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The applicability of stable carbon isotope analysis to measure the water use efficiency of Melaleuca Preissiana populations on the Swan Coastal Plain, Western Australia

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THE APPLICABILITY OF STABLE CARBON ISOTOPE ANALYSIS TO MEASURE THE WATER USE EFFICIENCY OF *MELALEUCA PREISSIANA* POPULATIONS ON THE SWAN COASTAL PLAIN, WESTERN AUSTRALIA.

by

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A Thesis Submitted in Partial Fulfilment of the

Requirements for the Award of

Bachelor of Science (Environmental Management) Honours

At the Faculty of Communications, Health and Science, Edith Cowan University,

Joondalup.

Date of submission: 29th November, 2000.

ABSTRACT

Stable carbon isotope ($\delta^{13}C$) analysis is a tool used to understand the water use efficiency (WUE) of plants. The advantage of measuring the carbon isotope composition in plant tissue over other measures of WUE (such as the ratio of photosynthesis to transpiration) is that it provides a *time-integrated*, rather than an *instantaneous* measure of plant-water relations. The purpose of this study was to evaluate the applicability of using $\delta^{13}C$ analysis in natural populations of the fringing tree *Melaleuca preissiana* based on short and long term sampling methods. There were three components to this study; a controlled, glasshouse experiment which established whether a relationship existed between $\delta^{13}C$ and instantaneous WUE in *M. preissiana*, and two field studies which evaluated the use of time-integrated, short and long term sampling methods of $\delta^{13}C$ in *M. preissiana* populations along a hydrological gradient.

The glasshouse experiment compared measures of instantaneous WUE and time-integrated $\delta^{13}C$ of *M. preissiana* seedlings undergoing a drying regime (two five-week drying intervals) to those in a control (regular watering). The seedlings undergoing the drying regime became more water use efficient (using instantaneous measures) as soil moisture decreased. Time-integrated $\delta^{13}C$ measurements of the same seedlings also increased significantly over the duration of the experiment. This established the positive relationship between $\delta^{13}C$ and instantaneous WUE in *M. preissiana*, and supported the inverse relationship between water availability and $\delta^{13}C$, which has been documented by many researchers. Light intensity was also a likely factor influencing the $\delta^{13}C$ discrimination of the seedlings. Future studies need to account for light variations when isolating water availability as the primary factor affecting $\delta^{13}C$.

Short term sampling for $\delta^{13}C$ of natural *M. preissiana* populations involved collecting recently-formed xylem tissue for isotopic analysis. Trees situated along a hydrological gradient (from areas of high to low relative water availability) at three wetlands were sampled for $\delta^{13}C$. $\delta^{13}C$ of trees decreased significantly from areas of low to high water availability. Seasonal sampling also showed that $\delta^{13}C$ of the trees decreased significantly from the dry to wet season. This suggested that short term $\delta^{13}C$ measurements indicated a physiological response in WUE in *M. preissiana,* due to spatial and temporal variations in water availability.

Long term $\delta^{13}C$ measurements involved sampling annual growth ring tissue from the same *M. preissiana* populations sampled for short term $\delta^{13}C$. This provided a record of the trees WUE across annual periods of growth. Direct relationships between ring $\delta^{13}C$ and historical water availability were not significant, however $\delta^{13}C$ of annual rings did indicate a physiological response to past, episodic events such as fire and prolonged drought periods.

From these results, it was concluded that short and long term $\delta^{13}C$ measurements of *M. preissiana* populations were indicative of spatial and temporal variations in water availability. It was supported that the degree of isotopic discrimination in *M. preissiana* was inversely related to water availability due to the various physiological processes occurring during carbon assimilation. The applicability of the approaches used for measuring $\delta^{13}C$ would increase by accounting for tree morphological factors such as age, size and health, and "external" environmental factors such as light intensity, all of which additionally influence $\delta^{13}C$ discrimination.

DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

i.   incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;

ii. contain any material previously published or written by another person except where due reference is made in the text; or

iii. contain any defamatory material.

Signature_ —

Date____ —

ACKNOWLEDGEMENTS

Finally, I would like to acknowledge the support and understanding provided by my friends and family. The particular interest, advice, tolerance, and friendship offered by my fellow honours colleagues Robyn Loomes, Kelli O'neill and Tara Reid made a difficult year seem not so hard after all!

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CHAPTER 1: INTRODUCTION

The relationship existing between plant physiology and the surrounding biophysical environment is highly complex and encompasses many interactive processes. Plant water relations involve the physiological functioning of the plant in response to water availability (Baird & Wilby, 1999). There are a variety of methods available for measuring plant water relations, many of which involve measuring the plant's physiological responses to changes in water supply on an instantaneous or short-term basis. One measure is the photosynthetic water use efficiency (WUE), which refers to the plant's capacity to conserve or retain water, and is defined by the ratio of net photosynthesis to transpiration (Lajtha & Marshall, 1994). This is a useful tool for understanding factors such as water stress in plants. However, traditional measures of WUE are instantaneous in nature, and therefore are subject to high diurnal variation (Lajtha & Marshall, 1994). A relatively recent tool adopted by scientists to understand the WUE in plants is measuring the abundance of stable carbon isotopes ($\delta^{13}C$) contained within plant tissue. This provides a longer, more time-integrated measure of plant WUE, and therefore a more reliable understanding of the plant's physiological use of water.

An understanding of plant water relations using measures such as WUE are becoming of increasing importance in the scientific community due to the detrimental effect that a depleting water supply has on the health and survival of plant communities. Increasing anthropogenic activity on the Swan Coastal Plain (SCP), Western Australia, has altered natural water regimes, and consequently resulted in the gradual drying of wetland

ecosystems (Froend *et al*, 1993). Surrounding vegetation has responded differently to these changes in water availability. Knowledge of the physiological responses of plant populations to variations in water availability around wetlands is a valuable tool for incorporating the vegetation component into wetland management.

The paperbark *Melaleuca preissiana* (Shauer) is a common tree species occurring around lake margins. Its distribution on the SCP is related to areas of high groundwater availability, and its health and survival are likely to be dependent on its physiological use of water. It is for this reason that *M. preissiana* is an ideal species to study physiologically using stable carbon isotope analysis, in its response to fluctuating water availability on the SCP.

1.1 CARBON DISCRIMINATION IN PLANTS

Approximately 98.89% of all carbon in nature exists as ^{12}C and the remaining 1.11% of carbon is ^{13}C (Boutton, 1991a). These two isotopes exist as a ratio (^{13}C/^{12}C) in natural materials as a result of isotopic fractionation during physical, chemical and biological processes. ^{13}C is discriminated *against* in biological systems because of its lower reactivity during these processes (Farquar *et al*, 1982). For instance, when conditions are optimal, ^{12}C will be used in preference to ^{13}C in biological systems (McNulty & Swank, 1995). However, when conditions are less than optimal, more ^{13}C may be used as alternative. This affects the ^{13}C/^{12}C ratio contained within living matter.

The isotopic compositions of carbon are often referred to as delta values (δ^{13}C), specifying

the $^{13}C/^{12}C$ ratio of a material relative to a standard. This is calculated by the equation:

$$\delta^{13}C\ (\permil) = [R_{sample} / R_{standard} - 1] \times 10^3$$

where $\delta^{13}C$ is the parts per thousand (‰) difference between the ^{13}C content of a sample to a standard, and R is the mass ratio of the sample or standard gas (Farquar *et al*, 1992). $\delta^{13}C$ values are expressed relative to the calcium carbonate standard Pee Dee Belemnite (PDB), which has a $^{13}C/^{12}C$ ratio of 0.0112372 (Boutton, 1991a). The $\delta^{13}C$ value of a material therefore indicates whether the sample has a higher or lower $^{13}C/^{12}C$ ratio than PDB.

$\delta^{13}C$ values of natural materials range from 0‰ to −110‰. The $\delta^{13}C$ value for carbon dioxide in the atmosphere lies at about -7.8‰, indicating the smaller proportion of ^{13}C relative to ^{12}C. This value is influenced by the atmosphere – ocean exchange of CO_2, global patterns of photosynthesis and respiration by plants, and human activities (Boutton, 1991b).

The $\delta^{13}C$ signature contained by plant tissue is a result of carbon uptake during photosynthesis, and is highly dependent on its photosynthetic pathway. Plants utilising the C_3 pathway of photosynthesis discriminate against the heavier isotope through two components. The diffusion of CO_2 from the atmosphere into the plant via the stomatal openings on the leaf's surface causes a fractionation of approximately -4.4‰. More evidently, isotopic discrimination of carbon occurs during carbon fixation, when C_3 plants reduce CO_2 to a 3-C compound via the enzyme ribulose-1,5 bisphosphate (RuP_2) carboxylase (otherwise known as rubisco). Carbon fixation results in a fractionation of about −27‰ (Ehleringer, 1993). The $\delta^{13}C$ signature of a C_3 plant expressed during photosynthesis is demonstrated by the equation:

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$$\delta^{13}C_{leaf} = \delta^{13}C_{atmosphere} - a - (b-a)c_i/c_a$$

where $\delta^{13}C_{atmosphere}$ is $-7.8‰$, a is the fractionation caused by atmospheric CO_2 diffusion ($-4.4‰$), b is the fractionation caused by carbon fixation ($-27‰$) and c_i/c_a is the ratio of intercellular to atmospheric CO_2 concentration (Farquar $et\ al$, 1992). The balance between a and b yields leaf $\delta^{13}C$ values of approximately $-27‰$. Plants utilising C_4 and CAM (Crassulacean Acid Metal) photosynthetic pathways differ slightly due to the different enzymatic processes occurring during carbon fixation. C_4 and CAM plants average $\delta^{13}C$ values of $14‰$ and $11‰$ respectively (Lajtha & Marshall, 1994).

1.2 $\delta^{13}C$ AND PHOTOSYNTHETIC WATER USE EFFICIENCY IN PLANTS

One of the most common biological uses of carbon isotope ratios is as a measure of photosynthetic water use efficiency (WUE) of C_3 plant species. WUE is defined as the ratio of net photosynthesis (A) to transpiration (E). Physical factors such as temperature, solar irradiation, nutrients and water availability influence the isotopic ratio via their effect on the intercellular CO_2 concentration during photosynthesis (Saurer $et\ al$, 1995). Water availability in particular is a major factor determining the $\delta^{13}C$ value of a plant due to its direct relationship with CO_2 exchange (Livingston & Spittlehouse, 1996). In conditions of low water availability, such as a drought, stomatal closure occurs. As a result, water loss is reduced and there is a decrease in intercellular carbon levels. This causes an increase in the CO_2 concentration gradient moving into the leaf (A) and an overall increase in A/E (Lajtha & Marshall, 1994). The isotopic response is a higher $^{13}C/^{12}C$ or more positive $\delta^{13}C$. When water availability is high, leaf stomatal conductance increases, resulting in higher

intercellular carbon levels. Therefore the ratio of *A/E* decreases due to the abundance of water and carbon, resulting in lower $^{13}C/^{12}C$, or more negative $\delta^{13}C$. This negative relationship between WUE and $\delta^{13}C$ has be experimentally demonstrated for numerous species (e.g. Farquhar *et al*, 1982; Ehleringer & Cooper, 1988; Körner *et al*, 1991; Leavitt, 1992; Stewart *et al*, 1995; Livingston & Spittlehouse, 1996; Berry *et al*, 1997; Damesin *et al*, 1997; Walcroft *et al*, 1997; MacFarlane & Adams, 1998; Pate & Arthur, 1998).

Traditional WUE measures have relied on gas-exchange analysis to measure *A* and *E*. This technique measures WUE on an instantaneous basis, indicating the leaf WUE for that moment in time. This provides limited insight into the overall productivity and fitness of the individual due to the rapid variations in photosynthesis and transpiration rates in response to environmental conditions (Ehleringer, 1993). Long term estimates of WUE are difficult to make using this method because gas exchange rates change over the life time of the leaf (Lajtha & Marshall, 1994). Carbon isotope analysis is a more appropriate and efficient means of determining the long-term WUE of plant tissue. $\delta^{13}C$ is recorded in all plant tissue as a time-averaged estimate of WUE over the lifetime of the tissue. By measuring $\delta^{13}C$, spatial and temporal variability is reduced by integrating over all seasons of carbon gain (Leffler & Evans, 1999). Additionally, it is possible to sample many plants simultaneously for isotopic compositions, which allows for a greater degree of replication. This is not possible using standard gas exchange techniques (Boutton, 1991b).

1.3 APPLICATIONS OF STABLE CARBON ISOTOPE MEASUREMENTS IN PLANTS

Due to the proven link between $\delta^{13}C$ and WUE, and their direct relationship with environmental variables, it is possible to make the assumption that $\delta^{13}C$ measurements in most C_3 species can be applied as actual measurements of WUE. Additionally, $\delta^{13}C$ values contained in plant tissue can be indicative of the environmental conditions occurring during tissue formation. These applications of $\delta^{13}C$ measurements have been widely adopted by researchers and are reviewed below.

Leaf tissue is one of the most common plant tissues used for $\delta^{13}C$ analysis and its measure of WUE is dependent on the age of the leaf (McNulty & Swank, 1995). This is a particularly useful measure for assessing the WUE of annual and perennial species. In an agronomic context, the $\delta^{13}C$ of annual crop species (such as wheat) has been used to assist in the selection of water use efficient individuals in breeding programs (Farquar & Richards, 1984; Matus *et al*, 1997). There is also extensive literature available on the use of $\delta^{13}C$ to assess the WUE of perennial desert plants, which are highly dependent on the availability of water for growth and survival (e.g. Ehleringer & Cooper, 1998).

There are variations existing in leaf $\delta^{13}C$ and light intensity gradients. Berry *et al* (1997) found that in a forest ecosystem where light is a limiting factor, leaf tissue sampled near the forest floor had lower $\delta^{13}C$ values than samples located near the top of the canopy where light is abundant. This reflects the strong influence that light intensity has on C_i/C_a and subsequently $\delta^{13}C$. Branch length is also a contributing factor in determining leaf $\delta^{13}C$,

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where the foliage from long branches of *Pinus sp.* was found to be isotopically heavier than the foliage from shorter branches due to the effect of reduced hydraulic conductance (Warren & Adams, 2000). Other factors such as air temperature and atmospheric pressure were found by Körner *et al* (1991) to affect leaf ^{13}C discrimination in alpine plant species.

Wood is an alternative tissue used for the sampling of ^{13}C. The carbon contained in stem wood integrates the variability in leaf carbon assimilation to reveal long term trends in $\delta^{13}C$ (Walcroft *et al*, 1997). Similarly to leaf tissue, wood tissue contains a record of $\delta^{13}C$ as a time-averaged estimate of the WUE of the plant over the lifetime of the tissue (Leffler & Evans, 1998). The tissue from annual growth rings contained in a tree stem persists indefinitely. Therefore, one annual ring contains a time-averaged, integrated record of the $\delta^{13}C$, or WUE, of the tree during that period of growth. Recent studies have investigated changes in the $\delta^{13}C$ of annual ring tissue of trees and related these to historic environmental conditions including water availability. Water availability influences the $\delta^{13}C$ of wood by altering leaf stomatal conductance and intercellular carbon levels (McFarlane & Adams, 1998). This in turn influences the supply of photosynthates and auxins to the cambium of the tree. Therefore, growth rings exhibiting more positive $\delta^{13}C$ signatures usually developed during years when water availability was low (e.g. low precipitation, low soil moisture content, high temperature), and more negative $\delta^{13}C$ values occurred during "wetter" years when water availability was higher (Lipp *et al*, 1991; Leavitt, 1992; McNulty & Swank 1995; Saurer *et al*, 1995; Livingston & Spittlehouse, 1996; Walcroft *et al*, 1997).

$\delta^{13}C$ signatures have also been correlated with tree growth, with the ^{13}C content of tree rings being more negative during years when basal area increment was high (Leavitt & Long, 1986; Leavitt & Long, 1989; Dupouey *et al*, 1993; McNulty & Swank, 1995). This suggests that more negative $\delta^{13}C$ in wood tissue forms during years in which the environmental conditions were more favorable for tree growth. Macfarlane & Adams (1998) found that this theory had important implications for the management of *Eucalyptus globulus* plantations in the southwest of Western Australia, where $\delta^{13}C$ could be used to infer the appropriate conditions required for rapid tree growth.

As an alternative, Pate & Arthur (1998) investigated novel means of obtaining $\delta^{13}C$ signatures using tissue types other than wood and leaf material, to obtain water relations data for *E. globulus* stands. As well as collecting samples from mature leaf and wood tissue, $\delta^{13}C$ signatures were analysed from phloem sap obtained through cambial-deep incisions made into the tree's trunk, and nascent xylem tissue taken from outer cambium layers of the tree stem. These sampling techniques proved to be simple and reliable means of obtaining time-integrated measures of $\delta^{13}C$.

To date, there have been very few studies in wetland environments focused on the use of $\delta^{13}C$ analysis *within* tree populations to gain an understanding of their response to spatial and temporal variations in water availability through time. Leffler & Evans (1999) recently investigated the environmental parameters affecting $\delta^{13}C$ in riparian tree populations of *Populus fremontii* in the United States. For this species, stream flow was found to be the major factor determining its water use efficiency through time. This fact alone is an

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important implication for improving the management of not only populations of this species, but also the riparian ecosystem. The high occurrence of wetland ecosystems throughout the SCP and their decreasing trends in water availability provide reason for requiring an understanding of the physiological responses of fringing tree species.

1.4 SIGNIFICANCE AND AIMS OF THIS STUDY

The commonly occurring tree species *M. preissiana* that fringes lake margins on the Swan Coastal Plain is responsive to fluctuations in water availability. Froend *et al* (1993) recognise that the recruitment of the species is not a common occurrence, and rather is a response to episodic events such as fire and raised groundwater levels. There is very little research focused on the water use efficiency of fringing tree species around wetlands on the SCP and their response to fluctuations in water availability. In order to gain an understanding of the complex relationship between plant physiology and water availability in a wetland environment, it is of significance to apply $\delta^{13}C$ measurements to fringing tree populations such as *M. preissiana.*

The overall aim of this study was to evaluate the applicability of stable carbon isotope analysis as a time-integrated measure of the water use efficiency of natural populations of *M. preissiana* on the Swan Coastal Plain, and discuss its relevance to wetland management. The purpose was to report findings regarding the variations in $\delta^{13}C$ occurring within *M. preissiana* populations and assess the applicability of the approaches used by identifying the qualities and constraints involved. Specific objectives are as follows:

a) Assess the reliability of $\delta^{13}C$ to measure the water use efficiency of *M. preissiana* in a controlled environment;

b) Evaluate the applicability of time-integrated, short term $\delta^{13}C$ measurements in understanding the water use efficiency of natural populations of *M. preissiana* in a wetland environment;

c) Evaluate the applicability of time-integrated, long-term $\delta^{13}C$ measurements in understanding the historical water use efficiency of natural populations of *M. preissiana* in a wetland environment.

These objectives are addressed in separate chapters in this report, and are discussed in terms of the relevance of $\delta^{13}C$ analysis as a tool in wetland management on the SCP.

CHAPTER 2: GLASSHOUSE EXPERIMENT

2.1   INTRODUCTION

The ability of $\delta^{13}C$ to indicate the water use efficiency of a plant in response to water availability has been well documented in the existing literature (e.g. Ehleringer & Cooper, 1988; Körner *et al*, 1991; Leavitt, 1993; Stewart *et al*, 1995; Livingston & Spittlehouse, 1996; Berry *et al*, 1997; Damesin *et al*, 1997; Walcroft *et al*, 1997; MacFarlane & Adams, 1998; Pate & Arthur, 1998). Farquar *et al* (1982) demonstrate the theory of carbon isotope discrimination in C_3 plants on the basis of the activity of the primary carboxylating enzyme rubisco during photosynthesis. Rubisco discriminates against the heavier isotope ^{13}C more so during carbon fixation when water availability is high, due to the higher intercellular carbon levels and higher abundance of $^{12}CO_2$ entering the leaf. When water availability is low, and stomatal conductance decreases, the lower supply of $^{12}CO_2$ entering the leaf means that rubisco must fix a higher proportion of $^{13}CO_2$ than preferred. Therefore, a relationship exists, where higher $^{13}C/^{12}C$ (or $\delta^{13}C$) indicates higher water use efficiency, and low $\delta^{13}C$ indicates lower water use efficiency. Both $\delta^{13}C$ and WUE are inversely related to water availability.

The relationship between $\delta^{13}C$, WUE and water availability has been experimentally demonstrated for numerous tree species, many of which are Northern Hemisphere softwood varieties. In terms of native Australian trees, a number of *Eucalyptus* species have also been shown to demonstrate this relationship (Le Roux *et al*, 1996; Macfarlane & Adams, 1998;

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Pate & Arthur, 1998; Pate *et al*, 1998). However, there is no proven evidence that fringing wetland tree species in the southwest of Western Australia exhibit this relationship, and there have been no previous studies investigating $\delta^{13}C$ and WUE in *Melaleuca preissiana*.

This study attempts to prove that a relationship exists between $\delta^{13}C$ and WUE in *M. preissiana* in water-limiting conditions. By investigating this relationship between plant $\delta^{13}C$ and WUE in a controlled environment (e.g. a glasshouse), the knowledge can be applied to naturally occurring populations of *M. preissiana* in wetland environments.

The aim of this study was to assess whether $\delta^{13}C$ is a reliable measure of WUE in *M. preissiana* under experimental conditions. A number of specific research objectives were investigated. These were as follows:

i. Investigate the variations in the instantaneous gas exchange rates (A, E, and g_s) of *M. preissiana* in response to water availability;

ii. Investigate the variations in the instantaneous WUE of *M. preissiana* in response to water availability;

iii. Investigate the variations in the $\delta^{13}C$ of *M. preissiana* in response to water availability;

iv. Examine the relationship between $\delta^{13}C$ and instantaneous WUE in *M. preissiana*;

v. Identify the external factors (other than water availability) that influence $^{13}C/^{12}C$ in *M. preissiana*.

2.2 MATERIALS AND METHODS

A controlled glasshouse experiment was constructed to confirm that a significant relationship existed between the $\delta^{13}C$ signature and photosynthetic WUE of *M. preissiana*. It was necessary to examine this relationship in a controlled environment before sampling natural populations of the species in order to identify the qualities and constraints involved with using this approach.

2.2.1   Description of the Experiment

This experiment involved studying 144 six-month *M. preissiana* seedlings, which were divided into a control and a treatment, with 72 plants in each (Plate 2.1). Seedlings in the control were maintained under glasshouse conditions for ten weeks, with temperature moderated at approximately 22°C and watering twice daily. Each plant received approximately 330ml of water each day. Plants in the treatment underwent a drying regime, where plants were maintained under similar glasshouse conditions to the control, however watering was excluded for two five-week periods. This was achieved by constructing a "mini glasshouse" within the glasshouse, where the 72 seedlings in the treatment were surrounded by clear, plastic drop sheets attached to the ceiling, in order to exclude watering from surrounding sprinklers. Five weeks of drying resulted in the seedlings displaying signs of water stress such as wilting and discolouration of leaves. This time period also resulted in significant drying of surface and bottom soil in the pots. After the five-week drying period, seedlings were watered to saturate soils and were dried for another five weeks. All

seedlings were potted in 180mm standard black pots using white, coarse grained sand, and a thin layer of pea gravel to line the bottom of the pots to assist in drainage. Additionally, at the time of potting each seedling was fertilised with one tablespoon of native slow release fertiliser. During the 10 week period, a series measurements were taken from plants in both control and treatment to test the hypothesis that photosynthetic WUE and $\delta^{13}C$ signatures are related in *M. preissiana*.

Plate 2.1: Set up of *M. preissiana* seedlings in glasshouse. 72 seedlings contained in control and treatment. Watering was excluded periodically from the treated seedlings, which were enclosed by clear, plastic sheeting (pictured in background). Controlled seedlings (pictured in foreground) were watered daily.

2.2.1.1 Experimental Design

In order to test for the relationship between $\delta^{13}C$ and WUE of the seedlings, a variety of instantaneous and integrated measures were taken including soil moisture content, instantaneous gas exchange and WUE, xylem pressure potential (XPP), $\delta^{13}C$ using foliar

samples and $\delta^{13}C$ using nascent xylem tissue samples. The timing of the experiment was designed so that the treated seedlings were dried to the extent that they showed signs of water stress, in order to enable repeatability of the experiment and ensure sufficient sampling for $\delta^{13}C$. During this period the series of measurements were taken over regular intervals to quantitatively show the physiological response of the treated seedlings to the drying regime in comparison to the control. The timing of measurements for the glasshouse experiment is presented in Table 2.1.

Table 2.1: Design and timing of various measurements for 10-week glasshouse experiment. The treatment underwent a drying regime where *M. preissiana* seedlings were dried for five weeks, rewatered once, then dried again for 5 weeks. The control seedlings were watered regularly for five minutes twice daily. The numbers within the table indicate the number of seedlings used per control/treatment during each week, for each particular measure. X indicates the weeks during which watering occurred for the control and treatment. Week 0 refers to the time period prior to the commencement of the experiment.

| | Week Number | | | | | | | | | | |
	0	1	2	3	4	5	6	7	8	9	10
Watering – Control	X	X	X	X	X	X	X	X	X	X	X
Watering – Treatment	X					X				*	
Soil Moisture Content	3		3		3	3	3	3		3	
Gas exchange measurements		12	12		12	12		12	12		12
XPP measurements	6			6			6			6	
Nascent xylem tissue samples for $\delta^{13}C$	6				6				6		
Foliar tissue samples for $\delta^{13}C$	12		12		12				12		12

* A light rewatering occurred in the treatment in week nine. This was to prolong the experiment to ensure sufficient sampling of $\delta^{13}C$ during week 10.

Repeated measures were carried out on seedlings measured for instantaneous gas exchange and seedlings sampled for $\delta^{13}C$ from foliar tissue. This is because of the non-destructive nature of these methods, which therefore allowed for repeated sampling/measuring of the same seedlings. Xylem pressure potential (XPP) measures and sampling for $\delta^{13}C$ from nascent xylem tissue caused some destruction, and therefore could not be repeatedly measured/sampled from the same seedlings. Number allocation of replicate seedlings used

for each measure was based on the total number of seedlings (72 seedlings each for the control and treatment) and the largest number of replicates were allocated to measures that were considered to be of importance in meeting the aims of the experiment. Irregular sampling over weeks occurred due to weather conditions (gas exchange measurements required full sunlight) and due to the supply of tissue for the sampling of $\delta^{13}C$ (after prolonged drying, seedlings in the treatment irregularly produced new foliage).

2.2.1.2   Sampling/Measurement Techniques

2.2.1.2.1 *Soil moisture*

At one to two weekly intervals, the soil from three replicate pots in the control and treatment were measured for moisture content. Approximately 30 grams of soil from the surface, middle and bottom of the pots were weighed, oven dried for 48 hours at 105°C and weighed again in order to compare wet and dry weights, and calculate the % moisture of the soil. The resulting soil saturation values for top, middle and bottom of each pot were averaged in order to determine the mean soil moisture content (%).

2.2.1.2.2 *Instantaneous gas exchange measurements*

In both control and treatment, gas exchange measures were taken on weekly to fortnightly intervals on selected seedlings in order to determine changes in instantaneous WUE over the duration of the experiment. This involved using a portable differential infra-red gas exchange analyser (IRGA) (model LCI-3, Analytical Development Co., Hoddesdon, UK) to sample a combination of new and mature leaves from 12 seedlings for measuring leaf photosynthesis rate (A μmol m^{-2}s^{-1}), transpiration rate (E mol m^{-2}s^{-1}), stomatal conductance

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$(g_s$ mol m^{-2}s$^{-1})$ and intercellular carbon level $(C_i \mu$mol mol$^{-1})$. WUE was calculated based on the ratio of *A/E*. Measurements were taken mid-morning (10am – 11am) in sunny conditions.

2.2.1.2.3 *Xylem pressure potential*

The xylem pressure potential (XPP) was measured for selected plants in both the control and the treatment. This is a technique of measuring plant water stress using a plant water status chamber (Model 3000, Soil Moisture Equipment Corp, Santa Barbara, California). The method consisted of removing a single stem (approximately 20cm length) from the seedling and sealing it inside the pressure chamber. Pressure was applied inside the chamber until sap exuded from the cut end of the stem. The pressure (MPa) required for exudation was measured, providing an indication of the negative pressure of the moisture stress within the plant at the time of cutting (Milburn, 1979). Three replicates from the control and treatment were measured for xylem pressure potential at pre-dawn (6:00am) and midday (12:00pm), at two to three weekly intervals.

2.2.1.2.4 *δ^{13}C measurements*

Plant tissue was taken from selected seedlings in order to sample δ^{13}C signatures using two short-term sampling methods. Integrated, short-term methods for measuring δ^{13}C involved sampling recently formed plant tissue, in order to obtain a time-averaged measure of WUE of the plant for the duration of tissue formation. Therefore, the new tissue formed over two weeks (such as new leaf matter) contained an integrated δ^{13}C value indicative of the WUE of the plant over the two week period. The two methods adopted for measuring short-term

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$\delta^{13}C$ measurements are described below.

2.2.1.2.4.1 Foliar sampling

In both control and treatment, the most recently developed leaves (1mm-2mm in length) were removed, dried at 40°C for 24 hours and subjected to $\delta^{13}C$ analysis. The resulting delta signatures represented an average, time-integrated measure of the plant's WUE for the lifetime of the leaf, presumably one to two weeks. This was conducted fortnightly in order to gain $\delta^{13}C$ signatures of the plant for the duration of the experiment.

2.2.1.2.4.2 Nascent xylem tissue sampling

In all seedlings, nascent stem xylem tissue was collected for $\delta^{13}C$ analysis. The thin layers of bark and cambium were removed from the stem using a scalpel blade, and the thin, gelatinous layer of recently formed xylem tissue was collected and placed into vials containing 80% ethanol for a period of three weeks, during which the ethanol was replaced three times. The xylem tissue was oven dried at 75°C for 48 hours after which the remaining woody residue was subjected to $\delta^{13}C$ analysis. Sampling was conducted every four weeks in order to account for the time taken for the phloem-derived carbon that supplied the cambium to become incorporated into the xylem tissue (Pate & Arthur, 1998). Therefore the $\delta^{13}C$ value derived from this tissue represented the WUE of the seedling from approximately four weeks ago.

2.2.1.3 $\delta^{13}C$ Analysis

Samples of dried nascent xylem tissue and foliar tissue were ground to a fine pulp manually. Wood was weighed to 1-1.5mg samples ($\pm$ 0.1mg) and placed in small foil

capsules. The $\delta^{13}C$ signature of the samples was determined using a ^{13}C analyser mass spectrometer (ANCA-GSL, Europa Scientific, Crewe, UK). Wood samples were analysed relative to the PDB standard using a scientific flour (40.37%C, $\delta PD4$ -25.34; Europa Scientific) as the laboratory standard. Whole wood samples were analysed for $\delta^{13}C$. Chapter 4 identifies a method for extracting the crude cellulose from wood for analysis, in order to reduce isotopic variations that may occur due to different proportions of lignin and hemicelluloses occurring within different samples. The strong positive relationship existing between the $\delta^{13}C$ of whole wood and $\delta^{13}C$ of cellulose in *M. preissiana* nascent xylem tissue of mature trees (proven in Chapter 4) meant that the cellulose extraction procedure was unnecessary. $\delta^{13}C$ signatures were expressed in parts per thousand (‰).

2.2.1.4   Data Analysis

To understand the changes occurring in seedling gas exchange, instantaneous WUE, XPP and $\delta^{13}C$ over the duration of the experiment, measurements were analysed descriptively in order to detect trends. Average values and standard error for each measure were calculated and displayed graphically over time in order to determine the physiological changes occurring in the treated seedlings in comparison to the control. Additionally, repeated measures analysis of variance (ANOVA) using SPSS Version 10.0 were used to identify significant differences in $\delta^{13}C$ and instantaneous WUE of the *M. preissiana* seedlings according to experiment (control and treatment) and to weeks (each sampling period). Two-way ANOVA was conducted to identify *overall* differences occurring between experiments and between weeks. If a significant interaction existed between the two factors, individual

samples t-tests were conducted to examine where the differences existed between individual sampling periods. Additionally, one-way ANOVA identified *specific,* rather than *overall,* differences existing over the duration of the experiment within both the control and the treatment. The Bonferroni test was applied to make post-hoc comparisons where significant relationships existed. Before all analyses, Levene's test for equality of variances and Mauchly's Test of Sphericity were conducted to test for the relevant assumptions, and appropriate data transformations were made if necessary. $\delta^{13}C$ and WUE were used for ANOVA due to the emphasis of this study being on the relationship between the two.

2.3   RESULTS

2.3.1   Soil Moisture

Over each five week drying interval, it was found that on average, the soil moisture of the treated seedlings fell from approximately 11% by weight at the beginning of the experiment (week 0) to below 2% after five weeks of drying (week 5 and week 9) (Figure 2.1). In comparison, over the duration of the experiment, the mean soil moisture content of the control fluctuated slightly, with values ranging from 10.4% (week 2) to 12.4% (week 4).

Figure 2.1: Mean Soil Moisture Content of *M. preissiana* seedlings under glasshouse conditions. Control seedlings were watered twice daily and treated seedlings underwent a drying regime where
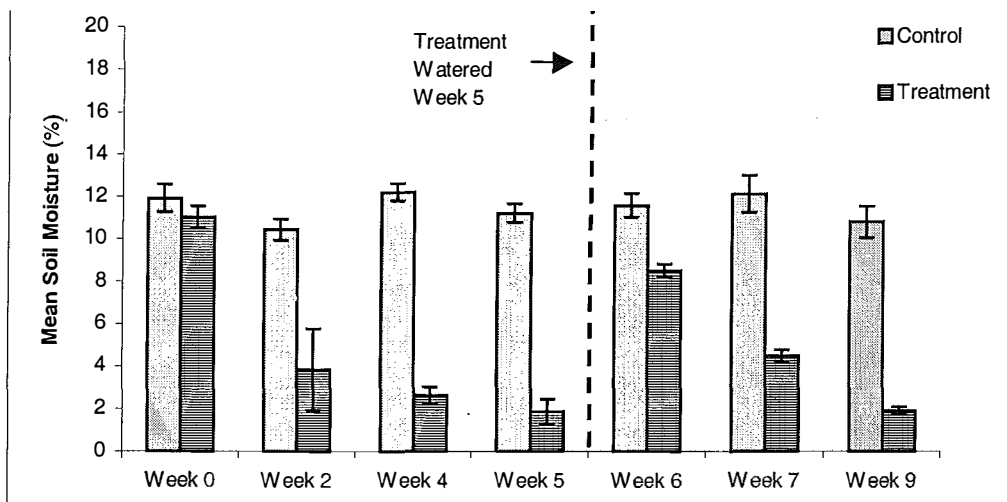
seedlings were dried for five weeks then rewatered. Mean soil saturation values represent average moisture content of soil taken from the top, middle and bottom of three seedling pots. Error bars represent the standard error within means.

2.3.2   Physiological Changes in *M. preissiana* Seedlings

2.3.2.1   Instantaneous Gas Exchange Measures

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The average rate of photosynthesis (*A*) for the watered seedlings remained fairly constant throughout the duration of the experiment with rates ranging from $0.25\mu\text{mol m}^{-2}\text{s}^{-1}$ (week 10) to $1.08\mu\text{mol m}^{-2}\text{s}^{-1}$ (week 8) (Figure 2.2). The rate of photosynthesis of the treatment was smaller than that of the control in the first five weeks of the experiment, with the seedlings displaying the lowest *A* values in week 5a (before watering) and 5b (after watering) of $0.20\mu\text{mol m}^{-2}\text{s}^{-1}$ and $0.11\mu\text{mol m}^{-2}\text{s}^{-1}$ respectively. This corresponded to the period during which the seedlings may have been most water-stressed. Following week 5, photosynthesis rates increased up to $1.37\mu\text{mol m}^{-2}\text{s}^{-1}$ in week 8.
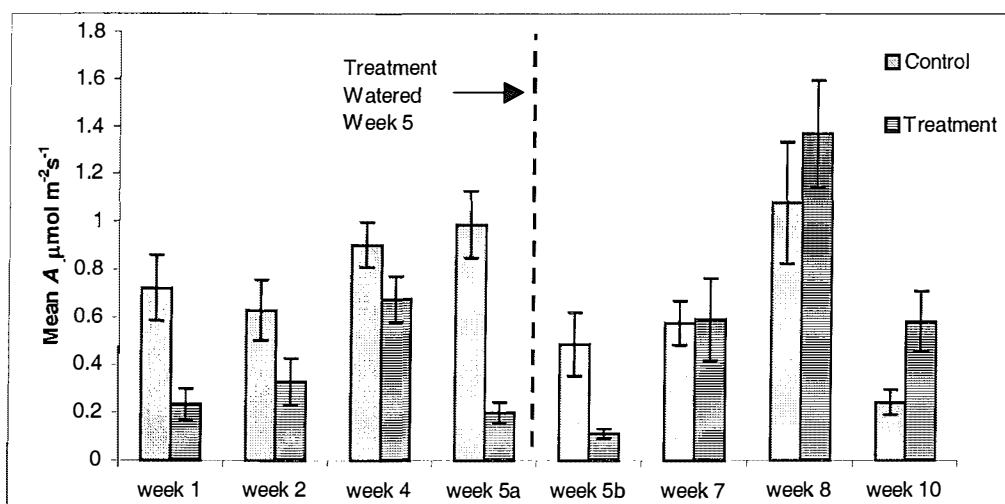
Figure 2.2: Mean photosynthesis rates (*A*) of *M. preissiana* seedlings under glasshouse conditions. Control seedlings were watered twice daily and treated seedlings underwent a drying regime where seedlings were dried for five weeks then rewatered. Mean *A* values represent average photosynthesis rate taken from 12 seedlings. Week 5a refers to measurements taken in week 5 *prior* to rewatering; week 5b refers to measurements taken in week 5 *following* rewatering. Error bars represent the standard error within means.

