The Living Murray Hattah Lakes Intervention Monitoring

Black Box Reproduction and Tree Health

Annual Report 2017-2018

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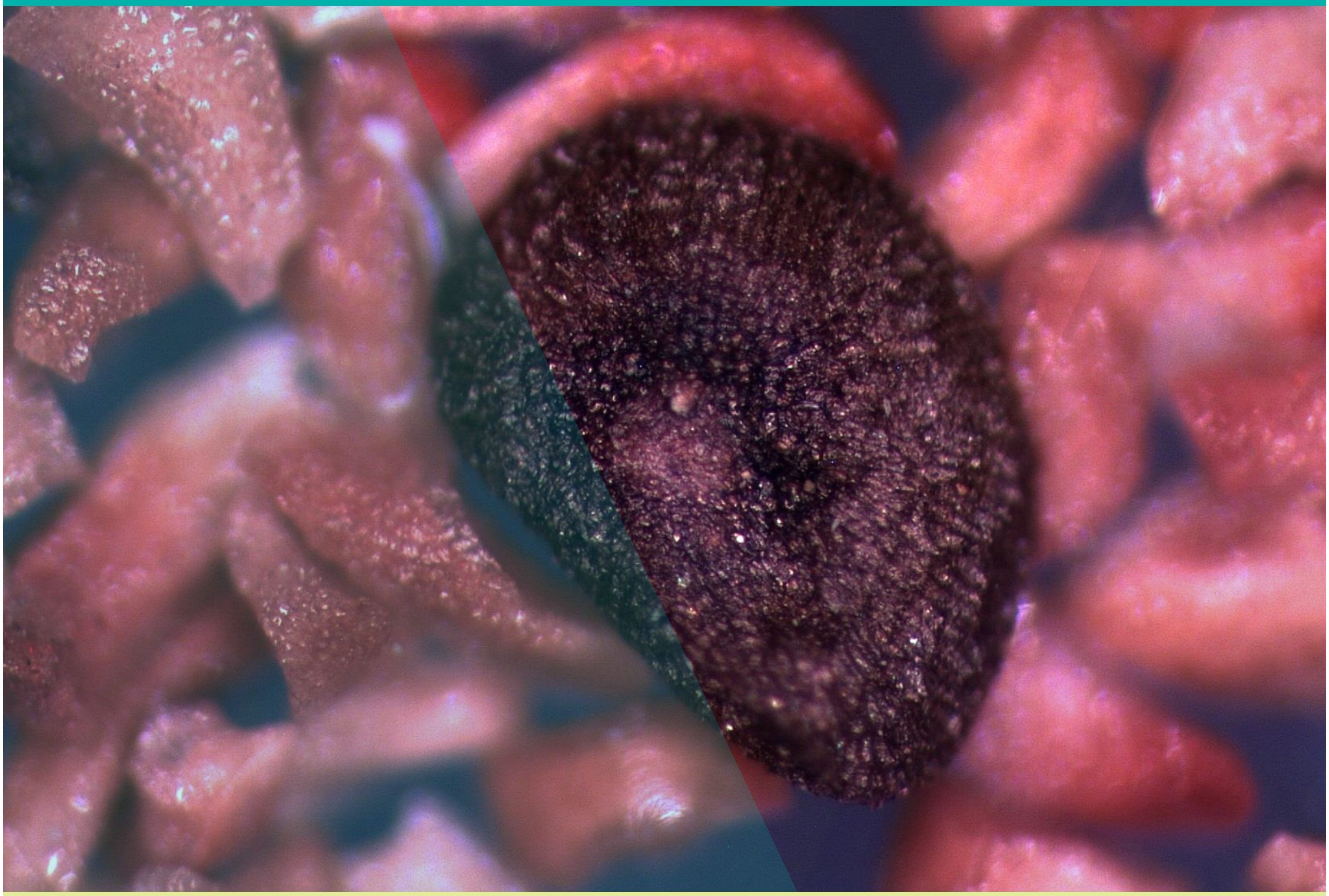
June 2018



Arthur Rylah Institute for Environmental Research
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Front cover photo: A viable Black Box (*Eucalyptus largiflorens*) seed among numerous unfilled seeds (i.e. chaff). Photo credit: B Farmilo.

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Summary

Context

The health of Black Box (*Eucalyptus largiflorens*) tree populations are strongly influenced by the timing, frequency and duration of natural flooding and environmental watering events. In the Hattah Lakes Icon Site, the combined effects of drought and the historical over-use of water resources have led to Black Box plant communities varying in condition and health, with the majority in poor or average health. Previous research has shown that environmental watering can improve floodplain tree health. However, the response of Black Box to environmental watering has been relatively under-studied. This monitoring program investigates the response of Black Box trees to environmental watering using an adaptive framework. This approach will provide an improved understanding of the response of Black Box populations to environmental watering. Information generated by the project will contribute to The Living Murray program which aims to restore the health of Murray River ecosystems through targeted environmental watering events.

Aims

This monitoring program has expanded from a pilot study in 2014, to a scientifically robust monitoring program with the inclusion of six new sites in 2017-2018. The monitoring aim is to document the outcomes environmental watering events to the health of Black Box trees to enable improvements in the effectiveness of future environmental watering scenarios. A set of key ecological questions were developed to (i) guide the approach to monitoring, and (ii) ensure program outcomes can be used to improve the health of the floodplain Black Box communities. The monitoring program assesses the health and reproductive output of Black Box trees at both the (i) tree-level (individuals; monitored monthly) and (ii) site-level (populations; monitored annually).

Findings

This monitoring program demonstrates a trend for environmental watering to lead to an improvement in the health and reproductive capacity of Black Box trees at the Hattah Lakes Icon Site. In particular:

- Black Box reproductive output (fruit and seed density) and health (broad tree health, crown extent and density) are higher in sites that receive environmental watering compared to unwatered control sites,
- Measures of reproduction and health are dynamic over time, suggesting the timing of environmental watering could be important to demonstrate a positive effect on Black Box communities,
- Black Box peak seed release is likely to occur between October and December, but requires further investigation.

The findings to date are only a trend as the monitoring program has been limited by a lack of site replication and monitoring points. The trends identified here will be validated in subsequent years following the recent expansion of the pilot study to a fully replicated, statistically robust monitoring program. Project findings can then be applied more widely across Black Box populations throughout semi-arid floodplains of the Murray Darling Basin.

Recommendations

Continued monitoring of all eight sites is required to produce management recommendations at the Hattah Lakes Icon Site and to fill key ecological knowledge gaps in relation to Black Box tree health and reproductive output. Specifically, two main recommendations are (in priority order):

1. Obtain consistent resources to ensure sites are monitored biomonthly and to effectively achieve project outcomes. Since the monitoring project commenced in 2014, monthly or bimonthly monitoring of Black Box seed fall has been inconsistent often due to project resources and funding cycles. This severely limits the utility of the project in achieving the aims.
2. Investigate Black Box seed viability and germination rates to determine if seed is capable of germinating and producing new plants.

1 Introduction

1.1 Project context

The Murray-Darling Basin (MDB) supports over 30,000 wetlands and rivers that provide important habitat for a wide variety of plants and animals (Ralph and Rogers 2011). However, in recent decades the health of the Murray-Darling Basin has declined due to the combined effects of drought and the over-use of water resources (Adamson *et al.* 2009). Hence, the effective management of biodiversity across the MDB requires additional water resources – via environmental watering – to restore ecosystem function (Reid and Brooks 2000).

The Australian Government implemented The Living Murray (TLM) program in 2003 to restore the health of Murray River ecosystems (a dominant feature of the Murray-Darling Basin) through targeted environmental watering events. The Hattah Lakes Icon Site is one of six target areas in the TLM program selected for its significant environmental values and potential for effective restoration (MDBA 2013). The TLM program is co-ordinated by the Murray-Darling Basin Authority (MDBA) in partnership with national and state governments, and has the long-term goal of achieving a healthy working Murray River system for the benefit of the environment and all Australians (MDBA 2013).

Health of the two dominant floodplain trees (River Red Gum (*Eucalyptus camaldulensis*) and Black Box (*Eucalyptus largiflorens*)) at the Hattah Lakes Icon Site are strongly influenced by the timing, frequency and duration of natural and environmental watering events (Rogers 2011). The majority of floodplain research on trees in south-eastern Australia has focussed on the commercially valuable River Red Gum (Rogers 2011). However, the response of Black Box to environmental watering has been relatively under-studied (George 2004). Black Box communities vary in condition and health across the Hattah Lakes Icon Site, with the majority in poor or average health (Cunningham *et al.* 2009, 2011). Preliminary studies have shown that environmental watering events can improve Black Box health (Akeroyd *et al.* 1998). However, Black Box regeneration response has been relatively low, with TLM condition monitoring indicating low numbers of seed germination when compared to River Red Gum (Walters *et al.* 2011).

