Literature review and experimental design to address retaining floodwater on floodplains and flow enhancement hypotheses relevant to native tree species

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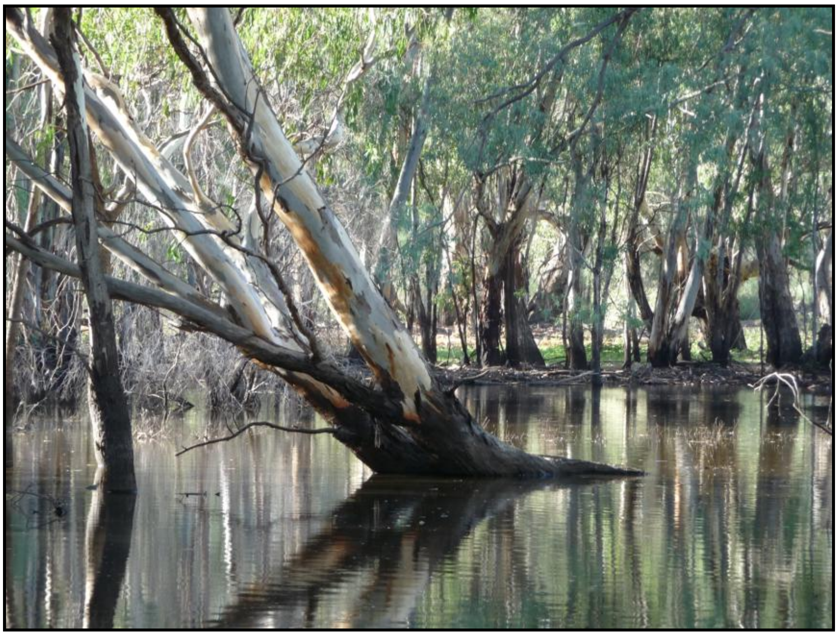

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The Murray-Darling Freshwater Research Centre

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MURRAY–DARLING BASIN AUTHORITY

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A report prepared for the Murray-Darling Basin Authority by The Murray-Darling Freshwater Research Centre.

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Cover Image: River red gum (Wallpolla Island, February 2009)
Photographer: Caitlin Johns

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Contents

1 Introduction

1.1 Project background

Floodplain and riparian ecosystems of the Murray-Darling Basin (MDB) are largely dominated by four native tree species. These are river red gum (*Eucalyptus camaldulensis* Dehnh.) which is the most wide-spread within the Basin, black box (*Eucalyptus largiflorens* F. Muell.) which is found predominantly in the south and west along the Murray River, coolibah (*Eucalyptus microtheca* F. Muell) which occurs in the north and west of the Basin, particularly in the Darling system, and river cooba (*Acacia stenophylla* A. Cunn. ex Benth.) which is a smaller species widespread in the western areas of the MDB (Cunningham *et al.* 1981; Marcar *et al.* 1995; Roberts and Marston 2000). Willows (*Salix* spp.) are the dominant exotic tree species that occur in these ecosystems, and are found throughout the MDB, primarily in permanently inundated or groundwater fed areas (Cremer *et al.* 1999; Roberts and Marston 2000). These species, along with other notable, but less widely distributed and less flood-dependent tree species, such as silver wattle (*Acacia dealbata* Link.), river oak (*Casuarina cunninghamiana* Miq.) and tea trees (*Melaleuca* spp.) (Roberts and Marston 2000), perform a range of critical roles in floodplain ecosystems. These roles include influencing primary production rates, micro-climate regulation, erosion control and the provision of habitat for fauna, including birds.

The development of the Intervention Monitoring Assessment Framework (IMAF) (McCarthy *et al.* 2006), associated with The Living Murray (TLM) Icon Site management, have identified knowledge limitations regarding the response of native and exotic tree species to water management interventions. Of particular interest are tree responses to two types of intervention, retention of floodwater on floodplains and flow enhancement. These interventions are defined as follows:

'Retaining floodwater on floodplains – This intervention involves use of regulators or small weirs to retain floods on the floodplain following flood recession. The source of the water is either natural floodwater or environmental water allocations. This intervention may act as a substitute for some of the functions of a long flood. This intervention can also be used to direct water to areas that would not otherwise have been inundated. While an efficient use of water, its river connection is limited to initial delivery and, in some cases, where the retained water can be released after several months ponding.' (Source: The Living Murray Outcomes Evaluation Framework (MDBC 2006).

'Flow enhancement – Involves manipulation of flow hydrographs through either release of environmental water allocations from storages or the use of storages to re-regulate either surplus flows or environmental water allocations (or combinations of both)'(MDBC 2006).

The following hypotheses, relating to native trees, arose directly from the IMAF development process:

1. *'Suitable habitat for native tree species will be created or maintained through retaining floodwater on floodplains.'*

2. *'The health and growth of native tree species will increase through retaining floodwater on floodplains.'*

3. *'Native tree species will germinate and recruit through retaining floodwater on floodplains.'*

4. *'Suitable habitat for native tree species will be created or maintained through flow enhancement.'*

5. *'The health and growth of native tree species will increase through flow enhancement.'*

6. *'Native tree species will germinate and recruit through flow enhancement.'*

7. *'Suitable habitat for weed vegetation will be created or maintained through retaining floodwater on floodplains.'*

Information on the validity of these hypotheses is required for improved decision-making, investment and monitoring of TLM sites, and for Basin-wide management.

1.2 Aims

The broad aim of this project is to review the effects that management interventions, in particular flow enhancement and retaining floodwater on the River Murray floodplain, will have on creation and maintenance of habitat suitable for germination, growth, health and recruitment of native tree and weed species. For each of the hypotheses above, this report aims to:

- Summarise existing knowledge.

- Highlight knowledge gaps which may need to be addressed through further monitoring or experimentation.

- Identify sources of research data that could help inform or strengthen any relevant future project.

- Develop appropriate experimental designs for future monitoring project(s) or experiments to address the knowledge gaps identified.

1.3 Scope

1.3.1 Selection criteria for inclusion of native tree and weed species

The following species were selected for inclusion in this literature review: river red gum (*E. camaldulensis*), black box (*E. largiflorens*), river cooba (*A. stenophylla)* and willows (*Salix* spp.). These species were selected because they have broad distributions, and are occurring throughout the River Murray floodplain, particularly within TLM Icon Sites. They primarily occur in riparian or floodplain habitats, which will be affected by the management interventions of flow enhancement or retaining floodwater on floodplains. River red gum and black box are the most common and dominant tree species in these habitats at the TLM Icon Sites, and river cooba is also very common, particularly along the mid to lower reaches of the River Murray (Henderson *et al.* 2008; McCarthy *et al.* 2008; AVH 2009). Willows are the only invasive exotic tree species currently known to present a threat to riparian and floodplain ecosystems at TLM Icon Sites, and are therefore the only tree weed considered in this review.

River red gum (*E. camaldulensis*) is a medium to large tree (20-45 m) possessing a short, thick trunk and large spreading crown (Marcar *et al.* 1995; Harden 2002). Northern and southern forms are recognised, although some gradation does occur between the two (Marcar *et al.* 1995). It is the southern form that is found throughout the MDB (Marcar *et al.* 1995). In the MDB, river red gum typically occurs as a dominant species in grassy woodland or forest communities (Cunningham *et al.* 1981; Harden 2002). This species is found primarily along riverbanks and on floodplain areas subject to frequent or periodic flooding (Cunningham *et al.* 1981; Harden 2002). In this part of its range, river red gum predominantly occurs on heavy grey clay soils, although some stands can be found lining sandy creeks and watercourses (Cunningham *et al.* 1981).

Black box (*E. largiflorens*) is a medium tree (10-20 m) with a short trunk, drooping branches and open crown that is typically wide-spreading and irregular in shape (Cunningham *et al.* 1981; Marcar *et al.* 1995; Harden 2002). In some flat, poorly drained areas, black box occurs in almost pure stands, while in other areas it occurs as a local community dominant in woodland or in open woodland, often mixed with other eucalypts and acacias (Cunningham *et al.* 1981; Marcar *et al.* 1995; Harden 2002). This species typically occurs on periodically inundated floodplains and often occurs in areas with poor drainage, along dry lake margins and in drainage lines and depressions (Cunningham *et al.* 1981). While often found in association with river red gum black box generally occurs at higher elevations, in less frequently inundated areas (Cunningham *et al.* 1981; Jensen 2008). Black box is most frequently found on grey clay loam soils, although it occasionally occurs on dark grey, self-mulching clays and less frequently, on fine red-brown sands (Marcar *et al.* 1995).

River cooba (*A. stenophylla*) is a small to medium-sized tree (5-15 m), either single-stemmed or multi-stemmed close to the ground, with an erect to spreading habit and pendulous branches which form a rounded crown (Jolly and Walker 1996; Roberts and Marston 2000). The phyllodes of river cooba are characteristically long and narrow (0.1-0.8cm × 15-40 cm) (Marcar *et al.* 1995; Harden 2002). River cooba is widespread throughout the MDB, and is common along the margins of watercourses, river floodplains and depressions, although also occurring more generally on plains and gentle slopes (Marcar *et al.* 1995; Harden 2002). This species often occurs in ribbon-like stands fringing watercourses, or as a component of eucalypt woodland or forest close to rivers (Cunningham *et al.* 1981; Marcar *et al.* 1995). River cooba is mainly found on grey cracking clays, fine-textured alluvial soils and red sandy clays (Marcar *et al.* 1995).

Willows are exotic to Australia with over 100 taxa (including various species, varieties and hybrids) introduced since European settlement, including tree and shrub forms (Harden 2000; Cremer 2003). They have been planted for various purposes including ornamental value, provision of shade and shelter, or riverbank stabilisation (Cremer *et al.* 1995; Cremer 2003). Willows are now listed among Australia's Weeds Of National Significance, due to their invasive habit and the deleterious effects they can have on riparian and aquatic ecosystems (Cremer 2003). As a result of historical practices and their invasive nature, willows are now widespread throughout southern Australia. The negative impacts of willows include very high rates of water extraction (Doody *et al.* 2006), erosion (caused by the obstruction and diversion of flow), disruption of aquatic food webs and displacement of native vegetation (Cremer 2003; Kennedy *et al.* 2003; Stokes 2008). Willows are found lining the banks of rivers, streams and water bodies, and in wet habitats where there is permanency of water, particularly during the growing season.

1.3.2 Aspects of flow regime to be considered

For each of the selected species, the effects of the following flow regime components will be considered in terms of their effects on tree health and growth, reproduction, dispersal, germination, seedling establishment and recruitment:

- Timing of watering events (i.e. in what season is watering best conducted?)

- Frequency of watering events (i.e. how often do sites require watering, and how long can plants and their dispersal mechanisms (e.g. seeds) tolerate dry periods?)

- Inundation period (i.e. how long should trees be inundated to stimulate growth and reproduction?)

- Depth of inundation (i.e. what depth do the different tree species prefer?)

- Rate of change (i.e. how rapidly the water level rises and falls).

- Transmission velocity (i.e. the advantages and disadvantages of retaining water on the floodplain when compared against floodwater flowing through the floodplain).

1.3.3 Definitions

The terms health and recruitment are used in the hypotheses to be addressed. We define these terms as follows.

'Health' or tree 'condition' can be defined and assessed in a number of different ways, varying according to species and the purposes of data collection. However, a set of standard tree condition assessment guidelines are currently under development for river red gum and black box at TLM Icon Sites (MDBA in prep.), designed specifically for monitoring tree responses to differences in flow regime. Tree condition is measured in terms of crown extent and density, bark condition, new tip growth, epicormic growth and leaf die off (MDBA in prep.). Standard condition assessment protocols have not been developed for other tree species occurring at TLM Icon Sites.

'Recruitment' refers here to the completion by an individual tree of all of the growth stages, from germination through to reproductive maturity.

1.4 Approach and methods

A literature review was conducted to address the first two aims outlined in section 1.2; i.e. to provide an overview of existing knowledge and to highlight knowledge gaps. The review process was necessarily brief, owing to the time constraints placed on this project, and primarily targeted work conducted in the last 5-10 years. Key sources were the reviews and syntheses by Roberts and Marston (2000) and Rogers (in prep.), with additional information sourced from recent research publications, theses and expert opinion.

A workshop was conducted to address the other aims of section 1.2 on the 11th and 12th May 2009; i.e. to identify additional sources of research data that could help inform or strengthen any relevant future project, to develop conceptual models, and to develop appropriate experimental designs for future monitoring project(s), or experiments to address the knowledge gaps identified. Workshop participants were as listed in Table 1.

Table 1: List of workshop participants and their organisations

Invitee	Organisation
Cherie Campbell	MDFRC
Tanya Doody	CSIRO
Ben Gawne	MDFRC
Caitlin Johns	MDFRC
Stuart Little	MDBA
Heather McGinness	CSIRO
Ian Overton	CSIRO
Christine Reid	MDFRC
Jane Roberts	Independent consultant
Kerrylee Rogers	DECC
Neil Sims	CSIRO

At the workshop, the literature review was revisited, and additional information sources identified. Conceptual models were developed focusing on the water requirements of different tree growth stages, and identifying linkages between the various flow regime components and tree responses.

To help identify relevant knowledge gaps and rank these in order of priority for research, a list was made of the assumptions summarised in each of the models. These assumptions were then phrased in the form of hypotheses, relating to the effects of flow regime on trees. Workshop participants gave each of these hypotheses a score (from 1-5) against three criteria; 'confidence' in the hypothesis according to expert opinion, the quantity and quality of 'evidence' already available to support the hypothesis, and the 'criticality' of the hypothesised relationship in terms of sustaining a process required for survival and/or recruitment. Knowledge gaps were ranked in order of priority for future investigation based on these scores. Finally, workshop participants were asked to put forward methodologies that could be used to address knowledge gaps.

1.5 Report structure

The main body of this report is comprised of the results of the literature review and conceptual models, summarising tree responses to flow management. The review and models consider the water requirements of trees during four life cycle phases;

- maintenance of established tree health and growth (including seed production),
- seed dispersal,
- seed germination, and
- seedling establishment.

Information on the effects of different flow regime characteristics on these growth phases are summarised for each species. Conceptual models are then included for each of these four phases. These models summarise linkages between flow regime characteristics and tree responses and are presented as flow diagrams. Since successful tree recruitment requires that the habitat requirements of every one of these growth phases is met, from the dispersal of seed from existing individuals through to the reproductive maturation of new recruits, recruitment is considered on a holistic basis rather than being addressed in any one specific section of this report.

Other outputs from the workshop have been summarised in the appendices to this report. These include a list of the hypotheses summarised in the models, scores used to identify knowledge gaps and to rank the importance of related hypotheses for further consideration, and methodologies proposed for addressing these hypotheses.

2 Literature review

2.1 Water requirements for riparian and floodplain tree growth and health

2.1.1 River red gum (*Eucalyptus camaldulensis)* growth and health

Established eucalypts have dimorphic root systems, with both shallow lateral roots and a large taproot capable of penetrating to some depth (Burgess *et al.* 2001). This root structure allows river red gum to opportunistically access surface soil water, resulting from episodic rainfall events or overbank flows, along with deeper soil water and groundwater (Mensforth *et al.* 1994; Burgess *et al.* 2001). River red gums progress through sapling and pole tree growth stages after seedling establishment, before they reach their mature growth form (George *et al.* 2005). Saplings are generally short, with narrow, pointed crowns, while pole trees are taller with crowns that are fuller and more rounded, but not as full and wide as those of mature trees (George *et al.* 2005). By the time the sapling stage has been reached, river red gums have well developed root systems, capable of accessing both groundwater and shallow soil moisture (George *et al.* 2005).

River red gums are not strongly adapted to water stress (Roberts and Marston 2000). Although occurring in semi-arid areas, the availability of floodwater is typically important for maintenance of river red gum growth and health, with numerous studies indicating that healthy *E. camaldulensis* are situated in areas that receive frequent surface flooding (Roberts and Marston 2000; Rogers in prep.). Between floods, river red gum survival is largely determined by the availability (or non-availability) of freshwater from other sources, along with reductions in canopy water use which are achieved by shedding leaves (Roberts and Marston 2000). In the semi-arid regions, rainfall is low and variable, and is usually insufficient to maintain river red gum health and growth (Roberts and Marston 2000).

In areas with low rainfall and infrequent flooding, groundwater may provide the most temporally stable water resource (Mensforth *et al.* 1994; Burgess *et al.* 2001). The salinity of available groundwater is important for the maintenance of river red gum growth and health because this species appears to utilise groundwater, regardless of the availability of other water sources. For example, Mensforth *et al.* (1994) found that *E. camaldulensis* used groundwater in preference to freshwater from a permanent stream, even when growing within 30m of the stream channel, when groundwater was moderately saline (EC ~40 dS/m), and when freshwater was available at the soil surface (0.05-0.15 m). Growth reductions have been reported for *E. camaldulensis* at salinities around EC_e (i.e. EC of a saturated soil paste) of 5 dS/m or above, with survival affected around EC_e 10-15 dS/m (Marcar *et al.* 1995), and signs of water stress occurring when saline groundwater (EC >20 dS/m) intruded into the root zone (Mensforth *et al.* 1994; Burgess *et al.* 2001). Despite being somewhat groundwater dependent, replenishment of soil water from less saline sources, including overbank flows, bank recharge or rainfall, can help to reduce water stress in mature trees caused by excessive groundwater salinity. This is because the root structure of *E. camaldulensis* allows individuals to simultaneously access these other water sources (Mensforth *et al.* 1994; Burgess *et al.* 2001).

The time required for river red gum saplings to reach reproductive maturity is likely to vary substantially according to habitat conditions and tree growth rates. No studies appear to have established a maturation time for river red gum (George 2004). However, under favourable growing conditions most eucalypts begin to produce seed when aged between 20-40 years (George 2004). Estimates of longevity for *E. camaldulensis* range from 150 up to 1000 years (George 2004). These time periods are also likely to vary substantially according to tree condition and growth rates (Smith and Long 2001; George *et al.* 2005).Where dense even-aged cohorts of seedlings/saplings occur, active growth of individuals is delayed until after self-thinning, whereby weaker trees are suppressed and die (Smith and Long 2001; George *et al.* 2005). The period of time that it takes for self-thinning to occur can have a considerable effect on tree maturation time. The effects of water availability on rates of self-thinning have not been determined for *E. camaldulensis*.

Reproduction and seed set

The phenology of flowering and seed production vary geographically in *E. camaldulensis*. In the lower Murray valley, *E. camaldulensis* flowering typically occurs in December, with buds generally formed in January-February, and then retained on the tree until they open the following summer (Jensen *et al.* 2006). However, in the Barmah Forest located in the mid-reaches of the River Murray, flowering usually occurs earlier from late spring to mid summer. Fruit formation occurs after pollination, with fruits retained on the tree for 1-2 years before seed fall occurs (Jensen *et al.* 2006).

Buds are often shed in response to excessively dry conditions, post-bud initiation and pre-flowering, hence the size of the flower crop is determined by water availability in the 12 months prior to flowering (Jensen *et al.* 2006). Fruits are also shed during excessively dry periods to reduce resource use by trees, and these fruit losses can cause substantial reductions in seed yield. Therefore, seed yields are affected by water availability over the 24 to 36 months prior to seed fall (Jensen *et al.* 2006). Tree condition has an effect on reproductive output, with healthy trees producing more fruit and shedding larger quantities of seed than those with lower canopy vigour (George *et al.* 2005).