The mean transpiration rates (*E*) of the *M. preissiana* seedlings in the control were also relatively consistent throughout the ten-week experiment. With the exception for weeks 7 and 10 where transpiration rates of the seedlings were lower than other weeks, *E* values ranged from $2.65\text{mol m}^{-2}\text{s}^{-1}$ in week 5b to $3.16\text{mol m}^{-2}\text{s}^{-1}$ in week 1 (Figure 2.3). In comparison, the seedlings undergoing the drying regime displayed lower transpiration rates

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than the control in most weeks. In week 5, both before and after watering, seedlings were transpiring at their lowest levels at less than $1 \text{mol m}^{-2}\text{s}^{-1}$. The low rates of photosynthesis and transpiration in week 5a (before rewatering) corresponded to the low soil moisture content of the treated seedlings. However, re-watering (week 5b) did not appear to cause an immediate increase in either of the gas exchange rates.

Figure 2.3: Mean transpiration rates (E) of *M. preissiana* seedlings under glasshouse conditions. Control seedlings were watered twice daily and treated seedlings underwent a drying regime where
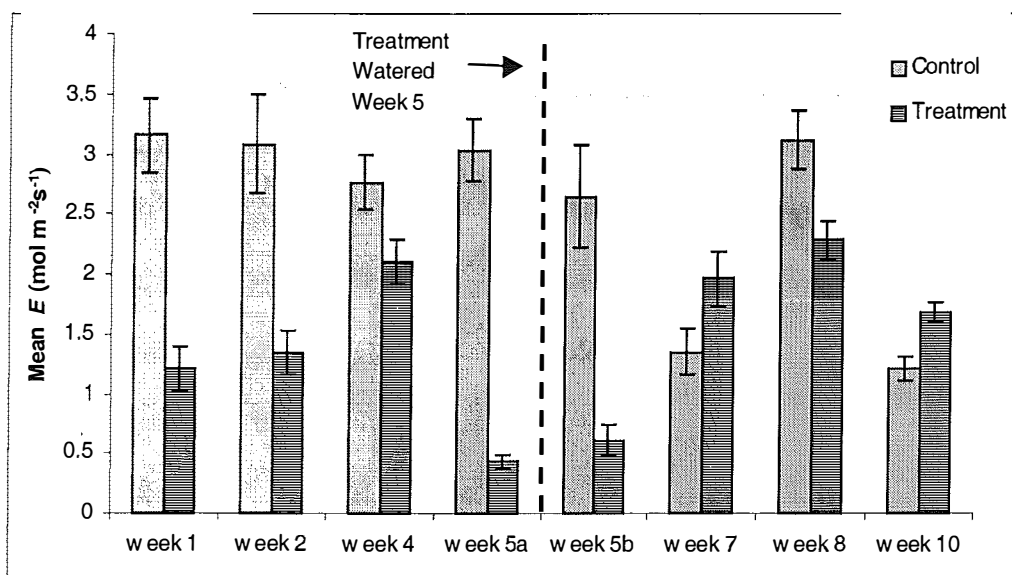
seedlings were dried for five weeks then rewatered. Mean E values represent average transpiration rate taken from 12 seedlings. Week 5a refers to measurements taken in week 5 *prior* to rewatering; week 5b refers to measurements taken in week 5 *following* rewatering. Error bars represent the standard error within means.

There were no obvious trends or differences in the average intercellular carbon levels (C_i) of both watered and dried seedlings (Figure 2.4). The mean C_i levels of the treatment fluctuated above and below that of the control over the duration of the experiment. The dried seedlings displayed the lowest carbon levels of $340 \mu\text{mol mol}^{-1}$ in week 5 following re-watering, while the control were of the highest C_i of $355 \mu\text{mol mol}^{-1}$ in week 2.
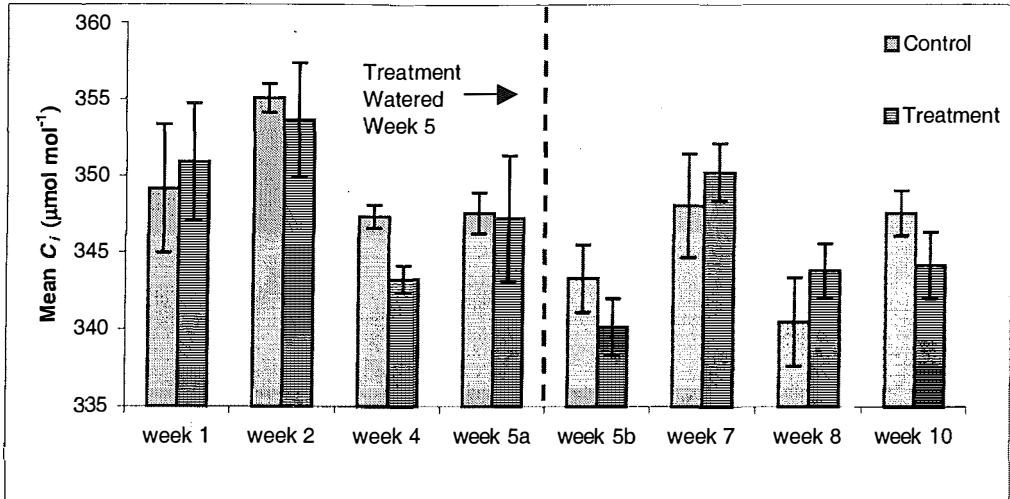
Figure 2.4: Mean intercellular carbon levels (C_i) of *M. preissiana* seedlings under glasshouse conditions. Control seedlings were watered twice daily and treated seedlings underwent a drying regime where seedlings were dried for five weeks then rewatered. Mean C_i values represent average intercelluar carbon level taken from 12 seedlings. Week 5a refers to measurements taken in week 5 *prior* to rewatering; week 5b refers to measurements taken in week 5 *following* rewatering. Error bars represent the standard error within means.

The watered seedlings displayed variations in stomatal conductance throughout the duration of the experiment, with weeks 2, 4 and 5a supporting the highest g_s values, of greater than 0.4mol m^{-2}s^{-1} (Figure 2.5). For the first five weeks of the drying regime, the treated seedlings displayed lower g_s values than the control, with values decreasing below 0.05mol m^{-2}s^{-1} both before and after rewatering in week five. Weeks 7-10 saw a rise in stomatal conductance for the seedlings under the drying regime with rates increasing to a maximum of 0.38mol m^{-2}s^{-1} in week 8.

Figure 2.5: Mean stomatal conductance (*g_s*) of *M. preissiana* seedlings under glasshouse conditions.
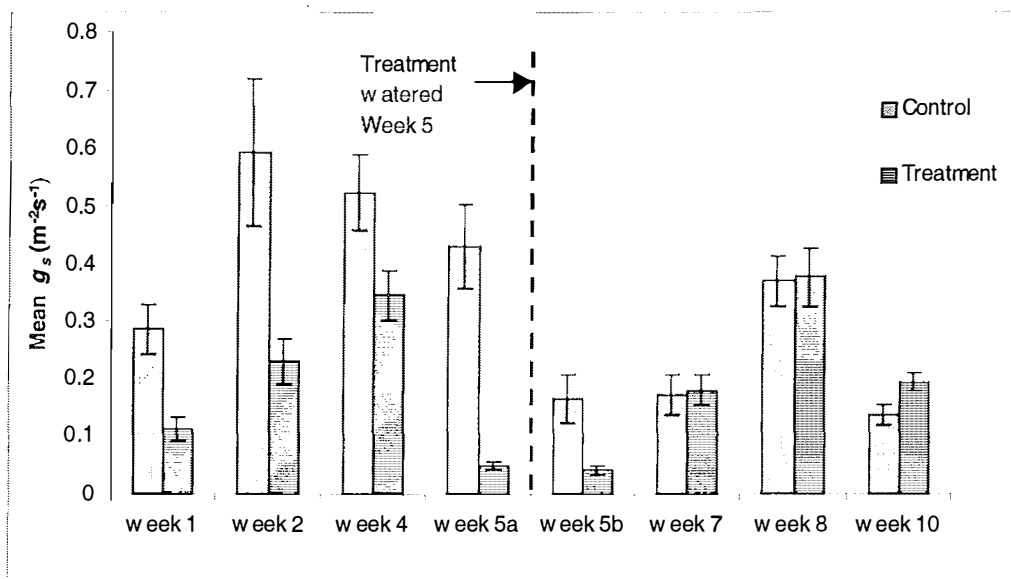
Control seedlings were watered twice daily and treated seedlings underwent a drying regime where seedlings were dried for five weeks then rewatered. Mean g_s values represent average stomatal conductance taken from 12 seedlings. Week 5a refers to measurements taken in week 5 *prior* to rewatering; week 5b refers to measurements taken in week 5 *following* rewatering. Error bars represent the standard error within means.

Water use efficiency was calculated by the ratio of photosynthesis rate to transpiration rate (*A/E*) for each seedling. Figure 2.6 shows that the mean water use efficiency of the control remained fairly consistent throughout the experiment, with ratios ranging from 0.21 in week 10 to 0.47 in week 7. The error bars in Figure 2.6 show that the degree of error within the control mean was higher than that of the treatment. It was found that as soil moisture decreased with drying, WUE of the treatment increased, with the seedlings being most water use efficient in week 5a (before rewatering) with an *A/E* ratio of 0.48. Following rewatering, WUE decreased to 0.27 in week 5b, then proceeded to increase as soil moisture progressively decreased for the second drying interval.
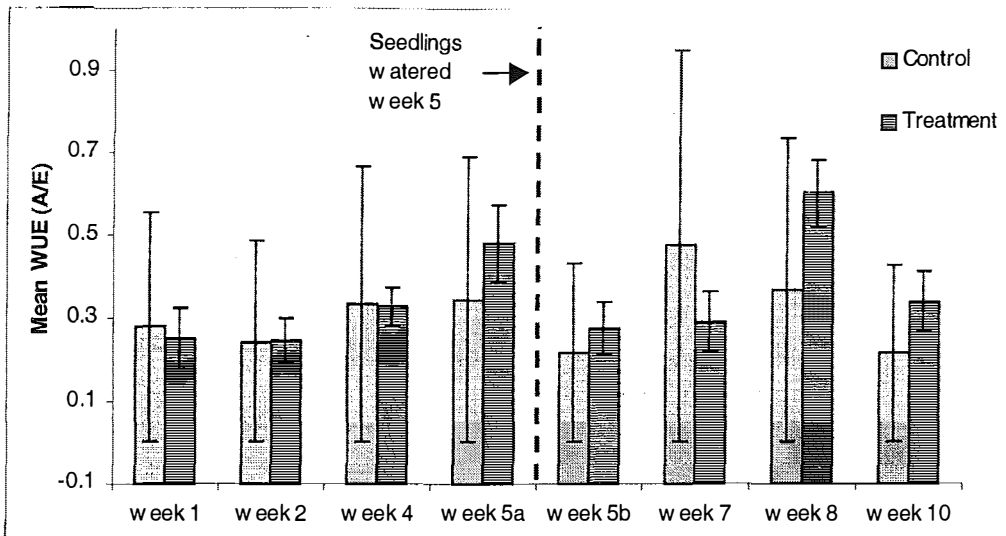
Figure 2.6: Mean water use efficiency (WUE) of *M. preissiana* seedlings under glasshouse conditions. Control seedlings were watered twice daily and treated seedlings underwent a drying regime where seedlings were dried for five weeks then rewatered. Mean WUE values represent the ratio of average photosynthesis rate: transpiration rate. Week 5a refers to measurements taken in week five *prior* to rewatering; week 5b refers to measurements taken in week five *following* rewatering. Error bars represent the standard error within means.

Analysis of variance was used to further understand the effects of the drying regime on the instantaneous WUE of the *M. preissiana* seedlings and to determine whether differences in WUE between the control and treatment over the ten week experiment were significant. Two-way ANOVA found that there was an overall difference in WUE over weeks, however, no significant difference between the control and treatment (Table 2.2). There was no significant interaction between the two, therefore it could be concluded that time was independently affecting the WUE of the *M. preissiana* seedlings. In order to identify the *specific* differences in WUE over time, one-way ANOVA was conducted to investigate differences existing in WUE *within* both the control and the treatment. It was found that the seedlings in the control did not differ significantly in instantaneous WUE over time, while the seedlings in the drying regime did (Table 2.2). The Bonferroni post-hoc test confirmed that these differences were significant between week 2 and week 5a and week 2

and week 8, with the seedlings being significantly less water use efficient in week 2 (p<0.05).

Table 2.2: Results of repeated measures ANOVA testing differences in *M. preissiana* instantaneous WUE (*A/E*) between experiment (treatment and control) and between sampling period (weeks). Two-way ANOVA was conducted to test for the overall effects of week and experiment on WUE. Week*Experiment refers to the interaction between the two factors. One-way ANOVA was conducted to test for differences in WUE over the experiment, within the treatment and the control.

Two-way repeated measures ANOVA					
Differences in WUE between weeks and experiment					
Factor	**d.f.**	**Mean Square**	**F-Value**	**P-Value**	
Week	7	0.187	3.544	0.001	*
error	154	0.005			
Experiment	1	0.009	1.600	0.219	NS
error	22	0.005			
Week*Experiment	7	0.009	1.800	0.091	NS

One-way repeated measures ANOVA					
Differences in WUE within experiment, between weeks.					
Factor	**d.f.**	**Mean Square**	**F-Value**	**P-Value**	
Control	7	0.009	1.973	0.070	NS
error	77	0.005			
Treatment	7	0.188	3.250	0.004	*
error	4	0.423			

NS   Not statistically significant (p>0.05)
*   Statistically significant (p<0.05)

2.3.2.2   Xylem Pressure Potential

The pre-dawn measurements of XPP, representing the time of day when seedlings were least water stressed, were used for result analysis. Figure 2.7 shows that the XPP of the control seedlings remained higher than that for the treatment, with pressures ranging from -0.4mPa to -0.27mPa. The treated seedlings displayed a progressive decrease from -0.3mPa in week 0 to -0.65mPa in week 8 during which the soil dried twice.
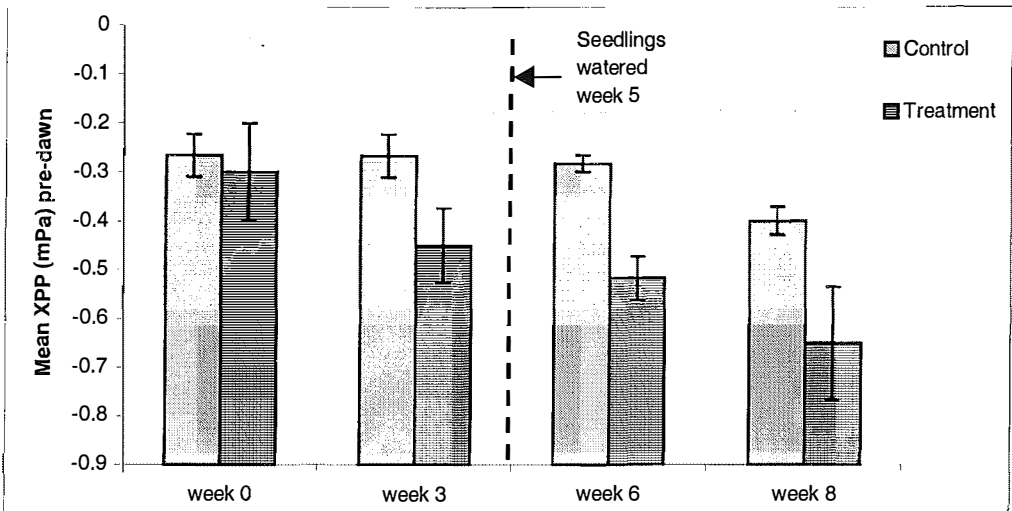
Figure 2.7: Mean xylem pressure potential (XPP) of *M. preissiana* seedlings under glasshouse conditions. Control seedlings were watered twice daily and treated seedlings underwent a drying regime where seedlings were dried for five weeks then rewatered. Mean XPP values represent the mean xylem pressure potential taken from 3 seedlings at pre-dawn. Week 5a refers to measurements taken in week 5 *prior* to rewatering; week 5b refers to measurements taken in week 5 *following* rewatering. Error bars represent the standard error within means.

2.3.2.3 $\delta^{13}C$ Measurements

2.3.2.3.1 *Foliar sampling*

Sampling at two to three week intervals found that the mean foliar $\delta^{13}C$ measurements of the controlled seedlings remained relatively consistent throughout the experiment, with values ranging from −27.38‰ (week 0) to −26.61‰ (week 4). Surprisingly, the treated seedlings that underwent the drying regime were found to have isotopically lighter $\delta^{13}C$ signatures on average for all sampling periods (Figure 2.8). This was unexpected considering the positive relationship that was proven in previous studies to exist between water use efficiency (*A/E*) and $\delta^{13}C$.

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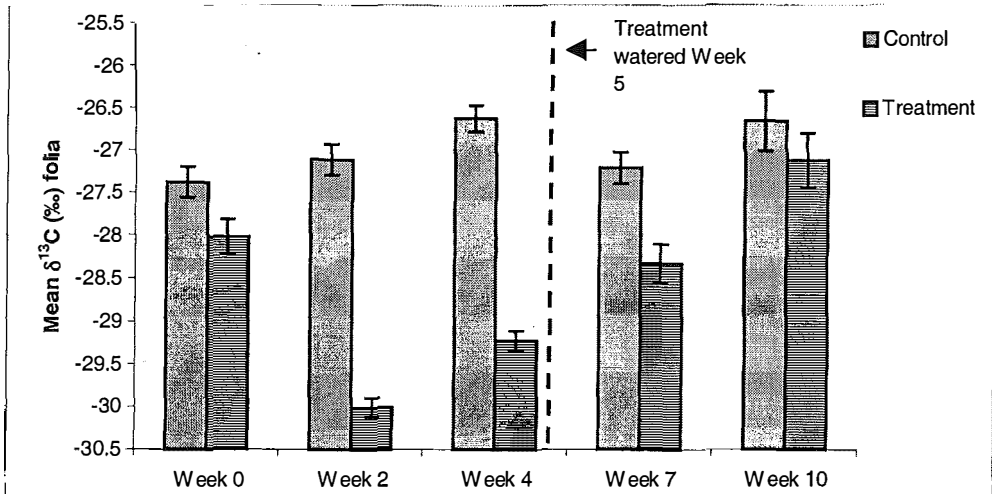
Figure 2.8: Mean $\delta^{13}C$ of *M. preissiana* foliar tissue based on a 10-week glasshouse experiment. Control seedlings were watered twice daily and treated seedlings underwent a drying regime where seedlings were dried for 5 weeks then rewatered. Mean $\delta^{13}C$ refers to the mean discrimination between ^{13}C and ^{12}C measured from 12 seedlings. Error bars refer to the standard error within treatment/control.

The mean $\delta^{13}C$ of the treated seedlings was similar to that for the treatment at the beginning of the experiment (week 0). After the initial two weeks of drying, there was a decrease in $\delta^{13}C$ from $-28.0‰$ to $-30.0‰$. As the soil moisture content of the treatment progressively decreased, the $\delta^{13}C$ of seedlings increased to $-27.1‰$ in week 10, with the rewatering event in week 5 not appearing to affect changes in foliage $\delta^{13}C$ of the treated seedlings.

Two-way analysis of variance found that overall, there were significant differences in foliar $\delta^{13}C$ of seedlings over time, and between the control and treatment (Table 2.3). There was a significant interaction between the two factors, indicating that $\delta^{13}C$ of the *M. preissiana* seedlings also differed due to the combination of time and experiment type. In order to understand this interaction, independent samples T-Tests were conducted to make comparisons in $\delta^{13}C$ between the control and treatment within individual sampling periods. It was found that foliar tissue $\delta^{13}C$ of the *M. preissiana* seedlings was significantly lower in

the treatment than the control during weeks 2, 4 and 7, however not significantly different prior to the start of the experiment (week 0) and during the last sampling period (week 10). One-way ANOVA was conducted to further understand the differences in foliar $\delta^{13}C$ of the seedlings over time within the control and treatment (Table 2.3). This found that the $\delta^{13}C$ of seedlings in the control did not change significantly over time, while the treated seedlings did. The Bonferroni post hoc test confirmed that the $\delta^{13}C$ of seedlings in the treatment differed significantly ($p<0.05$) according to the mean values depicted in Figure 2.8 between *all* sampling periods, with the exceptions being between week 0 and week 7, and week 0 and week 10, where $\delta^{13}C$ values were not significantly different.

Table 2.3: Results of repeated measures ANOVA testing differences in *M. preissiana* foliage $\delta^{13}C$ between experiment (treatment and control) and between sampling period (weeks). Two-way ANOVA was conducted to test for the overall effects of week and experiment on $\delta^{13}C$. Week*Experiment refers to the interaction between the two factors. One-way ANOVA was conducted to test for differences in $\delta^{13}C$ over the experiment, within the treatment and the control.

Two-way repeated measures ANOVA Differences in foliar $\delta^{13}C$ between weeks and experiment					
Factor	d.f.	Mean Square	F-Value	P-Value	
Week	4	8.575	17.826	<0.001	*
error	88	0.481			
Experiment	1	71.828	57.369	<0.001	*
error	22	0.005			
Week*Experiment	4	7.714	16.036	<0.001	*

One-way repeated measures ANOVA Differences in foliar $\delta^{13}C$ within experiment, between weeks.					
Factor	d.f.	Mean Square	F-Value	P-Value	
Control	4	1.393	2.857	0.074	NS
error	44	0.487			
Treatment	4	14.897	31.382	<0.001	*
error	44	0.475			

NS   Not statistically significant (p>0.05)
*   Statistically significant (p<0.05)

2.3.2.3.1 Nascent Xylem Tissue Sampling

The four-weekly sampling of the nascent xylem tissue found that the $\delta^{13}C$ signatures of the control and treatment were similar to those measured from foliage tissue (Figure 2.9). The mean $\delta^{13}C$ of the control displayed a slight decrease over the duration of the experiment. The seedlings in the treatment displayed a similar mean $\delta^{13}C$ signature than the control in week 0. After the initial four weeks of drying, $\delta^{13}C$ decreased with a drop from $-27.9‰$ in week 0 to $-30.2‰$ in week 4. Week 8 saw an increase in $\delta^{13}C$ of seedling xylem tissue (Figure 2.9).
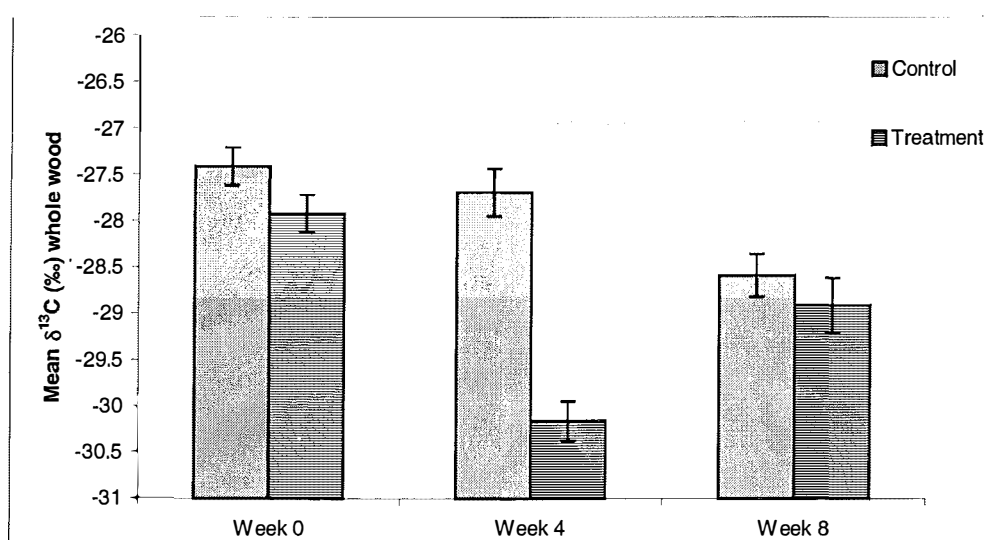
Figure 2.9: Mean $\delta^{13}C$ of *M. preissiana* nascent xylem tissue based on a 10-week glasshouse experiment. Control seedlings were watered twice daily and treated seedlings underwent a drying regime where seedlings were dried for 5 weeks then rewatered. Mean $\delta^{13}C$ refers to the mean discrimination between ^{13}C and ^{13}C taken from 6 seedlings. Error bars refer to the standard error within treatment/control.

Two-way analysis of variance found that overall, nascent xylem tissue $\delta^{13}C$ differed significantly over the duration of the experiment and between the treatment and control (Table 2.4). There was a significant interaction between the two factors, and independent samples t-tests confirmed that the difference in $\delta^{13}C$ of the *M. preissiana* seedlings between the control and treatment occurred during week 4, with $\delta^{13}C$ of the control being

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significantly higher in the control (p<0.05). One way ANOVA, found that xylem $\delta^{13}C$ varied significantly over time within both control and treatment (Table 2.4). The Bonferroni post hoc test revealed that nascent xylem tissue $\delta^{13}C$ of the control decreased significantly between week 0 to week 8, and decreased significantly in the treatment between week 0 and week 4 (p<0.05).

Table 2.4: Results of repeated measures ANOVA testing differences in *M. preissiana* xylem tissue $\delta^{13}C$ between experiment (treatment and control) and between sampling period (weeks). Two-way ANOVA was conducted to test for the overall effects of week and experiment on $\delta^{13}C$. Week*Experiment refers to the interaction between the two factors. One-way ANOVA was conducted to test for differences in $\delta^{13}C$ over the experiment, within the treatment and the control.

		Two-way repeated measures ANOVA Differences in xylem $\delta^{13}C$ between weeks and experiment			
Factor	**d.f.**	**Mean Square**	**F-Value**	**P-Value**	
Week	2	5.571	18.037	<0.001	*
error	20	0.309			
Experiment	1	10.896	28.133	<0.001	*
error	10	0.387			
Week*Experiment	2	4.204	13.587	<0.001	*

		One-way repeated measures ANOVA Differences in xylem $\delta^{13}C$ within experiment, between weeks.			
Factor	**d.f.**	**Mean Square**	**F-Value**	**P-Value**	
Control	2	2.285	9.705	0.005	*
error	10	0.235			
Treatment	2	7.500	19.562	<0.001	*
error	10	0.383			

NS	Not statistically significant (p>0.05)
*	Statistically significant (p<0.05)

2.3.3 Soil Moisture, Water Use Efficiency and $\delta^{13}C$

Figure 2.10 combines the soil moisture, WUE and foliar $\delta^{13}C$ measurements of the treated *M. preissiana* seedlings to descriptively illustrate the changes that occurred between the three measures over the duration of the experiment. The short sampling period and the

limited number of measures made during this period means that the relationship between measures could not be demonstrated statistically via correlation. However, observation shows that as the soil moisture decreased progressively, WUE of the treated seedlings increased, and $\delta^{13}C$ of foliar tissue dropped (with the exception of week 0) (Figure 2.10).
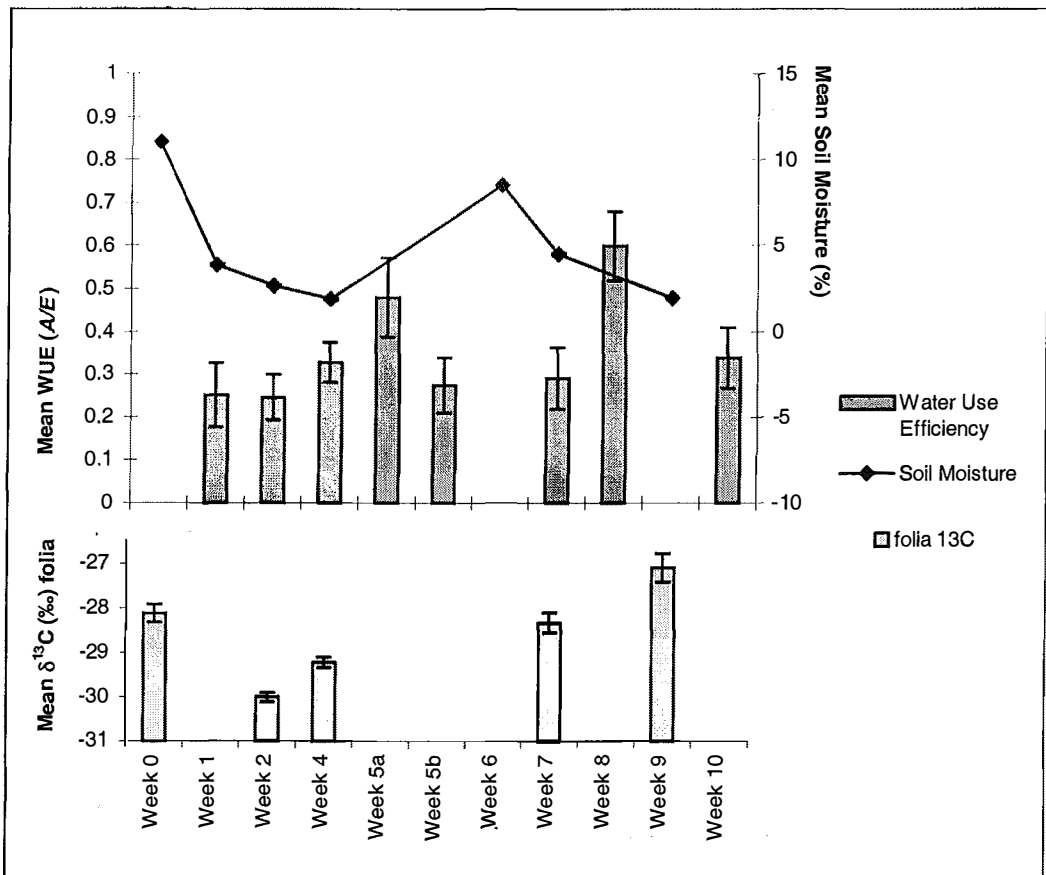
Figure 2.10: Mean water use efficiency, soil moisture and foliar $\delta^{13}C$ of *M. preissiana* seedlings under the drying regime, where seedlings were dried for five weeks then rewatered.

2.4 DISCUSSION

The relationship existing between $\delta^{13}C$ and photosynthetic water use efficiency in *M. preissiana* was partly demonstrated by the results obtained in the glasshouse experiment. The experiment attempted to eliminate the effects from all variables other than water availability on isotopic discrimination, however, the unexpected differences between the $\delta^{13}C$ of the control and treatment suggest that other variables may have been present.

2.4.1 Water availability and instantaneous gas exchange

The various instantaneous gas exchange measures followed similar trends for the duration of the drying experiment. The mean rates of photosynthesis, transpiration and stomatal conductance of the seedlings undergoing the drying regime were lower than those in the control for the first five weeks of drying. Farquar *et al* (1989) recognise that as a physiological response to drought, plants commonly show a simultaneous decrease in A, E and g_s in order to survive the effects of reduced water availability. C_i varied between the control and the treatment over the duration of the experiment, possibly due to the longer time period required for carbohydrate fractions within leaves to indicate water stress (Farquar *et al*, 1989).

The dramatically lower A, E and g_s values measured from the treatment in week 5 in comparison to the control expressed the severe state of water stress that the seedlings were under as the soil moisture content approached zero. Rewatering in week 5 did not induce an

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immediate response in the instantaneous gas exchange rates measured from treated seedlings, however by week 7 (two weeks after rewatering), the rates of A, E and g_s increased to higher than those measured in the control. The higher rates of gas exchange were unexpected, and may possibly be a result of diurnal variability, which affects the reliability of the IRGA measurements. It is also possible that the age of the leaves sampled for instantaneous gas exchange may have affected the values measured. The restricted water supply for the treated seedlings caused an obvious reduction in plant growth. The higher abundance of new growth formed by the seedlings in the control resulted in the instantaneous gas exchange rates being taken from a higher proportion of newly developed leaves than from the treatment. Cavender-Bares & Bazzaz (2000) found that the photosynthetic capacity of plants increased with age, with more mature leaves exhibiting higher A values than juvenile leaves. Similarly, Damesin et al, (1997) found that mature leaves display more negative $\delta^{13}C$ signatures than leaves that are recently formed, which could be attributed to higher rates of photosynthesis, transpiration and stomatal conductance.

2.4.2   Water availability and instantaneous water use efficiency

By calculating water use efficiency from the instantaneous gas exchange measures, one can reduce the variations that are likely to occur. This is because WUE incorporates a ratio comparing the plant's rate of photosynthesis to its rate of transpiration. The instantaneous WUE calculated for the treated seedlings was found to be negatively associated with soil moisture, where the WUE of the treated seedlings increased significantly during the drying

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regime, and decreased when soil moisture was replenished. Figure 2.6 shows that rewatering in weeks 5 and 9 induced a negative response in terms of seedling WUE, illustrating the instantaneous basis in which this variable was measured.

The relationship between water availability and WUE can be understood by considering the combined physiological responses of the seedlings to a reduced water supply. Reduced stomatal conductance in dry conditions leads to a reduction in water loss (E) and a reduction in intercellular carbon levels. The assimilation of carbon (A) increases due to the reduction in the diffusive supply of carbon, resulting in an overall increase in A/E, or an increase in water use efficiency (Lajtha & Marshall, 1994). The opposite occurs when water availability is high, and the ratio of A/E decreases, leading to a decrease in WUE.

The xylem pressure potential of the seedlings that underwent the drying regime also suggested that WUE and water availability for *M. preissiana* might have been inversely related. Fitter & Hay (1989) suggested that pressure potential be used as an indicator of plant water stress (rather than WUE), and derived three degrees of water stress in plants. Mild stress was measured in plants with XPP around −0.5mPa, moderate stress measured between −0.5mPa to −1.2mPa and severe stress measured up to −1.5mPa. The progressive decrease in XPP of the seedlings in the treatment suggested that the seedlings were of moderate stress by the end of the experiment when the water supply was lowest, while the control remained less than mildly stressed. This was due to water stress being associated with a rapid loss of leaf turgor and an increase in leaf wilting as a result of increased drought conditions (Baird & Wilby, 1999).

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2.4.3 Water availability, WUE and $\delta^{13}C$

These instantaneous measures of plant water use efficiency and plant water stress of the *M. preissiana* seedlings that experienced the drying regime in comparison to those that were regularly watered suggested that a decrease in soil moisture resulted in the plants becoming more water use efficient and more water stressed. The $\delta^{13}C$ signatures measured from new foliar tissue and nascent xylem tissue indicated a similar response to the drying regime. However, it is recognised that outside factors (other than water availability) may have affected isotopic discrimination. While the isotopic discrimination displayed by the controlled seedlings fluctuated around a mean value of approximately -27‰ and -28‰ for the foliar and xylem tissues respectively, the treated seedlings exhibited significantly more negative $\delta^{13}C$ than the control for the duration of the experiment as water availability decreased. This was unexpected due to the proven negative relationship that exists between water availability and $\delta^{13}C$ documented by many researchers (e.g. Farquhar *et al*, 1982; Ehleringer & Cooper, 1988; Körner *et al*, 1991; Leavitt, 1992; Stewart *et al*, 1995; Livingston & Spittlehouse, 1996; Berry *et al*, 1997; Damesin *et al*, 1997; Walcroft *et al*, 1997; MacFarlane & Adams, 1998; Pate & Arthur, 1998). However, by examining exclusively the isotopic discrimination displayed by the treatment (with the exception of week 0), $\delta^{13}C$ was found to increase significantly as water availability decreased. This corresponds to the theory that as water availability in an environment decreases, WUE and $\delta^{13}C$ increase due to the reduced discrimination against the heavier isotope during carbon assimilation (Boutton, 1991b). The rewatering events in weeks 5 and 9 did not induce a significant response in $\delta^{13}C$. This enforces the fact that isotopic measurements are not

instantaneous, and act as a time-integrated measure of WUE.

The more positive $\delta^{13}C$ values of the controlled seedlings receiving a regular water supply and of the treatment in week 0 before the drying regime commenced indicates that other variable/s may have been affecting the isotopic discrimination of the seedlings. The lower light conditions experienced by the treated seedlings were probably the dominant environmental factor causing the variation in $\delta^{13}C$ between the control and treatment. The clear, plastic sheeting surrounding the glasshouse area that contained the treated seedlings (in order to exclude watering from surrounding sprinklers) shaded these seedlings from the morning and midday sun. These less than optimal conditions were difficult to overcome due to limitations in glasshouse space. However, the lower light intensity reaching the treatment explained the higher levels of isotopic discrimination that occurred when water availability was limited. Studies investigating the changes in $\delta^{13}C$ along light gradients in forest ecosystems from the canopy to forest floor, found that $\delta^{13}C$ of leaves increased with canopy height due to the increase in light intensity (Berry *et al*, 1997). This pattern has been explained by lower light conditions influencing a decrease in intercellular carbon levels influenced, and therefore similarly influencing a decrease in $\delta^{13}C$ (Lajtha & Marshall, 1994). Although there were no significant trends found between the C_i of the *M. preissiana* seedlings of the control and the treatment, lower levels were displayed by the treatment during most sampling periods. Therefore, the more positive $\delta^{13}C$ displayed in week 0 and by the control may have been attributed to higher light intensity and subsequently higher levels of C_i.

