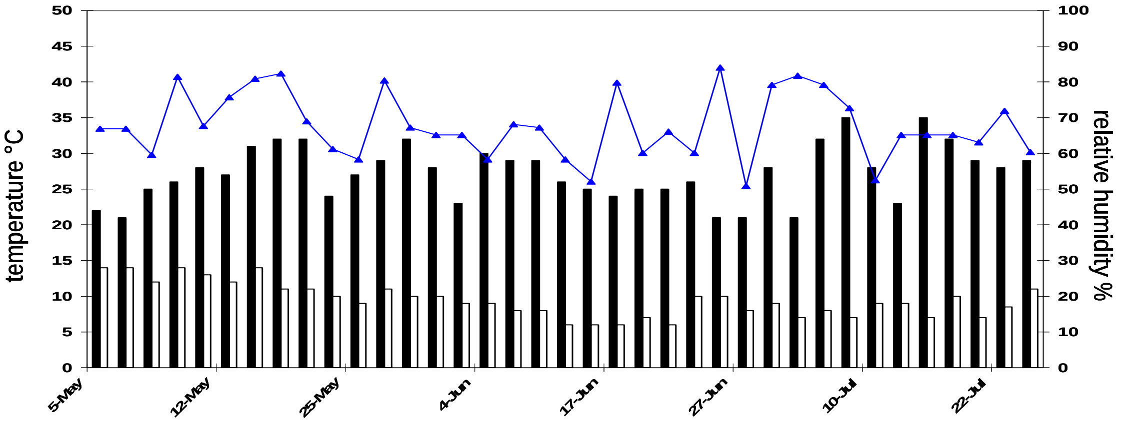

Figure 8.2 Glasshouse conditions for the soil moisture experiment over 12 weeks, showing minimum (range 6-14°C, white bars, left Y-axis) and maximum temperatures (range 21-35°C, black bars) and relative humidity (range 51-84%, line, right Y-axis).

Results

The responses of the seeds and cuttings to the six water regimes are presented in Figures 8.3-8.6 (river red gum (>500 µm), black box, lignum seeds and lignum cuttings respectively). A single river red gum seedling germinated from all samples >200 µm, so these results were not plotted.

No eucalypt or lignum seedlings grew in the soil control (no seeds added), confirming that all seedlings of these species germinated from added seeds. No eucalypt or lignum seedlings (or any other species) germinated in the dry control (Figures 8.3-8.6), confirming the need for soil moisture to trigger germination. No lignum cuttings survived beyond 11 d in dry treatments. Some apparently dry samples initially registered very low soil moisture content values, but all dried out completely within 11 d.

Significant numbers of seedlings of all three species germinated in the controls (18-22 red gum seedlings, 38-42 black box, and 15-18 lignum) confirming the expected positive response to sustained soil moisture. A corroborative test indicated the potential number of viable eucalypt seeds per sample was much higher than estimated in the experiment design (30), with 38 river red gum seedlings (n = 3) and 129 black box seedlings (n = 3). Control pots also germinated significant numbers of seedlings of other species previously found in sampling of the soil seed bank, particularly native terrestrial annuals of the chenopod family (Jensen *et al.*, 2007a; *see Chapter 4*).

Red gum responded best to treatments including rain events (150 mL single watering), with highest mean seedling germination numbers in the rain treatment (2 separate rain events), but with rapid decrease in surviving seedlings as soil moisture content declined (Figure 8.3). Black box samples responded in a similar pattern but with approximately twice as many seedlings compared to red gum (Figure 8.4). Lignum seeds responded more positively to flooding (extended inundation) than to rain (Figure 8.5). Lignum cuttings preferred flooded conditions up to 45 d and moist conditions (in both initial phase of rain treatment and control) (Figure 8.6).

Black box and lignum seeds demonstrated an ability to germinate underwater, with black box settling onto the substrate and surviving underwater, while lignum seeds floated and developed roots and shoots (Figures 8.4, 8.5). River red gum seeds imbibed water and floated, surviving where they were able to settle onto moist substrate, although a few individuals did persist under shallow water (Figure 8.3).

Both river red gum and black box had higher numbers of seedlings in response to the rain treatment, which provided a moist soil substrate and then gradually dried out, compared to flood (inundated soil) or control (constant moisture) (Figures 8.3, 8.4). Lignum seeds responded differently, with higher numbers of seedlings under constant moisture conditions, compared to rain treatments (Figure 8.5).

Deferred germination occurred in all three species of seeds, with new batches of seedlings germinating from the same samples when a new set of favourable conditions arose (Figures 8.3-8.5). River red gum germinated approximately 50% of potential seedlings in the first event, while black box germinated about 30%.

Lignum cuttings did not respond well to treatments generally, with all samples declining in health, and many dying, possibly since the experimental set up was not conducive to setting roots. However, a sufficient number maintained shoots and demonstrated a pattern indicating a better response in constant moisture compared to flood (Figure 8.6).

Lignum cuttings proved difficult to sustain under experimental conditions, even though all cuttings had struck either roots or shoots or both. The health (greenness) of cuttings was sustained longest under flood treatments, with yellowing after 45 d (Figure 8.6). In the flood/rain treatment, the cuttings appeared to be brown and dead at the end of the flood (48 d), but responded to rain on day 55 and produced new shoots. A similar decline in health was noted in the flood treatment and the rain/flood treatment, with an improvement in health as the flooded conditions phased into moist conditions. The most successful sample was two cuttings from a female bush, under constant moisture. One cutting produced two strong shoots (reached 190 mm height), the other had 9 shoots varying from 100 mm to 210 mm. The latter cutting developed a total of 73 flowers which developed and shed seeds within the 78 days of the experiment.

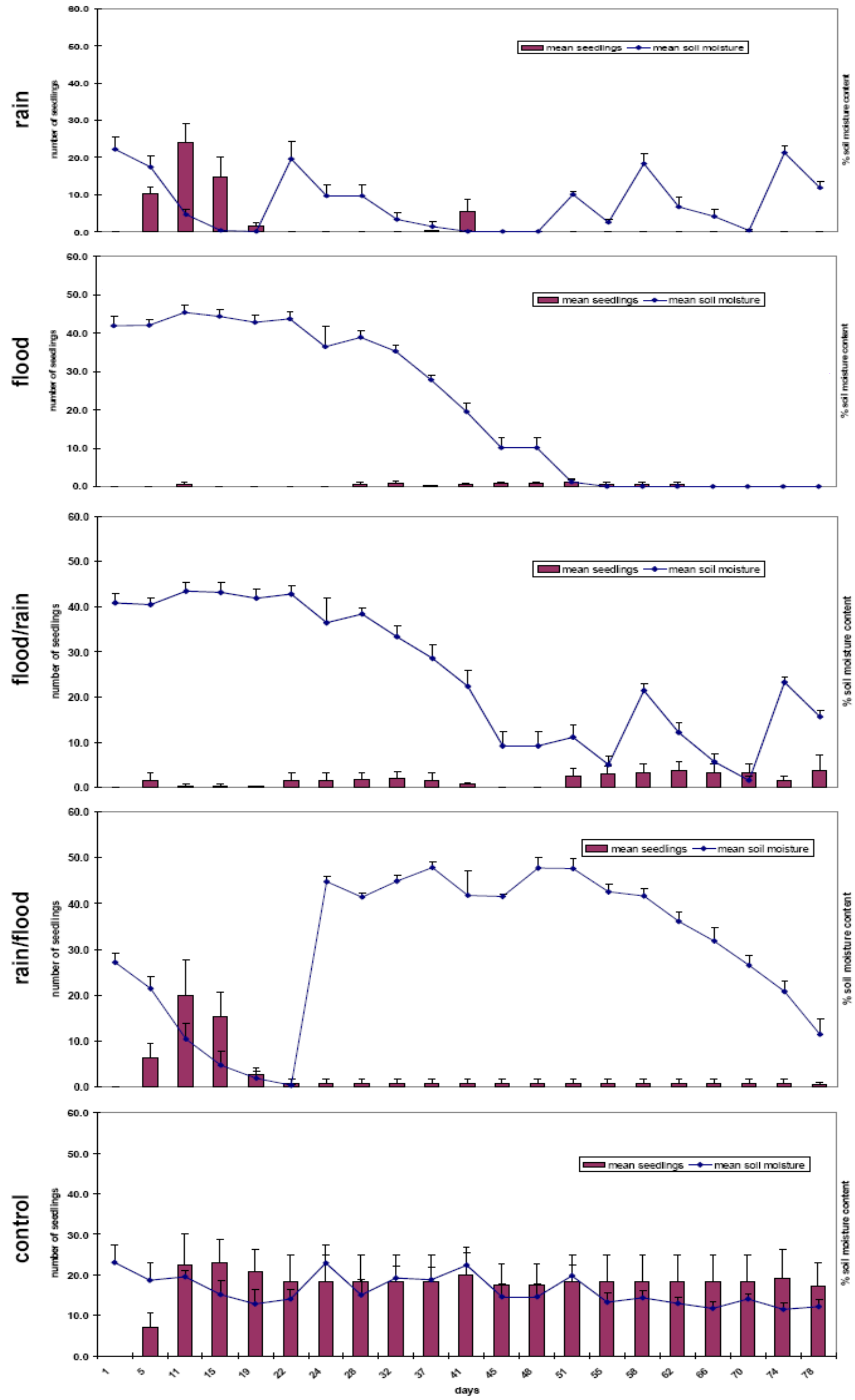

Figure 8.3 Response of large river red gum seeds (>500µm) to rain, flood, rain/flood, flood/rain and control treatments, showing the highest number of seedlings in response to rain, but not surviving drying below 10% soil moisture. Note second germinations from the same seed source following a second wetting event (rain, flood/rain). There is a high number of seedlings in response to constant moisture, but insignificant germination under flooded conditions (coloured bands).

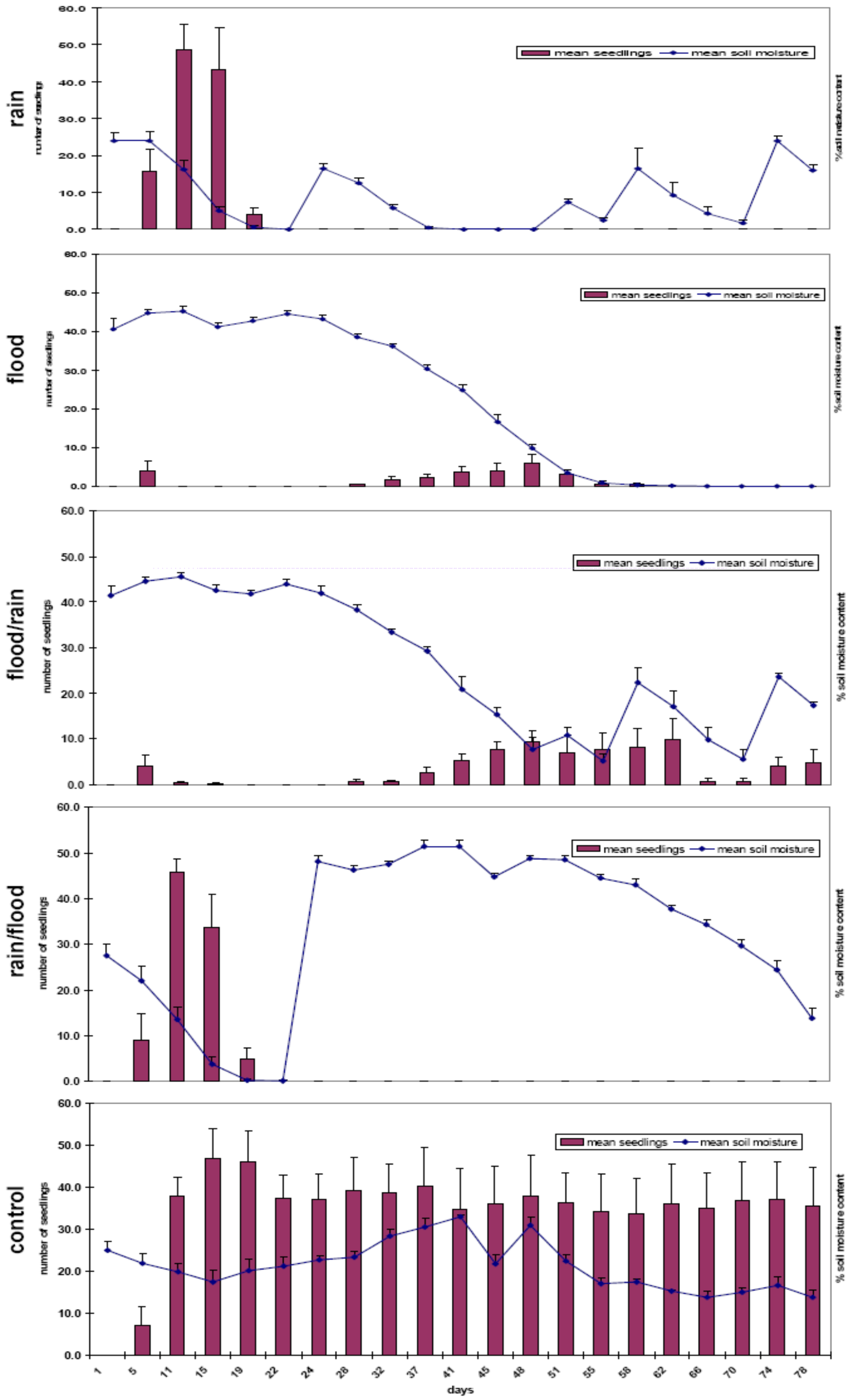

Figure 8.4 Response of black box seeds to rain, flood, flood/rain, rain/flood, and control treatments, showing the highest number of seedlings in response to rain, but not surviving drying below 10% soil moisture. Note second and third germinations from the same seed source following subsequent wetting events (flood, flood/rain). There is a higher number of black box seedlings in response to constant moisture, compared to red gum. (Coloured bands = flooded conditions).

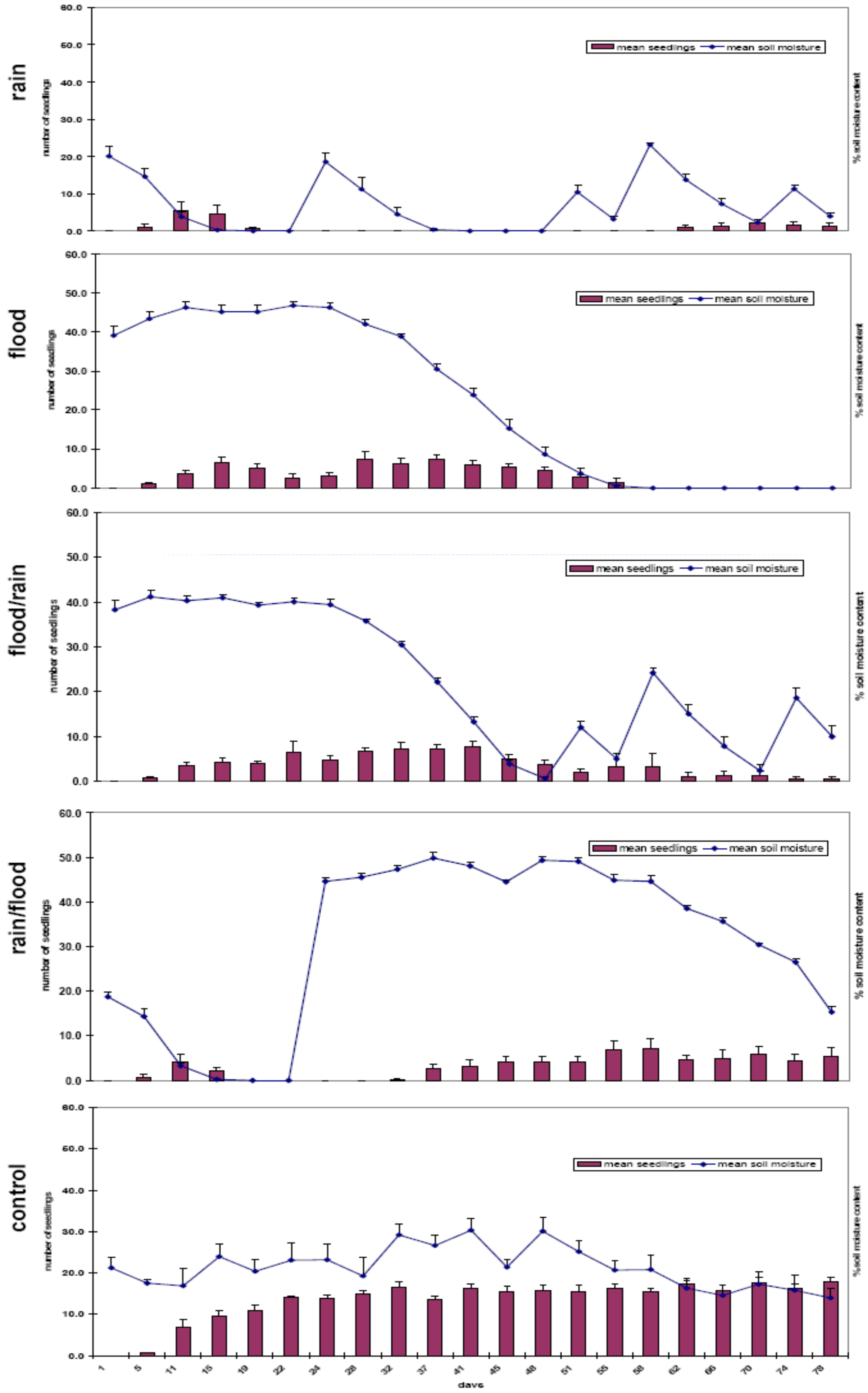

Figure 8.5 Response of lignum seeds to rain, flood, flood/rain, to rain/flood and control treatments, showing the higher number of seedlings in response to flood, but not surviving drying below 10% soil moisture. Note second germinations from the same seed source following subsequent wetting events (rain/flood, rain, flood/rain). Seedlings germinating under flooded conditions float until the flood recedes (coloured bands = flooded conditions). The highest number of seedlings germinated in response to constant moisture.