Effects of flooding regime on river red gum growth and health

i) Ideal timing of flood events

Timing of floods may not be a critical factor affecting *E. camaldulensis* survival. This species tolerates flooding at any time of the year by increasing canopy transpiration rates (Heinrich 1990, cited by Rogers (in prep.)). However, summer flooding may result in greater biomass production (Robertson *et al.* 2001). Higher rates of wood production were recorded during summer flooding in the Barmah Forest compared to spring flooding or no flooding, despite the fact that natural flooding usually occurs there in winter-spring (Rogers in prep.).

The timing of flood events may also have an effect on reproductive output. Buds are shed in excessively dry conditions, post-bud initiation and pre-flowering and watering at this stage may help to increase bud retention (Jensen 2008). Watering 12 months after above-average rain may also help to help maintain bud crops and aerial seed banks initiated in the previous year, if subsequent conditions are dry (Jensen 2008).

ii) Ideal flood frequency

The ideal frequency of flooding for maintenance of tree growth and health is likely to vary on a site by site basis. Flood frequency affects tree growth and health through its effects on soil moisture and groundwater availability. However, the effects of flood frequency on soil moisture and groundwater availability are likely to vary according to site-specific factors, including rainfall, soil properties (such as permeability and salinity) and geomorphology (Holland *et al.* 2006). Differences in tree density between sites will affect the frequency of flooding required to maintain tree growth and health, with forests requiring more water than woodlands (Paul *et al.* 2003). Other aspects of flooding regime, such as timing and duration of surface floods, also affect the frequency of flooding required to water trees effectively. For example, Robertson *et al.* (2001) found that one large flood event every three years had a similar effect on *E. camaldulensis* growth as short spring floods occurring regularly over a number of years.

While the range of flooding frequencies tolerated by *E. camaldulensis* varies according to other site and flooding regime characteristics, healthy populations of *E. camaldulensis* generally occur in areas with a flooding frequency of approximately once every 1-3 years (Roberts and Marston 2000), with interflood dry periods ranging from 5-15 months (Rogers in prep.). Interflood periods greater than 2 years are expected to lead to a decline in tree condition, and without adequate water, individuals usually take around 5 years to die (I. Overton pers. comm.).

Maximum interflood period tolerated varies depending on tree health prior to flooding, with stressed trees being less resilient than healthy trees. While stressed trees may demonstrate a rapid increase in canopy condition post-flooding, this response may be short-lived and occur at the expense of sapwood maintenance. Resilience to moisture stress is related to ability to maintain sapwood area (Doody *et al.* in prep.). Once trees demonstrate a decline in sapwood area due to prolonged water stress, top-up watering is likely to be required for the next 2-3 years before sapwood extent is fully restored (T. Doody pers. comm.).

The period from bud initiation until seed fall is between 2-3 years (Jensen *et al.* 2008a). To maximise seed production, flood frequencies should be sufficient to maintain tree growth and health over this time.

iii) Ideal inundation period

As for flooding frequency, the ideal inundation period for health and growth varies according to local site conditions. In addition, ideal inundation periods vary according to inundation frequency and timing. Short duration floods typically have a positive effect on tree growth and health, however, this effect can be short-lived, depending on soil properties (Bacon *et al.* 1993). Longer duration floods may be more effective at replenishing deep soil moisture, but can eventually cause stress due to the development of root zone anoxia. Historical records for Barmah Forest show that *E. camaldulensis* stands occur in areas that would have been naturally flooded for periods ranging from approximately 1-7 months (Roberts and Marston 2000; Rogers in prep.).

Soil properties such as permeability, water-holding capacity and salinity affect the minimum duration of flooding required to water trees effectively, as do other factors affecting water availability, such as distance from channel, site inundation history and rainfall. For example, short duration (30-45 days) floods significantly improved tree growth over large areas in the Barmah-Millewa Forest complex, recharging soil moisture >38m from flooded channels where soil permeability was high due to sand content (Bacon *et al.* 1993). However, at sites with lower soil permeability, lateral seepage was much lower and bank recharge only reached trees within 7.5m. At these less sandy sites a 60-80 day channel inundation period was required before subsurface seepage reached trees at 3 m distance (Bacon *et al.* 1993; Roberts and Marston 2000).

While longer duration floods may be more effective at replenishing soil moisture reserves, *E. camaldulensis* trees do not tolerate permanently inundated conditions (Roberts and Marston 2000). Some variability has been reported in the maximum inundation periods tolerated by *E. camaldulensis.* Death of established *E. camaldulensis* trees occurred after 4 years of continuous flooding at Barmah Forest (Chesterfield 1986), while established trees continued to survive after 3-4 years of flooding behind Hay Weir (Bren 1987). In general, established *E. camaldulensis* can tolerate periods of continuous flooding up to 2-4 years before showing signs of stress (Roberts and Marston 2000). Such flooding durations should be considered as extreme rather than desirable for management purposes.

iv) Ideal depth of inundation

Flood depth is unlikely to have a direct effect on the growth and health of mature trees.

v) Ideal rate of change

Rates of change in water depth are unlikely to affect tree health and growth directly. However, rate of change may affect tree health and growth indirectly, through the relationship between rate of fall and inundation period.

vi) Ideal transmission velocity

Transmission velocity is unlikely to affect the health and growth of mature *E. camaldulensis,* unless sufficient to cause bank erosion and undercutting. The dissolved oxygen (DO) content of flood water often declines rapidly when ponded, driven by warm temperatures and the breakdown of organic matter (Unger *et al.* 2009). However, while it has been hypothesised that reduction in the dissolved oxygen content of flood water may lead to a reduction in oxygen availability in underlying soils, data from a recent field experiment suggest otherwise (Unger *et al.* 2009). While soil oxygen availability decreased over time in all flooded treatments, there was no difference in soil oxygen content between soils that had been subjected to five weeks of stagnant ponded surface water, and soils subjected to five weeks of pumped surface flows (Unger *et al.* 2009).

2.1.2 Black box (*Eucalyptus largiflorens)* growth and health

As for *E. camaldulensis*, *E. largiflorens* progresses through sapling and pole tree growth stages after seedling establishment, before reaching the mature growth form (George *et al.* 2005). By the sapling stage, individuals have well developed root systems, with shallow lateral roots and a deep taproot (George *et al.* 2005). Black box, like river red gum, is opportunistic in the water sources used, accessing both groundwater and shallower soil moisture, derived from rainfall, flooding and bank recharge (Jolly and Walker 1996).

Eucalyptus largiflorens populations typically occur at higher elevations on the floodplain that are flooded less frequently, than those occupied by *E. calmaldulensis*. This distribution reflects a higher tolerance to drought and lower tolerance to waterlogging in black box compared to river red gum (Roberts and Marston 2000). During dry periods, black box trees are able to reduce canopy transpiration rates considerably, and survive by shedding leaves and lowering stomatal conductance (Jolly and Walker 1996).

Eucalyptus largiflorens is one of the more salt-tolerant eucalypts and, in the absence of fresher soil water, can meet up to 100% of water requirements by using moderately saline groundwater (<40 dS/m) (Thorburn *et al.* 1993; Jolly and Walker 1996; Holland *et al.* 2006). Despite their relatively high tolerance of salinity, increased accumulation of salt in the soil profile, due to river regulation, is the primary factor contributing to deaths of black box on the lower River Murray (Jolly and Walker 1996). While *E. largiflorens* can use moderately saline groundwater, increases in salinity reduce the ability of roots to take up water, with tree growth rates declining as a consequence (Akeroyd *et al.* 1998). At salinities between EC 40-60 dS/m, water is no longer available to black box (Roberts and Marston 2000; Holland *et al.* 2006).

While water availability is important for black box growth and health, it is the interaction between water regime and salinity that has proved critical in the Murray valley. For example, a three-fold reduction in flood frequency due to river regulation has contributed to the accumulation of salt in the soil profile beneath *E. largiflorens* woodlands on the Chowilla floodplain, resulting in tree deaths (Roberts and Marston 2000; Rogers in prep.).

Depth to groundwater and the salinity of groundwater may be used as predictors of black box growth and health, with dieback observed when saline water tables (EC >40 dS/m) occur within 4m of the soil surface (Jensen *et al.* 2008b). However, in some areas the growth and health of *E. largiflorens* is maintained despite high groundwater salinity (EC >40 dS/m), through the use of fresher, deep soil water, overlying the saline groundwater (Holland *et al.* 2006). Rainfall, surface flooding and bank recharge are important for maintaining deep soil moisture reserves (Akeroyd *et al.* 1998; Holland *et al.* 2006). For example, horizontal bank recharge was found to be important for the maintenance of *E. largiflorens* populations growing within 50m of the Chowilla Anabranch (Holland *et al.* 2006).

As for other tree species, maturation time will vary substantially according to growth rate. Under favourable growing conditions, most eucalypts begin to produce seed when aged between 20-40 years (Akeroyd *et al.* 1998). However, no known studies have established the time necessary for reproductive maturation in *E. largiflorens* (George 2004). The period of time that it takes for self-thinning and sapling release to occur, has a considerable effect on maturation time in *E. largiflorens*. For example, some *E. largiflorens* trees, believed to have germinated on the Chowilla floodplain in response to the 1956 flood, remain at the sapling stage (approximately 1.5m height and only 2cm diameter) after more than 50 years (George 2004). The effects of water availability on rates of self-thinning and sapling release have not been investigated. Estimates of longevity for *E. largiflorens* range up to at least 300 years (I. Overton pers. comm.).

Reproduction and seed set

The number of flower buds produced by black box trees is affected by water availability, with a 170% increase recorded at one site following a high rainfall period (Jensen *et al.* 2008a). Buds are retained on trees for up to 12 months before flowering, and may be shed during this time in response to poor conditions (Jensen *et al.* 2008a). Therefore, the size of the flower crop is determined by water availability over the year leading up to flowering (Jensen *et al.* 2008a).

The timing of flowering varies geographically in *E. largiflorens*. Flowering is reported to occur mainly between August and January (Briggs and Townsend 1993, cited in Rogers, In prep.; Roberts and Marston 2000). However, an earlier flowering peak (May to October) has been reported for trees on the lower Murray floodplain compared with those elsewhere (George *et al.* 2005; Jensen *et al.* 2008a). Flowering occurs over a more protracted period in black box than in river red gum, with flowers observed in all months except February and March during 2004-2006 on the lower Murray floodplain (Jensen *et al.* 2008a). While most trees flowered in summer on an annual cycle, others flowered in winter each year (Jensen 2009). This spreading of reproductive effort may allow flowering to occur in response to water availability regardless of time of year.

Fruits are produced soon after pollination, however, *E. largiflorens* exhibits serotiny, with fruits retained on trees for up to 24 months before valves open and seeds are shed (Jensen *et al.* 2008a). Fruit and seed yields are therefore affected by water availability in the 24-36 months prior to seed fall (Jensen *et al.* 2008a). Fruit and seed yields are also affected by parent tree vigour, with less vigorous trees producing lower yields (Jensen 2008). Peak seed fall typically occurs several months after peak flowering, and coincides approximately with peak flooding throughout the Murray-Darling Basin (Rogers in prep.). Peak seed fall on the lower River Murray floodplain was observed in October to March during 2004-2006 (Jensen *et al.* 2008a).

Effects of flooding regime on black box growth and health

i) Ideal timing of flood events

No clear relationship has been observed between growth rate and temperature (as a surrogate for seasonality, and thus timing) in *E. largiflorens* (George 2004). However, this may be due to other factors limiting growth. This species makes use of water for growth opportunistically, whenever it becomes available (George 2004).

Bud initiation may occur in response to watering throughout the year, however, maximum bud loads are likely to result from watering timed to coincide with natural peaks in reproductive allocation (Jensen 2008). These appear to coincide with natural peak flooding periods (Rogers in prep.). Top-up watering to avoid moisture stress in the 24 months after watering, or after an above-average rainfall event, should ensure maximum bud retention, flower loads and seed production (George *et al.* 2005).

ii) Ideal flood frequency

The frequency of flooding required to maintain adequate moisture availability, and control salinity, will vary according to site geomorphology and soil properties, as well as flood durations. Flood frequencies recommended for growth and health of *E. largiflorens* range from 1 in 3 years to 1 in 10. On the Chowilla floodplain natural flood frequencies in the *E. largiflorens* zone would have been approximately 1 in every 2-5 years, with flood durations of 2-4 months (Akeroyd *et al.* 1998). Healthy individuals have been recorded in areas flooded for 4-6 months every 4-5 years (Roberts and Marston 2000). However, flood frequencies of 1 in 7-10 years may be tolerated if flood duration is not also affected (Roberts and Marston 2000). Stands can tolerate more frequent flooding if flood duration is reduced. For example, a healthy population of *E. largiflorens* has been observed in an area that has received short waterings from irrigation drainage, annually, over the past 10-15 years (H. McGinness pers. comm.).

The maximum interflood period tolerated depends on the availability and salinity of alternative water sources. One population of *E. largiflorens* on the Chowilla floodplain has survived for more than 30 years without surface flooding (I. Overton pers. comm.).

As for river red gum, the period from bud initiation until seed fall in black box is between 2-3 years (Jensen *et al.* 2008a). To maximise seed production, flood frequencies should be sufficient to maintain tree growth and health over this time.

iii) Ideal inundation period

Ideally flooding should last long enough for infiltration to replenish soil moisture, but should not continue until trees become stressed due to the development of root zone anoxia (Roberts and Marston 2000; Jensen 2008; Rogers in prep.). Ideal inundation periods will vary between sites, with infiltration rates affected by soil properties, landscape position and inundation history. *Eucalyptus largiflorens* on the Chowilla floodplain tolerated flooding for periods of 32-78 days without showing signs of anoxia (Roberts and Marston 2000; Rogers in prep.). However, dying trees have been recorded in areas where water was ponded twice for 12-18 months, and at Nearie Lake, continuous flooding for 13 months caused acute stress, including canopy yellowing (Jolly and Walker 1996; Akeroyd *et al.* 1998).

iv) Ideal depth of inundation

Inundation depth is unlikely to affect the health and growth of established trees directly, although the interaction between depth and duration will affect soil and groundwater recharge.

v) Ideal rate of change

Rates of change in water depth are unlikely to affect tree health and growth directly. However, the rate of change may affect tree health and growth indirectly, through the relationship between rate of flood-fall and inundation duration.

vi) Ideal transmission velocity

Whether water is ponded or flowing is unlikely to make a difference to the health and growth of established trees.

2.1.3 River cooba (*Acacia stenophylla)* growth and health

Little information is available on the water requirements of *A. stenophylla.* However, this species is considered to be both drought and flood tolerant (Marcar *et al.* 1995). A recent study measuring transpiration of *A. stenophylla* on the Bookpurnong floodplain has shown that high transpiration rates occur where water availability is high (river bank) with very low transpiration rates in environments where water is limited (Doody *et al.* in press). *Acacia stenophylla* is reported to grow well where shallow groundwater is available (Boxshall and Jenkyn 2001, cited in Rogers In prep.). The distribution of *A. stenophylla* overlaps those of both *E. camaldulensis* and *E. largiflorens* (Henderson *et al.* 2008; McCarthy *et al.* 2008), suggesting that water requirements for *A. stenophylla* growth and health lie somewhere between these two species. *Acacia stenophylla* does not appear to require surface flooding for maintenance of growth and health, provided adequate groundwater or rainfall is available (T. Doody pers. comm.). This species exhibits some tolerance toward salinity. Marcar *et al.* (1995) report that growth is reduced in *A. stenophylla* when salinity reaches EC 10-15 dS/m, with survival rates declining at salinities higher than EC 15 dS/m. Doody *et al.* (In press) observed *A. stenophylla* surviving at salinities as high as 40 dS/m, although health was very poor and transpiration rates very low.

Reproduction and seed set

Flowering has been reported to occur in *A. stenophylla* from March to August, with seeds maturing between October-December (Marcar *et al.* 1995; Harden 2002). However, flowering has also been observed in *A. stenophylla* at other times of year (T. Doody pers. comm.), possibly as an opportunistic response to water availability.

Effects of flooding regime on river cooba growth and health

i) Ideal timing of watering events

The effect of flood timing on river cooba growth and health is unknown.

ii) Ideal frequency of watering events

The effect of flood frequency on river cooba growth and health is unknown.

iii) Ideal inundation period

The maximum inundation period tolerated by *A. stenophylla* is unknown. Healthy individuals have been observed growing on river banks, with some roots subjected to prolonged waterlogging (T. Doody pers. comm.). Large and healthy established trees occur around the edges of Yanga Lake which, until recently, was permanently inundated (H. McGinness pers. comm.). However, individuals at these sites may have some roots in the soil that was not waterlogged, which could offset the effects of low oxygen availability experienced by some roots.

iv) Ideal depth of inundation

Flood depth is unlikely to have a direct effect on the growth and health of mature trees unless they are overtopped.

v) Ideal rate of change

As for other tree species.

vi) Ideal transmission velocity

As for other tree species, quantification of velocities on growth and health are not known.

2.1.4 Willow (*Salix* spp.*)* growth and health

Willows (*Salix* spp.) are deciduous and follow a distinct annual growth cycle, growing actively in spring and summer then shedding leaves in autumn and remaining dormant until the following spring (Karrenberg *et al.* 2002). There are many willow taxa present in Australia and the specific water requirements of most of these have not been investigated in depth (J. Roberts pers. comm.). Willows are generally vulnerable to water stress, requiring high levels of soil water or access to groundwater at least through the growing season (Roberts and Marston 2000; Doody *et al.* 2006). Willows generally prefer stable water- levels and may not tolerate large water table fluctuations, particularly rapid drawdown (Stokes 2008). They can exhibit low water use efficiency and be prone to drought-induced leaf abscission (Stokes 2008), with massive leaf-falls observed when root zones are allowed to dry (T. Doody pers. comm.).

Willows are typically more tolerant of flooding than drying. For example, established *Salix nigra* can tolerate periodic inundation of the root crown equivalent to 82% of the growing season (Stokes 2008). Adventitious roots are produced when willow stems are immersed in water (Stokes 2008). Willows also produce aerenchymal tissue; a special type of open tissue that serves as a conduit for oxygen from the shoot to the root system, allowing normal root metabolic activity to occur under anoxic soil conditions (Stokes 2008). Flooding can increase the production of aerenchymal tissue, as demonstrated for *S. nigra* when cuttings were subjected to periodic flooding (Li *et al.* 2006, cited by Stokes 2008). Tolerance toward flooding does vary between species, making it difficult to make accurate generalisations about inundation tolerance for willows as a group (Karrenberg *et al.* 2002). The water requirements of willows are likely to vary according to annual growth cycles, decreasing once leaves are shed (Karrenberg *et al.* 2002).

Reproduction and seed set

Willows can reproduce either vegetatively or via seed. Willows are largely dioecious, with individuals producing flowers of only one sex. Most cultivars in Australia were originally imported and planted as cuttings of only one or two clones at a time (Cremer 2003). The resulting stands were initially believed capable of spreading only via vegetative means, being comprised of single sex individuals, or of opposite sex individuals with non-overlapping flowering times (Cremer *et al.* 1995; Cremer 2003). However, since 1995 it has become apparent that seed production is occurring in many populations. New plantings and expansion of existing populations have led to closer proximity of the sexes and increased potential for cross-pollination (Cremer 2003). Monoecious individuals, which bear flowers of both sexes and are often capable of producing viable seed, have also appeared, often as a result of hybridisation, and are now widespread (Cremer 2003). Unlike *Eucalyptus* spp., willows reach reproductive maturity rapidly, producing flowers and viable seed within 2-3 years after germination (Cremer 2003).