2.4.4   Conclusion

The precise nature of isotopic studies means that methods applied to experimental design, sample collection and analyses techniques require high accuracy in order to reduce error (Boutton, 1991a). It has been acknowledged that this experiment may have been limited by light variations, which occurred in an otherwise controlled environment. However, by examining the *rate* of change in the $\delta^{13}C$ of the treated seedlings over the duration of the drying regime, it can be concluded that the $\delta^{13}C$, instantaneous WUE and water stress of *M. preissiana* seedlings became more positive as water availability decreased. However, the differences in $\delta^{13}C$ noted *between* the control and the treatment during the experiment suggested that external factors (other than water availability) might have also been inducing these differences. By eliminating light variations, the findings of this experiment may be improved in order to investigate the primary isotopic response of this species to water availability. However, being a study to assess the *applicability* of $\delta^{13}C$ analysis in measuring the WUE of *M. preissiana*, this experiment succeeded in proving that the instantaneous water use efficiency of the species was a response to water availability, and with all other factors constant, $\delta^{13}C$ can be used to indicate this response on a short term basis.

CHAPTER 3: FIELD SITE SELECTION

3.1 SITE SELECTION

Three wetlands situated within the Perth region of the Swan Coastal Plain were selected for the field component of this study: Lake Jandabup, Thomsons Lake and Banganup Lake. The location of the lakes is shown in Figure 3.1. All three sites were selected on the basis of having a large sampling population of *M. preissiana* occurring along a topographical gradient. The gradient was assumed to represent a gradient in depth to groundwater, inundation frequency, and therefore water availability. Site selection was also considered according to the availability of historical ground and surface water monitoring data. It is acknowledged that emphasis was placed on obtaining and processing data from Banganup Lake. This lake has one of the most significant populations of *M. preissiana* occurring on the Swan Coastal Plain (Froend *et al*, 1993). Additionally, the pristine nature of Banganup Lake means that external factors caused by anthropogenic disturbances were minimised, and tree water relations could be isolated as the major factors affecting $\delta^{13}C$. Data obtained from Thomsons and Jandabup Lakes were used to provide back-up to findings made at Banganup Lake, to allow comparisons between the three lakes, and to sample the variability occurring within the Swan Coastal Plain. Below is a background to the characteristics of the Swan Coastal Plain, as well as a description of the three study sites.
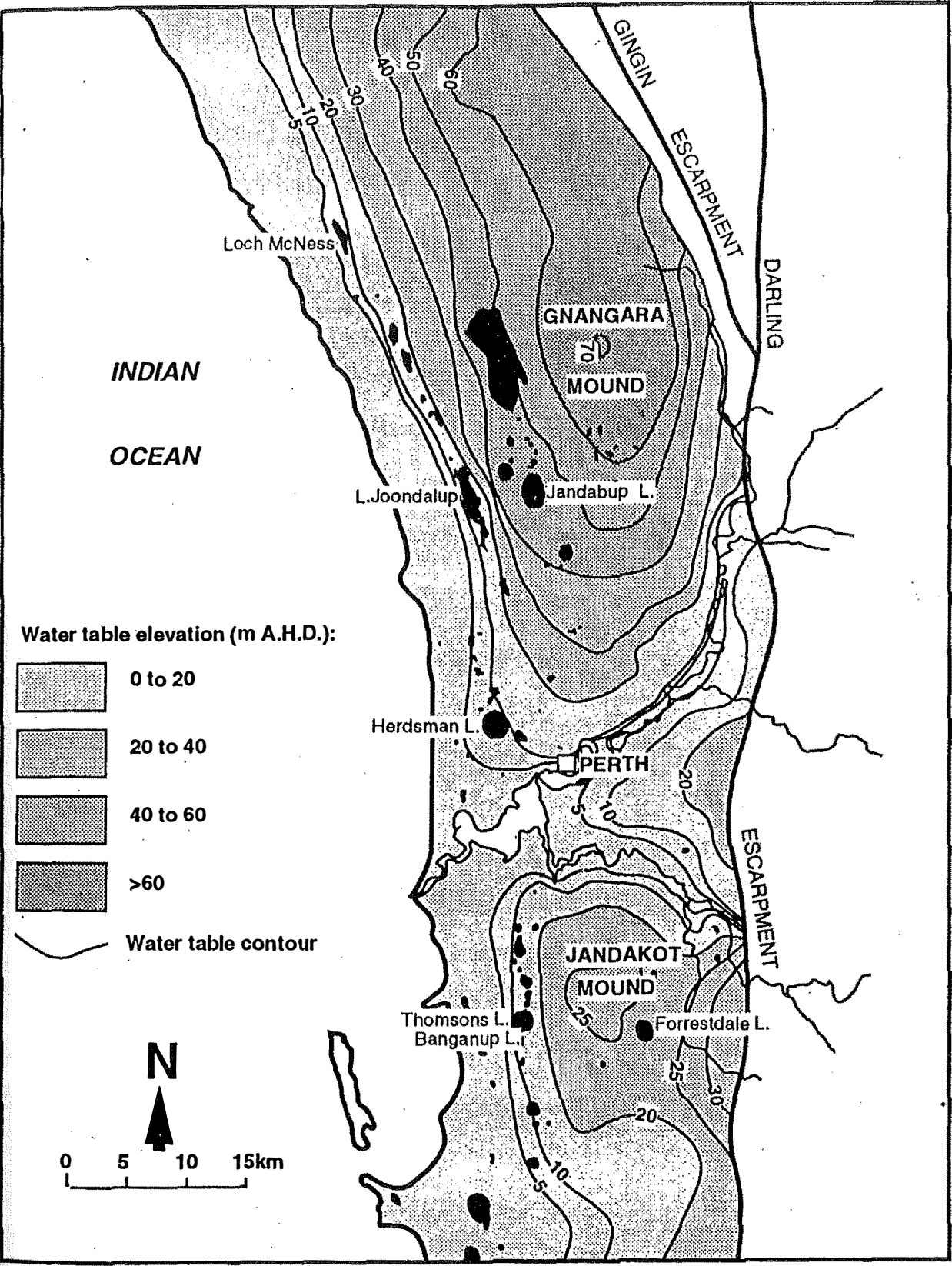
Figure 3.1: Location of the three study wetlands within the Perth Region of the Swan Coastal Plain: (from north to south) Lake Jandabup, Thomsons Lake and Banganup Lake. Location of the Gnangara and Jandakot Groundwater Mounds and other SCP wetlands are also shown. Adapted from Froend *et al* (1993).

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3.2   SITE DESCRIPTION

3.2.1   Description of the Swan Coastal Plain

3.2.1.1   Location

The Swan Coastal Plain was defined by Seddon (1972) as being the coastal plain along the west coast of southwest Australia, extending from Geraldton in the north, to Dunsborough in the south. It covers an expanse of 550km of coastline, and at its maximum, extends eastward for 35km to the Darling Scarp (Balla, 1994). The Perth Region lies within the Swan Coastal Plain, includes most of the Perth metropolitan area, and covers an area of approximately 4000km². (Davidson, 1995).

3.2.1.2   Climate

The climate of the Perth Region is typically Mediterranean with hot, dry summers and cool, wet winters. The hot, dry summers are a result of a series of anti-cyclones (high-pressure zones) that pass over the region during summer (Davidson, 1995). The cool, wet winters are associated with the subpolar, low-pressure cells that cross the region as cold fronts (Davidson, 1995). The average annual rainfall for the Perth region is 769mm. A majority of Perth's rainfall occurs during the period of April – October, with the summer months being virtually dry.

3.2.1.3 Geomorphology

The Swan Coastal Plain consists of four major landforms that run parallel to the coastline. The most easterly landform is known as the Pinjarra Plain, which is an alluvial plain located at the foot of the Darling Scarp (Seddon, 1972). The three successive landforms, the Bassendean, Spearwood and Quindalup dunes, consist as a series of dune systems that formed during periods of higher sea levels. Wetlands occurring on the Swan Coastal Plain mostly lie in the interdunal swales of the Bassendean Dune System, in the interbarrier depressions between the Spearwood Dune and Bassendean Dune Systems, and within the Spearwood Dune System (Arnold, 1990)

3.2.1.4 Hydrology

The Perth Region contains a very large and renewable groundwater resource (Davidson, 1995). The Gnangara and Jandakot Mounds exist as two shallow, unconfined groundwater mounds occurring to the north and south of the Perth metropolitan area respectively (Figure 3.1). The lakes occurring on the groundwater mounds are surface expressions of the underlying unconfined aquifer and their water levels vary with that of the water table (WAWA, 1991a). The lakes are directly recharged by rainfall infiltration, surface runoff and artificial drainage, while discharge is comprised of evapotranspiration, drainage and groundwater outflow (WAWA, 1991). The lakes reach their maximum levels at the end of winter as a response to the winter rains and drop to minimum levels at the end of summer (Froend *et al*, 1993). Over 80% of wetlands on the SCP are seasonal sumplands or damplands, and only contain water during these winter months (Davidson, 1995). A

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combination of low rainfall and decreasing groundwater levels in the Perth Region have

seen lake levels and the period of inundation decrease gradually (Davidson, 1995).

3.2.1.5   Vegetation

The vegetation of the Swan Coastal Plain is highly diverse and consists of many different

community types. Beard (1990) identified the vegetation of the Perth region as being part

of the Drummond Botanical Subdistrict. This is mainly comprised of *Banksia* woodland

located on leached sands, with *Melaleuca* swamps occurring in poorly drained soils, and

woodlands of tuart (*Eucalyptus gomphocephala*), jarrah (*E. marginata*) and marri

(*Corymbia calophylla*) occurring on less leached soils (Beard, 1990). Areas of open water

are commonly bordered by the paperbark *Melaleuca rhaphiophylla*, which can survive

seasonal waterlogging (Seddon, 1972), and accompanied by a belt of sedges and/or rushes

extending into the water such as *Baumea articulata* and the introduced bulrush *Typha*

orientalis. Seasonally inundated swamps are often fringed by the paperbark *M. preissiana*,

along with *Banksia littoralis* and *E. rudis* (Beard, 1990).

3.2.1.5.1 Melaleuca preissiana (Shauer)

Melaleuca preissiana (common name, moonah) is a paperbark tree, growing 9-13m high

(Froend *et al*, 1993) (Plate 3.1). It has subterminal white flowering spikes and white papery

bark, both of which resemble that in *M. rhaphiophylla* (Seddon, 1972). Flowering occurs

from November to January in most areas, and sometimes extends into March (Marchant *et*

al, 1987). *M. preissiana* is most common growing around the fringing area of freshwater

swamps on the Swan Coastal Plain, although it also occurs in low lying depressions in

moister soils (WAWA, 1991b). Unlike *M. rhaphiophylla*, *M. preissiana* will not survive when regularly inundated, and grows several metres back from the wet depression (Seddon, 1972; Froend *et al*, 1993). The two *Melaleuca* species often occur in succession in wetland environments.

Plate 3.1: Photograph of a *Melaleuca preissiana* individual growing on the margin of Lake Jandabup.

3.2.2   Description of Study Sites

3.2.2.1   Banganup Lake

Banganup Lake is an ephemeral wetland, situated within the Harry Waring Marsupial Reserve at 32°10'S, 115°50'E. The lake is located on the Jandakot Groundwater Mound and forms part of the Beeliar wetland chain (Froend *et al*, 1993).

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3.2.2.1.1 History of Banganup Lake

Banganup Lake is located to the adjacent south of Thomsons Lake and remains as one of the least disturbed wetlands within the Perth region. There is no record of previous horticultural activities occurring within the lake or the lake margins, however, dairy, cattle and horse grazing were prevalent during the 1930s, and timber felling of banksia and jarrah for firewood occurred during the 1930s and 1940s (Wilkins, 1992). Fires were an occurrence in summer and remained uncontrolled until the Jandakot Bush Fire Brigade formed in the late 1960s. In 1970, Banganup Lake was included in the 253.7ha Harry Waring Marsupial Reserve, located to the south of Russell Road. The purpose of the reserve was primarily to establish and maintain a quokka colony translocated from Rottnest Island, and was vested in the Western Australian Wildlife Authority and the Minister for Fisheries and Fauna (Wilkins, 1992). An electrified, 2 metre high wire fence enclosed the reserve to prevent the entry of dogs, cats, rabbits and foxes. This fence controlled predators, and limited the dispersal of species and as a result, in the 1970s and early 1980s the reserve experienced excessive overgrazing (Wilkins, 1992). Wildfires occurring in 1977 and 1978 exacerbated the problem, and it is believed that the combination of competitive grazing and fox predation caused the quokka population to "crash" in 1983-1984 and never recover.

Since then, the Reserve changed its lease to the University of Western Australia Zoology Department and the Department of Conservation and Land Management (CALM). It is managed as an A class reserve and forms part of the eastern chain of wetlands in the Beeliar Regional Park (WAWA, 1991; DPUD, 1992). Banganup Lake is believed to be the least disturbed in this chain of wetlands (WAWA, 1991).

3.2.2.1.2 Current management issues

Management of the Harry Waring Marsupial Reserve is currently undertaken by the Reserve Warden. This involves rabbit, fox and feral cat control, weed control, fence maintenance, firebreak maintenance, fire fighting and control of research activity (Wilkins, 1992). The three areas of highest concern for management for Banganup Lake include:

3.2.2.1.2.1 Fire

Wilkins (1992) identified that fire is excluded from the Reserve, in order to preserve the fauna population for research purposes. This has resulted in an increased fuel load in the Reserve, and surrounding Banganup Lake. Consequently, previous wildfires have been of high intensity, and caused considerable damage to the vegetation (Wilkins, 1992; Froend *et al*, 1993). The most recent fire recorded in the HWMR was in March, 1994 ("Arsonists Suspected", 1994). The last recorded fire prior to 1994 was in March, 1977, which scorched the fringing *M. preissiana* and *E. rudis* tree canopies and the *B. articulata* stands of the lake bed (Froend *et al*, 1993).

3.2.2.1.2.2 Disease

Jarrah dieback (*Phytopthora cinnamomi*) has not been recorded as present in the fringing areas of Banganup Lake. However, the canker fungus (*Botryophaeria*) is suspected to be invading the reserve, which is evident from dead branches observed on some *M. preissiana* individuals fringing Banganup Lake (Wilkins, 1992). This has not been confirmed, however *M. preissiana* is known to be highly susceptible to the fungus.

3.2.2.1.2.3 Water regimes

The seasonality of Banganup Lake's water levels currently remains unaffected by artificial drainage lines and industrial or urban effluent inflow. However, its close proximity to

Thomsons Lake means that the wetland is affected by its groundwater management, aimed at maintaining low water levels for waterbird habitat (WAWA, 1991). Additionally, in 1991, the EPA approved the Stage 2 expansion of the Jandakot Groundwater Scheme. This involved increasing groundwater abstraction for public water supply from 4 to 8 million cubic metres per year by creating a new line of 13 wells to the west of the existing well field (WAWA, 1991). The most expected effect of this increase in groundwater abstraction is the drawdown of the local water table. Drawdowns of greater than 2m in depth can be a threat to phreatophytic vegetation, and have an affect on areas located more than 1km radius from the well (WAWA, 1991). Stage 2 proposed to place wells at least 300m away from wetlands in order to reduce the drawdown effect on lake levels. Wilkins (1992) predicted that as a result of the Stage 2 expansion of the Jandakot Groundwater Scheme, Banganup Lake would experience a 0.20m reduction in groundwater levels at the end of summer, as well as a prolonged dry period.

3.2.2.1.3   Current environment at Banganup Lake

Banganup Lake is a shallow basin, lined with sandy peat sediment. Its area totals 37.5 ha, however much of this is covered by either sedgeland dominated by *Baumea articulata*, or *Melaleuca preissiana/Eucalyptus rudis* woodland. There is very little open water. The Lake is seasonal, remaining dry for 10 – 11 months during the year (WAWA, 1991). Froend *et al* (1993) found variations in water levels during 1986-1990 to range from 0.9m above the lakebed in the wettest months, to 1.2m below in the driest months.

The sediment of Banganup Lake consists of a peat/sand (Froend *et al*, 1993). The lake bed and surrounding areas are comprised of a peaty or black friable silt, are abundant in organic

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matter and variable in soil content (Wilkins, 1992). The sediment of the surrounding eastern portion of the Reserve consists of highly leached, deep gray sands. To the west, the soil formed as part of the Karrakatta soil association, being a pale, yellow-brown sand derived from Tamala limestone (Wilkins, 1992).

The population of *M. preissiana* at Banganup Lake is one of the most significant on the Swan Coastal Plain. It is the dominant fringing tree species, occurring with *Eucalyptus rudis* and *Banksia littoralis.* It forms a dense, closed canopy in fringing areas (Wilkins, 1992). In the surrounding woodland, dryland species include *B. menziesii, B. attenuata, Allocasuarina fraseriana* and *E. marginata. M. preissiana* occurs in distinct age group bands (Figure 3.2). Surrounding the northern to eastern lake margins is a juvenile band, aged by Froend *et al* (1993) at 23 years. This band established after the reserve was subjected to a bushfire in March 1977. The combination of the fire in 1977, and declining lake levels and rainfall during the late 1970s, would have induced the establishment of seedlings at lower elevations. A young parental band of trees aged 69 – 75 years surrounds the juvenile band. These trees were likely to have established during the period of 1925 – 1930 when rainfall was higher. With the clearance of land in the Jandakot region in the 1890s, and the high rainfall in the 1920s, lake levels would have risen, generating a mass recruitment of individuals in more elevated areas (Froend *et al*, 1993). Along the outskirts of the young parental band occur old parental trees, with ages estimated to be greater than 200 years (using tree diameter as an indicator). This structured nature of the age distribution at Banganup Lake suggests that recruitment of *M. preissiana* is episodic rather than a common occurrence. The sequence of ages down the elevational and hydrological gradients may represent a species response (through recruitment) to long term changes in

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water availability and fire regimes (Froend *et al*, 1993).
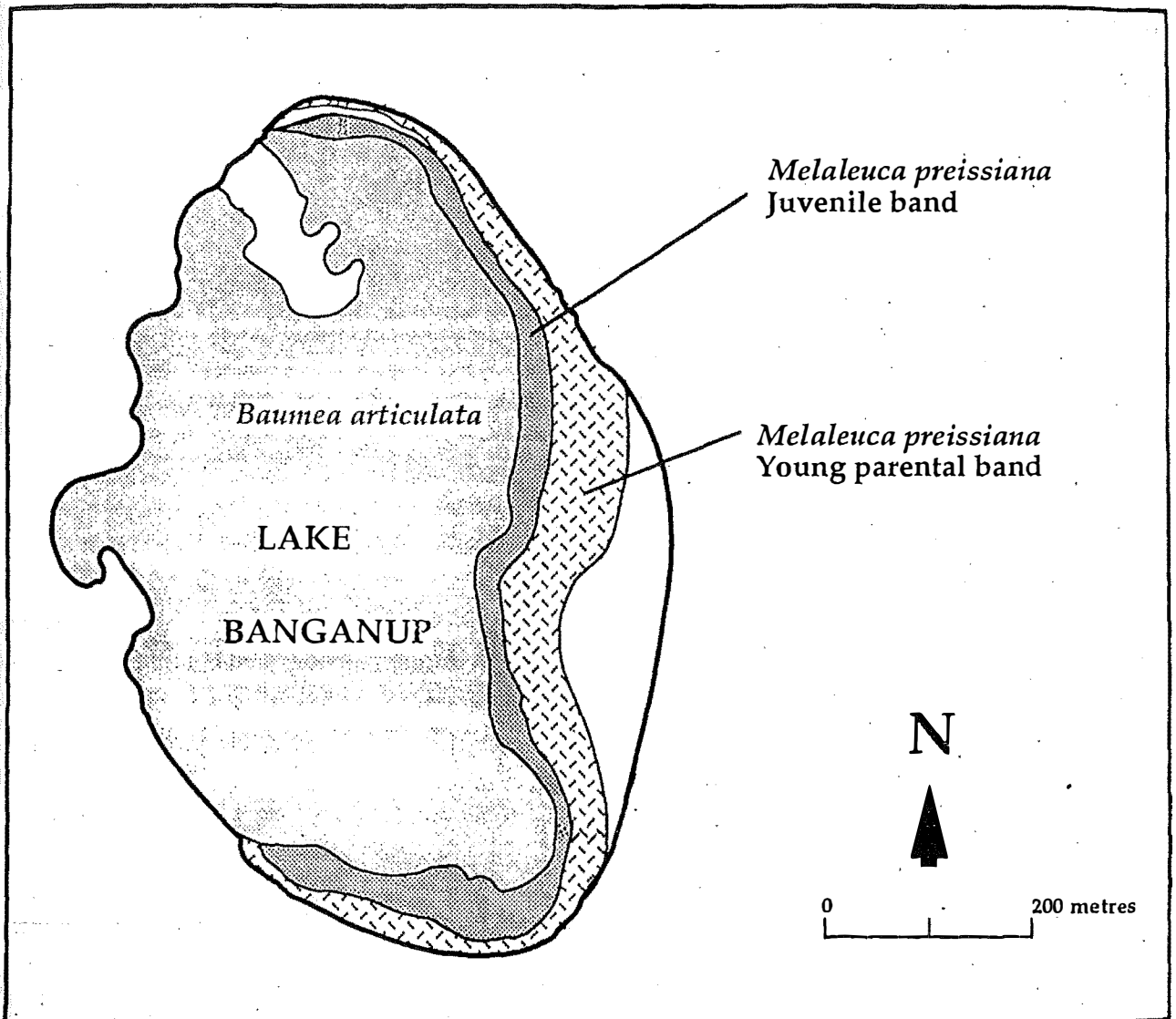
Figure 3.2: Spatial distribution of the juvenile and young parental bands of *M. preissiana* at Banganup Lake. Old parental trees were scattered throughout the upper slope. The bands were assumed to have established as a response to fire, and/or during periods of high groundwater levels. Map adapted from Froend *et al* (1993).

3.2.2.2   Thomsons Lake

Thomsons Lake is a large circular basin located 34km south west of Perth, and is situated to the north of Banganup Lake. The lake occupies a depression between the Bassendean and Spearwood dune systems. The junction between the two dune systems is marked by the Beeliar chain of wetlands, in which Thomson Lake is the largest (Crook & Evans, 1981). The Lake is currently an A class reserve and is managed by the Department of Conservation and Land Management. The overall management objective for Thomsons Lake is to "*protect the ecological character of the lake and, in particular, its importance as a waterbird habitat*" (WAWA, 1991).

The lake covers an expanse of 253.7 ha, with an extensive fringe of sedgeland comprising of *B. articulata* and *Typha orientalis,* occupying 101.2ha (Froend *et al*, 1993). Variations in water levels have been dramatic in the past, with the water regimes changing from permanent to seasonal. A ring of large, old *M. preissiana* individuals on the upper slope suggests a higher water table existed sometime in the past (WAWA, 1991). Local residents reported that the lake dried during the 1940s and was completely covered in reeds (Wilkins, 1992). Since then, the maximum recorded lake depth was 3.3m in the 1970s, and has since varied from being 0m in drier years, to 1.2m above the lake bed during wetter years.

Thomsons Lake is currently affected by rural and urban drainage. Water drains into the lake from the north, from Lake Kogolup, and from agricultural land from the east. This has subsequently caused nutrient enrichment by nitrogen and phosphorus draining into the lake.

Surface water inputs via drains from surrounding areas are causing an increase in the mean water level, and it is possible that without the drainage system, the water regime at Thomson Lake's would reflect that found at Banganup Lake (Froend *et al*, 1993). CALM's management strategy is to maintain a seasonal water regime at the lake in order to provide optimal bird breeding habitats (WAWA, 1991). Sudden, unexpected rises in water levels may occur due to artificial sources, however, these are minimised under CALM's management criteria for Thomsons Lake (EPA, 1989). In 1987, the South Jandakot Management Plan proposed to construct the South Jandakot Branch Drain to assist in the drainage management of the Beeliar Wetlands (EPA, 1987). The development of this drain has been deferred until construction of the Thomsons Lake Main Sewer commences. Development of the sewer is dependent on the rate of urban development, and the subsequent deferral of drain construction may result in increased lake water levels (EPA, 1996).

Thomsons Lake occupies the 509ha Thomsons Lake Nature Reserve. This reserve supports a wide variety of vegetation zones, identified in Crook & Evans (1981). *M. preissiana* occurs predominantly around the north, east and west lake margins in association with *E. rudis, B. menziesii, B. illicifolia, B. attenuata* and *B. littoralis*. Similarly to Banganup Lake, a fox-proof fence surrounds the Reserve, however public access is not restricted.

The fire history at Thomsons Lake Reserve is particularly extensive, with the vegetation being severely damaged by frequent fires (Crook & Evans, 1981). The first recorded fire occurred to the south of the lake during 1965 and 1966. The 1970s saw a series of extensive and hot fires occurring throughout the entire Reserve, with the entire Reserve burned at

least twice from 1965-1980 (Crook & Evans, 1980). The regular burning of vegetation has resulted in thinning and damage to woodland canopies and in tree mortality. The recruitment of grasses and weeds has also become a problem due to fire (Crook & Evans, 1981). At present, fire management and suppression at Thomsons Lake is undertaken by CALM (DPUD, 1992).

3.2.2.3   Lake Jandabup

Lake Jandabup is located 22km north of Perth, 9km east of the coastline, and is situated on the Gnangara Groundwater Mound. Similarly to Thomsons and Banganup Lakes, Lake Jandabup lies in the depression between the Spearwood and Bassendean dune systems (Davis & Rolls, 1987). The Lake occupies a shallow (1.5m) north-south oval basin about 3km long and 2km wide, and covering an area of 330h. (Allen, 1979).

The Lake is a surface expression of the Gnangara Mound groundwater flow system, with groundwater inflows occurring from the east, and outflows occurring from the lake's southwest margin (Allen, 1979). Rainfall does add a significant volume of water to the Lake, however losses by evapotranspiration are greater, and account for 90% of the total recharge (Allen, 1979). The Lake does not dry completely in summer, however seasonal patterns show a gradual decrease in water table levels as a result of regional groundwater abstraction, maturing of near-by pine plantations and the artificial maintenance of water levels (Froend *et al*, 1993). Previous shorelines can be recognised, which is evident by the changes in slope and vegetation, and suggest that the lake is progressively becoming drier. Open water is restricted to an area of 1.2km², and is bounded by *Baumea articulata* and

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Leptocarpus sp. (Froend *et al*, 1993). The centre of lake bed is covered with organic sediment, which is mainly diatomite, while the periphery consists of carbonaceous sands (Allen, 1979).

The vegetation of Jandabup Lake consists of a few species of aquatic plants in the areas of open water, broad zones of reeds which border the open water and extend to the shores of the lake, and a narrow zone of sedgeland/grassland in areas of occasional inundation (Allen, 1979). Large trees are sparse and occur amongst the low scrubland located on the older, more elevated shoreline. The remaining fringing tree communities consist predominantly of *E. rudis*, *M. preissiana* and *M. rhaphiophylla* in areas of seasonal inundation (WAWA, 1995). Only 25% of the littoral zone surrounding the lake remains undisturbed and, although remnant areas of terrestrial vegetation are few, the populations of *M. preissiana* are of significance (Froend *et al*, 1993).

The central area of the lake is vested as an A-Class Reserve, managed by CALM and the City of Wanneroo. 21% of the perimeter is privately owned, and has been cleared for horticulture, grazing and housing (Ryder, 1993). The high diversity of macrophytes, aquatic invertebrates and waterbirds suggests that Lake Jandabup is of high conservation value (WAWA, 1995). In order to protect these values, CALM's specific management objectives include maintaining the bird wading habitat, sedge habitat and fringing woodlands and ensuring a high species richness of aquatic invertebrates (WAWA, 1995).

CHAPTER 4: TIME-INTEGRATED, SHORT TERM $\delta^{13}C$ MEASUREMENTS USING NASCENT XYLEM TISSUE

4.1 INTRODUCTION

The application of $\delta^{13}C$ analysis in understanding plant physiological responses to variations in water availability is of use in the management of natural ecosystems where the water resource is of significance to the plant community. Studies investigating the relationship between $\delta^{13}C$ and environmental parameters in controlled experiments are difficult to apply to natural ecosystems due to the exclusion of the complex ecological interactions existing in nature (Bert *et al*, 1997). Plants respond physiologically to a variety of environmental factors, and their response is largely determined by morphological characteristics such as species type, growth strategy, plant size, age, and health. These are factors that cannot be controlled in the natural environment.

$\delta^{13}C$ measurements in plant populations have been used to understand how populations respond to natural variations in the environment. Variations in $\delta^{13}C$ has been measured in the field in relationship to light (Berry *et al*, 1997), climate (Lipp *et al*, 1991), temperature (Saurer *et al*, 1995), altitude (Körner *et al*, 1991) and water availability (Dupouey *et al*, 1993; Livingston & Spittlehouse, 1996; Damesin *et al*, 1997). A majority of these studies compare the carbon isotopic ratios *between* populations from contrasting environments. Leffler & Evans (1999) investigated the $\delta^{13}C$ dynamics *within* a single riparian tree population in terms of stream flow. The remarkable variations found in $\delta^{13}C$ between individuals of the same population were indicative of the heterogeneity that occurs along a hydrological gradient. The occurrence of *Melaleuca preissiana* in cohorts along some lake

margins suggests that similar heterogeneity may be present along hydrological gradients of water availability. $\delta^{13}C$ is a possible tool for measuring physiological heterogeneity within natural populations of *M. preissiana.*

The various methods of sampling plant tissue for $\delta^{13}C$ have been widely documented. All plant tissue contains a record of $\delta^{13}C$, which exists as a time-averaged measure of the plant's water use efficiency over the lifetime of the tissue. Pate & Arthur (1998) suggested a novel method for measuring short-term $\delta^{13}C$ in *Eucalyptus globulus* by sampling nascent xylem tissue taken from cambium layers of the tree stem. This was proven to be a simple and reliable means of obtaining time-integrated, short-term measures of $\delta^{13}C$.

It was proposed to investigate the applicability of a short term sampling method for $\delta^{13}C$ analysis in *M. preissiana* populations occurring along a hydrological gradient. The temporal and spatial variations in water availability experienced by Swan Coastal Plain wetlands are factors causing the heterogeneity in the distribution of *M. preissiana* individuals. By assessing the differences in short term $\delta^{13}C$ of populations of *M. preissiana*, it can be understood how seasonal fluctuations in water availability affect the water use efficiency of individuals located in different positions within the landscape.

There is a paucity of literature available on the physiological response of fringing tree species to variations in water availability in the southwest of Western Australia. Therefore, the objective of this study aimed at assessing the applicability of short-term $\delta^{13}C$ measurements in understanding the water use efficiency of natural populations of *M.*

preissiana in a wetland environment. Specific research objectives were as follows:

i. Identify whether a hydrological gradient of water availability exist within wetlands;

ii. Investigate the variations in short-term measures of $\delta^{13}C$ in *M. preissiana* individuals along a hydrological gradient;

iii. Investigate the variations in short-term measures of $\delta^{13}C$ in *M. preissiana* individuals between seasons;

iv. Examine the relationship between short-term $\delta^{13}C$ measures and instantaneous WUE of *M. preissiana* populations along a hydrological gradient;

v. Identify the external factors (other than water availability) that influence short-term $\delta^{13}C$ in natural *M. preissiana* populations.

4.2   MATERIALS AND METHODS

4.2.1   Field Sampling Design

At the three study wetlands: Banganup, Thomsons and Jandabup Lakes, three transects were marked in various positions in order to sample the variability occurring throughout the sites. Transects were of various lengths, ranging from 50m to 200m, each starting from the water's edge and extending radially toward the dryland vegetation. A total of nine *M. preissiana* trees were selected at each wetland, with three located along each of the three transects. These were labelled A, B and C. Tree A was located on the lower slope and closest to the water's edge. Tree B was located mid-slope, and Tree C was located on the upper slope with furthermost distance from the lake (Figure 4.1).
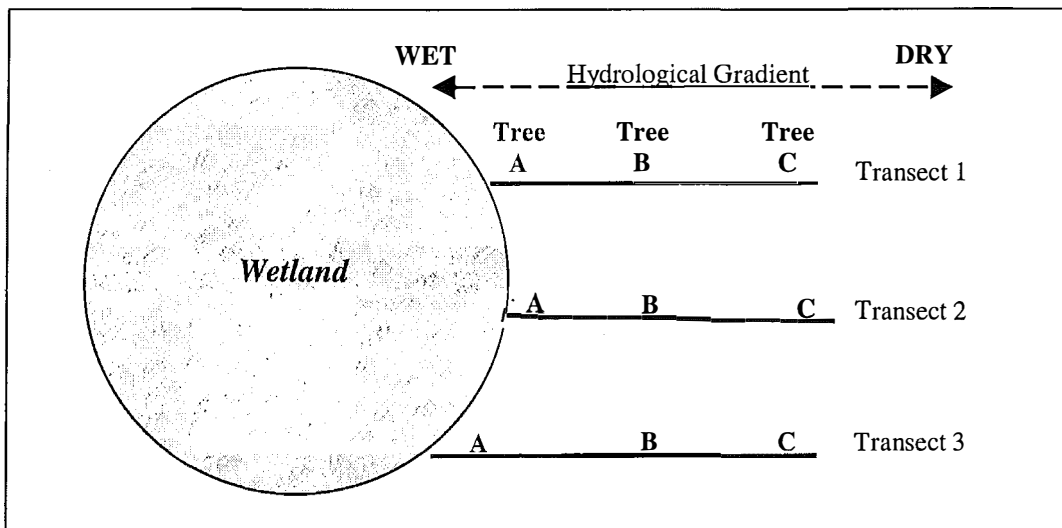
Figure 4.1: Field Sampling Design showing hydrological gradient, transect and tree positions. Trees located on the lower slope (A position) were assumed to have highest relative water availability, and trees on the mid and upper slopes (B and C positions) had moderate and lowest relative water availability respectively.

Each transect represented an assumed hydrological gradient, with the trees located on the

lower slope (A position) located in the area of highest relative water availability, the trees located on the mid slope (B position) located in an area of moderate water availability and the trees located on the upper slope (C position) located in the area of lowest relative water availability. The position of transects at Banganup, Thomsons and Jandabup Lakes is shown in the aerial photographs in Plates 4.1, 4.2 and 4.3 respectively. Effort was made to spread transect locations throughout the fringing wetland environment in order to sample the variations in $\delta^{13}C$ throughout the entire wetland. For Banganup Lake, the bands of *M. preissiana* occurred continuously around the north, east and south margins, making it possible to do so adequately. At Thomsons Lake and Lake Jandabup, the occurrence of *M. preissiana* populations suitable for this study were limited in comparison to Banganup Lake, and therefore transect location could not adequately cover the expanse of the lake margins. The small area of remnant vegetation surrounding Lake Jandabup meant that the location of the three transects were within 200m of each other.

Trees were selected on the basis being of good health and where possible, trees of the same size/age were selected at each wetland. Tree height, diameter at breast height (DBH) and vigour (based on the crown classification scale procedure shown in Figure 4.2 (Ladd, 1994)) were measured. The trees were used as subjects for sampling, with each tree sampled for time-integrated, short term $\delta^{13}C$ measurements of nascent xylem tissue.

Plate 4.1: Aerial photograph of Banganup Lake. Numbers refer to location of the three transects. Scale 1:5000. Photograph taken in February 2000. Source: Department of Land Administration.
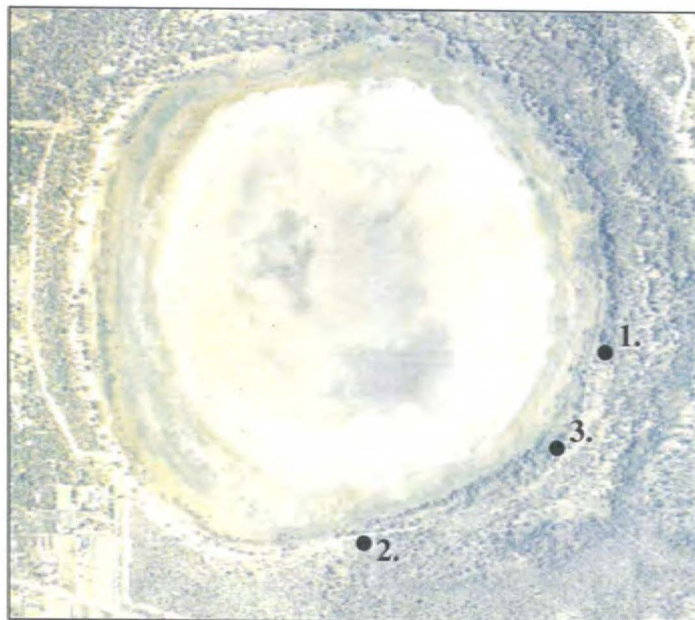
Plate 4.2: Aerial photograph of Thomsons Lake. Numbers refer to location of the three transects. Scale 1:10 000. Photograph taken in February 2000. Source: Department of Land Administration.

Plate 4.3: Aerial photograph of Lake Jandabup. Numbers refer to location of the three transects. Scale 1:10 000. Photograph taken in January 1999. Source: Department of Land Administration.

9	7	5	3	1
Very dense leaf clumps distributed over crown. Very little light penetrating clumps.	Dense leaf clumps unevenly distributed over crown.	Clumps of average density equally distributed or dense clumps unevenly distributed.	Clumps sparse and poorly spread.	Very few leaves anywhere in crown.

