Recruitment of long-lived floodplain vegetation:

Literature review

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Recruitment of long-lived floodplain vegetation: Literature review

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Contents

List of Figures

List of Tables

Executive summary

The 'Recruitment of Long-lived Floodplain Vegetation' component of the MDB EWKR Vegetation Theme aims to increase our understanding of how recruitment and seedling establishment occurs in response to varying watering regimes. Four woody floodplain species, River Red Gum, Black Box, Tangled Lignum and Coolibah, were identified as key target species within the Murray–Darling Basin to investigate the drivers of sustainable populations within four field-based research sites, Upper Murray, Lower Murray, Macquarie Marshes and the Lower Balonne floodplain.

Species-specific literature reviews where undertaken to assess and collate existing information available about the recruitment and establishment of seedlings. This review focused on understanding how flow and non-flow drivers influence recruitment and seedling establishment responses and the response to water regimes over multi-year timeframes for the key target species.

For the four key species identified, the aims of this report were to:

- summarise existing knowledge of seedling establishment requirements
- conceptualise the processes that lead to successful seedling establishment
- identify the key flow and non-flow drivers influencing successful establishment
- highlight knowledge gaps that can be addressed through mesocosm experiments
- develop appropriate experimental designs for prioritised mesocosm experiments to address the knowledge gaps identified.

A number of knowledge gaps where identified in relation to the four key species' recruitment and seedling establishment phases:

- Depth of inundation –
    - How do rates of rise and fall of floodwater affect soil moisture?
    - What are ideal and maximum flood depths?
- Duration of inundation –
    - What are the effects on germination success?
    - What are ideal and maximum flood durations?
- Sequence of and/or consecutive inundation –
    - Are there seasonality effects?
    - Are effects site specific?
    - Do multiple small and/or follow-up flooding benefit seedling establishments?
- Timing/season of inundation –
    - What are the effects in response to soil moisture persistence?
    - How are reproduction and seedling establishment, including survival and growth affected?
- Water stress
    - Are effects site specific?
- Soil salinity
    - Effects are unknown

In addition to the species-specific literature review, a review on the methods and experimental approaches conducted on woody floodplain species was undertaken to aid in the design of a mesocosm experiment. The review on experimental designs and use of mesocosm methods has highlighted possible designs/methods that can be used to address the key questions of long-lived vegetation, such as:

1. What is the relationship between soil moisture and seedling survival and root development?
2. What is the relationship between flow parameters such as duration and frequency (sequential, multi-year) and seedling survival and root development?
3. What is the critical time period between germination and successful seedling establishment and, therefore, what sequence of multi-year watering may be required to facilitate successful establishment?
4. How do stressors and threats (e.g. soil type, salinity, grazing pressure) modify the expected recruitment outcomes to flow regimes?

Understanding seedling root growth development and the drivers of population structure across multiple scales is fundamental to successful recruitment and seedling establishment. There is a critical time period between germination and seedling establishment, and so it is important to determine at what point seedling establishment is successful.

1 Introduction

1.1 Project background

The Vegetation Theme was established in the Murray–Darling Basin Environmental Water Knowledge Research (MDB EWKR) project to enable effective exploration of the effect of flow on aquatic and floodplain plants and an understanding of how stressors (e.g. land use, grazing, salinity and climate change) influence predicted outcomes from the use of environmental water for both understorey plant communities and long-lived woody vegetation (MDFRC 2015a). Aquatic and floodplain plants are critical components of floodplain and wetland ecosystems in that they provide refuge, breeding habitat and an important food source for a wide range of organisms, contribute to ecosystem services such as, nutrient and carbon cycling, water and sediment oxygenation, and have an intrinsic biodiversity value (MDFRC 2015a). Across the Murray–Darling Basin (MDB), the maintenance or improvement in the 'health' of aquatic and floodplain vegetation is a priority objective of the majority of management plans through the delivery of environmental water (MDFRC 2015a).

The Priority Research Question agreed to by the Steering Committee (Burns & Gawne 2014) for the Vegetation Theme will aim to address:

> *'What are the drivers of sustainable populations and diverse communities of water-dependent vegetation?'*

This question seeks to explore the key functional processes that drive outcomes for water-dependent vegetation populations and communities, as well as the situations under which each of these processes become limiting (Burns & Gawne 2014). From this process, seedling establishment was identified as being a priority for water managers, and recent literature reviews have identified successful establishment as a knowledge gap (Casanova 2015). It was felt that datasets looking specifically at establishment responses were likely to be limited (pers. comm. EWKR Vegetation Theme Leadership Group), and that focusing studies on seedling responses was an appropriate way to ensure this priority research question was addressed.

1.2 MDB EWKR research sites

The MDB EWKR project identified four sites (Table 1) for the focus of research activities, noting non-field-based activities are not defined by the boundaries of the four sites (Burns & Gawne 2014). The sites needed to be able to provide opportunities to address priority questions at both the area and basin scales, yet not all priority research topics need to be explored at all four sites. For more information relating to the selection and description of research sites, refer to Burns and Gawne (2014) and MDFRC (2015b).

Table 1. MDB EWKR field research sites.

Basin region	Research site	Incorporating area
Southern Basin	Upper Murray	Centred around Barmah–Millewa Forest and potentially including lower reaches of adjacent tributaries (Goulburn and Campaspe) and parts of the Edward–Wakool system
	Lower Murray	Centred around the Chowilla–Lindsay–Wallpolla Floodplain and potentially including the Riverland Ramsar site and adjacent floodplain systems and river reaches
Central Basin	Macquarie Marshes	Focusing on the floodplain wetlands of the Macquarie Marshes, which form north of Marebone Weir on the Macquarie River, including parts of the Macquarie Marshes Ramsar site and Nature Reserve
Northern Basin	Lower Balonne floodplain	Focusing on the Narran Lakes ecosystem, which includes the Narran Lakes Nature Reserve in the north, Narran Lake in the south and the surrounding floodplain in-between.

1.3 Key species

The recruitment and establishment of long-lived woody vegetation seedlings was specifically identified as a priority in most workshops with managers. Four key woody floodplain species were identified as target species, consisting of three eucalypt tree species, River Red Gum (*Eucalyptus camaldulensis* Dehnh.), Black Box (*Eucalyptus largiflorens* F.Muell.) and Coolibah (*Eucalyptus coolabah* Blakely & Jacobs), and one native floodplain shrub species, Tangled Lignum (*Duma florulenta* Meissner) (Burns & Gawne 2014). This is due to their significant role as major structural components of floodplain communities in the MDB and because expected outcomes for these species are specifically mentioned in the Murray–Darling Basin Authority's (MDBA) Basin-Wide Environmental Watering Strategy (BEWS) (MDBA 2014).

River Red Gum has been well studied in the southern MDB, and its ecology is better known than any other riparian tree in Australia (Roberts & Marston 2011). However, flow regime requirements and the influence of flow on seedling recruitment and establishment are less well understood for Black Box, Coolibah and Lignum. As such, it is anticipated that more effort will be expended on the latter three species. These four species occur throughout the MDB, although populations are not consistent at all of the MDB EWKR research sites, and thus reviews and mesocosm experimental designs will reflect this distribution (Table 2).

Table 2. Species distribution within the Murray-Darling Basin, based on the Atlas of Living Australia (X = present in MDB EWKR research site).

Sites Key species	Upper Murray	Lower Murray	Macquarie Marshes	Lower Balonne floodplain
River Red Gum	X	X	X	X
Black Box	X	X	X	X
Coolibah			X	X
Tangled lignum	X	X	X	X

While adult population survival and condition are among the better understood aspects of vegetation ecology (certainly in relation to River Red Gum and Black Box), seedling establishment and recruitment are not as well understood, yet these remain central to environmental watering decisions. Major knowledge gaps identified in relation to the four key species recruitment and seedling establishment phases include:

- How do vegetation responses vary with water regimes and among sites across the Basin?
- How do required water regimes vary with plant condition or age?
- How do required water regimes vary between years (e.g. with respect to antecedent conditions, benefits of cumulative events)?

1.4 Aims and key research questions

The Vegetation Theme aims to improve the capacity to predict vegetation outcomes in response to the delivery of environmental water through an enhanced understanding of how flow and non-flow variables influence vegetation responses (Burns & Gawne 2014). Specifically, the proposed research questions are:

1. What flow regimes best support recruitment within populations of long-lived floodplain vegetation species?
    - o How significant are the individual drivers (habitat availability, connectivity – dispersal) on recruitment?
    - o How do key drivers interact to influence outcomes?
    - o How should flows be managed to enhance drivers and thereby recruitment?
    - o How do the characteristics of sites (soil type, climate etc.) influence these flow requirements?
2. How do threats impact on the drivers and recruitment outcomes?

The aim of this population recruitment priority research topic is to understand the drivers of population recruitment and seedling establishment for key species across multiple scales (Burns & Gawne 2014). Priority threats identified in Burns and Gawne (2014) as most significant in terms of their potential impact on vegetation outcomes, include:

Flow independent threats

- Invasive species
- Grazing
- Habitat loss/land use

Flow related threats

- Climate change
- Groundwater/salinization
- Water quality

For the four key species identified, the aim of this report was to:

- summarise existing knowledge of seedling establishment requirements
- conceptualise the processes that lead to successful seedling establishment
- identify the key flow and non-flow drivers influencing successful establishment
- highlight knowledge gaps which can be addressed through mesocosm experiments
- develop appropriate experimental designs for prioritised mesocosm experiments to address the knowledge gaps identified.