1.2 Black Box reproductive cycle

Black Box trees are long-lived (250 years; Snowball 2001) and typically occur along more elevated (i.e. drier) parts of the floodplain that are only periodically flooded (Cunningham *et al.* 1981). Flowering in Black Box is prolific (hence the species name '*largiflorens*'; Cunningham *et al*. 1981) and occurs primarily between August and January (Brooker and Kleinig 1983, Roberts and Marston 2000, George *et al.* 2005), but can vary depending on geographic region. Flower buds may be retained in the canopy for up to 12 months before flowering (Jensen *et al.* 2007). Black Box trees have an aerial seed bank with minimal soil stored seed (Jensen *et al*. 2007). Seeds are retained in the canopy for 24 months until conditions for seed fall occur (Dexter 1967, Jensen *et al.* 2007). Seed fall occurs any time of the year but predominantly from February to April (George 2004) and from October through to March in the lower Murray region (Jensen *et al*. 2007). Black Box produce large numbers of small seeds (484 germinants/gram of seed and chaff mixture; Gunn 2001). However, the duration of seed fall and the factors inducing seed fall are relatively unknown (George 2004), although tree health is an important variable influencing seed production (George 2004, Jensen *et al.* 2007).

1.3 Monitoring Black Box health and seed fall

In 2014 a monitoring program was established to investigate the influence of environmental watering on floodplain Black Box tree health and seed release (Moxham *et al.* 2014). The program contributes to evaluating the TLM overarching ecological objective (MDBA 2013): to *restore communities of wetland and terrestrial plant assemblages by maintaining sustainable populations of River Red Gums and Black Box communities* (MDBC 2006).

The program investigates the following key evaluation questions:

1. Does Black Box tree health increase in response to environmental watering over time?
2. Do measures of Black Box tree reproductive output increase in response to environmental watering over time?
3. Does Black Box aerial seed fall increase in response to environmental watering over time?
4. Does understorey vegetation condition change in response to environmental watering over time?

The monitoring program was initially implemented as a one-year pilot study to examine the effect of the 2014/15 environmental watering event on Black Box health and seed fall. The program was designed to require minimal financial investment, involve limited human resources and be expandable over time to incorporate new sites and questions. The pilot study established two monitoring sites (watered and unwatered) in the northern Hattah Lakes system, and in 2016 two extra sites were also established (totalling two watered and two unwatered sites). In 2018 to increase scientific rigor and progress the pilot study into a more comprehensive monitoring program, four sites (two of each treatment) were established in the southern Hattah Lakes system.

This report outlines the monitoring program activities for the 2017/18 financial year and presents trends in Black Box health and seed fall in response to environmental watering events over the last five years.

2 Methods

A full description of the monitoring protocol is provided in Moxham *et al.* (2014). The monitoring design, sampling methods, and monitoring schedule are briefly summarised below.

2.1 Design

In 2014, two 0.5 ha monitoring sites were established in the northern lakes section of the Hattah Lakes Icon Site to examine Black Box aerial seed fall in response to an environmental watering event in Spring 2014 (i.e. 45 m inundation) (Table 1). The sites were divided into two treatments:

1. Watered treatment site – Mournpall Track
2. Unwatered control site – Bitterang Stop Bank

Black Box tree health was consistently poor across both sites, which ensured the best chance of detecting changes in the health and reproductive output of Black Box in response to watering.

At each monitoring site two scales of assessment were established:

- Site level assessments of tree health and understorey floristics (assessed annually), and
- Target tree-level assessments (eight trees/site) examining seed fall, reproduction and tree health (assessed monthly – where possible)

Environmental watering

A series of large environmental watering events have been implemented over the last five years (2014-2018) targeting not only the River Red Gum plant communities, but also the higher elevation (to 45 m) Black Box floodplain plant communities. Environmental watering events occurred in the Spring/Summer of 2014/15, 2016/17 and 2017/18 and this was the first time the higher floodplain has been inundated since the 1990s.

2.2 New Sites

Northern lakes

Based on recommendations in the 2016 annual progress report (Moxham *et al.* 2016) two additional monitoring sites were incorporated into the project in the northern lakes system (one watered and one unwatered site; Table 1). These additional sites were established prior to a second environmental watering event in spring/summer 2016/17 and the data generated have been included in this report. However, the new watered site (Bitterang Track) was only partially watered during this event. As a result, the data generated has been separated to ensure the full effects of the watering event are realised.

Table 1. Complete list of monitoring sites showing when sites were established, treatments, spatial arrangements and location information

Lake Section	*Site name	Treatment	Date established	Zone	Easting	Northing
Northern Lakes	Mournpall Track	Watered	May 2014	54	624729	6163717
	Bitterang Stop Bank	Unwatered	May 2014	54	626906	6165206
	Bitterang Track	Watered^	February 2017	54	626530	6164151
	Boolca Track	Unwatered	February 2017	54	623478	6169781
Southern Lakes	Stockyard Track Sth	Watered	April 2018	54	627384	6152886
	Stockyard Track Nth	Watered	April 2018	54	625652	6152586
	Brockie Track	Unwatered	April 2018	54	628320	6152522
	Nip Nip Track	Unwatered	April 2018	54	628502	6153036

* Note: some site names have been modified to ensure they are informative and consist. A lookup table showing the site names used in previous reports is in Appendix 1; Table A1.1.

^ Bitterang Track was only partially watered.

Southern lakes

Based on recommendations in the 2017 annual progress report (Farmilo *et al.* 2017) four additional sites (two watered and two unwatered sites) were added in April 2018 to establish a fully replicated monitoring program across the Hattah lakes Icon site (Figure 1 and 2; Table 1).

The monitoring program now has eight sites across the northern and southern lakes system, which increases scientific rigor and the capacity to provide management recommendations across the Icon Site.

The data from the southern lakes sites are not reported on in the body of the report because these new sites, currently have insufficient data to warrant analysis (data from these sites are provided in Appendix 2).