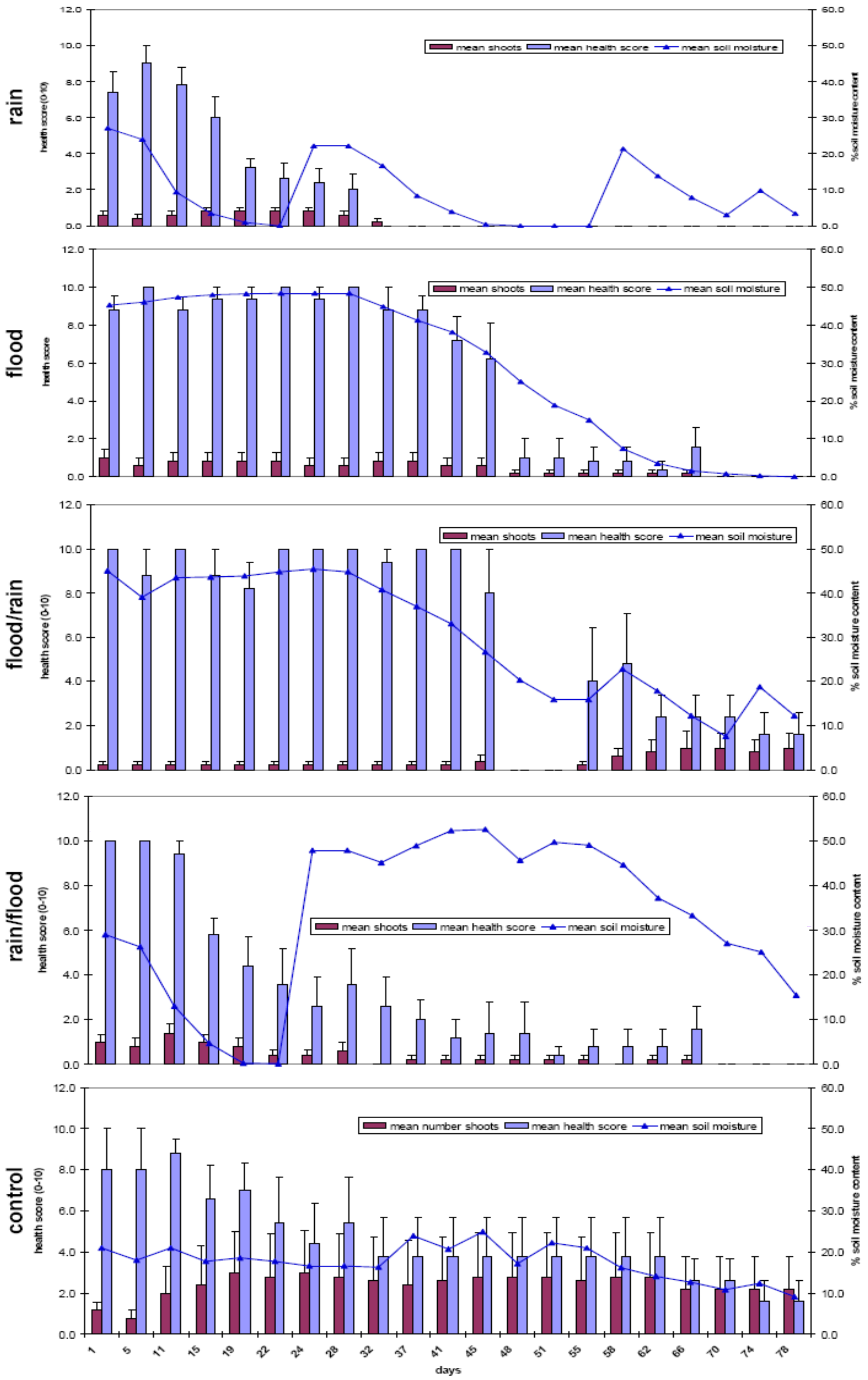

Figure 8.6 Response of lignum cuttings to rain, flood, flood/rain, rain/flood and control treatments, showing the higher number of shoots in response to rain, but not surviving flooded conditions (coloured bars) or drying below 10% soil moisture. Note recovery of health and shoot production from apparently dead cuttings following exposure to air as sample reduced to below 20% moisture (flood/rain). The highest number of shoots emerged and continued to grow in response to constant moisture.

The maximum soil moisture content measured for the floodplain clay soil was 59% in a flood treatment, with most values for flood treatments in the range 40-55%. Water-logged samples were 30-40% and controls (moist conditions) were generally in the range 15-25% (Table 8.1, Figure 8.7). As the soil dried, it began to crack and crumble, making use of the Theta Probe sensor difficult. Readings below 3% were inconsistent using the Theta Probe, which relies on uniform contact between the soil and its 5 probes to generate the reading. Readings which were 'under range' were scored as 0.01. There was considerable variation between values assigned using the visual soil moisture ratings system and the soil moisture content measurements, particularly in the moist and drying categories, although the median values were in the expected ranges. Based on the appearance of drying of soil and wilting of seedlings, the critical range of soil moisture to maintain seedlings is when the visual rating drops from 3 to 1, which occurs below 10% soil moisture content (Figure 8.7). Death of seedlings occurred very rapidly once wilting began (<5% moisture), with seedlings dead in 1-2 days.

Visual soil rating	0(dry)	1(drying)	2(N/A)	3(moist)	4(wet)	5(inundated)
Maximum % soil moisture	27	18.9	0	38.5	53.5	59
Minimum %	0	0	0	0	8.6	15
Median %	0.01	1.75	0	16.53	36.6	44.65

Table 8.1 Variations in soil moisture content values measured against visual soil ratings. Note that category 2 was not scored, owing to the difficulty in distinguishing degrees of moist soil visually.

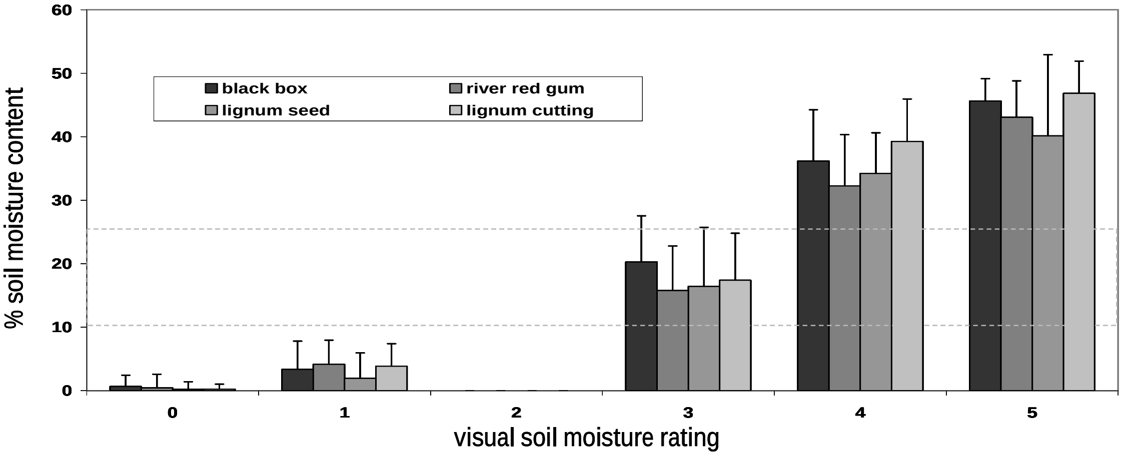

Figure 8.7 Comparison of % soil moisture content (Y-axis) and visual soil moisture ratings (0-5, X-axis) indicates that dry conditions (rating=0) occur at <5% soil moisture content, drying soil (1) 5-10%, moist soil (3) 15-25%, water-logged conditions (4) occur in the range 30-40% and inundated conditions (5) 40-55%. The shaded band at 10-25% indicates the soil moisture zone where best growth responses were recorded.

Discussion

While the link between flooding and recruitment for eucalypts in the Murray Valley is well-documented (Bren, 1988; Dexter, 1970; George, 2004), the mechanisms for sustaining soil moisture for seedlings within this process have had less attention. This has become much more critical in the context of a floodplain severely deprived of water by over-allocation of resources. It has been estimated that, under the current regime of diversions, the floodplain may contract to 30% of its original area (Walker & Thoms 1993, Roberts 2003). As discussed in Chapter 2, the likely timing of germination is more likely to coincide with hotter, drier conditions, with less soil moisture to sustain seedlings through their first summer.

The importance of sustained soil moisture in the period following germination of eucalypt and lignum seedlings is emphasised by these experimental results, which indicate that stressed seedlings may perish in 1-2 days if water-stressed at critical early stages. Under current conditions, with no significant water sources likely to be available, no seedlings (including those triggered by experimental watering programs since 2004 and the wetter than average season in 2005) are likely to survive predicted hot dry conditions in summer 2007-08 to reach reproductive age (>10 y).

It was found that there was a germination response by both eucalypt species and lignum seeds within 5 days to each moisture regime, confirming the hypothesis that such a response would occur. Survival of seedlings required maintenance of soil moisture above 10%, and seedling death occurred if moisture dropped below 5%. The health score for lignum cuttings declined under all treatments, although the average number of shoots remained stable in the controls (constant moisture). Interestingly, both red gum and black box responded with higher numbers of germinants to rain treatments (once only application of 150 mL), compared to the simulated flood (inundated to 10 mm for 4 weeks). It appears to be a requirement that red gum in particular can settle out of inundated conditions onto exposed moist soil within 3-4 days of germination. Black box needs to settle within 10 days of germination. These results indicate the very transient nature of suitable conditions for germination success and potential recruitment success.

It appears likely that rain-triggered germination of both eucalypt species may be occurring relatively frequently, following rain events >5 mm (Jensen *et al.*, 2007b). However, the lack of surviving recruits observed on the floodplain indicates that there is almost no survival without follow-up sources of water. This suggests the impact from the loss of small and medium floods from the floodplain has been under-estimated, with no significant recruitment accumulating from either rain-triggered germination or flood-triggered germination. Related findings in this study suggested that rain-triggered germination may be the source of scattered, low-density ‘maintenance’ recruitment which complements the larger ‘boom’ recruitment associated with flood events (Jensen *et al.*, 2007b). With flood-triggered recruitment also suppressed, the rate of recruitment would be inadequate to sustain Lower Murray eucalypt woodland communities into the future, as already reported for the Banrock Station floodplain (George, 2004).

Smaller river red gum seeds (<500 µm) apparently do not contribute to seedling germination in the field, even though these seeds contributed 10-20% of viable seed counts (*see Chapter 6*). Seedlings from the smaller seeds were observed to be smaller and had slower growth rates in germination trials, suggesting that they are not significant, compared to the larger seeds with high germination rates and rapid growth rates to produce healthy seedlings.

The results for lignum indicate a preference for flooded conditions, with seedlings distributed by hydrochory. There was a significant response by lignum seeds to flooding, with germinating seeds floating on the water surface until able to settle onto moist soil. The results here indicate that lignum seeds can survive flooded conditions for a period of at least 45 d, provided that they gain access to a moist soil surface to strike roots and develop into surviving seedlings. Only one occurrence of 10 lignum seedlings was located during 30 months of field observations in this study, including a season of above average rainfall and prolific seeding by lignum bushes. It appeared that the seeds had been distributed to this location by hydrochory during a watering event. No instances of successful recruitment of lignum have been reported in the literature. One previous record indicated that lignum germinated after good rain in winter, but subsequently was overcome by fungus in the damp, relatively cold conditions (Chong, 2002).

The volumes of water available and the constraining logistics of providing water to floodplain sites limit the potential effectiveness of environmental watering events. Previous recommendations for management of environmental flows presumed that over-bank flows would be manipulated under river management to provide enhanced environmental benefit, but it appears unlikely in the short term that over-bank flows can occur due to the severe drought and serious water shortages for consumers. Another option to consider is to apply limited quantities of water through direct irrigation of germinated seedlings, rather than watering to fill wetland sites (a form of flood irrigation requiring larger volumes of water. The results here suggest that applications of 5 mm at weekly intervals would be sufficient to maintain soil moisture content at 10%. Previous results confirm this, and suggest that 100 mm at fortnightly intervals could be equally effective (*see Chapter 4*).

These experimental results emphasise the importance of managing all available sources of water to maintain soil moisture during critical stages, particularly during the first summer after germination has been triggered. It appears critical to maintain soil moisture content above 10% for up to 2 years, until seedlings can develop a tap root to source water from fresh water lenses overlying the saline regional groundwater (George, 2004). It was observed that 6 surviving seedlings out of 360 germinants on a strandline on Chowilla Floodplain were able to survive for at least 27 months with stunted habits during summer when soil moisture declined to 1.9%, then grew from 10 mm to 100 mm high in 4 weeks following rain, when soil moisture increased to 38% (Jensen *et al.*, 2007b). This suggests that the seedlings, which germinated at the high water mark of a watered site in June (winter), had sufficient time and water to develop deep enough roots to reach stored soil moisture at deeper levels. Excavation at the site showed soil moisture was ‘under range’ (< 3%) to 100 mm, suggesting that the surviving seedlings had developed root systems reaching soil moisture sources deeper than 100 mm.

Experimental data here confirm that minimum soil moisture levels of >10% are required for early seedling survival, reinforcing earlier results (*see Chapter 4*). Management of the sequence of rainfall, flooding and artificial watering may provide opportunities to sustain adequate soil moisture for successful small recruitment events. This suggests a useful basis for timing managed watering events to support recruitment. Artificial watering offers some flexibility in timing but limited duration and volumes. Volumes of water available under regulation are much less than under natural floods, and there are constraints on delivery of water to higher elevations on the floodplain. The effectiveness of ‘environmental flows’ for recruitment of eucalypts could be increased by linking the timing of late spring-early summer watering with local rainfall, to sustain soil moisture above 10% for as long as possible during seedling development in the first summer immediately following germination.

In the absence of available water for delivery of environmental flows, another option to consider is irrigation, with application rates equivalent to 5 mm per week or 10 mm per fortnight sufficient to support seedlings through their first summer, until a tap root develops or local rain events provide an alternative source of surface soil moisture.

Soil moisture availability has been shown to be critical for successful plant recruitment elsewhere in semi-arid zones (Cooper *et al.*, 1999). Conclusions from this experiment and field observations during the overall study indicate that a succession of flooding or rainfall events over a season or consecutive years may be required to maximise seed production and recruitment. Single, short-lived watering events clearly are not sufficient to sustain germinants through dry periods. Given that synchrony between peak seed fall and peak flood flows has been disrupted by river regulation and extractions, environmental flows could be managed to enhance the effectiveness of any available water which coincides with the natural seasonal peak of late spring-early summer.