Vegetative reproduction can occur via canopy fragments, layering or suckering. Most tree willows in Australia produce branches with fragile bases, and these can snap off and take root in wet areas (Cremer *et al.* 1999). In contrast, layering occurs when branches still attached to the tree come in contact with the ground and produce adventitious roots (Cremer *et al.* 1999). *Salix exigua* and *S. nigra* produce suckers (shoots produced from roots), however, most willows do not (Cremer 2003). Layering and suckering are most likely to occur during spring-summer when plants are actively growing. Canopy fragments may be produced at any time in response to flooding, wind damage or other disturbances, however, establishment from fragments is less likely in winter when willows are not actively growing.

Sexual reproduction follows a more specific time schedule. Flowering occurs in spring, with most willows in Australia flowering for approximately three weeks during September-October (Cremer 2003). Flowering times vary within this period according to species or variety. Willows are predominantly insect-pollinated, although wind pollination may also occur (Cremer 2003). Pollination has been recorded over distances of up to 1km (Cremer 2003). Willows hybridise readily, with cross-pollination often resulting in viable seed (Cremer 2003). Fruits ripen and seeds mature approximately one month after flowering (Cremer 2003).

Effects of flooding regime on willow growth and health

i) Ideal timing of watering events

Availability of permanent water. Ideally access to shallow groundwater throughout the growing season is desirable. Flooding assists with the replenishment of groundwater.

ii) Ideal flood frequency

As above.

iii) Ideal inundation period

As above.

iv) Ideal depth of inundation

Constant access to shallow groundwater, avoiding complete inundation of the root crown.

v) Ideal rate of change

As for other species. Rate of change is unlikely to affect growth and health of established trees directly.

vi) Ideal transmission velocity

As for other species, i.e. transmission velocity is unlikely to directly affect the growth and health of established trees. Quantification of velocities on growth and health are not known.

2.1.5 Conceptual model - Effects of flooding regime on tree growth and health

A single model is sufficient to describe the main relationships between the various characteristics of flooding regime, and growth and health responses for the tree species considered here; namely *E. camaldulensis*, *E. largiflorens*, *A. stenophylla* and *Salix* species (Figure 1). While these species differ in their individual flooding requirements (Table 2) and degree of tolerance toward salinity, anoxia and other factors that limit plant growth, the main factors affecting growth and health are held in common between species. The tree growth and health model is made up of five components; responses, primary controls, flooding regime characteristics, modifying factors and potential stressors. These are explained further below.

Tree growth and health responses

- *Growth and health (leaves, roots and sapwood)*

Leaf, sapwood and root growth and health responses to watering can differ within as well as between species. Canopy condition alone may not be indicative of tree health and ability to recover from water stress. For example, some drought-stressed black box trees exhibit low canopy cover, but retain a higher proportion of sapwood than other trees, which invest their resources in canopy maintenance at the expense of sapwood (I. Overton pers. comm.). Recovery of sapwood requires a much longer period of time than recovery of the tree canopy. Sapwood thickness is more indicative of drought resilience than canopy condition.

Tree root growth patterns change according to water availability and water table depth. Trees need to have roots growing at an appropriate depth to access available water resources. In drier areas, trees typically invest in deeper roots in order to access deep soil moisture and groundwater reserves. After watering a time-lag may occur before a visible improvement in canopy growth is seen as new surface roots are produced to intercept the available soil moisture. Conversely, in areas prone to waterlogging, trees invest in surface roots at the expense of deeper roots. This can increase the risk of tree fall (Marcar *et al.* 1995).

- *Reproduction*

While flowering may occur in response to stress, flower and seed production generally increase with tree health.

Primary controls

- *Soil moisture and groundwater*

Flow enhancement and retention of floodwater on floodplains will largely affect floodplain tree health and growth indirectly, via their effects on water availability. The availability of water to established trees is determined by soil moisture content, groundwater depth, and the salinity of soil and groundwater resources. The quantity of water required for tree health and growth varies according to species and tree density.

Flooding regime characteristics

Four flooding regime characteristics are likely to affect the growth and health of established trees; timing, depth, duration and frequency of flooding. The effects of these flooding regime characteristics vary according to species, as summarised in Table 2.

- *Timing*

Temperature and day length vary according to a seasonal cycle. These two factors control evaporation rates. Thus the timing of water application will affect ratios of soil moisture recharge to evaporative loss. Temperature and day lengths also affect tree growth rates through their effects on physicochemical processes. In particular, rates of photosynthesis are controlled by interactions between light availability, water availability and temperature. Therefore growth responses may be greater in spring-summer than during autumn-winter.

The extent of reproduction is affected by season as well as tree condition. Flowering is often triggered by a seasonal cue, which is commonly temperature or day length. These response cues may be relatively ‘hard-wired’, restricting the ability of species to reproduce at other times of year regardless of water availability. In such situations, if the goal is to enhance reproductive output, the optimum time for water application will coincide with the time of year when that species begins allocating resources toward reproduction. Additional watering may be required to support reproduction through hot dry summers.

- *Depth*

The depth of water applied is not likely to have any direct effect on established tree growth and health, provided that the leaves remain above water to enable photosynthesis and gas exchange. However, interactions between depth and duration of flooding may affect the extent of soil moisture and groundwater recharge.

- *Duration*

The duration of flooding will affect the extent of infiltration and subsequent availability of soil moisture and groundwater to floodplain trees. Flood duration also affects oxygen availability in the tree root zone, with gradual depletion occurring over time. Species vary in their tolerance toward anoxia.

- *Frequency*

The frequency of flooding necessary to maintain soil moisture reserves will vary according to various factors, including flood duration, temperature, rainfall and soil properties. If flooding is too infrequent, then soil moisture will become limiting. If flooding occurs too frequently, then waterlogging will occur and oxygen will become limiting. Tree condition prior to watering may affect the number of watering events required to ensure recovery, with stressed trees requiring multiple waterings before sapwood recovery occurs.

Site-specific modifiers

The effects that an individual watering event, with any given combination of characteristics, will have on water availability, oxygen availability and salinity are not uniform and simple to predict. A number of site-specific characteristics, such as soil properties, geomorphology, inundation history, rainfall history and land use can act as modifiers, affecting the outcomes of water application.

- *Inundation history*

Soil moisture and groundwater recharge rates are affected by previous watering history, including rainfall and flooding. If recently watered, then less water may need to be applied to meet the needs of tree populations. Some soil types can become hydrophobic if they are allowed to dry excessively, limiting future infiltration. Alternatively, when heavy clay soils are allowed to dry out and crack, these cracks may increase infiltration rates, allowing water to penetrate deeper into the soil profile and further from the boundary of the flooded area.

- *Geomorphology*

Landscape position can affect rates of rainfall and floodwater infiltration, with higher infiltration rates occurring in topographic depressions (Holland *et al.* 2006). Landscape position, underlying geology and soil types affect groundwater distribution and flow paths (Holland *et al.* 2006).

- *Soil properties*

Soil properties affect both infiltration rates and water-holding capacity. Infiltration and evaporation rates are generally higher for sandy soils than for soils high in clay content, although water can sometimes infiltrate to deep soil layers in clay soils relatively quickly via macropores (Bramley *et al.* 2003). While a shorter flood duration is required to replenish soil moisture in sandy habitats, a higher watering frequency may be required to maintain soil moisture levels (Holland *et al.* 2006), with the reverse applying to soils high in clay content. Sodic soils disperse when wet, exhibiting low permeability and low infiltration rates (Marcar *et al.* 1995). Sodicity is a common feature in saline soils (Marcar *et al.* 1995). Soil salinity will also affect the flooding regime required to maintain tree growth and health. Infiltration of surface water may reduce the salinity of water in the tree root zone by leaching salts down the soil profile, thereby increasing water availability to trees. However, where shallow saline water tables occur, excessive infiltration may push saline groundwater closer to the soil surface, reducing water availability to trees and resulting in declining tree growth and health.

- *Land use*

Soil moisture and groundwater recharge rates are affected by vegetation cover and composition, which determines root depth and density and water use rates. Some land uses may result in soil compaction, which increases runoff and reduces recharge rates.

- *Rainfall*

Rainfall events can reduce surface flooding requirements for tree growth and health.

Potential stressors

- *Temperature*

High temperatures lead to soil moisture loss through increased levels of evaporation and evapotranspiration. When subjected to water stress, trees shed leaves to reduce evaporative water loss, resulting in reduced growth rates.

- *Salt*

Salinity affects tree growth and health in three main ways. High salt concentrations lead to water stress, with osmotic effects reducing the ability of tree roots to extract water from the soil, regardless of soil water abundance (Marcar *et al.* 1995). Uptake of salts by tree roots can lead to direct cell damage (Marcar *et al.* 1995). High salinities can also lead to nutrient deficiencies due to reduced root growth, uptake of salts in preference to nutrient ions (such as nitrogen, potassium and phosphorus) or disruption of nitrogen fixation by legumes such as river cooba (Marcar *et al.* 1995).

- *Nutrients*

Nutrient limitation may restrict the growth of trees at some sites.

- *Oxygen*

Waterlogging can lead to an oxygen deficit in the root zone. Trees vary in their ability to tolerate waterlogging, with tolerant species exhibiting specialised adaptations, including root air channels (aerenchyma) and adventitious roots, which are produced close to or above the soil surface (or above the waterline e.g. mangrove roots) in some species adapted to regular inundation (Marcar *et al.* 1995). Oxygen deficiencies, produced by excessive waterlogging, cause reductions in shoot growth, root growth and viability, premature leaf loss, reduced nutrient uptake and reduced ability to exclude salt (Marcar *et al.* 1995).

- *Competition*

Competition with other trees (including competition within or between species) and with understorey species for resources, including water, light, nutrients and space, can limit tree growth.

- *Herbivory/Pathogens*

Insect or pathogen attack may lead to reductions in tree growth and health. New growth produced in response to water availability may be more attractive to insect herbivores than older growth. Trees stressed by salinity and waterlogging are more susceptible to damage by insect pests and pathogens (Marcar *et al.* 1995)**.**

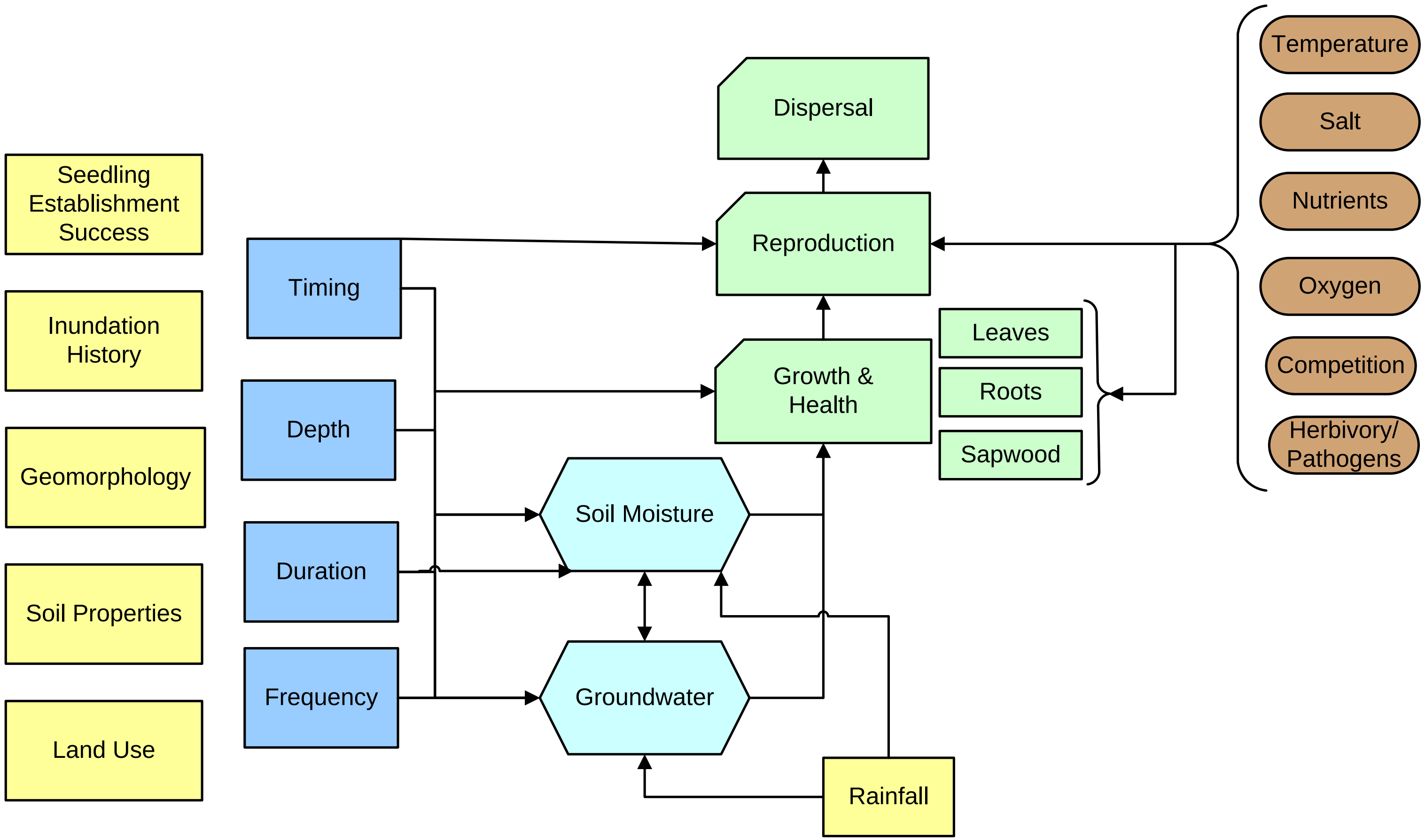

Figure 1: Conceptual model summarising the main relationships between flooding regime characteristics and tree growth and health. Yellow boxes are modifying factors, blue boxes indicate flooding regime characteristics, light blue hexagons are primary controls, green boxes are response components and brown ovals are potential stressors

Table 2: Summary of species flooding requirements for maintenance of growth and health

Species	Ideal flood timing	Ideal flood depth	Maximum flood depth	Ideal flood duration	Maximum flood duration	Ideal flood frequency	Maximum flood frequency	Ideal inter-flood dry period	Maximum inter-flood dry period	Notes
Eucalyptus camaldulensis	[1]Winter-spring	No direct impact	No direct impact	[2]2-8 months	[2]2-4 years	[2]1-3 years	[2]Variable	[2]5-15 months	[2]2-3 years	[1]Flood timing is more critical for reproduction and seed set than for tree growth and survival. [2]Varies according to other aspects of flooding regime, tree health and site factors (soils, geomorphology, rainfall, salinity, etc).
Eucalyptus largiflorens	[1]Winter-spring	No direct impact	No direct impact	[2]2-4 months	[2]>4 months	[2]2-5 years	[2]Variable	[2]Variable	[2]7-10 years	[1]Flood timing is more critical for reproduction and seed set than for tree growth and survival. [2]Varies according to other aspects of flooding regime, tree health and site factors (soils, geomorphology, rainfall, salinity, etc).
Acacia stenophylla	Not known	Not known	Not known	Not known	Not known	Not known	Not known	Not known	Not known	
Salix spp.	Year round	[1]Shallow	[2]Not known	Permanent	Permanent	Continuous	Continuous	None	[3]Variable	[1]Require continuous access to shallow groundwater, but does not tolerate permanent inundation of the root crown. [2]Likely to vary between species, and with flood duration. [3]Tolerance to drying may vary between species.

2.2 Water requirements for propagule dispersal

2.2.1 River red gum (*Eucalyptus camaldulensis)* seed dispersal

River red gum exhibits serotiny, with seeds retained in the canopy in an aerial seed bank and released after approximately 1-2 years (George 2004; Jensen *et al.* 2008a). Serotiny is a strategy used by some species to protect seed from predation, or to ensure that seeds are released during favourable conditions for dispersal, germination and seedling establishment (Jensen *et al.* 2008a). Like other eucalypts, this species does not produce large persistent soil seed banks, with few viable seeds found in the soil beneath mature trees regardless of tree health and seed yield (Jensen *et al.* 2008a). Low numbers of viable seeds in the soil seed bank may indicate high rates of seed predation or a short viability period.

Seed dispersal mechanisms used by river red gum include amenochory (wind dispersal) and hydrochory (water dispersal). While wind usually disperses eucalypt seed within a distance of 1-2 times tree height (George 2004), the seeds of river red gum can float for 10 or more days and may be carried considerable distances by flowing water (Pettit and Froend 2001). Therefore, flow velocities and the magnitude or spatial extent of flooding may affect the distance from the parent plant that seeds are carried. Hydrochory may be the most important dispersal mechanism for river red gum. Concentrated deposits of river red gum seed can be found at flood strandlines (Pettit and Froend 2001). In a soil seed bank experiment, significantly more *E. camaldulensis* seeds germinated from flood debris than from equivalent soil samples collected under trees on the Blackwood River in Western Australia (Pettit and Froend 2001).

Effects of flooding regime on river red gum seed dispersal

i) Timing of watering events

Timing watering so that flood recession coincides with peak seed fall is likely to maximise *E. camaldulensis* seed dispersal and increase the probability that seeds are deposited on moist ground, suitable for subsequent germination and seedling establishment (Pettit and Froend 2001). In the lower Murray region most fruit open over 1-2 months during summer. However, seed fall occurs earlier in the Barmah Forest, reaching its peak in spring (Dexter 1978, cited in Roberts and Marston 2000). Some seed fall also occurs throughout the year when fruit or branches become damaged or break off (Jensen 2009).

Peak seed fall appears to occur in *E. camaldulensis* populations approximately at the time that spring-summer floods would have been occurring at those sites under unregulated river flow conditions (Roberts and Marston 2000; Rogers in prep.). This may be a natural adaptation designed to maximise seed dispersal during favourable conditions for subsequent germination and seedling establishment.

Flooding may trigger seed release in *E. camaldulensis,* however more research is required to support this. Extensive seed fall was recorded from healthy trees at Banrock Station when the wetland was flooded for several days (George 2004). Seed fall in healthy trees occurred two months after rainfall, while trees in poor condition dropped seed almost immediately after rainfall occurred (George 2004). Therefore, seed fall may or may not be initiated by surface water availability.

ii) Frequency of watering events

Seeds in the soil seed bank appear to be short-lived, with Pettit and Froend (2001) reporting a decline from around 40% germination after seed fall, to 4-5% of germination after 6 months of burial. This indicates that seed dispersal needs to occur during or soon before the season when conditions will be conducive to germination and seedling establishment. A single watering event timed to occur during peak seed fall should maximise seed dispersal.

iii) Inundation period

Seeds of *E. camaldulensis* float for up to approximately 10 days, and may be dispersed in floodwaters during that time according to water currents and wind direction (Pettit and Froend 2001). Floating seeds begin to germinate and sink after 6-9 days (at 20^{o}C) (Pettit and Froend 2001). Inundation period may not affect the deposition of seeds in strandlines at the waters edge. However, longer periods of flooding may reduce the survival of seeds remaining in the water column. The relationship between seed survival and inundation period is not known.

iv) Depth of inundation

Since seeds of *E. camaldulensis* float, water depth is unlikely to affect dispersal success directly. However, since depth is linked to the magnitude, or spatial extent of a flood, depth may indirectly affect the distances seeds are carried.

v) Rate of change

Rates of depth change may affect seed dispersal. Steady water levels may lead to deposition and survival of seeds predominantly around the high water mark, if *E. camaldulensis* seeds do not tolerate long periods of submergence. However, if flood waters are receding during seed dispersal, seed could potentially survive and be deposited over a greater range of elevations (Cremer *et al.* 1995).

vi) Transmission velocity

Faster flow rates can be expected to result in dispersal of floating seed over greater distances (Groves *et al.* 2009). Allowing water to flow through the floodplain may allow movement of seed between populations. Genetic robustness of populations may therefore be increased through flow enhancement, compared to floodwater retention, due to increased connectivity between sites, particularly longitudinal connectivity. The quantitive effects of flow velocity on seed dispersal are not known.