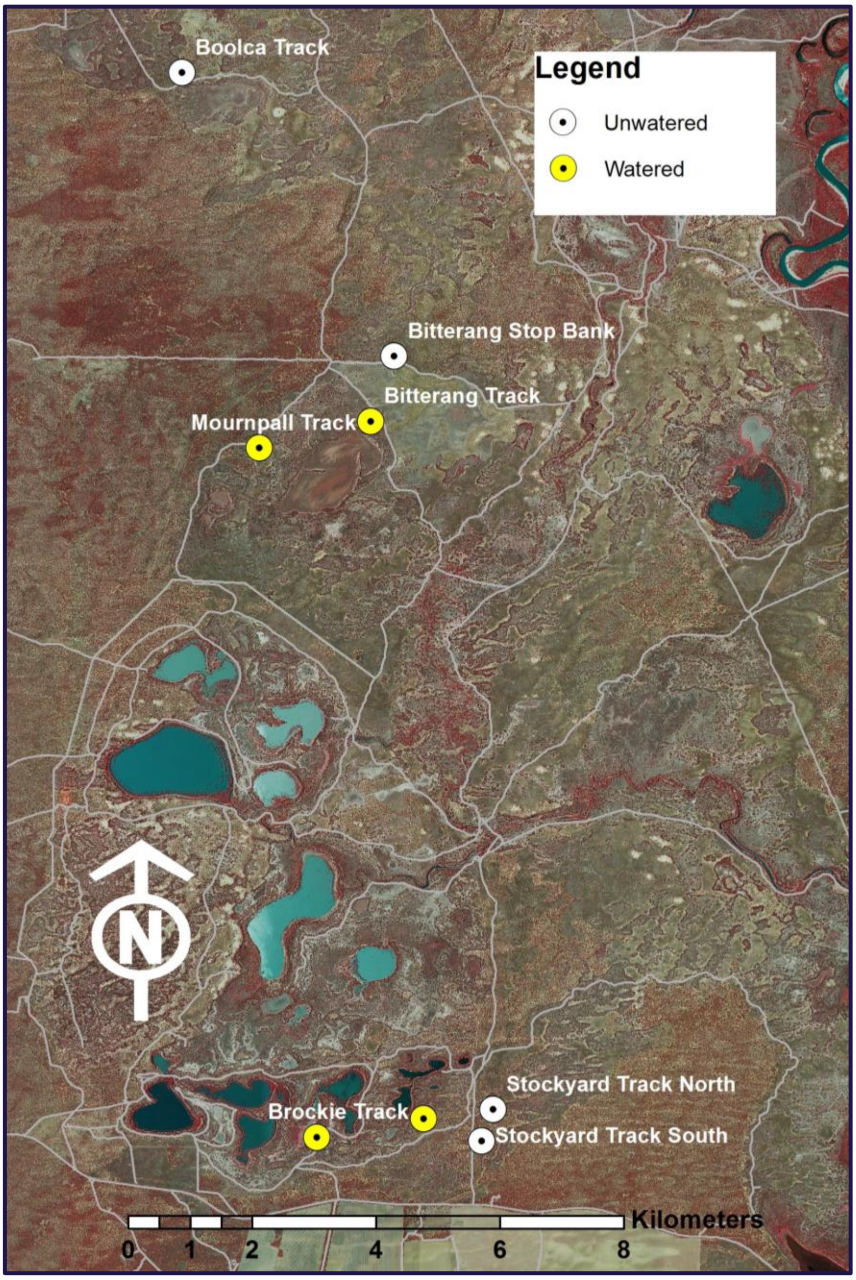

Figure 1. Map of the Hattah Lakes Icon Site showing monitoring sites**.**

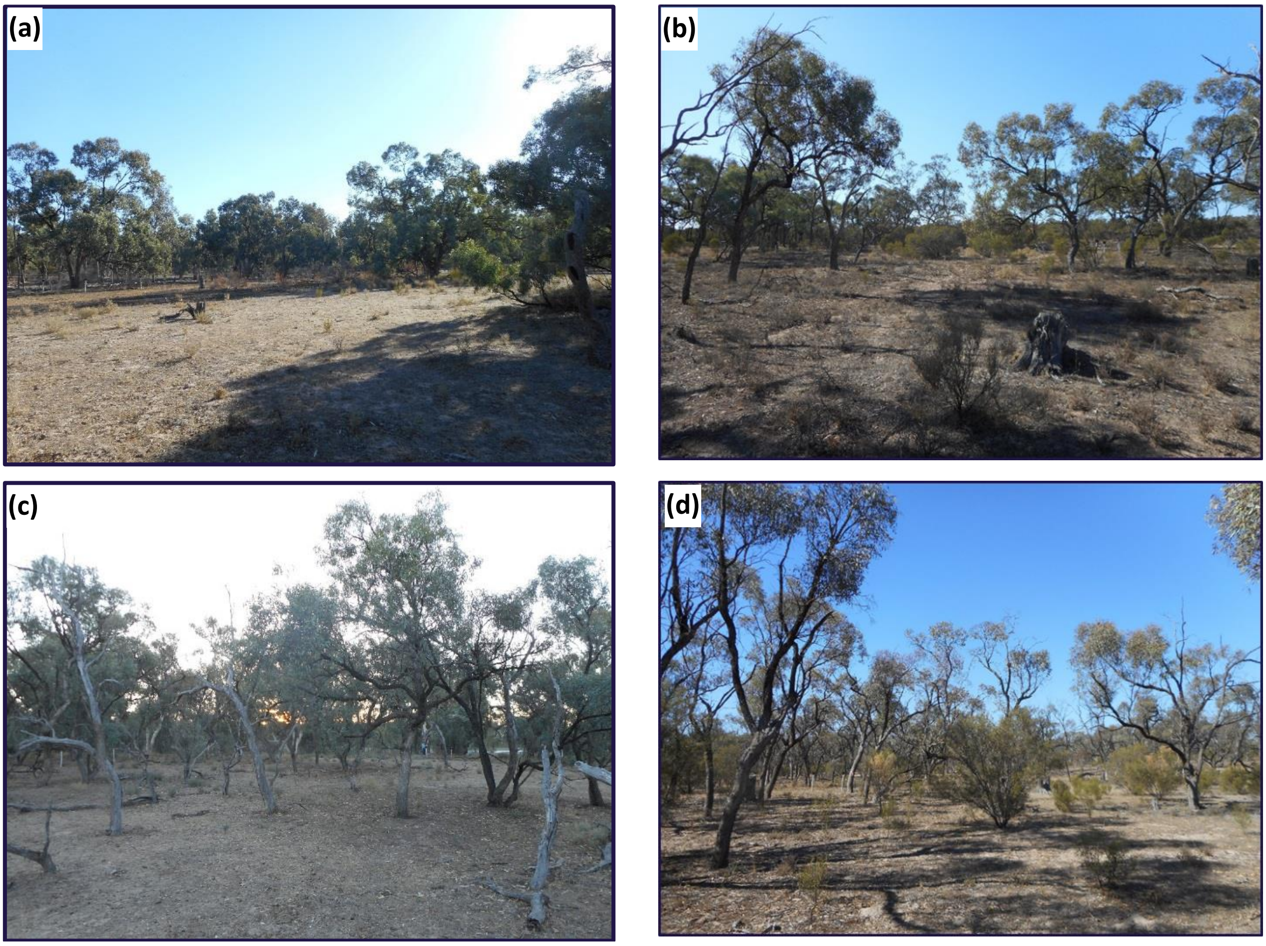

Figure 2. The four recently established monitoring sites in the Southern Lakes system of the Hattah Lakes Icon Site. Photos show (a) Nip Nip Track [watered], (b) Stockyard Track South [unwatered], (c) Brockie Track [watered] and (d) Stockyard Track North [unwatered].

2.3 Site-level assessments

Site level assessments were undertaken annually to identify broad changes in tree health and understorey condition. Since the establishment of the monitoring project, annual monitoring has been conducted in late May to early April.

Understorey floristics

Understorey floristic assessments were conducted to quantify the understorey condition of the monitoring sites, and to monitor any change in this condition in relation to environmental watering. A floristic survey recording all plant species present and their relative abundance (direct percent live cover estimated to the nearest 5%) was undertaken within two 15 m x 15 m quadrats (225 m^2) at the north-west and south-east corners of each monitoring site. The direct percent cover of bare ground, litter, logs and biological soil crust were also estimated.

Stand level tree health

Stand level tree health assessments (i.e. all trees [n = 31 – 66] within each 0.5 ha site) were undertaken to gain an understanding of the overall response of tree health to environmental watering.

Two measures of tree canopy vigour were assessed across the site: (1) the broad tree health measure – the percentage of the entire tree crown containing live leaves (four categories), and (2) the TLM crown extent measure – the percentage of the assessable tree crown in which there are live leaves (seven categories; MDBA 2010). In addition, each Black Box tree in the monitoring site was assessed based on the following criteria: tree status (alive or dead), diameter at breast height (DBH) of the main trunk, number of main branches, broad tree health, and nine TLM tree health measures (MDBA 2010).

Site photo points

Permanent photo points provide a useful visual representation of changes in tree stand health. At each site a permanent photo point was established from a central star picket located at the site edge orientated toward the centre (Figure 3).

2.4 Target tree-level assessments

Target tree health

Target tree-level assessments were conducted on a monthly to quarterly basis (Figure 3) to determine fine-scale changes in tree health and seed fall in response to environmental watering.

Within each monitoring site eight target Black Box trees were randomly selected to monitor seed fall, reproduction (TLM reproduction extent score; Moxham *et al.* 2014) and tree health (broad tree health measure) in response to environmental watering. Three seed fall traps were established at the canopy edge of target trees. To maximise seed capture, traps were located on the leeward side of the prevailing wind (south-westerly in this region; Bureau of Meteorology, Ouyen 2018).

After seed collection, the material from each of the three seed traps, per tree, was pooled. Buds, flowers and fruits were quantified for each sample. Following this, samples were sieved (2 mm mesh) and seeds were quantified under a dissecting microscope.

Target tree photo points

In addition, during each seed fall collection, photo points and a rapid tree health assessment were undertaken which included recording the abundance of buds, flowers and fruits (based on a 0-3 scoring system; i.e. TLM Reproduction Extent Score; Moxham *et al.* 2014).