1.5 Purpose and approach

There is general consensus that the flow regime requirements of seedling establishment and recruitment differ to the requirements of adult survival and condition, and are not as well understood (MDFRC 2015a). Seedling establishment is reliant on the availability of viable seeds, appropriate conditions for germination, and ongoing suitable conditions for the growth and establishment of seedlings (MDFRC 2015a). Species-specific literature reviews were undertaken to assess and collate the existing information available about the recruitment of seedlings of the four key species. This brief review acknowledges the recent work of others and draws heavily on their findings to avoid duplication (Casanova 2015; Johns *et al.* 2009; Roberts & Marston 2011; Rogers & Ralph 2011). The review also includes an assessment of experimental techniques that have been used to assess seedling responses, to ensure that the techniques applied in the MDB EWKR project build on the knowledge of previous work.

This literature review will aid in the development of a pilot study to test techniques for establishing seedlings, including seedling root development in mesocosm tanks, and to test techniques to apply different flow and non-flow variables to undertake these mesocosm experiments. An experimental design will be finalised, based on the results of the pilot study, to determine flow and non-flow variables, levels of manipulation, interactions between variables and required replication. Assessment of seedling responses will include seedling survival, root development, above and below-ground biomass in relation to soil moisture and linked flow parameters (duration, frequency, sequencing of events) and stressors (grazing pressure, soil salinity, soil type).

Outputs from this component will be used to inform water regimes and complementary management of tree and Lignum seedlings through:

- a literature review report summarising the current knowledge of seedling recruitment
- an experimental design report.

1.6 What is a seedling?

The germinant-seedling stage is possibly the most vulnerable life-history stage for most plant species (Capon 2012; Holloway *et al.* 2013; Johns *et al.* 2009), with root growth regarded as an important factor in seedling survival (Schütz *et al.* 2002). Despite the importance in understanding seedling development and establishment, there is no consensus on what constitutes a seedling or when it ceases to be a seedling. Studies have defined seedlings as; 1) a young plant that has developed from a seed (Fenner 1987), 2) is formed following the radicle that grows into the soil as the root and the plumule that grows away from the soil towards light as a shoot (Shivanna & Tandon 2014) or 3) is a non-reproductive plant (Hanley *et al.* 2004).

A plant is no longer considered to be a seedling when; 1) it has emerged from the soil surface until the end of its exponential growth (Sattin & Sartorato 1997); 2) when seed reserves are exhausted (Hanley *et al.* 2004); 3) when the seedling changes its dependence from seed resources to external resources (Soriano *et al.* 2013); or 4) based on seedling height (Shivanna & Tandon 2014). The seedling establishment phase for eucalypt species defined by Johns *et al.* (2009) is the period of growth from production of the first true leaves until sapling stage, when the root systems has developed sufficiently to access moisture from sources other than surface flows, including deep soil moisture and/or groundwater resources.

The definition of what constitutes a seedling for floodplain shrubs differs to that for floodplain trees, and the height or age of when Lignum reaches maturity or ceases to become a seedling is unknown. Lignum has the ability to develop from a seed or vegetatively reproduce (Casanova 2015; Holloway *et al.* 2013), with vegetative reproduction occurring more than sexual reproduction (Cale 2009; Capon *et al.* 2009) and new plants striking from nodes on roots or on branches once they come into contact with the soil (Jensen 2008). Consequently, determining seedling morphology is problematic.

For the purpose of this review, a seedling has been defined as a young plant, starting from the production of the first true leaves and ceasing based on the seedling height of 0.6–1.3 m tall (Capon 2012; Fox *et al.* 2004; George 2004; Johns *et al.* 2009).

1.7 Conceptualisation

Conceptual models focusing on flow and non-flow variables of the four key species in relation to recruitment (i.e. germination) and seedling establishment identify linkages between various flow regime components and species responses. Single models for recruitment (Figure 1) and seedling establishment (Figure 2), each, are sufficient to describe the linkages for these species.

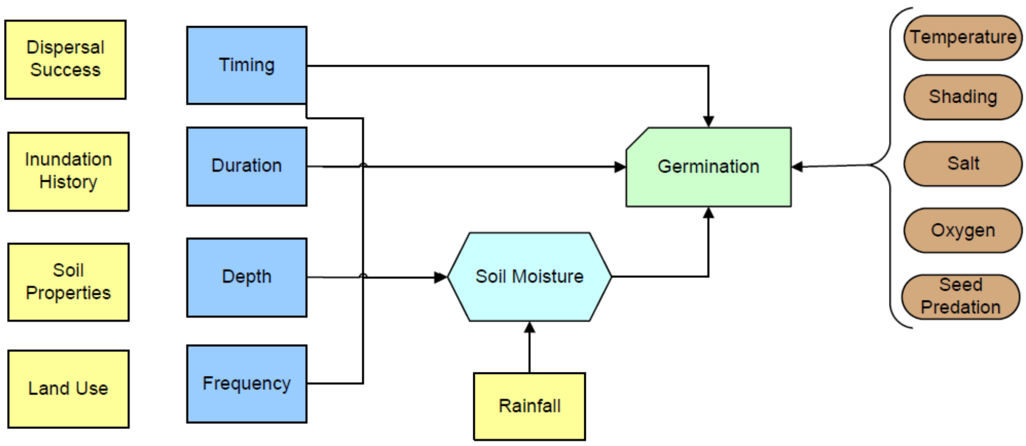

Figure 1. Conceptual model summarising the main relationships between flow and non-flow variables and germination. Blue boxes indicate flow variables, hexagons are primary controls, yellow boxes are modifying factors, green boxes are response components and brown ovals are non-flow (potential stressor) variables (Johns *et al.* 2009).

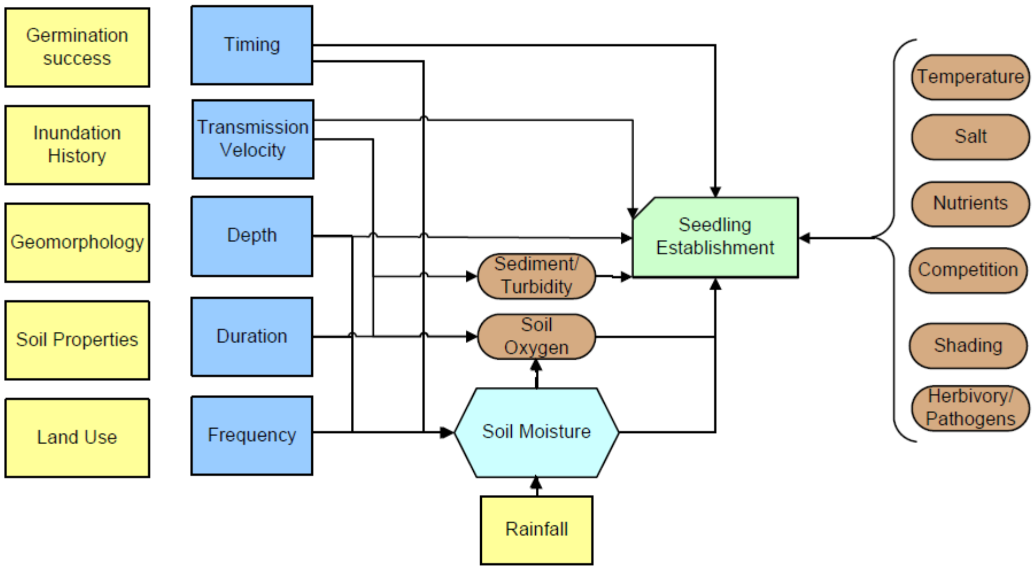

Figure 2. Conceptual model summarising the main relationships between flow and non-flow variables and seedling establishment. Blue boxes indicate flow variables, hexagons are primary controls, yellow boxes are modifying factors, green boxes are response components and brown ovals are non-flow (potential stressor) variables (Johns *et al.* 2009).

2 Black Box: *Eucalyptus largiflorens*

2.1 Introduction

Black Box is a small to medium tree, 10–20 m tall, with a large spreading crown and drooping branches that forms open woodlands on floodplains and on the fringes of ephemeral lakes and water courses (Cunningham *et al.* 1992). It is one of the dominant floodplain tree species throughout the MDB, although its distribution is largely confined to the MDB (Atlas of Living Australia as cited in Casanova 2015). This species typically occurs at higher elevations on the floodplain and displays a low tolerance to waterlogging (Roberts & Marston 2011). It can withstand long periods without floods by shedding leaves, reducing canopy transpiration rates and lowering stomatal conductance (Jolly & Walker 1996).

Black Box trees are important components of riparian zones, providing habitat as well as carbon and nutrient inputs via litter fall (Bogenhuber & Linklater 2012). These ecosystem engineers (sensu Jones *et al.* (1994)) also stabilise banks and regulate water movement through the soil (Bramley *et al.* 2003; Colloff & Baldwin 2010). The condition or health of riparian trees, therefore, influences floodplain function, and it has been suggested that tree condition, population structure and recruitment may be a useful substitute for ecosystem resilience (Colloff & Baldwin 2010).

