Recruitment of long-lived floodplain vegetation: Mesocosm study experimental design

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Recruitment of long-lived floodplain vegetation: Mesocosm study experimental design

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1 Introduction

Seedling recruitment was identified as being a priority for water managers and recent literature reviews have revealed a gap in the knowledge regarding recruitment success (Burns & Gawne 2014; Casanova 2015). It was felt that datasets looking specifically at recruitment responses were limited (pers. comm. Environmental Water Knowledge and Research (EWKR) Vegetation Theme Leadership Group), and that focusing studies on seedling responses was an appropriate way to ensure this priority research topic was addressed.

One way to investigate seedling establishment under controlled (or partially controlled) conditions is through mesocosm studies. Mesocosm studies provide a powerful means of quantifying causal relationships in a controlled environment. This study will focus on the responses of seedlings to a sequence of flooding and drying treatments. Work will be undertaken within experimental/laboratory set-ups, so there will be no specific work undertaken at MDB (Murray–Darling Basin) EWKR research sites.

Four woody floodplain species, River Red Gum (*Eucalyptus camaldulensis* Dehnh.), Black Box (*Eucalyptus largiflorens* F.Muell.) and Coolibah (*Eucalyptus coolabah* Blakely & Jacobs), and one native floodplain shrub species, Tangled Lignum (*Duma florulenta* Meissner), were identified as the key target species (Burns & Gawne 2014). Seedling-specific literature reviews were undertaken to assess and collate existing information about the recruitment of seedlings of the four key species (Durant *et al.* 2016). The information collated from the literature review, as well as expert discussions and input through workshops, teleconferences and emails, forms the basis of this experimental design.

The experiment will focus on addressing the primary question:

'What is the relationship between flow parameters such as duration, frequency and interflood-dry period (sequential, cumulative events) and establishment?'

It will also address the following secondary questions:

1. How important are patterns of root development to overall growth and survival in changing conditions?
2. How do sequential flooding and drying events affect seedling growth?
3. How does the initial condition of seedlings affect their response to a flooding/drying treatment?

Four key woody floodplain species were identified as target species, consisting of three eucalypt tree species, River Red Gum (*Eucalyptus camaldulensis* Dehnh.), Black Box (*Eucalyptus largiflorens* F.Muell.) and Coolibah (*Eucalyptus coolabah* Blakely & Jacobs), and one native floodplain shrub species, Tangled Lignum (*Duma florulenta* Meissner) (Burns & Gawne 2014).

These four species occur throughout the MDB, although populations are not consistent at all of the MDB EWKR research sites (Table 1). Due to logistical constraints, the same provenance will be used for individual species with no reference to the actual MDB EWKR research sites.

Table 1. Species distribution within the MDB based on the Atlas of Living Australia (X = present at the MDB EWKR research site).

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

2 Summary of literature reviewed

A variety of literature sources were reviewed, relating directly or indirectly to the key questions and/or key species, and these have been used to form the basis of the mesocosm study experimental design. For more information about the literature reviewed on recruitment of long-lived floodplain vegetation and experimental designs, refer to Durant et al. (2016). Appendix A provides a summary of germination and seedling establishment attributes and watering requirements compiled from the literature review.

3 Experimental design

Appendix B shows a summarised version of experimental studies considered in the literature review, which forms the basis of designs and considerations in this experimental design. Knowledge gained from this literature review in combination with that regarding the current state of knowledge about the four key species, was applied to form the detailed experimental design methods described below.

3.1 Seeds source and germination

The seeds of the four species will be of known providence; however, they will not have been collected from the four MDB EWKR research sites. It is understood that in order to have an accurate representation of the key species, collection of seeds from the four MDB EWKR research sites would be preferential. However, the collection of seeds from the sites is an enormous task, and is dependent on variables such as the timing and availability of seed fall. It was determined that hydrology would be the focus of the experiment, and that the aims of this experiment would be best answered via the use of a seed source.

The germination of the four key species will be sub-contracted to a commercial nursery, who will source and grow the seedlings to the required height and/or age. Seeds will be germinated in standard horticultural germination media. Once the seedlings have grown approximately three leaves, they will be transplanted into the experimental pots (750 mm lengths of 90 mm diameter PVC pipe). After a maximum of six weeks, these pots will be deployed into the treatment tanks. Regular watering will be applied for a minimum period of two weeks to acclimatise seedlings.

3.2 Soil type

Site-specific soil characteristics play an important role in seedling establishment. Soil properties such as water holding capacity, particle size, porosity, nutrient status and organic matter will all influence plant productivity. This experiment is not investigating the effects of soil on plant production. As a result, a standard floodplain soil will be collected from the Murray River floodplain near Albury. Soil particle size (% sand and % clay), water holding capacity and the total carbon content will be determined prior to the commencement of the experiment.

The soil will not be sterilised prior to the seedling being transplanted. The main reason for soil sterilisation is to remove the seed bank of plants that may germinate during the experiment and compete with the target plant. The process of soil sterilisation can change the chemical and physical makeup of the soil. It also destroys much, if not all, of the soil micro flora and fauna, and thus may have implications for nutrient cycling. For the purpose of this study, it has been decided that pots will have non-target plants removed on a weekly basis rather than soil sterilisation.