Chapter9 Application to Management of Environmental Flows

Introduction

Rehabilitation of the health of floodplain vegetation has become a priority management objective associated with the provision of environmental flows (MDBC, 2003). Management of environmental flows for ecosystem repair in the Murray Valley is a practical example of the ecohydrological principle of using functional relationships between hydrology and biota for control of ecosystem processes (Zalewski & Robarts, 2004). It has been suggested that, in the absence of detailed data to apply to these general models, the natural hydrograph can supply a template for river management in the Murray Valley (Jensen, 2002a; Walker, 2002).

Given widespread acceptance of the hypothesis that changed water regime, specifically reduced flood frequencies, later timing and drier floodplain conditions, is the cause of decline in riverine and floodplain ecosystem health, the provision of environmental flows is proposed as the management solution to reverse this decline. Priority management questions therefore centre on identification of the key elements of the local water regime to be delivered to a target site to generate desired outcomes. The first step was a review by a scientific panel of the flow requirements for the River Murray from Lake Hume to Wellington (approximately 2,000 km) (Thoms *et al.*, 2000). A separate scientific panel examined the flow needs for the terminal Lower Lakes and Coorong, owing to their very different hydrological characteristics (Jensen *et al.*, 2000). In principle, all of these recommendations were based on partial reinstatement of small to medium floods, which are missing or greatly reduced under regulated river conditions. Proposals for environmental flow management aim to make most effective use of available water, estimated at 20% of natural flows (Jensen *et al.*, 2000; Thoms *et al.*, 2000). Management questions require predictions of local ecological responses to different water regimes under possible flooding scenarios (Roberts *et al.*, 2001).

However, recommendations for the return of 3,000 GL annually as environmental flows (Jones *et al*. 2002) have translated to much lower volumes in a staged process, starting with 500 GL and eventually reaching 1500 GL by 2014. Thus, much smaller volumes are available and choices have been made in selecting priority habitats and species.). The definition of environmental flows for the Murray-Darling Basin encompasses the provision of water allocations designed to create micro-habitat suitable for recruitment of key species. The application of the first environmental flows is being developed as part of the *Living Murray Program*, which includes the Chowilla Floodplain as part of one of its six selected icon sites (http://www.mdbc.gov.au The initial allocation of 500GL, to be delivered by 2009, will be allocated to six icon sites currently suffering decline. The Chowilla Floodplain in the Lower Murray Valley (one of the study sites for this project) has been classified a ‘Significant Ecological Asset’ under the MDBC Living Murray program to receive an allocation of environmental flows (DLWBC, 2004).

Environmental Flow Needs

Work on the opportunities for managing environmental flows for the Lower Murray has been developed since 1993 (Jensen, 1998; Jensen *et al.*, 2003; Jensen *et al.*, 2000; Jensen & Nicholls, 1997; Jensen *et al.*, 1997; Jensen *et al.*, 1994; Roberts *et al.*, 2001; Thoms *et al.*, 2000). These recommendations for changes in hydrological management are all based on proposals to restore key elements of the natural hydrograph, particularly relating to spring freshes and flood events in the river mainstream, and the frequency with which they reach floodplain wetlands. From the increasing body of scientific work, the message has become more urgent to introduce environmental considerations such as natural variations in levels, natural flooding frequencies and distribution, and fish passage. The potential impact on biodiversity through the loss of intermittent drying cycles in formerly temporary wetlands now inundated by raised river levels has also been highlighted, and recommendations made for intermittent drying cycles with natural seasonality.

The importance in the Murray Valley of the flood pulse and its wetting and drying effect in maintaining biodiversity and in triggering regeneration and breeding has been well demonstrated (Boulton & Lloyd, 1991; Pressey, 1987, 1990; Roberts *et al.*, 2001; Thoms *et al.*, 2000; Walker, 1986, 1990; Walker *et al.*, 1994; Walker *et al.*, 1992). The key role of wetting and drying cycles in recruitment emerged in the 1980s (Pressey, 1987). Early work on this topic by Australian wetland ecologists included a study by (Briggs & Maher, 1985), which suggested that, in floodplains of the Murray Darling Basin, essential nutrients become adsorbed onto water-logged clay substrates, requiring a period of desiccation and re-wetting for their release into the food chain. These findings then led to recommendations for drying and re-wetting permanently inundated wetlands to increase their productivity, in surveys of wetland status and condition (Pressey, 1986; Thompson, 1986).

In the 830 km lower reach of the River Murray below the Darling River junction at the town of Wentworth (*see Figure 3.1*), natural drying cycles occurred characteristically for at least 2-4 months in autumn-winter, with re-filling ideally in spring-summer (Pressey, 1987). The minimum drying period allows complete drying and cracking of the wetland bed. The minimum period of inundation would allow the completion of some life cycles, with 4 months inundation or longer required for completion of life cycles in a full wetland community. These conclusions apply in reverse to droughted wetlands higher on the floodplain, which are not flooded often enough under regulated flow conditions. The ideal time for wetting is during spring and summer, to coincide with the natural pattern of flow peaks in the unregulated river system (Jensen, 2001, 2002b).

Based on monitoring of several wetlands in the Lower Murray Valley with managed water regimes, a baseline biennial operating regime is being recommended for wetlands which have been permanently inundated at pool level under regulated flow conditions (Jensen, 2002b). This wetland water regime is based on a wet phase of 12-14 months maximum fully inundated, and 6 months maximum dry phase. With the transition phases between wet and dry states, this provides a 2-year cycle, with a dry period every second year (Figure 9.1).

The timings recommended for wetting and drying cycles in wetlands of the Murray Valley are based on the Guidelines for Hydrological Management (Tucker *et al.*, 2002), and monitoring results for Little Duck Lagoon, in the Riverland region, under a wetting and drying regime (Jensen, 2001, 2002b).The extended wet period is designed to maximise diversity of aquatic macroinvertebrates (water animals) and macrophytes (water plants). It was found that a longer than annual cycle was required to attain maximum numbers of individuals and numbers of species. The maximum set on the dry period is designed to limit biomass production on the wetland bed, as excessive build up of biomass in the dry phase can lead to oxygen deprivation and ‘black water’ events during re-filling, which will kill fish (Jensen, 2002b).

The wetting and drying cycle is a guide, which will vary with seasonal conditions. For any given wetland, there should be a drying cycle of at least 2 months, at least once every three years on average. The managed cycle should take account of rainfall and floods, to maximise the benefit from soil moisture to support regeneration, and to maximise the duration of flooding. The wetland should be filled at least once every two years, on average, and dried at least once every three years (Jensen, 2001).

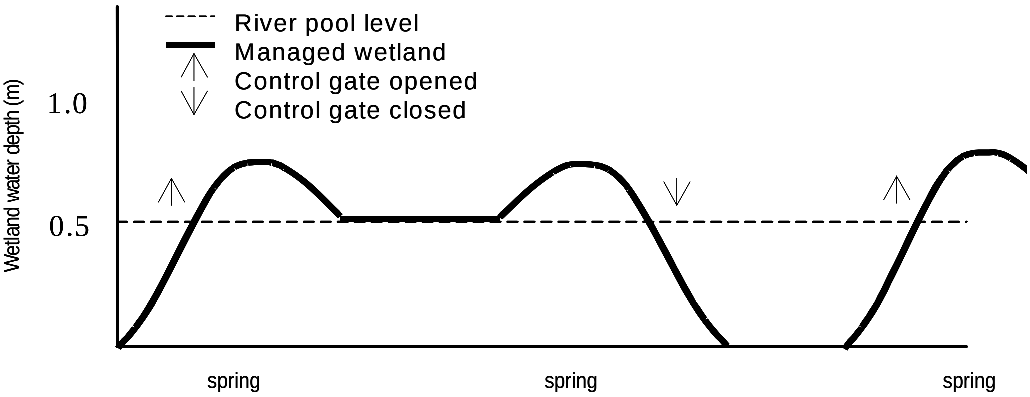

Figure 9.1 Generalised 2 year wetting and drying cycle suitable for Lower Murray wetlands which had been kept permanently inundated due to river regulation (Jensen, 2002a, b).

These operating guidelines would apply during regulated flows. In the event of a natural flow peak in the river, all controls should be opened to maximise the benefit of the natural event. This would allow free exchange of water and biota between the river and the wetlands. Once the high flows have receded, the control structures can be reinstated and the managed regime resumed at the appropriate season (Jensen, 2001, 2002b, 2004).

Constraints on Delivery of Environmental Flows

'Environmental flows' are strictly limited by volume and delivery constraints within the operating infrastructure of the Murray-Darling Basin. The environmental flow volumes proposed to be delivered from 2009 are less than 5% of storage volumes, and can generally only be provided with limited duration and area of inundation. Timing may be dictated by management arrangements, such as surplus irrigation water becoming available in May-June (winter), which does not coincide with natural water regimes with a natural peak in October-November (spring). During regulated flow conditions in the Lower River Murray (up to 25,000 ML d^{-1}, but usually <7,000 ML d^{-1}), when water is confined to the main channel and connected wetlands, water can only be delivered by gravity flows through existing natural flow paths, or by pumping at selected sites (areas inundated ranging from 6 ha to 37 ha).

It is not possible to create over-bank flows or widespread inundation unless there is a flow peak of >25,000 ML d^{-1} in late spring, which can be enhanced or mitigated by approximately 10% through manipulation of release sequences from upstream storages and of weir heights. At the majority of watered sites, engineering structures are required to move water and retain it within the site, thus introducing additional barriers to flow and fish movement.

Results

Remedial projects have presumed that the floodplain soil seed bank contains viable seeds of these species, and that watering will automatically result in germination, but there have been few investigations of germination or survival to maturity. This study showed that, contrary to assumptions by water managers, neither red gum nor black box seeds are well-represented in the soil, and that these species rely instead on aerial (canopy) seed banks (*see Chapter 4*). The phenology and patterns of seed rain from river red gum and black box have been described, and factors affecting recruitment evaluated (*see Chapters 5 & 6*). For river red gum, there is evidence of a pattern of short summer flowering in alternate years, with trees in the same community on alternate cycles, ensuring some flowers in each year. Black box trees have longer seasonal periods of flowering, with trees apparently peaking annually in either summer or winter. Seed rain for each species shows similar annual patterns. For lignum, there is a pattern of peak seed production in October-November, linked to significant winter and spring rainfall events. These patterns vary regionally, and would need to be checked before formulating a managed watering regime for local sites.

The phenological patterns for the three key species, red gum, black box and lignum, all coincide with the natural pattern of higher rainfall (May-Nov) and likely flooding (Oct-Dec) (Figure 9.2). The responses are complicated by the biennial cycles in most red gums, and the alternate summer or winter flowering peaks in black box. Lignum appears to be consistent with its strongest flowering response in Oct-Nov, but capable of light flowering in response to rainfall events at any season. All species have the capacity to respond to water availability with ongoing light seed rain in the eucalypts, and rapid flowering and seeding, or clonal growth, in lignum. The model suggested in Chapter 2 (*see Figure 2.4*), with serial events of 2-4 years required to sustain germination and survival of seedlings to tap root stage, was supported by the field data.

The strategy of alternate biennial cycles in the red gums appears to be a mechanism to maximise the ability of the population to capitalise on floods with frequencies up to 1 in 2-3 y, which is the most common location for this species on the floodplain. The strategy of either winter or summer flowering peaks in black box would provide the ability to flower in response to winter rains in years between floods, since this species tends to be located in areas where flood frequencies are greater than 1 in 6-8 y. Lignum has the capacity to respond quickly to any available water, but the conditions required for sexual recruitment were not fully elucidated in this study, as germination of seedlings appeared to be very sparse. The few seedlings found survived but their development was seriously compromised by heavy kangaroo grazing. Clonal reproduction appears to be the dominant reproductive strategy in existing plants, but no new clonal reproduction was observed during the study more investigation is required to clarify the relative roles of sexual and asexual reproduction.

Jensen *et al.* (2007) suggested that, given the trend of drier conditions on the floodplain, timing environmental flows to augment rainfall or flooding could increase rates of seedling survival, hence recruitment. Small, targeted watering events could be timed to maintain soil moisture levels to sustain particular phenological phases. These data, and the phenological observations described here, suggest that a plan for managed watering events could be:

- 1-2 months after spring rain, to support seedlings (Oct-Dec)
- 1-2 months after small floods (>40,000 ML d^{-1}), to support seedlings (Dec-Feb)
- at flowering/bud set (Dec-Feb, for both species, May-Jul for black box on winter cycle)
- at peak seed rain (Dec-Feb for river red gum; May-Jul and Nov-Jan for black box)
- 12 months after above-average rain to maintain bud crops and aerial seed banks (Mar-Jun), and in subsequent years if conditions are dry.

These recommendations apply to health trees and trees with low-medium stress. Severely stressed trees warrant special consideration, with regard for possible negative impacts, including top-flooding in later watering events. Watering of stressed river red gums at Monoman Billabong (Chowilla) in March 2004 produced aseasonal fresh leaves, but several trees died while still flooded (Dr T Wallace, SA Department of Water, Land & Biodiversity Conservation, pers. comm.). Stressed trees in this study showed more frequent but lower volume seed rain than healthy trees, for example in comparing seed release for healthy and stressed trees at Clarks Floodplain (*see Figure 6.3*).

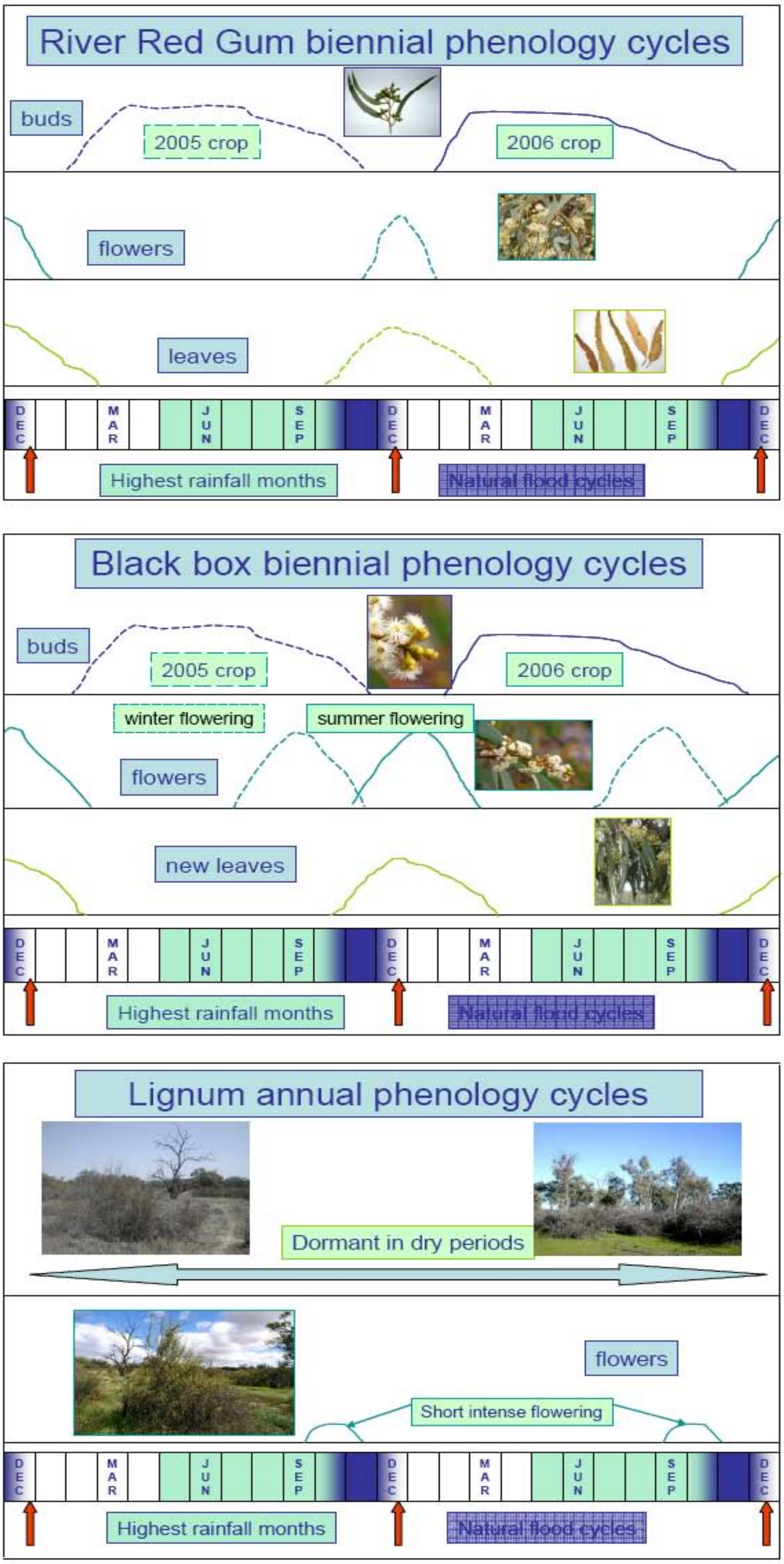

Figure 9.2 Watering events can be timed to value-add on local rainfall events, and to support development of bud crops in healthy trees. The key timing is around December (red arrow), when it is most likely that soil moisture levels will begin to decline following seasonal rain and/or flood inundation. Watering should be timed to maintain soil moisture for germinating seedlings for as long as possible as conditions dry out over summer.