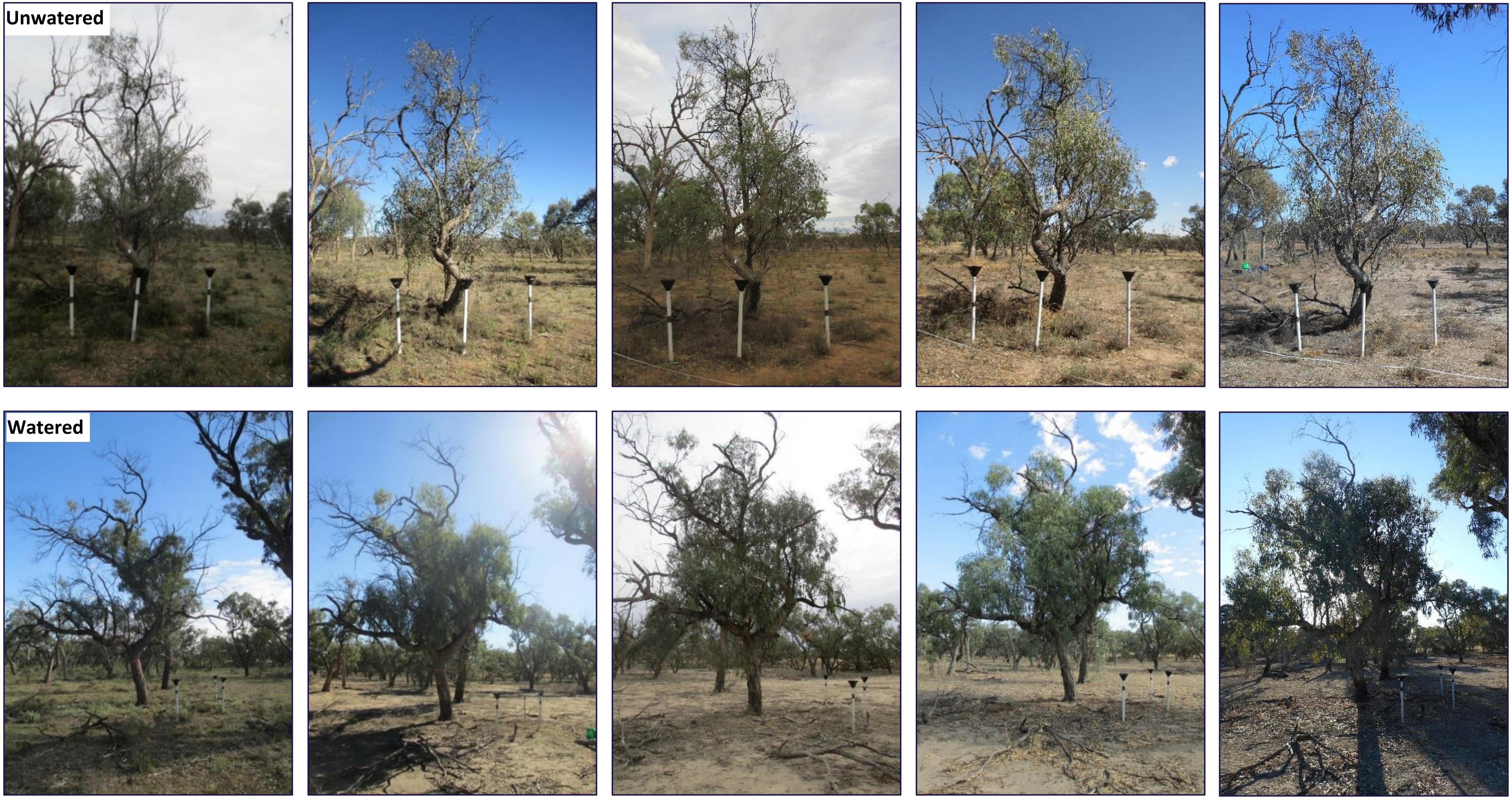

Figure 3. A time-series (2014-2018 left to right) of photographs taken of a target tree from Bitterang Stop Bank unwatered site (top row) and Mournpall Track watered site (bottom row) over the duration of the monitoring project. Photos demonstrate the increase canopy health at watered sites and the arrangement of seed traps.

2.5 Seed fall monitoring schedule

The original monitoring design and schedule involved the monthly collection of seed and rapid assessment of tree health and reproduction by regional staff and/or volunteers. However, due to resourcing issues this did not occur consistently over the duration of the project. Sites were established in May 2014 before the first environmental watering event. In February 2015, funding was reallocated from this project to cover the 2014-15 vegetation offset monitoring, a statutory requirement of the Hattah works. This led to the temporary cessation of seed fall monitoring between February – September 2015. Monitoring of seed fall traps resumed in September 2015 for two months (until November 2015). The monitoring has since been conducted by ARI scientists who monitored the seed fall traps monthly from January – June 2016 (funded), in September 2016 (unfunded), and bimonthly in January, March and May 2017 (funded). Following the implementation of a large environmental watering event in Spring/Summer 2017, bimonthly monitoring was inhibited over the period September – December 2017, when resources were re-allocated. Following this, bimonthly monitoring has been conducted by ARI scientists in January (to clean and reset traps), February, March and April 2018. The next scheduled monitoring event is in June 2018.

2.6 Analysis

Due to the multiple environmental watering events that have occurred since the project began and the addition of six new sites at different stages of the project (February 2017 and April 2018) the initial Before-After Control Impact design that was used in previous annual reports cannot be used here. Instead, additive models that explore the overall changes in response to environmental watering (irrespective of time), and the overall changes through time (irrespective of watering) are used to give insights into the role of environmental watering on improving the health of Black Box floodplain trees. However, with the addition of the new sites and adequate collection of data through time, a robust analysis that includes interactive effects (to determine the effect of watering in relation to the time of monitoring) will be possible in future reports.

All analyses were constructed in a Bayesian framework in the statistical program *R* (R Core Team 2017) and using the package ‘brms’ (Bürkner 2017). Where appropriate statistical models were developed for a selection of response variables (i) seed and fruit density (collected monthly); and (ii) tree health, crown extent, crown density, reproductive output (scores, collecting annually).

All other potential response variables (e.g. floristics, other TLM measures, flower and bud density) were considered either under-replicated, under-sampled, or reflect very little variation over time or in relation the imposed watering treatments. These variables are contained within the database and will be presented in future reporting.

A detailed description of the approach to data analysis is presented in Appendix 3.

All model results are provided in Appendices 4 and 5.

3 Results

3.1 Results interpretation and context

The current approach to modelling can determine the strength of a difference between treatments (irrespective of time), and differences over time (irrespective of treatment). Statistical models were developed for tree health and reproductive measures. However, these results should be interpreted as trends at this stage due to the low site replication. Descriptive changes in understorey vegetation provide trends only. Where modelling has been conducted and descriptive statistics have been generated, most outputs (figures and tables) appear in Appendices 4 and 5 for brevity. It is anticipated that with the inclusion of monitoring data from the four new southern lakes sites, and additional data from the existing northern lakes sites, a comprehensive data analysis can be undertaken in the future.

3.2 Site-level assessments (annual)

Black Box reproductive extent

On average, reproductive extent was higher at sites that have had environmental watering and was dynamic over time (Appendix 4, Table A4.1).

Black Box tree health

All measures of Black Box tree health were higher in the watered site compared to the unwatered controls (Appendix 4, Table A4.1). The effect of the environmental watering on Black Box tree health varied depending on the sampling measure (Appendix 4, Table A4.1). Modelling also suggests that crown density, crown extent and broad tree health were dynamic over the monitoring period.

Understorey vegetation

Understorey vegetation and ground layer changed over the monitoring period reflecting a response to flooding events (i.e. the wet/dry floodplain cycles) and annual rainfall. Native plant species richness approximately halved over the five-year monitoring period, regardless of watering treatment (appendix 4, Table A4.3). The decline in native richness ceased in unwatered sites (i.e. has not declined further), but continued to decline in the watered site, following the flood event in 2017/18 (Appendix 4, Table A4.3). Exotic species richness has recovered in unwatered sites in 2018 following a decline in 2015; however, it continues to decline in the watered site (Appendix 4, Table A4.3).

The abundance (cover) of grasses and forbs declined over the monitoring period regardless of environmental watering (Appendix 4, Table A4.2). Alternatively, shrub abundance displayed a stronger decline in watered sites over the monitoring period due to inundation.

Bare ground cover increased over the monitoring period at all sites; however, the increase was more dramatic at the watered site (Appendix 4, Table A4.4). Litter cover increased in unwatered sites, and to a lesser extent in the watered site (Appendix 4, Table A4.4). In contrast, cover of soil crust disappeared completely in response to watering (and is yet to recover), while it increased moderately during the same period at the unwatered site (Appendix 4, Table A4.4).

3.3 Target Tree-level assessments (monthly)

Black Box reproductive output

The number of Black Box buds, flowers, fruits and seeds released from the canopy was consistently higher at the watered site, at almost all monthly monitoring events (Figure 4a-d) and these responses were validated by the reproduction scores (Appendix 4, Table A4.5). In addition, a strong difference was observed between the treatments, when averaged across the monitoring period (inset figures; Figure 4), with density of fruits and seeds higher in the watered site (Appendix 5, Table A5.1). This trend mirrors the TLM reproduction extent scores (Appendix 5, Table A5.1). Peak fruit and seed release occurred between October and December (Figure 4c, d). However, due to a large amount of missing data over this peak period (particularly in 2016 and 2017) this claim cannot be made with a high level of certainty.