3.3 Experimental pots

The design for the experimental pots is based on the common design approaches identified in the literature review (Appendix B), with one plant per pot. A pot is 75 cm in length and 90 mm diameter PVC pipe cut in half and taped back together. One end of the pipe will remain open (for the plant to grow up through) while the other end will be closed with a lid that has drainage holes. The benefit of splitting the PVC pipe in half is it aids in the removal of the plant at the time of sacrificial harvesting, providing an opportunity to distinguish the root length and/or depth in the chamber and preventing overall damage to the specimen. These pots/pipes will then be deployed vertically into ~1 m high outdoor circular fibre glass tanks at Wonga Wetlands, Albury. Temperature and rainfall at the site will be recorded.

3.4 Hydrological treatments

Four hydrological treatments (and potentially five treatments) (Table 2) have been identified to answer the questions:

- How do sequential flooding and drying events affect seedling growth?
- How does the initial condition of seedlings affect their response to a flooding/drying treatment?

Treatment 5 will only be added if there are sufficient plants available/alive after the establishment phase.

Table 2. Sequences of flooding/drying identified for the mesocosm study. Green indicates a drying treatment (where pots will be watered with 5 cm of water in the bottom of tanks), while blue indicates shallow flooding (2–3 cm) above the top of the pot. Red lines indicate sacrificial and observational harvesting, and the thick black lines indicate observational measurements.

	Time (Weeks)																						
Trea-tments	Establi-shment		Early Phase										Late Phase										
Week no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1																							
2																							
3																							
4																							
5																							

The experiment will be divided into three stages: establishment, and early and late development. Once seeds have germinated and reached a set age (≤ 6 weeks), seedlings will be deployed into the treatment tanks, and water levels will be maintained in the tanks 15 cm from the top of each pot for a minimum period of 2 weeks to acclimatise seedlings (establishment phase) after transportation from the commercial nursery. Treatments 1 and 2 will be flooded in the early phase for four weeks. Treatment 1 will be sequentially flooded in the late phase, while treatment 2 remains dry and treatment 5 remains constantly flooded (Table 2). Treatments 3 and 4 will remain dry in the early phase for 10 weeks. In the late phase, treatment 3 will be flooded, while treatment 4 continues to remain dry and treatment 5 continues to remain flooded (Table 2).

Floods will be imposed to a depth of 2–3 cm above the top of the pot. The length of a flood (4 weeks) has been based on the literature and known tolerance of each species (Table 3). In the absence of known tolerance levels, timing has been selected for ease of sampling based on information for species with known tolerance levels. Plants will be monitored on a weekly basis and the flooding duration may be shortened in response to high plant stress. If the flood duration is reduced, the duration of the early phase flood will become the duration of flood imposed in the late phase flooding events. The duration of flooding will be consistent across all species to allow for comparison.

Table 3. Optimal times each species can withstand flooding and/or waterlogging based on seedlings at ~2 months of age (Durant *et al.* 2016). ? equates to information unknown.

Species	Min. duration	Tolerance levels	Max. duration	Flood depth
Black Box	< 30 days	4 weeks	30–60 days	4 cm
Coolibah	?	?	?	?
River Red Gum	?	4 weeks	6 weeks	20–30 cm
Tangled Lignum	?	4–6 weeks	?	5–15 cm

To stimulate the drying period, a step down lowering rate will be applied. This step down method will be applied by lowering water in the holding tanks by approximately 15 cm a week over a 4 week period until 5 cm of water remains at the base of the pot, which will allow water movement through the soil via capillary action. If the plants become water stressed at a particular drawdown height, water will be maintained at that height and drawdown will cease, but no manual surface watering will be applied. Plants will be exposed to natural rainfall during the drying phase, as the experiment is being conducted in an outdoor facility.

3.5 Sampling and variables

Sampling time or length of experiment period in the literature ranged from 1–6 months growth time, with sampling events occurring daily, weekly, fortnightly, monthly, 3-monthly or at the end of the experiment. The length of the experiment influenced the number of sampling events required. Many different combinations of sampling events can be applied, but the inclusion of a greater number of sacrificial sampling events requires a greater monitoring effort and more seedlings.

A combination of sacrificial harvesting (three occasions) and observational monitoring (five occasions) will be undertaken through the experiment (Table 4). Sacrificial harvesting will occur at the end of the establishment phase and before the early phase, at the end of the early phase and before the late phase, and at the end of the late phase. Observational monitoring will be undertaken at the same time as sacrificial sampling and at every treatment change, as indicated by the bold black lines in Table 2. At each observation/harvest, 12 replicates (1 replicate = 1 pot = 1 plant) per species per treatment combination will be measured. As there are a different number of treatments during each phase of the experiment, there will be a different number of plants sampled at each sampling occasion, with a higher number of plants sampled during the late phase (Table 4). Treatment 5 will consist of a maximum of 16 plants per species. Based on both the literature and tolerance levels to waterlogging, it is not expected that these plants will survive past the early phase. If plants are still alive at the end of the early phase, we will have to consider the sampling replication at the end of the early phase. Variables to be measured are shown in Table 5.

Table 4. Number of replicates required to be harvested or observed per species during each phase of the experiment. Numbers in brackets will be applied in the scenario that there are sufficient plants to include the fifth (flooded) treatment.

Phase	Number of treatments and treatment type	Replicates per treatment per species	Plants harvested or observed per species within each phase
Establishment	1 – Drying	12	12
Early	2 – Drying – Flooded – Flooded then drying	12	24 (36)
Late	3 – Drying followed by drying – Flooded followed by flooded (only 4 reps) – Flooded then drying followed by flooded then drying – Flooded then drying followed by drying – Drying followed by flooded then drying	12	48 (52)
		Total plants per species required	84 (100)

Table 5. Method of sampling and associated variables. √ represents the sampling method applicable to the variable being tested.