Watering of stressed trees should follow the phenological cycle and natural water regime, to avoid a stress response to watering at other seasons which may be unproductive, depleting scarce physiological resources without leading to regeneration (Lichtenthaler, 1996).

Discussion

Environmental flows as presently allocated for the River Murray under the Living Murray Program comprise a small fraction of natural flow regimes and are too small to deliver on the scale of the natural hydrograph. It is therefore useful to consider ways to optimise environmental responses within the constraints on volumes and delivery.

The key floodplain species of concern in this study are river red gum, black box and lignum. The two former are the dominant perennial trees species and the latter is the dominant perennial shrub species of the Lower Murray Valley floodplain, and continued successful recruitment of these species is a reasonable indicator of satisfactory health in floodplain vegetation. It is assumed here that the desired outcome is successful recruitment of the two eucalypt species and lignum (as appropriate), so advice on appropriate application of environmental flows is based on minimum hydrological conditions required for seed fall, germination and survival of seedlings.

It has been noted from European examples that it is harder to rehabilitate low-dynamic systems dominated by biota, than high-dynamic systems dominated by hydrology (Tockner & Stanford, 2004). In the case of the River Murray, it could be classified as a low-dynamic system dominated by hydrology, and it will certainly be difficult to rehabilitate without the ability to restore the natural hydrology to any significant degree. It will be important to reinstate elements of the hydrological and hydraulic diversity to the ecosystem (Quinn & Hillman, 2004). However, the processes for application of environmental flows in order to trigger successful recruitment are not well understood, particularly at the level of detail required for precise engineering and manipulation of flows to provide suitable micro-conditions at stressed sites, with measurable results in improved vegetation health.

It is essential that appropriate, measurable targets are set before environmental flows are introduced, so that monitoring results can inform the management feedback loop for adjustment (Walker, 2002). While this is stated repeatedly in reports, very few projects have appropriate monitoring targets which are tied to their management objectives (Streever, 1997). The indicators selected need to be sensitive to hydrological and ecological changes (Tockner & Stanford, 2004). One suggestion is to define what species and what life stages are expected to present over a specified time scale (Buijse & Coops, 2004). In the case of the highly variable conditions in the Lower Murray valley, the time scale would need to extend over at least one flood cycle, which could be 5-10 years.

Monitoring in this study of responses to the *Living Murray Program* watering trials has noted new leaves, flowering from existing bud crops, new bud crops and germination of new seedlings. A clear biennial phenological cycle has been observed, with flowering and new leaf development peaking in December. Individual trees operate on alternate biennial cycles, so that each December some are flowering. The bud crop is set by environmental conditions in the preceding year, and buds persist for up to 12 months. Factors found to affect the response to watering events included winter-spring rainfall in the previous year, timing of watering, health of the tree, repetition of watering events, and removal of grazing stock. It is suggested that watering events can be timed to value-add on local rainfall events, and to support development of bud crops in healthy trees. However, watering of stressed trees should follow the phenological cycle, to avoid a stress response which may be unproductive.

The importance of local rainfall has been highlighted, for example with red gum seedlings emerging after above average spring rainfall in 2005, and lignum flowering densely and producing seeds. There appears to be a time lag of 12 months from higher rainfall events to flower and fruit production in red gum and bud and flower production in black box. These responses support the conclusion that the annual rainfall threshold for recruitment of eucalypts on the Lower Murray floodplain is about 300 mm (George *et al.*, 2005), or 116% of average regional rainfall, as observed in 2005. Conversely, drought conditions in 2006 were associated with abortion of immature fruit in red gum and suppressed leaf growth in black box (Jensen *et al.*, 2007). It is anticipated that the mature fruit crop and seed rain in summer 2007 is likely to be reduced in response to drought conditions 12 months prior, in 2006.

In both eucalypts, seedling survival appears to limit recruitment and depends on soil moisture, hence rainfall and flooding (Jensen *et al.*, 2008d). In addition, water-stressed trees are producing significantly less seed, thus limiting potential germination. Jensen (2002) suggested that environmental flows should be timed to duplicate flow peaks (post-regulation in early-mid summer) to stimulate germination, but the limited volumes of water available and the costly logistics of delivering water to floodplain sites would limit their effectiveness. The possibility of flood events in the mainstream to support inundation of floodplain sites with environmental flows remains unlikely for the next several years, due to the ongoing effects of over-allocation and drought.

Thus, options for irrigation of sites in critical need of watering should be explored, based on the findings that 5 mm weekly (Jensen *et al.*, 2008d) or 10 mm every 2 weeks (Jensen *et al.*, 2008a) is sufficient to maintain soil moisture in the critical 10-20% range. The guidelines suggested here may prove useful as an alternative approach for timing of very limited environmental flows designed to promote increased seed production, by watering to promote flowering and bud set, by matching peak soil moisture with peak seed rain to promote germination, and by maintaining soil moisture levels to increase survival of seedlings germinated by natural watering.

Ideally, for effective environmental flows, water volumes need to be allocated in late spring – early summer, at sufficient scale and duration to mimic former small to medium floods. These would need to inundate significant areas of the floodplain for at least four weeks, in order to provide sufficient soil moisture to sustain germinating seedlings through subsequent hot and dry summer conditions. Until sufficient volumes for effective environmental flows can be made available, the guidelines above can be used to maximise effectiveness of very limited environmental watering.

Chapter10 Conclusions

Introduction

River ecosystems are in decline world-wide due to changes in water regimes which have disrupted regeneration and recruitment of many species of flora and fauna. In the Lower Murray valley, floodplain vegetation particularly is affected. The survival of seedlings and mature trees is threatened by reduced frequency and duration of flood events, changes in the seasonal timing of peak and low flows, disappearance of fresh water lenses over-lying saline groundwater (with subsequent intrusion of saline water into root zones), increased moisture stress due to drier conditions, and external factors such as grazing. The decline threatens the viability of populations, and highlights the necessity for recruitment when conditions are suitable.

Vegetation health is declining rapidly, particularly in river red gum communities (*Chapters 2 & 3*). The death rate of mature trees has accelerated since 2000, as the period without effective inundation on the floodplain has lengthened to 11 years by December 2007. During the course of this study, a serious regional drought has exacerbated the decline of water sources due to over-allocation, with no likelihood of over-bank flows reaching the floodplain for at least the next 1-2 years, and possibly up to 7 years, based on climatic history of past droughts (Prof M Young, University of Adelaide, pers comm.).

In response to the decline in vegetation health, environmental flows are proposed, which ideally aim to simulate elements of natural flooding cycles (*Chapter 2*). However, severe limitations are imposed on the scale and duration of inundation by water availability and physical ability to deliver water to the floodplain in the absence of flow events in the mainstream and under a current severe regional drought.

This study has considered key factors in the process of successful recruitment in dominant floodplain plant species, to determine the availability and seasonal patterns of seed sources, and the process of initiating germination and supporting the survival of seedlings, with the aim of informing effective application of environmental flows. The findings of the study are summarised firstly by Chapter, and then evaluated in relation to the hypotheses advanced in Chapter 1.

Floodplain Soil Seed Bank: Status and Response to Flooding (Chapter 4)

The floodplain soil seed bank was dominated by the seeds of native annual species, primarily terrestrial ground cover and small shrub species, with small numbers of riparian and semi-aquatic species. The dominant tree species on the floodplain, river red gum, had only a sparse and patchy presence in the soil seed bank and was transient rather than persistent. However, it appears that the numbers of transient seeds in the soil seed bank are sufficient for annual maintenance recruitment of river red gum, which would supplement mass recruitment triggered by flooding. There was some evidence of ant granivory of red gum seeds. While this would not affect long-term recruitment in naturally-balanced conditions, the majority of river red gums are now in a very stressed state, with significant reduction of seed rain. In addition, as it has been suggested that some granivorous ant species are expanding as the floodplain dries out, the impact of ant granivory may have an increasingly negative impact on seed survival and potential recruitment.

Black box seeds were not present in any soil seed bank samples, not even those taken directly under healthy trees. This indicates that seeds are highly transient and not persistent, with losses possibly due to ant granivory.

Lignum seeds were also transient rather than persistent, and were not found in any soil seed bank samples, including samples taken under lignum bushes during seed production. Achenes recovered from the soil surface had been stripped of their seeds, with the granivores almost certainly being ants.

Germination and survival in river red gum and black box

Phenological Patterns (*Chapter 5*)

Within the context of changed (reduced) hydrological regimes and drier floodplain conditions, distinctive phenological patterns are still occurring, with healthy eucalypts showing seasonal patterns of bud, flower and fruit production which appear to be adapted to natural (pre-regulation) cycles of rainfall and flood peaks for the Lower Murray floodplain. Many of these activities peak in December and appear to be timed to coincide with natural flood peaks in October-December. Approximately 50% of river red gums at each sample site were on opposite biennial cycles, thus about half of the trees flower in a given year, while most black box were on annual cycles which peaked in either summer or winter (December for river red gums, either December or May-June for black box). These strategies would ensure that some flowers and seed are available each summer in river red gum, coinciding with flood events, and in either summer or winter in black box, coinciding with flood events or winter rain.

The size of the flower crop in eucalypts appeared to be set by soil moisture levels 12 months previously, with the phenological cycle of bud development, maturation, retention, flowering, fruit set and maturation of seed taking two years. In years of increased moisture stress, trees shed buds and immature fruit, resulting in significantly reduced crop volumes. Red gums appeared to be more sensitive than black box to climatic conditions, with a larger volume of mature fruit in 2006 following above average rainfall in 2005, while crop volumes in black box were relatively consistent each year.

Seed Release Patterns and Germination (*Chapter 6*)

In spite of changed hydrological and floodplain conditions, the production and release of seed from aerial seed banks is still occurring, with healthy eucalypts producing large quantities of seed in seasonal patterns which appear to be adapted to natural (pre-regulation) cycles of rainfall and flood peaks for the Lower Murray floodplain. Peak river red gum and black box (summer) seed fall in December is timed to coincide with natural flood peaks in October-December, and black box seed fall peaks in May-July (winter), coinciding with winter rains.

Aerial Seed Bank

Both river red gum and black box exhibited serotiny, with aerial seed banks holding mature closed fruit on the tree for up to two years. Thus, these species do have persistent seed banks. The volume of viable seed was high in healthy trees but there was a significant reduction in seed volume in stressed river red gums and black box (up to an order of magnitude). With more than 75% of trees stressed, this is a very significant potential impact on the volume of available seed.

The timing of seed release in river red gum was variable between trees and between sites, but there was a distinct peak in seed fall in summer, particularly in December but as late as March for some trees. The majority of river red gums were on biennial cycles, with individual trees on opposite cycles, and only a minority on annual cycles. Seed fall in black box peaked over a longer period, with some trees peaking in December, and others in winter (May-July). Most black box were on annual cycles, peaking either in summer or winter, with a minority on biennial cycles. For trees operating on the even biennial cycle, seed volumes in 2004 were much larger than in 2006, suggesting a reduction in response to drought conditions.

Seedling Germination

Ongoing light seed rain was observed from eucalypts throughout sampling but no new germinants were found in the field unless significant rainfall or watering had occurred. Germination of river red gums associated with the above average rainfall year in 2005 occurred at Monoman Island Horseshoe Billabong. Germination associated with watering events occurred at Monoman Island Horseshoe Billabong, Pilby Creek floodplain and Clarks Floodplain sites. Surprisingly, little germination of river red gums was observed at Pipeclay Lagoon or Werta Wert Lagoon at the sampling sites, in spite of apparently ideal conditions, but regeneration may have occurred at other locations on these wetlands which were not observed.

River red gum was observed to germinate in winter following local rainfall, when average daily temperatures were 5.7-17°C. Thus, although this species has optimum germination rates above 30°C, it can germinate all year round if there is sufficient soil moisture. It also suggests that a key sequence for successful germination may be rain-triggered germination in winter-early spring, with soil moisture for survival of the seedlings then sustained by flood events or further rainfall in late spring-early summer. This provides a second mechanism for germination throughout the year, in addition to germination triggered by flood events and sustained by replenished soil moisture reserves.

Both red gum and black box demonstrated a capacity for deferred germination, with not all viable seeds germinating on the first opportunity. However, while survivorship of seeds in soil for several months has been demonstrated, this could only occur in field conditions where seeds are protected from ant predation, such as in strandline mulch or under small woody debris.

Both river red gum and black box seeds can float, with black box tending to sink within 1-2 days and starting to germinate in water within 3 days, surviving for up to 10 days. Red gum continued to float for up to 10 days, and started to germinate from 7 days. Similar responses were seen in soil moisture experiments, with red gum seeds not responding to flood conditions lasting for 28 days, but black box responding where the flood receded within 10 days after germination. This suggests that seeds being dispersed by hydrochory would need to reach the edge of the water body within 10 days of immersion, to set roots into moist soil.

No black box seedlings were found at any sampling sites. Examples of existing cohorts of black box saplings on the floodplain indicate that a specific event with suitable conditions (i.e. sustained soil moisture following rain or flood) is required for successful recruitment of significant numbers of seedlings.

Grazing Impacts

Sheep grazing of river red gum seedlings was measured at two Chowilla sites, with less than 5% survival of seedlings. Once sheep were removed, new seedlings showed 80-100% survival, with losses caused by desiccation, exacerbated by insect herbivory. Seedlings in a strandline along a constructed bank, initially grazed down to the surface by sheep, grew strongly from the surviving root systems 18 months later after significant local rain.

Kangaroo grazing of river red gum seedlings was not significant on a newly germinated strandline in the absence of sheep, with light pruning of seedling tips but 100% survival of all seedlings. In contrast, kangaroo grazing of the only patch of lignum seedlings found at Chowilla was very heavy, with extensive pruning severely limiting seedling development to less than 100 mm in height over 12 months. Some plants had up to 20 grazed shoots, although with 90% survival of seedlings and repeated growth of new shoots.

Although not directly measured in this study, ant granivory of eucalypt seeds and lignum seeds from the soil surface was thought to be very high, based on observations of stripped lignum achenes (seed cases), eucalypt seed chaff discarded at ant holes, and the lack of eucalypt and lignum seeds in the persistent soil seed bank.

Strandlines

Several natural strandlines were monitored in the field, with a low rate of successful germination and survival. At Pilby Creek floodplain, 100% survival of seedlings in strandlines coincided with the removal of sheep, in conjunction with good rain and two inundation events. At Monoman Island Horseshoe Billabong, in a strandline 60 m long, 6 seedlings survived for 23 months from 360 original seedlings. The surviving seedlings remained very stunted (<50 mm) under grazing pressure and very dry conditions for 15 months, putting on a major spurt of growth to treble in size (>150 mm) after local rain 18 months later. At Werta Wert Lagoon, only 10 seedlings germinated on four natural strandlines and none survived. Only 2 seedlings were observed in an experimental strandline planted with seeds, while a duplicate set of pots in controlled glasshouse conditions produced over 50 seedlings.