Growth, flowering and germination in Black Box tend to occur in pulses in response to flooding (Casanova 2015; Roberts & Marston 2011). Reductions in flood frequency are, therefore, likely to reduce growth rates, flowering frequency and recruitment, and subsequently lead to declines in community viability (SKM & Roberts 2003). Although the species is drought hardy and salt tolerant, recent work has drawn attention to reductions in the crown condition and community viability (i.e. insufficient recruitment) of Black Box along the lower Murray River floodplain (George *et al.* 2005; Henderson *et al.* 2010; Lane & Associates 2005; Wallace 2009). These changes have been attributed to increases in soil salinity associated with shallower water tables and reductions in flood frequency (MDBC 2002; Slavich *et al.* 1999).

It is generally accepted that flooding is necessary for Black Box trees to maintain condition, and probably to recruit (Roberts & Marston 2011). Black box also responds to wet conditions (high rainfall and/or flooding) through improvement in tree condition and germination (Bogenhuber *et al.* 2013; Henderson *et al.* 2014a, b; Jensen 2008; Treloar 1959).

2.2 Water requirements for seedling establishment

Black Box seed germination and seedling establishment is reliant on natural flooding events (Holland *et al.* 2013), and subsequent local flooding or rainfall (Dexter 1967 as citied in (George *et al.* 2005; Jensen *et al.* 2008). Maintaining a constant soil moisture level of 10–25% is critical for seedling survival (Jensen 2008). Germination and, therefore, seedling establishment generally occur in a belt along the flood high-water line (Cunningham *et al.* 1992). With germination cued by sunlight and a temperature range of 15–35°C, pre-regulation spring floods would have provided Black Box seedlings with moist growing conditions into summer (Rogers & Ralph 2011).

However, flood duration and drawdown rates are critical. Black Box seeds are able to germinate underwater, but seedlings do not tolerating waterlogging and are unlikely to survive complete immersion unless it is only for a brief duration (Cunningham *et al.* 1992; Jensen 2008). Establishing seedlings experience slow growth when flooded to a depth of 5 cm (Heinrich 1990 as cited in Johns *et al.* (2009)).

Seedling establishment in the first year of growth is largely inhibited by competition for soil moisture, or drought and grazing pressure (Duncan *et al.* 2007; Llewelyn *et al.* 2014; Treloar 1959). Grazing, particularly by sheep rather than cattle, along with burrowing of rabbits distributing the soil profile have been observed to restrict Black Box regeneration (Victoria. 1990).

Seedling establishment to sapling stage or to develop a sinker root takes up to two years, under optimal conditions (George 2004). Newly germinated seedlings are susceptible to frost and heat injury (Johns *et al.* 2009). However, Morris (1984) showed that irrigated two-year-old Black Box seedlings displayed higher tolerance to frost damage than other trees and shrubs. Black Box saplings and mature trees have a relatively high tolerance to saline ground water (<40 dS/m); however, highly saline groundwater reduces the ability of roots to take up water, consequently reducing growth rates (Akeroyd *et al.* 1998; Jolly & Walker 1996). A study on irrigated Black Box seedlings noted high mortalities in the first two years at a site with sodic soils overlying highly saline groundwater within two metres of the surface (Morris 1984). Follow-up flooding, shortly after germination, may be required to provide sufficient soil moisture and nutrients for seedling establishment (George 2004).

2.3 Knowledge gaps

A number of studies and extensive reviews have been conducted on Black Box, covering aspects of life history attributes including recruitment and seedling establishment in relation to water requirements. While Black Box are considered to recruit episodically with floods, this is not the sole reason for regeneration and studies have shown trees can tolerate a range of wet-dry and fresh-saline conditions (Roberts & Marston 2011). Thus, further research questions regarding the specific effects that flooding and/or local rainfall has on soil moisture and the timing/seasonality of these effects, are worthy of consideration:

- If flooding in winter–late spring with water receding into early summer provides optimal moist conditions for germination and seedling establishment, what depth of inundation is required to maintain 10–25% soil moisture and avoid a prolonged drawdown rate causing waterlogging or complete immersion?
- Alternatively, is it better to have multiple short, shallow inundation periods?

- At what age/height or length of time can a seedling tolerate waterlogging or does age/height not matter?

3 Coolibah: *Eucalyptus coolabah*

3.1 Introduction

Coolibah are among the most common trees in arid riverine environments in the north-west of the MDB (Roberts & Marston 2011; Rogers & Ralph 2011). They dominate infrequently inundated floodplains of northern rivers such as the Darling and Gwydir (Roberts & Marston 2011; Rogers & Ralph 2011). Coolibah are medium sized trees, 15–20 metres tall, that vary in shape from erect to spreading (Cunningham *et al.* 1992; Harden 2002; Roberts & Marston 2011). They provide important habitat and shelter for animals on the floodplain and have cultural and heritage significance (Roberts & Marston 2011).

There have been very few ecological studies of Coolibah, and the majority of the studies undertaken to date have been conducted in the Lake Eyre Basin (Roberts & Marston 2011); e.g. regeneration and growth (Roberts 1993) and water sources (Costelloe *et al.* 2008). There are three sub-species of Coolibah in the MDB: *E. coolabah* ssp. *coolabah*, *E. coolabah* ssp. *exerata* and *E. coolabah* ssp. *arida* (Roberts & Marston 2011). For the purposes of this literature review, Coolibah is considered only at the species level, as ecological differences between sub-species are not well established (Roberts & Marston 2011).

3.2 Water requirements for seedling establishment

Flooding is likely to be important for reproduction and seedling establishment of Coolibah trees (Roberts & Marston 2011). A sequence of floods, or flood and wet years, may be necessary to ensure seedlings are well established (Li & Wang 2003; Roberts & Marston 2011; Tuomela *et al.* 2001). Coolibah trees occur on different soil types and on different parts of the floodplain with great variability in flood frequency among sites (Rogers & Ralph 2011). Coolibah trees on the Gwydir are flooded on average every 10–20 years, while Coolibah trees on the Cooper Creek have reportedly been flooded one in every five to six years (Rogers & Ralph 2011). It is likely that successful large-scale regeneration events are dependent on floods and are, therefore, episodic (Roberts & Marston 2011). In New South Wales, it is possible that only six major regeneration events have occurred in the last 105 years (Kerle 2005). The ideal and maximum flood duration and depth for reproduction and regeneration of Coolibah trees are unknown (Rogers & Ralph 2011).

Flowering times vary between regions and across years, and reproductive efforts may be lowered by stressors such as soil salinity and water stress (Roberts 1993; Roberts & Marston 2011). Germination requirements are not well understood, although it has been suggested that Coolibah trees are adapted to regenerate after late summer flooding, based on temperatures required for germination (Roberts & Marston 2011). The effect of season and flood timing on reproduction and seedling establishment are unknown. Successful recruitment may require protection from grazing (Roberts 1993).

3.3 Knowledge gaps

Coolibah trees are quite salt-tolerant (Costelloe *et al.* 2008; Roberts & Marston 2011). On the floodplain of the Diamantina River, mature Coolibahs are growing and using groundwater at salinities of at least 20 000–30 000 mg chloride (Costelloe *et al.* 2008). It is not known if Coolibahs in the MDB have similar tolerances (Roberts & Marston 2011). While mature Coolibahs are able to utilise saline groundwater, higher soil salinities (e.g. ≥ 0.2 dSm^{-1}) may lower their reproductive output (Roberts 1993). The effect of (soil) salinity on seedling establishment is unknown and should be investigated. It is also unknown at what stage (and over what timeframe) Coolibah seedlings transition from reliance on surface and sub-surface soil moisture to developing roots that are able to access and utilise groundwater, including saline groundwater.

In general, further research into the flood responses and water requirements of Coolibah at a range of sites and in all subspecies is required (Roberts & Marston 2011; Rogers & Ralph 2011). Very little is known about seedling establishment requirements of Coolibah, and thus, many research questions need to be addressed, including:

- Is the timing, season or duration of a flood important for seedling establishment?
- Are multiple small floods required (i.e. to trigger flowering, provide sufficient soil moisture for germination and follow up shallow flooding to promote seedling establishment)?
- What are the soil moisture requirements for seedling establishment?
- How critical are depth and duration of flooding for seedling establishment?
- What are the depth and duration limits that seedlings can tolerate?
- What impact does grazing have on seedling establishment?
- What impact does soil salinity have on seedling establishment and how does it affect the flow requirements for establishment?
- What is the critical time period between germination, seedling development and successful establishment?
- At what stage (and over what timeframe) do seedlings go from relying on surface and sub-surface soil moisture to developing roots that are able to access and utilise groundwater, including saline groundwater?