Variables	Observational monitoring	Sacrificial harvesting
Seedling height Measured to the shoot tip (±1 mm)	√	
Soil moisture Surface soil moisture content as % volume determined via use of a soil moisture probe (Lynch 2006)	√	
Leaf numbers Leaf number calculated on leaves with a minimum length of 1 cm (Mahoney & Rood 1991)	√	
Mortality Number of seedlings that die recorded by date/time	√	
Root depth/length On day of harvest soil root column is exposed and length of root system measured to its lowest point in the soil (Neave & Florence 1994)		√
Biomass of shoot, root and leaf components Separation of roots from stem/leaves at the root-shoot junction and weighed separately after being dried in oven at 70 °C temperature for 48 hours (Horton & Clark 2001)		√
Leaf area Place leaves on scanner and determine area via image analysis (e.g. Bioscan Image analyser; Capon at al. 2009)		√

3.6 Block design and allocation of plants/treatments to tanks

Due to logistical constraints (large pot size and associated plant weight), it is not possible to randomise the allocation of treatments among different experimental tanks. As a result, each tank will be assigned to a treatment for the duration of the experiment. The current design is for the four treatments. This may change slightly (by the addition of an extra three tanks) if there are sufficient plants alive at the end of the establishment phase to include a fifth treatment. Twelve tanks will be used for the duration of the experiment, with three tanks randomly allocated to each treatment (Figure 1).

At the commencement of the experiment, each tank will have 28 plants/pots (seven plants of each species) randomly allocated to each of the 12 tanks. All surplus plants will be randomly placed into holding tanks and treated the same way as the experimental tanks. If it is found that plants have died or are visibly unwell at the end of the establishment phase, they will be replaced with plants from the holding tanks. Plant mortality (if observed) is likely to occur as a result of transportation/shock from being moved from a nursery to the open air research facility. At this stage it will be determined if there are sufficient plants remaining to run the fifth treatment, the holding tanks will become the fifth treatment. Four plants will be harvested at the end of the establishment phase from each of the treatment tanks (excluding the holding tanks) (one plant of each species). Monitoring during and sampling at the end of the early phase, where there are two (or three) treatments, eight plants will be harvested from each tank (two plants of each species). Monitoring during and sampling at the end of the late phase, where there are four (or five) treatments, 16 plants (or all remaining for the flooded treatment) will be harvested from each tank (four plants of each species). The tank numbers will be recorded to allow statistical testing to determine if there is a tank effect.

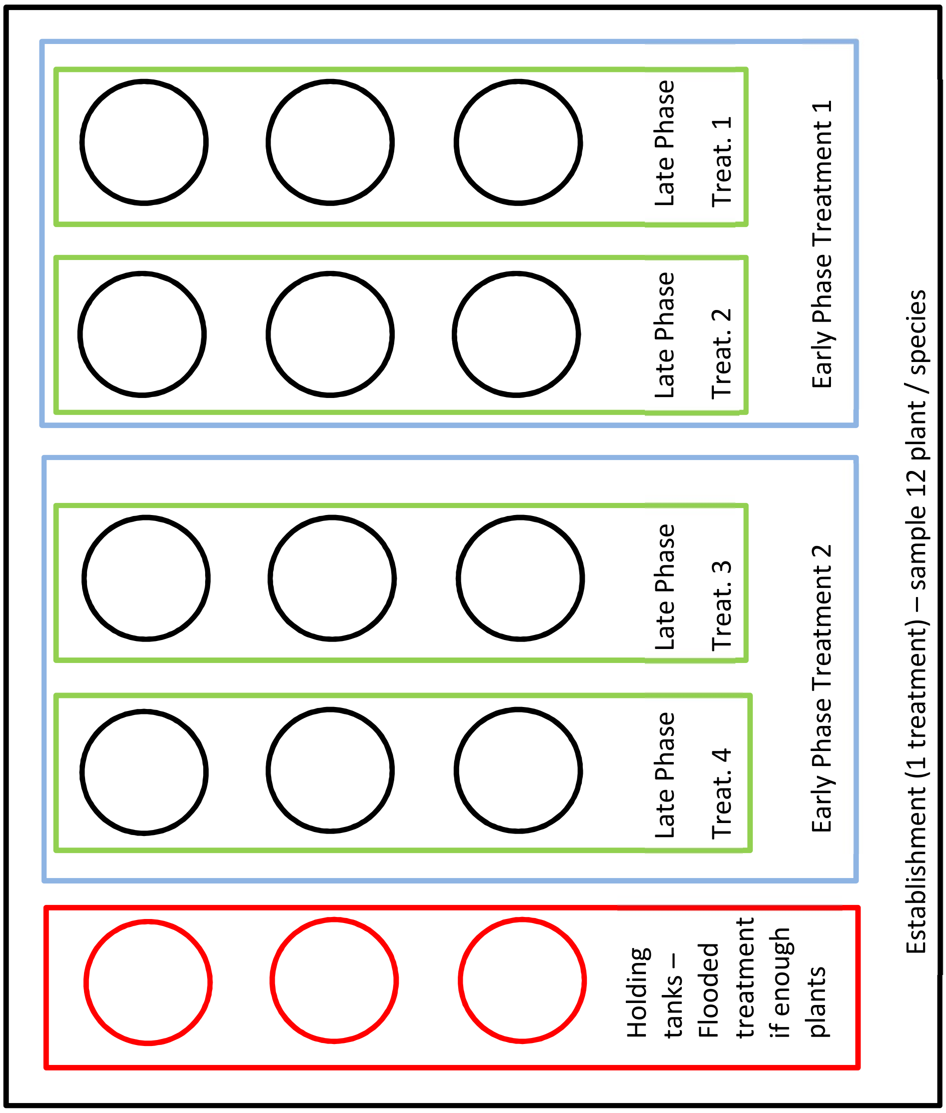

Figure 1 . Schematic diagram of experimental tanks and allocation of tanks to treatments.