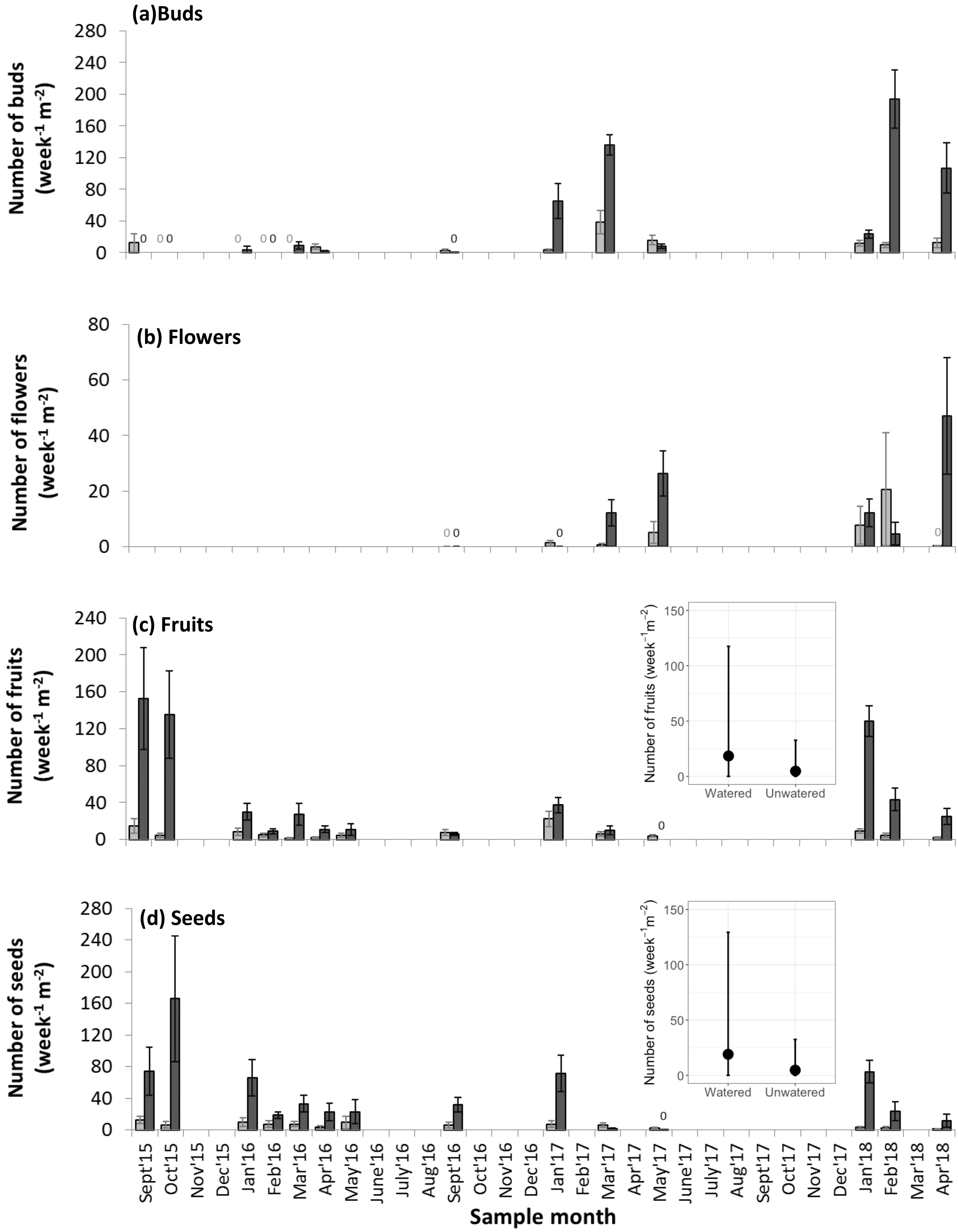

Figure 4. The mean (± standard error) number of (a) buds, (b) flowers, (c) fruits and (d) seeds for the eight target Black Box trees between September 2015 – April 2018 at the unwatered (light grey) and completely watered (dark grey) monitoring sites (not including partially watered site(Bitterang Track) and recently established sites (i.e. Southern Lakes); see Appendix 1). Inset figure shows model predictions between the two treatments (holding month constant).

Black Box tree health

In all sample months tree health at the watered site was equal to, or higher than, the health of trees in the unwatered control site (Figures 5 and 6). These trends were supported by modelling which suggested that environmental watering had a positive influence on the health of the eight target trees over the monitoring period (Appendix 4, Table A4.1).

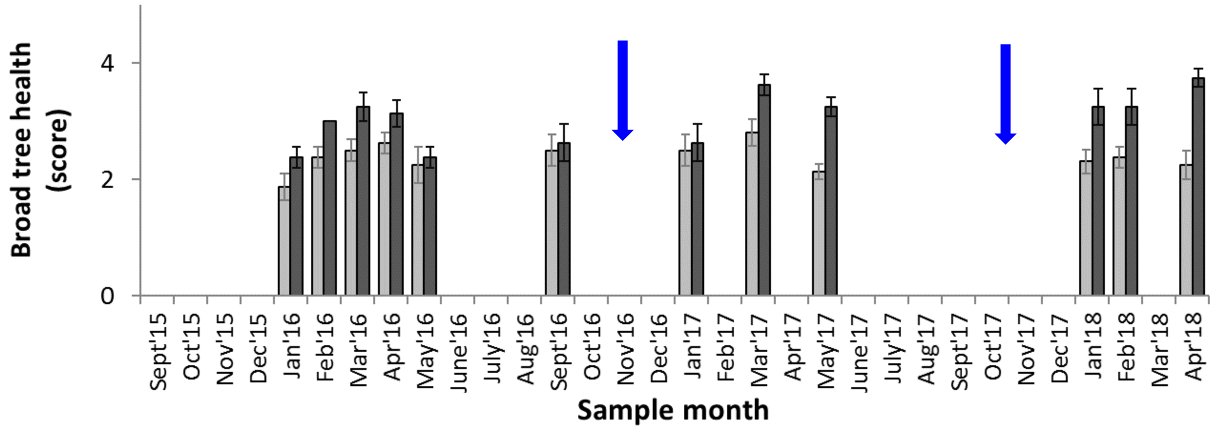

Figure 5. The mean (± standard error) broad tree health score (Scores: 1 - < 25%; 2 - 25-50%; 3 - 50-75%; 4 - > 75%) for each month sampled at the unwatered (light grey) and watered (dark grey) monitoring sites (not including partially watered site (Bitterang Track) and recently established sites (i.e. Southern Lakes; see Appendix 1). Blue arrows indicate the two flood events in 2016/17 and 2017/18. Sites established in 2014 were flooded in spring/summer 2014/15.

Figure 6. The Bitterang Stop Bank unwatered site (top) and the Mournpall Track watered site (bottom), in 2014 (left) and in 2018 (right). Note the improvement in tree canopy at the watered site and the drying of the understorey at the unwatered sites and the removal of understorey at the watered site by extensive flooding a few months beforehand.

4 Discussion

To date, the findings from this monitoring program confirm the effectiveness of environmental watering in meeting conservation management objectives for improving the health and reproductive output (including seed release) of Black Box tree populations. Although the current analysis is somewhat statistically limited (i.e. poor replication), this program has started to fill key ecological knowledge gaps in relation to Black Box tree health and reproductive output.

4.1 Tree health responses to environmental watering

Black Box tree health at both the site-level, and the target tree-level was higher in sites that received substantial environmental water (i.e. completely watered sites). In addition, findings at the site-level were validated with other measures of tree health with both crown density and crown extent showing a substantial increase at completely watered sites. As tree-level data were collected more frequently, trends in tree health suggest a dynamic response that varies throughout a year. The tree-level results suggest that environmental watering may provide conditions for improved tree health particularly over summer and autumn, whereas spring monitoring (represented by a single monitoring event) indicates that tree health is much more similar between the two treatments. Regardless, these results suggest that the multiple watering events that have been implemented during the monitoring project can have substantial positive benefits for Black Box tree health. However, effects of the flood regime (frequency, duration and timing) on the Black Box life cycle and health require further investigation.

4.2 Tree reproductive output responses to environmental watering

An increase in the health of Black Box trees in response to environmental watering should improve plant growth, and by extension, reproductive output (Akeroyd *et al.* 1998, George 2004, Walters *et al.* 2011). This monitoring program detected a persistently higher rate of reproductive extent, fruit fall and seed fall at the watered site compared to the unwatered control sites. This is attributed to environmental watering. However, the exact watering regime or aspects of the regime that influence this outcome require further examination. For example, the findings here could also be attributed to adequate duration of the flooding events (not too long as to detrimentally impact tree health; not too short to prevent positive impacts on tree health), or the frequency of events (three flood events in five years). Furthermore, the timing of environmental water occurred when Black Box trees were experiencing one of the most severe droughts in recorded history (i.e. the Millennium drought). Thus, drought stressed trees may take longer to respond to flooding or may require repetitive flood events, as implemented in this instance, to improve tree health in the long term.