2.2.2 Black box (*Eucalyptus largiflorens)* seed dispersal

Black box exhibits serotiny, with seeds stored in the tree canopy for 12-24 months before they are released (Jensen 2009). Seeds of this species appear to be transient, with few viable seeds found in the soil seed bank (Jensen 2009). There is little information available on the effects of flooding regime on *E. largiflorens* seed dispersal. However, tree condition has been reported to have a considerable effect on both the volume of seed released, and the timing of seed release (Jensen *et al.* 2008a). For example, stressed trees (n = 3) at Banrock Station on the lower River Murray floodplain produced a light continuous seed fall, with maximal output of 5-60 seeds/m^2 per month only. A healthy tree at this site released up to 2 356 seeds/m^2 per month, with a distinct peak in seed release occurring during October-March (Jensen *et al.* 2008).

Seed dispersal in *E. largiflorens* is likely to occur via a combination of amenochory (wind dispersal) and hydrochory (water dispersal). Amenochory typically disperses eucalypt seed within a distance of approximately 1-2 times tree height (Jensen *et al.* 2008a). Hydrochory may lead to the concentration of *E. largiflorens* seed in flood strandlines. While *E. largiflorens* seeds float, the buoyancy period in this species is relatively short with seeds beginning to sink after approximately 4 hours (Jensen 2008). It is not known how long *E. largiflorens* seeds remain viable under water. Seedlings largely occur after flooding, however, it is not known if flooding triggers seed release.

Effects of flooding regime on black box seed dispersal

i) Ideal timing of watering events

Timing of watering so that flood recession coincides with the time of peak seed fall should lead to dispersal of larger numbers of seed away from parent trees and increase the probability that seeds are deposited on moist ground, suitable for subsequent germination and seedling establishment. In healthy stands, maximum seed fall usually occurs in summer, with a second, smaller, peak occurring in winter (George 2004; Jensen 2008). Since reproductive effort is spread throughout the year in black box populations, some seed is likely to be available for dispersal regardless of the timing of watering events.

As for river red gum, maximum seed fall in black box appears to occur approximately at the time that spring-summer floods would have occurred under natural, unregulated river flow conditions (Rogers in prep.). This may be a natural adaptation designed to maximise seed dispersal during favourable conditions for subsequent germination and seedling establishment. It is not known whether watering triggers seed release in black box.

ii) Ideal flood frequency

A single watering event timed to occur during peak seed fall should maximise seed dispersal.

iii) Ideal inundation period

The effects of inundation period on seed survival and dispersal are not known.

iv) Ideal depth of inundation

Water depth is unlikely to affect dispersal success directly. However, since depth is linked to the magnitude, or spatial extent of a flood, depth may indirectly affect the distance from the channel that seeds are carried.

v) Ideal rate of change

Rates of rise and fall of floodwaters may affect seed dispersal, depending on how long *E. largiflorens* seeds survive underwater, depth itself is inconsequential, it is the dispersal and drowning of seeds that are the important factors here. Stable water levels may lead to deposition and survival of seeds predominantly around the high water mark, if seeds do not tolerate long periods of submergence. However, if flood waters are receding during seed dispersal, seed may survive and be deposited on moist soils, over a greater range of elevations (Cremer *et al.* 1995).

vi) Ideal transmission velocity

Faster flow rates can be expected to result in dispersal of floating seed over greater distances (Groves *et al.* 2009). Allowing water to flow through the floodplain may allow movement of seed between populations and help maintain the genetic diversity of stands. Quantification of velocities on seed dispersal are not known.

2.2.3 River cooba (*Acacia stenophylla)* seed dispersal

Large numbers of seed are commonly observed germinating along strandlines after flooding, indicating that hydrochory is an important seed dispersal mechanism for *A. stenophylla* (Cunningham *et al.* 1981).

Effects of flooding regime on river cooba seed dispersal

i) Ideal timing of watering events

The effect of flood timing on seed dispersal is not known. Seed maturation occurs from October-December in *A. stenophylla* (Marcar *et al.* 1995). However, no information was found on the timing of seed fall.

ii) Ideal frequency of watering events

The effect of inundation frequency on river cooba seed dispersal is not known.

iii) Ideal inundation period

The effect of inundation period on seed survival and dispersal is not known.

iv) Ideal depth of inundation

The effect of water depth on river cooba seed dispersal is not known.

v) Ideal rate of change

The effect of rate of change on river cooba seed dispersal has not been investigated. However, if flood waters are receding during seed dispersal, seed may survive and be deposited over a greater range of elevations (Cremer *et al.* 1995).

vi) Ideal transmission velocity

The effect of transmission velocity on seed dispersal is not known. Quantification of velocities on seed dispersal are also not known.

2.2.4 Willow (*Salix* spp.*)* seed and stem fragment dispersal

Willows shed their seed during October-November, approximately one month after flowering (Cremer 2003). The primary dispersal mechanism is amenochory, with seeds each possessing a pappus of cottony hairs that helps them to drift for kilometres at a time on only a slight breeze. The relative importance of hydrochory for seed dispersal is uncertain but could be important since the pappus gives the seed some buoyancy. This buoyancy is limited as the pappus detaches easily unless the water surface remains still (Cremer 2003). However, seeds and seedlings can be carried downstream and deposited along with sediment at flood strandlines (Cremer *et al.* 1995).

Willow populations can spread quite rapidly by seed. Most seedlings appear within 100m of their seed source, however, individuals regenerating from seed have been found up to 2.9km downstream of the nearest known female (Cremer *et al.* 1995). *Salix nigra* spread 50-100km in various directions by seed during the 30 years after it was planted at Tumut (Cremer 2003). While seed dispersal can result in very rapid rates of spread, most populations spread to new areas via the dispersal of stem fragments. These are commonly broken off during storms and floods, and may travel some distance downstream (Cremer *et al.* 1995; Cremer 2003). References to specific distances for dispersal of stem fragments were not found.

Effects of flooding regime on willow seed and stem fragment dispersal

i) Ideal timing of watering events

Flooding is unlikely to contribute to seed dispersal unless it coincides with seed release (October-November). Dispersal of canopy fragments by water may occur at any time.

ii) Ideal flood frequency

Seeds are primarily wind-dispersed, and are released only once per year, therefore inundation frequency will not affect seed dispersal. Dispersal of canopy fragments in flowing water may occur at any time.

iii) Ideal inundation period

Inundation period is unlikely to affect seed dispersal, but is likely to affect subsequent germination and establishment success. Longer inundation periods are likely to favour dispersal of canopy fragments.

iv) Ideal depth of inundation

Water depth is unlikely to affect seed dispersal, but is likely to affect subsequent germination and establishment success.

v) Ideal rate of change

If flood waters are receding during seed dispersal, seed may survive and be deposited over a greater range of elevations (Cremer *et al.* 1995).

vi) Ideal transmission velocity

Higher velocity flows are more likely to result in detachment and dispersal of viable willow stem fragments.

2.2.5 Conceptual model - Effects of flooding regime on propagule dispersal

The effects of flooding regime on river red gum, black box, river cooba and willow propagule dispersal are summarised in Figure 2. The relative importance of flooding regime for propagule dispersal varies between species (Table 3). Willows differ from the other species because they can be spread via dispersal of both stem fragments and seed. Therefore, a component has been added to the model to summarise the effects of flooding on stem fragment dispersal for willows (Figure 2).

Seed and stem-fragment dispersal response components

- *Seed fall*

The timing of seed fall appears to be largely governed by seasonal cues. Seed fall may or may not be triggered by flooding in river red gum, black box and river cooba.

- *Fragmentation*

Willow stem fragments are usually produced as a result of wind or flood damage, but it is suspected that high transmission velocities of floodwaters would also contribute to dispersal of plant fragments.

- *Dispersal*

Dispersal refers here to the movement of propagules from the parent tree to a separate location.

- *Settlement*

Settlement refers here to deposition of propagules on a potential growth substrate while still viable. Seed buoyancy, seed longevity underwater and rates of rise or fall of floodwaters will each affect the likelihood of settlement.

Flooding regime characteristics

- *Timing*

When hydrochory is the primary dispersal mechanism, flood timing can have a large effect on the effectiveness of seed dispersal. This is particularly relevant when seed fall is restricted to a particular time of year, when seeds are short-lived, and/or where seed predation rates are high. Hydrochory appears to be a more important dispersal mechanism for seeds of river red gum, black box and river cooba than it is for willows, which primarily disperse via amenochory. Timing of flood recession to match periods of peak seed fall (Table 3) should maximise seed dispersal. The timing of peak seed fall varies geographically, but for river red gum and black box appears to coincide with the general timing of floodwater recession under historical (pre-regulation) flooding regimes.

- *Velocity*

Under high flow velocities, willow branches are more likely to be dislodged and carried downstream. Seeds and stem fragments are also likely to be carried greater distances under conditions of high flow velocity (Groves *et al.* 2009).

- *Magnitude*

The magnitude, or spatial extent, of flooding will have an effect on the distance that propagules are carried, and the position of the flood strandline.

- *Longitudinal connectivity*

The maintenance of longitudinal connectivity between sites, by allowing water to flow freely between them, may increase the potential for propagules to spread into new areas away from the parent tree population.

- *Rate of change*

The effects of rates of rise and fall on propagule dispersal will vary according to how long propagules remain buoyant, and how long they survive underwater. For example, propagules with a short buoyancy period, and a short life-span underwater, have a high risk of mortality when water levels are rising. Receding water levels can ensure survival and deposition of propagules over a broader range of elevations (Cremer 1995). However, if flood recession is too rapid, soil moisture levels may drop too rapidly for successful germination and seedling establishment.

Site-specific modifiers

- *Reproductive success*

The abundance of seed available will determine the amount that can be dispersed. Willow trees usually produce either male or female flowers, therefore seed production depends on the presence of opposite sex individuals within close proximity. It is recommended that a distance of more than two kilometres be maintained between male and female willow trees to prevent pollination and seed production (Cremer 2003). Seed output in the other tree species is more likely to be governed by inundation history, salinity and other factors affecting tree growth and health.

- *Geomorphology*

Landform may have an affect on patterns of propagule deposition.

- *Habitat structure*

The presence of obstacles such as vegetation, rocks or large woody debris can also alter patterns of propagule deposition, by trapping seed or stem fragments.

- *Land use*

Drainage lines, earth banks and other water management structures may block or alter patterns of water movement and propagule dispersal.

Potential stressors

- *Seed predation*

Seed predators may remove or damage seed before or after dispersal by flood waters, or other applicable dispersal mechanisms.

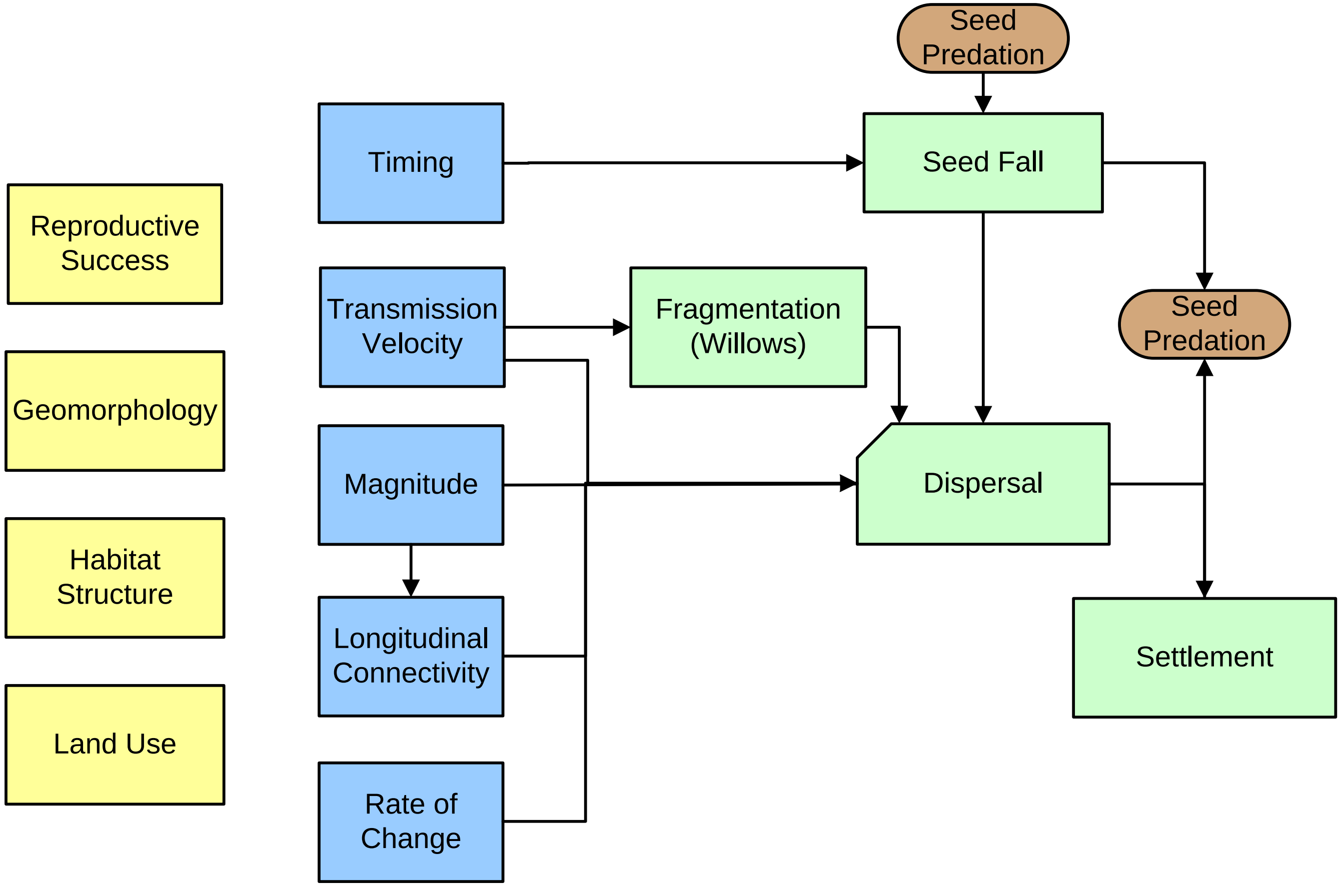

Figure 2: Conceptual model summarising the main relationships between flooding regime characteristics and propagule dispersal. Blue boxes indicate flooding regime characteristics, green boxes are response components, yellow boxes are modifying factors and brown ovals are potential stressors

Table 3: Summary of species flooding requirements for propagule dispersal

Species	Ideal flood timing	Ideal flood depth	Maximum flood depth	Ideal flood duration	Maximum flood duration	Ideal flood frequency	Maximum flood frequency	Ideal inter-flood dry period	Maximum inter-flood dry period	Notes
Eucalyptus camaldulensis	[1]Receding in spring-early summer	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[1]Ideal watering time for dispersal is during peak seed fall. A single flood event, timed to coincide with peak seed fall, will ensure dispersal. [2]The distances seeds are dispersed by floods and where they settle are likely to vary according to flood extent, velocity and connectivity between sites, rather than other aspects of flooding regime.
Eucalyptus largiflorens	[1]Receding in spring-early summer	[2]No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	[1]As above.
Acacia stenophylla	Not known	Not known	Not known	Not known	Not known	Not known	Not known	Not known	Not known	
Salix spp. (seeds)	[1]October-November	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[1]Willow seed is primarily wind-dispersed, and largely independent of flooding regime. However, flood events can move both seed and seedlings into new areas. [2]As above.

Species	Ideal flood timing	Ideal flood depth	Maximum flood depth	Ideal flood duration	Maximum flood duration	Ideal flood frequency	Maximum flood frequency	Ideal inter-flood dry period	Maximum inter-flood dry period	Notes
Salix spp. (stem fragments)	[1]Year round	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[2]No direct impact	[1]Dispersal of stem fragments may occur during flooding at any time, but is most likely to occur during times of high flow. [2]As above.

2.3 Water requirements for germination

2.3.1 River red gum (*Eucalyptus camaldulensis)* germination

Germination is largely driven by water availability, however, temperature, oxygen and light availability also affect germination success. Germination is not dependent on flooding in *E. camaldulensis* and may occur in response to rainfall alone, provided sufficient soil surface moisture is available (Roberts and Marston 2000). Rain events >5mm can be sufficient to trigger germination on the lower River Murray floodplain (Jensen 2009). Individuals germinating after rainfall are more scattered than those germinating after flooding, since floods typically lead to dense concentrations of seed being deposited in strandlines (Pettit and Froend 2001). The optimum temperature for germination was reported to be 35^{o}C, under laboratory conditions (Grose and Zimmer 1958). However, germination does occur at lower temperatures and can occur in winter (Roberts and Marston 2000). Light is a requirement for germination in *E. camaldulensis* (George 2004). *Eucalyptus camaldulensis* seeds will germinate readily on moist soil or while floating, but will not germinate underwater (Jensen 2009).

Effects of flooding regime on river red gum germination

i) Timing of watering events

Flood timing does affect germination success. In the southern part of the Basin, low temperatures result in fewer seeds germinating when flood recession occurs in winter, with those that germinate being susceptible to frost injury (Roberts and Marston 2000). Conversely, if flood recession occurs in summer germination may be higher but heat and water stress can lead to massive seedling mortality (Roberts and Marston 2000). Winter floods receding in spring-early summer provide optimal conditions for *E. camaldulensis* germination and early seedling establishment (Dexter 1967; 1978, cited in Roberts and Marston, 2000). Floods which recede immediately before peak seed fall may help to increase numbers of seeds germinating (Jensen *et al.* 2008a).

ii) Frequency of watering events

A single watering event is sufficient to trigger germination. The long-term success of these germinated trees will rely on other factors. Ideal inter-flood periods can only be determined by locally assessing the soil moisture content required by species, on both a spatial and temporal basis.

iii) Inundation period

Most *E. camaldulensis* seeds germinate within 10 days under favourable moisture conditions (Jensen *et al.* 2008a). Therefore, to trigger germination, soil moisture need only last for approximately 10 days. However, subsequent seedling establishment will require moisture availability over a longer time period.

Eucalyptus camaldulensis seeds dispersed in water will germinate while floating, but lose buoyancy as the hypocotyl develops (Pettit and Froend 2001). Germinants are unlikely to survive prolonged inundation (Pettit and Froend 2001). However, the specific inundation period tolerated after germination is not known.

iv) Depth of inundation

Water depth does not affect germination in *E. camaldulensis* because the seeds of this species are buoyant and will germinate while floating (Pettit and Froend 2001). However, after germination, the seeds sink and are unlikely to survive for a prolonged period underwater (Pettit and Froend 2001).

v) Rate of change

Rate of change will affect germination primarily via its effect on surface soil moisture, which is also affected by soil characteristics and evaporation rates. Optimum conditions for germination are likely to occur after flood recession when moist sediments are exposed.

vi) Transmission velocity

Transmission velocity is unlikely to affect germination rates directly. However, germinants are not likely to survive for extended periods underwater. Germination occurs most successfully in flood debris lines and on moist sediments exposed after flooding (Pettit and Froend 2001).