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4 Appendix A: Summary of germination and seedling establishment attributes and watering requirements

Summary of germination and seedling establishment attributes and watering requirements. Blue is for refereed scientific literature; red is for reviews and books; grey is for published reports, proceedings and theses (grey literature).

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

	Water stress	• Requires flooding and/or local rainfall (Casanova 2015)	• Not tolerate waterlogging or complete immersion (Johns *et al.* 2009) • Soil moisture of 10–25% is critical (Jensen 2008) • Intolerant of drought (Casanova 2015) • Slow drawdown rates detrimental to establishment as seedling do not tolerate extended periods of waterlogging (Johns *et al.* 2009) • Flowing water may lead to dislodgement or burial (Johns *et al.* 2009) • Artificial flood not so useful (Casanova 2015)
	Timing/season of inundation	• Requirements 15–35 °C for germination (Casanova 2015; Rogers & Ralph 2011) • Inundation receding in spring–early summer provides moist conditions (Johns *et al.* 2009; Rogers & Ralph 2011; Holloway *et al.* 2013) • Local rainfall in spring–summer (Casanova 2015)	• Floods in winter–late spring optimal (Johns *et al.* 2009) • Flood recession in spring to summer to provide moist conditions (Holloway *et al.* 2013; Casanova 2015) or local rainfall (Casanova 2015) • Grow in summer after shedding old leaves and bark (Casanova 2015) • Newly germinated seedlings susceptible to frost and heat injury (Johns *et al.* 2009) • Follow-up inundation in same season as germination or following season (Holloway *et al.* 2013) • Timing should be sufficient to ensure maintenance of soil moisture in the first summer after germination (Johns *et al.* 2009)
	Grazing pressure	• Vulnerable (Casanova 2015)	• Vulnerable, seedlings are grazed (Casanova 2015) • Grazing, particularly by sheep (and more so than cattle and rabbit burrowing), restricts establishment and impacts soil structure (Smith & Smith 1990)
	Soil salinity		• Salinity tolerant (related to ground and surface water) (Casanova 2015) • Sodic soils overlying highly saline groundwater causes high mortality in first 2 years (Morris 1984)

Coolibah	Depth of inundation	• Most likely to be influential on wet soils following floods or rainfall (Roberts & Marston 2011) • Not critical to seed germination (Holloway *et al.* 2013) • Requires moist soil (Holloway *et al.* 2013)	
	Duration of inundation		• Longer flood = fewer seedlings (Casanova 2015)
	Sequence of and/or consecutive inundation	• Follow-up floods in summer of first year thought to increase recruitment rates (Roberts & Marston 2011)	• Regular rainfall required for establishment (but saturated soil following inundation might be adequate) (Casanova 2015) • Follow-up rainfall or shallow inundation in summer of first year (or second year) thought to increase seedling recruitment rates (Roberts & Marston 2011) • Sequence of floods or flood and wet years may be necessary (Li & Wang 2003; Tuomela *et al.* 2001)
	Water stress	• Seeds take two weeks to germinate (Casanova 2015)	
	Timing/season of inundation	• Fluctuating temperature 15–30 °C for germination, vulnerable to frost, adapted to regeneration after late summer flooding (Capon *et al.* 2009). • Flood recession in spring to provide warm and moist conditions (Holloway *et al.* 2013) • Timing not critical (Holloway *et al.* 2013)	• Shade or protection from summer heat required (Casanova 2015) • Flood recession in spring to provide warm and moist conditions (Holloway *et al.* 2013) • Flood summer–late summer (but other factors important e.g. rainfall) (Casanova 2015)
	Grazing pressure	• Successful recruitment may require protection from grazing (Roberts 1993)	• Seedlings die from herbivory (Casanova 2015) • Grazing, seasonal conditions and competition from grass (not so important) effects (Casanova 2015)
	Soil salinity	• Reproductive effort may be lowered by soil salinity (Roberts 1993; Roberts & Marston 2011)	• Are salt-tolerant in the Diamantina River region i.e. utilise saline groundwater (Costelloe *et al.* 2008; Roberts & Marston 2011)