Dead branches

9	7	5	3	1
No visible dead branches or branchlets except thin twigs inside new leaf development and lowest branches being shed.	Dead branchlets evident on close inspection. Not all over crown.	Small branches dead, not all over crown. Easily observed but not seriously affecting crown.	Large and/or small branches dead over part of crown. Serious.	Large and small branches dead over most of the crown.

Epicormic growth

5	4	3	2	1
Nil epicormic growth with normal foliar growth concentrated at the branch extremities	Slight in part of crown only.	Moderate over most of crown.	Severe on crown or stem.	Severe on crown and stem.

Figure 4.2: Crown Assessment Procedure used to determine tree vigour. The assessment was based on observations of crown density, dead branches and epicormic growth. Trees were given a vigour score from 3-23, where a score of 23 indicated high vigour. Adapted from Ladd (1994).

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4.2.2 Time-Integrated, Short Term $\delta^{13}C$ Measurements

4.2.2.1 Nascent Xylem Tissue Sampling

The methods for sampling xylem tissue followed those outlined by Pate & Arthur (1998) and were similar to those used in the glasshouse experiment (Chapter 2). Recently formed xylem tissue located directly under the cambium of the tree contained a $\delta^{13}C$ signature reflecting the environmental conditions experienced by the plant over the past month. Trees at each of the three study sites were sampled for their $\delta^{13}C$ signature using this method three times during the year (in April, June and August) to account for seasonal variations. In each tree, a square window of bark approximately 10cm x 10cm in area was removed at breast height (1.3m) to just beneath cambium level. The thin, gelatinous layers of recently formed xylem tissue were scraped off and collected into vials containing 80% ethanol. This process is pictured in Plate 4.4.
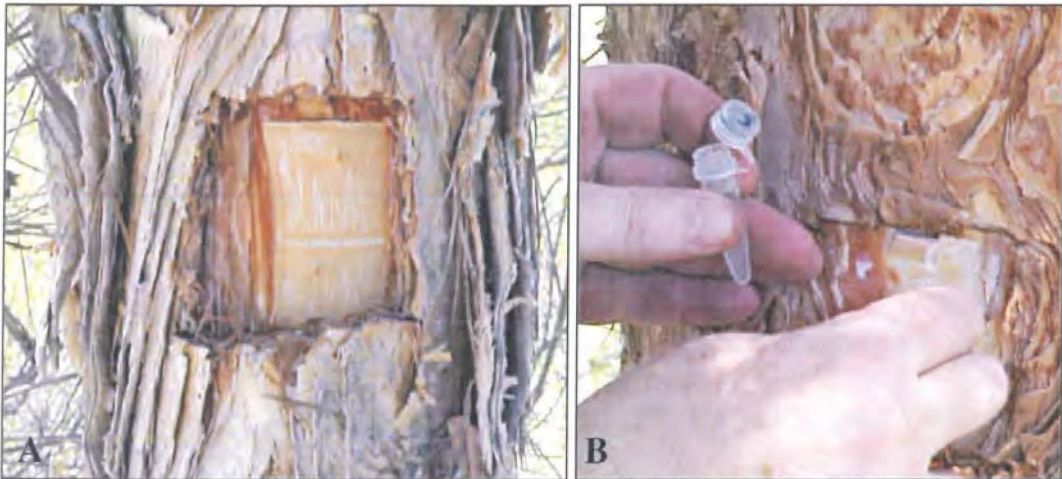
Plate 4.4: Photographs showing the technique used for sampling nascent xylem tissue from *M. preissiana* individuals. A 10cm square piece of bark was removed to cambium level (A). Using a razor blade, the thin, gelatinous layer of recently formed xylem tissue was removed and collected into vials containing 80% ethanol solution (B). Samples were dried and analysed for $\delta^{13}C$. This method was based on the technique outlined by Pate & Arthur (1998).

The xylem tissue was washed with 80% ethanol solution three times over three weeks to remove contaminating solutes and resins which may alter the $\delta^{13}C$ of the wood. The remaining insoluble residue was oven dried at 75°C for 48 hours and subjected to $\delta^{13}C$ analysis.

4.2.2.2   Cellulose Extraction

Wood is comprised of three main components: lignin, cellulose and hemicellulose (Rowell, 1984). These components exist in wood in different proportions, depending on the number of biochemical reactions occurring within the plant. It is assumed that the number of reactions is positively correlated with the extent of discrimination between ^{12}C and ^{13}C (i.e. the greater the number of reactions, the greater the discrimination). The formation of cellulose during photosynthesis requires fewer reactions than those required for the formation of lignin and hemicellulose. For this reason, the $\delta^{13}C$ of cellulose is similar to the $\delta^{13}C$ of the whole plant (MacFarlane *et al*, 1999). The $\delta^{13}C$ of hemicellullose in wood differs from that of cellulose by ±1.5‰, while lignin is 2 – 4‰ lighter than cellulose. The ratio of lignin to cellulose has been found to vary within a single plant, both radially and vertically along the stem (Wilson & Grinsted, 1977). The lignin-to-cellulose ratio also varies within an individual growth ring, with the early wood containing a higher proportion of lignin, and therefore being isotopically heavier, than the late wood (Wilson & Grinsted, 1977). By analysing the $\delta^{13}C$ of only cellulose, these variations are minimised.

Nascent xylem tissue collected from the first sampling period (April, 2000) was treated to extract the cellulose from the whole wood for $\delta^{13}C$ analysis. The $\delta^{13}C$ signatures of the cellulose samples were compared to the $\delta^{13}C$ from the same, untreated sample of whole wood to determine whether the procedure was necessary. The methods for extracting the cellulose from the samples in this study followed the modified diglyme-HCl procedure introduced in McFarlane *et al* (1999). Following sample collection, wood samples were weighed, and treated to extract crude cellulose from whole wood. The resulting residue after extraction consisted of crude cellulose, which was subjected to $\delta^{13}C$ analysis. Comparisons were made to compare the $\delta^{13}C$ signatures of whole wood and cellulose from the same sample using the Pearson correlation coefficient. If a significant correlation ($p < 0.05$) existed between the $\delta^{13}C$ value of whole wood and cellulose, it was deemed unnecessary to extract the cellulose from the remaining samples, as the $\delta^{13}C$ of crude cellulose would not be significantly different to that of whole wood.

4.2.2.3 $\delta^{13}C$ Analysis

The process of analysing samples for $\delta^{13}C$ follow that outlined in Chapter 2. $\delta^{13}C$ signatures were expressed in parts per thousand (‰).

4.2.3   Instantaneous WUE Measurements

Instantaneous gas exchange measurements for each tree at Banganup Lake were conducted once per week for the four weeks prior to sampling nascent xylem tissue, in order to

compare values to the monthly integrated $\delta^{13}C$ value. IRGA equipment was used to measure the leaf photosynthesis rate (A μmol m^{-2}s^{-1}), transpiration rate (E mol m^{-2}s^{-1}), stomatal conductance (g_s mol m^{-2}s^{-1}) and intercellular carbon level (C_i μmol mol^{-1}) by sampling intact foliar exposed to sunlight. Foliage from each tree was sampled three times and an average for each tree calculated. WUE was measured using the ratio A/E, or the ratio of net photosynthesis to transpiration (Lajtha & Marshall, 1994). Measurements were conducted in sunny conditions, during mid-morning (between 10am and 11am).

4.2.4 Water Availability

To relate the $\delta^{13}C$ signatures of the nascent xylem tissue to the tree's water availability, it was necessary to collect data from different variables such as meteorological parameters and lake water levels. This involved collecting water level monitoring data for the twelve months from September 1999 to August 2000 from the Water and Rivers Commission for each wetland. Lake surface water level monitoring data were collected from staff gauges and groundwater level data collected from near-by monitoring bores. These data provided information regarding the water availability at the site level during the latter half of 1999 and during 2000.

Additionally, the meteorological data for the Perth region from September 1999 to August 2000 were collected from the Bureau of Meteorology. These data included the total monthly rainfall and mean monthly maximum temperature. The truncated period of sampling for water availability from 1999-2000 was carried out in order to understand the

fluctuations that occurred over a 12-month period, and to account for variations in xylem tissue $\delta^{13}C$ that may have been a result of changes in water availability occurring during the latter half of 1999.

In order to examine the water availability at the individual tree scale, the depth to groundwater next to each tree was measured by augering to the top of the underlying, unconfined aquifer. At Lake Jandabup, where all trees were located in close proximity to one another due to the small area of remnant bushland, augering occurred adjacently to the trees in transect 1 only. These data were extrapolated to incorporate the depth to groundwater of trees from transects 2 and 3. An elevational gradient was measured using theodolite equipment in order to depict the slope of the land's surface in relationship to the water table below, in metres Australian Height Datum (AHD). Using a period of seven years (from 1993-2000 because this was the minimum period of time that groundwater was monitored for between the three sites), mean water table levels, minimum water table levels and maximum water table levels were calculated for each transect. Mean water levels were calculated by averaging the monthly groundwater readings for the seven-year period. Minimum water levels were calculated by averaging the groundwater readings taken during the dry season of each year (March, April and May). Maximum water levels were calculated by averaging the groundwater readings taken during the wet season of each year (August, September and October). The values were calculated in accordance to current groundwater levels (taken by augering) by constructing elevational gradient diagrams for each transect at each site showing the extremes in groundwater level changes experienced by the trees occurring along the transect.

To investigate the combination of water availability parameters measured on a monthly basis over 2000, a water availability index (WAI) was derived, which combined total rainfall, mean maximum temperature, mean relative humidity, mean lake levels and mean groundwater levels for each tree during each sampling period. The methodology used to derive the index is detailed in chapter 5, where it was used to investigate historical water availability on an annual basis. The same method was applied, however, monthly values were calculated rather than annual values.

4.2.5   Data Analysis

The $\delta^{13}C$ signatures measured for each tree during each sampling period were descriptively analysed and illustrated graphically in order to display any differences occurring in $\delta^{13}C$ of trees between transect positions and between sampling periods. Results were statistically analysed using SPSS Version 10.0 to perform two-way repeated measures analysis of variance (ANOVA) where p=0.05. Differences in the xylem tissue $\delta^{13}C$ of *M. preissiana* populations at each wetland were tested between transect positions, and between sampling periods. The Bonferroni test was applied to make post hoc comparisons where significant relationships existed. Before all analyses, Levene's test for equality of variances and Mauchly's Test of Sphericity were conducted to test for the relevant assumptions, and appropriate data transformations were made if necessary. Gas exchange results obtained from IRGA measurements were correlated with $\delta^{13}C$ using Pearson's correlation coefficient in order to assess whether a significant relationship (p<0.05) between the two existed. The

distance to groundwater and water availability index were also correlated with $\delta^{13}C$ for each tree during the months that sampling took place. Comparisons were not made between wetlands. This is because it was unlikely that the sampled hydrological gradients were consistent between the three wetlands.

4.3 RESULTS

4.3.1 Transect location

4.3.1.1 Banganup Lake

The *M. preissiana* trees selected for this study in the "A" position (located closest to the water's edge) were relatively smaller than the "B" and "C" trees located further upslope. Table 4.1 presents the size pattern at Banganup Lake, with the trees in A position being the shortest in height, trees on the mid slope being tallest, and the height of trees on the upper slope in C position lying between the two. Tree B in transect 1 was recorded as particularly tall, reaching over 21m in height, which exceeds all height descriptions for this species (Seddon, 1972; Marchant *et al*, 1987; Froend *et al*, 1993). In terms of tree diameter (DBH), it was found that trees in the C position were largest in diameter, followed by the trees in B position, then the trees located on the lower slope.

Table 4.1: Height, DBH and Vigour of study trees at Banganup Lake. Diameter was measured at breast height (1.3m). Where multiple stems were present, diameter was recorded from the largest stem. Vigour measurements were based on the crown classification technique outlined by Ladd (1996). Trees labelled according to lake, transect number and transect position.

	Tree	Height (m)	DBH (cm)	Number Stems	Vigour score (3 - 23)
Transect 1	BL1a	11.26	15.9	1	14
	BL1b	21.23	36.4	1	17
	BL1c	13.52	74	1	14
Transect 2	BL2a	8.602	12.8	1	18
	BL2b	15.88	35.8	1	17
	BL2c	15.36	58.75	1	12
Transect 3	BL3a	6.26	13.6	1	16
	BL3b	11.6	25.6	1	17
	BL3c	8.09	31.3	1	14

Vigour scores of the trees within the sampling population remained relatively constant, ranging from 12 (tree 2c) to 18 (tree 2a).

4.3.1.2   Thomsons Lake

Tree heights at Thomsons Lake were generally found to be shorter than the trees at Banganup Lake in all transect positions. Trees in position C appeared to be tallest at the lake, with the height of tree 1c being 11.13m (Table 4.2). In terms of tree diameter, the trees located furthermost from the water's edge (position C), were larger than the trees located downslope in both B and A positions.

Table 4.2: Height, DBH and Vigour of study trees at Thomsons Lake. Diameter was measured at breast height (1.3m). Where multiple stems were present, diameter was recorded from the largest stem. Vigour measurements were based on the crown classification technique outlined by Ladd (1996). Trees labelled according to lake, transect number and transect position.

	Tree	Height (m)	DBH (cm)	Number Stems	Vigour score (3 - 23)
Transect 1	TL1a	3.91	15.85	1	13
	TL1b	4.47	19.8	1	17
	TL1c	4.85	37.5	1	10
Transect 2	TL2a	5.24	23.5	1	18
	TL2b	7.12	15.5	1	15
	TL2c	8.55	46	1	19
Transect 3	TL3a	5.64	34.5	3	18
	TL3b	5.05	18	3	12
	TL3c	11.13	48	1	10

The vigour of individuals varied, with trees 1c and 3c having low vigour scores of 10 out of a possible 23. All trees were single-stemmed with the exceptions of trees A and B from transect 3, which were both triple-stemmed.

4.3.1.3   Jandabup Lake

Tree heights at Jandabup Lake were the shortest of the three wetlands, with tree heights ranging from 3m (tree 2a) to 9.1m (tree 1c) (Table 4.3). Diameters ranged from 20.5cm (tree 2a) to 55.5cm (tree 1c). Tree vigour remained fairly constant between transect positions. All trees in Transect 1 were multiple stemmed (with tree 1b having 8 stems at breast height), while transect 2 and 3 contained all single-stemmed trees.

Table 4.3: Height, Diameter and Vigour of study trees at Lake Jandabup. Diameter was measured at breast height (1.3m). Where multiple stems were present, diameter was recorded from the largest stem. Vigour measurements were based on the crown classification technique outlined by Ladd (1996). Trees labelled according to lake, transect number and transect position.

	Tree	Height (m)	DBH (cm)	Number Stems	Vigour score (3 - 23)
Transect 1	JL1a	5.4	21	2	13
	JL1b	6.3	22	8	17
	JL1c	9.1	55.5	2	16
Transect 2	JL2a	3	20.5	1	17
	JL2b	3.8	25	1	15
	JL2c	6.7	46	1	14
Transect 3	JL3a	3.1	22	1	16
	JL3b	6.5	39	1	19
	JL3c	7.1	34.25	1	14

4.3.2   Hydrological Gradients along Transects

4.3.2.1   Banganup Lake

By calculating the mean, average minimum and average maximum groundwater levels experienced at Banganup Lake (based on monitoring data from 1993-2000), it was found that there existed an annual 0.93m range in groundwater levels. For all three transects, the

mean groundwater level was found to lie directly below (less than 0.1m) the level augered to in July 2000, indicating that at the time of augering, groundwater levels had only just risen above the mean level. Figures 4.3, 4.4 and 4.5 show the elevational diagrams of transects 1, 2 and 3 respectively at Banganup Lake. The three positions A, B and C are described below in terms of annual groundwater fluctuations.

The combination of groundwater levels measured in 2000 and the monthly groundwater data monitored from a nearby bore during 1993-2000, showed that the trees located on the lower slope (A position) were of shallowest depth to groundwater. The three A trees experienced a mean annual depth to groundwater ranging from 0.45m and 0.5m. Maximum groundwater levels were reached during August-October and rose to ground level. Minimum levels dropped approximately 0.9m under ground during March-May.

The trees located on the mid slope ranged in depths to groundwater from an average of 1.3m to 1.6m annually. Groundwater rose to a maximum depth during the winter months from 0.8m to 1.2m below ground, and dropped to a depth between 1.7m and 2.1m.

The C trees positioned on the upper slope were located with furthermost distance from the lake's edge. These trees were elevated between 2 to 4m above the trees located on the mid and upper slopes. On average, the annual depth to groundwater for the C trees ranged from 2.9m (tree 2c) to 4.4m (tree 1c). Maximum levels rose to depths between 2.5m and 3.9m, and minimum levels dropped to depths between 3.4m and 4.8m below ground.
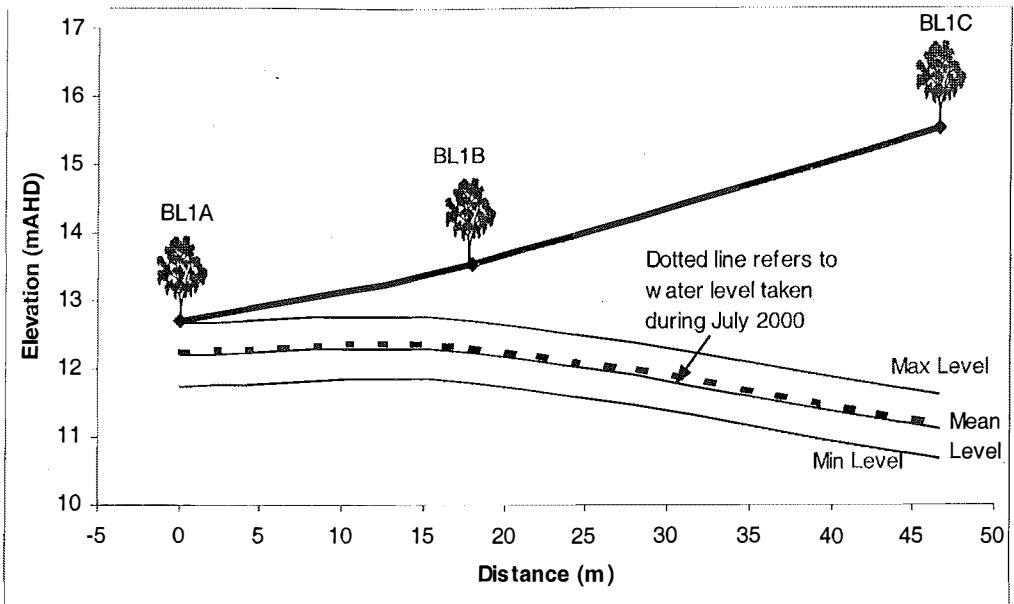
Figure 4.3: Elevational Gradient of Transect 1, Banganup Lake, showing position of trees along transect and depth to groundwater. Dotted line refers to water levels taken during July 2000. Mean levels were calculated from monitoring data taken from bore LB1 during the period 1993 to 2000. Minimum water levels refer to the mean taken from the dry season each year (March, April, May); Maximum water levels refer to the mean taken from the wet season each year during this period (August, September, October). Trees not pictured to scale.
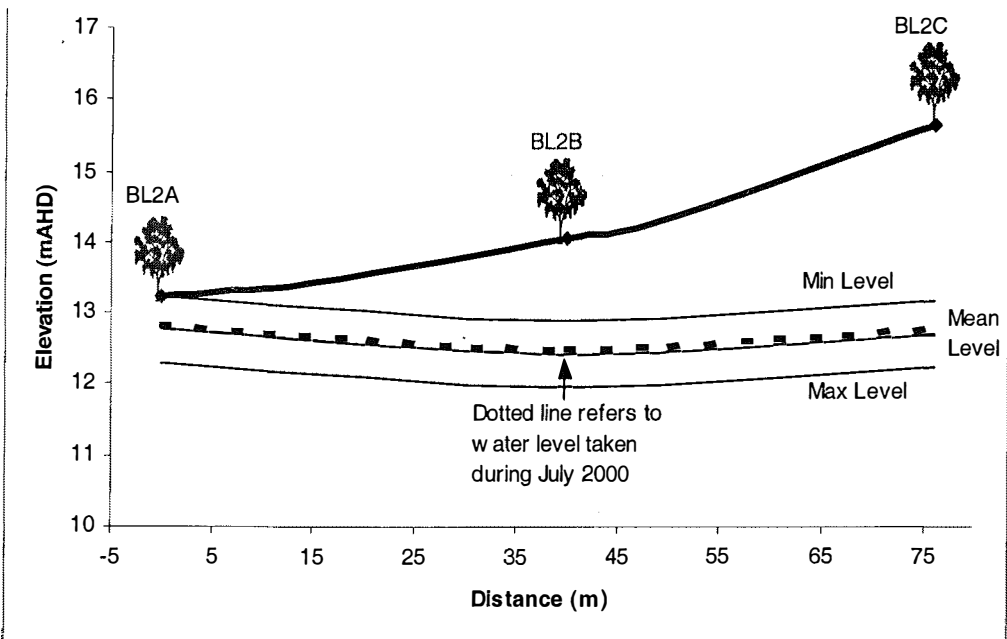
Figure 4.4: Elevational Gradient of Transect 2, Banganup Lake, showing position of trees along transect and depth to groundwater. Dotted line refers to water levels taken during July 2000. Mean levels were calculated from monitoring data taken from bore LB1 during the period 1993 to 2000. Minimum water levels refer to the mean taken from the dry season each year (March, April, May); Maximum water levels refer to the mean taken from the wet season each year during this period (August, September, October). Trees not pictured to scale.
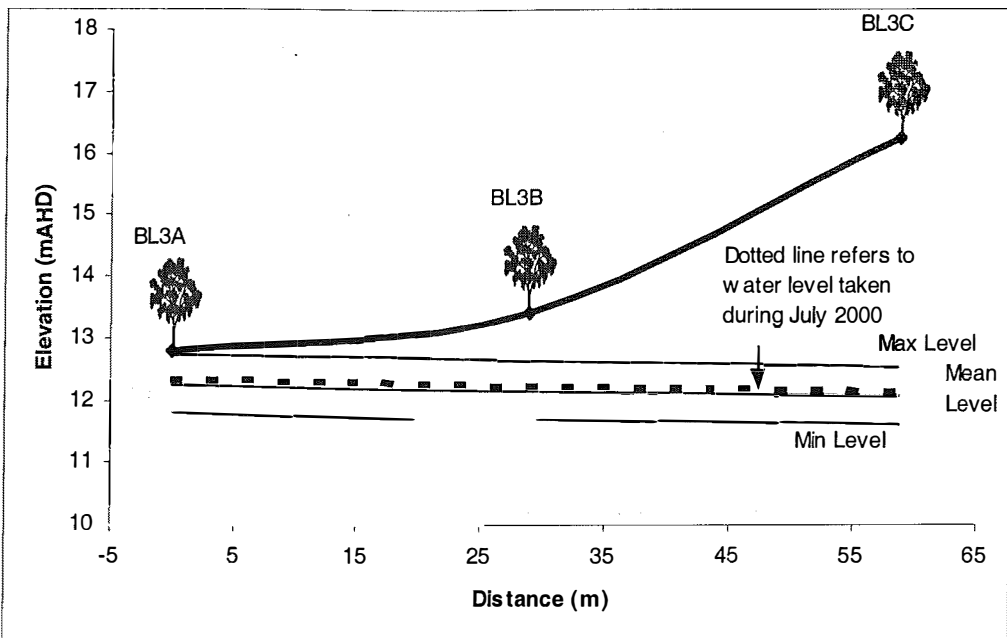
Figure 4.5: **Elevational Gradient of Transect 3, Banganup Lake, showing position of trees along transect and depth to groundwater. Dotted line refers to water levels taken during July 2000. Mean levels were calculated from monitoring data taken from bore LB1 during the period 1993 to 2000. Minimum water levels refer to the mean taken from the dry season each year (March, April, May); Maximum water levels refer to the mean taken from the wet season each year during this period (August, September, October). Trees not pictured to scale.**

4.3.2.2   Thomsons Lake

It was found that groundwater levels for Thomsons Lake had an annual range of 0.58m, with maximum levels occurring during September-November and minimum levels occurring from March-May (Figures 4.6-4.8). The groundwater level measured during July 2000 at each tree was 0.04m below the average minimum groundwater level expected, indicating that groundwater levels in July 2000 were fairly low in comparison to the expected levels calculated from the period 1993-2000. This may be due to the rapid decrease in groundwater levels during this period (Chapter 5, Figure 5.9). In terms of depth to groundwater and inundation period experienced by the trees at Thomsons Lake, the conditions were somewhat "wetter" than those experienced at Banganup Lake.

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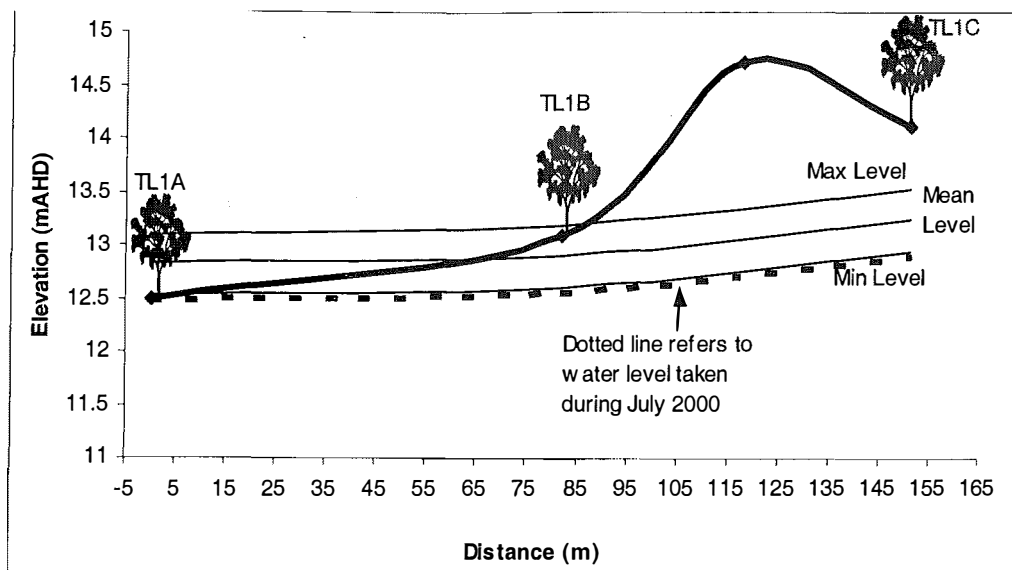
Figure 4.6: Elevational Gradient of Transect 1, Thomsons Lake, showing position of trees along transect and depth to groundwater. Dotted line refers to water levels taken during July 2000. Mean levels were calculated from monitoring data taken from bore TM10C during the period 1993 to 2000. Minimum water levels refer to the mean taken from the dry season each year (March, April, May); Maximum water levels refer to the mean taken from the wet season each year during this period (September, October, November). Trees not pictured to scale.
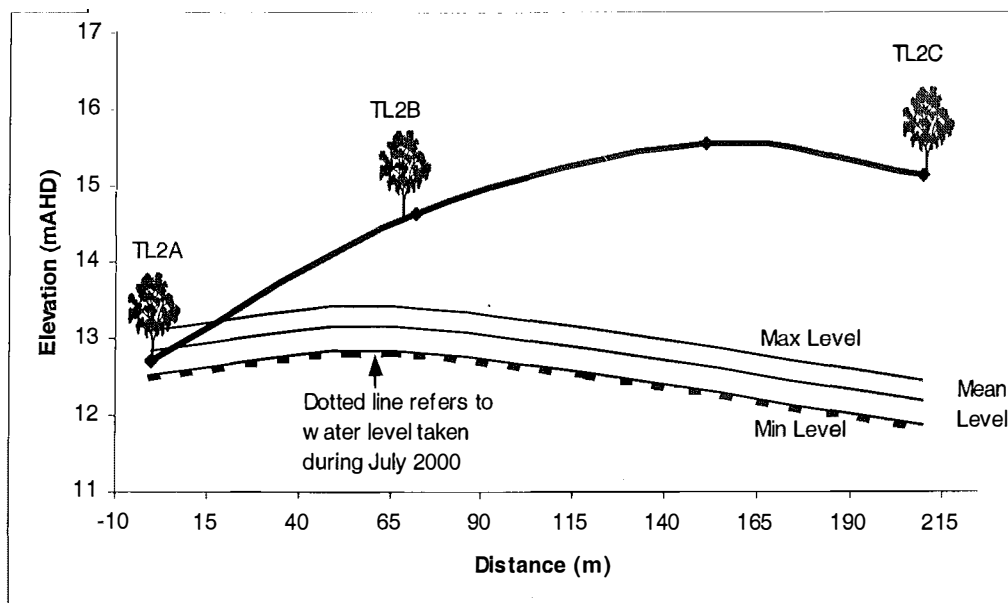
Figure 4.7: Elevational Gradient of Transect 2, Thomsons Lake, showing position of trees along transect and depth to groundwater. Dotted line refers to water levels taken during July 2000. Mean levels were calculated from monitoring data taken from bore TM10C during the period 1993 to 2000. Minimum water levels refer to the mean taken from the dry season each year (March, April, May); Maximum water levels refer to the mean taken from the wet season each year during this period (September, October, November). Trees not pictured to scale.

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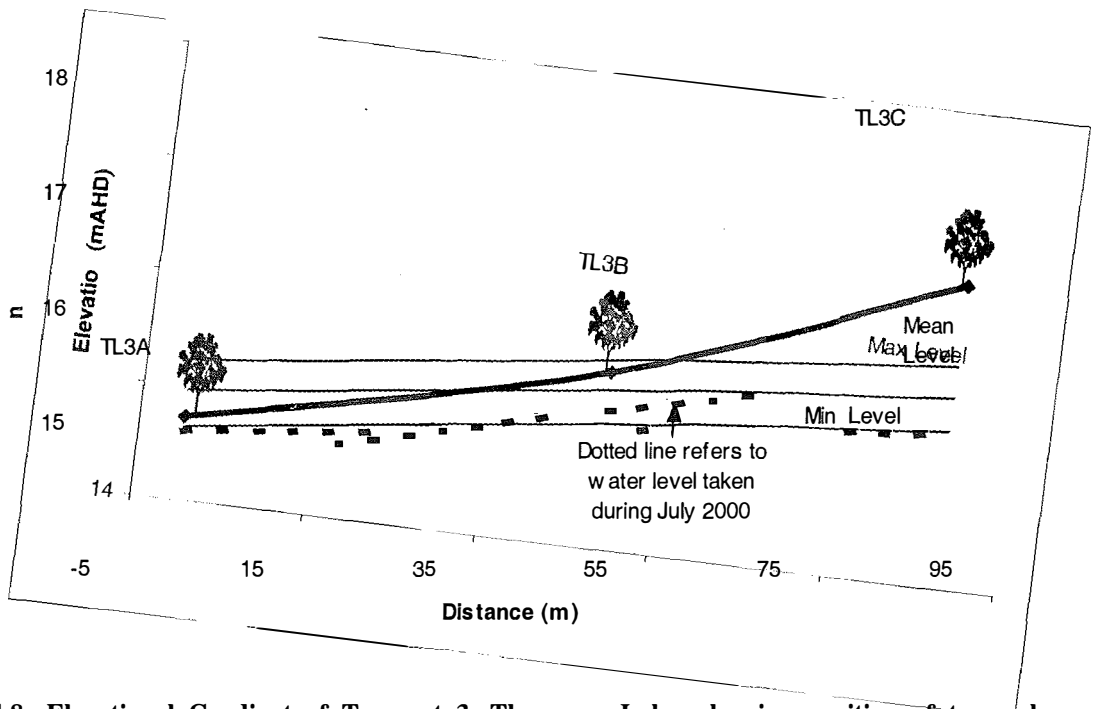
Figure 4.8: Elevational Gradient of Transect 3, Thomsons Lake, showing position of trees along transect and depth to groundwater. Dotted line refers to water levels taken during July 2000. Mean levels were calculated from monitoring data taken from bore TM10C during the period 1993 to 2000. Minimum water levels refer to the mean taken from the dry season each year (March, April, May); Maximum water levels refer to the mean taken from the wet season each year during this period (September, October, November). Trees not pictured to scale.

The trees located on the lower slope experienced seasonal inundation, based on the data monitored from a nearby bore during 1993-2000. Tree 1a experienced groundwater rising to 0.34m above ground on average, and Tree 2a experienced a mean depth to groundwater of 0.14m above the land's surface. During the winter months, inundation occurred, with levels rising between 0.41m and 0.61m, and the drier months saw groundwater dropping, with trees 1a and 3a remaining slightly inundated, and tree 2a experiencing groundwater levels dropping to 0.16m below ground.

The trees located on the mid slope at Thomsons Lake experienced a range in the depth to groundwater and inundation period. Tree 1b inundated seasonally, with annual depths to groundwater rising to 0.11m above ground during the winter months, and falling to 0.47m underground in summer. Tree 2b experienced the driest condition of the trees at this

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position, with a mean depth to groundwater being 1.58m underground, and depths ranging from 1.23m to 1.81m annually. The annual groundwater regime at tree 3b fell between trees 1b and 2b, with the mean depth to groundwater being 0.34m below ground.

The trees in the C position at Thomsons Lake also experienced a variety of annual groundwater regimes. The annual depth to groundwater at tree 1c ranged from 0.59m to 1.16m, at tree 2c the annual water level ranged from 2.69m to 3.26m below ground and at 3c, the range in water levels fell between 0.7m and 1.2m below ground. Despite the high variations in depth to groundwater experienced by the replicate trees within transect positions at Thomsons Lake, comparisons between different transect positions were made by taking average values of the replicates, in order to reduce the effects of this variation.

4.3.2.3   Lake Jandabup

Due to the small area of remnant terrestrial vegetation existing at Lake Jandabup, groundwater levels were measured at transect 1 trees only, and data relating to groundwater levels were extrapolated to include all trees. Physical measurements to the groundwater at Lake Jandabup were impossible to make due to the layer of hard pan rock intercepting the auger before the water table was reached. For this reason, the depths to groundwater were estimated by using the data from a near by bore, and assuming that the water table levels measured remained constant throughout the landscape. This method found that the average range in annual groundwater levels at Lake Jandabup (from 1993-2000) was 0.8m, with minimum water levels occurring from March-May and maximum levels occurring from September-November (Figure 4.9).
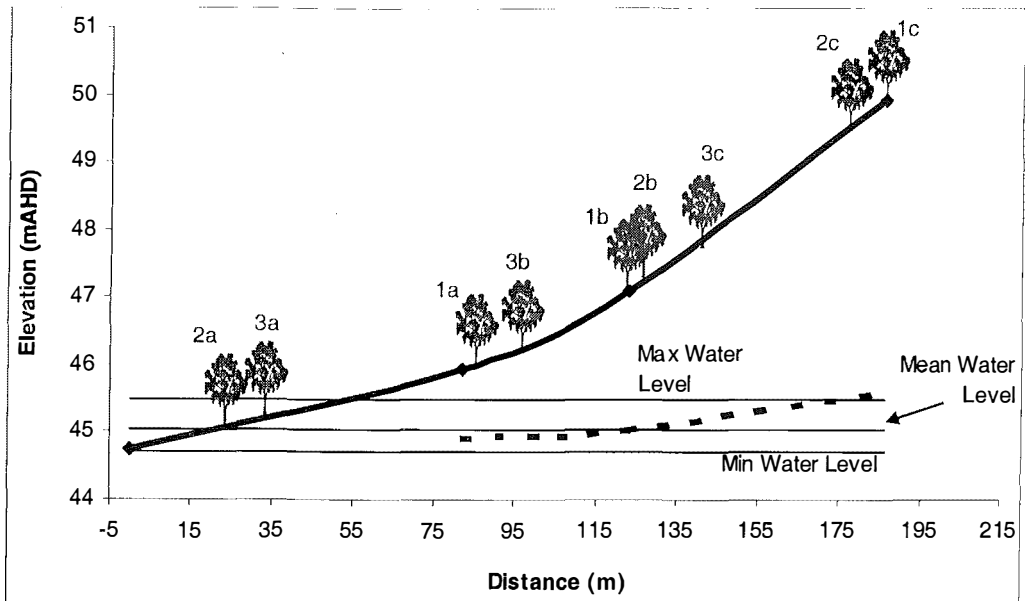
Figure 4.9: Combined Elevational Gradient based on measurements taken from Transect 1, Lake Jandabup, showing position of all trees along gradient and depth to groundwater. Dotted line refers to layer of hard pan rock measured during July 2000. Mean levels were calculated from monitoring data taken from bore JB12A during the period 1993 to 2000. Minimum water levels refer to the mean taken from the dry season each year (March, April, May); Maximum water levels refer to the mean taken from the wet season each year during this period (September, October, November). Trees not pictured to scale.

Figure 4.9 shows that the nine study trees occurred along the hydrological gradient in four apparent "patches". Trees 2a and 3a occurred closest to the water's edge, with mean depths estimated at being between 0.1m and 0.2m below ground. At maximum, these trees were inundated with groundwater, with levels rising from 0.2-0.4m above the surface. Minimum levels were found to fall from 0.4m-0.6m below ground. The second patch of trees located on a similar elevation were trees 1a and 3b. Tree 1a was found to lie approximately 0.9m above the water table on average, with levels rising to 0.45m below ground in the wet season and falling below 1.2m underground in the dry season. It is predicted that tree 3b experienced similar conditions to this. Trees 1b, 2b, and 3c were located further along the gradient. The annual groundwater level averaged 2.1m below ground for tree 1b. Levels were predicted to rise in the wet season to 1.6m under ground, and fall in the dry season to

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2.4m below ground. It was assumed that trees 2b and 3c also experienced this groundwater levels regime. Trees 1c and 2c were located the furthermost away from the water's edge, with 1c elevated 2.8m above 1b. Groundwater levels were 4.9m below the land surface in this position, with the dry season seeing the water table drop to 5.2m below ground, and rise to 4.4m below ground in the wet season.