2.3.2 Black box (*Eucalyptus largiflorens)* germination

The germination requirements of *E. largiflorens* seeds are similar to those of *E. camaldulensis.* Rainfall events may trigger germination, however rainfall may not be adequate to support subsequent seedling establishment (Trelour 1959, cited by Rogers in prep.). Large scale flood events appear to be required to provide the soil moisture necessary for widespread germination and establishment of *E. largiflorens* (Trelour 1959, cited by Rogers in prep.). Prior to river regulation these large flood events occurred mainly in spring, on the lower and mid-River Murray floodplain, providing seedlings with moist conditions going into summer (Rogers in prep.). Germination is not limited to spring-summer and has been observed between May-October in the northern parts of the Basin (Roberts and Marston 2000). Light is required for germination of *E. largiflorens* seed (Grose and Zimmer 1957, cited in Rogers in prep.). *Eucalyptus largiflorens* seeds can germinate underwater. However, it is not clear how long *E. largiflorens* germinants can survive while submerged (Jensen 2008).

Effects of flooding regime on black box germination

i) Ideal timing of watering events

Germination may occur at any time with sufficient soil moisture availability, however, newly germinated seedlings are susceptible to frost and heat injury. The number of seeds germinating may be maximised by timing watering events to coincide with periods of peak seed rain (Jensen 2009). Watering in spring may ensure higher rates of seedling survival since extreme temperatures are less likely to occur during and after germination.

ii) Ideal flood frequency

A single watering event is sufficient to trigger germination.

iii) Ideal inundation period

No data were found on the effects of inundation period on viability of *E. largiflorens* seed, or survival of seed after germination. However, recently germinated seedlings are unlikely to survive prolonged inundation.

iv) Ideal depth of inundation

Germination can occur on moist soil or underwater. However, *E. largiflorens* regeneration after flooding is typically restricted to a narrow band along the flood high water mark (Cunningham *et al.* 1981). This pattern suggests a low survival rate for seeds that germinate underwater.

v) Ideal rate of change

Rate of change will affect germination primarily via its effect on surface soil moisture, which is also affected by soil characteristics and evaporation rates.

vi) Ideal transmission velocity

Transmission velocity is unlikely to affect germination rates directly. However, germinants are not likely to survive for extended periods underwater.

2.3.3 River cooba (*Acacia stenophylla)* germination

Prolific germination is often observed at the strandline following major floods, with floods appearing to enhance germination success (Cunningham *et al.* 1981). The effect of rainfall availability on *A. stenophylla* germination rates is not known.

Effects of flooding regime on river cooba germination

The effects of the various different characteristics of flooding regimes on river cooba germination have not been investigated to our knowledge.

2.3.4 Willow (*Salix* spp.*)* germination

Willow seeds are typically short-lived, surviving approximately 1-9 weeks when kept dry, at room temperature. Seeds require moist conditions and light, within this viability period, in order to germinate. When adequate moisture is available seeds germinate rapidly, within 1-2 days. Germination can even occur on the tree before seeds are shed, in response to rainfall. Willow seeds germinate when deposited on moist sediment, or while floating or even while underwater. Newly emerged seedlings are able to survive for up to a month while completely submerged. However, submerged seedlings will not grow until they are exposed to air. Growth of newly emerged seedlings is very slow, with root elongation rates of around 0.5mm/day. While willow seeds are short-lived and are only available in spring-early summer, stem fragments can be produced at any time of year, and these may sprout at any time throughout the active growth season in response to water availability (Cremer 1995; Cremer *et al.* 1995; Cremer *et al.* 1999; Cremer 2003).

i) Ideal timing of watering events

Viable seeds are only available for a short time during spring-early summer, therefore water must be available at this time for germination to occur. Stem fragments are most likely to sprout during spring-summer.

ii) Ideal frequency of watering events

A single watering event is sufficient to trigger germination.

iii) Ideal inundation period

One day of moisture availability is sufficient to cause seed germination. However, seedlings will not survive unless surface moisture remains available for some time. Newly-emerged seedlings do not survive longer than one month underwater.

iv) Ideal depth of inundation

Seeds will germinate either above or below the water surface, provided they are also exposed to light.

v) Ideal rate of change

Unlikely to affect germination unless light-availability is reduced, since seeds will germinate underwater. Newly emerged seedlings can be killed by either prolonged submergence or desiccation.

vi) Ideal transmission velocity

As for rate of change.

2.3.5 Conceptual model - Effects of flooding regime on germination

The effects of flooding regime on river red gum, black box, river cooba and willow seed germination are summarised in Figure 3. The effects of flooding on seed germination vary between species, as summarised in Table 4.

Germination response

- *Germination*

Germination is defined here as the period from radical (initial root) emergence, until the production of the first true leaves.

Primary control

- *Soil moisture*

The availability of soil surface moisture from rainfall or flooding is a primary factor affecting germination success. The various aspects of flooding regime can affect germination through their effects on surface moisture availability.

Flooding regime characteristics

- *Timing*

Seed availability varies for each species according to time of year, for example, willow seeds are available during spring only. Temperature also varies according to season, with germinants being susceptible to frost damage in winter and heat injury and desiccation in summer.

- *Duration*

Germination may occur in response to a single rainfall or flooding event. Seed buoyancy periods and survival times underwater determine maximum inundation periods tolerated.

- *Depth*

While river red gum and black box seeds can germinate while floating, and willow and black box seeds can germinate underwater, germinants must be deposited on exposed sediments above the waterline in order to survive.

- *Frequency*

Repeated flooding may lead to mortality of germinated seed, by restricting access to light and atmospheric gases or by dislodgement and stranding of germinants in unfavourable growth habitats.

Site-specific modifiers

- *Dispersal success*

If local seed production and dispersal rates are low, germination may be limited.

- *Inundation history*

Inundation history may affect soil moisture status, moisture-holding capacity and soil surface salinity.

- *Soil properties*

Soil properties such as sand, clay or organic matter content may affect moisture-holding ability. Excessive salinity or compaction may retard root development, limiting survival.

- *Land use*

Land use effects, such as soil compaction and disturbance, may also affect early survival, either directly or indirectly.

Potential stressors

- *Temperature*

Extreme temperatures can reduce germination rates, and/or increase mortality of germinants.

- *Shading*

Shading can reduce germination success. River red gum, black box and willow seeds require light to germinate.

- *Salinity*

Excessive salinity is likely to lead to moisture stress in germinants.

- *Oxygen*

Atmospheric gas exchange is required for growth after germination.

- *Seed predation*

Seed predation may reduce the availability of viable seed prior to germination.

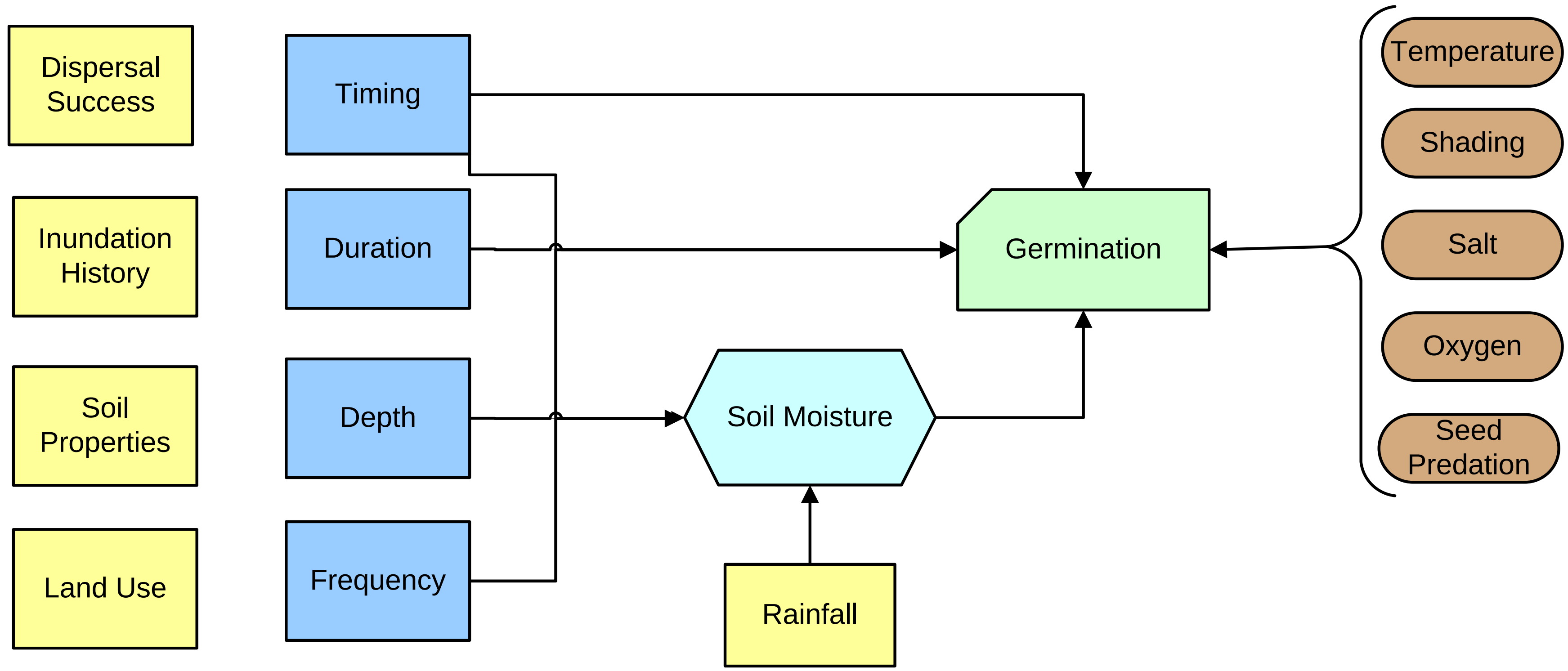

Figure 3: Conceptual model summarising the main relationships between flooding regime characteristics and germination. Blue boxes indicate flooding regime characteristics, hexagons are primary controls, yellow boxes are modifying factors, green boxes are response components and brown ovals are potential stressors

Table 4: Summary of species flooding requirements for germination

Species	Ideal flood timing	Ideal flood depth	Maximum flood depth	Ideal flood duration	Maximum flood duration	Ideal flood frequency	Maximum flood frequency	Ideal inter-flood dry period	Maximum inter-flood dry period	Notes
Eucalyptus camaldulensis	Receding in spring-early summer	Moist soil	[1]No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	Germination success is primarily controlled by seed availability and moisture availability after seed dispersal, with most seeds germinating within 10 days of watering. [1]Depth does not affect germination because seeds of this species will germinate while floating. However, depth will affect subsequent seedling survival and establishment, as will the other aspects of flooding regime.
Eucalyptus largiflorens	Receding in spring-early summer	Moist soil	[1]No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	[1]As above. Seeds may germinate underwater but are unlikely to survive prolonged immersion.
Acacia stenophylla	Not known	Moist soil	Not known	Not known	Not known	Not known	Not known	Not known	Not known	
Salix spp.	[2] October-November	Moist soil	[1]No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	No direct impact	[1]As above. [2]Willow seeds are viable for a short time only (1-9 weeks) after dispersal in spring. During this time seeds will germinate rapidly (typically within 1-2 days) in response to high moisture availability. Seeds will germinate while floating or underwater, however, seedlings will not grow further unless exposed to air within 1 month.

2.4 Water requirements for seedling establishment

2.4.1 River red gum (*Eucalyptus camaldulensis)* seedling establishment

Seedling survival in the first year after germination is a critical stage in river red gum stand regeneration, 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 River Murray floodplain (George 2004; Jensen *et al.* 2008a). 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.* 2008a).

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, then groundwater (George 2004; Jensen *et al.* 2008a). 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 *E. camaldulensis* seedlings invest significant resources into root growth with seedlings up to 23cm tall producing roots approximately four times plant height (Dexter 1978; Roberts and Marston 2000). Seedlings also develop resilience to stress at a relatively early stage; seedlings only 15cm were able to shed leaves under stress and recover from axillary buds (Roberts and Marston 2000).

Eucalyptus camaldulensis seedlings are vulnerable to the effects of flooding and do not tolerate prolonged immersion (Roberts and Marston 2000). However, seedlings do possess some adaptations that allow them to cope with anoxia caused by waterlogging, including adventitious root production and aerenchymatous tissue (Roberts and Marston 2000). Tolerance to flooding increases as seedlings become established, root systems extend and sapling height increases (Roberts and Marston 2000). Two month old seedlings can survive in waterlogged soils for one month without obvious effects on leaf number and height (Marcar 1993; Roberts and Marston 2000). Seedlings 50-60cm in height can survive extended flooding of 4-6 months, and complete submergence for several weeks, by shedding leaves (Roberts and Marston 2000).

Establishment times for *E. camaldulensis* seedlings vary according to growing conditions. Seedlings may establish within one year at temperate sites (George 2004). However, at Banrock Station, on the lower River Murray floodplain, seedlings were not considered fully established until 2-3 years of age and >1.3m height, since 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).

Effects of flooding regime on river red gum seedling establishment

i) Timing of watering events

Winter floods receding in spring-early summer provide ideal conditions for river red gum seedling establishment (Dexter 1967; 1978, cited in Roberts and Marston). 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). Ideally, adequate water to support seedlings through the first summer should be applied before germination (Roberts and Marston 2000). Flooding after germination may lead to seedling mortality due to burial, dislodgement or excessive immersion periods.

ii) Frequency of watering events

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 10cm during the first summer after germination (Jensen 2009). A follow-up watering may be required one year later to maintain seedlings while root systems develop further (George 2004).

iii) Inundation period

Tolerance to waterlogging increases with seedling height. Seedlings 50-60cm high can survive waterlogging (but not complete immersion) for 4-6 months (Roberts and Marston 2000).

iv) Depth of inundation

Complete immersion of seedlings should be avoided. Seedlings 50-60cm high ceased growing and shed their leaves after 1-3 weeks of immersion.

v) Rate of change

Because they do not tolerate prolonged periods of immersion, a rapid drawdown rate is preferable. However, soil moisture content should be maintained above 10% within the seedling root depth range.

vi) Transmission velocity

Flowing water may lead to dislodgement or burial of establishing seedlings.

2.4.2 Black box (*Eucalyptus largiflorens)* seedling establishment

Eucalyptus largiflorens seedling establishment is seldom supported by rainfall alone (Jensen 2008). Large scale regeneration of *E. largiflorens* appears to be confined to periods following major floods, when soil moisture is sufficient to ensure both germination and successful seedling establishment (Roberts and Marston 2000; Jensen *et al.* 2006). After flooding, seedling establishment typically occurs in a narrow band marking the high water line of the flood event that triggered germination (Cunningham *et al.* 1981). Distributions of established *E. largiflorens* stands on the lower River Murray floodplain are believed to mark the upper extent of historical floods (George *et al.* 2005; Jensen 2008).

Effects of flooding regime on black box seedling establishment

i) Ideal timing of watering events

Flooding should be timed to ensure surface moisture availability for germination in spring, as well as maintenance of shallow soil moisture over summer (George *et al.* 2005).

ii) Ideal flood frequency

Ideal watering frequency will vary between sites according to soil properties, evaporation rates and rainfall. At Banrock Station on the lower River Murray floodplain, the current flood frequency is too low to support seedling establishment rates high enough for maintenance of *E. largiflorens* stands (George *et al.* 2005). This area is now watered approximately once every 12 years, whereas under natural conditions the floodplain would have been inundated once every 3-4 years (George *et al.* 2005).

iii) Ideal inundation period

An extended period of inundation before germination and seedling establishment is desirable to ensure replenishment of soil moisture. However, floods during seedling establishment may be detrimental to seedling survival. Two month old *E. largiflorens* seedlings were able to tolerate waterlogging for one month (Heinrich 1990, cited by George 2004). Growth reductions occurred in 22 month old *E. largiflorens* seedlings subjected to longer periods of waterlogging, with signs of stress apparent after 70 days (McEvoy 1992, cited in Jolly and Walker 1996).

iv) Ideal depth of inundation

Black box seedlings are not adapted to waterlogging, and are unlikely to survive complete immersion unless brief (Jolly and Walker 1996). The survival period for *E. largiflorens* seedlings underwater is not known.

v) Ideal rate of change

If seedlings are inundated, slow drawdown rates will be detrimental to black box establishment because seedlings do not tolerate extended periods of waterlogging.

vi) Transmission velocity

Flowing water may lead to dislodgement or burial of establishing seedlings.

2.4.3 River cooba (*Acacia stenophylla)* seedling establishment

Surface floods may be required to provide sufficient soil moisture for *A. stenophylla* seedlings to establish at some sites. Despite prolific germination occurring in response to flooding, very few of these individuals typically survive (Cunningham *et al.* 1981). Additional watering or rainfall events may be necessary to ensure seedling establishment at some sites.

Effects of flooding regime on river cooba seedling establishment

The effects of different flooding regime characteristics on seedling establishment have not been investigated for river cooba, to our knowledge.

2.4.4 Willow (*Salix* spp.*)* seedling establishment

Seedling establishment is determined by seedbed conditions and ongoing moisture availability. Except for *S. cinerea*, establishment of willows from seed in Australia has been predominantly restricted to riparian sites, where bare sediments are kept wet for weeks to months after germination (Cremer *et al.* 1995; Stokes and Cunningham 2006). Because root growth rates are initially very slow (<1 mm/day), establishing seedlings are vulnerable to desiccation, and to dislodgement by rising flows (Cremer *et al.* 1999; Cremer 2003; Stokes 2008). Root growth rates increase with willow size. For example, the roots of six month old *S. nigra* cuttings, trimmed to 10cm, were able to elongate rapidly enough to survive a drawdown rate of up to 4cm per day (Stokes 2008). However, a drawdown rate of 8cm per day led to significant leaf loss (Stokes 2008).

If submerged, seedling growth ceases, however, growth can resume provided seedlings are exposed to air again within approximately one month (Cremer 2003). For example, 100% mortality was recorded for a stand of *S. nigra* seedlings submerged continuously for more than 30 days at Blowering Dam in their first growth season (Stokes 2008). The main potential limitations to willow seedling establishment are therefore lack of a suitable seedbed, rising or rapidly falling water levels after germination and occurrence of severe floods that wash seedlings away before they are sufficiently anchored (Cremer *et al.* 1995; Stokes and Cunningham 2006).

New individuals establishing from detached branch fragments appear to require similar conditions to seedlings for establishment. However, their larger size can make them less vulnerable to dislodgement. For example, *Salix fragilis* can establish midstream in relatively fast-flowing environments when branches become caught on rocks (Cremer 2003). Growth rates may not vary substantially between individuals establishing from seed and those establishing from branch fragments. A recent study of *S. nigra* establishing at Blowering Dam demonstrated no significant difference in the relationship between plant size and age between sexual and asexual individuals (Stokes 2008).

Effects of flooding regime on willow seedling establishment

i) Ideal timing of watering events

As root growth is initially slow, seedlings are vulnerable to water stress in the weeks to months following germination. Throughout this time the soil surface needs to remain constantly moist to ensure seedling survival (Cremer 2003).

ii) Ideal flood frequency

Soils must remain moist throughout seedling establishment.

iii) Ideal inundation period

Soils must remain moist throughout seedling establishment.

iv) Ideal depth of inundation

Seedlings of *Salix* spp. cease growing when submerged. However, they may resume growth if exposed to air within 30 days.

v) Ideal rate of change

Seedlings will not survive if drawdown rates exceed the rate of root descent. Root growth rates increase with seedling size (Cremer *et al.* 1999; Stokes 2008).

vi) Ideal transmission velocity

Rapid rates of flow may dislodge individuals and prevent establishment.