4 River Red Gum: *Eucalyptus camaldulensis*

4.1 Introduction

River Red Gum is the most widely distributed eucalypt in Australia (Brooker *et al.* 2002; Colloff 2014; Roberts & Marston 2011; Rogers 2011; Romanowski 2013), occurring across an area of approximately 5 million km^2 (Boland *et al.* 2006; Butcher *et al.* 2009) that encompasses most climatic zones (McDonald *et al.* 2009). This iconic eucalypt grows along thousands of kilometres of waterways and in intermittently flooded areas, such as on floodplains, and is particularly common around billabongs and other floodplain wetlands (Roberts & Marston 2011; Romanowski 2013). There are seven subspecies currently recognised, with three of these occurring in the MDB: *E. camaldulensis* ssp. *camaldulensis, E. camaldulensis* ssp. *actua,* and *E. camaldulensis* ssp. *arida* (Roberts & Marston 2011). This review concentrates on subspecies *camaldulensis* due to its occurrence mainly in and rarely outside the MDB, and disregards the other two subspecies due to their sporadic occurrences and apparent rarity within the Basin (Casanova 2015; Roberts & Marston 2011).

In the MDB, River Red Gums are dependent on flooding for recruitment and maintenance (Roberts & Marston 2011). The construction of dams, weirs and levees, and increases in water diversions/extractions has reduced the magnitude and duration of mid-range flows required to flood River Red Gums (Bren 1988; Kingsford 2000; Maheshwari *et al.* 1995; Walker 1985). River regulation-induced changes in flow regime are not uniform throughout the Basin. For example, changes in flow duration are minimal at Albury and become more pronounced further downstream, whereas changes in the seasonal distribution of monthly flows (i.e. winter–spring flows reduced and summer–autumn flows increased) are more pronounced at Albury than further downstream where inflows from tributaries augment winter–spring flows (Maheshwari *et al.* 1995). The implications of river regulation for River Red Gums are therefore site dependent and may differ markedly throughout the Basin.

The majority of studies have been done in the southern regions of the Basin on floodplain forests (Roberts & Marston 2011). Many of the early studies into the relationships between river flows and life-history processes of River Red Gum were done as part of the silviculture industry in the mid-Murray region (Dexter 1967, 1970, 1978). Due to the value of River Red Gum as a commercial resource, it has been well studied and its ecology is better known than for any other riparian tree in Australia (Roberts & Marston 2011). However, the knowledge generated in one part of the Basin may lack relevance or applicability elsewhere.

4.2 Water requirements for seedling establishment

Seedling survival in the first year after germination is a critical stage in River Red Gum stands, with the main factors affecting initial survival and establishment being soil moisture and seedbed conditions (Dexter 1967). Low density 'maintenance' seedling establishment can occur in response to above-average (>300 mm) annual rainfall on the lower Murray River floodplain (George 2004; Jensen *et al.* 2008). However, higher density establishment usually occurs in response to medium to large flood events, which are likely to recharge soil moisture reserves for some time afterward (George 2004; Jensen *et al.* 2008).

Seedlings are vulnerable to moisture stress; therefore, moisture must be maintained in the upper levels of the soil profile until seedlings produce sinker roots, allowing access to deeper soil moisture, and then groundwater (George 2004; Jensen *et al.* 2008). In a recent pot experiment, 10–20% soil moisture (volumetric moisture content) was found to be the minimum necessary to sustain seedling growth, with seedlings wilting and dying rapidly once soil moisture fell below 10% (Jensen 2008). During early establishment, River Red Gum seedlings invest more resources into developing roots than other riparian species (Chong *et al.* 2007), so when 23 cm tall, they can produce roots approximately four times plant height (Dexter 1978; Roberts & Marston 2011). Seedlings also develop resilience to stress at a relatively early stage; seedlings only 15 cm were able to shed leaves under stress and recover from axillary buds (Roberts & Marston 2011). Competition for moisture by other understorey vegetation and/or by overstorey trees can influence seedling survival (Roberts & Marston 2011).

River Red Gum seedlings are vulnerable to the effects of flooding and do not tolerate prolonged immersion (Roberts & Marston 2011). However, seedlings do possess some adaptations that allow them to cope with periods of anoxia associated with waterlogging, including adventitious root production and aerenchymatous tissue (Roberts & Marston 2011). Soil moisture is the most important factor for seedling establishment (Johns *et al.* 2009). Tolerance to drying increases as seedlings become established, root systems extend and sapling height increases (Roberts & Marston 2011). Two-month-old seedlings can survive in waterlogged soils for one month without obvious effects on leaf number and height (Marcar 1993; Roberts & Marston 2011). Seedlings 50–60 cm in height can survive extended flooding of 4–6 months, and complete submergence for several weeks, by shedding leaves (Roberts & Marston 2011).

Seedling establishment times for River Red Gum vary according to growing conditions. Seedlings may establish within one year at temperate sites (George 2004). Seedlings are thought to transition to juveniles somewhere between size weeks and 22 months after germination (Roberts & Marston 2011), however, at Banrock Station, on the lower River Murray floodplain, seedlings were not considered fully established until they were 2–3 years of age and >1.3 m in height (George 2004). Drought, lack of flooding and high soil and groundwater salinity at this semi-arid site contributed to extremely high mortality rates in the 2–3 years after germination (George 2004).

Winter floods receding in spring–early summer provide ideal conditions for River Red Gum seedling establishment (Dexter 1967; 1978, cited in Roberts and Marston 2011). Flooding at this time avoids exposure of seedlings to extreme temperatures, and ensures that surface moisture is available to support seedlings during initial root development (Dexter 1967; 1978, cited in Roberts and Marston 2011). Ideally, adequate water to support seedlings through the first summer should be applied before germination (Roberts & Marston 2011). Flooding after germination may lead to seedling mortality due to burial, dislodgement or excessive immersion periods (Johns *et al.* 2009).

Optimum watering frequency will vary between sites according to rainfall, inundation periods and other factors, but should be sufficient to maintain soil moisture levels above a minimum of 10–20% in the top 10 cm during the first summer after germination (Jensen 2008). A follow-up watering may be required one year later to maintain seedlings while root systems develop further (George 2004).

Tolerance to waterlogging increases with seedling height — seedlings 50–60 cm high can survive waterlogging (but not complete immersion) for 4–6 months (Roberts & Marston 2011). Complete immersion of seedlings should be avoided (Roberts & Marston 2011). Seedlings 50–60 cm high ceased growing and shed their leaves after 1–3 weeks of immersion (Roberts & Marston 2011). A rapid drawdown rate is preferable owing to the inability of seedlings to tolerate prolonged periods of immersion (Roberts & Marston 2011). However, soil moisture content should be maintained above 10% within the seedling root depth range (Jensen 2008). Flowing water may lead to dislodgement or burial of establishing seedlings (Johns *et al.* 2009). Seedlings can tolerate experimental waterlogging (surface inundation, not groundwater) with saline solutions equivalent to 1700 Na Cl (Roberts & Marston 2011).

Grazing by sheep, cattle and kangaroos have been noted to severely restrict River Red Gum regeneration, with cattle less destructive compared to sheep and rabbits (Victoria. 1990). This observation was based on the regeneration of River Red Gums occurring in protected areas (e.g. islands, reed beds or dense patches of Lignum) rather than in grazed areas (Victoria. 1990).

4.3 Knowledge gaps

Of the four floodplain species under consideration in this document, more is known about the requirements of River Red Gum seedlings. However, information is required to determine the effects of floodwater retention or flow enhancement on the habitat requirements for River Red Gum seedlings. Quantitative information on how the depth, duration and frequency of flood events affect soil moisture and groundwater levels and quality affect seedling growth and health, is currently limited.

Seedling establishment, rather than germination, is the critical stage in stand regeneration (ANBG 2004). The effects of environmental watering on the water quality and sediment type, may affect the health and growth of seedlings. From this, there may be site-specific requirements for seedlings based on the differences in the habitat of River Red Gum across the Murray–Darling Basin, and this may influence the way environmental water is delivered in different areas of the Basin.

5 Tangled Lignum: *Duma florulenta*

5.1 Introduction

Tangled Lignum (*Duma florulenta* (Meisn.) T.M. Schust; formerly known as *Muehlenbeckia florulenta* Meisn.) is considered one of the most ecologically significant floodplain shrubs in arid and semi-arid regions of Australia (Roberts & Marston 2011; Rogers & Ralph 2011). It dominates large areas of arid and semi-arid floodplain and is particularly common in the Murray–Darling and Lake Eyre basins (Campbell 1973; Capon 2005; Roberts & Marston 2011). Following favourable conditions such as flooding, Lignum can grow to three metres in diameter and form dense thickets (Cunningham *et al.* 1992; Jensen *et al.* 2006; Sainty & Jacobs 1981) and attain 1–3 m in height with persistent rootstock at least 2–3 m deep (Craig *et al.* 1991). This structure is significant as breeding habitat for many colonially nesting waterbirds (Maher & Braithwait 1992; Roberts & Marston 2011), including threatened species (Braithwaite 1976; Frith 1967; Rogers *et al.* 2004), and provides shelter for fish and aquatic invertebrates (Roberts & Marston 2011; Young 2001). During dry periods, the structure of Lignum facilitates the growth of floodplain understorey herbs (Roberts & Marston 2011).