Regeneration at strandline sites was thus inconsistent, with potentially productive sites not producing any seedlings. Some strandlines did produce seedlings, with very low survival rates under stock grazing and dry conditions, but high survival rates at sites with no sheep grazing and light kangaroo grazing. During dry conditions, surviving seedlings adopted a relatively dormant state once a root system had been established, then exhibited masting growth following significant rainfall 18 months later. The mulch provided by organic litter appeared to be effective only initially, as it did not persist due to the effects of wind, rain, sheet erosion or subsequent watering events.

Germination and survival in lignum (Chapter 7)

It was difficult to assess any long-term decline in health in lignum, owing its ability to enter a dormant state when water availability is low. Dormant lignum appeared virtually dead, with no green stems, but was able to respond within 2-4 weeks if water was applied to the base of the plant or within the root zone. Similar rapid responses were observed in soil moisture experiments in the glasshouse. Rainfall was an important contributor to soil moisture for lignum, with intense flowering following significant rains in winter-spring 2005, moderate flowering observed following rain in June 2007, and strong growth of vertical green stems in response to soil moisture in September 2007. However, only 10 new seedlings were found during 30 months across 8 sites, and no new cloned individuals.

Asexual Reproduction

Strong vegetative growth (from root clones and layering of stems) was noted at all sites. Growth of stems was the first response by lignum to available moisture, with multiple vertical stems growing upwards until their weight caused horizontal arching. New vertical stems subsequently grew up from nodes on the arched stems and then arched themselves, leading to the typical tangled habit of this species. In some instances, growth took the form of new stems growing directly from underground root nodes, giving a circle of vertical stems surrounding the parent bush.

Vegetative reproduction of the plant occurs as the arching stems strike roots from nodes when they touch soil and create a new plant. This process was facilitated by inundation, as adventitious roots were formed at nodes on arching stems when they dipped underwater for 3+ weeks. Thus arching branches would be ready to strike as soon as the receding water level allowed these roots to touch moist soil. A similar response was noted in laboratory conditions, with <15% success in striking cuttings set directly into pots, compared with >85% success in cuttings first placed in water and then transferred to pots after either roots or shoots appeared. However, no new cloned individuals were observed during the study at any sites, probably because the right combination of conditions to strike roots into moist soil did not occur.

Sexual reproduction

Lignum was found to hold its seed in a short-lived aerial seed bank (*serotiny*), and was not present in the persistent soil seed bank, with seeds vulnerable to ant granivory. Lignum appeared to be seasonal in seed production, with more intensive flowering in October (mid-spring) in response to either flooding or seasonal rainfall. However, it also demonstrated the ability to respond with light flowering to available moisture in any season. Little seed production was observed except when the volume of viable seed peaked after good winter-spring rains in 2005, with dense flowering following rapidly by seed production in October-November. Seed availability was very short (<4 weeks).

Only a few independent lignum seedlings were found at one site on the Chowilla floodplain. Their position and distance from adult lignum bushes indicated that the seeds were dispersed to this location by hydrochory. However, these seedlings were all severely affected by kangaroo grazing and unable to develop beyond 100 mm in height, with all branches trimmed to 10 mm.

Under glasshouse conditions lignum seeds showed a capacity for deferred germination, with a minority of seeds germinating later, following drying and re-wetting. Lignum seeds floated and germinated on the water surface, surviving for at least 28 days until the flood receded and they were deposited on moist soil.

Lignum Ploidy

An investigation of the chromosome structure of lignum was undertaken, to determine if this was a suitable tool to measure the ratio of sexual to asexual reproduction in lignum communities (Appendix I). A previous report that lignum is octoploid (Chong, 2002) was confirmed in a single sample. Numerous attempts were made to map ploidy in lignum populations for multiple Lower Murray Valley sites for this research and another concurrent study (Lynch, 2006). However, the process for preparation of samples for ploidy analysis had extremely low success in obtaining slides well-defined enough to count chromosomes, and was too time-consuming to be pursued in this study. Further pursuit of this issue requires a degree of skill and practice in plant physiology techniques.

Soil moisture availability required for recruitment (Chapter 8)

Soil moisture availability is critical for germination and subsequent survival of seedlings until they can establish a tap root to reach soil moisture reserves and shallow groundwater sources. Seedlings germinating on the floodplain were extremely sensitive to moisture stress, and seedlings died within 24 hours if soil moisture volume dropped below 10% in glass house conditions.

A rapid growth response to watering was observed in mature trees located in sandy soils, while the response was slower in clay soils. The quicker response in mature trees in sandy soils was counteracted as the wetlands with sandy beds dried out very quickly, while those with clay beds retained water for many months. Thus, water retention for seedling growth was higher in soils with greater clay content. Glasshouse experiments indicated that the critical level of soil moisture for maintaining seedlings in a healthy state is in the range 10-30% volume, and that once values dropped below 10%, seedlings began to wilt. Soil moisture values varied significantly, due to the highly variable clay content in soils, and visual estimates of soil moisture content proved unreliable.

Experimental results indicated that rain treatments generated more seedlings than flood, with river red gum germinating poorly in inundated conditions. Black box only benefited from floods if seeds were able to land on moist soil within 10 days of germination. Lignum responded equally well to flood or rain, owing to its ability to float and germinate in water, surviving for up to 28 days in inundated conditions. Conditions with moist soil in the range 10-30% generated the highest numbers of seedlings, indicating that soil moisture is the critical parameter in determining conditions suitable for germination.

Environmental Watering Guidelines (Chapter 9)

Soil moisture was found to be the critical factor in successful recruitment, using water from all available sources. Thus experimental watering should be timed to maximise the benefit of all available sources of water, to maintain soil moisture in the range 10-30%. Timing to coincide with maximum water availability and peak seed fall from the aerial seed bank will maximise the chances of germination. An important finding was that light seed rain is continuous, so some seed is always available and this can be sufficient, combined with hydrochory in flooded sites, to establish strandline germination.

Guidelines have been developed in the form of a watering calendar to indicate the combination of benefits which are possible, and for watering to ensure successful recruitment of river red gum, black box and lignum from aerial seed banks. The natural timing centred on December was confirmed as the most beneficial time for managed environmental watering, with maintenance of soil moisture >10% as the critical parameter to be monitored.

Responses to Experimental Watering Trials

This investigation was conducted at sites in the Lower Murray Valley which received water during the period of study, in order to observe field responses to experimental watering events and managed wetland water regimes. A significant benefit was noted in the health of mature river red gum trees in the vicinity of watering trials. Stressed trees responded with epicormic growth, and low volumes of seed in a more varied seasonal pattern. Increased seed rain was noted in stressed trees approximately 12 months after watering, but volumes were very significantly reduced compared to healthy trees, and the seasonal pattern of seed release was much more variable. A generation of new seedlings appeared within watered zones as the water receded, but their survival will depend on sufficient soil moisture during subsequent dry seasons. Significant germination occurred at the same sites in response to local rainfall events, and these seedlings could benefit from future local watering designed to maintain soil moisture levels.

Few black box trees were able to benefit from the watering trials as they were beyond the zone of influence of watered areas. Black box trees located near the watering sites showed less signs of stress, but significant areas of stressed black box trees occurred at higher elevations on the floodplain. Many of these areas have gone for more than 30 years without inundation, which last occurred in large flood events in 1973-75. Seed volumes were very low (<50 seeds m^{-2}, compared to >2,500 seeds m^{-2} in healthy trees). Flooding in 1996 which partially inundated the floodplain may have been detrimental to black box on higher elevations which were not inundated, forcing saline groundwater into their root zones.

For lignum, it was observed that benefits from the experimental watering trials were restricted to lignum bushes directly watered or in damp soil zones. The growth response by lignum to watering was rapid and sustained during watering period, but the return to dormancy was equally rapid once the water receded and the soil dried out. Lignum which was inundated for 24 months continued growth and flowering, but was unable to germinate in the inundated area. Experimental results indicate that seed shed from inundated bushes could have germinated and floated, then been moved to the edge of the water body by hydrochory, but no seedlings were located in the field at the inundated site at Brenda Park. A single group of seedlings were found at a Chowilla site that appeared to have been deposited by hydrochory during a watering event.

It should be noted that the volumes of environmental water applied in these trials are insignificant when compared to the volume and duration of over-bank flows such as would occur in a natural flood. The value of watering single confined water bodies for short durations is significantly less when compared with the environmental values accruing from broadscale watering of floodplain environments, including a full range of micro-habitats suitable for the full suite of associated animals and plants. However, in the absence of sufficient water to provide more natural flooding, managed watering may play a useful role in sustaining core remnants of floodplain habitat until the next flood event.

Response to Hypotheses

Five hypotheses were presented at the beginning of this study, to be evaluated on the basis of field and laboratory data. The findings are summarised below.

Hypothesis 1

River red gum, black box and lignum seeds are present transiently but not persistently in the soil seed bank.

The persistent floodplain soil seed bank was dominated by native annual species, with small numbers of riparian and semi-aquatic species. The dominant deep-rooted perennial species were not present in the persistent soil seed bank, but retained their seed in aerial seed banks, using serotiny as a strategy to reduce potential granivory on the soil surface. River red gum had a sparse, patchy presence in the soil seed bank, which tended to be higher in riparian zones under healthy mature trees. Black box and lignum were not found in any soil samples. It may be that the two eucalypt species only germinate when seeds fall directly onto moist soil for immediate germination, or onto a water surface, with hydrochory playing an active part in assisting germination and establishment of seedlings on suitable moist soil. Thus, watering should be timed to coincide with a good chance of local seed rain, as there is not a residual bank of seeds in the soil which will germinate after watering. The timing of the recession will be important, to expose moist soil suitable for germination during times of significant seed rain.

Hypothesis 2

There is an annual peak in volumes of seed rain in river red gum and black box, with greater volumes every alternate year.

A seasonal peak was found for both river red gums and black box. River red gum releases seed mostly on biennial cycles which peak in summer, while black box trees are most frequently on annual cycles which peak in either summer or winter. Both species time seed release to coincide with either rain or flood for germination, but this study suggests that rain is the primary contributor to soil moisture in the surface layers to trigger germination and to maintain seedlings through early stages, with flood replenishing fresh water lenses in the water table and soil moisture reserves to support seedling growth. A pattern was found in the phenological cycles of red gum that suggested correlation of high flowering volume with enhanced rainfall and abortion of immature fruit with drought in the previous season. This was particularly apparent with high numbers of flowers and mature fruit in red gum during 2006, in spite of drought conditions, but reflecting good rainfall in the previous year. However, numbers of immature fruit declined in the subsequent cycle during 2006 as they were aborted in response to the dry conditions and seed volumes in the summer 2006 peak were much lower than in 2004. Black box appeared to be less responsive to conditions in the previous year, with less variation in numbers of fruit produced.

Hypothesis 3

River red gum release seed in seasonal patterns likely to coincide with floods, and black box release seed in seasonal patterns likely to coincide with rainfall, reflecting their respective adaptations to potential water sources and suitable recruitment conditions.

The seasonal pattern for seed release in river red gum peaked in summer, suggesting coincidence with soil moisture from flood inundation which naturally occurred in October-December. Black box trees peaked either in summer or in winter, suggesting that some trees are keyed to floods, and some are keyed to rainfall. As these trees are found at higher elevations with lower flood frequencies, they rely on rainfall between floods to maintain soil moisture reserves, and sudden death in local populations has been linked to extended rainfall droughts during long periods without floods. The alternative seasonality option suggests a mixed strategy to ensure that some black box trees will be able to reproduce in the intervals between larger floods.

Hypothesis 4

Sexual reproduction (seed set) in lignum requires over-bank flows, whereas asexual reproduction (clonal growth) may be promoted by significant seasonal rainfall events.

The evidence was inconclusive on the link between water source and either sexual reproduction (seed set) or asexual reproduction (clonal growth) in lignum. Seed production appeared to be highly responsive to seasonal rainfall, but only 10 surviving seedlings were observed at one location throughout the study, and these appeared linked to hydrochory and watering. Vegetative reproduction appeared to be unsuccessful in dry conditions. Plants inundated for several months developed roots on nodes of arched branches which dipped underwater. However, when plants were continuously flooded, there was no opportunity for these branches to reach exposed soil and set roots. These results indicate that flooding may be a necessary condition for both sexual and asexual reproduction. Early results indicated that female bushes were located closer to water than male bushes at Brenda Park (similar to some *Atriplex* species), but analysis of the full data set did not reinforce this pattern over wider scales. Some locations at Brenda Park appeared to have concentrations of plants of one gender or the other, but this distribution pattern was not consistent. It was easier to find plants of opposite gender adjacent to each other at Chowilla locations, but this was not conclusive. Investigations with ploidy to trace the regeneration mode of individual plants were unsuccessful due to the extreme difficulty in producing consistent slides of chromosomes with clear enough definition to count.

Hypothesis 5

Maintenance of a minimal level of soil moisture (suggested >30%) will sustain eucalypt and lignum seedlings through the vulnerable stage of relying on soil moisture in surface layers while developing ‘sinker’ roots to deeper soil moisture reserves.

The minimum level of soil moisture required to maintain germinants through the vulnerable early stage prior to development of ‘sinker’ roots (up to 2 y) was found to be significantly less than postulated, being in the range 10-30%, with 10% the critical minimum level. The maximum level of soil moisture recorded in the floodplain clays was 60%, so the ideal for sustaining seedlings is approximately 30% of the maximum level. It was found that river red gum and black box have similar requirements for soil moisture. Both responded better to rain simulations than flood simulations, but black box was able to benefit from flooding if seeds germinated in the last 10 days before the flood receded, so that germinated seeds were deposited on soil with around 30% soil moisture content.

Both species had the capability for deferred germination from the same seed source, offering opportunities for germination if suitable conditions occurred again at a later time. Lignum seeds had a similar preference for soil moisture in the range 10-30% and the capability for deferred germination, but also had the capability of germinating and floating on the surface of flood waters for at least 28 days (the duration of the flood treatment) until deposited on moist soil and taking root. Lignum cuttings also responded positively to moist soil, provided cuttings were first struck in water (either roots or shoots) before being laid on the soil surface in treatment pots.

Further Research

A number of significant questions arise from this research which should be investigated further, including:

- field testing of the proposed watering timetable, particularly for river red gum, and evaluation of effects on health of mature trees and survival of seedlings
- evaluation of possible negative effects of watering events on germinating seedlings (stress response in mature trees in poor health, top-flooding of new seedlings by subsequent watering, a-seasonal timing of watering)
- field testing of matching watering with local rain events >5 mm to extend period of soil moisture >10%, and evaluation of survival rate of seedlings and effect on soil moisture
- field testing ability of serial environmental watering to maintain soil moisture >10%
- investigation of relative significance of ant predation on eucalypt and lignum seeds with reduced recruitment rates and changed water regime, relative to natural conditions, and evaluation of suggested increase of granivorous ant species under drier conditions
- evaluation of health and status of lignum communities in response to drier floodplain conditions, to determine if the population is declining
- investigation of relative proportion of sexual and asexual reproduction in lignum, possibly using ploidy to separate plants by reproductive mode, and to assess whether the gender balance and distribution has been altered by changed water regimes
- development of a computer model of recruitment in red gum and black box, based on data from this study and also findings from a previous study in the Lower Murray Valley (George, 2004). This could interface with the Floodplain Inundation Model (Overton, 2005).

Chapter11 References

Allison, G.B., Cook, P.G., Barnett, S.R., Walker, G.R., Jolly, I.D., & Hughes, M.W. (1990). Land clearance and river salinisation in the Western Murray basin, Australia *Journal of Hydrology*, **119**, 1-20.

Andersen, A.N. (1989). How important is seed predation to recruitment in stable populations of long-lived perennials? Oecologia, 81, 310-315.

Andersen, A.N. (1991). Seed harvesting by ants in Australia. In Ant-Plant Interactions (eds C.R. Huxley & D.F. Cutler), pp. 493-503. Oxford University Press, Oxford.

Andersen, A.N. (2003). Ant biodiversity in arid Australia. Records of the South Australian Museum Monograph Series, 7, 79-92.

ARMCANZ/ANZECC (1966). National Principles for the Provision of Water for Ecosystems. Agricultural Resource Management Council of Australia and New Zealand, and the Australian and New Zealand Environment and Conservation Council, Canberra.