Black Box peak seed release is likely to occur between October and December, which aligns roughly with that in the lower Murray region (Jensen *et al*. 2007). However, key phases of seed production (October-December) continue to go unmonitored (sometimes due to the presence of standing water) and therefore are artificially deflating the impact of watering on seed production. The increase in seed production is suspected to be in response to the environmental watering, as the production of seeds correlates with the expected delay period predicted for floodplain trees in response to flooding. That is, Black Box trees frequently exhibit a two-year delay in the production of fruits and seeds (George 2004, Jensen *et al.* 2007). Therefore, it is suggested that environmental watering has resulted in an increase in the release of seed from the Black Box canopy. However, the fate of these seeds (i.e. do they survive?) is unknown. Due to the limited nature of the current data, future monitoring over the next 12-24 months will help fill this knowledge gap.

Other reproductive measures were collected more frequently (i.e. measures at the tree-level) and tell a slightly different story. At different times throughout the monitoring period, both reproduction scores (i.e. buds, flowers, fruits) and reproductive material (i.e. buds, flowers, fruits and seeds) were higher or lower at watered sites, compared to unwatered control sites. This could be described as an asynchronous response to watering, whereby all reproduction measures still occur irrespective of watering, but the timing and magnitude of change between watered and unwatered sites varies over time. This variation within a year is interesting and has implications for ecosystem function (i.e. food source for fauna). This monitoring program provides evidence to suggest that the watered and unwatered sites have slight differences in the timing of bud, flower and fruit production. Such changes could have impacts on gene flow across the landscape (Fuchs *et al.* 2003; Moore *et al.* 2005) and could also affect those organisms reliant on consistent supply of resources provided by Black Box (e.g. nectar, pollen). Therefore, it is important that in the future these sites are monitored at much more consistent intervals (i.e. bimonthly), so this response can be quantitatively assessed.

4.3 Understorey responses to environmental watering (trends only)

Understorey vegetation and ground layer changes over the monitoring period reflected responses to flooding events (i.e. the wet/dry floodplain cycles) and annual rainfall. An overall decline in vegetation cover was observed over the monitoring period, irrespective of environmental watering, reflecting the annual rainfall availability. The region had below average rainfall in 2016 and 2018, furthermore the rainfall in the three months preceding sampling also influences results (BOM 2018). The decline of terrestrial dry functional groups, such as shrubs, and the increase in bare ground reflect the dynamics of the wet/dry floodplain cycle (Blom and Voesenek 1996, Capon *et al.* 2009, Moxham *et al.* 2017).

Interestingly, the changes in bare ground were almost entirely offset by changes in litter cover, which showed a much stronger increase in unwatered sites compared with watered sites. These changes make sense given the strong capacity for environmental water to redistribute resources (e.g. leaf litter) around the landscape (Pettit and Naiman 2006) and the subsequent creation of bare ground patches. Although soil crust cover appears to be dynamic over time (as evidenced by the dramatic changes in cover in the unwatered sites) the complete loss of cover in watered sites suggests environmental watering still has a strong influence. Greater replication at the site-level will soon be realised with the additional six sites incorporated into the study (two in 2017 and four in 2018), and therefore these relationships and the responses to environmental watering will be better understood.

4.4 Statistically robust monitoring

Based on recommendations in the 2016 annual report (Moxham *et al.* 2016) two additional monitoring sites in the northern lakes system were incorporated into the project in February 2017 (one watered Bitterang track and one unwatered control site, Boolca Track). However, the Bitterang Track site was only partially inundated in recent flooding event in Spring/summer 2017/18, and this site may need to be relocated in 2018/19.

Following the recommendations in the 2017 annual report (Farmilo *et al.* 2017), four additional monitoring sites in the southern lakes system were incorporated in the project in April 2018 ensuring broader representation of the Hattah Lakes Icon Site (two watered and two unwatered control sites).

These additional sites form the baseline – or pre-watering – data for any future flooding events. These sites will be used in subsequent reporting to strengthen the capacity of the project to investigate the role of environmental watering on Black Box reproduction and canopy health.

4.5 Conclusion

This monitoring program demonstrates a trend that Black Box tree health and reproductive output can improve in response to environmental watering and provides important information for managers of the Hattah Lakes Icon Site. Changes due to environmental watering include an increase in Black Box canopy health and reproductive output; in particular, the release of fruits and seeds stored in the canopy. At Hattah Lakes, Black Box peak seed release is likely to occur between October and December, but requires further investigation. The findings to date are only a trend, as the monitoring program has been limited by a lack of site replication and monitoring points. The trends identified here will be validated in subsequent years following the recent expansion of the pilot study to a fully replicated, statistically robust monitoring program. This will fill key knowledge gaps and enable recommendations to managers about how to improve Black Box community health and survival. Project findings can then be applied more widely across the Hattah Lakes Icon Site and Black Box populations throughout semi-arid floodplains of the Murray Darling Basin.

4.6 Recommendations

The monitoring program has been running for five years and has contributed to informing a key knowledge gaps in relation to the impact of environmental watering on the seed release and health of Black Box trees. A series of recommendations are made below that could increase the capacity of the program to make useful management recommendations:

1. **Resources for consistent monitoring:** Since the monitoring project inception in 2014, monthly or bimonthly monitoring of Black Box seed fall has been inconsistent due to project resources and funding cycles. This severely limits the usefulness of the project in achieving its aims. It is recommended that consistent resources are obtained to ensure sites are monitored bimonthly, and project outcomes are achieved efficiently. This is important to gain insights into the temporal dynamics of tree health and reproduction, and the relationship with environmental watering.
2. **Seed viability:** It is unknown if the Black Box seeds being produced are viable (i.e. can they germinate and produce new plants?). Conducting germination trials in a glasshouse or in controlled temperature growth cabinets can determine seed viability and germination rates. Germination triggers could also be examined (e.g. soil moisture levels).
3. **Inundation levels and duration:** Black Box may respond more positively to inundation levels, and periods of inundation. Therefore, a complementary Black Box population dynamics and reproductive output project, that incorporates a gradient of inundation levels could be undertaken. This could prove to be informative to managers involved in determining optimum flooding regimes to be used during environmental watering.

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Appendices

Appendix 1 – Site names

Table A1.1 Site names and codes lookup table for comparison with previous reports and datasets

Old Site Name	Current Site Name	Current Site Code
Bitterang Stop Bank	Bitterang Stop Bank	NL_Bitterang Stop Bank_UW
Boolca dry	Boolca Track	NL_Boolca Track_UW
Southern Lakes Control 1	Stockyard Track South	SL_Stockyard Track South_UW
Southern Lakes Control 2	Stockyard Track North	SL_Stockyard Track North_UW
Bitterang 45m	Mournpall Track	NL_Mournpall Track_W
New Bitterang	Bitterang Track	NL_Bitterang Track_W
Southern Lakes Watered 1	Nip Nip Track	SL_Nip Nip Track_W
Southern Lakes Watered 2	Brockie Track	SL_ Brockie Track_W

NL – Northern Lakes; SL – Southern Lakes; UW – Unwatered (Control site); W – Watered (Treatment site)

Appendix 2 – New Sites (Southern Lakes) Descriptive Statistics

Table A2.1 Tree demographics for the first annual assessment of the four new sites included in 2018

Site	Adult Trees	Saplings	Seedlings
Stockyard Track South	72	0	0
Stockyard Track North	123	42	0
Nip Nip Track	44	0	0
Brockie Track	70	0	0

Table A2.2 Tree health scores for the first annual assessment of the four new sites included in 2018. Scores represent means (± standard error).

Site name	Broad tree health (score)	TLM Crown Extent (score)	TLM Crown Density (score)	TLM Reproduction Extent (score)
Stockyard Track South	2.61 ±0.15	3.19 ±0.12	3.05 ±0.11	0.65 ±0.10
Stockyard Track North	1.79 ±0.08	2.34 ±0.06	2.24 ±0.05	0.08 ±0.03
Nip Nip Track	2.97 ±0.18	3.66 ±0.23	3.55 ±0.21	1.52 ±0.20
Brockie Track	2.18 ±0.10	3.03 ±0.10	2.88 ±0.10	0.31 ±0.09

Table A2.3 Understorey plant cover for the first annual assessment of the four new sites included in 2018. Scores represent means (± standard error).