Despite its recognised ecological significance, Lignum is an understudied species (Capon *et al.* 2009). The limited published literature has considered Lignum with respect to: flooding and soil correlations (Craig *et al.* 1991), seed banks (Chong & Walker 2005), germination and growth (Jensen 2008), seedling response to water regimes (Capon *et al.* 2009), gender distribution (Lynch 2006), or as part of broad vegetation community studies (Capon 2005).

5.2 Water requirements for seedling establishment

Lignum seedling establishment across the MDB is variable and not well understood (Roberts & Marston 2011). It has been suggested that initial seedling development may require consecutive floods; one to promote flowering and seed set, then one to promote germination (Rogers & Ralph 2011). Floodplain wet and dry phases do appear to provide important cues for Lignum. The wet phase (i.e. flood inundation) promotes vigorous growth in mature Lignum plants (Campbell 1973; Craig *et al.* 1991; Jensen 2008) and generates seed setting and germination in water or on wet mud (Campbell 1973; Chong & Walker 2005). Damp conditions associated with the drying phase (i.e. following floodwater recession) are fundamental in facilitating seedling growth (Capon *et al.* 2009).

In laboratory experiments, Lignum seedlings demonstrated considerable tolerance to a range of hydrological conditions (Capon *et al.* 2009; Lynch 2006), with damp conditions promoting the greatest growth (Capon *et al.* 2009). Root depth of seedlings under damp and drying conditions grew rapidly, almost tripling in length after 2–4 months, whereas flooding, waterlogging and dry conditions significantly impeded seedling growth (Capon *et al.* 2009). Successful root depth may have important implications for the survival of mature Lignum plants. Mature Lignum plants are estimated to have roots to more than three metres deep, which could enable them access to groundwater during times of low soil moisture or drought (Craig *et al.* 1991).

Although Lignum seedlings demonstrated tolerance of both flooding and drying, Capon *et al.* (2009) noted that these stressors on seedling establishment in the field are likely to be exacerbated by additional pressures such as grazing. Young leaves found on Lignum seedlings are considerably more palatable than mature plants and the authors suggest that this could partially explain the rarity of Lignum seedlings in the field. Lynch (2006) commented on the lack of seedlings recorded in her field surveys investigating growth responses to soil moisture. Jensen (2008) recorded seedlings at only one of three sites in her two and a half year study looking at the role of seed banks and soil moisture in Lignum recruitment. The seedlings were subject to grazing by kangaroos, but survived with stunted growth (Jensen 2008). Further investigation into the effects of grazing on Lignum seedlings would be beneficial to inform management of their survival and maintenance in the field.

5.3 Knowledge gaps

The importance of soil moisture on seedling growth has been identified in Capon *et al.* (2009) for Lignum seedlings from Narran Lakes in the northern MDB. It would be beneficial to determine if the soil moisture requirements for Lignum seedling growth and establishment were similar in other parts of the Basin. It would also be beneficial to assess how identified stressors such as grazing and salinity affect the soil moisture requirements for growth and establishment. While it is known that soil moisture is important for early seedling growth, it is unknown how flood depth and duration affect seedling survival and establishment. Seedling establishment requirements should be given priority for investigation due to the potential ecological consequences of shifts in floodplain vegetation communities (e.g. where seedlings will or will not establish as a result of altered hydrological regimes) (Capon *et al.* 2009).

Rogers and Ralph (2011) suggest that the ideal flood timing for reproduction and regeneration is spring/summer, increasing soil moisture conditions throughout the warmer summer months when growth of (mature) Lignum is at its greatest. It would be beneficial to determine if seedling growth rates are affected by temperature and flood timing (e.g. season). This knowledge would improve management practices (e.g. delivery of water) for seedling survival and growth.

Flooding may also be important in distributing genetic material (Roberts & Marston 2011) and for the (re)colonisation of habitats. Lignum is a dioecious plant and connection of the floodplain to wetlands and rivers during flood may play an important role in gender distribution. Lynch (2006) and Jensen (2008) investigated gender distribution; however, their studies were inconclusive and further investigation is required.

Given changes in flooding regimes throughout the MDB (e.g. as a result of river regulation and climate change), seedling establishment requirements should be given priority for investigation (Capon *et al.* 2009; Jensen 2008). The potential changes to where seedlings can or cannot establish as a result of altered flow regimes may shift floodplain vegetation communities, resulting in significant ecological consequences (Capon *et al.* 2009).

Lignum seedlings can survive inundation; however, inundation may delay seedling development, which could then hamper growth when conditions become favourable (e.g. damp soil as floodwater recedes) (Capon *et al.* 2009). The effect of flooding depth and duration on early seedling establishment requires further investigation. Lynch (2006) suggests that experimenting with varying levels of soil moisture for different periods of time would improve understanding of the factors that promote or inhibit Lignum growth response.

In addition to soil moisture, other factors worthy of investigation that could impact seedling establishment are salinity, soil nutrients, grazing and flood timing (i.e. season). The successful development of root systems is seen as important for the long term survival of Lignum and should be investigated further. Consideration should also be given to how long it takes for seedlings to reach maturity, as well as trends associated with soil moisture and plant gender.

6 Summary on key species

The key research questions relate to flow regimes, sustainable populations, stresses and threats on woody floodplain species in relation to recruitment and seedling establishment. The reviewed studies have concentrated on species in specific parts of the Basin and do not necessarily compare between populations. For example, the literature states that in the southern MDB, River Red Gum seedlings can withstand complete immersion during flooding for several weeks and waterlogging for two months, and that winter flooding maintains soil moisture to minimise the effects of extreme temperature stress during the spring–summer drawdown (Roberts & Marston 2011); however, it is unknown if similar effects occur in the northern MDB populations. While in the northern MDB, studies have identified that Lignum growth is impeded by waterlogging and dry conditions (Capon *et al.* 2009), yet the species can survive grazing pressure even with stunted growth (Jensen 2008). Again, it is unknown if these results are site specific or if similar situations occur in the southern MDB.

The influence of flow and non-flow variables on recruitment and seedling establishment vary between the four key species. Current knowledge and corresponding knowledge gaps are summarised in Table 3.

Table 3. Summary of germination and seedling establishment attributes, watering requirements and identified knowledge gaps. Blue is for refereed scientific literature; Red is for reviews and books; Grey is for published reports, proceedings and theses (grey literature).

Key species	Process	Description		Knowledge gap
		Recruitment (germination)	Seedling establishment	
Black Box	Depth of inundation	• Most likely on moist–wet soils (Johns *et al.* 2009; Holloway et al 2013) • No direct impact on depth as seeds will germinate while floating or underwater (Jensen 2008; Johns *et al.* 2009) • High rainfall and/or flooding increases germination (Jensen 2008)	• Not tolerate waterlogging, unlikely to survive prolonged immersion (Jensen 2008; Johns *et al.* 2009) • Slower growth when flooded to 5 cm (Johns *et al.* 2009; Casanova 2015) • Recommended flood depth 4 cm (Casanova 2015) • Ideal depth less than total seedling height (Johns *et al.* 2009)	• Rates of rise and fall of floodwater that affect seed settlement are unknown (Johns *et al.* 2009) • Survival of seedlings underwater • Limited and/or unpublished data on flood depth effects on soil moisture (Johns *et al.* 2009)
	Duration of inundation	• No direct impact on duration, but unlikely to survive prolonged immersion (Johns *et al.* 2009) • Seeds die if submerged for >10 days (Casanova 2015)	• Ideal <30 days, maximum 30–60 days depending on seedling size (Johns *et al.* 2009) • Two-month-old plants can tolerate waterlogging for 1 month (Johns *et al.* 2009; Casanova 2015) • Signs of stress from waterlogging after 70 days at 22 months of age (Johns *et al.* 2009) • Duration should be sufficient to ensure maintenance of soil moisture (Johns *et al.* 2009) • Flood duration 4 weeks after 2 months of age (Casanova 2015)	• Limited and/or unpublished data on flood duration effects on germination (Johns *et al.* 2009)
	Sequence of and/or consecutive inundation events	• Requires follow up water (Casanova 2015)	• Follow up watering, whether rainfall or shallow inundation in the first or second year expected to improve establishment (Holloway *et al*. 2013). • Summer after germination (or local rainfall) (Casanova 2015)	• Unknown flood seasonality effects on seed fall and reproduction (Johns *et al.* 2009)