River Red Gum	Depth of inundation	• Moist soils required (Johns *et al.* 2009) • Germination success primarily controlled by seed availability and moisture availability after seed dispersal — most seeds germinate within 10 days of watering (Johns *et al.* 2009) • No direct impact on depth as seeds can germinate while floating (Johns *et al.* 2009) • Flooding after germination may lead to mortality (burial, dislodgement or immersion periods) (Johns *et al.* 2009)	• In southern MDB: do not tolerate waterlogging and complete or prolonged immersion (Roberts & Marston 2011) • Depth will affect subsequent seedlings survival and establishment (Johns *et al.* 2009) • On moist soil following flood recession • Tolerance to waterlogging increases with seedling height • Shallow flooding (20–30 cm) preferable to avoid over-topping seedlings in first year (Holloway *et al.* 2013)
	Duration of inundation	• No direct impact (Johns et al 2009). • Seeds die after 10 days of immersion (Casanova 2015)	• In southern MDB: 2-month-old plants can withstand waterlogging for 1 month, 50–60 cm plants can survive for 4–6 months flood, but only several weeks if completely submerged (Roberts & Marston 2011) • Maximum duration 1–6 months depending on seedling size (Holloway *et al.* 2013) • Susceptible to prolonged flooding (Roberts & Marston 2011) • Four-to-six weeks is adequate, but longer can be tolerated depending on age and if totally submerged (Holloway *et al.* 2013)
	Sequence of and/or consecutive inundation		• In southern MDB: follow-up watering 1 year after germination (George 2004) • Requires watering 1–2 months after spring rain or small flood (Casanova 2015) • Sufficient to maintain soil surface moisture during first year and needs adequate moisture in the second season (Johns *et al.* 2009) • Follow-up flood to recharge soil moisture is desirable in same year as germination or following year (Holloway *et al.* 2015)
	Water stress	• Germinate within 5 days given adequate moisture (Holloway *et al.* 2013) • Soil moisture required >10% (Holloway *et al.* 2013)	• In southern MDB: soil moisture levels 10–25% in top 10 cm ideal (Jensen 2008; Holloway *et al.* 2013) • In southern MDB: low density response to above-average (>300 mm) annual rainfall, with higher establishment

		• Seeds require imbibing (saturation) and light to break dormancy (Casanova 2015)	occurring in response to medium-to-large flood events — recharges soil moisture (George 2004; Jensen *et al.* 2008) • Inhibited by drought conditions, develops adventitious roots in response to flooding (Casanova 2015) • Rapid drawdown rate preferable as intolerant of prolonged periods of immersion (Roberts & Marston 2011) • Competition for moisture by other understorey and/or overstorey vegetation • Maintenance of soil moisture within first year is critical (Holloway *et al.* 2013) • Seedlings wilt and die rapidly once soil moisture falls below 10% (Jensen 2008)
	Timing/season of inundation	• Flood receding in spring–early summer preferred (Johns *et al.* 2009) • Rates limited by low temperatures and light availability (Holloway *et al*. 2013) • Require adequate moisture and day time temperature >30 °C for germination (Holloway *et al* 2013) • Optimal temperature 35 °C (11–34 °C) (Casanova 2015) • Adequate water applied before germination (Roberts & Marston 2011)	• In southern MDB: winter flood receding in spring/early summer maintains soil moisture and avoids extreme temperatures for seedling survival (Roberts & Marston 2011) • Sensitive to frost • Flooding after germination may lead to seedling mortality due to burial, dislodgement or excessive immersion periods (Roberts & Marston 2011) • Recession spring/early summer (or sufficient rainfall), artificial watering to extend effect (Casanova 2015)
	Grazing pressure (and competition)	• Seeds removed by ants (Casanova 2015)	• Seed predation varies through the year, lowest under sheep grazing, highest in ungrazed, high under cattle grazing (Casanova 2015) • Compete with reeds and weeds (Casanova 2015) • Increased during flood (cattle, kangaroos, rabbits) (Casanova 2015) • Grazed more during drought (Casanova 2015) • Grazing (sheep, cattle and kangaroos) severely restrict regeneration (cattle are less destructive) (Smith & Smith 1990)
	Soil salinity		

Tangled Lignum	Depth of inundation	• In water (while floating) or wet mud, occurs after flooding (Campbell 1973; Chong & Walker 2005; Holloway *et al.* 2013)	• Damp conditions promote growth, while flooding, waterlogged and dry conditions impede growth (Capon *et al.* 2009) • Damp conditions associated with drying phase facilitate growth (Capon *et al.* 2009) • Depth of flood seedling establishment < 15 cm (Casanova 2015)
	Duration of inundation		• In northern MDB: 2–4 months rapid growth in damp and drying, while flooding, waterlogging and dry conditions impede growth (Capon *et al.* 2009) • Flood duration 3 months (Casanova 2015)
	Sequence of and/or consecutive inundation	• May require consecutive floods, one to promote flowering and seed set and one to promote germination (Rogers & Ralph 2011)	• Needs flood once in 12–18 months of 5–15 cm for 4–6 weeks in late spring/summer (Casanova 2015) • Spreads predominantly via vegetative growth, particularly in more frequently flooded areas (Casanova 2015) • Follow up flood 9–12 months after germination (Casanova 2015)
	Water stress	• Germination occurs within 14 days of dispersal (6–12 days) (Casanova 2015)	• Soil moisture known for northern MDB; damp conditions, can survive flooding (Capon *et al.* 2009). • More tolerant of drying than flooding (Capon *et al.* 2009) • Opportunistic and rapid growth under optimal experimental conditions (Holloway *et al.* 2013)
	Timing/season of inundation	• Flood timing spring/summer preference (Rogers & Ralph 2011) • Rates are temperature dependent (Holloway *et al.* 2013) • Season appears to be critical for germination (late summer to autumn) (Casanova 2015) • Appears to recruit continuously (Casanova 2015)	
	Grazing pressure	• Vulnerable to ant predation (Casanova 2015)	• Can survive grazing but at stunted growth (Jensen 2008)

			• Vulnerable to grazing (Capon *et al.* 2009) • Grazing and competition pressure unknown (Casanova 2015)
	Soil salinity		

5 Appendix B: Summary of methods from relevant reviewed literature to aid in mesocosm designs

Summary of methods from the literature used to aid in the mesocosm design.