4.3.3   Water Availability in 2000

4.3.3.1   Climate

Total monthly rainfall and mean maximum monthly temperature were calculated for the 12-month period between September 1999 – August 2000, as this was the likely period to affect the $\delta^{13}C$ signature obtained from samples of nascent xylem tissue. From September 1999 to August 2000, Perth's average monthly maximum temperature remained fairly similar to that of the mean temperature expected for that month. Figure 4.10 shows that only in November and December 1999 did the temperature increase considerably above the mean. The monthly rainfall received during this period did deviate from the mean, with January receiving 102.4mm of rain compared to the average level of 9.2mm. July also received more rainfall than expected, totalling 231mm compared to the average of 161mm.
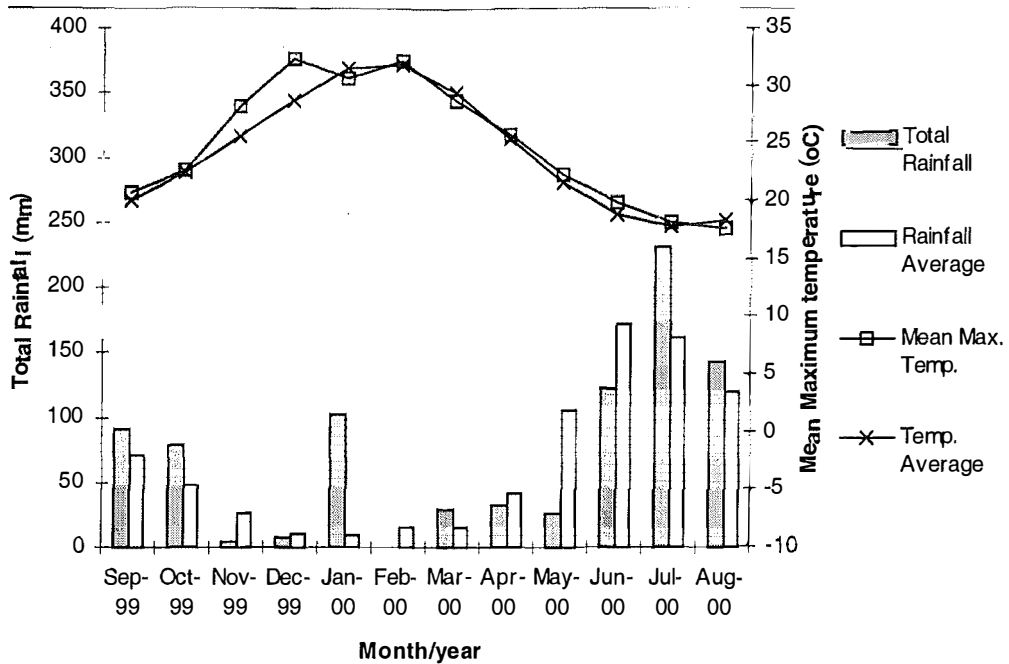
Figure 4.10: Perth's total monthly rainfall and mean maximum temperature, September 1999 - August 2000. Monthly averages are included, based on data between 1944 - 2000.

4.3.3.2 Hydrology

4.3.3.2.1 Banganup Lake

From September 1999 to June 2000, Banganup Lake remained dry with water levels never exceeding the lake bed. July 2000 saw a small rise in lake levels with the lake inundating to a depth of 0.08m (Chapter 5, Figure 5.4). The decreasing trend in groundwater levels at Banganup Lake since 1993 saw October 1999 peaking at 12.59mAHD, which was 0.7m lower than levels in October 1994. However, 2000 saw a rise in groundwater levels, with levels rising to 12.78mAHD in August (Chapter 5, Figure 5.6).

4.3.3.2.2 Thomsons Lake

The surface water monitoring data was available until February 2000, and these data show

that Thomsons Lake was dry from September 1999 to February 2000 (Chapter 5, Figure 5.7). Groundwater levels reached a maximum in 1999 during November, peaking at 12.44mAHD. Levels declined to 11.48mAHD in May 2000, and then rose in June 2000. Lake levels would be expected to peak during September to November 2000

4.3.3.2.3 Lake Jandabup

January 2000 saw surface lake levels dropping to a minimum for the 1999/2000 dry season of 44.3mAHD. The water depth during this period remained just below 0.5m, which was relatively high compared to the previous year where the lake filled to just 0.24m in February (Chapter 5, Figure 5.1). Lake levels filled gradually until August, after which depths rose rapidly to over 1m. Groundwater levels did not rise until July 2000.

4.3.4   Time-Integrated, Short Term $\delta^{13}C$ Measurements from Nascent Xylem Tissue

The preliminary trial of extracting cellulose from whole wood samples of xylem tissue occurred using the samples taken in April 2000. It was found that a strong, positive correlation existed between the $\delta^{13}C$ of whole wood and the $\delta^{13}C$ of the same sample consisting of crude cellulose (Figure 4.11). This strong relationship has been demonstrated in other studies (e.g. Livingston & Spittlehouse, 1996; Macfarlane *et al*, 1999; Warren & Adams, 2000) and indicates that reliable data can be obtained from whole wood samples for *M. preissiana*. For this reason, all subsequent $\delta^{13}C$ analyses of nascent xylem tissue were performed on whole wood.
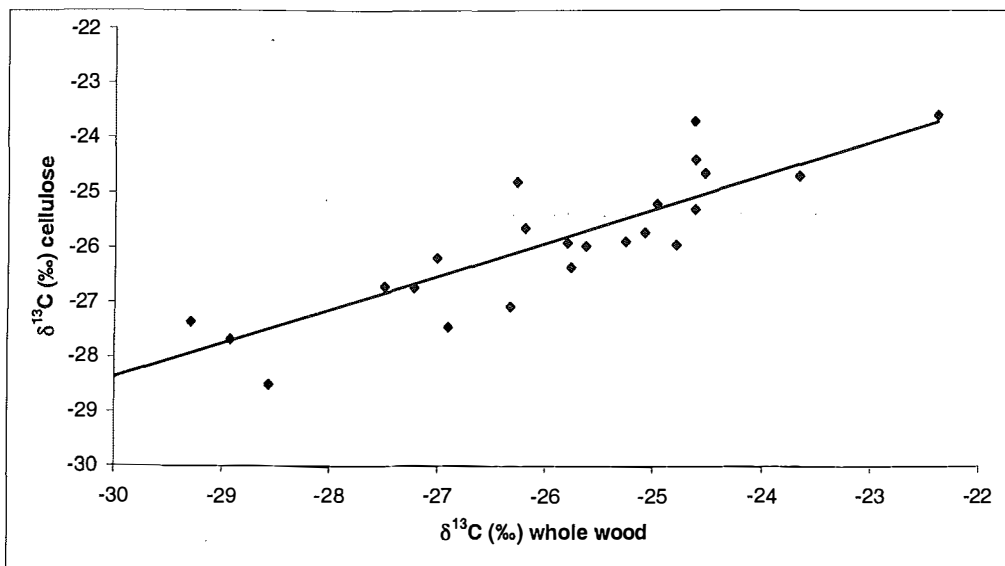
Figure 4.11: Scattergraph of δ¹³C of cellulose versus δ¹³C of whole wood of *M. preissiana* (including line of best fit) taken from nascent xylem tissue sampled during April 2000. Each value represents a value taken from a single sample. Points represent trees from the three wetlands. A significant correlation (r = 0.87) existed between whole wood and cellulose δ¹³C.

4.3.4.1 Transect position, month and δ¹³C

4.3.4.1.1 Banganup Lake

The mean $\delta^{13}C$ signatures of trees at all sites appeared to become more negative from position C (with greatest distance from the lake's edge) to position A (closest to the lake's edge) and from April to August. Banganup Lake best exhibited these trends (Figure 4.12). In April, there was little difference in $\delta^{13}C$ between transect positions, however, trees in the "A" position displayed the most negative xylem tissue $\delta^{13}C$ values averaging –25.60‰, and C trees displayed the most positive signatures averaging –24.32‰. In June, these differences between positions became more pronounced, with the C trees showing little change in $\delta^{13}C$, however the B and A trees decreasing in $\delta^{13}C$, averaging –25.57‰ and –26.58‰ respectively. In August, the mean $\delta^{13}C$ of all trees decreased more so,

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corresponding to the increase in rainfall during August.
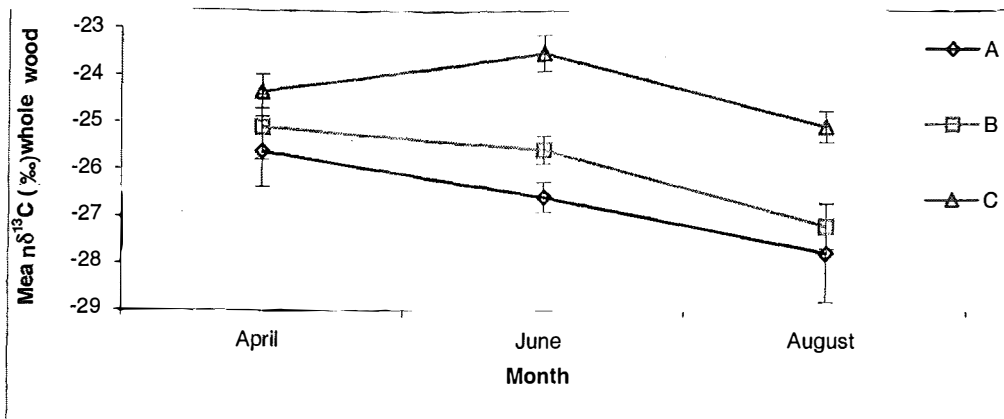
Figure 4.12: Mean δ¹³C and standard error of nascent xylem tissue at Banganup Lake during 2000. Line graph shows mean changes in δ¹³C from April-June-August according to transect position.

Two-way analysis of variance, which investigated the effect of month and transect position on δ¹³C of the *M. preissiana* population at Banganup Lake, found that both factors significantly affected nascent xylem tissue δ¹³C (Table 4.4). The Bonferroni post-hoc test proved that this difference existed between the trees located on the lower slope (position C)

Table 4.4: Results of repeated measures ANOVA testing differences in *M. preissiana* δ¹³C between months and transect position at Banganup Lake. Two-way ANOVA was conducted to test for the overall effects of month and position on δ¹³C. Month*Position refers to the interaction between the two factors.

Two-way repeated measures ANOVA					
Differences in δ¹³C between time and transect position					
Factor	**d.f.**	**Mean Square**	**F-Value**	**P-Value**	
Month	2	7.640	16.888	<0.01	*
error	12	0.452			
Position	2	13.077	6.378	0.033	*
error	6	2.050			
Month*Position	4	0.774	1.711	0.212	NS

NS   Not statistically significant (p>0.05)
*   Statistically significant (p<0.05)

and the upper slope (position A) and between the month of August with April and June (p<0.05). There was no significant interaction between the two, and therefore it could be

concluded that these factors were independently affecting the $\delta^{13}C$ signatures of the *M. preissiana* population at Banganup Lake. The Bonferroni post hoc test confirmed that these differences occurred between the months of April and August and June and August, with the $\delta^{13}C$ values of the trees being significantly lower in August. There was no significant difference between xylem tissue $\delta^{13}C$ in April and June. Differences in $\delta^{13}C$ between transect positions existed between positions A and C, with the trees located on the upper slope being isotopically heavier than those on the lower slopes.

4.3.4.1.2 *Thomsons Lake*

Thomsons Lake reflected similar differences in xylem tissue $\delta^{13}C$ of *M. preissiana* to those observed at Banganup Lake. Figure 4.13 shows that there was a general decrease in $\delta^{13}C$ from April to August by trees from all positions. Similarly to Banganup Lake, C trees did not show a high degree of change between April and June (in contrast $\delta^{13}C$ increased during this period), however, as the wet season progressed between June and August, $\delta^{13}C$ became more negative. The $\delta^{13}C$ activity of the *M. preissiana* trees in the B position at Thomsons Lake was of particular interest. In April, the mean $\delta^{13}C$ of the trees in this "central" B position was similar to that of the C trees located further most from the water's edge. These trees were more than 1.2‰ heavier than the A trees. In comparison, in August when water availability was higher, the mean $\delta^{13}C$ signature of the B trees was more similar to that of the A trees located closest to the water's edge. A and B trees during August were found to be more than 2.7‰ more negative than the C trees.
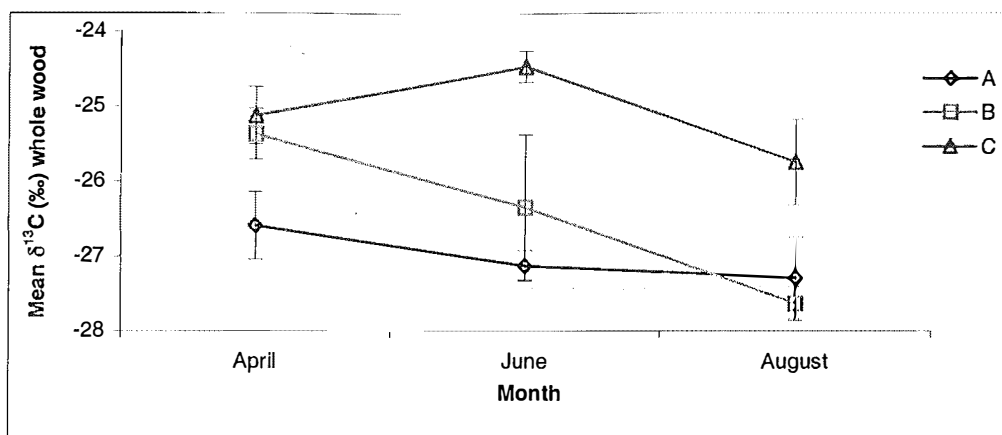
Figure 4.13: Mean δ^{13}C and standard error of nascent xylem tissue at Thomsons Lake during 2000. Line graph shows mean changes in δ^{13}C from April-June-August according to transect position.

Two-way analysis of variance showed that as with Banganup Lake, transect position and month both influenced independent, significant responses in xylem tissue δ^{13}C at Thomsons Lake (Table 4.5). The Bonferroni post-hoc test revealed that these differences existed between the months of April and August, where δ^{13}C was significantly higher in April ($p<0.05$). In terms of transect position, the trees located on the A position were less depleted in δ^{13}C than those on the C position, where water availability was lower ($p<0.05$).

Table 4.5: Results of repeated measures ANOVA testing differences in *M. preissiana* δ^{13}C between months and transect position at Thomsons Lake. Two-way ANOVA was conducted to test for the overall effects of month and position on δ^{13}C. Month*Position refers to the interaction between the two factors.

Two-way repeated measures ANOVA Differences in δ^{13}C between time and transect position					
Factor	**d.f.**	**Mean Square**	**F-Value**	**P-Value**	
Month	2	3.541	5.434	0.021	*
error	12	0.652			
Position	2	8.545	9.897	0.013	*
error	6	0.863			
Month*Position	4	0.997	1.530	0.255	NS

NS   Not statistically significant (p>0.05)
*   Statistically significant (p<0.05)

4.3.4.1.3 Jandabup Lake

The results obtained from the $\delta^{13}C$ of nascent xylem tissue at Lake Jandabup also reflected the trends found at Banganup and Thomsons Lakes. From April to August, the $\delta^{13}C$ of trees at the three transect positions became more negative as water availability at the sites increased (Figure 4.14). June and August showed the most prominent variations, with the A trees being more depleted in $\delta^{13}C$ than the B trees, which in turn were more depleted than the C trees.
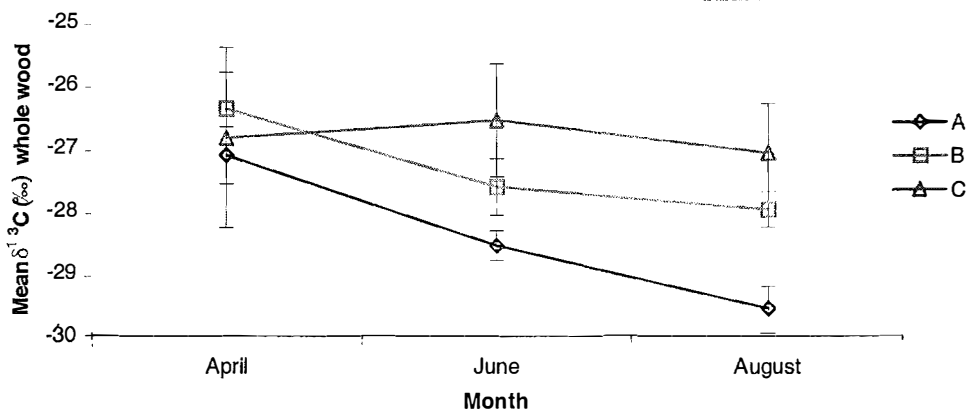
Figure 4.14: Mean $\delta^{13}C$ and standard error of nascent xylem tissue at Lake Jandabup during 2000. Line graph shows mean changes in $\delta^{13}C$ from April-June-August according to transect position.

Two-way analysis of variance proved that a significant difference in $\delta^{13}C$ of the *M. preissiana* population at Lake Jandabup existed between months, however there was no significant change in $\delta^{13}C$ between positions along the hydrological gradient (Table 4.6). The Bonferroni post hoc test confirmed that this difference existed from April to August and from June to August, with the $\delta^{13}C$ signatures of the *M. preissiana* population at Lake Jandabup being significantly higher in April and June than in August. The $\delta^{13}C$ signatures obtained from Lake Jandabup for all trees appeared to be more negative than those obtained from the other two wetlands, with mean $\delta^{13}C$ signatures never exceeding -26‰.

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Table 4.6: Results of repeated measures ANOVA testing differences in *M. preissiana* $\delta^{13}C$ between months and transect position at Lake Jandabup. Two-way ANOVA was conducted to test for the overall effects of month and position on $\delta^{13}C$. Month*Position refers to the interaction between the two factors.

	Two-way repeated measures ANOVA				
	Differences in $\delta^{13}C$ between time and transect position				
Factor	d.f.	Mean Square	F-Value	P-Value	
Month	2	5.118	11.418	0.002	*
error	12	0.448			
Position	2	5.871	1.646	0.268	NS
error	6	3.546			
Month*Position	4	1.111	2.477	0.1	NS

NS   Not statistically significant (p>0.05)
*   Statistically significant (p<0.05)

4.3.4.2 $\delta^{13}C$ and Instantaneous Water Use Efficiency

Gas exchange measures were carried out for the month prior to both the June and August sampling for $\delta^{13}C$ at Banganup Lake. The weekly measurements made during these months were averaged in order to relate directly with the month-averaged $\delta^{13}C$ signature obtained from the same tree. For the five gas exchange measurements (A, E, C_i, g_s, A/E), the $\delta^{13}C$ of xylem tissue proved to be insignificantly correlated with all measures (p<0.05). For example, the instantaneous WUE (A/E) of *M. preissiana* individuals at Banganup Lake measured during June and August were not well correlated with the $\delta^{13}C$ of trees in the A position (r = 0.18; p>0.05), and were negatively correlated with the $\delta^{13}C$ of trees in the mid (r = -0.82; p<0.05) and upper slopes (r = -0.31; p<0.05) (Figure 4.15). This negative correlation between $\delta^{13}C$ and instantaneous WUE was unexpected due to the widely documented *positive* relationship existing between the two (Ehleringer & Cooper, 1988; Körner *et al*, 1991; Leavitt, 1993; Stewart *et al*, 1995; Le Roux, 1996; Livingston &

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Spittlehouse, 1996; Berry *et al*, 1997; Damesin *et al*, 1997; Walcroft *et al*, 1997; MacFarlane & Adams, 1998; Pate & Arthur, 1998).
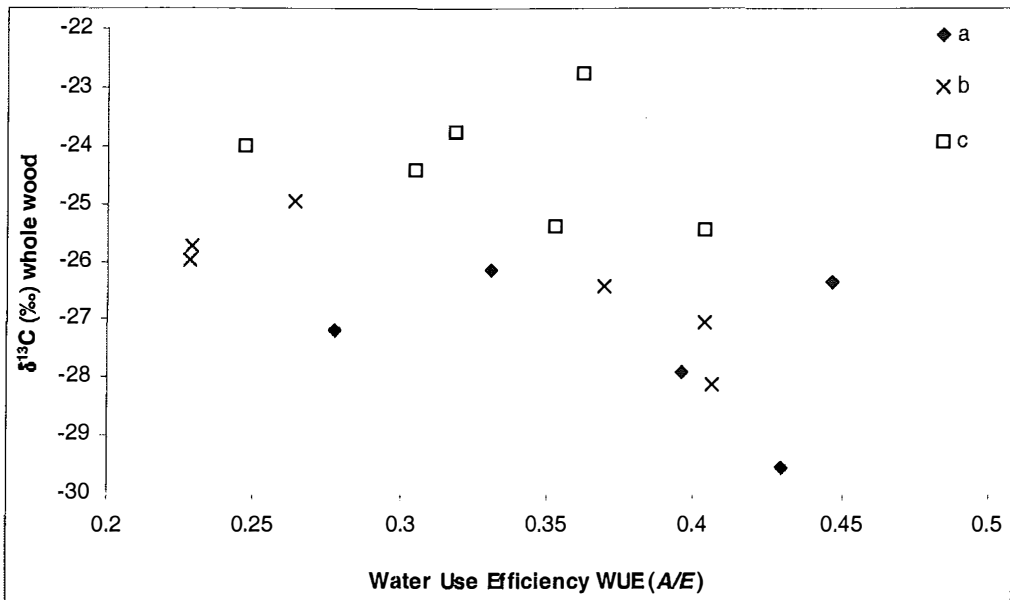
Figure 4.15: Scatterplot showing the trend between instantaneous water use efficiency (*A/E*) and δ¹³C of nascent xylem tissue for *M. preissiana* at Banganup Lake. Values based on measurements taken during June and August 2000. Correlation coefficient between WUE and "a" trees (♦) was 0.18 "b" trees (x) was -0.82 and "c" trees (□) was -0.31.

4.3.4.3 $\delta^{13}C$ and Water Availability

The water availability index, which combined winter and summer rainfall, humidity, temperature, groundwater and surface water, was found to be negatively correlated with the $\delta^{13}C$ of *M. preissiana*. Figure 4.16 combines the water availability index for the months April, June and August in 2000 with $\delta^{13}C$ of the trees for all sites. It was found that there was a significant, negative correlation between the $\delta^{13}C$ of xylem tissue and the water availability index of the B trees, (r = -0.83; p<0.05). $\delta^{13}C$ of trees on the lower slope (A position) formed a moderate (and insignificant) negative correlation (r = -0.64; p>0.05) with the water availability at each site. The $\delta^{13}C$ of C trees was not significantly correlated

with low water availability (r = -0.32; p>0.05).
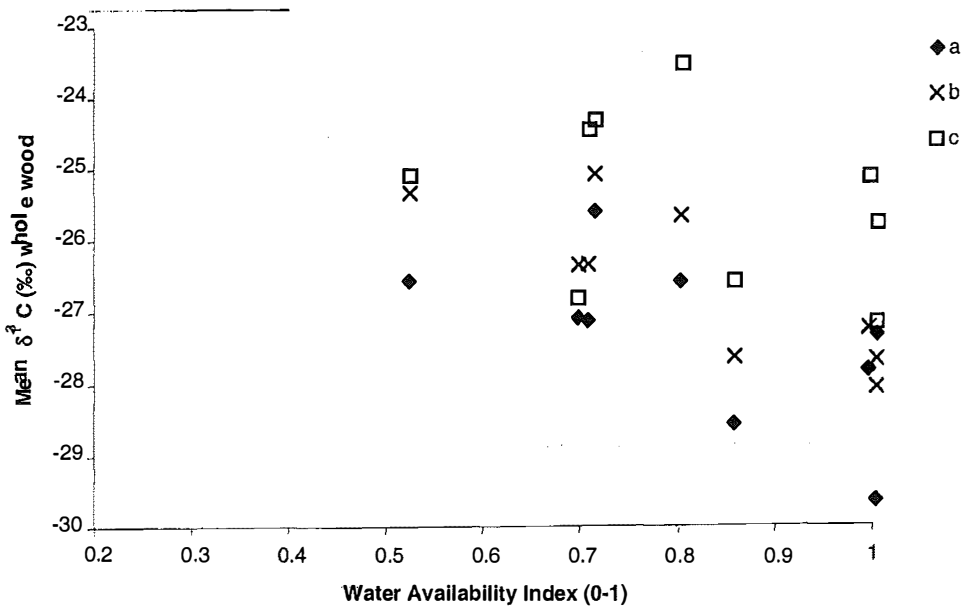
Figure 4.16: Scatterplot showing the relationship between seasonal xylem tissue $\delta^{13}C$ of *M. preissiana* at all sites, and the water availability index for corresponding seasons (April, June, August 2000). The correlation coefficient between the index and $\delta^{13}C$ of "A" trees (♦) was -0.64, "B" trees (×) was -0.83 and C trees (□) was -0.32.

By correlating the depth to groundwater experienced by each tree at Banganup and Thomsons Lakes (Lake Jandabup could not be correlated due to the lack of accurate groundwater data available for each individual tree) with its corresponding $\delta^{13}C$ signature obtained during April, June and August, it was found that a significant, positive relationship existed between the two (Banganup Lake: r = -0.76; p<0.05; Thomsons Lake: r = -0.71; p<0.05). Figure 4.17 shows that for Banganup and Thomsons Lakes, trees with the greatest access to the groundwater supply (i.e. the lowest depth to groundwater) had more negative $\delta^{13}C$ values. This is also reflective of the negative correlations existing between tree $\delta^{13}C$ and the water availability index.
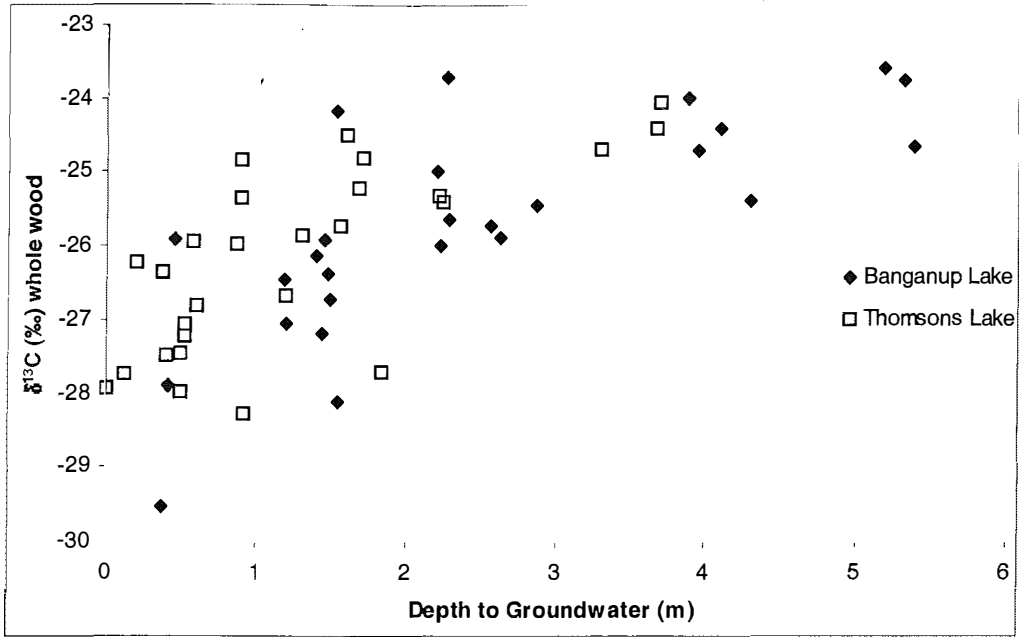
Figure 4.17: Scatterplot showing the relationship between tree depth to groundwater and $\delta^{13}C$ of nascent xylem tissue of *M. preissiana* at Banganup (♦) and Thomsons (□) Lakes during 2000. $\delta^{13}C$ values represent samples taken during April, June and August 2000. Correlation coefficient between groundwater depth and $\delta^{13}C$ at Banganup Lake was 0.76, and at Thomsons Lake was 0.71.

In general, these results conclude that as water availability at the sites increased spatially and temporally, *M. preissiana* discriminated more so against the heavier isotope, and therefore exhibited more negative, short term $\delta^{13}C$ signatures.

4.4   DISCUSSION

The $\delta^{13}C$ of *M. preissiana* at the three wetlands on the Swan Coastal Plain were interpreted in association with the water availability parameters measured at the time of sampling, in order to understand how the species responded physiologically to changes in water availability in the natural environment. Additionally, this information can be used to determine whether the water availability of a site induced a response in terms of instantaneous water use efficiency, and whether this is indicative of $\delta^{13}C$.

4.4.1   Water availability

The seasonal trend in water availability at Banganup Lake, Thomsons Lake and Lake Jandabup were similar, with water availability being highest during the latter part of winter and early spring, and lowest during late summer and early autumn. This is due to the Mediterranean climate of the Swan Coastal Plain, with the warm, dry summers and cool, wet winters being the principal forces determining ground and surface water levels (Froend *et al*, 1993). Groundwater levels reflect a two to three month lag behind cumulative rainfall. Therefore, recharge to the shallow, unconfined aquifer occurring during April to October generally sees a progressive rise in groundwater levels. Levels peak in September-October as a response to winter rains, and drop to a minimum during March-April due to the dry summer (Froend *et al*, 1993). Being surface expressions of the unconfined groundwater table, the lakes studied showed seasonal variations in surface water levels that reflected those of the groundwater. Banganup Lake showed seasonal drying during summer, and

expressed high annual variation in groundwater levels of nearly 1m. Thomsons Lake also dried periodically, and displayed smaller annual variation in groundwater levels of 0.6m. This is possibly due to the fact that lake levels are currently managed under CALM Water Level Criteria in order to prevent flooding and excessive periods of drying (EPA, 1989). Lake Jandabup is permanently inundated and experiences annual variations in groundwater of approximately 0.8m.

The water availability occurring *within* the wetland sites was assumed to be distributed along a gradient (represented by the transects), where water availability was highest in areas closest to the lake's margin, and lowest on the upper slope with furthermost distance from the lake. The depth to groundwater taken from the three positions along each transect supported this assumption. At Banganup Lake, the trees on the lower slope (the A position) became close to inundated during winter, while groundwater levels dropped 1m in summer. Thomsons Lake saw trees on the lower slope being inundated for a majority of the year, and the A trees at Lake Jandabup from transects 2 and 3 also become seasonally inundated. Trees on the mid slope were found to experience a mean depth to groundwater ranging from 0.5m to 1.85m (both at Thomsons Lake), with two of the B trees at Thomsons Lake experiencing inundation during winter. The mean depths to groundwater for trees located on the upper slope (the C position) ranged from 1.2m (Thomsons Lake) to 4.3m (Banganup Lake). Moisture measurements were not taken from the surrounding soil of each tree, however, it was assumed that soil moisture increased down the soil profile to the saturated zone. Therefore the trees with greatest access to the groundwater supply (trees in the A position) would similarly have greatest access to the water contained in the soil's upper horizons.

4.4.2 Time-Integrated Short Term $\delta^{13}C$ Measurements of Nascent Xylem Tissue

4.4.2.1 $\delta^{13}C$ variations along the hydrological gradient

The *M. preissiana* individuals occurring along the water availability gradient within the three wetlands were also found to display a gradient in carbon isotope discrimination. The short-term method of sampling for $\delta^{13}C$, which provided an integrated, one-month value, became more negative from the upper slope (C position) to the lower slope (A position). That is, the $\delta^{13}C$ of *M. preissiana* decreased as water availability increased within the wetlands. This pattern was most pronounced at Banganup and Thomsons Lakes, where significant increases in $\delta^{13}C$ were noted in trees from the A to the C positions. $\delta^{13}C$ was not found to differ significantly between trees on the mid slope to those on the upper and lower slopes. This decrease in $\delta^{13}C$ in areas of increasing water availability has been likewise documented in other studies. Ehleringer & Cooper (1988) found that $\delta^{13}C$ became more negative in species located in microhabitats of higher water availability. Garten Jr. & Taylor Jr. (1992) similarly discovered that the $\delta^{13}C$ of various tree species was more positive in xeric sites in comparison to mesic sites, while trees along a riparian zone were found to discriminate against the heavier isotope more so in zones of high stream flow than in zones of low stream flow (Leffler & Evans, 1999). This negative relationship between water availability and $\delta^{13}C$ has been documented by various researchers where variations in water availability are investigated in terms of factors such as irrigation regimes, rainfall gradients, climate types and habitat types (Handley *et al*, 1994; Saurer *et al*, 1995; Stewart *et al*, 1995; Le Roux *et al*, 1996; Livingston & Spittlehouse, 1996; Damesin *et al*, 1997;

Walcroft et al, 1997; Pate & Arthur, 1998). The theory behind this relationship is that water availability limits the stomatal conductance of the tree, and when water availability is low, there is a decrease in transpiration rates, and an overall increase in A/E or WUE (Ehleringer & Cooper, 1988). The decrease in stomatal control in turn, leads to reduced discrimination between ^{13}C and ^{12}C, and therefore higher, or more positive, $\delta^{13}C$ (Handley et al, 1994).

4.4.2.2 $\delta^{13}C$ variations between seasons

The relationship between water availability and $\delta^{13}C$ within the wetland sites was also proven by the seasonal changes in $\delta^{13}C$ occurring over 2000. There was a trend observed at all lakes, where the $\delta^{13}C$ values of the $M.$ $preissiana$ populations decreased significantly from April to August. The trees at Banganup and Jandabup Lakes also exhibited this significant decrease from June to August. The decrease in $\delta^{13}C$ from April to August may be indicative of the increase in water availability due to the onset of winter rainfall. In particular, Perth received above average rainfall during June 2000, which may have influenced the negative response in $\delta^{13}C$ of the $M.$ $preissiana$ populations during August. This seasonal pattern of $\delta^{13}C$ has similarly been found by other researchers, where $\delta^{13}C$ is most negative during the growth period when water availability is highest, and is most positive during the hot, dry summer-autumn period (Leavitt & Long, 1986; Livingston & Spittlehouse, 1996; Walcroft et al, 1997; Macfarlane & Adams, 1998). This annual trend in $\delta^{13}C$ is illustrated by the stylised pattern of $\delta^{13}C$ of $E.$ $globulus$ wood during one growth season (Macfarlane & Adams, 1998) (Figure 4.18).
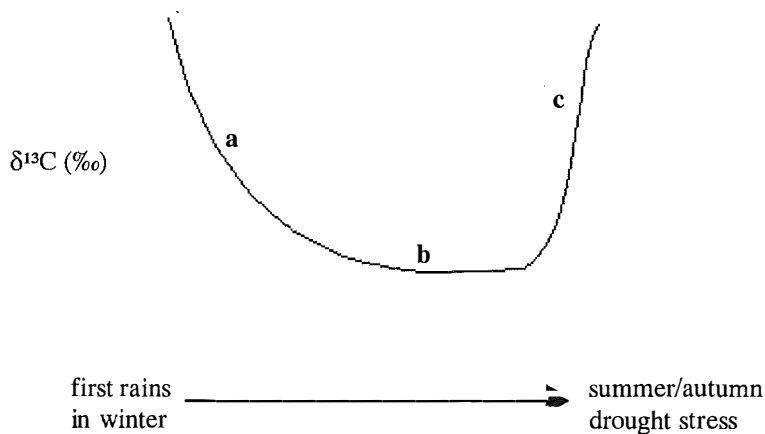
Figure 4.18: Stylised pattern of changes occurring in $\delta^{13}C$ of wood during one growth season. Wood formed during the start of winter (a) still carries a $\delta^{13}C$ record of low WUE from the previous summer. $\delta^{13}C$ formed in wood during the growth period (b) is most negative and indicative of low WUE. Wood formed during late summer/early autumn (c) is most positive in $\delta^{13}C$ and indicates relative drought stress (adapted from Macfarlane & Adams, 1998).

The strong, negative correlation between $\delta^{13}C$ and the water availability index of the *M. preissiana* populations sampled during April, June and August during 2000 incorporated both spatial (transect position) and temporal (season) variations in $\delta^{13}C$. The high correlation between the index and $\delta^{13}C$ therefore reinforces the inverse relationship between water availability and $\delta^{13}C$. The strong positive correlation existing between the depth to groundwater and $\delta^{13}C$ for Thomsons and Banganup Lakes measured in April, June and August indicates that the depth to groundwater experienced by each tree may be a primary factor influencing its carbon isotope discrimination. This is expected due to the preference of *M. preissiana* to grow in areas of elevated groundwater levels (Seddon, 1976).

Trees located on the upper slope (C position) were found to respond more slowly to the annual rise in water availability than the trees on the lower and mid slopes in terms of $\delta^{13}C$.

At the three wetlands, the $\delta^{13}C$ of the nascent xylem tissue of the C trees did not show a decline in $\delta^{13}C$ until August, while trees in the A and B positions were found to show a continual decline in $\delta^{13}C$ from April to August as water availability increased. This was similarly reflected by the relationship between the water availability index and $\delta^{13}C$, where the trees in the C position were not significantly correlated with the increasing water availability. Froend *et al* (1993) recognise that these old, parental trees show a slower response to changes in groundwater in terms of root distribution and growth due to their reduced plasticity (e.g. they are less able to alter the root system to "follow" a declining water table). Additionally, the larger size of the individuals located on the upper slope supported a larger leaf area than the smaller trees, and probably transpired more water on an individual plant basis (Donovan & Ehleringer, 1992). This suggests that the larger trees use water more conservatively in the drier months than smaller trees in order to maintain a larger leaf area, and therefore may not respond isotopically to the influx of water until late winter, when groundwater levels maximise.