			• Frequency of inundation variable depending on a site's soil properties, evaporation rates and rainfall (Johns *et al.* 2009)	
	Water stress	• Requires flooding and/or local rainfall (Casanova 2015)	• Intolerant of waterlogging or complete immersion (Johns *et al.* 2009) • Soil moisture of 10–25% is critical (Jensen 2008) • Intolerant of drought (Casanova 2015) • Slow drawdown rates are detrimental to establishment as seedlings do not tolerate extended periods of waterlogging (Johns *et al.* 2009) • Flowing water may lead to dislodgement or burial (Johns *et al.* 2009) • Artificial flood not so useful (Casanova 2015)	
	Timing/season of inundation	• Requirements 15–35°C for germination (Rogers & Ralph 2011; Casanova 2015) • Inundation receding in spring–early summer provides moist conditions (Johns *et al.* 2009; Rogers & Ralph 2011; Holloway *et al.* 2013) • Local rainfall in spring–summer (Casanova 2015)	• Floods in winter–late spring optimal (Johns *et al.* 2009) • Flood recession in spring to summer to provide moist conditions (Holloway *et al.* 2013; Casanova 2015), or local rainfall (Casanova 2015) • Grow in summer after shedding old leaves and bark (Casanova 2015) • Newly germinated seedlings susceptible to frost and heat injury (Johns *et al.* 2009) • Follow-up inundation in same season as germination or following season (Holloway *et al.* 2013) • Timing should be sufficient to ensure maintenance of soil moisture in the first summer after germination (Johns *et al.* 2009)	• Limited and/or unpublished data on flood seasonality effects on soil moisture persistence (Johns *et al.* 2009)
	Grazing pressure	• Vulnerable (Casanova 2015)	• Vulnerable, seedlings are grazed (Casanova 2015) • Grazing, particularly by sheep (and more so than cattle and rabbit burrowing), restricts	

			establishment and impacts soil structure (Victoria. 1990)	
	Soil salinity		• Salinity tolerant (related to ground and surface water) (Casanova 2015) • Sodic soils overlying highly saline groundwater cause high mortality in first 2 years (Morris 1984)	• Impact of soil salinity
Coolibah	Depth of inundation	• Most likely occur on wet soils following floods or rainfall (Roberts & Marston 2011) • Not critical to seed germination (Holloway *et al.* 2013) • Requires moist soil (Holloway *et al.* 2013)		• What are the ideal and/or maximum flood depth requirements (Rogers & Ralph 2011)
	Duration of inundation		• Longer flood = fewer seedlings (Casanova 2015)	• What are the ideal and/or maximum flood duration requirements (Rogers & Ralph 2011)
	Sequence of and/or consecutive inundation events	• Follow-up floods in summer of first year thought to increase recruitment rates (Roberts & Marston 2011)	• Regular rainfall required for establishment (but saturated soil following inundation might be adequate) (Casanova 2015) • Follow-up rainfall or shallow inundation in summer of first year (or second year) thought to increase seedling recruitment rates (Roberts & Marston 2011) • Sequence of floods or flood and wet years may be necessary (Li & Wang 2003, Tuomela *et al.* 2001)	• Are multiple small floods required to provide sufficient soil moisture for germination and is follow-up shallow flooding needed to promote seedling establishment?
	Water stress	• Seeds take two weeks to germinate (Casanova 2015)		• Soil moisture requirements for seedling establishment
	Timing/season of inundation	• Fluctuating temperature 15–30°C for germination, vulnerable to frost, adapted to regeneration after late summer flooding (Capon *et al.* 2009).	• Shade or protection from summer heat required (Casanova 2015) • Flood recession in spring to provide warm and moist conditions (Holloway *et al.* 2013)	• Effects of season and flood timing on reproduction and seedling establishment are unknown

		• Flood recession in spring to provide warm and moist conditions (Holloway *et al.* 2013) • Timing not critical (Holloway *et al.* 2013)	• Flood summer–late summer (but other factors important e.g. rainfall) (Casanova 2015)	
	Grazing pressure	• Successful recruitment may require protection from grazing (Roberts 1993)	• Seedlings die from herbivory (Casanova 2015) • Grazing, seasonal conditions and competition from grass effects (not so important) (Casanova 2015)	
	Soil salinity	• Reproductive effort may be lowered by soil salinity (Roberts 1993; Roberts & Marston 2011)	• Are salt-tolerant in the Diamantina River region i.e. utilise saline groundwater (Costelloe *et al.* 2008; Roberts & Marston 2011)	• Impact of soil salinity • Is this site specific?
River Red Gum	Depth of inundation	• Moist soils required (Johns *et al.* 2009) • Germination success primarily controlled by seed availability and moisture availability after seed dispersal — most seeds germinate within 10 days of watering (Johns *et al.* 2009) • No direct impact on depth as seeds can germinate while floating (Johns *et al.* 2009) • Flooding after germination may lead to mortality (burial, dislodgement or immersion periods) (Johns *et al.* 2009)	• In southern MDB: not tolerate waterlogging and complete or prolonged immersion (Roberts & Marston 2011) • Depth will affect subsequent seedlings' survival and establishment (Johns *et al.* 2009) • On moist soil following flood recession • Tolerance to waterlogging increases with seedling height • Shallow flooding (20–30 cm) preferable to avoid over topping seedlings in first year (Holloway *et al.* 2013)	• Rates of rise and fall of floodwater that affect seed settlement are unknown (Johns *et al.* 2009) • Limited and/or unpublished data on flood depth effects on soil moisture (Johns *et al.* 2009)
	Duration of inundation	• No direct impact (Johns et al 2009) • Seeds die after 10 days of immersion (Casanova 2015)	• In southern MDB: 2-month-old plants can withstand waterlogging for 1 month; 50–60 cm plants can survive flooding for 4–6 months, but only for several weeks if completely submerged (Roberts & Marston 2011) • Maximum duration 1–6 months depending on seedling size (Holloway *et al.* 2013) • Susceptible to prolonged flooding (Roberts & Marston 2011)	• Limited and/or unpublished data on flood duration effects on germination (Johns *et al.* 2009)

			• Four-to-six weeks is adequate, but longer can be tolerated depending on age and if totally submerged (Holloway *et al.* 2013)	
	Sequence of and/or consecutive inundation events		• In southern MDB: follow-up watering 1 year after germination (George 2004) • Requires watering 1–2 months after spring rain or small flood (Casanova 2015) • Sufficient to maintain soil surface moisture during first year and needs adequate moisture in the second season (Johns *et al.* 2009) • Follow-up flood to recharge soil moisture is desirable in same year as germination or following year (Holloway *et al.* 2015)	
	Water stress	• Germinate within 5 days given adequate moisture (Holloway *et al.* 2013) • Soil moisture required >10% (Holloway *et al*. 2013) • Seeds require imbibing (saturation) and light to break dormancy (Casanova 2015)	• In southern MDB: soil moisture levels 10–25% in top 10 cm ideal (Jensen 2008; Holloway *et al.* 2013). • In southern MDB: low density response to above-average (>300 mm) annual rainfall, with higher establishment occurring in response to medium-to-large flood events — recharges soil moisture (George 2004; Jenson *et al.* 2008) • Inhibited by drought conditions, develops adventitious roots in response to flooding (Casanova 2015) • Rapid drawdown rate preferable as intolerant of prolonged periods of immersion (Roberts & Marston 2011) • Competition for moisture by other understorey and/or overstorey vegetation • Maintenance of soil moisture within first year is critical (Holloway *et al.* 2013) • Seedlings wilt and die rapidly once soil moisture falls below 10% (Jensen 2008)	Is this site specific?

	Timing/season of inundation	• Flood receding in spring–early summer preferred (Johns *et al.* 2009) • Rates limited by low temperatures and light availability (Holloway *et al.* 2013) • Require adequate moisture and day time temperature >30°C for germination (Holloway *et al* 2013) • Optimal temperature 35 °C (11–34 °C) (Casanova 2015) • Adequate water applied before germination (Roberts & Marston 2011)	• In southern MDB: winter flood receding in spring/early summer maintains soil moisture and avoids extreme temperatures for seedling survival (Roberts & Marston 2011) • Sensitive to frost • Flooding after germination may lead to seedling mortality due to burial, dislodgement or excessive immersion periods (Roberts & Marston 2011) • Recession spring/early summer (or sufficient rainfall), artificial watering to extend effect (Casanova 2015)	• Limited and/or unpublished data on flood seasonality effects on soil moisture persistence (Johns *et al.* 2009)
	Grazing pressure		• Seed predation varies through the year, lowest under sheep grazing, highest in ungrazed conditions, high under cattle grazing (Casanova 2015). • Compete with reeds and weeds (Casanova 2015) • Increased during flood (cattle, kangaroos, rabbits) (Casanova 2015) • Grazed more during drought (Casanova 2015) • Grazing (sheep, cattle and kangaroos) severely restrict regeneration (cattle are less destructive) (Victoria. 1990)	
	Soil salinity			• Impact of soil salinity
Tangled Lignum	Depth of inundation	• In water (while floating) or wet mud, occurs after flooding (Campbell 1973; Chong & Walker 2005; Holloway *et al.* 2013)	• Damp conditions promote growth; flooding, waterlogged and dry conditions impede growth (Capon *et al.* 2009) • Damp conditions associated with drying phase facilitate growth (Capon *et al.* 2009) • Depth of flood seedling establishment < 15 cm (Casanova 2015)	• Impact of depth?