Arthington, A.H., Bunn, S.E., Pusey, B.D., Bluhdorn, D.R., King, J.M., Day J, A., Tharme, R., & O'Keeffe, J.H. (1991). Development of an Holistic Approach for Assessing Environmental Flow Requirements of Riverine Ecosystems. In Water Allocation for the Environment (eds J.J. Pigram & B.P. Hooper), pp. 69-76. Centre for Water Policy Research, UNE, University of New England, Armidale, New South Wales.

Ashton, D.H. (1979). Seed harvesting by ants in forests of Eucalyptus regnans F Muell in central Victoria. Australian Journal of Ecology, 14, 265-277.

AWE (1999). *Bookpurnong/Lock 4 Preliminary Land and Water Management Plan*. Report for the Loxton-Bookpurnong Local Action Planning Committee. Australian Water Environments, Adelaide.

AWE (1999). *Clarks Floodplain Investigations*. Report for the Loxton-Bookpurnong Local Action Planning Committee. Australian Water Environments, Adelaide.

Baskin, C.C. & Baskin, J.M. (1998). Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination Academic Press, San Diego.

Bayley, P.B. (1991). The flood-pulse advantage and the restoration of river-floodplain systems. *Regulated Rivers: Research & Management*, **6**, 75-86.

Beauchamp, V. B. (2001). Impacts of Flow Diversion and Regulation on Successional Processes in Cottonwood and Willow Dominated Riparian Forests, Verde River, Arizona. Arizona State University.

Berens, V, White, M G, Souter, N J, Jolly, I D, Holland, K L, McEwan, K, Hatch, M A, Fitzpatrick, A D & Munday, T J (2007). Surface water, groundwater and ecological interactions along the River Murray. A pilot study of management initiatives at the Bookpurnong Floodplain, South Australia. Proceedings of the XXXV IAH Congress, Groundwater & Ecosystems. Lisbon, Portugal.

Boland, D.J., Brooker, M.I.H., Chippendale, G.M., Hall, N., Hyland, B.P.M., Johnston, R.D., Kleinig, D.A., & Turner, J.D. (1984) *Forest Trees of Australia.* CSIRO, Melbourne.

Bonis, A., Lepart, J., & Grillas, P. (1995). Seed bank dynamics and coexistence of annual macrophytes in a temporary and variable habitat. Oikos, 74, 81-92.

Boudell, J.A. & Stromberg, J.C. (2008). Flood pulsing and metacommunity dynamics in a desert riparian ecosystem. Journal of Vegetation Science, 19, 373-380.

Boulton, A.J. & Jenkins, K.M. (1997). Flood regimes and invertebrate communities in floodplain wetlands. In Wetlands in a Dry Land: understanding for management (ed W.D. Williams). Environment Australia, Canberra.

Boulton, A.J. & Lloyd, L.N. (1991). Macroinvertebrate assemblages in floodplain habitats of the lower River Murray, South Australia. *Regulated Rivers: Research & Management*, 6, 183-201.

Bren, L.J. & Gibbs, N.L. (1986).Relationships between flood frequency, vegetation and topography in a river red gum forest. *Australian Forest Research*, 16, 357-370.

Bren, L.J. (1988a) Effects of River Regulation on Flooding of a Riparian Red Gum Forest on the River Murray, Australia. *Regulated Rivers: Research & Management*, 2, 65-77.

Bren, L.J. (1988b) Flooding characteristics of a riparian Red Gum forest. *Australian Forest Research*, 51, 57-62.

Briggs, S.V. & Maher, M.T. (1985). Limnological studies of waterfowl habitat in south-western New South Wales. II Aquatic macrophyte productivity. *Australian Journal of Marine and Freshwater Research*, 36, 707-715.

Brock, M.A. & Casanova, M.T. (1997). Plant life at the edges of wetlands: ecological responses to wetting and drying patterns. In *Frontiers in Ecology: Building the Links* (ed N.L. Klomp, I), pp. 181-192. Elsevier Science, Oxford.

Brock, M.A. & Crossle, K. (2002). Seed-bank and vegetation responses to timing, duration, and frequency of flooding in temporary wetlands. *Verh Internat Verein Limnol*, 28, 1756-1761.

Brock, M.A. & Rogers, K.H. (1998). The regeneration potential of the seed bank of an ephemeral floodplain in South Africa. *Aquatic Botany*, 61, 123-135.

Brock, M.A. (1998). Are temporary wetlands resilient? Evidence from seed banks of Australian and South African wetlands. In *Wetlands for the Future*, Intecol V International Wetlands Conference (ed J.A. McComb A J & Davis). Gleneagles Publishing Adelaide, Perth, Western Australia.

Brock, M.A., Nielsen, D.L., Shiel, R.J., Green, J.D., & Langley, J.D. (2003). Drought and aquatic community resilience: the role of eggs and seeds in sediments of temporary wetlands. *Freshwater Biology*, 48, 1207-1218.

Brock, M.A., Theodore, K., & O'Donnell, L. (1994). Seed-bank methods for Australian wetlands. *Australian Journal of Marine & Freshwater Research*, 45, 483-493.

Buijse, T. & Coops, H. (2004). Rehabilitation of dynamic processes in large-river floodplains. In *7th Intecol International Wetlands Conference* (eds J.T.A. Verhoeven, E. Dorland & M. Coemans). Utrecht University, Utrecht, Holland.

Bunn, S.E. & Arthington, A.H. (2002). Basic principles and ecological conequences of altered flow regimes for aquatic biodiversity. *Environmental Management*, 30, 492-507.

Busch, D.E. & Smith, S.D. (1995). Mechanisms associated with decline of woody species in riparian ecosystems of the southwestern U.S. *Ecological Monographs*, 63, 347-370.

Caldwell Connell Engineers Pty Ltd (1981). Katarapko Evaporation Basin Environmental Study. Engineering and Water Supply Department, Adelaide.

Capon, S. J. (2004). Germination responses to flooding in desert floodplain plants. In *7th Intecol International Wetlands Conference* (eds J.T.A. Verhoeven, E. Dorland & M. Coemans). Utrecht University, Utrecht, Holland.

Capon, S.J. & Brock, M.A. (2006). Flooding, soil seed bank dynamics and vegetation resilience of a hydrologically variable desert floodplain. Freshwater Biology, 51, 206-223.

Casanova, M.T. & Brock, M.A. (2000). How do depth, duration and frequency of flooding influence the establishment of wetland plant communities? *Plant Ecology*, 147, 237-250.

Chong, C. & Walker, K.F. (2005). Does lignum rely on a soil seed bank? Germination and reproductive phenology of *Muehlenbeckia florulenta* (Polygonaceae). *Australian Journal of Botany*, 53, 1-9.

Chong, C. (2002). Achene germination and reproductive phenology of Muehlenbeckia florulenta (Polygonaceae). BSc (Hons), University of Adelaide, Adelaide.

Clark, J.S., Macklin, E., & Wood, L. (1998). Stages and spatial scales of recruitment limitation in southern Appalachian forests. *Ecological Monographs*, 68, 213-235.

Close, A. (1990). River Salinity. In *The Murray* (eds N. Mackay & D. Eastburn). Murray-Darling Basin Commission, Canberra.

Cooper, D. J., D. M. Merritt, D. C. Andersen, and R. A. Chimner. (1999). Factors controlling the establishment of Fremont cottonwood seedlings on the upper Green River, USA. *Regulated Rivers: Research & Management* 15: 419-440.

Corenblit, D., Tabacchi, E., Steiger, J., & Gurnell, A.M. (2007). Reciprocal interactions and adjustments between fluvial landforms and vegetation dynamics in river corridors: a review of complementary approaches. *Earth-Science Reviews*, 84, 56-86.

Craig, A.E., Walker, K.F., & Boulton, A.J. (1991). Effects of edaphic factors and flood frequency on the abundance of lignum *Muehlenbeckia florulenta* Meissner: Polygonaceae on the River Murray floodplain South Australia. *Australian Journal of Botany*, 39, 431-443.

Cremer, K. W. (1977). Distance of seed dispersal in eucalypts estimated from seed weights. *Australian Forest Research* 7: 225-228.

Cremer, K.W. (1965). How eucalypt fruits release their seed. *Australian Journal of Botany*, 13, 11-16.

Cremer, K.W. (1965a). Effects of fire on seedshed from *Eucalyptus regnans*. *Australian Forest Research*, 29, 252-262.

Cunningham, G.M., Mulham, W.E., Milthorpe, P.L., & Leigh, J.H. (1981). *Plants of Western New South Wales.* NSW Government Printing Office, Sydney.

Cunningham, S.C., Mac Nally, R., White, M., Read, J., Baker, P.J., & Griffioen, P. (2007). Assessing the current condition of river red gum along the Victorian Murray River: from ground survey to validated map. In Proceedings of *Place and Purpose: Spatial models for natural resource management and planning*. Bendigo, Victoria.

Curtis, D. (1985). Phenology and establishment of selected eucalypts from the northern tablelands of NSW. MSc thesis, University of New England, Armidale.

Dexter, B.D. (1967). Flooding and regeneration of river red gum, *Eucalyptus camaldulensis*, Dehn., Rep. No. Forests Commission Bulletin No 20. Forests Commission, Melbourne.

Dexter, B.D. (1970). Regeneration of river red gum *Eucalyptus camaldulensis* Dehnh. M Sc thesis, University of Melbourne, Melbourne.

Dexter, B.D. (1978). Silviculture of the river red gum forests of the central Murray floodplain. *Proceedings of the Royal Society of Victoria*, 90, 175-191.

DLWBC (2004). Environmental Flows for the River Murray: South Australia's framework for collective action to restore river health. SA Department of Land, Water and Biodiversity Conservation, Adelaide.

Edgar, J.G. (1977). Effects of moisture stress on germination of *Eucalyptus camaldulensis* Dehnh. and *E regnans* F. Muell. *Australian Forest Research*, 7, 241-5.

Eldridge, S. (1991). The Role of Salinisation in the Dieback of Black Box (*Eucalyptus largiflorens* F Muell) on the Floodplain of the River Murray, South Australia. Honours thesis, University of Adelaide, Adelaide.

Eldridge, S.R., Thorburn, P.J., McEwan, K.L., & Hatton, T.J. (1993). Health and structure of *Eucalyptus* communities on Chowilla and Monoman Islands of the River Murray floodplain, South Australia, Rep. No. 93/3. CSIRO Division of Water Resources, Adelaide.

Evans, R., Brown, C., & Kellett, J. (1990). Geology and Groundwater. In *The Murray* (eds N. Mackay & D. Eastburn). Murray-Darling Basin Commission, Canberra.

Fenner, M. (1985) Seed Ecology Chapman & Hall, London.

Florence, R. G. (1996). *Ecology and Silviculture of Eucalypt Forests*. CSIRO Publishing, Collingwood, Australia.

Frears, A.P. (2001). An ecological evaluation of the proposal for rehabilitation of Reedy Creek lagoon, a wetland of the River Murray, South Australia. Honours thesis, University of Adelaide, Adelaide.

George, A.K. (2004). Eucalypt regeneration on the Lower River Murray Floodplain, South Australia. PhD thesis, The University of Adelaide, Adelaide.

George, A.K., Walker, K.F. & Lewis, M.M. (2005). Population status of eucalypt trees on the River Murray floodplain, South Australia. *River Research & Applications* 21, 271 282.

Good, M.J. (1989). Investigations into the relationships between the health of black box (*Eucalyptus largiforens*) and edaphic and hydrological factors on Katarapko Island South Australia. Honours thesis, University of Adelaide, Roseworthy.

Greenslade, P.J.M. (1979). *A Guide to Ants of South Australia*. South Australian Museum, Adelaide.

Grose, R.J. & Zimmer, W.J. (1958). Some laboratory germination responses of the seeds of river red gum, *Eucalyptus camaldulensis* Dehn. syn. *Eucalyptus rostrata* Schlecht. *Australian Journal of Botany*, 6, 131-153.

Grose, R.J. (1962). Germination responses of seeds of Victorian eucalypts. In Proceedings of ANZAAS Jubilee Congress, Sydney.

Gunn, B. (2001). *Australian tree seed centre operations manual.* CSIRO Forestry and Forest Products, Kingston, ACT, Australia.

Gurnell, A.M., Thompson, K., Goodson, J., & Moggridge, H. (2008). Propagule deposition along river margins: linking hydrology and ecology. *Journal of Ecology*, 96, 553-565.

Heinrich, P.A. (1990). The eco-physiology of riparian river red gum (*Eucalyptus camaldulensis*). Unpublished report to the Australian Water Resources Advisory Council. Forestry Section, University of Melbourne, Melbourne.

Holland, K. (2002). Black Box (*Eucalyptus largiflorens*) Tree Health and Groundwater Discharge on the lower River Murray Floodplain: Linking Spatial and Temporal Scales. PhD thesis, Flinders University, Adelaide.

Hollingsworth, I.D., Meissner, A.P., & Davies, G. (1990). A Reconnaissance Soil Survey of the Chowilla Anabranch System of the River Murray in South Australia and New South Wales. SA Dept of Agriculture, for the Murray Darling Basin Commission.

Jacobs, M.R. (1955) *Growth habits of the eucalypts* Forestry and Timber Bureau, Canberra.

Jacobs, T. (1990). River regulation. In *The Murray* (eds N. Mackay & D. Eastburn). Murray-Darling Basin Commission, Canberra.

James, C. & Capon, S.J. (2004). Diversity of plants in ephemeral floodplain-wetland systems -- a spatial study in north-western NSW, Australia. In 7th Intecol International Wetlands Conference (eds J.T.A. Verhoeven, E. Dorland & M. Coemans). Utrecht University, Utrecht, Holland.

James, C.S., Capon, S.J., White, M.G., Rayburg, S.C., & Thoms, M.J. (2007). Spatial variability of the soil seed bank in a heterogeneous ephemeral wetland system in semi-arid Australia. *Plant Ecology*, **190**, 205-217.

Jansen, A. & Robertson, A.I. (2001) Relationships between livestock management and the ecological condition of riparian habitats along an Australian floodpain river. *Journal of Applied Ecology*, **38**, 63-75.

Jarvis, J.C. & Moore, K.A. (2008). Influence of environmental factors on *Vallisneria americana* seed germination. *Aquatic Botany*, **88**, 283-294.

Jensen, A.E. (1983). *Lake Merreti, an environmental baseline study*. Department of Environment and Planning, Adelaide.

Jensen, A. (1986). Inland Waters. In *A Land Transformed. Environmental Impact in South Australia* (eds C. Nance & D. Speight). South Australian Department of Environment and Planning, Adelaide.

Jensen, A. (1998a). Rehabilitation of the River Murray: identifying causes of degradation and options for bringing the environment into the management equation. In *Rehabilitation of Rivers* (eds L. De Waal, A. Large & M. Wade), pp. 215-236. J Wiley & Sons, Cambridge.

Jensen, A. (1998b). Factors Affecting Regeneration of Floodplain Plant Species in the Rehabilitated Bulyong Island Evaporation Basin. In *Fitting a few more pieces to the Mysteries of the Murray jigsaw* (eds A. Jensen, K. Nicholls & R. Seaman). South Australian Department of Environment and Natural Resources, Berri, South Australia.

Jensen, A. (2001). Applying ecohydrology to on-ground management of wetlands and floodplains -- 'learning by doing'. In *The Application of Ecohydrology to Water Resources Development & Management*. Proceedings of the Final Conference of the First Phase of the IHP-V Project on Ecohydrology, Venice (eds M. Zalewski & D. Harper). Polish Academy of Sciences, Warsaw.

Jensen, A. (2002a). Applying ecohydrology to on-ground management of wetlands and floodplains -- 'learning by doing'. *Ecohydrology & Hydrobiology*, **2**, 67-78.

Jensen, A. (2002b). Repairing wetlands of the Lower Murray: Learning from restoration practice. *Journal of Ecological Management & Restoration*, **3**, 5-14.

Jensen, A. (2004). Making wetlands more fish friendly. In *Native Fish Habitat Rehabilition and Management in the Murray-Darling Basin* (ed M. Lintermans), pp. 66-76. Murray Darling Basin Commission, Albury.

Jensen, A., Harper, M., Sharley, T., & Seekamp, J. (2003). Critical Flows Package. Environmental Flow Management Strategy to Restore 50% of Murray Valley Floodplain Below Wentworth (Lock 10 to Barrages). SA Department of Water, Land and Biodiversity Conservation and Murray-Darling Basin Commission, Adelaide.