2.4.5 Conceptual model - Effects of flooding regime on seedling establishment

The effects of flooding regime on river red gum, black box, river cooba and willow seedling establishment are summarised in Figure 4. Moisture requirements and tolerance of waterlogging and immersion vary according to species and seedling size, as summarised in Table 5.

Seedling establishment response

- *Establishment*

The seedling establishment phase, for eucalypt species, is defined here as 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. Establishment may occur more rapidly in willows, which reach reproductive maturity in 2-3 years under favourable conditions (Cremer 2003), than in river red gum and black box, which can take multiple years to reach sapling stage (George 2004). However, the establishment requirements of willow differ from those of river red gum and black box. Willow seedlings require constant access to saturated soil to establish (Cremer 2003). Due to their low water use efficiency and slow initial rates of root growth (Cremer *et al.* 1999; Doody *et al.* 2006), willow establishment is generally restricted to areas within the riparian zone, near the waterline (Cremer 2003). No specific information was found on the establishment requirements of river cooba.

Primary control

- *Soil moisture*

Soil moisture is the most critical factor affecting seedling establishment. During the establishment phase sufficient soil moisture should be maintained within the seedling root zone to support survival and growth. Soil moisture content and depth are affected by a number of factors, including flooding regime and site characteristics.

Flooding regime characteristics

- *Timing*

Flooding should be timed to ensure moisture availability during early establishment stages. Maintenance of soil moisture over summer is important due to high evaporation rates at this time. However, complete immersion should be avoided while seedlings are small.

- *Velocity*

Fast flows may lead to seedling dislodgement. Flow rates will also affect turbidity. The dissolved oxygen content of ponded water is typically less than that of flowing water. However, it is not clear whether differences in dissolved oxygen content due to flow affect soil oxygen content and seedling growth and health.

- *Depth*

Flood depth during seedling establishment may affect seedling survival directly, as seedlings do not tolerate prolonged immersion. Interactions between flood depth, duration and frequency will also affect soil moisture content.

- *Duration*

The duration of flooding will affect the extent of infiltration, and subsequent availability of soil moisture to seedlings. Flood duration also affects oxygen availability in the seedling root zone, with species varying in their tolerance toward anoxia.

- *Frequency*

The frequency of flooding necessary to maintain soil moisture reserves will vary according to other aspects of flooding regime, as well as site-specific modifying factors.

Site-specific modifiers

- *Germination success*

Germination success will determine the maximum number of individuals available to establish at a site.

- *Inundation history*

Site inundation history will affect the subsequent flooding regime required to provide sufficient soil moisture for seedling establishment.

- *Geomorphology*

Landscape position can affect rates of rainfall and floodwater infiltration, which will affect soil moisture availability for seedling establishment.

- *Soil properties*

Soil properties will affect infiltration rates and moisture-holding capacity.

- *Land use*

As for other models.

- *Rainfall*

As for other models.

Potential stressors

- *Temperature*

Young seedlings are vulnerable to heat injury and frost damage. High temperatures lead to increased evaporation and evapotranspiration, which may lead to depletion of soil moisture in the seedling root zone.

- *Salt*

Excessive salinity can reduce the ability of seedlings to take up soil moisture, cause direct cell damage, and lead to nutrient deficiencies, reducing seedling growth.

- *Nutrients*

Low nutrient availability may limit seedling growth.

- *Competition*

Competition with other tree and understorey species for resources, including water, nutrients and space, can limit seedling growth and may prevent establishment.

- *Shading*

Seedlings require light to carry out photosynthesis. Shading from other plants may reduce seedling growth and establishment success.

- *Sediment/Turbidity*

Deposition of sediments, particularly increased in turbid waters, on submerged leaves may reduce the photosynthetic capacity of seedlings post-inundation.

- *Soil oxygen*

Anoxic conditions in the root zone can reduce seedling growth and survival.

- *Herbivory/Pathogens*

Seedlings are more vulnerable to damage by grazing animals than established trees, due to their low canopy height. Insect pests and pathogens may reduce seedling growth and survival rates. Grazing management may be required in conjunction with flooding to ensure higher rates of seedling survival (Meeson *et al.* 2002).

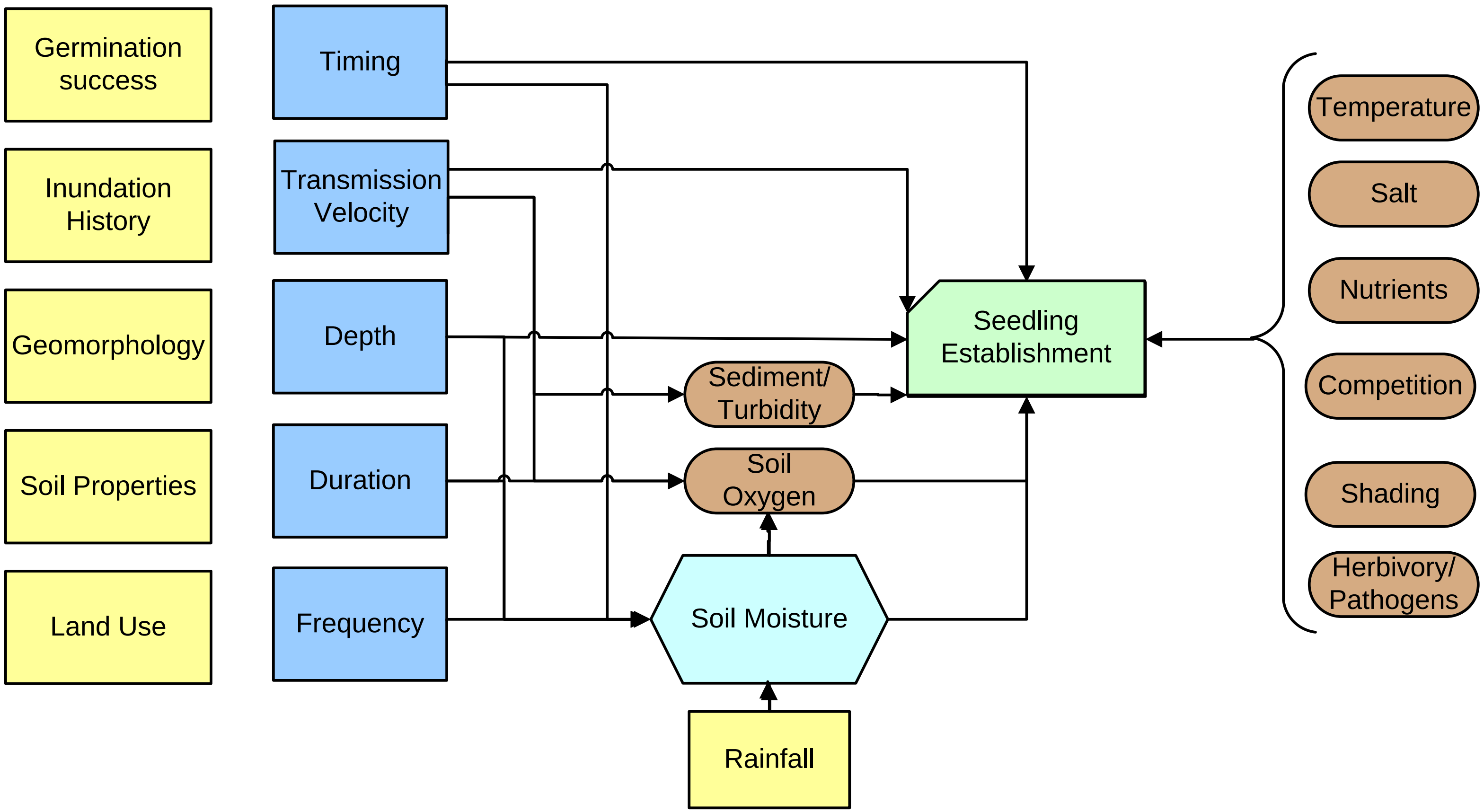

Figure 4: Conceptual model summarising the main relationships between flooding regime characteristics and seedling establishment. Blue boxes indicate flooding regime characteristics, hexagons are primary controls, green boxes are response components, yellow boxes are modifying factors and brown ovals are potential stressors

Table 5: Summary of species flooding requirements for seedling establishment

Species	Ideal flood timing	Ideal flood depth	Maximum flood depth	Ideal flood duration	Maximum flood duration	Ideal flood frequency	Maximum flood frequency	Ideal inter-flood dry period	Maximum inter-flood dry period	Notes
Eucalyptus camaldulensis	[1]Winter-late spring	[2]Less than total seedling height	[2] Variable	Variable	1-6 months, depending on seedling size	Sufficient to maintain soil surface moisture during first year	Variable	Variable	Variable	[1] Flooding timing and duration should be sufficient to ensure maintenance of soil moisture (at least 10-20%) in the first summer after germination. Establishment times vary and further watering may be required. [2] While seedlings have some tolerance to waterlogging, they can tolerate complete immersion for short periods only (1-3 weeks, for seedlings 50-60cm in height).
Eucalyptus largiflorens	[1]Winter-late spring	[2]Less than total seedling height	[2]Less than total seedling height	<30 days	Variable. 30-60 days depending on seedling size	Variable	Variable	Variable	Variable	[1] Flooding timing and duration should be sufficient to ensure maintenance of soil moisture in the first summer after germination. Establishment times vary and further watering may be required. [2] Seedlings do not tolerate prolonged waterlogging, and complete immersion should be avoided.
Acacia stenophylla	Not known	Not known	Not known	Not known	Not known	Not known	Not known	Not known	Not known	
Salix spp.	Year round	Moist soil	Less than total seedling height	Permanent	Permanent	Constant	Constant	None	Variable	Stable water levels are critical during the first year of growth. Small seedlings can survive for one month underwater, but will not grow while submerged.

3 Knowledge gaps

This conceptual model development enabled the simplification of the relationships between the maintenance of tree species through their various life stages and flooding regime characteristics. However, the long-term viability of these species is not solely limited to these relationships. Complex interactions between a range of abiotic and biotic factors ultimately determine whether trees will prosper in the short-term or long-term, including soil type, salinity, climate, herbivory, seed predation, inter-specific and intra-specific competition and others. Site-specific factors in particular, including soil types, geomorphology and inundation history, can also have a major influence on the effectiveness of water delivery to trees.

It is not the characteristics of a singular flooding event, with the options of ponding or maintaining flow, that should be the critical consideration in water management. Instead the focus needs to be on determination of a temporal sequence of flooding events that will suit the needs of native trees on a site-specific basis. This requires knowledge on the water requirements of trees at all growth stages, from germination to maturity. It also requires understanding and quantification of the links between flooding regime and soil moisture and groundwater availability, since these are two of the most critical determinants of tree growth and survival.

There are a number of gaps in our understanding of these links, and their implications for native tree species. Our level of knowledge varies between species and at present is somewhat skewed in favour of *E. camaldulensis*, with less information available on *E. largiflorens* and *Salix* species, and very little on *A. stenophylla*. In the following sections, relationships between flooding regime characteristics and tree species and growth stage responses are presented in tables. These relationships are grouped according to the IMAF hypotheses they are most closely associated with. In each table our current state of knowledge of these relationships is also indicated, using the following categories:

U = Unknown. This category relates to relationships that have not been researched, or for which there are no quantitative data or published information available**.**

G = Grey. This category relates to relationships for which there is strong observational evidence, but limited and/or unpublished data only.

K = Known. This category applies to relationships that are well supported, and widely accepted, based on published data and other sources.

The following abbreviations are used in the tables to indicate the three native tree species under consideration in this section of the report:

RRG = River red gum (*E. camaldulensis*),

BB = Black box (*E. largiflorens*),

RC = River cooba (*A. stenophylla*).

A number of knowledge gaps were identified through the literature review and workshop. The main knowledge gaps and high priority areas for future research, as determined by the Project Team, are also listed under the various IMAF hypotheses that they relate to. The focus here is on those relationships that fall into the more weakly supported knowledge categories, particularly those placed into the U category, that were also considered to be critical for determining the long-term viability of native tree populations. However, it is strongly suggested that more time and research be undertaken to address other knowledge gaps, particularly those in the G category. Several potential experimental designs proposed by the Project Team for addressing identified knowledge gaps are included in the appendices, along with a list of further information sources potentially relevant to further research projects.

3.1 Identification of knowledge gaps relating to IMAF hypotheses

- **Hypothesis 1: 'Suitable habitat for native tree species will be created or maintained through retaining floodwater on floodplains.'**

- **Hypothesis 4: 'Suitable habitat for native tree species will be created or maintained through flow enhancement.'**

Table 6: Conceptual model relationships relevant to Hypotheses 1 & 4 and current knowledge status

Relationship	Species and Current Knowledge Status		
	RRG	BB	RC
Flood duration affects soil moisture	G	G	U
Flood duration affects groundwater	K	K	U
Groundwater affects adult health and growth	K	K	U
Soil moisture affects adult health and growth	K	K	U

High priority knowledge gaps:

Whether floodwater retention or flow enhancement occurs, the information required to determine effects of these interventions on habitat suitability for native tree species relates to how effective these interventions are at achieving soil moisture and groundwater recharge, within the ranges that these species can tolerate. Quantitative information on how the depth, duration and frequency of flood events affect soil moisture and groundwater levels is currently limited. Information on the effects of other site-specific factors in regard to soil moisture and groundwater recharge is also limited. Further research is required to address these limitations, as well as to quantify the effects of soil water and groundwater availability on germination, seedling establishment and tree growth and health (including reproduction) in *E. camaldulensis*, *E. largiflorens* and *A. stenophylla*.

- **Hypothesis 2: 'The health and growth of native tree species will increase through retaining floodwater on floodplains.'**

- **Hypothesis 5: 'The health and growth of native tree species will increase through flow enhancement.'**

Table 7: Conceptual model relationships relevant to Hypotheses 2 & 5 and current knowledge status

Relationship	Species and Current Knowledge Status		
	RRG	BB	RC
Flood frequency affects sapwood production	G	G	U
Groundwater affects adult health and growth	K	K	U
Soil moisture affects adult health and growth	K	K	U

High priority knowledge gaps:

Some information is available on relationships between soil moisture and groundwater availability and the growth and health of *E. camaldulensis* and *E. largiflorens*, but there is very little information on the water requirements of *A. stenophylla*. The data available show that these species are opportunistic in their water use, using shallow and deep soil moisture and groundwater when available. Salinity also substantially affects tree water use. Before accurate predictions can be made about the effects of flooding on tree growth and health, consideration of the availability of other water sources including groundwater, the effects of salinity, and the influence of other site-specific factors (e.g. soil properties) on recharge rates is required.

Some general parameters describing the flooding requirements of *E. camaldulensis* and *E. largiflorens* are provided in summary tables in this report. However, these are very broad, based on information from a variety of different sites, and describing a very wide range of potential flooding regime scenarios. As such, these parameters do not take into account the differences in specific flooding requirements that will be applicable at the individual, community or site-scale. The determination of appropriate flooding regimes for ensuring the long-term viability of native tree populations requires site-specific details on soil moisture and groundwater availability, salinity and other relevant factors.

It is not clear whether floodwater retention and flow enhancement will have differing effects on tree health and growth and further to this, it has not been tested. Existing monitoring data show that floodwater retention may lead to lower water quality (e.g. reduced dissolved oxygen levels and increased concentration of salts) compared to flow enhancement. Experimental evidence suggests that soil oxygen levels may not vary detectably according to transmission velocity. However, the effects of differences in water quality resulting from ponding (compared to maintenance of flow) on health and growth of established trees has not been determined directly.

- **Hypothesis 3: 'Native tree species will germinate and recruit through retaining floodwater on floodplains.'**

- **Hypothesis 6: 'Native tree species will germinate and recruit through flow enhancement.'**

Table 8: Conceptual model relationships relevant to Hypotheses 3 & 6 and current knowledge status

Relationship	Species and Current Knowledge Status		
	RRG	BB	RC
Flood extent affects seed dispersal	U	U	U
Flood seasonality affects seed fall	U	U	U
Flood seasonality affects reproduction	U	U	U
Longitudinal connectivity affects dispersal	U	U	U
Rates of rise and fall affect seed settlement	U	U	U
Flood duration affects germination	G	G	U
Soil moisture affects reproduction (flower to seed drop)	G	G	U
Flood seasonality affects seedling growth and health	G	G	U
Flood seasonality affects germination	G	G	U
Flood depth affects seedling health and growth	K	K	G
Soil moisture affects seedling growth	K	K	G
Soil moisture affects germination	K	K	G

High priority knowledge gaps:

Information on how flooding interventions are likely to affect reproductive output, the timing of seed fall, and the effectiveness of seed dispersal and settlement is currently lacking. Research is required into the effects of flood timing (or seasonality) on reproduction, seed fall, germination and seedling growth. The effects of flood duration on seed viability and germination are currently unknown, as are the effects of rates of flood water of rise and fall on the dispersal of native tree seeds, germination success and early seedling survival. It is hypothesised that a slower rate of fall may lead to distribution of seed more broadly across the floodplain, however, this is based largely on assumption. Flow enhancement may also lead to greater distribution of seed than flood water retention due to increased connectivity between sites, however, further information is also required to test this hypothesis. Under flow enhancement, high flow rates may scour sediments and impede the establishment of seedlings, therefore the effects of transmission velocity on early life stages should also be considered.

- **Hypothesis 7: 'Suitable habitat for weed vegetation will be created or maintained through retaining floodwater on floodplains.'**

This report has assessed the current status of knowledge on the effects of flooding interventions (floodwater retention and flow enhancement) on willow dispersal and establishment. No table has been included in this section, as it was considered that sufficient information is available to address the main hypotheses relating to effects of floodwater retention on willows. Willows require continuous access to surface water or shallow soil moisture during the growing season. Early life stages are less tolerant of drying than established trees, with successful germination and seedling (or stem fragment) establishment unlikely to occur unless the soil surface is kept continuously moist. In the event of flood water retention, the invasive spread of *Salix* spp. would be limited to areas of permanent water or areas where the water table is shallow enough for the tree to access it permanently. Long-term survival is likely to remain restricted to areas of the River Murray riparian zone with a relatively constant water regime.

Potential detrimental effects of the two flooding interventions, relating to spread of *Salix* species include the risks that:

- if water retention is permanent, *Salix* spp. could invade and spread, and

- flow enhancement (in conjunction with longitudinal or lateral connectivity) could make it possible for the spread of willow fragments to disperse into new areas of permanent water supply.

High priority knowledge gaps:

It is important that the distribution of *Salix* spp. and their potential for spread be researched prior to flooding interventions. This investigation should consider the likelihood of seed production (based on distribution of male and female individuals), degree of longitudinal and lateral connectivity provided by flooding interventions, and the relative permanence of water availability in sites subjected to flooding interventions.

3.2 Other conceptual model relationships and knowledge gaps requiring consideration

Table 9: Conceptual model relationships relating to hydrology and current knowledge status

Relationship	Species and Current Knowledge Status		
	RRG	BB	RC
Flood seasonality affects soil moisture persistence	U	U	U
Flood depth affects soil moisture	U	U	U
Flood frequency (inter-flood frequency) affects soil moisture	G	G	U
Flood frequency (inter-flood frequency) affects groundwater	K	K	U

High priority knowledge gaps:

The relationships identified in the table above are important to the maintenance of native tree population viability, since these are the hydrological processes which determine whether the water requirements of trees are met. Research into the impacts of flooding interventions on soil moisture and groundwater is required on a site-scale basis, and in consideration of temporal, climatic, geomorphologic and other conditions. This will lead to enhanced knowledge of the processes required to effectively recharge soil moisture across the range of conditions in which these floodplain tree species occur. Moving away from generic flooding regime ranges (e.g. 1 in 2 year flooding) towards an understanding of the relationships that underpin long-term viability of tree communities is vital, particularly the relationships between trees, soil moisture content and groundwater availability. An increased level of understanding of these relationships will ensure that the most intelligent approach is taken toward planning flooding interventions.