Site name	Forb	Grass	Shrub	Tree
Stockyard Track South	8.75 ±2.75	0.5 ±0	24.75 ±1.75	12.5 ±2.5
Stockyard Track North	0.25 ±0.25	0.25 ±0.25	21.25 ±0.25	8.5 ±1.5
Nip Nip Track	3.75 ±1.75	0.25 ±0.25	19.25 ±6.75	15 ±5
Brockie Track	15.75 ±12.25	0.5 ±0.50	20 ±80	25.25 ±0.25

Table A2.4 Plant species richness for the first annual assessment of the four new sites included in 2018. Scores represent means (± standard error).

Site name	Native	Exotic
Stockyard Track South	10.5 ±0.5	0 ±0
Stockyard Track North	7.5 ±0.5	0 ±0
Nip Nip Track	9 ±0	0 ±0
Brockie Track	12 ±1	1.5 ±1.5

Table A2.5. Ground cover for the first annual assessment of the four new sites included in 2018. Scores represent means (± standard error).

Site name	Bare Ground	Litter	Log	Soil Crust
Stockyard Track South	27.5 ±7.5	25 ±5	0 ±0	47.5 ±12.5
Stockyard Track North	25 ±5	20 ±10	0 ±0	55 ±15
Nip Nip Track	40 ±20	55 ±15	0 ±0	15 ±5
Brockie Track	12.5 ±2.5	82.5 ±2.5	3.5 ±1.5	2.5 ±2.5

Table A2.6. Reproduction scores for the first annual assessment of the four new sites included in 2018. Scores represent means (± standard error).

Site name	Buds (score)	Flowers (score)	Fruits (score)
Stockyard Track South	1.38 ±0.18	0 ±0	1.38 ±0.42
Stockyard Track North	0.13 ±0.13	0 ±0	0 ±0
Nip Nip Track	1.63 ±0.26	0.5 ±0.19	2.13 ±0.4
Brockie Track	0.5 ±0.19	0.75 ±0.37	1.63 ±0.46

Appendix 3 – Data Analysis

Site-level assessments

Ordinal regression was used to compare broad tree health, TLM crown extent, TLM crown density, and TLM extent reproduction scores in relation to the watering treatment (watered (complete), water (partial) and unwatered) and the year of sampling (2014, 2015, 2016, 2017 and 2018). Separate ordinal regression models were used to test if there was any difference in health measures of Black Box trees over time and in relation to the watering treatments. Broad tree health, TLM crown extent, TLM crown density, and TLM extent reproduction were all integer response variables. All models were constructed in a Bayesian framework in the statistical program *R* (R Core Team, 2017) and using the package “brms” (Bürkner 2017). A stopping-ratio logit model (Agresti 2002) was used in the ordinal regression. Four chains were used, each with 2,000 iterations and a burn-in of 1000, without thinning, and convergence was checked. Convergence was defined as having all Gelman and Rubin’s convergence diagnostic potential scale reduction factors being less than 1.05 (Gelman et al. 2004). A parameter was considered to have sufficient evidence of an impact on the model if the lower and upper 95% credible interval for a parameter excludes zero. The lower and upper 95% credible interval is constructed from the posterior distribution for that parameter.

Floristic and ground cover data were not analysed using statistical approaches due to the scarcity of replicates (i.e. only two large quadrats per site). Therefore, trends were interpreted based on mean and standard error of the mean, hence any claims made in relation to these trends should be treated with caution.

Target tree-level assessments

A hierarchical Bayesian modelling framework was employed to explore temporal changes (monthly sampling) in the number of seeds and fruits in relation to the complete watering treatment (partially watered sites were not included in the analysis). A grouping term reflecting the repeated nature of sampling (target trees repeatedly sampled) was included in all models. All response variables were non-negative continuous values representing the density of seed or fruit per week per m^2. Models employed a hurdle gamma distribution to account for zero-inflation of the response variable. Plots of residuals and fitted values were inspected for each response variable to ensure model adequacy.

Appendix 4 – Descriptive Statistics

Table A4.1 Tree health scores (2014-2018) for the four existing sites established prior to 2018. Scores represent means (± standard error).

Treatment	Year	Broad tree health (score)	TLM Crown Extent (score)	TLM Crown Density (score)	TLM Reproduction Extent (score)
*Unwatered	2014	2.43 ±0.11	3.5 ±0.16	3.5 ±0.15	1.1 ±0.12
	2015	1.46 ±0.11	2.58 ±0.15	1.58 ±0.13	0.65 ±0.11
	2016	2.46 ±0.22	3.67 ±0.3	1.83 ±0.13	0.92 ±0.15
	2017	2.44 ±0.16	3.58 ±0.2	2.84 ±0.17	1.2 ±0.14
	2018	2.18 ±0.11	3.89 ±0.21	3.27 ±0.21	0.96 ±0.12
Watered (complete)	2014	2.1 ±0.15	3.37 ±0.21	2.9 ±0.16	1.17 ±0.14
	2015	2 ±0.16	3.44 ±0.18	2.44 ±0.18	2.28 ±0.14
	2016	2.93 ±0.14	4.57 ±0.19	2 ±0.1	1.75 ±0.11
	2017	3.47 ±0.13	4.63 ±0.15	3.53 ±0.18	2.44 ±0.12
	2018	3.37 ±0.12	5.26 ±0.17	5.11 ±0.2	2.63 ±0.12
Watered (partial)^	2014	NA	NA	NA	NA
	2015	NA	NA	NA	NA
	2016	NA	NA	NA	NA
	2017	1.89 ±0.12	2.81 ±0.17	3.24 ±0.2	0.76 ±0.14
	2018	2.21 ±0.13	4.05 ±0.27	3.59 ±0.15	0.1 ±0.05

* Based on two sites: Bitterang Stop Bank (2014-2018) and Boolca Track (2017-2018)
^ Bitternag Track was only partially watered.

Table A4.2 Plant cover (2014-2018) for the four existing sites established prior to 2018. Scores represent means (± standard error).

Treatment	Year	Forb (%)	Grass (%)	Shrub (%)	Tree (%)
*Unwatered	2014	79.25 ±20.64	10 ±1.44	53 ±0.58	7.5 ±1.44
	2015	4.75 ±0.72	2.5 ±0.29	66 ±2.89	10 ±0
	2016	3.25 ±1.3	0 ±0	31.5 ±0.87	12.5 ±1.44
	2017	14.38 ±4.11	7.5 ±1.06	43 ±2.01	16.25 ±3.15
	2018	13.38 ±4.48	4 ±2.04	31.13 ±4.95	13.75 ±2.39
Watered (complete)	2014	63.5 ±15.5	13.25 ±7.25	49.75 ±5.25	12.5 ±2.5
	2015	22.5 ±3.5	1.75 ±0.75	1.25 ±0.25	15 ±0
	2016	8.75 ±4.25	2.75 ±0.25	6.75 ±0.25	12.5 ±2.5
	2017	17.75 ±2.25	10.5 ±4.5	17.5 ±12.5	15 ±0
	2018	6.5 ±1	1 ±0	1.5 ±1	22.5 ±2.5
Watered (partial)	2014	NA	NA	NA	NA
	2015	NA	NA	NA	NA
	2016	NA	NA	NA	NA
	2017	5.25 ±1.75	0.75 ±0.75	130 ±11	37.5 ±7.5
	2018	7.5 ±1	0 ±0	40.75 ±10.25	10 ±5

* Based on two sites: Bitterang Stop Bank (2014-2018) and Boolca Track (2017-2018)

Table A4.3 Species richness (2014-2018) for the four existing sites established prior to 2018. Scores represent means (± standard error).

Site name	Year	Native	Exotic
*Unwatered	2014	18 ±2	4 ±1
	2015	11.5 ±1.5	0 ±0
	2016	10.5 ±0.5	0 ±0
	2017	12.25 ±1.11	0.75 ±0.48
	2018	12.5 ±2.1	4 ±0.41
Watered (complete)	2014	23 ±1	2.5 ±1.5
	2015	13 ±3	2 ±1
	2016	12.5 ±0.5	0 ±0
	2017	14 ±3	1 ±1
	2018	8.5 ±1.5	1 ±0
Watered (partial)	2014	NA	NA
	2015	NA	NA
	2016	NA	NA
	2017	11.5 ±1.5	1 ±0
	2018	12.5 ±0.5	1 ±0

* Based on two sites: Bitterang Stop Bank (2014-2018) and Boolca Track (2017-2018)

Table A4.4 Ground cover (2014-2018) for the four existing sites established prior to 2018. Scores represent means (± standard error).