Jensen, A., Harvey, P., Good, M., Tucker, P., & Long, M. (2000). River Murray Barrages Environmental Flows. An evaluation of environmental flow needs in the Lower Lakes and Coorong. Murray-Darling Basin Commission, Canberra.

Jensen, A., Marsh, F., & Nichols, S. (1999). *Brenda Park/Scotts Creek Wetland Complex Management Plan*. For the Brenda Park/Scotts Creek Wetland Rehabilitation Group. Wetland Care Australia, Berri, South Australia.

Jensen, A. & Nicholls, K. (1997). Flow Management Options for the Lower Murray below Wentworth. Wetlands Management Program, SA Department of Environment and Natural Resources, Adelaide.

Jensen, A., Nicholls, K., & Nicolson, C. (1997). *Flow Management Options for the Lower Murray below Wentworth. Analysis of Options and Impacts for each Reach*. Wetlands Management Program, SA Department of Environment and Natural Resources, Adelaide.

Jensen, A., Nicolson, C., & Carter, J. (1994). Preservation and Management of Natural Wetlands in the South Australian Murray Valley. *Water Science Technology*, 29, 325-333.

Jensen, A., Seekamp, J., Harper, M., & Sharley, T. (1998). *Chowilla Ecological Assessment*. Wetland Care Australia, Berri, South Australia.

Jensen, A.E., Nichols, S., Nicol, J.M., & Turner, R. (2002). *Clarks Sandbar Management Strategy*. Wetland Care Australia, Berri, South Australia.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2006). The Secret Life of Tangled Lignum, Muehlenbeckia florulenta (Polygonaceae): little known plant of the floodplains. In Wetlands of the Murrumbidgee River Catchment (eds I Taylor, P. Murray & S. Taylor), 79-85. Murrumbidgee Catchment Management Authority, Leeton, NSW.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2007). Using phenology to determine environmental watering regimes for the River Murray floodplain, South Australia. In *Australian Rivers: making a difference* (Eds A.L. Wilson, R.L. Dehaan, R.J. Watts, K.J. Page, K.H. Bowmer & A. Curtis). Proceedings of 5th Australian Conference on Stream Management, 175-180. Charles Sturt University, Albury, New South Wales.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2008a). Smart Environmental Watering: getting most benefit from scant flows for floodplain trees (River Murray, South Australia). In (Eds) Daniell, T., Lambert, M. & Leonard, M., *Proceedings of Water Down Under 2008 Conference*, 15-17 April, Adelaide, 1426-1437. Engineers Australia, Melbourne, Australia.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2008b). The role of seed banks in restoration of floodplain woodlands. *River Research & Applications*, *Special Issue: Proceedings of Riverine Ecohydrology Conference 2006, Stirling Scotland,* 24 (2008), 632-649.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2008c). The floodplain seed bank of a regulated lowland river: composition and responses to wetting treatments for the River Murray floodplain, Australia. *In prep*.

Jensen, A.E., Walker, K.F., & Paton, D.C. (2008d). Soil Moisture for Seedling Survival: a key factor in woodland decline on the Lower Murray, Australia. *In prep*.

Jolly, I., Walker, G., Thorburn, P., & Hollingsworth, I. (1992). The cause of black box decline in the Chowilla Anabranch region, South Australia and possible ameliorative approaches. In *Catchments of Green: a national conference on vegetation and water management*, pp. 33-41. Greening Australia, Adelaide.

Jones, G. (2002). Setting Environmental Flows to Sustain a Healthy Working River. In *Watershed*, Vol. February 2002.

Jones, G., Hillman, T., Kingsford, R.T., McMahon, T.A., Walker, K.F., Arthington, A.H., Whittington, J., & Cartwright, S. (2002). *Independent Report of the Expert Reference Panel on Environmental Flows and*

Water Quality Requirements for the River Murray System. Cooperative Research Centre for Freshwater Ecology, Canberra.

Junk, W.J. & Wantzen, K.M. (2004). The Flood Pulse concept: new aspects, approaches and applications -- an update. In *Second International Symposium on the Management of Large Rivers for Fisheries* (eds R.L. Welcomme & T. Petr), Vol. 2: 111-149. Food and Agriculture Organization & Mekong River Commission, Bangkok.

Junk, W.J., Bayley, P.B., & Sparks, R.E. (1989). The flood pulse concept in river-floodplain systems, Vol. 106: 110-127. Canadian Special Publications in Fisheries and Aquatic Sciences.

Kiddle, G. (1987). River red gum (*Eucalyptus camaldulensis*) regeneration along the River Murray in South Australia. Department of Environment and Planning, Adelaide.

King, S.L. & Keeland, B.D. (1999). Evaluation of Reforestation in the Lower Mississippi River Alluvial Valley. *Restoration Ecology*, 7, 348-359.

Klute, A. (1986). *Methods of Soil Analysis: Part 1-Physical and Mineralogical Methods* Soil Science Society of America, Madison, Wisconsin, USA.

Knutson, M.G. & Klaas, E.E. (1998). Floodplain Forest Loss and Changes in Community Composition and Structure in the Upper Mississippi River: A Wildlife Habitat at Risk. *Natural Areas Journal*, 18, 138-150.

Lamont, B.B. & Enright, N.J. (2000) Adaptive advantages of aerial seed banks. *Plant Species Biology*, 15, 157-166.

Lange, R.T. (1969). The piosphere: sheep track and dung patterns. *Journal of Range Management*, 22, 396-400.

Lange, R.T. & Purdie, R. (1976). Western myall (*Acacia sowdenii*), its survival prospects and management needs. *Rangeland Journal*, 1, 64-69.

Leck, M.A. & Brock, M.A. (2000). Ecological and evolutionary trends in wetlands: evidence from seeds and seed banks in New South Wales, Australia and New Jersey, USA. *Plant Species Biology*, 15, 97-112.

Leckie, S., Vellend, M., Bell, G., Waterway, M.J., & Lechowicz, M.J. (2000). The seedbank in an old-growth, temperate deciduous forest. *Canadian Journal of Botany*, 78, 181-192.

Li, E.H., Lui, G.-H., Yuan, L.Y., & Li, S.C. (2008). The seed-bank of a lakeshore wetland in Lake Honghu: implications for restoration. *Plant Ecology*, 195, 69-76.

Lichtenthaler, H.K. (1996). Vegetation stress: an introduction to the stress concept in plants. *Journal of Plant Physiology*, 148, 4-14.

Lopez, O.R. & Kursar, T.A. (2007). Interannual variation in rainfall, drought stress and seedling mortality may mediate monodominance in tropical flooded forests. *Oecologia*, 154, 35-43.

Lynch, C. (2006) Reproduction in tangled lignum (*Muehlenbeckia florulenta* Meissn. Polygonaceae): gender balance and growth responses to soil moisture. Honours thesis, University of Adelaide, Adelaide.

Mackay, N. & Eastburn, D. (1990). *The Murray* Murray Darling Basin Commision, Canberra.

Maheshwari, B.L., Walker, K.F., & McMahon, T.A. (1993). *The impact of flow regulation on the hydrology of the River Murray and its ecological implications*. Centre for Environmental and Applied Hydrology, University of Melbourne and Department of Zoology, University of Adelaide, Melbourne.

Maheshwari, B.L., Walker, K.F., & McMahon, T.A. (1995) Effects of Regulation on the Flow Regime of the River Murray, Australia. *Regulated Rivers: Research & Management*, 10, 15-38.

Mahoney, J. M., and S. B. Rood (1992). Response of a hybrid poplar to water table decline in different substrates. *Forest Ecology and Management* 54:141-156.

Margules and Partners, Smith, P., Smith, J., & Department of Conservation Forests and Lands (1990). *River Murray Riparian Vegetation Study*. Murray-Darling Basin Commission, Canberra.

McArthur, A.J. (2003). Ants and Acacias. *South Australian Naturalist*, 78, 3-7.

McEwan, K., Holland, K., Smitt, C., & Jolly, I. (2003). Results of a groundwater/soil survey to assess the impacts of land and water management on the River Murray Floodplain. CSIRO Land and Water, Adelaide.

MDBC (2003). Preliminary Investigations into Observed River Red Gum Decline along the River Murray below Euston. Rep. No. Technical Report 03/03. Murray-Darling Basin Commission, Canberra.

MDBC (2005). Survey of River Red Gum and Black Box health along the River Murray in New South Wales, Victoria and South Australia -- 2004, Rep. No. 06/05. Murray-Darling Basin Commission, Canberra.

MDBC (2006). *River Murray System -- Drought Update*. 06/20954, Murray-Darling Basin Commission, Canberra.

Meeson, N., Robertson, A.I., & Jansen, A. (2002). The effects of flooding and livestock on post-dispersal seed predation in river red gum habitats. *Journal of Applied Ecology*, 39, 247-258.

Mensforth, L.J., Thorburn, P.J., Tyerman, S.D., & Walker, G.R. (1994). Sources of water used by riparian *Eucalyptus camaldulensis* overlying highly saline groundwater. *Oecologia*, 10, 21-28.

Mensforth, L.J., Thorburn, P.J., Tyerman, S.D., & Walker, G.R. (1992) The sources of water used by river red gum (Eucalyptus camaldulensis) along an outer creek of the Chowilla anabranch system. In *Ecotones at the river basin scale - global land/water interactions* (ed A. Jensen). South Australian Department of Environment and Natural Resources in association with UNESCO Ecotones Research Project, 'Role of land/ inlandwater ecotones in landscape management and restoration', Barmera, South Australia.

Micklin, P.P. (1988). Desiccation of the Aral Sea: a water management disaster in the Soviet Union. *Science*, 241, 1170-6.

Mitsch, W.J. & Gosselink, J.G. (1993). *Wetlands*. John Wiley & Sons, New York.

Naiman, R.J., Fetherston, K.L., McKay, S., & Chen, J. (1997). Riparian forests. In *River Ecology and Management: Lessons from the Pacific Coastal Ecoregion* (eds R.J. Naiman & R.E. Bilby), pp. 289-323. Springer-Verlag, New York.

Naiman, R.J., Magnuson, J.J., McKnight, D.M., & Standford, J.A. (1995). *The freshwater imperative: a research agenda*. Island Press, Washington (D C).

Nicol, J., Muston, S., D'Santos, P., McCarthy, B., & Zukowski, S. (2007) Impact of sheep grazing on the soil seed bank of a managed ephemeral wetland: implications for management. *Australian Journal of Botany*, 55, 103-109.

Nicol, J.M., Ganf, G.G., & Pelton, G.A. (2003). Seed banks of a southern Australian wetland: the influence of water regime on the final floristic composition. *Plant Ecology*, 168, 191-205.

Nielsen, D.L. & Brock, M.A. (2004). Impacts of increasing salinity on Australia wetland seed and egg banks. In 7th Intecol International Wetlands Conference (eds J.T.A. Verhoeven, E. Dorland & M. Coemans). Utrecht University, Utrecht, Holland.

Nilsson, C., Gardfjell, M., & Grelsson, G. (1991). Importance of hydrochory in structuring plant communities along rivers. *Canadian Journal of Botany*, 69, 2631-2633.

Nilsson, C., Reidy, C.A., Dynesius, M., & Revenga, C. (2005). Fragmentation and Flow Regulation of the World's Large River Systems. *Science*, 308, 405-408.

O'Dowd, D.J. & Gill, A.M. (1984). Predator satiation and site alteration following fire: mass reproduction of alpine ash (*Eucalyptus delegatensis*) in southeastern Australia. *Ecology*, 65, 1052-66.

O'Malley, C. & Sheldon, F. (1990). *Chowilla Floodplain Biological Study*. Nature Conservation Society of South Australia Inc, Adelaide.

Overton, I.C. (2005). Modelling floodplain inundation on a regulated river: integrating GIS, remote sensing and hydrological models. *River Research & Applications*, 21, 991-1001.

Page, M.J., Newlands, L., & Eales, J. (2002). Effectiveness of three seed-trap designs. *Australian Journal of Botany*, 50, 587-594.

Paton, D.C., Crossfield, E.L., Hurrell, B., & Rogers, D.J. (2004). *Floral resources used by the South Australian Apiary Industry*. Rep. No. Project No DEH-1A. Rural Industries Research and Development Corporation, Canberra.

Pearcy, R.W., Ehleringer, J.R., Mooney, H.A., & Rundel, P.W. (1989). *Plant Physiological Ecology: Field methods and instrumentation.* Chapman and Hall, London.

Pettit, N.E. & Froend, R.H. (2001). Availability of seed for recruitment of riparian vegetation: a comparison of a tropical and a temperate river ecosystem in Australia. *Australian Journal of Botany*, 49, 515-528.

Pickett, S.T.A. & McDonnell, M.J. (1989). Seed bank dynamics in temperate deciduous forests. In *Ecology of soil seed banks* (eds M.A. Leck, V.T. Parker & R.L. Simpson), 123-148. Academic Press, San Diego.

Pigram, J.J. & Hooper, B.P. (1992). *Water Allocation for the Environment*. Proceedings international seminar and workshop, Nov 1991 Centre for Water Policy Research, University of New England, Armidale, New South Wales.

Poff, N.L., Allan, J.D., Bain, M.B., Karr, J.R., Prestegaard, K.L., Richter, B.D., Sparks, R.E., & Stromberg, J.C. (1997). The Natural Flow Regime. A paradigm for river conservation and restoration. *BioScience*, 47, 769-784.

Poiani, K.A. & Johnson, W.C. (1988) Evaluation of the emergence method in estimating seed-bank composition of prairie wetlands. *Aquatic Botany*, 32, 91-97.

PPK (1998). Assessment of the impact of the Bookpurnong/Lock 4 Irrigation District on floodplain health and implications for future options. Loxton to Bookpurnong Local Action Planning Committee, Adelaide.

Pressey, R.L. (1986). *Wetlands of the River Murray*. Rep. No. RMC Environmental Report 86/1. River Murray Commission, Canberra.

Pressey, R.L. (1987). *The Murray Wetlands in South Australia: Management considerations and research needs*. Background Paper No 5, Murray Valley Management Review. Department of Environment and Planning, Adelaide.

Pressey, R.L. (1990). Wetlands. In *The Murray* (eds N. Mackay & D. Eastburn). Murray-Darling Basin Commission, Canberra.

Puckridge, J.T., Sheldon, F., Walker, K.F., & Boulton, A.J. (1998). Flow variability and the ecology of large rivers. *Australian Journal of Marine & Freshwater Research*, 49, 55-72.

Pudney, S. (1998) Seed dispersal in Eucalyptus camaldulensis: implications for natural regeneration. Honours thesis, The University of Adelaide, Adelaide.

Quinn, G. & Hillman, T. (2004). Responses of floodplain wetlands to seasonal flooding and the role of environmental flows. In *7th Intecol International Wetlands Conference* (eds J.T.A. Verhoeven, E. Dorland & M. Coemans). Utrecht University, Utrecht, Holland.

Rea, N. & Ganf, G.G. (1994). The influence of water regime on the performance of aquatic plants. *Water Science Technology*, 29, 127-132.

Richardson, C.J. & Hussain, N.A. (2006). Restoring the Garden of Eden: An Ecological Assessment of the Marshes of Iraq. *BioScience*, 56, 477-489.

RMCWMB (2002). *Water Allocation Plan for the River Murray Prescribed Watercourse*. River Murray Catchment Water Management Board, Berri, South Australia.

Roberts, J. & Marston, F. (2000). *Water regime of wetland and floodplain plants in the Murray-Darling Basin*. Rep. No. Technical Report 30/00. CSIRO Land & Water, Canberra.

Roberts, J. (2003). Floodplain forests and woodlands in the southern Murray-Darling Basin. Australian Conservation Foundation, Canberra.

Roberts, J., Bourman, B., Close, A., Erdmann, B., Hillman, T., Jones, G., Ganf, G., Geddes, M., Koehn, J., Newman, B., Paton, D., Suter, P., & Thoms, M. (2001). *A Scientific Panel Assessment of Modelled Flow and Technical Options for the River Murray*. Murray-Darling Basin Commission, Canberra.