4.4.2.3 $\delta^{13}C$ variations and instantaneous WUE

The gradient in $\delta^{13}C$ that was found to occur at Banganup Lake was not reflected in the WUE measures made during June and August. There was no significant relationship existing between WUE and $\delta^{13}C$, and the relationships between $\delta^{13}C$ and *A, E, g_s* and *C_i* along the hydrological gradient were not of significance. The failure of *M. preissiana* at Banganup Lake to display this relationship may have been due to several factors. The sampling size (three replicates per transect position, per lake) may have been too small to

adequately measure gas exchange and instantaneous water use efficiency. The relatively short hydrological gradient sampled may have also been a factor affecting the results. At transect 2 at Banganup Lake, there was a maximum of 75m distance between the tree in the A position and the tree in the C position. Although the trees occurring along the gradient at Banganup Lake *did* show significant differences in $\delta^{13}C$, the variations in water availability along the gradient may not have been adequate in inducing a significant change in instantaneous WUE between trees. It is also possible that the instantaneous WUE measurements made at Banganup Lake were a reflection of the diurnal variations occurring at the time of measurement rather than a reflection of the plant's actual water status. These factors are difficult to account for in the natural environment and the short hydrological gradient upon which sampling was based may not have been adequate to induce an instantaneous response in plant WUE. Additionally, the morphological characteristics of the different trees sampled may have caused variations in the instantaneous measurements. Size and age differences between the trees were observed, however, Donovan & Ehleringer (1992) recognise that larger trees are generally more water use efficient than smaller, juvenile trees. This relationship was not observed for *M. preissiana* during the sampling period.

4.4.2.4 $\delta^{13}C$ variations and other factors

It is possible that the variations observed in $\delta^{13}C$ at the three wetlands were related to external factors other than water availability. One of these factors could be the light, or irradiance levels reaching the trees. The trees located on the lower and mid slopes at

Banganup Lake formed a part of two bands of trees. The A trees were part of a dense, juvenile band surrounding the lake's margin, which recruited after a fire in 1977. The B trees were also included in a thick, outer band, which was likely to have established 60-70 years ago when rainfall was high. The C trees were scattered throughout the surrounding woodland on the upper slopes (Froend *et al*, 1993). These dense bands of *M. preissiana* provided a highly competitive environment for the trees in the A and B positions at Banganup Lake, where light and water both may have been limited resources. Berry *et al* (1997) found that plants receiving limited light expressed more negative $\delta^{13}C$ signatures. This was unlikely to be the case in this study, because the *M. preissiana* populations at Thomsons Lake and Lake Jandabup displayed similar trends in $\delta^{13}C$ along the hydrological gradient. These populations were of relatively low density in comparison to the population at Banganup Lake, where it was possible that light and water were limited by competition.

Another possibility for the variations measured in $\delta^{13}C$ at the wetlands over 2000 could be tree age and/or size. By examining the measurements made of tree size (Tables 4.1, 4.2 and 4.3) it is clear that generally, the size and subsequent age of trees increased with distance up the slope from positions A to C. This size/age distribution of *M. preissiana* individuals around the wetlands is likely to be a response to changes in hydrology over time. This size gradient observed at Banganup Lake was found to be inversely associated with $\delta^{13}C$, with the larger, older trees displaying more positive $\delta^{13}C$ values than the smaller, juvenile trees on the lower slope.

Sanquist *et al* (1993) identified that a relationship exists where younger trees generally

exhibit more negative $\delta^{13}C$ signatures than older trees due to having lower A/E ratios (therefore lower WUE) and more rapid growth rates during the establishment stage of the plant's life. This conforms to the findings of Donovan & Ehleringer (1992) who suggested that instantaneous WUE was higher in larger trees due to the higher content of water transpired in total by the tree. The $\delta^{13}C$ variations measured along the hydrological gradient at the three wetlands do reflect these findings of Sanquist *et al* (1993), where the smaller trees (located in the A position of the transects) discriminated against the heavier isotope more so than the larger trees in the C position.

Similarly, Francey & Farquar (1982) identified a possible "juvenile effect" that exists, where an age-related physiological factor affects the discrimination of carbon in plants, with younger plants discriminating against the heavier isotope more so than older trees, and therefore exhibiting more negative $\delta^{13}C$ values. This is due to smaller trees reassimilating the respired CO_2 retained under the canopy. This is relevant in plant communities such as forests, where smaller juveniles are distributed throughout stands of parent trees. However, the *M. preissiana* individuals at the three wetlands studied were either sparsely distributed (Thomsons Lake and Lake Jandabup) or distributed in even-aged stands (Banganup Lake), suggesting that the "juvenile effect" was unlikely to play a role in $^{13}C/^{12}C$ discrimination.

4.4.3 Future Applications for Time-Integrated, Short Term $\delta^{13}C$ Measurements

This field-based approach to assessing the applicability of $\delta^{13}C$ analysis in natural *M. preissiana* populations in the short term was effective in establishing a relationship between

carbon isotope discrimination and water availability along a short, natural gradient. However, there were limitations associated with the sampling techniques and field methodologies selected. One of these limitations involved the assumption of a hydrological or water availability gradient. This assumption that water availability was greatest near the edge of the wetland and decreased with distance up the slope was made on the basis on the depth to groundwater measured along the slope, and the inundation period of each transect position. Exact measures of water availability involving the soil stratigraphy and soil moisture content were not made and therefore this assumption could not be quantified. However, the distribution of *M. preissiana* around wet depressions on the Swan Coastal Plain suggests that the species is dependent on groundwater availability and that its root system utilises the water contained in the groundwater. Small trees are often associated with lower water availability due to their smaller root systems not accessing the soil-water supply during the growth season (Donovan & Ehleringer, 1992). This is probably not the case for the *M. preissiana* populations studied, with the smaller trees being located on areas of relatively higher groundwater levels than the larger trees that had larger root systems that could access the deeper water table. Therefore, despite the fact that water availability was not quantitatively measured, the assumption that it formed a gradient along the rising elevation could be substantiated in terms of depth to groundwater.

As mentioned earlier, the age and size of individuals can impact $\delta^{13}C$. The distribution of *M. preissiana* populations at the wetlands made it impossible to select trees of similar age, size and health at each transect position. For this reason, some of the trends observed in $\delta^{13}C$ along the hydrological gradient at the wetlands may have been influenced by the age

or size of the tree in addition to water availability. Differences in $\delta^{13}C$ observed in trees of different sizes were likely to be due to the differences in their physiological responses to changes in water availability, with larger trees responding more efficiently to the water supply than smaller trees with higher growth rates (Donovan & Ehleringer, 1992).

Another factor that may have influenced the $\delta^{13}C$ signatures derived from the *M. preissiana* populations was the type of plant tissue sampled for $\delta^{13}C$ analysis. It has been documented that there are a variety sampling methods that can reliably indicate the short-term WUE of plant in terms of $\delta^{13}C$. This study focused on the analysis of recently developed xylem tissue, which provided an approximate one-month measure of the tree's WUE (Pate & Arthur, 1998). Perhaps more widely documented is the sampling of leaf tissue for $\delta^{13}C$, which provides a $\delta^{13}C$ value representative of the plant's WUE for the lifetime of the leaf. Damesin *et al* (1997) found that problems existed with the sampling of foliar tissue due leaf age, with leaves showing little change in $\delta^{13}C$ after maturity. Therefore, the most reliable method of sampling leaf tissue for $\delta^{13}C$ analysis involves the sampling of juvenile leaves. Due to the sampling period of this study (April to August), it was impossible to sample juvenile leaves of *M. preissiana* because of its delayed growth season commencing in November (Marchant *et al*, 1987). Another short term method for measuring $\delta^{13}C$ is by sampling phloem translocate (Pate & Arthur, 1998; Pate *et al*, 1998). This method requires extensive prior sampling to ensure "phloem bleeding" in a species. Due to the time limitations of the project, this method was not investigated, however, it certainly holds potential for use in future studies. A comparison of the $\delta^{13}C$ signatures obtained in *E. globulus* from different plant tissue (foliar, nascent xylem, phloem sap, and stem wood)

found that nascent xylem tissue $\delta^{13}C$ provided reliable, short term measures of WUE (Pate & Arthur, 1998), and hence its application in this study.

The mean $\delta^{13}C$ measurements made from the nascent xylem tissue of the *M. preissiana* individuals selected along the transects were subject to error due to the small number of replicates. Limitations in time and sampling costs meant that only three replicates for each transect position could be measured from three wetlands on the Swan Coastal Plain. Although significant differences were measured between trees experiencing different water availabilities, the accuracy of the predictions would increase with an increased number of replicates. Ideally, several transects would be constructed at each wetland, and longer hydrological gradients sampled in order to investigate changes in $\delta^{13}C$ and instantaneous WUE along a more significant hydrological gradient. Additionally, several wetlands on the Swan Coastal Plain would be selected for the study, with sampling accounting for wetlands of various water regimes in order to compare $\delta^{13}C$ variability between lakes.

The failure of *M. preissiana* to exhibit trends in instantaneous WUE may be due to the short gradients sampled, which may have represented insignificant variations in water availability. Otherwise, it may be accounted for by the variable nature of instantaneous measures, as they present only a "snapshot" of the tree's physiological response to the environmental conditions during that moment in time. Slight diurnal changes such as increased cloud cover or a change in atmospheric temperature may alter the WUE of the tree during that instantaneous moment, therefore providing results which do not truly represent the plant's physiological response to water availability. A method recommended

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to indicate water use efficiency or water stress in plants is to measure leaf water potential or xylem pressure potential (mentioned in Chapter 2). This is defined by the free energy per unit volume of water (Fitter & Hay, 1987). Generally, leaf water potential falls during a drought and increases when the water supply is abundant due to changes in the hydraulic conductance throughout the plant (Baird & Wilby, 1999). Because this method is based on measuring the water pressure moving through the plant, it is reliable in indicating its instantaneous water stress, without incorporating the diurnal variations such as those measured by IRGA equipment. This method could be incorporated with the IRGA-based measures and $\delta^{13}C$ measures to provide a more complete understanding of how *M. preissiana* responds physiologically to changes in water availability.

CHAPTER 5: TIME INTEGRATED, LONG TERM $\delta^{13}C$ MEASUREMENTS USING GROWTH RING TISSUE

5.1   INTRODUCTION

The use of $\delta^{13}C$ as a time-integrated measure of plant water use efficiency can be extended by sampling older tissue in order to get a long-term measure of WUE. The $^{13}C/^{12}C$ ratio contained in tree rings represents a time-averaged measure of the plant's water use efficiency during a season of growth (Leffler & Evans, 1999). Cellulose is not transferred between the annual growth rings formed in trees, therefore, annual events are recorded permanently within the $\delta^{13}C$ signatures of individual tree rings (Tans *et al*, 1978; Walcroft *et al*, 1997). For this reason, tree ring $\delta^{13}C$ is used to reveal long-term trends in plant physiological responses to various environmental conditions, and can be used as an indicator to monitor past changes in climate and atmospheric CO_2 levels (Dupouey *et al*, 1993).

Many researchers have found that $\delta^{13}C$ contained in tree ring wood is representative of the water availability of the site during a season of growth (E.g. Wilson & Grinsted, 1977; Depouey et al, 1993; McNulty & Swank, 1995; Livingston & Spittlehouse, 1996; Walcroft et al, 1997; Macfarlane & Adams, 1998; Pate & Arthur, 1998; Leffler & Evans, 1999). Other factors affecting growth ring $\delta^{13}C$ include climate, atmospheric CO_2 concentration, nutrient availability, pollution and growth (McNulty & Swank, 1995). One alternative to understanding past environmental conditions is by measuring radial tree growth. Annual variations in water availability influence tree growth rates, with positive correlations

existing between water availability with tree height and diameter (Livingston & Spittlehouse, 1996). However, $\delta^{13}C$ values contained within annual tree rings are believed to be less sensitive to random environmental effects than radial growth (Dupouey *et al*, 1993) and therefore better indicators of the plant's response to environmental conditions.

Many tree ring $\delta^{13}C$ studies have been conducted in controlled environments such as plantations, where environmental variations are minimised and trees sampled are of known ages (E.g. Macfarlane & Adams, 1998; Pate & Arthur, 1998). These studies prove to be difficult to apply to natural ecosystems. McNulty & Swank (1995) recognise that patterns in annual wood tissue $\delta^{13}C$ are often difficult to link with ecosystem parameters due to the complexity of, and variations in, natural ecological processes.

The variations in the hydrological regimes over the Swan Coastal Plain have impacted upon, and changed, wetland environments. The combination of reduced rainfall and declining groundwater levels have been the cause of the "drying" effects of wetlands on the SCP in terms of reduced water levels and prolonged drying periods (Froend *et al*, 1993). Banganup, Thomsons and Jandabup Lakes all show signs of declining water levels over time, which is indicative of the structure of the vegetation community (E.M. Mattiske & Associates, 1988; Froend et al, 1993). Investigating how plant populations occurring at wetlands responded physiologically to changes in the past will enable managers to monitor and understand the impacts of future activities on the health and survival of the vegetation. *Melaleuca preissiana*, being a dominant fringing tree species around wetlands on the SCP, and being responsive to changes in hydrology in terms of population distribution and

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recruitment (Froend *et al*, 1993), provides an ideal species in which to study the relationship between long term plant physiology and water availability using tree ring $\delta^{13}C$ measurements.

Following the short-term measurements of $\delta^{13}C$ made in *M. preissiana* populations by sampling recently formed tissue, it was proposed to make long term $\delta^{13}C$ measurements by sampling annual ring tissue of trees located along a hydrological gradient. The applicability of this choice of method will be determined by whether the long-term patterns of $\delta^{13}C$ sampled across tree rings are reflective of water availability parameters.

The objective of this study was to assess the applicability of long-term $\delta^{13}C$ measurements (by sampling growth ring wood) in understanding the water use efficiency of natural populations of *M. preissiana* on the SCP. Specific research objectives were as follows:

i.   Identify and describe how the water availability changed over time in three wetlands on the SCP;

ii. Investigate the variations in long-term measures of $\delta^{13}C$ in *M. preissiana* individuals along a hydrological regime;

iii. Investigate the variations in long term measures of $\delta^{13}C$ in *M. preissiana* individuals between annual growth seasons;

iv. Determine whether long-term $\delta^{13}C$ measures of *M. preissiana* relate to past water availability parameters along a hydrological gradient;

v.   Identify the external factors (other than water availability) that influence growth ring $\delta^{13}C$ in natural *M. preissiana* populations.

5.2   MATERIALS AND METHODS

5.2.1   Site Selection and Field Sampling Design

The field sampling design for this component of the study followed that used for the time-integrated, short term $\delta^{13}C$ method of sampling nascent xylem tissue. The same sites, transects and trees were used for this study (Chapter 4, Figure 4.1).

5.2.2   Integrated, Long Term $\delta^{13}C$ Measurements

5.2.2.1   Growth Ring Tissue Sampling

In order to obtain a time-averaged record of the historical growing conditions at each study site, individual growth rings for each tree were analysed for $\delta^{13}C$. At every site, cores were removed from the trunk of each tree at breast height (1.3m) using a 1.6mm diameter whole saw with a hand battery drill. Tree coring involved removing a small area of the bark layers and drilling from the edge radially toward the centre of the main stem. The core was removed and the exposed area of wood was treated with a bitumen-based wounding spray. Resulting tree cores were approximately 10mm in diameter and 40mm in length. Each core was collected into vials containing 80% ethanol for three weeks in order to remove contaminating solutes. Cores were oven-dried at 35°C for 24 hours and then sanded to enable visibility of growth rings by using a combination of coarse and fine-grained sandpaper. Plate 5.1 shows a longitudinal cross section with visible growth rings of the core

from tree 1a at Banganup Lake.
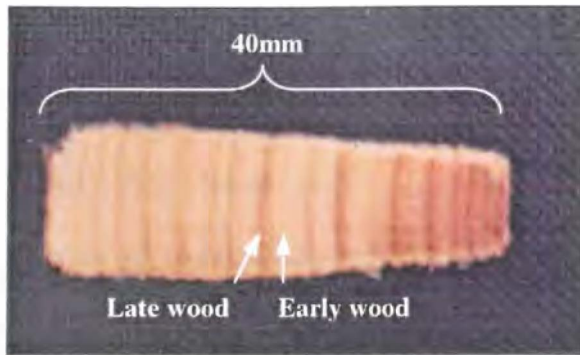
Plate 5.1: Longitudinal cross section of core taken from tree 1a (age 23 years) from Banganup Lake. Core was sanded to enable visibility of growth rings. Annual growth rings were identified as containing vessels of early wood formed during the growth period (late spring/early summer) and late wood formed during winter.

The cores were divided under a dissecting microscope into individual growth rings. Division into rings occurred along the layer of latewood using a scalpel blade, with care being taken to ensure that each ring contained early *and* late wood, therefore representing an annual cycle of growth. For cores where ring sizes were very small (e.g. the core from tree 3c at Banganup Lake contained over 40 rings in one 40mm long core), the cores were divided into equally spaced samples of wood, each sample representing 5% of the total core length. This is similar to the method adopted by Pate & Arthur (1998) for dividing trunk sections of *E. globulus*. Each wood sample was ground into a fine pulp using a 1.2mm mesh tissue grinder and subjected to $\delta^{13}C$ analysis.

5.2.2.1.1 Core aging and growth ring analysis

Cores were viewed under a dissecting microscope to define growth rings. One ring of wood was considered to consist of late *and* early wood, therefore representing one yearly cycle of growth. This pattern of early and late wood represents the seasonal climatic conditions of the site, with the large, thin-walled early wood vessels forming during the growth season,

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and the smaller, thick-walled late wood forming when growth slows down during the winter months (Mitchell Beazley Publishers Ltd, 1976). In most cases, one year of growth is represented by one tree ring, however, aberrations from this seasonal growth cycle do occur (e.g. from fire, insect attack, prolonged drought) and may result in the formation of "false rings" (Rowell, 1984). This was accounted for in the division of the *M. preissiana* growth rings. The thin layer of small, thick-walled late wood xylem cells was used to define the boundary of each growth ring. Growth rings were counted and measured to the nearest 0.01mm using a 0.05mm dial calliper (Mitutoyo, Japan) to estimate the approximate years of growth contained in each core. Cores were found to contain from 6 to 40 years of growth depending on tree age and size. In order to ensure that growth rings approximated one annual growth cycle, entire cross sections of trunk were removed from two, additionally selected *M. preissiana* trees of known ages at 20cm above ground level at Banganup Lake, which established after the 1977 fire (Froend *et al*, 1993). Two cores were taken from the same trees at breast height. Growth rings from both cores and cross sections (three radii from each cross section) were measured and compared in order to ensure the representation of cores to display annual growth rings.

5.2.2.1.2 *Basal area increment measurements*

Annual tree growth was related to the corresponding $\delta^{13}C$ value for that year. To do this, it was necessary to calculate the basal area increment (BAI) for each tree. This involved using the width of each tree ring to determine the area of wood produced during that year at breast height (1.3m). This was calculated by using the tree DBH, and the length of the core to determine the diameter, and the subsequent area of the remaining cross section of stem

not included by the core. Each ring width from the core (starting from the oldest ring located closest to the centre of the stem) was then added to the diameter of this smaller cross section, and the area of the new, larger cross section was then calculated. This new area value (with the growth ring added) was subtracted from the previous area value (without the growth ring added) to determine the area of wood growth produced during that year. This process continued until all growth rings for the tree were accounted for. This technique results in *estimates only* of BAI. It is acknowledged that the core represented only one small section of the stem's cross section, and ring widths displayed by the core may be poor representations of the width of the *entire* ring. The width of an entire ring is rarely constant and depends on the tree's position in the environment and its exposure to different conditions (Mitchell Beazley Publishers Ltd., 1976).

5.2.2.2   Cellulose Extraction

The cellulose extraction method followed that described in Chapter 4. Due to the variation in ages of growth ring tissue, it was unlikely that the cellulose content in all growth rings were equal. Macfarlane *et al* (1999) recognise that much of the $\delta^{13}C$ variation within trees is due to the presence of resins and oils in untreated wood occurring in different proportions in various parts of the tree. In order to reduce variability in $\delta^{13}C$ due to these factors, the cellulose extraction technique was carried out for all samples from all sites, regardless of whether there was a significant difference between the whole wood and cellulose $\delta^{13}C$ of the samples.

<u>5.2.2.3 $\delta^{13}C$ Analysis</u>

All growth ring wood samples were subjected to $\delta^{13}C$ analysis. A 1.2mm mesh tissue grinder was used to grind individual wood samples into a fine pulp. Similarly for the nascent xylem tissue technique, wood samples were weighed to 1-1.5mg samples ($\pm$ 0.1mg). The procedure used for analysing samples for $\delta^{13}C$ was outlined in Chapter 2. Resulting $\delta^{13}C$ signatures were expressed in parts per thousand (‰).

5.2.3   Water Availability

Similarly to the short term sampling method, it was necessary to collect historical data regarding the water availability of the three sites to compare to the long term $\delta^{13}C$ values. This involved collecting meteorological data for the Perth region since 1950 such as the total annual rainfall, the total number of annual rain days and average maximum temperature and mean relative humidity. Data were also obtained from the Water and Rivers Commission for the monthly surface water and groundwater levels for each of the three wetlands over the past 30 years. At Banganup and Thomsons Lakes, where lake inundation was a seasonal event in winter and spring, the number of months per year when inundation occurred was also calculated.

In order to combine the effects of each of the water availability parameters studied above, a water availability index (WAI) was calculated annually for each wetland. This involved combining the total rainfall, mean maximum temperature, mean relative humidity, mean

groundwater levels and mean lake surface water levels during the dormant period (June-August) and the growing season (October-February) of each year. The yearly monitoring data for each parameter in both the dormant and growing seasons were listed. For each parameter, the season containing the highest value (or for temperature, the year containing the lowest value) was given a value of 1. This indicates the year of highest water availability for that particular parameter. Each of the other years were designated a value lower than 1 as a proportion of the highest value. For each year, the values for all parameters during both the dormant and growing seasons were averaged in order to obtain a final, combined annual index of water availability. Growth seasons were expressed rather than single years, with one growth season incorporating the water availability during the dormant period of the prior year (June-August) with the growth period of the following year (October-February). For example, the growth season of 1993-1994 combined the water availability data from June-August in 1993 and October-February in 1994. Higher index values indicated seasons of high water availability, while lower index values indicated seasons of lower water availability. The information regarding the historical water availability of the wetlands provided an understanding at the *site* scale.

Similarly with the nascent xylem tissue sampling method, it was necessary to gain an understanding of the water availability at the *individual tree* scale. This involved relating the groundwater levels read from monitoring bores to the groundwater level read from physical measurements made at each tree during the same month. By doing this, it was possible to estimate the subsequent historical changes that each tree may have experienced over time.

5.2.4   Data Analysis

Resulting $\delta^{13}C$ of growth rings were analysed descriptively for each tree by graphically displaying the changes in tree $\delta^{13}C$ signatures between rings, and linking ring numbers with corresponding years of growth. Mean $\delta^{13}C$ values of corresponding tree rings were calculated for each transect position and for each wetland, in order to obtain an averaged, schematic overview of how $\delta^{13}C$ changed over time. For each tree, the $\delta^{13}C$ signature resulting from the estimated year of growth was correlated with the historical water availability data including the mean, minimum and maximum rainfall, the number of rain days, the mean maximum temperature, and mean, minimum and maximum groundwater and surface water levels. All measures were correlated using the Pearson correlation coefficient (p=0.05) on an annual basis, and during the growth season of the tree (October-February). Other factors such as BAI, period of lake inundation and changes in groundwater level at the individual tree scale were also examined descriptively in order to find any evidence of particular historical events reflected in tree $\delta^{13}C$ signatures. Results were investigated at the individual tree scale, transect positions scale and at the site scale.

5.3 RESULTS

5.3.1 Changes in Historical Water Availability

5.3.1.1 Climate

5.3.1.1.1 Rainfall (1951 – 1999)

For the period of 1951 – 1999, Perth experienced a decreasing trend in annual total rainfall (Figure 5.1). Rainfall in the Perth region was typically highest during winter (the wettest months being June and July) and lowest in summer (the driest months being January and February). During the monitoring period, Perth's annual rainfall peaked in 1955 at 1164.7mm, which was over double that of the average rainfall expected. 1973, 1986 and 1988 also displayed peaks in total rainfall, and 1992 experienced the highest total rainfall for Perth since 1965, receiving 960mm. Perth's total rainfall reached its lowest during 1969, receiving only 523.8mm due to its relatively dry winter. 1975 – 1985 was also a period of low rainfall.

5.3.1.1.2 Temperature(1951 – 1999)

Based on average annual maximum temperature, Perth's climate was progressively warming over time (Figure 5.2). Perth experienced its highest temperature maximums during summer (warmest months being January and February) and its lowest temperature maximums during winter (the coolest months being July and August). 1968 had a very low annual temperature of only 22.4°C, which was nearly two degrees lower than the mean. In contrast, 1972 was a much warmer year based on its mean annual maximum temperature,

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being 25.6°C. Following this, 1975-1978 were also warm years, with the four consecutive years being warmer than the average. Perth's temperatures fluctuated above and below the mean until 1994, and have since remained above the average.

5.3.1.1.3 Humidity (1951 – 1999)

By examining the average relative humidity from 1951-1999 (Figure 5.3), it is clear that there was a decreasing trend over the period, with the average annual humidity reaching a maximum in 1955 of 54.8% and falling to a minimum of 42.6% in 1999. Relative humidity in Perth was generally highest in winter (June-August) with the mean humidity in July being 60.4%. January-March was the period of lowest humidity on average, with the mean humidity in February being 35%.
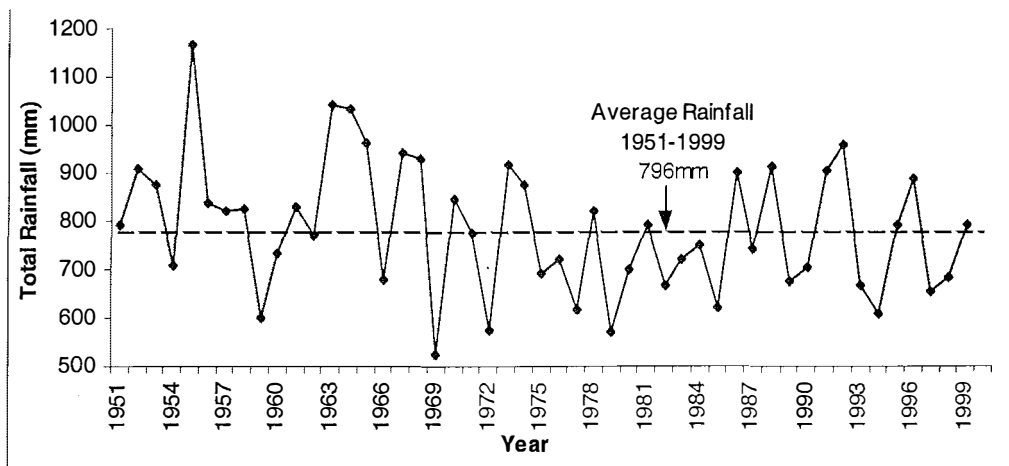
Figure 5.1: Total Annual Rainfall for the Perth Region 1951-1999. Dashed line shows Perth's average rainfall over the period of 796mm.
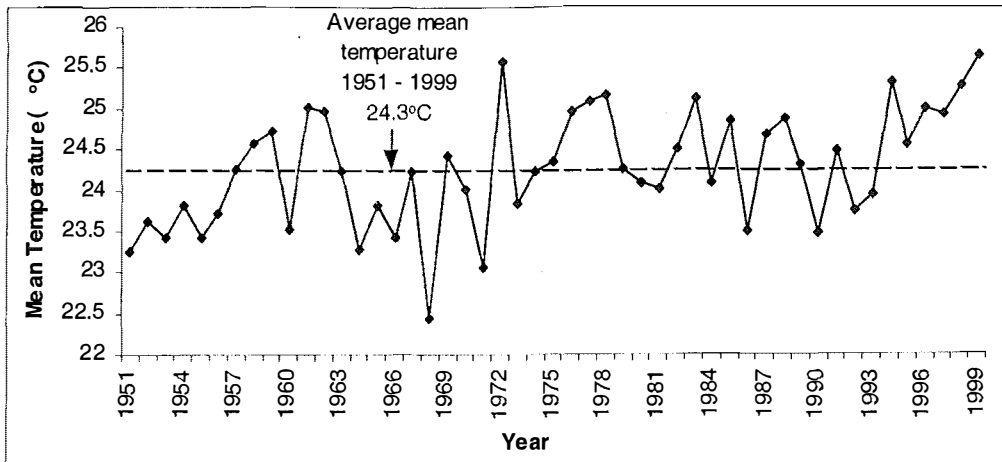
Figure 5.2: Mean annual temperature for the Perth Region 1951-1999. Dashed line shows Perth's average temperature over the period of 24.3°C.
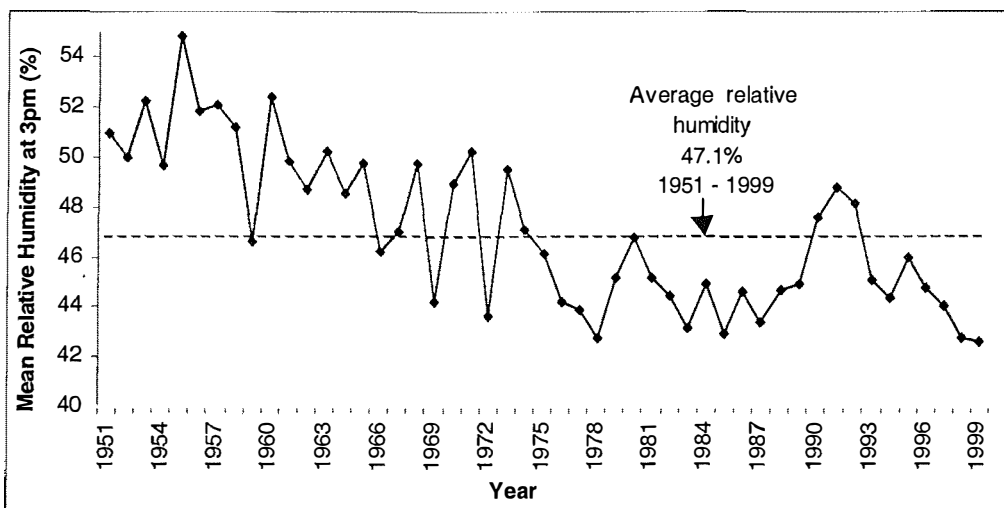
Figure 5.3: Mean annual relative humidity at 3pm for the Perth Region 1951-1999. Dashed line shows Perth's average humidity over the period of 47.1%.

5.3.1.2   Hydrology of Banganup Lake

5.3.1.2.1   Surface water

The surface water levels of Banganup Lake have been recorded historically since 1963, however, regular monthly monitoring did not occur until 1974. Since 1989, surface water levels were only recorded during periods of lake inundation. Therefore, when the lake dried surface levels were recorded at the lake bed level (12.696mAHD). This resulted in the

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flattening of the hydrograph for the lake after 1989 (Figure 5.4). For this reason, it was difficult to accurately interpret long term changes in groundwater levels without exact records of the level when the lake dried. It does appear however, that until 1988, Banganup Lake was becoming drier. Lake inundation has fluctuated over time, occurring between 0 and 12 months per year. During 1974-1976, Banganup Lake inundated for 12 months annually (Figure 5.5). However, following this "wet" period, the Lake inundated seasonally, with the average number of months of inundation calculated to be 3.875. 1979 was a dry year, being inundated for only 2 months, and with lake depths reaching a maximum of only 0.1m. In 1983, Banganup Lake experienced a decrease in water levels, with levels dropping to 11.12mAHD (1.5m below the lake bed) in May. Following the "dry spell" in the 1980s, Banganup Lake experienced a rise in water levels from 1988-1996. In 1992, water levels peaked at 13.42mAHD (over 0.7m in depth), and the lake remained flooded for 10 months. From 1997, Banganup Lake water levels dropped again, with the lake drying for the 12 months during 1999.

5.3.1.2.2 Groundwater

With Banganup Lake being an expression of the unconfined aquifer, its fluctuations in surface water levels reflected those of the groundwater. The Water Corporation established groundwater monitoring bores in 1993 and data are available from this year onwards. Figure 5.6 shows that the period of 1993-1999 displayed a decreasing trend in groundwater levels, with maximum levels dropping from 13.4mAHD in 1994 to 12.6mAHD in 1999.
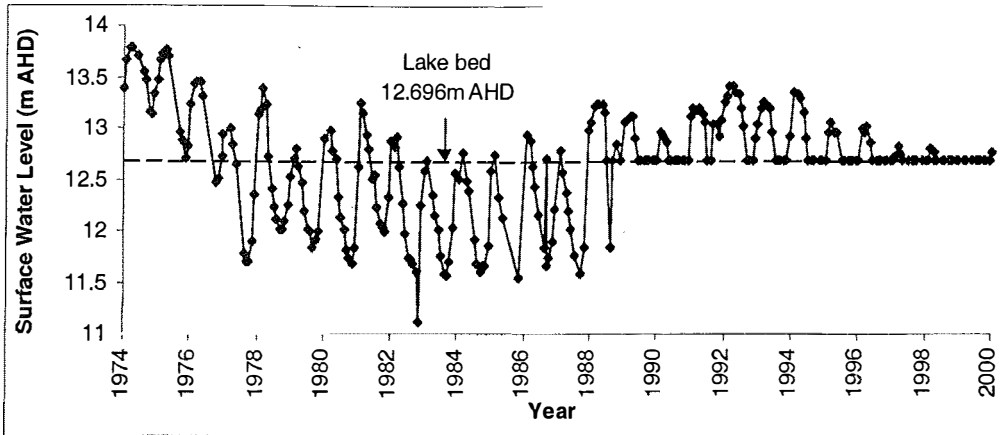
Figure 5.4: Hydrograph of Banganup Lake showing historical changes in surface water levels based on monitoring data from 1974-2000. From March 1989, lake drying was recorded as being at the lake bed level (12.696m AHD)
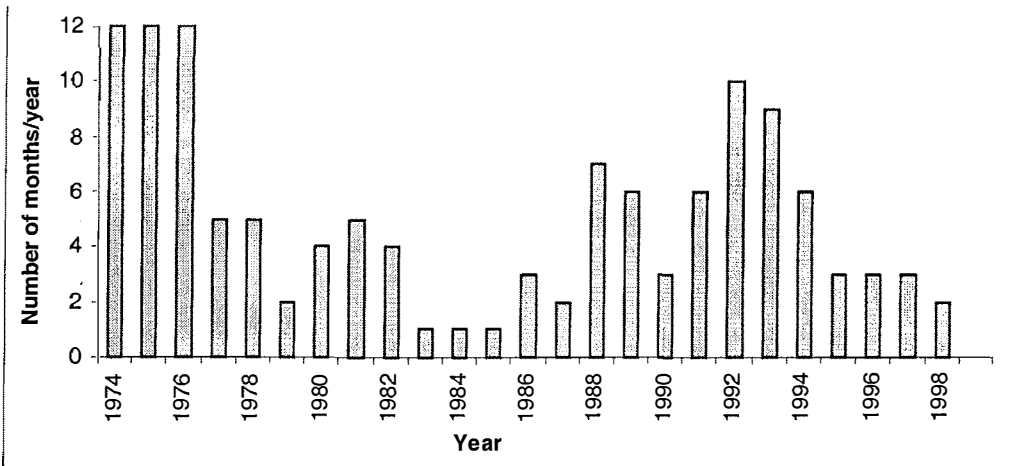
Figure 5.5: The number of months per year that Banganup Lake is inundated from 1974-1999. Mean number of months per year is 3.9
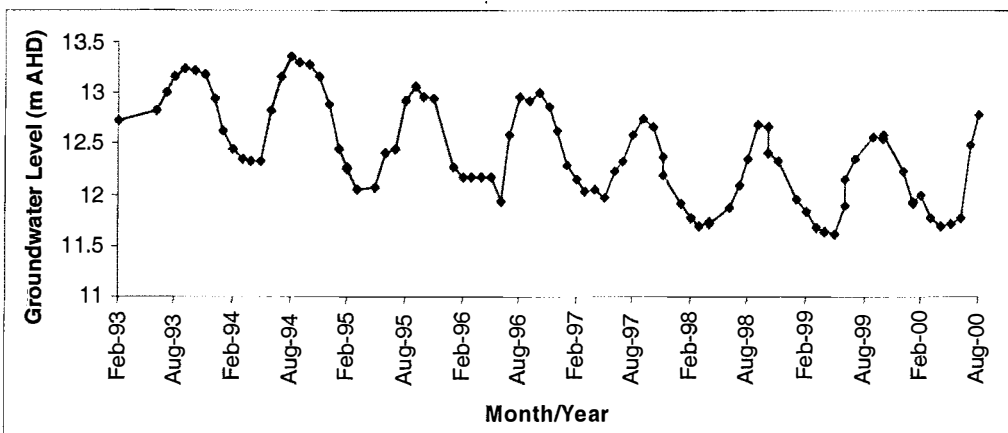
Figure 5.6: Hydrograph of Banganup Lake showing changes in groundwater levels since 1993 based on monitoring data from bore LB1.