Treatment	Year	Bare Ground (%)	Litter (%)	Log (%)	Soil Crust (%)
*Unwatered	2014	7.5 ±1.44	10 ±0	8 ±4.04	12.5 ±4.33
	2015	27.5 ±1.44	32.5 ±10.1	7.5 ±4.33	13.5 ±6.64
	2016	45 ±2.89	22.5 ±4.33	11 ±5.2	0 ±0
	2017	46.25 ±15.05	87.5 ±28.47	12.5 ±1.77	20.5 ±9.84
	2018	18.75 ±6.57	71.25 ±5.54	4 ±2.27	1.25 ±1.25
Watered (complete)	2014	17.5 ±12.5	10 ±5	7.5 ±2.5	20 ±15
	2015	82.5 ±2.5	15 ±0	7.5 ±2.5	0 ±0
	2016	77.5 ±2.5	22.5 ±2.5	7.5 ±2.5	0 ±0
	2017	70 ±0	30 ±0	10 ±5	0 ±0
	2018	77.5 ±2.5	22.5 ±2.5	4 ±1	0 ±0
Watered (partial)	2014	NA	NA	NA	NA
	2015	NA	NA	NA	NA
	2016	NA	NA	NA	NA
	2017	27.5 ±2.5	105 ±30	1 ±1	25 ±10
	2018	15 ±5	45 ±30	2.5 ±0.5	32.5 ±32.5

* Based on two sites: Bitterang Stop Bank (2014-2018) and Boolca Track (2017-2018)

Table A4.5 Reproduction/Health scores (2014-2018) for the four existing sites established prior to 2018. Scores represent means (± standard error).

Treatment	Sample month	Buds (score)	Flowers (score)	Fruits (score)	Broad tree health (score)
*Unwatered	Sept'15	NA	NA	NA	NA
	Oct'15	NA	NA	NA	NA
	Jan'16	0.75 ±0.16	0 ±0	1.25 ±0.25	1.88 ±0.23
	Feb'16	1.25 ±0.31	0 ±0	1.25 ±0.41	2.38 ±0.18
	Mar'16	1.5 ±0.33	0 ±0	1.38 ±0.38	2.5 ±0.19
	Apr'16	1.63 ±0.5	0.75 ±0.37	0.5 ±0.19	2.63 ±0.18
	May'16	1.75 ±0.37	1.13 ±0.4	1 ±0.27	2.25 ±0.31
	Sept'16	1.5 ±0.38	0.5 ±0.27	0.5 ±0.19	2.5 ±0.27
	Jan'17	1.88 ±0.23	0.13 ±0.13	0.75 ±0.25	2.5 ±0.27
	Mar'17	1 ±0.18	0.06 ±0.06	0.56 ±0.18	2.81 ±0.23
	May'17	1.75 ±0.21	0.25 ±0.19	0.63 ±0.2	2.13 ±0.13
	Jan'18	0 ±0	0 ±0	1 ±0.27	2.31 ±0.2
	Feb'18	0.13 ±0.09	0 ±0	1 ±0.24	2.38 ±0.18
	Apr'18	0.44 ±0.18	0 ±0	0.81 ±0.19	2.25 ±0.25
Watered (complete)	Sept'15	NA	NA	NA	NA
	Oct'15	NA	NA	NA	NA
	Jan'16	0.63 ±0.26	0 ±0	1.88 ±0.13	2.38 ±0.18
	Feb'16	0.5 ±0.19	0.5 ±0.27	2 ±0	3 ±0
	Mar'16	0 ±0	0.63 ±0.26	2.63 ±0.18	3.25 ±0.25
	Apr'16	0.5 ±0.19	0.25 ±0.16	1.75 ±0.25	3.13 ±0.23
	May'16	0.5 ±0.19	0 ±0	2.38 ±0.26	2.38 ±0.18
	Sept'16	1.88 ±0.23	0 ±0	0.25 ±0.16	2.63 ±0.32
	Jan'17	2 ±0.19	0 ±0	0.13 ±0.13	2.63 ±0.32
	Mar'17	2 ±0.27	2.13 ±0.3	1 ±0	3.63 ±0.18
	May'17	0 ±0	0.88 ±0.23	2.75 ±0.16	3.25 ±0.16
	Jan'18	2.63 ±0.18	0 ±0	0.75 ±0.25	3.25 ±0.31
	Feb'18	2.88 ±0.13	0.13 ±0.13	0.63 ±0.18	3.25 ±0.31
	Apr'18	2.38 ±0.32	2.38 ±0.26	2.25 ±0.25	3.75 ±0.16
Watered (partial)	Sept'15	NA	NA	NA	NA
	Oct'15	NA	NA	NA	NA
	Jan'16	NA	NA	NA	NA
	Feb'16	NA	NA	NA	NA
	Mar'16	NA	NA	NA	NA
	Apr'16	NA	NA	NA	NA
	May'16	NA	NA	NA	NA
	Sept'16	NA	NA	NA	NA
	Jan'17	NA	NA	NA	NA
	Mar'17	0.63 ±0.26	0.38 ±0.26	0.25 ±0.16	3.25 ±0.16
	May'17	0.5 ±0.19	1 ±0.19	0.75 ±0.25	2.38 ±0.18
	Jan'18	0 ±0	0 ±0	0 ±0	3 ±0.19
	Feb'18	0 ±0	0 ±0	0.13 ±0.13	3.13 ±0.23
	Apr'18	0 ±0	0 ±0	0.13 ±0.13	2.5 ±0.33

Appendix 5 – Model outputs

Table A5.1 Model estimates for all models reported in body of report. Parameters include cut-off points for ordinal data (e.g. Minimal to Sparse cut-off) and predictor variables (i.e. Year and Treatment).

			^95% credible interval	
Response variable	**Parameter**	**Estimate**	**Lower**	**Upper**
Site-level assessments				
Crown density	Minimal to Sparse cut-off	-2.367	-2.877	-1.871
	Sparse to Sparse-Medium cut-off	-1.051	-1.468	-0.646
	Sparse-Medium to Medium cut-off	0.027	-0.377	0.449
	Medium to Medium-Major cut-off	1.336	0.849	1.839
	Medium-Major to Major cut-off	1.326	0.599	2.058
	2015	-2.042	-2.656	-1.469
	2016	-2.17	-2.753	-1.587
	2017	0.01	-0.423	0.456
	2018	0.854	0.428	1.321
	Watered (fully)	0.715	0.398	1.041
	Watered (partial)	0.061	-0.35	0.468
Crown extent	Minimal to Sparse cut-off	-2.456	-3.027	-1.922
	Sparse to Sparse-Medium cut-off	-1.025	-1.445	-0.627
	Sparse-Medium to Medium cut-off	-0.378	-0.796	0.028
	Medium to Medium-Major cut-off	0.838	0.378	1.288
	Medium-Major to Major cut-off	2.132	1.561	2.711
	Major to Maximum cut-off	3.408	2.432	4.518
	2015	-0.726	-1.279	-0.189
	2016	0.781	0.264	1.298
	2017	0.493	0.048	0.925
	2018	1.454	0.984	1.931
	Watered (fully)	0.788	0.469	1.11
	Watered (partial)	-0.492	-0.918	-0.071
Extent reproduction	Absent to Scarce cut-off	-0.203	-0.638	0.246
	Scarce to Common cut-off	0.95	0.494	1.417
	Common to Abundant cut-off	3.043	2.404	3.745
	2015	0.29	-0.337	0.912
	2016	0.103	-0.447	0.689
	2017	1.469	0.943	2
	2018	0.743	0.245	1.26
	Watered (fully)	2.011	1.592	2.444
	Watered (partial)	-1.907	-2.449	-1.369
Broad tree health	<25% to 25-50% cut-off	-0.931	-1.377	-0.495
	25-50% to 50-75% cut-off	0.337	-0.08	0.755
	50-75% to >75% cut-off	2.156	1.592	2.726
	2015	-1.324	-1.96	-0.694
	2016	0.618	0.029	1.195
	2017	0.962	0.462	1.459
	2018	0.775	0.296	1.266
	Watered (fully)	1.121	0.754	1.497
	Watered (partial)	-0.867	-1.318	-0.38

Table A4.1 (continued)				
Tree-level assessments				
Fruit density	Intercept	2.403	1.894	2.854
	Watered (complete)	1.264	0.62	1.763
	Month	-0.013	-0.026	0.001
Seed density	Intercept	1.808	1.091	2.511
	Watered (complete)	1.45	0.446	2.369
	Month	-0.027	-0.041	-0.013

^ 95% credible intervals indicate the strength for a difference between different level of predictor variables against the baseline levels (i.e. Year – 2014, Treatment – Unwatered). If 95% CI does not include 0, a strong case for a difference can be implied.

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