	Reference	Factors	Treatments	Sampling period	Method/design
Inundation/flow regime (including depth, duration, timing and sequence)	Li and Wang (2003)	Basal diameter, shoot height, stem cross sectional area. Leaf area. Biomass- leaves, stem and roots. Root length. Stomatal density and guard cell length. Leaf water potential. Carbon isotope on leaf. Evaporation of water loss from soil surface.	Three water treatments: 100 (well watered), 50 (water stress) and 25% (water stress) field capacity. One species: *E. microtheca*.	5 months.	POTS: pots enclosed in plastic bags. 5L pot. SOIL: sand with slow release fertiliser.
	Tuomela *et al.* (2001)	Total plant biomass. Allocation of dry matter to roots and shoots. Specific leaf area (SLA). Water use and long term water use efficiency. Carbon isotope. Temperature and relative humidity. Height, xylem diameter above root collar, total leaf area, total leaf shot and root dry weight.	Three watering treatments: field capacity, 50 and 20% field capacity.	5 months; Temperature and RH daily. Control field capacity watered every 2 days at dusk. Two-day cyclical watering regime for other two treatments. Invasive harvest at 3, 4 and 5 months — 4 reps.	POTS: 2 L pot. SOIL: commercial peat-sand mix with fertiliser. Fertilised twice daily. SET UP: naturally lit glasshouse minimum temperature 17 °C.
	Awe *et al.* (1976)	Seedling height, basal stem diameter, number of leaves on stem and on branches, number of branches and number of internodes. Length of roots, root and shoot dry weight.	Three watering treatments: prolonged drought, progressive drying out, continued moisture.	80 days: Observations made every 10 days.	POTS: PVC pipes 12 cm diameter, 1 m in length, open ended, pipes halved lengthwise then sealed back with insulating tape and copper wire. One open end enclosed with nylon mesh.

			Three plant species: *E. camaldulensis*, *E. saligna* and *E.. pilularis.*		SOIL: 3:1 mixture to represent natural riverbank of floodplain soil. SET UP: pots placed in small plastic buckets on coarse gravel, under controlled temperatures between 15 and 30 °C.
	George (2004)	Germination methods, seedling height, time growth stages, flowering, seed fall, abundance and diversity, tree aging.	Field survey — 127 plots along 21 transects. Two species — River Red Gum and Black Box.	Germination — 3 weeks.	Transects perpendicular to river. Diameter at Breast Height (DBH) for trees >1.3 m. Elevated seed traps to collect seeds. Germination via emergent method: CT room, 12 hour light/temperature. Temperatures 15 and 35 °C.
	Argus *et al.* (2015)	Root dry mass, above ground dry mass, root porosity, root anatomical measurements.	One species: *E. camaldulensis* subsp. *refulgens* Three treatments: flooding 2 cm, free draining, flooding 2 cm 88 days then free draining.	88 days and additional 35 days.	POTS: 4.25 L, 200 mm diameter. SOIL: coarse river sand. SET UP: glasshouse, temperature range of 15.9–27 °C.
	Maxwell *et al.* (2015)	Stem length, number leaves, biomass.	Two species: *Acacia stenophylla* and *Casurarina cunninhamiana.* 4 Treatments: control, flood then dried, fully submerged 15 cm & partially submerged 5 cm. 7-10 reps.	15, 30, 40, 50, 55, 70, 80 and 90 days.	POTS: 200 mm length, 50 mm diameter pipe with 90% shade cloth on bottom. SOIL: low grade coarse potting mix and potting sand ratio 10:1.

Water stress (i.e. soil moisture)	Jensen (2008)	Soil moisture, number of roots and shoots, length of shoots.	Three species: River Red Gum, Black Box and Lignum. Four watering treatments: rainfall, flood, rainfall followed later by flooding, flooding followed later by rain. Controls: constant dry, constant soil moisture. Five types of sediment. Five reps of each.	12 weeks	POTS: ice cream containers. SOIL: collected from floodplain sites. SET UP: glasshouse up to temperature 30 °C.
	Capon *et al.* (2009)	Seedling heights, root depths and leaf numbers. Total leaf area, biomass of shoot, root and leaf components.	Five watering treatments: deep flooding, shallow flooding, waterlogging, damp, drying. Two sediment types: clay and clay/river sand mix. One species: Lignum.	180 days (6 months). 4 harvest times: 30, 60, 120, 180 days.	POTS: PVC pipe 750 mm diameter, 30 cm in length. Open ended gauze at one end. SOIL: steam-sterilised clay collected from field site and mix of sterilised clay and river sand. SET UP: pipe sat in larger buckets. Temperatures averaged 17–37 °C.
	Capon (2012)	Number and type of seedlings, height, recruit type, distance to nearest adult plant, number of stems, % greenness, extant vegetation canopy, bare ground, leaf litter cover, soil composition	Four species: River Red Gum, Coolibah, River Cooba and Tangled Lignum. Transects: 50 m long perpendicular to river channel. Three monitoring trips.	6 months.	Transects perpendicular to river channel based on presence of seedling patches. Literature review — regeneration of plant species. Conceptual models generated.
	Neave and Florence (1994)	Biomass shoot and root. Length of root to lowest point in soil. Moisture content. Length of root through soil structure.	Soil structure divided into 3 lengths: top 20 cm, 20–40 cm and below 40 cm. Eight species: all eucalypts.	65 days.	POTS: PVC pipe 12 cm diameter, 1 m in length open ended. Pipe halved lengthwise and sealed back together, gauze at base.