	Duration of inundation		• In northern MDB: 2–4 months rapid growth in damp and drying conditions; flooding, waterlogged and dry conditions impede growth (Capon *et al.* 2009)	• Is this site specific?
	Sequence of and/or consecutive inundation events	• May require consecutive floods, one to promote flowering and seed set and one to promote germination (Rogers & Ralph 2011)	• Needs floods once in 12–18 months of 5–15 cm depth for 4–6 weeks in late spring–summer (Casanova 2015) • Spreads predominantly via vegetative growth, particularly in more frequently flooded areas (Casanova 2015) • Follow-up flood 9–12 months after germination (Casanova 2015)	
	Water stress	• Germination occurs within 14 days of dispersal (6–12 days) (Casanova 2015).	• Soil moisture known for northern MDB; damp conditions, can survive flooding (Capon *et al.* 2009). • More tolerant of drying than flooding (Capon *et al.* 2009) • Opportunistic and rapid under optimal experimental conditions (Holloway *et al.* 2013)	• Is this site specific?
	Timing/season of inundation	• Flood timing spring–summer preference (Rogers & Ralph 2011) • Rates are temperature dependent (Holloway *et al.* 2013) • Season appears to be critical for germination (late summer to autumn) (Casanova 2015). • Appears to recruit continuously (Casanova 2015)		• Temperature and flood timing (season) effects on seedling survival and growth
	Grazing pressure		• Can survive grazing, but growth is stunted (Jensen 2008) • Vulnerable to grazing (Capon *et al.* 2009) • Grazing and competition pressure unknown (Casanova 2015)	
	Soil salinity			• Impacts on soil salinity

7 Experimental designs and methods

7.1 Introduction

Mesocosms have been used as research tools for multiple experimental designs, because the physical dimensions, particularly length or shape, can be manipulated to provide researchers with a characterisation of the complexity or simplicity in the system they are trying to simulate or represent. Mesocosm studies provide a powerful means of quantifying causal relationships in a controlled environment and have the ability to focus specific variables, giving them the potential to be replicated in future research.

The focus of the review was on the four key species, River Red Gum, Black Box, Lignum and Coolibah, and on information relating to soil moisture, flow parameters (such as duration and frequency), stressors and threats to long-lived woody species. Information was compiled from various sources, a number of which do not relate to our key species, but that have a direct or indirect relationship to the key questions being asked in this MDB EWKR theme.

The most common reasons applied for conducting woody floodplain growth experiments in mesocosm designs relate to the ability to manipulate chosen variables (e.g. soil moisture or flooding), and to confirm predictions of effects on target-specific and/or single species. This review was undertaken to inform the selection of an appropriate mesocosm design to achieve the necessary answers in predicting the effects watering has on long-lived woody riparian species, specifically the four key species. The resulting analysis of literature was used to support the development of a pilot trial to test this design.

7.2 Quantitative design and facilities

Depending on the objectives of the studies reviewed, the number of replicated test systems and number of experimental treatment systems varied. Replication efforts were focused around increasing the assessment of variability, with the number of replicates ranging from two to 10, yet the more common approach was for four replicates. The experimental design considerations for the number of treatment units employed ranged from 1–5 for hydrological regimes, one or two soil/sediment types, and 1–6 months growth time with sampling events occurring daily, weekly, fortnightly, monthly, 3-monthly or only at the end of the experiment. The more commonly adopted treatment units approach was for three, four or five watering treatments, one sediment type (a mixture to represent natural soils at sample sites or floodplain soils) and approximately two growing months. The number of sampling events depended on the length of the experiment (growth time), yet the common approach with 2-monthly experiments was for observational monitoring weekly (e.g. number of stems, leafs, stem height) and harvesting/sampling at time of completion to record factors such as root depths and biomass.

The results of the review identified multiple structures and materials used to grow seedlings, ranging from ice cream containers (Jenson 2008) to more complexly designed apparatus's consisting of water wells connected to soil tubes with automated pump systems (Hughes *et al.* 1997). The most common design approach consisted of PVC piping or plastic cylindrical pots, with size dependent on the target species and/or treatments applied (ranging from 9 to 75 cm in diameter and 0.3 to 1.25 m in length). Popular methods were to split the pipes or cylindrical pots lengthwise in half, and then seal them back together (e.g. with insulating tape or cooper wire) with one end open (for plants to grow up through) and one end closed off using nylon mesh, gauze, or fibreglass with drainage holes, or by lining the pipes with polythene bags. The benefit of splitting the PVC pipe in half appears to aid in the removal of the plant at the time of harvesting, thus providing opportunity's to assess the root length and/or depth in the chamber whilst preventing overall damage to the specimen.

These pots/pipes were then deployed into systems that would maintain them in a vertical position to hold varying levels of water depending on the objective being tested, e.g. large buckets or galvanised tanks. The use of a glass or green house was preferred as it provided the ability to control environmental effects such as temperature and rainfall/watering. Outside facilities or field sites were also used; however, timing for the experiment was then dependant on and influenced by the season.

7.3 Soil moisture and flooding

A number of experiments have been conducted on soil moisture requirements for woody riparian species (Capon *et al.* 2009; Jensen 2008; Lynch 2006; Neave & Florence 1994), with up to five watering treatments over 10 to 24-week growth periods. In a laboratory experiment conducted by Capon *et al.* (2009), five watering treatments were applied to Lignum seedlings to investigate their response to flooding or drying, in two sediment types (clay and clay/sand). Root depth appeared to be the only variable affected by sediment type and this affect varied through time, and Lignum appeared to be more tolerant of drying then flooding.

Other experiments have looked at the effect of seedling growth from watering regimes of daily or weekly watering (Lynch 2006; Neave & Florence 1994) to determine the effects soil moisture has on root growth. Neave and Florence (1994) found that the treatment of drying sediment after a watering event produced eucalypt seedlings (including River Red Gum) with a larger root: shoot ratio then seedlings that were exposed to constant soil moisture. However, the root systems of seedlings in the constant soil moisture treatment were shallower than those in the dry treatment and contained more root weight in the upper part of the soil profile (Neave & Florence 1994). In comparison, Argus *et al.* (2015) study on *E. camaldulensis* subsp. *refulgens* states 32 days after flooding limits root growth, suggesting that early flood tolerance could be an adaptation to capitalise on scarce water resources, even though extensive adventitious roots developed in the seedlings in soil flooded for 88 days.

Li and Wang (2003) and Tuomela *et al.* (2001) investigated the growth of *E. microtheca* by subjecting seedlings to three water treatments, ranging from flooding to two levels of stress (soil retaining minimal moisture) over five months. When making comparisons between populations of seedlings, Tuomela *et al.* (2001) noted that root: shoot ratios were consistently higher in seedlings from seasonally dry sites compared to those from semi-arid sites, and Li and Wang (2003) similarly found that the morphological and physiological responses of *E. microtheca* to water availability differed among populations.

The timing or season is just as imperative when considering soil moisture and/or flooding. Jensen (2008) indicates eucalypt and Lignum seedlings, following germination on moist soil, may perish within 1–2 days of becoming water-stressed, which occurs when soil moisture drops below 10%. Thus, soil moisture-dependant species are unlikely to be able to survive predicted hot dry conditions that dry out the soil (Jensen 2008). Salazar *et al.* (2012) undertook a study in Brazil on woody species, and found that seedling establishment was low when covered with large quantities of litter, yet tree canopy cover actually facilitated seedling establishment by reducing stressful environmental conditions. Thus, shading effects on those hot dry days may facilitate establishment and recruitment.

Seedlings can be influenced directly or indirectly by flooding. Flooding of rivers, wetlands and floodplains has various effects on root development, by initiating a chain of reactions encompassing various physical, chemical and biological processes that lead to reduced soil conditions and implications for wetland and riparian species (Capon 2012; Pezeshki 2001). Moisture availability may also be a function of soil type, thus the germination and survival of seedlings may differ between soil types (Schütz *et al.* 2002).

Therefore, successful germination and/or establishment is not only connected to soil moisture (i.e. the duration (of flooding and drawdown), frequency or magnitude of flooding, rainfall availability or the occurrence of follow-up rain or flooding); rather, it also depends on the composition of the soil at each site, since this can influence the reactions each species may have to different treatments. These studies on the effects of soil moisture, flooding and populations on seedlings, highlight the importance of investigating site-specific requirements for species when climate, availability of water and soil types differ.

7.4 Multi-year watering

The literature acknowledges follow-up flooding or rainfall to maintain moisture in the soil as being important for seedling survival and germination success (George 2004; Jensen 2008). Awe *et al.* (1976) looked at root development of three eucalypt species (including River Red Gum) in a simulated design of prolonged drought and progressive drying out of the soil profile. This experiment was designed to simulate a natural situation of germinating seeds on saturated soil, followed by a drought where the soil moisture was allowed to progressively dry out. Results suggested that River Red Gum seedlings rapidly produce a massive root system when faced with a rapidly drying soil profile (Awe *et al.* 1976). However, this study saw seedlings watered for only three weeks with a total of 80 growth days (just under three months). Jensen (2008) showed that rainfall and flooding in the first year of establishment are essential, with subsequent follow-up rain/flood events over the next two years required for River Red Gum, Black Box and Lignum for successful germination and recruitment. However, Jensen's (2008) study on the response of the treatments (rainfall, flood, rainfall followed later by flooding, flooding followed later by rain, and constant dry to the germination of woody species) was only for a 12-week growth period (three months).

The literature revealed that experiments on seedling survival and root development have occurred over a six month period, and not over multi-year time scales. Statements have been made that in the first year of growing, seedlings require watering and/or follow-up rain or watering, but no studies were found to have extended longer than the first year of growth.