Robertson, H. & James, K. (2004). Wetland plant establishment from the seed bank of a degraded floodplain wetland: Kanyapella Basin, Australia. In *7th Intecol International Wetlands Conference* (eds J.T.A. Verhoeven, E. Dorland & M. Coemans). Utrecht University, Utrecht, Holland.

Robertson, P.A., Weaver, G.T., & Cavanaugh, J.A. (1978). Vegetation and Tree Species Patterns near the Northern Terminus of the Southern Floodplain Forest. *Ecological Monographs*, 48, 249-267.

Sedell, J.R., Richey, J.E., & Swanson, F. (1989). The river continuum concept: a basis for the expected ecosystem behavior of very large rivers? *Canadian Special Publications in Fisheries and Aquatic Sciences*, 106, 49-55.

Shafroth, P.B., Stromberg, J.C., & Patten, D.T. (2000). Woody riparian response to different alluvial water table regimes. *Western North American Naturalist* 60:66-76.

Shafroth, P.B., Stromberg, J.C., & Patten, D.T. (2002). Riparian vegetation response to altered disturbance and stress regimes. *Ecological Applications*, 12, 107-123.

Sharley, T. & Huggan, C. (1995). *Chowilla Resource Management Plan*. Final Report. Murray-Darling Basin Commission, Canberra.

Siebentritt, M.A., Ganf, G.G., & Walker, K.F. (2004). Effects of an enhanced flood on riparian plants of the River Murray, South Australia. *River Research & Applications*, 20, 765-774.

Slavich, P.G., Walker, G.R., Jolly, I.D., Hatton, T.J., & Dawes, W.R.J. (1999). Dynamics of *Eucalyptus largiflorens* growth and water use in response to modified watertable and flooding regimes on a saline floodplain. *Agricultural Water Management*, 39, 245-264.

Southgate, A. (1988). A Reconnaissance of the Ecology of Lignum (*Muehlenbeckia cunninghamii* (Meissner) F Muell.) on the River Murray Floodplain. Honours thesis, University of Adelaide, Adelaide.

Specht, R.L. & Wood, J.G. (1972). *The Vegetation of South Australia.* Government Printer, Adelaide.

Stanford, J.A., Ward, J.V., Liss, W.J., Frissell, C.A., Williams, R.N., Lichatowich, J.A., & Coutant, C.C. (1996). A general protocol for restoration of regulated rivers. *Regulated Rivers: Research & Management*, 12, 391-414.

Stone, M.G. (2001). The effect of water regime on seed bank dynamics of three lower River Murray wetlands. Honours thesis, University of Adelaide, Adelaide.

Streever, W.J. (1997). Trends in Australian Wetland Rehabilitation. *Wetlands Ecology and Management*, 5, 5-18.

Stroh, C.L., De Steven, D., & Guntenspergen, G.R. (2008). Effect of climate fluctuations on long-term vegetation dynamics in Carolina Bay wetlands. *Wetlands*, 28, 17-27.

Stromberg, J.C. (1993). Fremont cottonwood-Goodding willow riparian forests: a review of their ecology, threats and recovery potential. *Journal of Arizona-Nevada Academy of Science* 27:97-110.

Stromberg, J.C., Boudell, J.A., & Hazelton, A.F. (2008). Differences in seed mass between hydric and xeric plants influence seed bank dynamics in a dryland riparian ecosystem. *Functional Ecology*, 22, 205-212.

Stromberg, J.C., Lite, S., & Beauchamp, V. (2003) Managing stream flow regimes for riparian ecosystem restoration. In *Proceedings of 2003 Tamarisk Symposium*, Grand Junction, Colorado.

Thompson, K., & Grime, J.P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology* 67:893-921.

Thompson, M.B. (1986). *River Murray Wetlands: their characteristics, significance and management*. Nature Conservation Society of South Australia and the Department of Environment and Planning: Adelaide, Adelaide.

Thoms, M., Suter, P., Roberts, J., Koehn, J., Jones, G., Hillman, T., & Close, A. (2000). *Report of the River Murray Scientific Panel on environmental flows. River Murray - Dartmouth to Wellington* and the Lower Darling River. Murray-Darling Basin Commission, Canberra.

Tiver, F. & Andrew, M.H. (1997). Relative effects of herbivory by sheep, rabbits, goats and kangaroos on recruitment and regeneration of shrubs and trees in eastern South Australia. *Journal of Applied Ecology*, 34, 903-914.

Tiver, F. & Kiermeier, A. (2006) Survivorship of seedlings of false sandalwood (*Myoporum platycarpum*) in the chenopod rangelands grazed by sheep, kangaroos and rabbits at Whyalla, South Australia. *Austral Ecology*, 31, 376-387.

Tockner, K. & Stanford, J.A. (2002). Riverine flood plains: present state and future trends. *Environmental Conservation*, 29, 308-330.

Tockner, K. & Stanford, J.A. (2004). Biocomplexity of floodplains: defining reference conditions. In 7th Intecol International Wetlands Conference (eds J.T.A. Verhoeven, E. Dorland & M. Coemans). Utrecht University, Utrecht, Holland.

Treloar, G.K. (1959). Some factors affecting seedling survival of *Eucalyptus largiflorens* F Muell. *Australian Forest Research*, 23, 46-48.

Tucker, P. (2003). *Your Wetland: Supporting Information*. Australian Landscape Trust, Melbourne, Australia.

Tucker, P., Harper, M., Dominelli, S., van der Wielen, M., & Siebentritt, M. (2002). *Your Wetland: Hydrology Guidelines*. Australian Landscape Trust, Renmark, South Australia.

Turnbull, J. & Doran, J. (1987). Seed development and germination in the Myrtaceae. In *Germination of Australian Plant Seed* (ed P.J. Langkamp). Inkata Press, Melbourne.

van der Valk, A.G. & Davis, C.B. (1978). The role of seed banks in the vegetation dynamics of prairie glacial marshes. *Ecology*, 59, 322-335.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., & Cushing, C.E. (1980). The river continuum concept. *Canadian Journal of Fisheries & Aquatic Sciences*, 37, 130-137.

VEAC (2007). *River Red Gum Forests Investigation*. Draft Proposals Paper for Public Comment. Victorian Environmental Assessment Council, Melbourne.

Walker, K.F. (1986). The Murray-Darling River system. In *The Ecology of River Systems* (ed B.R.W. Davies, K F), pp. 631-659. Dr W Junk Publishers, Dordrecht, The Netherlands.

Walker, K.F. (1990). Mussels. In *The Murray* (eds N. McKay & D. Eastburn). Murray-Darling Basin Commission, Canberra.

Walker, K.F. (2002). Ecology and Hydrology. In *The Science of Environmental Water Requirements in South Australia*. Hydrological Society of South Australia Inc, Adelaide.

Walker, K.F. (2006). Serial weirs, cumulative effects: the Lower River Murray, Australia. In *The Ecology of Desert Rivers* (ed R.T. Kingsford), pp. 248-279. Cambridge University Press, Cambridge.

Walker, K.F., Boulton, A.J., Thoms, M.C., & Sheldon, F. (1994). Effects of water-level changes induced by weirs on the distribution of littoral plants along the River Murray, South Australia. *Australian Journal of Marine & Freshwater Research*, 45, 1421-38.

Walker, K.F., Sheldon, F. & Puckridge, J.T. (1995). A perspective on dryland river ecosystems. *Regulated Rivers: Research & Management*, 11, 85-104.

Walker, K.F. & Thoms, M.C. (1993). Environmental effects of flow regulation on the Lower River Murray, Australia. *Regulated Rivers: Research & Management*, 8, 103-119.

Walker, K.F., Thoms, M.C., & Sheldon, F. (1992). Effects of weirs on the littoral environment of the River Murray, South Australia. In *River Conservation and Management* (eds P.J. Boon, P. Calow & G.E. Petts). John Wiley & Sons Ltd, York, England.

Walkley, A. & Black, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, 37, 29-38.

Walters, A.C. & Mackay, D.A. (2003). An experimental study of the relative humidity preference and survival of the Argentine ant, *Linephithema humile* (Hymenoptera, Formicidae): comparisons with a native *Iridomyrmex* species in South Australia. *Insectes Sociaux*, 50, 355-360.

Ward, J.V. & Stanford, J.A. (1983). The serial discontinuity concept of lotic ecosystems. In *Dynamics of Lotic Ecosystems* (eds T.D. Fontaine & S.M. Bartell). Ann Arbor Science, Ann Arbor, Michigan.

Ward, J.V. & Stanford, J.A. (1989). Riverine ecosystems: the influence of man on catchment dynamics and fish ecology. *Canadian Special Publication of Fisheries and Aquatic Sciences*, 106, 56-64.

Ward, J.V. & Stanford, J.A. (1995). The serial discontinuity concept: extending the model to floodplain rivers. *Regulated Rivers: Research & Management*, 10, 159-168.

Ward, J.V., Tockner, K., & Schiemer, F. (1999b). Biodiversity of floodplain river ecosystems: ecotones and connectivity. *Regulated Rivers: Research & Management*, 15, 125-139.

Ward, J.V., Tockner, K., Edwards, P.J., Kollmann, J., Bretchko, G., Gurnell, A.M., Petts, G.E., & Rossaro, B. (1999a). A reference river for the Alps: the 'Fiume Tagliamento'. *Regulated Rivers: Research and Management*, 15, 63-75.

Ward, J.V., Tockner, K., Uehlinger, U., & Malard, F. (2001). Understanding natural patterns and processes in river corridors as the basis for effective river restoration. *Regulated Rivers: Research & Management*, 17, 311-323.

Webb, L. & Nichols, S. (1997). A Study of *Eucalyptus largiflorens* dieback adjacent to the Disher Creek Evaporation Basin: Executive Summary. Department of Environment and Natural Resources, Adelaide.

Wellington, A.B. & Noble, I.R. (1985). Seed dynamics and factors limiting recruitment of the mallee *Eucalyptus incrassata* in semi-arid, south-eastern Australia. *Journal of Ecology*, 73, 657-666.

Whittington, J. (2002). Working Rivers. In *Watershed*, Vol. February 2002.

Whittington, J., Coysh, J., Davies, P., Dyer, F., Gawne, B., Lawrence, I., Liston, P., Norris, R., Robinson, W., & Thoms, M. (2001). *Development of a Framework for the Sustainable Rivers Audit*. Final Report, Project R2004. CRC for Freshwater Ecology, for Murray-Darling Basin Commission.

Yates, C.J., Hobbs, R.J., & Bell, R.W. (1994). Factors Limiting the Recruitment of *Eucalyptus salmonophloia* in Remnant Woodlands. I. Pattern of Flowering, Seed Production and Seed Fall. *Australian Journal of Botany*, 42, 531-542.

Yates, C.J., Hobbs, R.J., & Bell, R.W. (1996). Factors Limiting the Recruitment of *Eucalyptus salmonophloia* in Remnant Woodlands. III Conditions Necessary for Seed Germination. *Australian Journal of Botany*, 44, 283-296.

Yates, C.J., Taplin, R., Hobbs, R.J., & Bell, R.W. (1995). Factors Limiting the Recruitment of *Eucalyptus salmonophloia* in Remnant Woodlands. II Post-dispersal Seed Predation and Soil Seed Reserves. *Australian Journal of Botany*, 43, 145-155.

Young, W. (2001). *Rivers as Ecological Systems: the Murray-Darling Basin*. Murray-Darling Basin Commission, Canberra.

Zalewski, M. & Robarts, R. (2004). Ecohydrology -- a new paradigm for integrated water resources management. In *SIL News*, Vol. 40, pp. 1-5.

Chapter12 Appendix I Ploidy Analysis for Lignum

Background

Flowering plants have evolved polyploid forms which are expected to result in enhanced competitive ability or ecological tolerance compared to parental diploid plants (Nakagawa, 2006). The distribution of polyploid plants is therefore expected to reflect their ecological preferences.

In trees of the Australian Casuarina genus, instances have been noted where this dioecious tree has an excess of female plants in its populations. Sexual populations may be either diploid or tetraploid but populations also exist with many sterile triploid plants, which are mainly female (Eckert *et al.*, 2003). The triploid plant can reproduce both sexually and apomictically (asexual reproduction through seed), as well as vegetatively (Barlow, 1959). There have been many studies of genetic diversity and the proportion of sexual and clonal progeny in plants populations to understand the effect of ecological factors on reproductive mode (Eckert *et al.*, 2003).

The dioecious species tangled lignum (*Muehlenbeckia florulenta*) reproduces by both asexual and sexual methods. As well as production, dispersal and germination of seed, this species can produce new individuals by layering of branches, or new shoots from root nodes. The asexual mode of reproduction may offer an advantage in the fluctuating water regimes of the floodplain habitat where it is a dominant perennial shrub. The balance between sexual and clonal reproduction varies in response to limiting ecological factors, as well as the relative production.

Instances have been found in the field where one gender dominates a local population. This suggests that the distribution of male and female lignum plants may be reflected in the ploidy of individual plants and local populations, and an assessment of the ploidy status of lignum could determine if plants have reproduced by sexual or clonal means.

Most *Muehlenbeckia* species have $2n$=20 chromosomes, so the finding of $2n$=80 by Conran (Chong & Walker, 2005) was unusual. This study sought to confirm the finding of octoploidy, as a potential tool to investigate the distribution of male and female plants.

Methodology

Initial tests were done on root tips from lignum cuttings struck first in water and then planted out in a soil mixture (1 floodplain soil : 1 gypsum : 2 potting mix) for three months.

Tips of sprouting roots were cut and immersed in reverse osmosis (RO) water in 1.5 mL Eppendorf® tubes, placed in a slurry of ice and water and refrigerated for 24 h. The purpose of this treatment was to shrink the chromosomes and stop cell division at metaphase. The water was then decanted and the root samples fixed in a fresh mixture of 3 methanol: 1 glacial acetic acid. The samples were stored in 70% methanol in a freezer.

The staining-followed-by-squashing method was used to prepare slides (Guerra, 1999). Samples were rinsed twice in RO water to remove the fixative. They were then immersed in 5 Molar HCl for 15-20 minutes maximum to dissolve pectins and soften cell walls, and to denature the cytoplasm. The HCl was decanted into water and samples were rinsed twice in RO water for 10 minutes to hydrolyse them.

The growing point (meristem) was excised from each root sample, excess liquid blotted off and the meristem placed on a microscope slide. A drop of Toluodine Blue stain was added, the specimen macerated in the stain and left for 1-2 minutes. The specimen was then mashed by tapping gently on the cover slip to spread the sample enough to reveal cells undergoing mitosis. Finally, even pressure was applied by pressing on filter paper padding over the cover slip to reduce the material in the sample to two dimensions.

Toluidine blue appeared to be too intense, colouring all material on the slide (0.1% toluodine blue in 10% ethanol). An alternative stain which was also tested was Aceto orcein, with 1 drop of lacto acetic orcein (45%) to fix the sample.

These tests failed to produce satisfactory samples of chromosomes in either toluodine blue or aceto orcein stains. Very few chromosomes were found, from over 20 attempts. Material from cuttings grown in RO water also failed to produce successful chromosome samples (Lynch, 2006). The slide preparation technique was checked by an expert, who also failed to obtain chromosome samples from this material (Dr J. Conran, University of Adelaide, pers comm.).

The process was repeated with cuttings placed in 10% Hoaglands solution to provide nutrients for growth. For the second experiment, lignum cuttings with at least 3 nodes were taken from the field and placed in 10% Hoaglands solution until roots sprouted. Samples were taken from male and female bushes at Chowilla Creek, Pilby Junction and three habitat locations at Brenda Park (wet zone, moist riparian zone and dry zone). Hoaglands solution consists of:

• 1M KNO3	(5 mL/L)
• 1M Ca(NO3)2	(5 mL/L)
• 1M MgSO4	(2 mL/L)
• 1M KH2PO4	(1 mL/L)
• 1000 micronuts	(1 mL/L)
• FeEDTA	(1 mL/L)

An alternative method was also tried, using Snow’s carmine stain (16:1 70% ethanol: 1NHCl + setol carmine). Samples were placed in RO water with one drop of this stain, and refrigerated for a minimum 14 d. The Snow’s carmine stain was more successful, and one sample was isolated for which *n*=80 could be counted.

Unfortunately, the process to fix the slide permanently in clear gel was not successful, and the sample was lost before it could be photographed.