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5.3.1.3   Hydrology of Thomsons Lake

5.3.1.3.1 Surface Water

The surface water levels for Thomsons Lake have been monitored since 1952, however regular monthly monitoring did not occur until 1971. From 1971 to 1978, Thomsons Lake flooded permanently, with levels reaching a maximum during July and August and a minimum during February and March (Figure 5.7). Levels peaked during 1973 at 14.1mAHD (2.3m depth). From 1978, Thomsons Lake inundated seasonally, with the lake flooding for 8.6 months each year on average (Figure 5.8). In February 1980, lake levels dropped to a minimum of 10.8mAHD (1m below the lake bed) and until 1992, maximum lake levels mostly remained under 13mAHD (greater than 1m depth). In 1989, Thomsons Lake inundated for the whole year, and up to 1998, dried once only.

5.3.1.3.2 Groundwater

Groundwater monitoring data for Thomsons Lake was available from 1985. In 1991 groundwater levels reached a minimum of 12.1mAHD, which was similarly reflected by surface water levels (Figure 5.9). In 1992, groundwater peaked, with the maximum level reaching 13.9mAHD during October. Following 1992, the annual groundwater levels of Thomsons Lake gradually decreased, with maximum levels measured in 1998 being 1.6m below those in measured in 1992.
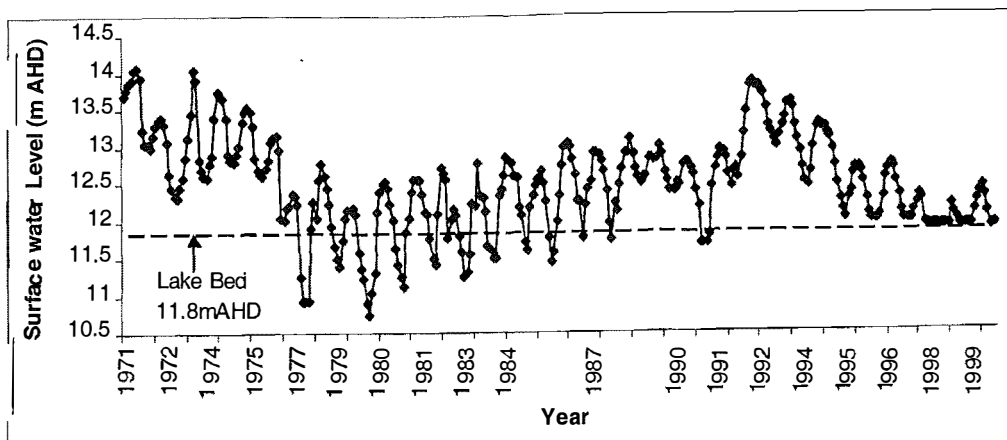
Figure 5.7: Hydrograph of Thomsons Lake showing historical changes in surface water levels based on monitoring data from 1971-2000.
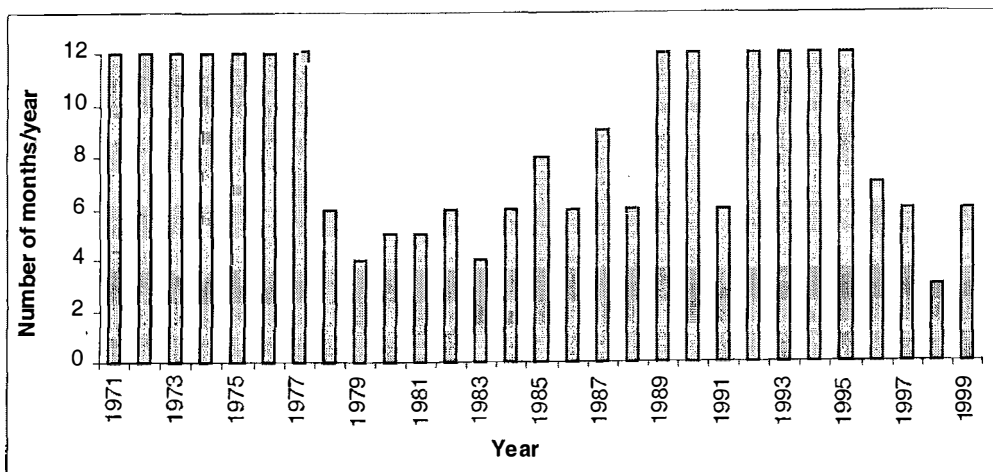
Figure 5.8: The number of months per year that Thomsons Lake is inundated from 1971-1999. Mean number of months per year is 8.6
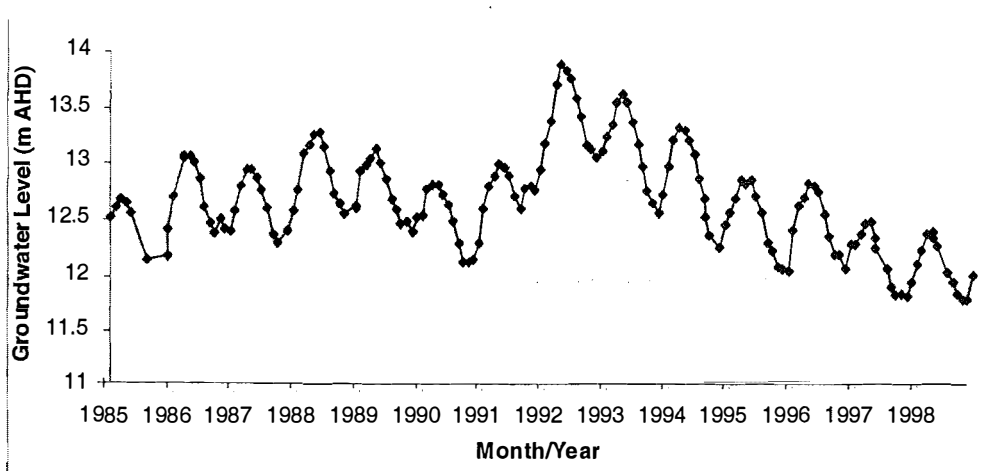
Figure 5.9: Hydrograph of Thomsons Lake showing historical changes in groundwater levels since 1985 based on monitoring data from Bore TM10C. Data were available up to June 1999.

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5.3.1.4   Hydrology of Lake Jandabup

5.3.1.4.1 Surface Water

The surface water data for Lake Jandabup were available from 1968, and levels were monitored on a monthly basis. From 1968 to 2000, the Lake's surface water levels declined, which is evident by the lake's hydrograph in Figure 5.10. The most rapid decrease was from 1968 where maximum lake levels declined from 46.8m AHD (water depth of 3m), to 45.1m AHD (water depth of 1.3m) in 1977. Lake Jandabup's water regime followed a seasonal trend, with water levels reaching a maximum in September-November, and falling to the minimum during March-May. Unlike the other two study lakes, Lake Jandabup remained permanently inundated, and never dried out completely. Minimum lake levels occurred in 1983, 1985, 1988 and 1991 and years when lake inundation peaked 1978 when depths rose to 1.5m; 1986 and 1987 when water depths rose to 1.3m, and 1992 when the maximum water depth was recorded in October as being 1.5m. 1997-1999 experienced low lake levels, with the maximum levels being less than 1m in depth.

5.3.1.4.2 Groundwater (1977-1999)

Groundwater levels for Jandabup Lake were monitored monthly since 1977. Over the monitoring period, groundwater levels decreased (Figure 5.11). Similarly to lake levels, groundwater levels peaked in 1978 and also during 1991-1993. Following 1993, groundwater levels decreased, with the lowest maximum level recorded during October 1998 being 45.3m AHD, which was approximately 1m lower than that recorded in 1992.

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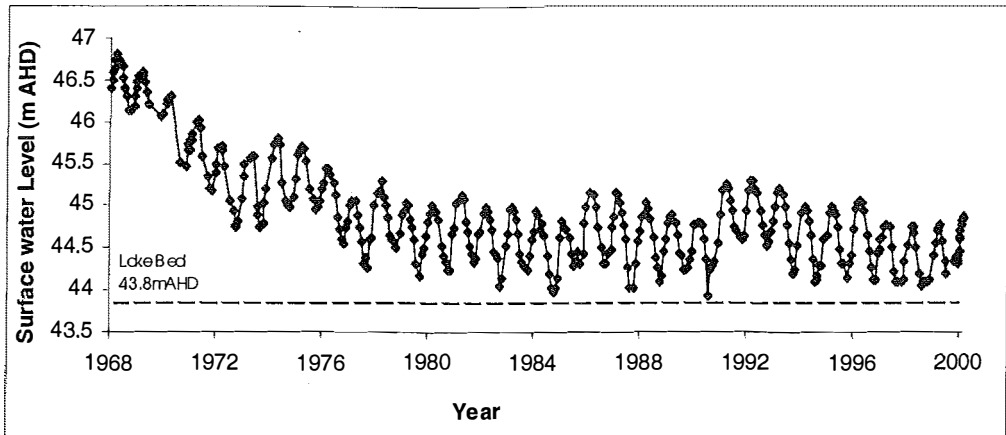
Figure 5.10: Hydrograph of Lake Jandabup showing historical changes in surface water levels based on monitoring data from 1968-2000.
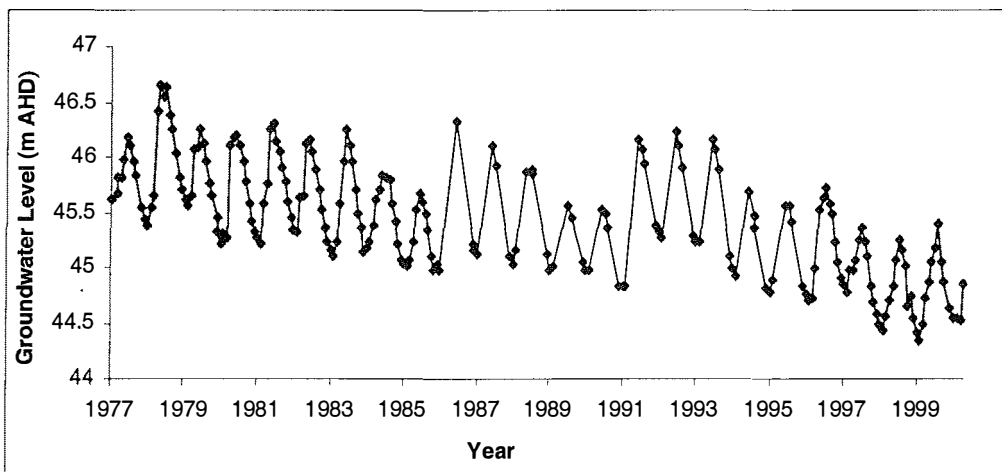
Figure 5.11: Hydrograph of Lake Jandabup showing historical changes in groundwater levels since 1977 based on monitoring data from Bore JB12A.

5.3.1.5   Water Availability Index

By combining the summer (the growth season) and winter values (the dormant season) for total rainfall, maximum temperature, relative humidity, surface water levels and groundwater levels into a water availability index (WAI), it was possible to study the combined effects from the key physical factors affecting water availability, on the physiology of the *M. preissiana* populations. The index for the three wetlands followed similar trends over the period of 1969-1999. This is most likely to be due to the strong

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emphasis that climate had on the overall index, with Perth's climatic data being applied to each lake. In most years, the water availability was highest at Lake Jandabup, while the index remained fairly consistent for Thomsons and Banganup Lakes (Figure 5.12). The growth period of 1971-1972 was found to be a peak year in water availability for the three lakes, which was also reflected by the lakes' water levels. The growth seasons of 1978-1979 and 1983-1984 both saw drops in water availability at the wetlands, which was also reflected by the low lake levels and low rainfall. Peak years of water availability at the wetlands based on the index were 1985-1986, 1991-1992 and 1995-1996. The highest peak in water availability occurred from 1991-1992 with the WAI of all lakes being higher than 0.95.

5.3.2   Core Aging and Growth Ring Analysis

5.3.2.1   Tree Rings in Cross Sections vs Cores

Entire cross sections of trunk were removed from two trees growing within the juvenile band of *M. preissiana* at Banganup Lake. These were known to have germinated after a fire in 1977, and were aged at 23 years (Froend *et al*, 1993). Both cross sections were found to contain 22 distinguishable rings, with the 23rd ring (probably formed at establishment in 1977) difficult to identify due to the high resinous content of the heartwood in the centre of the stem. Figure 5.13 compares the width of corresponding tree rings between one cross section and two cores taken from the same tree. The average width of tree rings from the cross section (taken from 3 radii sections) were found to have little standard error, indicating that growth remained relatively constant around the circumference of the stem.
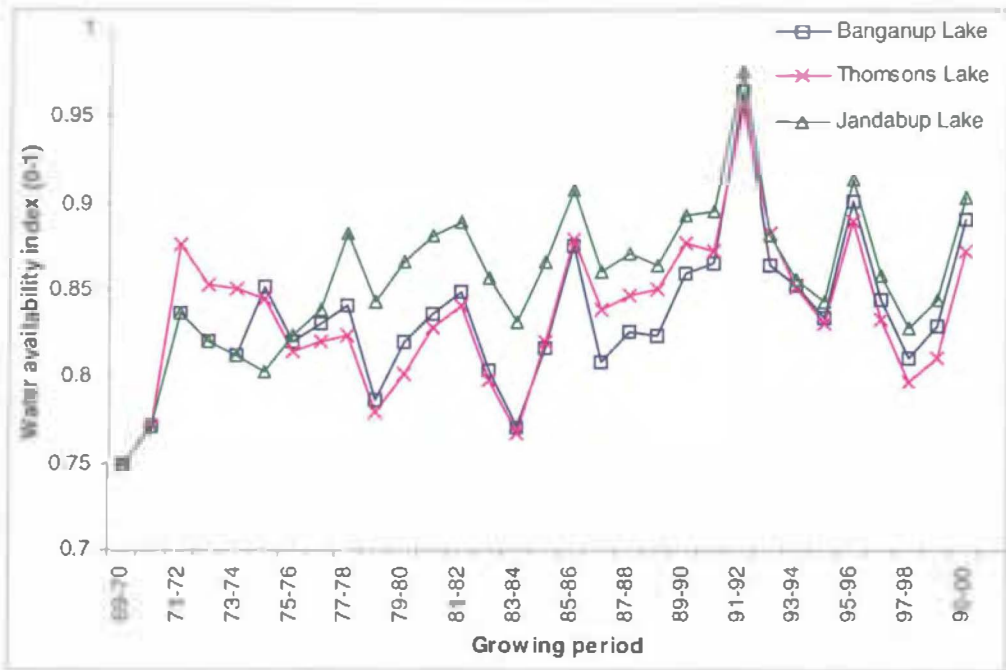
Figure 5.12: Changes in the water availability index from 1970-2000 at Banganup Lake, Thomsons Lake and Jandabup Lake. The index incorporates total rainfall, mean maximum temperature, mean humidity, mean groundwater levels and mean surface water levels in the dormant period (June-August) and the growing season (October-February) for *M. preissiana*. An index closer to 1 indicates higher water availability based on these parameters.
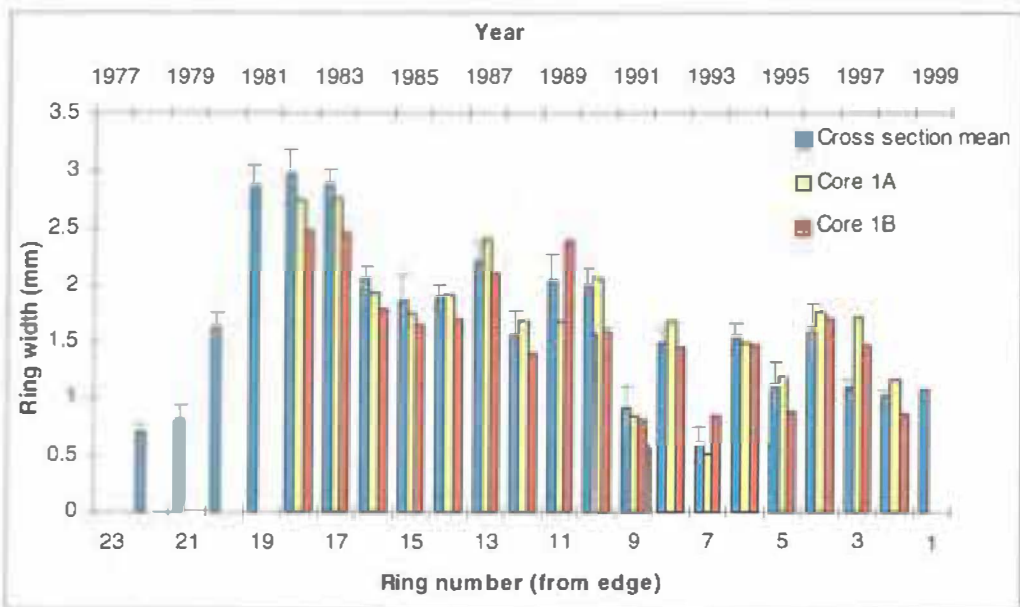
Figure 5.13: Width of tree rings taken from cross section 1. Mean refers to the average width of the tree ring taken from 3 radii across the cross section along with standard error bars. Cores 1a and 1b show the widths of corresponding rings taken from the two cores. Tree rings numbered from the edge of stem (bottom X-axis) and matched to corresponding years (top X-axis).

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The widths of the rings from the two cores were indicative of the corresponding rings occurring within the cross section. This is reinforced by the high, significant correlation (r = 0.96; p<0.05) existing between the average width of the tree rings contained in the cross sections with the average width of the rings contained in the cores (Figure 5.14). It was found that the latest ring displayed by the cores corresponded to the second ring displayed by the cross section, probably due to damage that occurred during coring, making visibility of the most recently formed ring difficult.
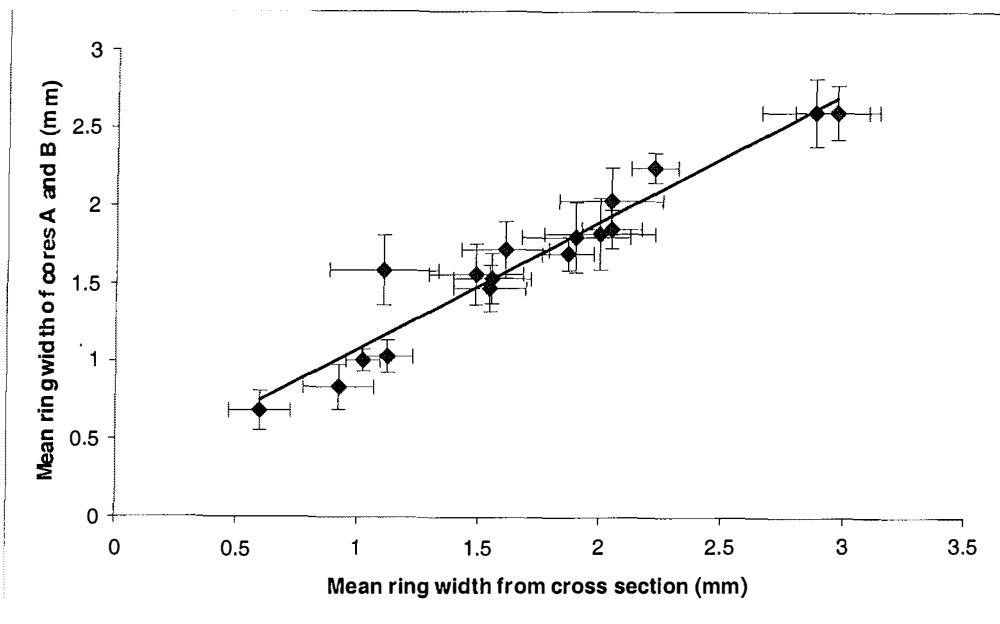
Figure 5.14: Scatterplot showing the relationship between the mean width of tree rings from *M. preissiana* cross section and the mean width of tree rings from cores A and B from measured from corresponding rings of the same tree. A significant, correlation existed (r = 0.96; p<0.05). Line of best fit and X and Y standard error bars are shown.

5.3.2.2   Aging of tree rings

The number of rings and the growth period displayed by each tree core are presented by Table 5.1. The cores at Banganup Lake generally represented the greatest number of years, ranging from core 3a displaying 14 years of growth (1985 – 1998) to core 3c displaying

over 40 years of growth. Trees located in the "A" position of the three transects at Banganup Lake displayed the fewest growth rings, while the trees from "B" and "C" positions exhibited the most rings, indicating that the trees may possibly be older. The cores at Thomsons Lake displayed similar trends, with the number of rings within cores ranging from 6 (tree 1a) to 22 (tree 2c). The Lake Jandabup cores similarly displayed these trends with the number of rings ranging from 6 (tree 2a) to greater than 40 (tree 2c).

Table 5.1: The study trees at each wetland, the number of rings contained within each core and the period of growth represented by the core

Site	Tree label	Number of rings in core	Approx. period of growth represented by core
Banganup Lake	1a	17	1982 – 1998
	1b	23	1976 – 1998
	1c	17	1982 – 1998
	2a	15	1984 – 1998
	2b	20	1979 – 1998
	2c	19	1980 – 1998
	3a	14	1985 – 1998
	3b	29	1970 – 1998
	3c	>40	*
Thomsons Lake	1a	6	1994 – 1999
	1b	7	1993 – 1999
	1c	11	1989 – 1999
	2a	12	1978 – 1998
	2b	18	1982 – 1999
	2c	22	1977 – 1998
	3a	10	1989 – 1998
	3b	16	1984 – 1999
	3c	19	1981 – 1999
Jandabup Lake	1a	19	1981 – 1998
	1b	17	1983 – 1999
	1c	24	1976 – 1998
	2a	6	1994 – 1999
	2b	24	1976 – 1999
	2c	>40	*
	3a	11	1989 – 1999
	3b	17	1983 – 1999
	3c	9	1991 – 1999

* Due to the small size and difficult visibility of growth rings, cores were divided into 5% intervals along the core following methods outlined by Pate & Arthur (1998).

5.3.3 Time-Integrated, Long Term $\delta^{13}C$ Measurements using Growth Ring Tissue

5.3.3.1 Transect position, year and $\delta^{13}C$

5.3.3.1.1 Banganup Lake

The $\delta^{13}C$ signatures of each tree ring for each tree are illustrated in Figures 5.15, 5.16, and 5.17 for transects 1, 2 and 3 at Banganup Lake respectively. In transects 1 and 2, the ring $\delta^{13}C$ of trees located on the lower slope (A position) were more negative than the trees on the upper slope (C position) during most periods sampled. The trees on the mid slope (B position) fluctuated between the two. This trend was also reflected in the results of the short term sampling method of measuring $\delta^{13}C$ at Banganup Lake, where $\delta^{13}C$ was significantly higher in trees of the C position than trees of the A position. The three trees from transect 1, tree 2c and tree 3b displayed an increase in $\delta^{13}C$ from the 18th ring to the 15th ring. This corresponded to the period between 1982 and 1985, during which rainfall was below the average for these four consecutive years. The 5th ring from the edge of the cores from all trees sampled at Banganup Lake displayed an apparent drop in $\delta^{13}C$, which corresponded to the period of growth during 1995. This is the year following the fire in March 1994. The trees proceeded to increase in $\delta^{13}C$ during the years following 1995. Trees 1a, 1b, 2a, 2b and 3a showed an immediate increase in $\delta^{13}C$, while the trees located on the upper slope continued to decrease for several years.
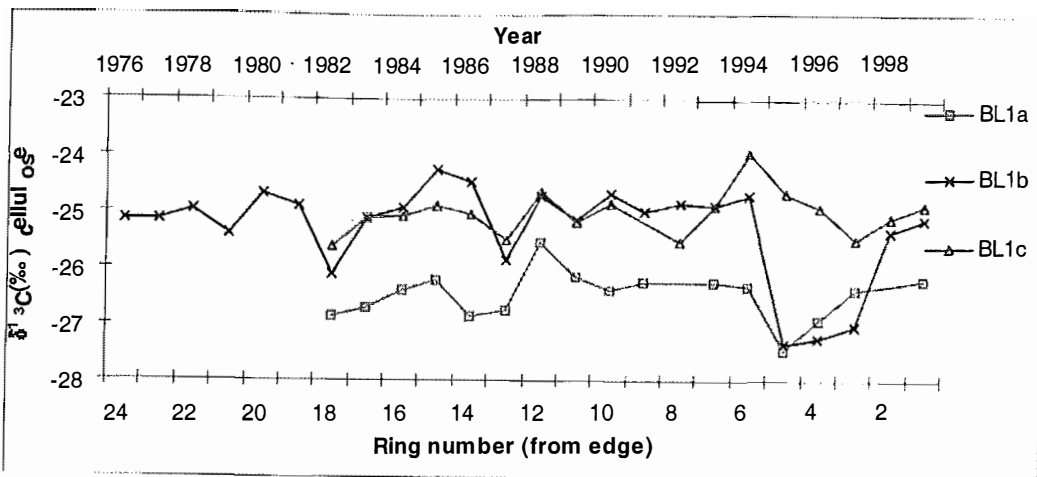
Figure 5.15: Transect 1, Banganup Lake
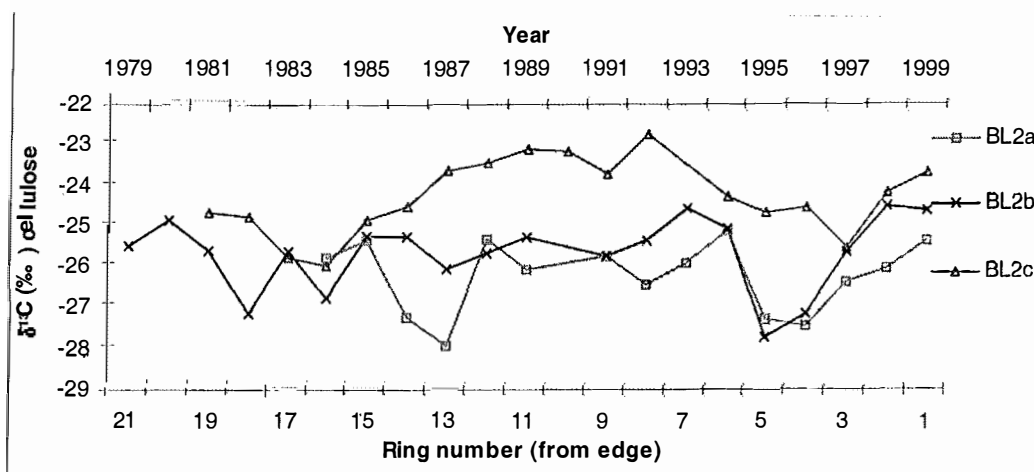
Figure 5.16: Transect 2, Banganup Lake
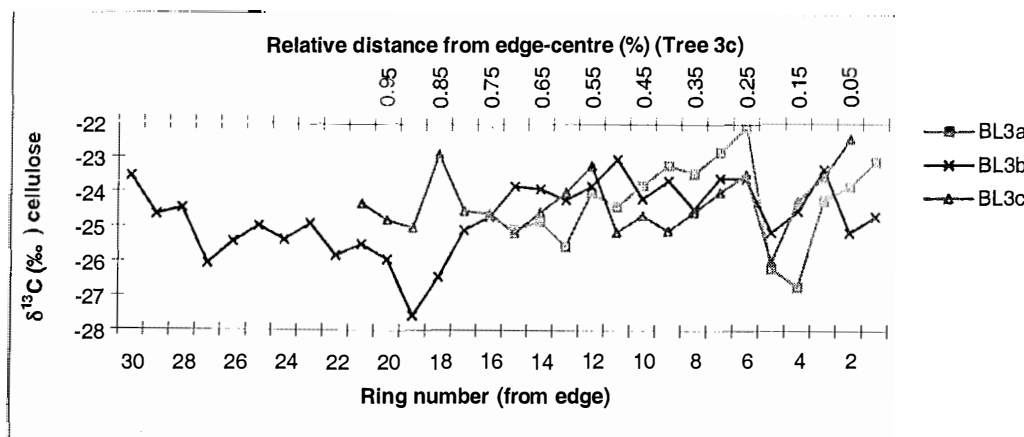
Figure 5.17: Transect 3, Banganup Lake

Figures 5.15, 5.16 and 5.17: Cellulose δ^{13}C of *M. preissiana* growth rings at Banganup Lake from transects 1, 2 and 3 respectively. Rings are numbered from the edge of the stem (bottom X-axis), and linked to the estimated year of corresponding annual growth (top X-axis). Values for tree 3c obtained from regular 5% divisions made along the length of the core.

By averaging the isotopic signatures of corresponding rings for trees located lower, mid and upper slopes, it was possible to obtain a schematic idea of how *M. preissiana* varied between different positions along the hydrological gradient. Figure 5.18a shows the average changes in $\delta^{13}C$ over the length of cores of trees located in the A, B and C positions at Banganup Lake. It was found that the trees in the A position were 1.20‰ more negative on average over the sampling period than trees in the C position, and 0.63‰ more negative than trees in the B position. Figure 5.18b displays a 3-point moving average of growth ring $\delta^{13}C$ for the same trees. Doing this removed the variation occurring between years in order to obtain a schematic overview of the long term water use efficiency of the trees at Banganup Lake. Trees in all three positions displayed an increase in $\delta^{13}C$ from the 16th ring. This corresponded to the period from the early to mid 1980s, when lake levels and the yearly inundation period dropped. $\delta^{13}C$ increased until the early 1990s, after which isotopic discrimination remained fairly constant. Following 1994 $\delta^{13}C$ dropped, however, proceeded to increase by the late 1990s. Figure 5.19 displays an overall average trend for $\delta^{13}C$ at Banganup Lake by taking average signatures of all trees sampled. The three point moving average for this trend shows that the *M. preissiana* population at Banganup Lake became more negative from the late 1970s to the mid 1980s. From this point onwards, the trees increased in $\delta^{13}C$ until the early 1990s where delta signatures levelled from -24‰ to -25‰. 1995 saw a rapid drop in $\delta^{13}C$ followed by a progressive increase in 1997, as trees became isotopically heavier.

Figure 5.18a: Mean and standard error cellulose $\delta^{13}C$ of growth rings for trees in positions A, B and C at Banganup Lake, showing ring number and year of annual growth.

Figure 5.18b: 3-point moving average of growth ring $\delta^{13}C$ for trees in positions A, B and C at Banganup Lake, showing ring number and year of annual growth. Moving average represents a schematic pattern of $\delta^{13}C$ experienced at the lake from 1970-1999.

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Figure 5.19: Mean and standard error cellulose δ¹³C of growth rings for all trees at Banganup Lake showing ring number and year of annual growth. Dotted line represents 3 point moving average, representing a schematic pattern of δ¹³C experienced at the lake from 1970-1999.

5.3.3.1.2 Thomsons Lake

Figures 5.20, 5.21 and 5.22 illustrate the changes in $\delta^{13}C$ over time for each tree in transects 1, 2 and 3 at Thomsons Lake respectively. A similar trend to that at Banganup Lake was observed at Thomsons Lake, with the trees in the A position having more negative $\delta^{13}C$ values than the trees located in the B and C positions during most growth periods sampled. The most apparent trend in ring $\delta^{13}C$ of *M. preissiana* individuals at Thomsons Lake was the drop in $\delta^{13}C$ displayed by trees in the 8th to 9th ring. This corresponded to the years 1991 to 1992, during which water availability reached a maximum at Thomsons Lake (based on WAI). The trees in transect 2 best reflected this drop in $\delta^{13}C$. Trees B and C from transect 3 both displayed a rise in $\delta^{13}C$ in the 7th ring, which corresponded to the annual growth period of 1993. This was followed by a drop in $\delta^{13}C$ in the 6th ring.

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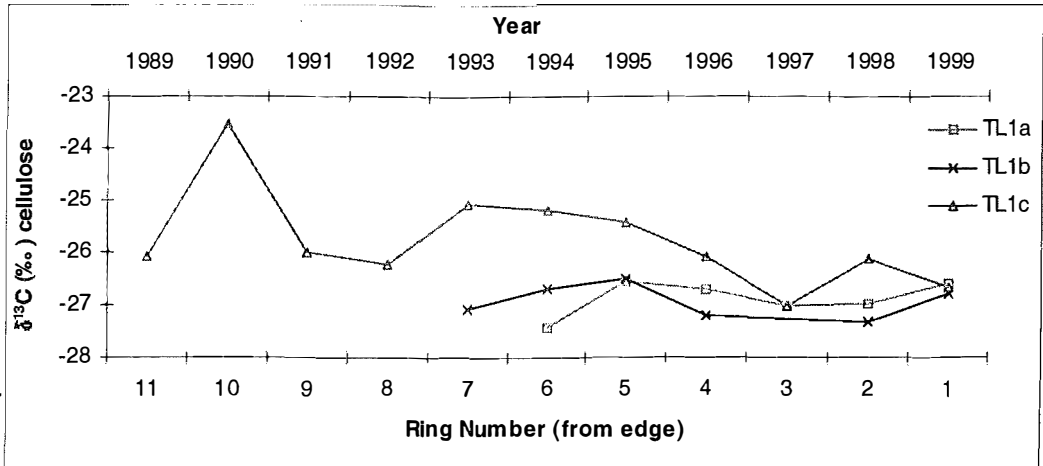
Figure 5.20: Transect 1, Thomsons Lake
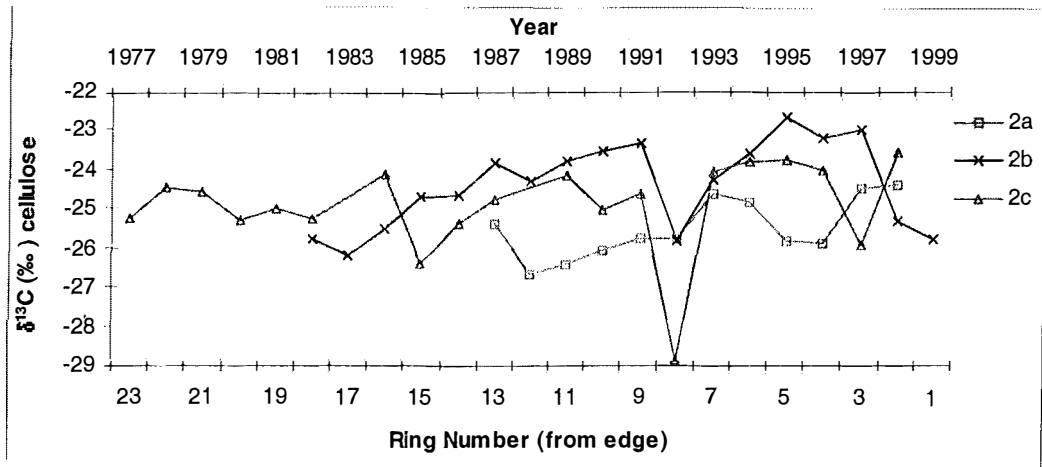
Figure 5.21: Transect 2, Thomsons Lake
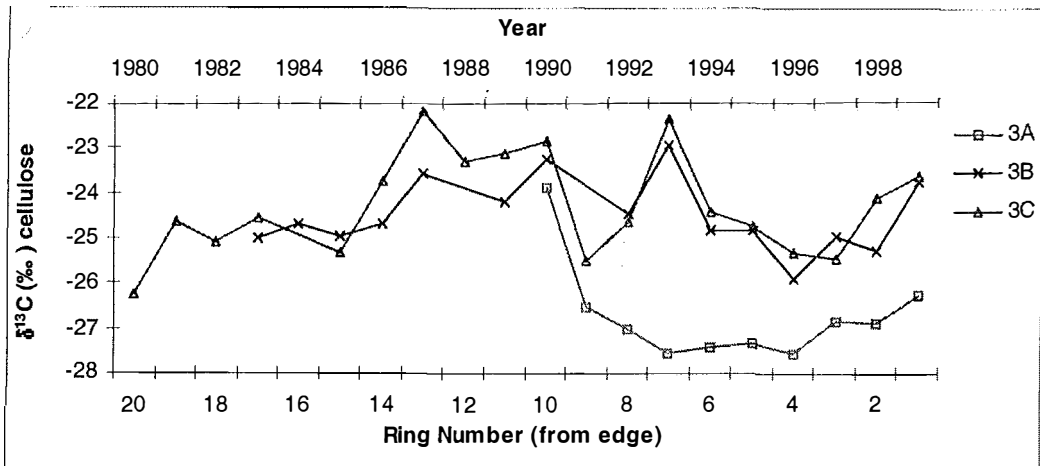
Figure 5.22: Transect 3, Thomsons Lake

Figures 5.20, 5.21 and 5.22: Cellulose δ¹³C of *M. preissiana* growth rings at Thomsons Lake from transects 1, 2 and 3 respectively. Rings are numbered from the edge of the stem (bottom X-axis), and linked to the estimated year of corresponding annual growth (top X-axis).