7.5 Stressors and threats

Stressors and threats to germination and establishment of woody species include (but are not limited to) grazing, salinity, soil type and climate change. From a physiological-ecology standpoint, the knowledge of the various soil compounds has critical implications for wetland plant functioning (Pezeshki 2001). The condition of the soil is influenced by nutrients received and lost through events such as in-channel and overbank flooding (Pezeshki 2001; Whitworth *et al.* 2012). The reduction of soil conditions, through droughts, or poorly timed or prolonged flood events, may lead to the inhibition of nutrient uptake and transport due to root dysfunction and/or death of plants (Pezeshki 2001).

Soil type has been looked at in relation to roots being able to penetrate heavy clays (Bell *et al.* 1993), the composition of the soil and effect it has on growth of eucalypt seedlings (Bennett *et al.* 1986), the soil's ability to maintain water (i.e. soil moisture) (Capon *et al.* 2009; Jensen 2008; Lynch 2006; Neave & Florence 1994), and the compaction of the soil as a result of grazing or heavy machinery use (Neave & Florence 1994). The sediment/soil type is just one part of the puzzle. The location of seedlings in the river/creek channel and the influence of drought, flooding and landform changes on the channel, will also affect woody riparian species' ability to survive, in relation to high water flows and the scouring of sediments (McBride & Strahan 1984). The location of species at specific elevations along a hydrological gradient generally reflect the water requirements and flood tolerance of that species and community, and thus, the soil moisture and water table depth that exists along the gradient determines the distribution of the vegetation community (Xu *et al.* 2015). Therefore, designing an experiment that considers the four key species residing within sites (i.e. MDB EWKR research site) at different elevations associated with different soil structures and nutrient deposition rates could be beneficial. In the absence of using sediment from the different sites and different elevations, simple chemical/physiological examination of the soil structure could be helpful.

Craig *et al.* (1991) compared Lignum plants under four levels of watering and four salinity levels over a 10-week period in plastic pots of native surface soil in a greenhouse, and found that Lignum cover was more strongly associated with soil hardiness (or compaction) and moisture rather than soil conductivity. Akilan *et al.* (1997) found that waterlogging River Red Gum plants with salt water over 16 weeks reduced shoot extension more than under freshwater waterlogged conditions; however, waterlogged freshwater plants produced adventitious roots just below the surface whereas no adventitious roots where formed in the salt water-affected plants.

Groundwater recharge or water table declines have been looked at internationally on other woody species (Horton & Clark 2001; Hughes *et al.* 1997; Mahoney & Rood 1991; Stella & Battles 2010). Even though these studies do not relate to the target species, the design may be beneficial if considering effects groundwater has on root development. Horton and Clark (2001) and Mahoney and Rood (1991) recorded that optimal growth and seedling survival in relation to groundwater decline was reached at ≤1 cm/day, while Hughes *et al.* (1997) measured highest growth rates in well-drained soils when water level decline rates were at 1 or 3 cm/day. Stella and Battles (2010) also considered water table declines as a stress on first year riparian seedlings (cotton wood and willows), by analysing the growth and below ground allocation response to water stress over 62 days. They found that water table recession had a strong negative influence on plant growth, with no evidence that plants increased below ground allocation in response to drawdown.

Shading effects require important considerations, as shading can impact or effect soil moisture and thus seedling establishment. A field study in Brazil investigating the importance of spatial variation in canopy cover and seasonal variation in the survival of seedlings of neotropical savannah woody species, indicated seedling establishment was low when covered with large quantities of litter (Salazar *et al.* 2012). Salazar *et al.* (2012) concluded that tree canopy cover reduces stressful environmental conditions, which, in turn, facilitates seedling establishment.

Grazing pressure on seedling establishments for the four key species is considered to be largely unknown. Cunningham *et al.* (1992) argued that the species are not readily grazed by stock except in times of drought and/or feed shortage. However, observational and anecdotal evidence suggests grazing on Lignum and River Red Gum seedlings does occur, by native animals (e.g. kangaroos) and domestic stock (e.g. sheep) (Capon *et al.* 2009; Jensen *et al.* 2008).

The re-sprouting ability of seedlings after a disturbance (e.g. grazing or flooding which results in loss of all stem and leaf material) has been investigated for northern Australian riparian tree species (Chong *et al.* 2007). To examine the disturbance, clipping was assigned to seedlings under six time treatments, and comparisons were made on seed size and seedling growth patterns and allocation to root mass and lateral root development. The results indicated that re-sprouting capacity was related to physiological and morphological specie traits rather than size or growth rates (Chong *et al.* 2007). In another grazing study that involved fenced, unfenced areas and cutting roots of woody species, the competition for resources between ground cover and woody vegetation, in the absence of grazing, was more evident (Smith *et al.* 2013).

The studies by Chong *et al.* (2007) and Smith *et al.* (2013) provide evidence of the indirect impacts grazing can have on establishment rates. Grazing can change/influence plant biomass (Reid *et al.* 2011), vegetation structure and community composition (Yates *et al.* 2000). Although in semi-arid regions, grazing has been shown to have a small influence on floodplain vegetation (excluding eucalypts) in comparison to flooding (Westbrooke *et al.* 2005). Cloven-hoofed livestock also affect the soil structure and soil regulatory processes, with soil compaction impeding root growth and thus the ability to provide plants with water and nutrients (Neave & Florence 1994; Yates *et al.* 2000). As a consequence, soil water availability may be limited in heavily grazed woodlands compared to in ungrazed woodlands, with implications for seedling establishment (Yates *et al.* 2000). Yates *et al.* (2000) concluded that livestock grazing in remnant *Eucalyptus salmonopholia* woodlands has impacted soil surface condition, and soil chemical, physical and hydrological properties, which subsequently have flow on effects to the restoration and establishment of plant species. Yates et al. (2000) found that heavily grazed woodland remnants were more susceptible to erosion due to loss of protective features such as perennial shrubs, woody debris and litter, and had higher concentrations of soil chemical properties (e.g. pH, nitrogen, EC, phosphorous, potassium) impacting nutrient status. Furthermore, rates of soil water infiltration were lower and soil temperatures were warmer when compared to remnants rarely grazed or ungrazed (Yates *et al.* 2000).

Looking beyond the seedling (and its potential root development and establishment rates) to the site-specific soil structure and associated impacts is just as important. Understanding impacts grazing has on the soil structure is also important to ensure soil water recharge, soil water storage and soil water availability are not declining in grazed areas to below-critical thresholds for seed germination and seedling establishment (Yates *et al.* 2000).

Conclusion

Understanding seedling root growth development is fundamental to successful seedling establishment. Environmental watering events should consider the successful establishment of seedlings that are able to withstand the next dry period. Putting energy and effort into the development of roots is likely to be the key way in which seedlings ensure they have access to soil moisture and potentially groundwater to enable them to survive between flows; however, very little is known about seedling root development. Understanding how different flow regimes affect seedling root development will inform the delivery of environmental watering events in terms of maintaining adequate soil moisture. Measuring the water regime parameters that affect soil moisture will help inform watering variables of frequency and duration of events. Understanding how certain non-flow drivers (e.g. grazing, soil type, soil salinity) affect the expected response of seedlings will allow water managers to alter watering events based on non-flow drivers of local relevance to their region and event, or to implement complementary management actions to mitigate these effects.

The literature highlighted a number of knowledge gaps in these areas for the establishment and recruitment of the four key species, including the lack of information between the northern and southern regions of the Basin (Table 3). The review on experimental designs and use of mesocosm methods has highlighted possible designs/methods (Table 4) that can be used to address the key questions of long-lived vegetation, such as:

1. What is the relationship between soil moisture and seedling survival and root development?
2. What is the relationship between flow parameters such as duration and frequency (sequential, multi-year) and seedling survival and root development?
3. What is the critical time period between germination and successful seedling establishment and, therefore, what sequence of multi-year watering may be required to facilitate successful establishment?
4. How do stressors and threats (e.g. soil type, salinity, grazing pressure) modify the expected recruitment outcomes to flow regimes?

Table 4. Identified knowledge gaps for recruitment and seedling establishment, based on this literature review of key species.

Process	Black Box	Coolibah	River Red Gum	Tangled Lignum
Depth of inundation	• Rates of rise and fall of floodwater • Flood depth effect on soil moisture	• Ideal and maximum flood depth	• Rates of rise and fall of floodwater • Flood depth effect on soil moisture • Site specific?	• Ideal and maximum flood depth
Duration of inundation	• Flood duration effects on germination	• Ideal and maximum flood duration	• Flood duration effects on germination • Site specific?	• Site specific? • Ideal and maximum flood duration
Sequence of and/or consecutive inundation events	• Flood seasonality effects on seed fall and reproduction	• Multiple small floods and/or follow-up shallow flooding for soil moisture effects on germination and seedling establishment	• Site specific?	
Timing/season of inundation	• Flood seasonality effects on soil moisture persistence	• Effect of season and flood timing on reproduction and seedling establishment	• Flood seasonality effects on soil moisture persistence • Site specific?	• Temperature and flood timing (season) effects on seedling survival and growth
Water stress		• Soil moisture requirements for seedling establishment	• Site specific?	• Site specific?
Soil salinity	• Unknown impact	• Unknown impact	• Unknown impact	• Unknown impact

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