3.3 Sources of data

The National Water Commission has funded a longer-term project which will comprehensively identify the data sources available on trees in the Murray-Darling Basin. This project is the National Water Commission's Ecological Outcomes Project. It may collect these known data sources or at the very least, identify issues with the collation and/or usage of such data. This project is also dealing with the Normalised Difference Vegetation Index (NDVI) time series for the Basin. NDVI measures and monitors plant growth (vigor), vegetation cover, and biomass production from multispectral satellite data. Both of these outputs would serve to confirm and extend the knowledge and project proposals discussed in this document (see Appendix 3, Project proposals 1 and 2 particularly).

The MDFRC is currently undertaking a study at Yanga National Park for the Department of Environment, Water, Heritage and the Arts on the soil characteristic changes across different drying regimes in a semi-arid floodplain ecosystem. The outputs from this project are:

- Investigation of small spatial changes in soil characteristics in response to flooding.
- Large field scale flooding manipulations investigating floodplain soils response to various wetting and drying cycles.
- Measure River red gum health across different flooding regimes and determine genetic variability, to determine if tree mortality with lack of flooding is causing in a decline in genetic diversity of Red gum trees.
- Measure soil meso- and micro-faunal diversity across different flooding regimes, using metagenomic procedures to determine if flooding history affects soil biological diversity.
- Determine the contribution of rainfall to surface soil moisture in semi-arid ecosystems.

This would give quantitative data relating to soil health, but also tree population genetic robustness, according to flooding regime. It will also give an account of rainfall in conjunction to flooding, and the combined influence on soil moisture, which was identified in this document but not explored by this project (See 3.2).

The MDFRC is commencing a 3 year study into the effects of willow removal on freshwater ecosystem dynamics, on behalf of the North East Catchment Management Agency. The outputs from this project (due in 2012) will help with better removal practices and the detrimental or beneficial effects that willows have in freshwater systems. This, along with a current literature review, should clarify some of the knowledge gaps identified in this project.

3.4 Project proposals

Four project proposals are provided in Appendix 3. They offer some direction to how the high priority knowledge gaps identified in this project could be addressed. Further project development must consider the available resources and viability of having these projects conducted. Such considerations need to include environmental water availability and feasibility of delivery, the scientific expertise and resources available to the project teams, site selection availability and feasibility of access, access to other resources to conduct the research (i.e. software, seeds, saplings etc), budgetary constraints and timing constraints.

Project proposal 1: The influence of soil moisture and ground water recharge on adult tree health and growth
This project addresses Hypotheses 2 and 5, and concurrently the knowledge gaps identified in Table 7:
- Flooding influences soil moisture that consequently affects adult tree health and growth, and
- Flooding influences groundwater that consequently affects adult tree health and growth.

Project proposal 2: Experimental design to determine the influence of longitudinal connectivity and rates of flood fall on seed dispersal
This project addresses Hypotheses 3 and 6, and concurrently the knowledge gaps identified in Table 8:
- Longitudinal connectivity affects dispersal
- Rates of rise and fall affect seed settlement
- Flood extent affects seed dispersal

Project proposal 3: Experimental designs for determining the effects of flood duration, soil moisture and flood seasonality on germination, reproduction and seed fall in native tree species
This project addresses Hypotheses 3 and 6, and concurrently the knowledge gaps identified in Table 8:
- Flood duration affects seed germination

- Soil moisture affects seed germination
- Soil moisture affects reproduction (flowering to seed fall)
- Flood seasonality affects seed fall
- Flood seasonality affects reproduction

Project proposal 4: Floodplain tree seedling survival and environmental watering

This project addresses Hypotheses 3 and 6, and concurrently the knowledge gaps identified in Table 8:

- Flood seasonality affects seedling growth and health
- Flood depth affects seedling health and growth
- Soil moisture affects seedling growth

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Appendix 1: Hypotheses as summarised in conceptual models for river red gum and black box

Key: RRG = river red gum, BB = Black box. The ratings provided were determined at the project workshop, based on a preliminary literature review and expert opinion (refer to section 1.4). Ratings vary from low (0) to high (5 or 6).

Species	Hypothesis	Confidence (0 to 5)	Evidence (Published, Grey literature, Experience, Opinion)	Evidence rating (0 to 5)	Criticality (0 to 6)
RRG	Flood seasonality affects seed fall	0	Unknown	0	6
BB	Flood seasonality affects seed fall	0	Unknown	0	6
RRG	Longitudinal Connectivity affects dispersal during floods	4	Opinion (Ian)	1	6
BB	Longitudinal Connectivity affects dispersal during floods	4	Opinion (Ian)	1	6
RRG	Rates of Fall affect dispersal	1	Opinion (whose??)	1	2
BB	Rates of Fall affect dispersal	1	Opinion (whose??)	1	2
BB	Flood duration affects germination	5	Experience, (theses?)	2	6
RRG	Flood duration affects germination	5	Experience, (theses?)	2.5	6
RRG	Flood Seasonality affects soil moisture persistence	4	Grey literature + ?	3	4
RRG and BB	Soil Moisture affects reproduction (flower to seed drop)	4	Thesis	3	4
BB	Flood Seasonality affects soil moisture persistence	4	Grey literature + ?	3	4
BB	Soil Moisture affects reproduction (flower to seed drop)	4	Thesis	3	4
RRG and BB	Soil Moisture affects Adult Growth and Health	5	Published	4	5
RRG and BB	Groundwater affects Adult Growth and health	5	Published	4	5
RRG and BB	Soil Moisture affects germination	5	Published	4	5
RRG	Flood seasonality affects seedling growth and health	4	Published and grey literature	4	4
RRG	Flood depth affects seedling growth and health	4	Published	4	4
RRG	Soil Moisture affects seedling growth	5	Published and theses	4	5
BB	Soil Moisture affects Adult Growth and Health	5	Published	4	5
BB	Groundwater affects Adult Growth and health	5	Published	4	5
BB	Soil Moisture affects germination	5	Published	4	5
BB	Flood seasonality affects seedling growth and health	4	Published and grey literature	4	4
BB	Flood depth affects seedling growth and health	4	Published	4	4
BB	Soil Moisture affects seedling growth	5	Published and theses	4	5

Species	Hypothesis	Confidence (0 to 5)	Evidence (Published, Grey literature, Experience, Opinion)	Evidence rating (0 to 5)	Criticality (0 to 6)
BB	Flood frequency affects sapwood growth	5	Unpublished data	2	4
RRG	Flood frequency affects sapwood growth	5	Unpublished data	2.5	4
RRG	Flood frequency (Interflood period) affects soil moisture	5	Grey literature and in press	3	4
BB	Flood frequency (Interflood period) affects soil moisture	5	Grey literature and in press	3	4
RRG	Flood duration affects groundwater	5	Published	4	4
BB	Flood duration affects groundwater	5	Published	4	4
RRG	Rates of rise and fall affect seed settlement	5	Unknown	5	4
BB	Rates of rise and fall affect seed settlement	5	Unknown	5	4
RRG	Flood Extent affects seed dispersal	5	Opinion and Experience	1.5	3
BB	Flood Extent affects seed dispersal	5	Opinion and Experience	2	3
RRG	Flood duration affects soil moisture	5	Grey literature + ?	3	3
RRG	Flood frequency (Interflood period) affects groundwater	5	Grey literature and in press	3	3
BB	Flood duration affects soil moisture	5	Grey literature + ?	3	3
BB	Flood frequency (Interflood period) affects groundwater	5	Published and model	4	3
RRG	Flood seasonality affects Germination	5	Experience (Theses?)	2	1
BB	Flood seasonality affects Germination	5	Experience (Theses?)	2.5	1
RRG	Flood Seasonality affects reproduction	5	Experience	2	0
RRG	Flood depth affects soil moisture	5	Experience	2	0
BB	Flood depth affects soil moisture	5	Experience	2	0
BB	Flood Seasonality affects reproduction	5	Unpublished data and experience	2.5	0

Appendix 2: Knowledge gaps

The following tables provide a summary of the relative priority rankings of hypotheses relating to river red gum and black box, as determined at the project workshop.

High – Medium priority hypotheses to be addressed:

Flooding regime characteristic	Will affect:
Flood seasonality	Soil moisture persistence (RRG, BB) Seedling growth & health (RRG, BB)
Longitudinal connectivity	Dispersal during flood (RRG, BB)
Rates of fall	Dispersal of seed (RRG, BB)
Flood duration	Germination (RRG, BB)
Soil moisture	Reproduction (flower to seed drop) (RRG, BB) Adult health & growth (RRG, BB) Germination (BB) Seedling growth & health (RRG, BB)
Groundwater	Adult health & growth (BB)
Flood depth	Seedling growth & health (RRG, BB)

Medium – Low priority hypotheses to be addressed:

Flooding regime characteristic	Will affect:
Flood frequency	Sapwood growth (RRG, BB) Soil moisture persistence (RRG, BB) Groundwater (RRG, BB)
Flood duration	Groundwater (RRG, BB) Soil moisture (RRG)
Rates of rise & fall	Seed settlement (RRG, BB)
Flood extent	Seed dispersal (RRG, BB)
Flood seasonality	Reproduction (RRG, BB) Germination (RRG, BB) Seed fall (RRG, BB)
Velocity	Dispersal (RRG, BB) Seedling growth & health (RRG, BB)

Appendix 3: Project proposals

Project Proposal 1:
The influence of soil moisture and ground water recharge on adult tree health and growth

Project Developed by: Neil Sims and Tanya Doody CSIRO

Recommended project duration: 2-3 years

Project Objectives
The experimental design addresses two of the high priority hypotheses relating to the influence of flooding on tree growth in the Murray Darling Basin:

1. Flooding influences soil moisture that consequently affects adult tree health and growth, and
2. Flooding influences groundwater that consequently affects adult tree health and growth.

The two hypotheses could be investigated concurrently. However, addressing these hypotheses requires work in two main areas:

A. Measuring the influence of flooding on soil moisture and groundwater, and
B. Measuring the influence of soil moisture and groundwater on adult tree health and growth.

Soil moisture and groundwater will be assessed quantitatively.

Background Information
This project recommends the use of satellite remote sensing methods to measure broad-scale tree response to differences in soil moisture and groundwater levels, which is ground-truthed on a site-scale.

Definitions of tree growth and health
Tree growth is measured as changes in the size of trees over time. These measurements often include parameters such as diameter at breast height (DBH) or total tree height. These metrics have been used as good indicators of tree growth for timber production. The limitation is that growth (girth) can change very slowly and may not be suitable for studies conducted over relatively short terms of up to only 1 or 2 years, for example. In addition these parameters cannot be directly correlated with pixel values in remotely sensed images.

Tree health can be more problematic to define. Two terms; ‘health’ and ‘condition’ are often used interchangeably, and the application of these terms is somewhat personal and/or situation dependent. Tree health assessments are usually based on quantitative physiological assessments of tree functioning (Christine Stone, Forestry NSW, *pers.comm*.), such as from assessments of the nutritional content of foliage. While these can be correlated with spectral data, this often requires expensive, time consuming and highly specialised processing of field and image data.

Tree condition is often based on visual assessment of the crowns, such as the colour and /or transparency of crowns in relation to a benchmark tree in good condition (Christine Stone, Forestry NSW, *pers.comm*.). Expert skills are required to accurately and consistently determine tree condition, but no further processing is usually required. Condition metrics correlate strongly with the physiological functioning of trees (e.g. crown transparency as an indicator of drought stress in eucalypts) and other indicators of stress such as increased susceptibility to insect attack. In addition, colour and defoliation are readily observed in satellite images.

One crown metric which may be interpreted to summarise a range of condition parameters is Leaf Area Index (LAI) or its surrogates such as plant area index or crown gap fraction. LAI is strongly correlated with tree growth, with higher LAI being associated with higher growth rates. LAI in eucalypts decreases under drought stress as trees shed their leaves, and increased susceptibility to pest attack via drought stress can also lead to reduced LAI. In addition LAI can (often) be readily correlated to reflectance values in image data in conifer and broad leaf species.

In consideration of the above, the proposed study design is as follows:

Methodology

Identify a suitable study area

Suitable study areas would include adult communities of one or more of the target tree species and a range of soil moisture and groundwater levels. These should occur in areas that will be inundated by controlled and measured flooding. Other site quality variables that may influence the relationship between flooding and soil moisture, including variations in soil depth and structure, should also be considered when identifying the appropriate study sites and plots within those sites.

Establish plots

Plots should be located to encounter a wide range of soil moisture and groundwater conditions, for example using a transect arrangement with plots located at various distances perpendicular to the channel. Plots along the transect should be placed at the very wettest and very driest extremes within the vegetation communities of interest with several plots in between. Plot centres should be marked with a (preferably differential) GPS.

Ideally, changes in soil moisture and groundwater conditions at the sites will occur over a long distance laterally from the channel, which will enable plots along the transects to be spaced about 150m apart (this can improve the accuracy of models against Landsat image data which has a pixel size of 30m by 30m). Alternatively, a series of individual plots (rather than transects) could be used to.

Plots should be located to encounter 3 or 4 levels of moisture stress along each transect. More levels of moisture stress may be targeted to increase the resolution of field data if desired, and these can be statistically grouped to correlate with wetness zones at a later stage. Indeed, higher resolution field data may enable a sensitivity analysis to be conducted to determine the minimum difference in soil moisture and groundwater levels that can be discerned from the images and/or the tree condition data.

Establishing the correct controls at both plot and transect level is essential for ensuring statistical rigour. This may include the replication of transects, in terms of the biogeographic setting and wetness levels (possibly a Reference Site approach), and also including monitored plots which receive no inundation.

The transect stratification is best suited to situations with a clear distinction from wet to dry conditions, such as adjacent to river banks with narrow riparian zones. In cases where groundwater and soil moisture conditions vary in a more complex pattern sites may have to be selected to encompass the maximum possible range of conditions. Appropriate replication and control plots must be established in these cases.

Field data collection

Field data collection should occur as close to the time of image data collection as possible. Soil moisture and groundwater measurements should be made at each plot.

Tree condition assessments including crown colour, transparency and obvious damaging agents should be collected. This information should be collected at the tree scale over an area not less than 20m in radius from each of the plot centres.

A minimum of 3 to 4 trees (ideally 10-20) should be assessed near each plot centre, which may require a much larger plot radius depending on the stand density. Trees included in the assessment should be marked so that repeated measurements can be made from the same trees at a later stage.

Upward-looking vertical hemispherical photography of the forest canopy should be collected. A minimum of 5 photos (maximum of 10) should be collected per plot at the plot centre and cardinal points on the plot boundary. Ideal conditions for hemispherical photography are under uniformly cloudy skies or at dawn and dusk.

Cover descriptions should also be collected. Data should include the dominant canopy, shrub and grass species including visual estimates of canopy cover to the nearest 5%, and ground cover descriptions including the proportion and colour of bare soil, and the proportion of litter.

Image data collection and analysis

Crown gap measurements should be made from the hemispherical photography using software such as the “Gap Light Analyser” (http://www.ecostudies.org/gla/), an open source and freely available software package which provides reliable and consistent results from good quality photography. Information from the photos should be collated at the plot scale. Useful data about canopy cover per plot may include mean canopy cover and some measure of cover variability within plots, such as the standard deviation of cover estimates for each plot.

Satellite reflectance data should be collected as close to the time of field data collection as possible. I would recommend acquiring Landsat 5 Thematic Mapper data for its low cost and moderate spatial and spectral resolution. Very high spatial resolution satellite data, such as from Quickbird which has a pixel size in the order of 2m, can complicate the analysis of plot-scale characteristics, and it includes less spectral information with which to differentiate conditions between pixels.

Satellite image data should be calibrated to reflectance if possible using sensor-specific calibration tools in an image processing package such as ENVI, and an atmospheric correction model. It may be possible to have this processing done by a third party organisation or prior to image delivery.

Pixel values extracted from the image(s) should include individual bands and a number of vegetation indexes, especially the normalised difference vegetation index (NDVI; (NIR-Red)/(NIR+Red) which is sensitive to differences in plant biomass and growth vigour. Pixel values may need to be averaged over a 3x3 pixel window around the plot centres if the accuracy of image registration and/or GPS locations of plot centres is questionable.

Interpretations of canopy condition from NDVI values are strongly influenced by the canopy density in each pixel. A given NDVI value in a pixel containing a single tree may indicate a tree in substantially better condition than the same NDVI value in a pixel with many trees. LiDAR data can be used in conjunction with NDVI data to determine canopy density (or crown transparency) and assist in the interpretation of tree condition. LiDAR generated topographic models would also assist in interpreting inundation conditions.

Modelling

The design above should enable a relationship between flow volumes, groundwater and soil moisture to be established at a range of distances from the channel.

Modelling of tree condition could be performed at 2 scales. A continuous-scale model of the association between crown condition (LAI) and soil wetness could be developed by comparing pixel values with soil wetness data at the individual plots using ordinary least squares regression (OLS) depending on the scale and range of values observed.

Alternatively, the LAI data could be used to train a classification of the image data to show spatially contiguous areas of different tree condition. A quantitative analysis of the relationship between condition classes and soil moisture and groundwater data could then be developed using the appropriate regression technique. Examination of the range of soil moisture and groundwater levels within each condition class would reveal the minimum differences in moisture levels that are discernible from remotely assessed measurements of tree condition.

Project Risks
None stipulated

Caveats
The establishment of proper controls is critical to the success of any sampling strategy, and therefore it is recommended that consultation with appropriately experienced researchers be conducted before this project proposal is implemented, to ensure the best resources and approaches (latest sound software and methods of application) is utilised.

Flood frequency is the primary driver of tree health on floodplains ecosystems. Consequently, any study examining the influence of flooding on tree growth and health via influences and groundwater or soil moisture must include flood frequency as a covariate.

Project proposal 2:
Experimental design to determine the influence of longitudinal connectivity and rates of flood fall on seed dispersal

Project Developed by: Ian Overton and Tanya Doody, CSIRO

Recommended project duration: not specified

Project Objectives

Two experimental designs are presented which evaluate the hypotheses that:

1. Connectivity (longitudinal floodplain) affects seed dispersal and
2. Rate of floodwater fall (recession) prior to a flood influences seed dispersal

Background Information

The first hypothesis can be investigated by collating information on flood extent and mapping the presence of juvenile tree vegetation for relevant target areas. If the presence of juvenile vegetation is related to flood extent boundaries, seed dispersal is linked to longitudinal or lateral connectivity and therefore maintaining irregular patterns of flood inundation is critical to establishment of future floodplain vegetation. Mapping of blockages to flooding (blockbanks, weirs etc) is also required.

The second hypothesis (that rate of fall has an influence on seed dispersal) can also be investigated using historic mapping of regeneration and flood extent under floods of different rates of fall when assessed by hydrograph shapes and local topography. A second method includes a field-based experiment to monitor the rate of fall after a flood event and assess vegetation regeneration in relation to the flood. If no relationship exists between regeneration and rate of flood fall, then seed dispersal of the three dominant floodplain species occurred irrespective of rate of decline. It is proposed that slow falling water levels will lead to regeneration at the outer most water extent as seed/seedlings are unable to survive in deep water and will not survive soils dried out too quickly.