			Two watering regimes: mechanically watering twice daily and bringing tubes to field capacity and then allowing them to dry. Four reps.		SOIL: sandy loam commercially purchased, fertiliser added.
	Lynch (2006)	Soil moisture, number of leaves, flowers.	Five readings of soil moisture: 0, 5, 10, 15 and 20 cm. Four watering treatments: 50, 500, 1000 ml and no water. One species: Lignum.	10 weeks. Non-invasive — weekly soil moisture, stem growth, number of leaves and flowers.	POTS: PVC pipe 90 mm diameter, 35 cm length. Bottoms covered with duct tape puncture for drainage. SOIL: 1:3:4 Couse sand: clay: potting mix. SET UP: glasshouse natural light.
	Jensen *et al.* (2008)	Soil moisture, wet and dry weights, surface soil salinity, pH and organic carbon.	Two species: River Red Gum and Black Box.		SET UP: in field within habitats.
	Florentine and Fox (2002)	Seedling height, number leaves, leaf dimensions, fresh and dry weight of root and shoot, leaf gas exchange	Three species: *E. victrix, E. terminalis, E. leucophloia.* Two water treatments: 1 cm deep, 15–20 mm deep (waterlogging).	32 weeks and 65 days (~9 weeks).	POTS: cylindrical 13 cm diameter, bottoms opening sealed with plastic draining tapes. SOIL: clay soil from site and red clay loan pH 7. SET UP: fibreglass tanks of 2.5 x 0.5 x 0.5 filled with rainwater to depth of 1 cm above pot soil level, outside in full sun.
	Schütz *et al.* (2002)	Root and hypocotyl lengths, fresh weight, soil moisture.	Five reps. Four species: all eucalypts. Two soil types: deep sand and lateritic loam.	14 days.	POTS: 80 mm diameter, 150 mm height with drainage holes, 700 ml soil material. SOIL: deep sand and lateritic loam.

			Two water treatments: full watering 250 ml, low watering 125 ml (below field capacity).		
Salinity (including soil, water and groundwater)	Akilan *et al.* (1997)	Shoot height. Leaf, steam and root dry weights, concentrations of Na and Cl in leaves and roots, water use, sap flow, stomatal conductance and net gas exchange.	Three treatments: freely drained control, waterlogging with fresh water, waterlogging with salt water. Two reps each. One species: River Red Gum, 2 clones.	16 weeks.	POTS: 255 mm diameter. Top of pot covered with black plastic. SOIL: composite mix peat/loam/sand with nutrients. SET UP: glasshouse.
	Craig *et al.* (1991)	Stem length.	Four water treatments: 10, 225, 450 and 900 ml. Four salinity treatments: 0, 250, 10 000 and 40 000 mgL^{-1}. Four reps. One species: Lignum.	10 weeks; weekly watering	POTS: 6L plastic pot SOIL: 3.6kg mixed native surface soil SET UP: glasshouse July - September Average temperature 13–27 °C.
	Horton and Clark (2001)	Mortality, plant height. Length of longest root, leaf area. Biomass of roots and shoots. Volumetric water content of substrate.	Four ground water decline rates: 0, 1, 2 and 4 cm/day. One sediment type: sand/gravel mix. Two species: *Salix* and *Tamarix*.	67 days. Non-invasive — twice weekly. One harvest.	POTS: PVC pipes see ref 13. SOIL: 3:1 volume of sand to river gravel mix (simulate natural river substrate). SET UP: 4 rhizopods (apparatus of PVC pipes and central reservoir). Consistent environmental conditions, 12 h light/dark cycle at night — 17 °C/30% RH, day — 24 °C/20% RH.

	Mahoney and Rood (1991)	Plaint height, leaf number. Length of major roots. Biomass of roots. Leaf area.	Five rates of water table decline: 0, 1, 2, 4 and 8 cm/day. Two species: Papules. One sediment type: sand/gravel mix.	46 days. Non-invasive — daily recording plant height and leaf number for 3 weeks, twice weekly thereafter. Each rhizopod water level adjusted 3 times daily.	POTS: 15 growth tubes (PVC pipe 8 cm diameter x 1.2 m long caped and sealed at bottom) around a central reservoir (PVC pipe 20 cm diameter x 1.2 m long sealed at bottom with fiberglass and fitted with drain valves and tygon tubes). Each growth tube connected to reservoir with tygon tubing. SOIL: base of each growth tube 10 cm coarse gravel. 1:2 mix of sand and gravel (simulate study area). SET UP: rhizopods — each rhizopod positioned in wooden frame.
	Hughes *et al.* (1997)	Shoot height, leaf number, leaf length and health. Leaf area, biomass shoot and root. Weight of nitrogen fixing nodules. Root length and depth.	Two sediment types: sandy silts, coarse sand/fine sand/gravel mix. Five water table drawdown rates: 3 cm/day, 3 cm/day + weekly rain application, 1 cm/day, 0.5 cm/day and 0 cm/day. One species: *Alnus incana*.	155 days (~5 months). Sampling at start, fortnightly for 14 weeks then 2 monthly intervals. Weekly watering.	POTS: rhizopods with terniometers to read soil suction. Sixteen growth tubes around central water well of 1.2 m height. SOIL: from river sites sandy silts and coarse/fine sand/gravel mix. Standard and uniform N-P-K soil nutrient prior to start applied. SET UP: pump system automatically applied to control drawdown rate. Five rhizopods in total. Greenhouse temp 15–30 °C.
	Stella and Battles (2010)	Root length, mortality, plant height, leaf length. Biomass of root, stem and leaf. Leaf tissue analyses — SLA, Carbon-	Three water table decline rates: 0, 1 and 3 cm/day.	62 days. Non-invasive daily monitoring of	POTS: PVC pipe 125 cm long x 3.2 cm diameter packed with sand and suspended in tanks.