Methodology

Hypothesis 1: Sample site identification using flood extents

Determine the scale of the area to be assessed and then observe hydrograph flow information for the location to determine the degree of flooding in the region in order to identify key flood events in a recent (~15 year) period. Remotely sensed satellite imagery such as Landsat can then be used to map the extent of each flood. Locations to be field assessed for presence/absence of juveniles, should be composed of several areas, some which did receive flood water and others that did not. Ideal locations are those which have received more than one flood with differing flood magnitudes of each event, creating different flood extents within the area. Impediments to flooding flooding extent is also to be mapped (natural or otherwise).

Hypothesis 1: Field mapping of terrestrial floodplain vegetation

To test the hypothesis in relation to the three dominant floodplain tree species (Red Gum, Black Box, River Cooba), field site locations are required which contain mixed species or are dominated by one of the three species, requiring multiple field locations. The field locations should occur in recently flooded and non-flooded areas. Once locations have been selected, the areas of differing age classes/species can be mapped onto a base aerial photograph by visual assessment and known flood boundaries (relating tree size to estimated tree age *i.e.* a five year tree old will be smaller than a ten years old tree), for later creation of a spatially mapped layer.

Identifying relationship between flood extent and vegetation distribution

Overlay the flood extent(s) of each field location as determined from satellite imagery with the mapped vegetation age classes/species. If seed dispersal were related to longitudinal connectivity, it would be expected that vegetation would have regenerated at the outer fringe of the flood extent. The presence of regeneration of vegetation in areas that have not flooded would indicate that seed dispersal is not affected by longitudinal connectivity.

Hypothesis 2: Method A

Using the field sites monitored in experimental design 1, the hydrograph can be interrogated to determine how quickly water levels dropped (flood receded). That information, in conjunction with a digital elevation model (generated from LiDAR data) providing elevation change across the flooded area, can be used to estimate an approximate rate of fall for target areas. Rate of fall can then be related to presence or absence of regenerated vegetation information obtained from the field mapping. Experimental design 1 could be planned to incorporate areas where different rates of water recession occurred, with comparison to non-flooded areas.

Hypothesis 2: Method B, watering experiment

This experiment relies heavily on an environmental water allocation to provide an overbank water supply to a river reach or wetland area at a time appropriate to maximise vegetation regeneration. The area also needs to be either mixed species composition to monitor the effect of rate of fall across the three species, or otherwise targeted to one of the three dominant tree species, and replicated at other areas to determine the relationship between seed dispersal and rate of fall per species.

Project Risks

None identified

Caveats

The influence of flood timing, subsequent flooding regimes, soil types, soil salinity and seed supply need to be considered.

- Seed supply is an important factor to consider as part of the experimental design. Jensen *et al*., 2008 report that Black Box tree condition has a considerable effect on both the volume of seed released, and the timing of seed release. Neither Red Gum nor Black Box maintain a large soil seed store.

- Timing of seed release/species/geographic location and flood timing are likely to be related. Flood timing therefore needs to be considered when undertaking the experiment. If a flood occurred and receded in winter and peak seed fall is in summer lack of regeneration may be due to lack of connectivity rather than connectivity not being related to seed dispersal.
- Inundation duration may also be an important factor as a long inundation post seed fall will reduce seed and seedling viability as a result of drowning.
- A further consideration is that older trees located further from the river channel and at higher elevations may be a similar size to younger trees located closer to the channel with more frequent inundation. Smaller tree size is the result of a potential lack of water availability over the ensuing time.
- Soil type might also have an influence on success of regeneration after seed dispersal. If regeneration is not successful it may be due to soil type rather than no relationship existing between seed dispersal and connectivity or rate of fall.

Project proposal 3:
Experimental designs for determining the effects of flood duration, soil moisture and flood seasonality on germination, reproduction and seed fall in native tree species

Project developed by: Caitlin Johns, MDFRC

Recommended Project Duration: not specified

Project Objectives

The experimental designs below are suggested to address the following hypotheses, which relate to the effects of floodwater retention and flow enhancement on tree seed production and seed germination:

1. Flood duration affects seed germination
2. Soil moisture affects seed germination
3. Soil moisture affects reproduction (flowering to seed fall)

The first two of these hypotheses can be tested using growth cabinet or glasshouse experiments. However, field monitoring before and after environmental watering will be required to address the final hypothesis.

Background Information

The focus of this project is on river red gum, black box and river cooba; native species that occur on the River Murray floodplain in areas likely to be affected by environmental watering interventions. While exotic willow species may also be affected by watering interventions at these sites these are not considered here because there is already substantial information available on willow tree reproduction, seed fall and the effects of flooding on germination. While seedlings of river red gum, black box and river cooba have been observed growing in flood strandlines and it has been demonstrated that both river red gum and black box can germinate in water, little is known about the specific effects of flood duration, flood timing or soil moisture levels on seed viability, germination rates or subsequent seedling survival for these species.

Some information is available on the reproductive cycles of river red gum, black box and river cooba. Flowering appears to occur in spring-summer for all three species, although some flowering can occur at other times of year, particularly for black box and river cooba. It is not clear whether or not flowering at other times can be triggered by high soil moisture availability. It is also known that reproductive cycles from bud initiation to seed fall occur over two to three years in river red gum and black box, and that developing buds and fruit can be shed over this time in response to water stress. However, the quantities of water required for fruit development have not been determined. Similarly, while it is known that river red gum and black box exhibit serotiny and retain their seeds in an aerial seed bank for up to two years before release, it is not known if flooding acts as a cue for seed fall. Less information is available on seed production in river cooba.

Methods

Hypothesis 1: Flood duration affects seed germination

The following growth cabinet experiment could be used to test this hypothesis. These methods would generate data on the effects of inundation duration on seed viability and seed germination as well as the longevity of any individuals that germinate in water.

Mature seed would be collected from river red gum, black box and river cooba. Seed fall is reported to peak in spring-summer for river red gum and black box, and seed maturation is reported in summer (December) for river cooba, therefore collection would need to occur at around these times. Seed should be collected at multiple sites for each species, to control for potential variability in seed viability between tree populations. Viability and germination rates would be tested for a weighed subsample of seed of each species soon after collection. This baseline data would be used to determine appropriate seed sample size for experimental replicates (this step is important for eucalypt seed lots, as seeds can be visually indistinguishable from chaff). Germination and viability tests would be carried out using Petri dishes set up in a growth cabinet. Light/dark periods and day/night temperature regime would be chosen to mimic typical conditions occurring in spring-summer, when conditions for germination are likely to be most favourable. Seeds would be kept on moist filter paper and germinated seeds would be counted and removed daily. Once germination stopped, all remaining seeds would be tested for viability by dissection.

To determine the effects of inundation duration on seed germination and survival, samples of seed would be subjected to a series of inundation treatments of different length (eg. 2 weeks, 4 weeks, 6 weeks, 8 weeks), as well as a control treatment without inundation. This control treatment would consist of seeds placed in a specimen jar, on moist filter paper only. The inundation treatments would be applied by placing seeds into specimen jars containing distilled water in a growth cabinet, with the same light/dark and temperature regime that was used to test initial seed viability. These treatments would be replicated, with at least five replicate jars per treatment × species combination. Treatment jars would be monitored throughout the experiment, with data collected on the presence/absence and duration of seed buoyancy (where applicable), germination rates (%), and the length of time that germinated individuals survive underwater. At the end of each respective inundation period, the samples from the relevant treatment (i.e. control, 2 weeks, 4 weeks, 6 weeks or 8 weeks) would be removed from the cabinet. Seed that failed to germinate underwater would then be placed on moist filter paper, to simulate drawdown conditions, and monitored for approximately 1-2 weeks to see if germination will occur under those conditions. The viability of any remaining seeds that have not germinated would then be tested by dissection.

The methods used above could also be extended, for those species able to germinate in water, to test the effects of other abiotic factors on germination and germinant survival while inundated. Relevant abiotic factors may include turbidity, dissolved oxygen (DO) levels, salinity or acidity. Light is a requirement for river red gum and black box germination, and for plant growth and survival in general. Therefore, reduced light levels due to high turbidity may reduce seed germination and/or seedling survival times during flooding, depending on whether these individuals sink or remain buoyant. A lack of oxygen for aerobic respiration can also limit seed germination underwater. Reductions in oxygen availability due to immersion can be enough to prevent germination for some species. For species that are able to germinate underwater, the reductions in dissolved oxygen (DO) that can occur through ponding and stagnation may have an effect on germination and/or seed viability. Increases in salinity and reductions in pH can also be detrimental for seed germination and survival. Treatments could be added to specifically test the effects of these factors. For example, the effects of a reduction in light availability on seed germination and the seedling survival time could be tested by adding replicates with a layer of shade cloth wrapped around those jars to reduce light penetration. The effects of high versus low oxygen availability could be tested by comparing germination between aerated and sealed flasks. The effects of salinity and pH on germination could also be tested by including additional jars representing a range of salinity and acidity levels, with adequate replication at each level.

Hypothesis 2: Soil moisture affects seed germination

This hypothesis could be addressed using a glasshouse experiment. As for assessment of the previous hypothesis, mature seed would be collected from river red gum, black box and river cooba and germination and viability rates would be tested prior to the experiment, to determine an appropriate seed sample size (by weight) for experimental replicates.

Containers of soil (approx. 4 L capacity) would be used as the experimental units, with soil heat-treated prior to the experiment to kill any pre-existing seeds. Germination would be tested across a range of fixed moisture level treatments, ranging from air-dried soil through to soil maintained at saturation point. These treatments would be replicated, with at least five containers per treatment. A soil moisture probe (or alternative methods, to be determined) would be used to monitor soil moisture content throughout the experiment, with additional water applied as required, to maintain stable moisture levels.

A fixed sample weight of river red gum, black box or river cooba seed would be spread over the surface of each container at the commencement of the experiment. The number of germinated seeds would be determined for each container at 2-3 day intervals. The effects of soil moisture on early seedling survival and growth could also be assessed if the experiment is extended (e.g. for several months). This could be achieved by marking the position of germinated individuals with a toothpick (to avoid recounting) then leaving these individuals *in situ* and monitoring their survival over time. Surviving individuals could be measured at the end of the experiment, to record the effects of soil moisture on height growth and leaf production.

For those species that will germinate in water (river red gum, black box and possibly also river cooba) it would be useful to test if those individuals that do germinate in water are able to survive and take root upon drawdown, or if they become stranded and dessicate as the water recedes. This could also be tested, using similar methods to those described above, but this time placing individuals germinated in water rather than ungerminated seed on the soil surface. Again, survival over time would be compared across a range of soil moisture treatments.

Hypothesis 3: Soil moisture affects reproduction (flowering to seed fall)

An assessment of the relationships between inundation soil moisture and tree reproductive output would involve monitoring of both of these variables at regular intervals over multiple years. The period from bud initiation to seed fall in river red gum and black box is 2-3 years. Soil moisture availability and reproductive output would therefore need to be monitored for more than three years in river red gum and black box to capture any relationship between these variables. Reproductive cycles may be shorter for river cooba.

Reproductive output, and the timing of seed fall can be determined by setting up seed traps beneath tree canopies and checking their contents regularly. Seed trap methods have been trialled previously for eucalypts. These may or may not require adjustment for use on river cooba. The timing of flowering can be determined visually during flowering as well as by the presence of flower parts in seed traps after flowering. Quantities of seed (combined with chaff for eucalypts) from trap samples can be determined by weighing after sieving to remove other canopy debris, and air-drying to minimise variability due water content. The relative quantities of seed and chaff for river red gum and black box can be determined using standard germination and viability-testing techniques, and the relative numbers of viable seeds compared. To determine whether watering triggers flowering and/or seed release, it would be useful to compare seed trap contents from sites that receive environmental water with seed trap contents from unwatered sites.

Some other variables are likely to interact with the effects of soil moisture on reproductive output, particularly groundwater depth and salinity and differences in initial tree condition. Groundwater resources may offset the effects of low soil moisture, while salinity may reduce the ability of trees to take up water from either soil moisture or groundwater. Therefore, both soil water and groundwater depth and salinity should be monitored throughout the study period. Tree condition can have a substantial effect on responses to watering, including reproductive output. To examine the effects of initial tree condition on flowering and seed production it may be useful to monitor trees that span a range of health categories (for example comparing trees considered to be in poor, intermediate and good health). These categories may be based measurements obtained using the standard TLM tree condition monitoring protocols currently being developed for MDBA. Since sapwood thickness has recently been found to be more indicative of tree drought resilience and recovery ability than canopy condition in black box (I. Overton *pers. comm.)*, this variable could also be useful for categorising tree condition. A minimum of three replicate trees per tree condition category, within each watering category (watered and unwatered sites) would be required for comparison.

Timeline

Timing	Hypotheses 1 & 2	Hypothesis 3
Spring (Sep – Nov)	• Seed collection – river red gum (RRG) and black box (BB)	• Monthly collection and analysis of seed trap contents (buds, flowers, fruit and seeds) along with soil moisture content and groundwater depth and salinity data is recommended. • Suggested duration ≥ 3 years (including at least one watering event).
Summer (Dec – Feb)	• Seed collection – river cooba (RC) • Germination & viability tests (RRG, BB, RC) • Set up glasshouse experiment (soil treatment etc)	
Autumn (Mar – May)	• Growth cabinet experiments (inundation and/or inundation × water quality treatments) • Glasshouse experiments (soil moisture × germination and seedling survival)	
Winter (Jun – Aug)	• Conclude experiments • Reporting • NB: If interested in moisture requirements during initial seedling establishment the glasshouse experiment may be extended until the end of the year. (In this case, deeper containers will be required to facilitate root growth.	

Project Risks: None stipulated

Caveats

Viable seed is required to test the first two hypotheses. While information is available on the timing of seed fall and appropriate methods for collecting river red gum and black box seed, less is known about the timing of seed release in river cooba. More fieldwork may be required to determine appropriate seed collection times and methods for this species.

More is known about the germination requirements of river red gum and black box seed than that of river cooba. If seed fails to germinate initially using standard methods, some form of pre-treatment may be required. Further testing would be necessary to determine an appropriate pre-treatment.

Seed traps set up to obtain seed and to monitor tree reproductive output at field sites may be at risk of vandalism if located on public land, particularly if they are located in readily accessed areas, or are visible from access tracks. They could also be damaged by falling limbs.

Soil moisture derived from localised rainfall or surface flooding is not the only factor likely to affect tree reproductive output. The effects of some variables, particularly groundwater depth and salinity, may be difficult to distinguish from those of surface moisture content. Adequate replication across multiple sites, representing a range of groundwater and surface water availabilities, will be necessary to detect any trends. Input from researchers experienced in this area would be required to develop an appropriate sampling design to address these issues.

Project proposal 4:
Floodplain tree seedling survival and environmental watering

Project Developed by: Dr Jane Roberts (private consultant) and Dr Heather McGinness (CSIRO)

Recommended project duration: 4 years

Project Objectives

This experiment asks: Assuming that a floodplain watering event has resulted in germination of floodplain tree seedlings, how does the frequency and timing of environmental watering of floodplain tree seedlings in their first year of growth affect their survival and growth (establishment) into their second and third years?

1. In the first year of seedling growth, is one environmental watering event sufficient, or are two watering events required?
2. If just one watering event is sufficient to ensure survival, does it matter during which 'season'? We suggest two 'seasons' of interest – August-October, during which time flooding would have occurred naturally; and March-May, during which time water is more likely to be available for environmental watering from sources such as irrigation drainage, environmental water purchase, end of season sale.
3. If seedling growth has ceased or seedling condition has declined, what is the ability of seedlings to recover from various states of decline with an exclusion of floodwater for up to 12 months

Methodology

Design

A fixed, replicated mesocosm field experiment at a single floodplain location, allowing inundation of planted seedlings under a range of treatments/conditions and at different times. Standardised initial watering (including standard depth and duration), followed by waterings at selected intervals and in selected seasons.

Location:

Single easily accessible floodplain location which has soil representative of several icon sites.

Treatments

Seedlings planted out in August-October. All mesocosms fully watered immediately post-planting. Follow-up watering times varied over the subsequent two years; three different combinations of August-October and/or March-May, to address frequency (0, 1 or 2) and if 1, then timing (mimic natural, likely watering time).

1) First follow-up watering in August-October one year after planting; 5 replicated mesocosms
2) First follow up watering in March-May following planting; 5 mesocosms
3) Follow-up watering in both March-May and August-October following planting; 5 mesocosms

Control mesocosms experience no follow-up watering; 5 mesocosms.

Total of 20 mesocosms; each containing min. 10 (preferably 20-30) seedlings of each tree species. Mesocosm is rectangular, with low earth retaining walls, capable of retaining shallow water; close enough to river to be pumped into. Mesocosms may need be contained within roo-proof exclosure. Floodplain location will need be flat, in order to standardise for depth.

After 2 years, all treatments undergo full watering to assess recovery (from lignotubers or otherwise). Assessments of seedling survival/mortality and growth conducted August, November, and March each year for two years. See Table 1 for timing detail.

Factors equal across treatments

Soil type (chosen to represent widespread floodplain soil types), water depth, water duration, full sun, rainfall, herbivore exclusion. No overtopping of seedlings by water.

Species

Black Box, Coolibah, and River Cooba (not River Red Gum: this species is already relatively well-researched).

Seedling growth stages

Two standard heights (e.g. 15cm and 30cm) grown and supplied by professional contractors (known provenance, and if possible, known age).

Analyses

Basic ANOVA; possibly also Bayesian approaches.

Opportunities

Such a mesocosm facility could be used also for other seedlings or young plants from species groups, such as shrubs and ground cover.

Timeline

Table 1: Design and timetable for seedling survival and growth experiment

Total project duration: 33 weeks spread over 4 years

- Project planning (site and water) negotiations, seedling and equipment orders: 4 weeks
- On-ground experiment set-up: 4 weeks
- Survival and growth measurements in-field + field prep + data entry and initial processing = 2 weeks per set of field measurements (8) = 16 weeks field work
- Dismantling the experiment: 1 week
- Data processing and analysis: 4 weeks
- Write-up: 4 weeks

Watering event indicated by filled cells. Survival and growth measurements indicated by *****					
START PROJECT					
Year 1 Experiment preparation	July	**A suitable site with a 3-year water supply must be negotiated and finalised before seedling orders are placed**			
		Equipment/machinery for setting up mesocosms must be booked			
		Seedling orders must be placed with nurseries *at least 12 months ahead*			
	Aug – Jun				
	Jul	On-ground setup: Build mesocosms, construct fences			
START EXPERIMENT		Control	Treatment 1	Treatment 2	Treatment 3
Year 2	Aug	**Seedlings planted and watered**			
	Sep	Site check			
	Oct				
	Nov	*****	*****	*****	*****
	Dec				
	Jan				
	Feb				
	Mar	*****	*****	*****	*****
	Apr				
	May				
	Jun				
	Jul				
Year 3	**Aug**	*****	*****	*****	*****
	Sep				
	Oct				
	Nov	*****	*****	*****	*****
	Dec				
	Jan				
	Feb				
	Mar	*****	*****	*****	*****
	Apr				
	May				
	Jun				
	Jul				
Year 4	**Aug**	*****	*****	*****	*****
	Sep				
	Oct				
	Nov	*****	*****	*****	*****
END EXPERIMENT	Dec	Dismantle the experiment: 1 week full time			
	Jan - May	Data processing and analysis: 4 weeks full time Write up: 4 weeks full time			
END PROJECT	Jun				

Project Risks: None specified

Caveats: None specified