		Nitrogen and stable carbon isotope ratio.	Three species: cottonwood and willows.	mortality, weekly height and length of longest leaf. Invasive sampling day 0, 18 and 49 when 4–8 live seedlings harvested.	SET UP: outside in late summer. Steel cylindrical tanks (125 cm deep x 61 cm diameter) with bottom drain and flexible discharge tube. Thirty-two to 41 seedlings. Tank water levels remained at 10 cm below soil surface for control and starting point for all other treatments.
Grazing (including shading and competition)	Salazar *et al.* (2012)	Light quantity and quality, soil nutrient availability, pH soil gravimetric water content and soil water potential. Quantify litter cover among vegetation types, effect of litter cover.	Three transects — 9 plots, 3 major vegetation types in field. Four monitoring times.	14 months.	In field: transects 1000 m length, 9 plots 20x20 m along each transect. Within each 20x20 m plot, 8 subplots of 1x1 m. All seedlings up to 30 cm tall tagged and identified.
	Chong *et al.* (2007)	Seed size, leaf developmental stage (number leaves, fresh shoot lengths, root length, number of lateral roots) biomass.	Three groups – clipping, biomass and control. Four species — *Melaleuca leucadendea, Asteromyrtus symphyocarpa, E. camaldulesis* var. *obtusa, tristaniopsis laurnia.* Six treatments: clipping at 5, 10, 15, 25, 40 and 60 days.	3 months.	POTS: seedling tubes (70x70x160 mm) SOIL: 6:3:2 steam sterilised sand/peat/perlite mix containing macro nutrients. SET UP: 5 PVC watering trays (1200x1200x150 mm) with 250 tubes per species (1000 seedling tubes).

	Good *et al.* (2014)	Biomass	Two treatments — without competition, with competition and clipping. Two species — *Paspalidium jubiflorum* and Coolibah. One water treatment — every 2 days with 500ml. Two clipping treatments to grass: clipped 5 cm above soil surface, clipped to maintain maximum growth of 30 cm.	2 months.	POTS; round — 20 cm diameter, 20 cm deep. SOIL: 2 cm sand at bottom, 2 cm from top of field soil.
	Smith *et al.* (2013)	Soil chemistry, groundcover.	Three treatments: grazing large herbivores, competition grazing exclusion. Habitat: woody encroachment with *E. populnea* subsp. *bimbil* and *E. intertexta* and Geijera, Dodonaea and Eremophila understorey.	Three, 16 and 30 months.	SET UP: in field, cut trenches recovered with soil surface.
Soil properties	Bennett *et al.* (1986)	Root and shoot biomass, root length and diameter. Plant height, number of leaves, and number of branches.	Two soil types: peat/sand 3:1 mix, forest soil from site. Two water treatments: 80% field capacity for forest soil, 100% for peat/sand mix. One species: Jarrah.	Four months; weekly sampling non-invasive of plant height. Sampling times at 0 then monthly, 5 reps each harvest.	POTS: undrained PVC pipes, 10.5 cm diameter and 40 cm deep, lined with polythene bags and about 50 ml of sand at bottom. SOIL: peat/sand 3:1 mix and forest soil from site. Soil surface covered with 30 g alkathene beans. Fertilised added twice weekly.

			Two cultures: seeds and micro-propagated plantlets.		SET UP: pH of soil adjusted to 6 and deionised water used. Pots placed in root cooling tank maintained at 20 °C in glasshouse with temperature range of 15–36 °C.
	Bell *et al.* (1993)	Plant height, diameter of trunk, leaf type, biomass of leaf and roots. Length and width area of leaf. Root length. Basic root shape, density, breadth.	Two soil treatments. One watering treatment. One species: River Red Gum. Three below ground root zones: upper 20 cm, 20–40 cm below, 40 cm below.	Six months.	POTS: in drums filled with sand, sand/clay mix — drums will drainage holes. SEEDS: had 5 months in jiffy pots before transferring to drums.

6 Appendix C: Proposed timing of experimental design

Proposed timeframe for experimental design

Week starting	**22/8**			**12/9**					**17/10**		**31/10**				**28/11**						**9/1**				**6/2**						**20/3**
Events: Week no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Seeds to nursery	■																														
Nursery time to grow seedlings	■	■	■	■	■	■	■	■																							
Pots cut/to nursery	■	■	■	■																											
Wonga clean up		■	■	■	■	■	■	■																							
Plant collection from nursery									■																						
Two-week establishment period									■	■																					
Establishment harvest											■																				
Change in treatment (observational)															■																
Early phase harvest & change in treatment																					■										
Change in treatment (observational)																									■						
Late phase harvest																															■

Key dates:

- Commencement of establishment period 17 October 2016
- Establishment harvest 31 October 2016
- Observational harvest/change in treatment 28 November 2016
- Early phase harvest and change in treatment 9 January 2017
- Observational harvest/change in treatment 6 February 2017
- Late phase harvest (end of experiment) 20 March 2017