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By examining the mean $\delta^{13}C$ signatures for each annual ring of trees in the A, B and C positions at Thomsons Lake, it was found that the lower slope trees (the A position) were on average 1.40‰ more negative over the growth period sampled than the trees from the other two transect locations (Figure 5.23a). For the trees in the B and C positions, the wood contained in the annual rings from the 1980s was progressively becoming more positive over time. A major drop in $\delta^{13}C$ of trees from the three positions occurred during 1992, which was the year during which WAI peaked. Following this sudden drop in $\delta^{13}C$, the trees from all transect positions became more positive during the next annual growth cycle. Figure 5.23b summarises this information in the three-point moving averages for the three positions along the hydrological gradient. By removing the variation shown in Figure 5.23a, Figure 5.23b shows that on average, trees in the A position were decreasing in $\delta^{13}C$ over time, while the $\delta^{13}C$ of trees in the B and C positions peaked in 1991 and 1989 respectively. These trees became more negative over the 2-3 years after this peak, and then both fluctuated to an average isotopic discrimination of approximately –25‰. The overall mean changes in $\delta^{13}C$ for the *M. preissiana* population sampled at Thomsons Lake over time is presented by Figure 5.24. There existed a slight trend where the $\delta^{13}C$ of the *M. preissiana* population was becoming more negative over time. The three-point moving average for this time series indicates that a slight rise in $\delta^{13}C$ was experienced during the mid to late 1980s. By the early 1990s, the $\delta^{13}C$ of the *M. preissiana* population began to decrease, and continued until the present. The error bars in Figure 5.24 show that there was a high degree of variation occurring within the *M. preissiana* population at Thomsons Lake.
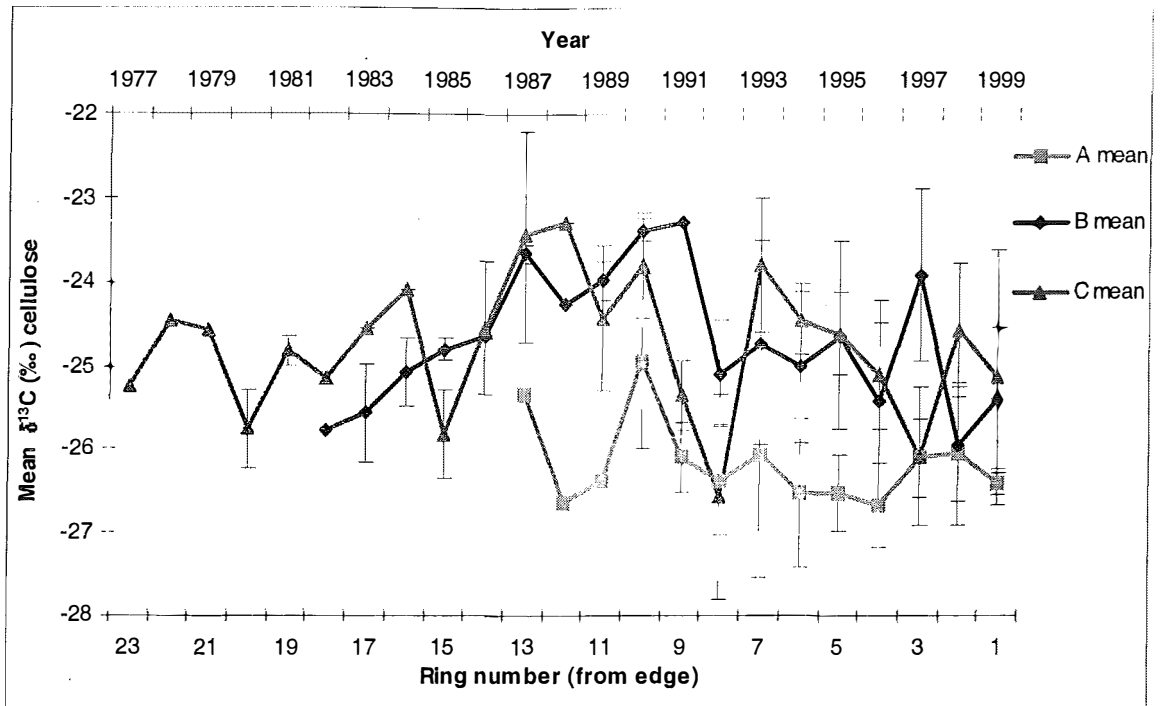
Figure 5.23a: Mean and standard error cellulose δ¹³C of growth rings for trees in positions A, B and C at Thomsons Lake, showing ring number and year of annual growth.
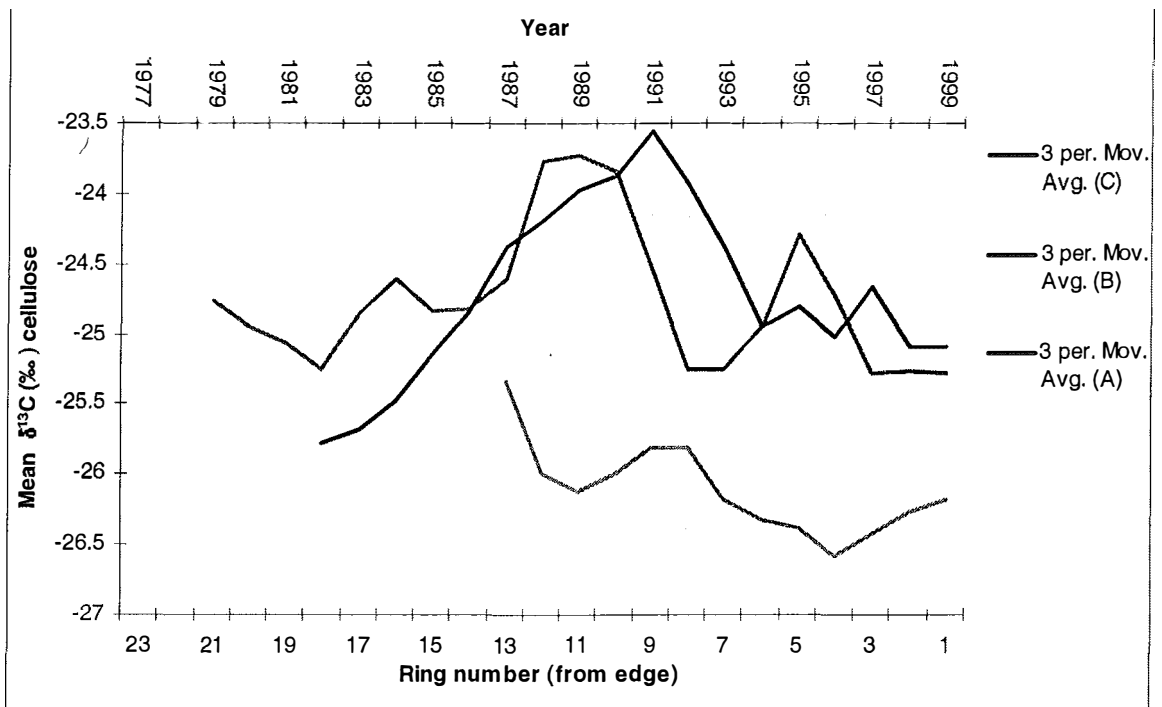
Figure 5.23b: 3-point moving average of growth ring δ¹³C for trees in positions A, B and C at Thomsons Lake, showing ring number and year of annual growth. Moving average represents a schematic pattern in δ¹³C experienced at the lake from 1977-1999.

Figure 5.24: Mean and standard error cellulose $\delta^{13}C$ of growth rings for all trees at Thomsons Lake showing ring number and year of annual growth. Dotted line represents 3 point moving average, representing a schematic pattern in $\delta^{13}C$ experienced at the lake from 1977-1999.

5.3.3.1.3 Lake Jandabup

In transect 1 at Lake Jandabup, tree 1c contained more positive $\delta^{13}C$ values in all growth rings than those contained in cores from trees 1a and 1b (Figure 5.25). This trend was also reflected in the xylem tissue $\delta^{13}C$ measured from the trees during 2000. Transects 2 (Figure 5.26) and 3 (Figure 5.27) failed to show this pattern, with trees located on the upper slope containing more negative ring $\delta^{13}C$ signatures than those on the mid and lower slopes. Over time, the nine trees sampled did not display apparent changes in $\delta^{13}C$. The fifth ring (year 1995, which was a year of high water availability (Figure 5.12)) contained in trees from transects 2 and 3 displayed a slight drop in $\delta^{13}C$. Trees from transect 1 and 3 displayed a gradual decrease in $\delta^{13}C$ from the ninth ring, which corresponded to the year 1991 when lake levels rose. The $\delta^{13}C$ fluctuations displayed between growth rings at Jandabup Lake were less prominent than those shown by the trees at Banganup and Thomsons Lakes.

135
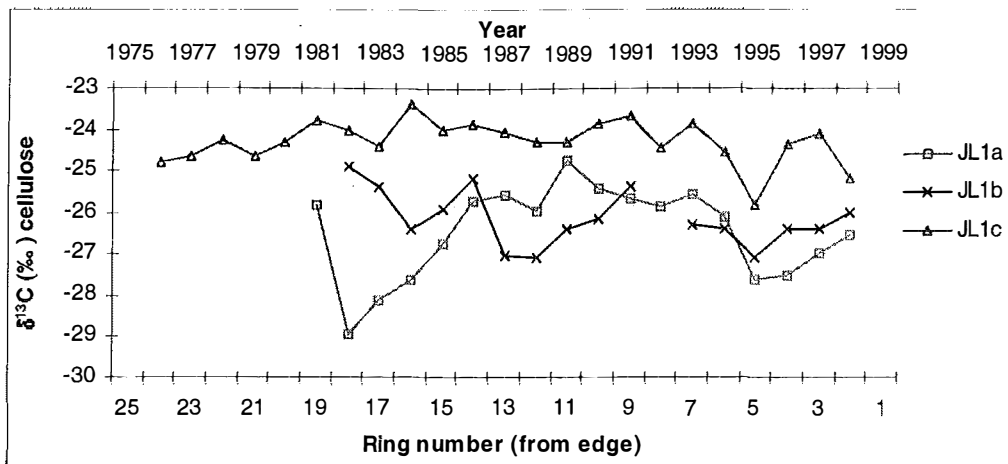
Figure 5.25: Transect 1, Lake Jandabup
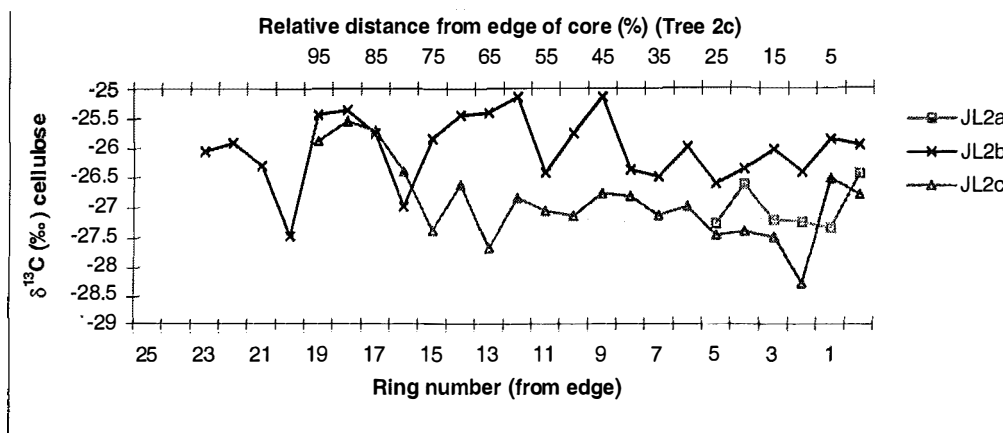
Figure 5.26: Transect 2, Lake Jandabup
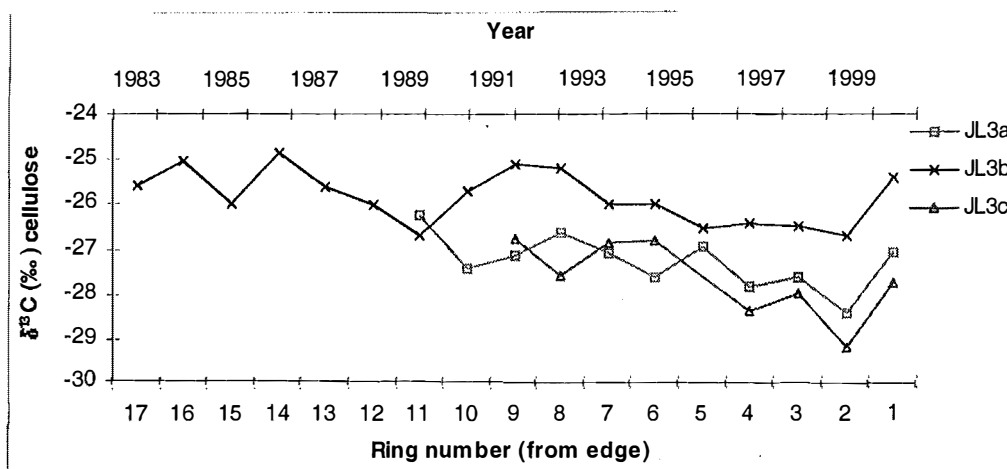
Figure 5.27: Transect 3, Lake Jandabup

Figures 5.25, 5.26 and 5.27: Cellulose $\delta^{13}C$ of *M. preissiana* growth rings at Lake Jandabup from transects 1, 2 and 3 respectively. Rings are numbered from the edge of the stem (bottom X-axis), and linked to the estimated year of corresponding annual growth (top X-axis). Values for tree 2c obtained from regular 5% divisions made along the length of the core.

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Figure 5.28a shows the mean trends in $\delta^{13}C$ of between growth rings from trees in the A, B and C positions at Lake Jandabup. Despite the unexpected results obtained from transects 2 and 3, it was found that on average, trees located on the lower slope were 1.85‰ more negative over the sampling period than the trees located on the upper slope and 0.75‰ more negative than the trees on the mid slope. In comparison to the results obtained from Banganup and Thomsons Lakes, there was a smaller degree of variation in $\delta^{13}C$ *between* rings at Lake Jandabup. The increase in $\delta^{13}C$ observed from the 18th to 14th rings for trees located in the A position was biased towards the $\delta^{13}C$ displayed by tree 1a, due to its core being the only one containing rings formed during this period. By observing the average, annual changes in $\delta^{13}C$ of trees in the three transect positions, it was apparent that $\delta^{13}C$ values of the *M. preissiana* population were becoming more negative over time. Figure 5.28b illustrates this relationship more so, with the three-point moving averages presenting a schematic overview of how the *M. preissiana* individuals changed over time at Lake Jandabup in terms of transect position. Trees in the C position of the transects showed an apparent decline in $\delta^{13}C$ over the time period sampled, while the trees from the B position remained fairly constant, with $\delta^{13}C$ signatures fluctuating around −26‰. The mean trend in $\delta^{13}C$ over time displayed by *all* trees at Lake Jandabup also showed that there was a decrease in the $\delta^{13}C$ of the *M. preissiana* population with time (Figure 5.29). This progressive drop in $\delta^{13}C$ was most apparent from the late 1980s onwards and corresponded to the period during which water availability at Lake Jandabup displayed several "peaks" (Figure 5.12).
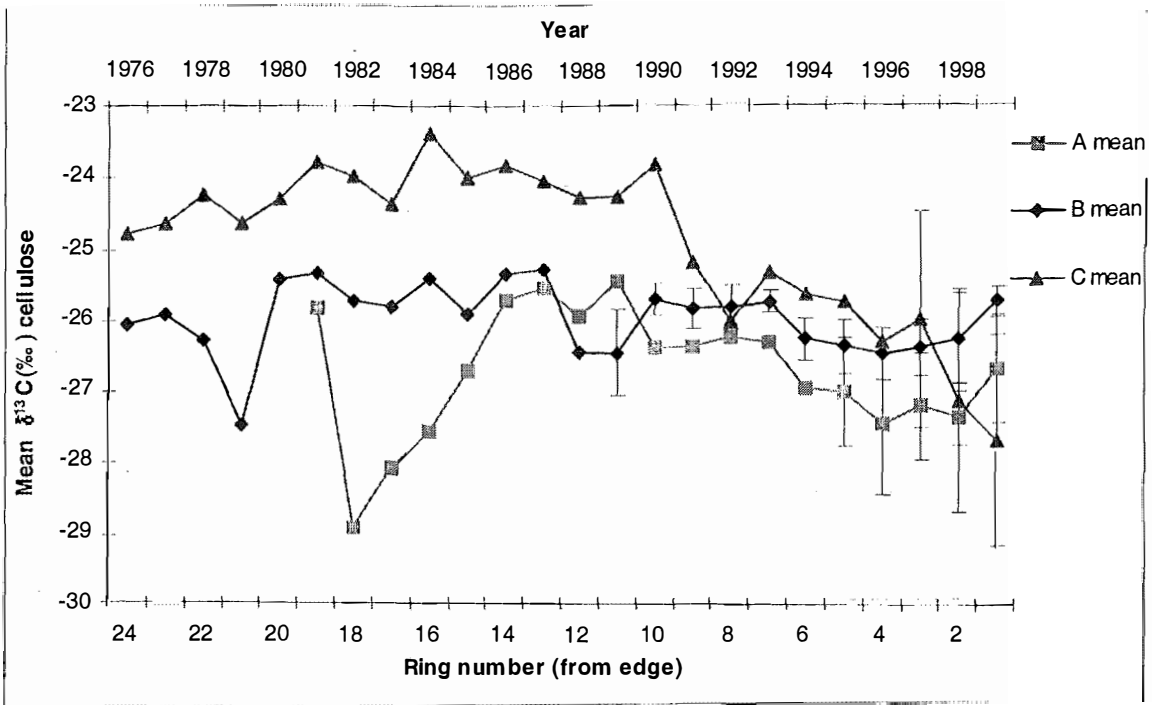
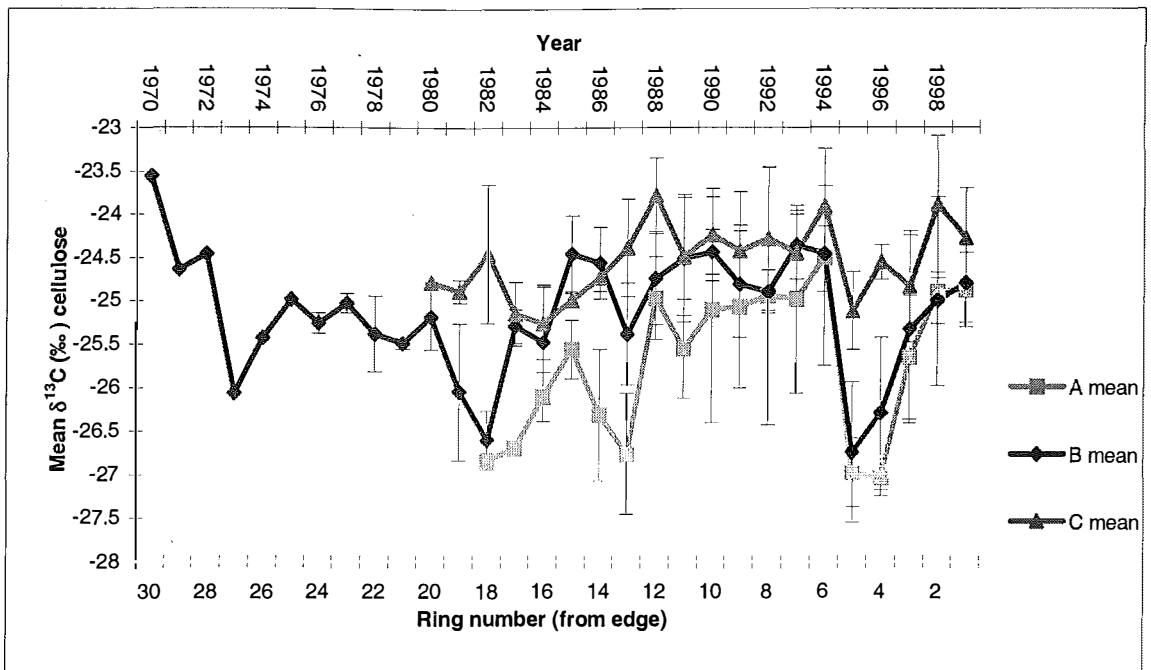**Figure 5.28a: Mean and standard error cellulose δ¹³C of growth rings for trees in positions A, B and C at Lake Jandabup, showing ring number and year of annual growth.**
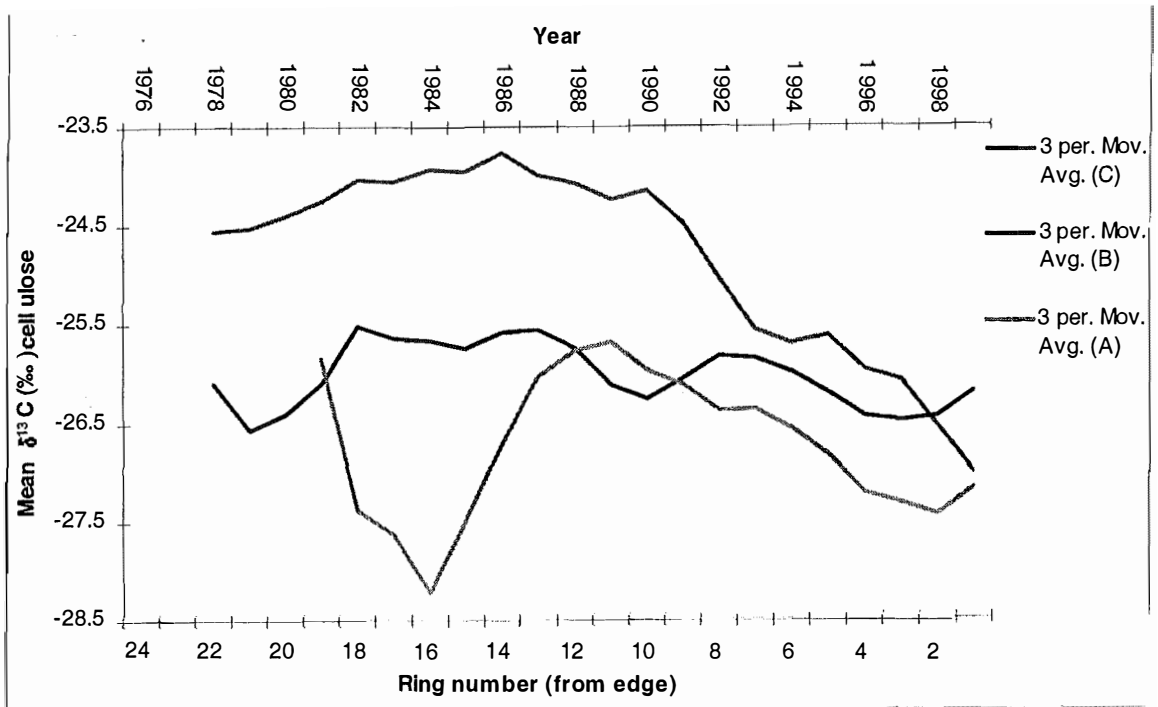
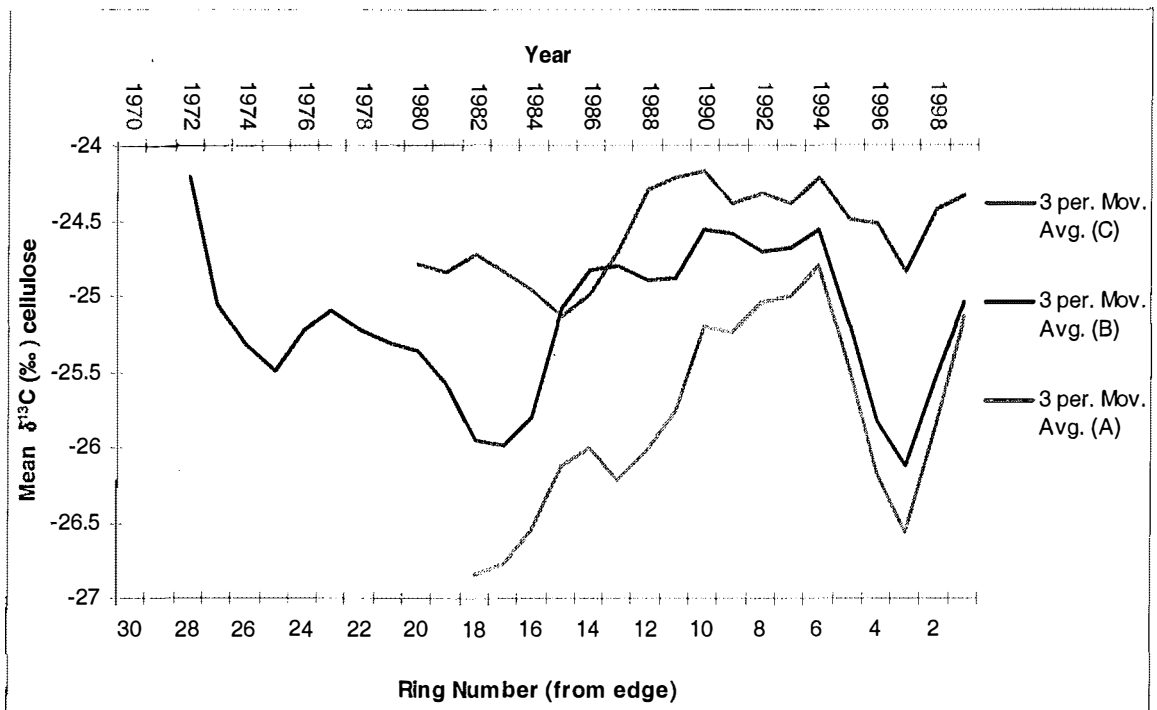**Figure 5.28b: 3-point moving average of growth ring δ¹³C for trees in positions A, B and C at Lake Jandabup, showing ring number and year of annual growth. Moving average represents a schematic pattern in δ¹³C experienced at the lake from 1976-1999.**

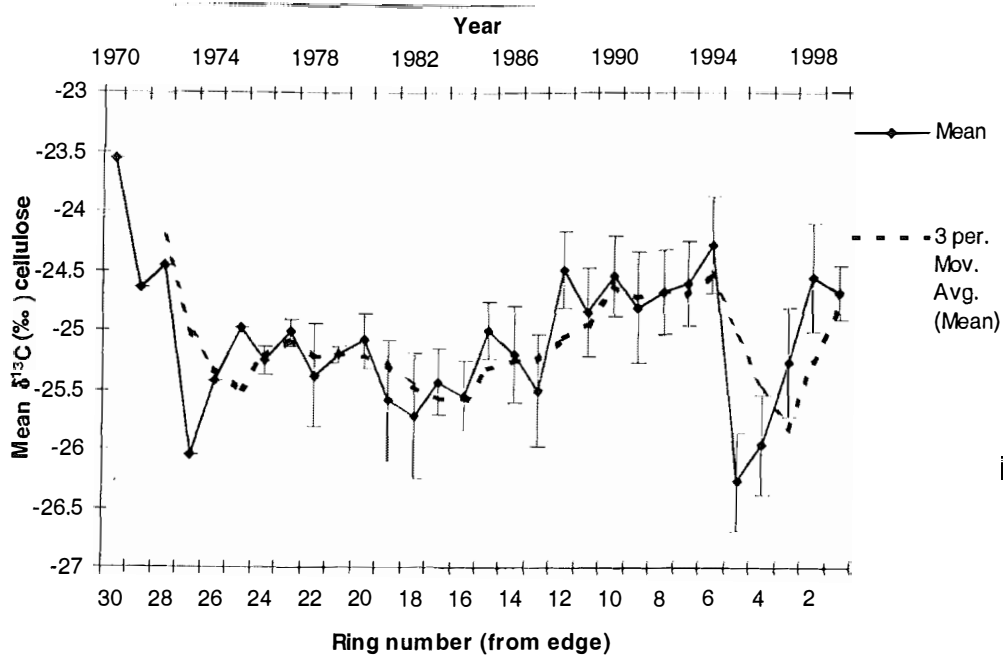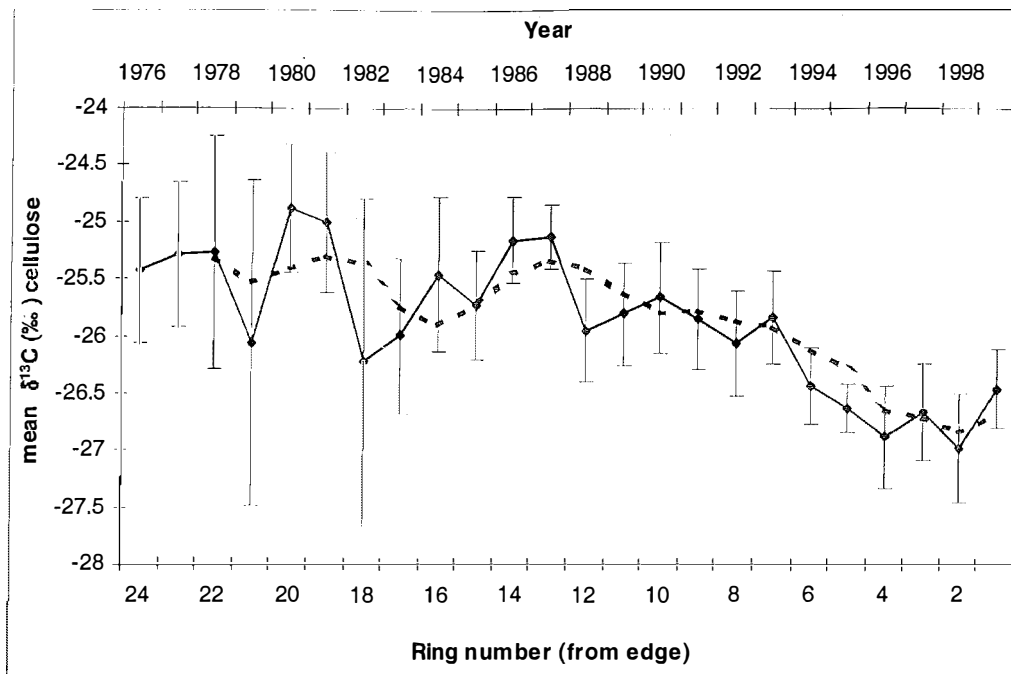**Figure 5.29: Mean and standard error cellulose δ¹³C of growth rings for all trees at Lake Jandabup showing ring number and year of annual growth. Dotted line represents 3 point moving average, representing a schematic pattern in δ¹³C experienced at the lake from 1975-1999.**

5.3.3.2 δ¹³C, Ring Area and Historical Water Availability

Correlation coefficients were calculated between ring δ¹³C, ring area and each of the water availability parameters (total rainfall, maximum temperature, relative humidity, surface and groundwater levels and WAI) for the annual period represented by each ring as well as for the time representing the growth period for the tree (October – February of each year). Very few significant correlations existed for all trees sampled ($p < 0.05$). Therefore, no distinct relationships could be identified that existed between water availability and ring δ¹³C of *M. preissiana* at the lakes over time. As an alternative, the time-series of historical tree δ¹³C was investigated descriptively in order to identify episodic periods in which δ¹³C appeared to be indicative of water availability.

5.3.3.2.1 Banganup Lake

Figure 5.30 plots the water availability index, mean growth ring area and mean $\delta^{13}C$ over time for trees located on the lower slope (A position) at Banganup Lake. It was found that from 1984 – 1990, there was a progressive increase in the average ring area formed by trees in the A position. This was accompanied by an increase in water availability during the same time period. $\delta^{13}C$ did not reflect this trend, with isotopic discrimination fluctuating between rings. The growth period of highest water availability (1991–1992) did not support rapid growth rates in terms of ring area, yet the small ring area formed during this year was accompanied by a more positive $\delta^{13}C$ value. The largest rings formed during 1995-1996, which was a period of high WAI. Low $\delta^{13}C$ signatures occurred during this growth season.

Figure 5.31 shows that variation existed between the mean ring area of trees in the B position and mean $\delta^{13}C$ of rings over the time period sampled. $\delta^{13}C$ steadily increased from 1980 – 1985, which corresponded to a period when lake levels were low. The water availability index and the mean ring area of trees did not reflect this trend. 1995-1996 was a period of high water availability, and was accompanied by high growth rates and low mean $\delta^{13}C$ signatures. The trees located mid slope at Banganup Lake expressed a rise in $\delta^{13}C$ in their recently formed rings (1996-1998). This was accompanied by a decrease in water availability and ring area during the same period.

The C trees at Banganup Lake did not exhibit distinct relationships between water availability, mean ring area and mean $\delta^{13}C$ (Figure 5.32). $\delta^{13}C$ and ring area fluctuated over the time period sampled and displayed high degrees of standard error.

140
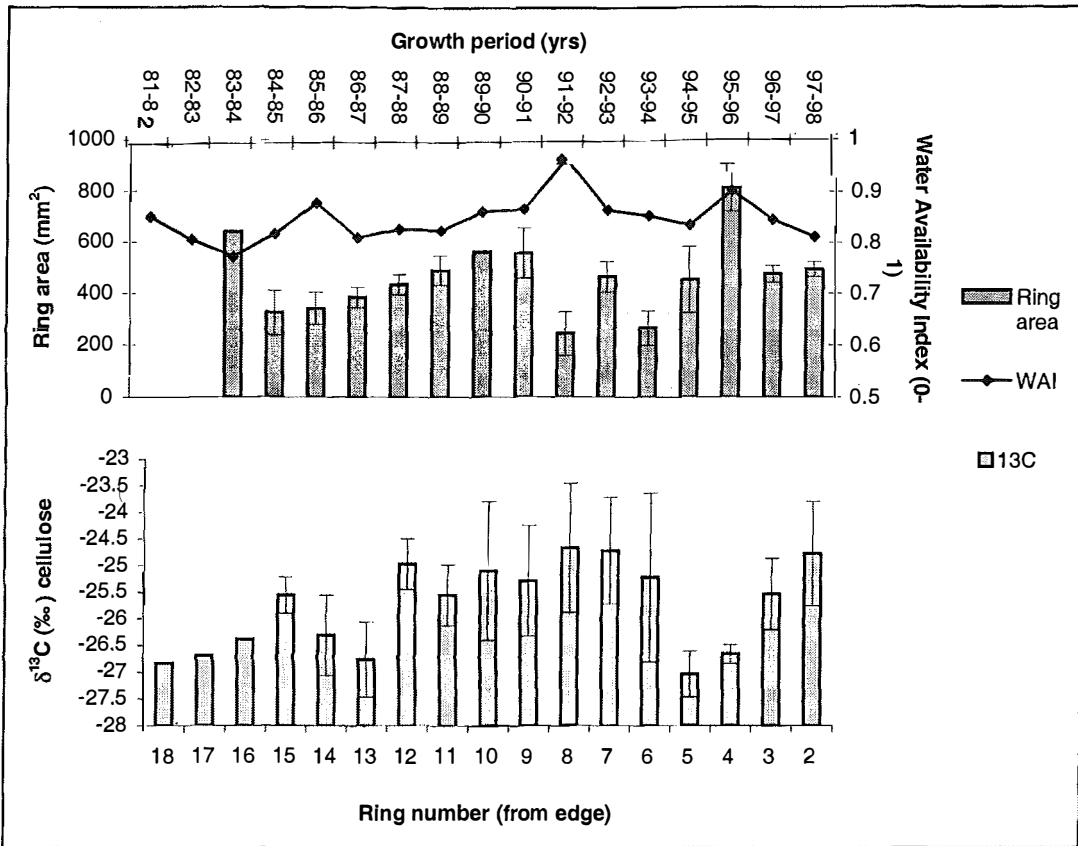
Figure 5.30: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean δ¹³C (lower Y-axis) of A trees at Banganup Lake. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.
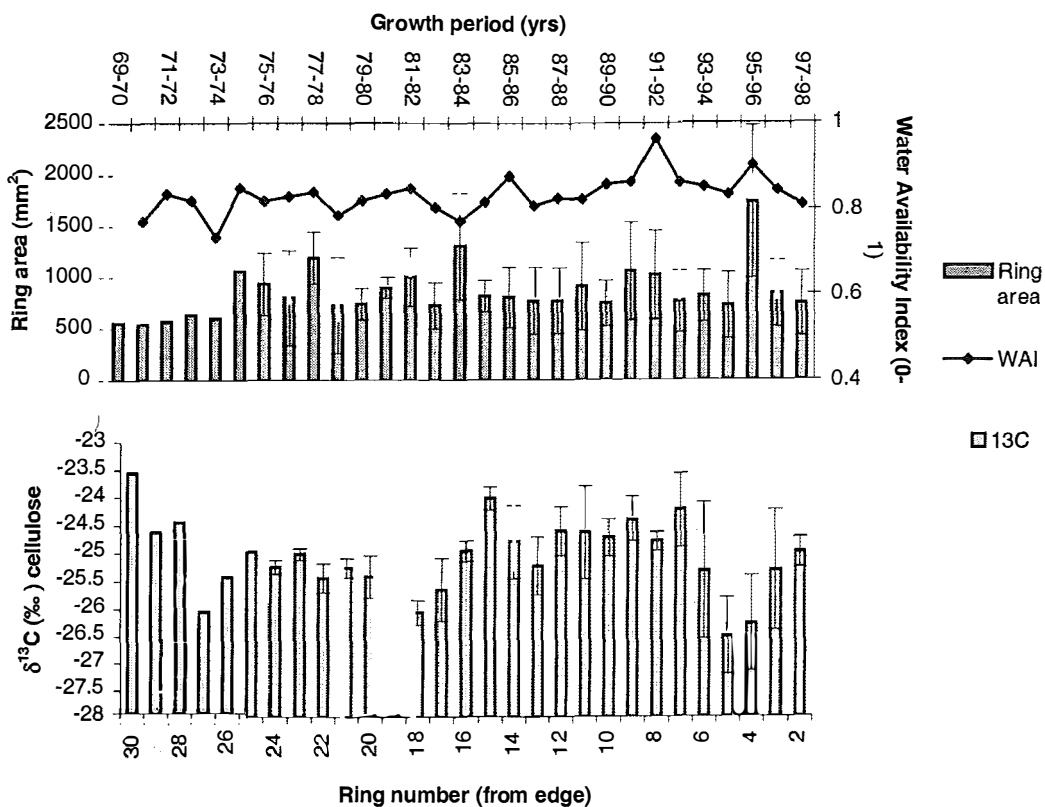
Figure 5.31: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean δ¹³C (lower Y-axis) of B trees at Banganup Lake. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.

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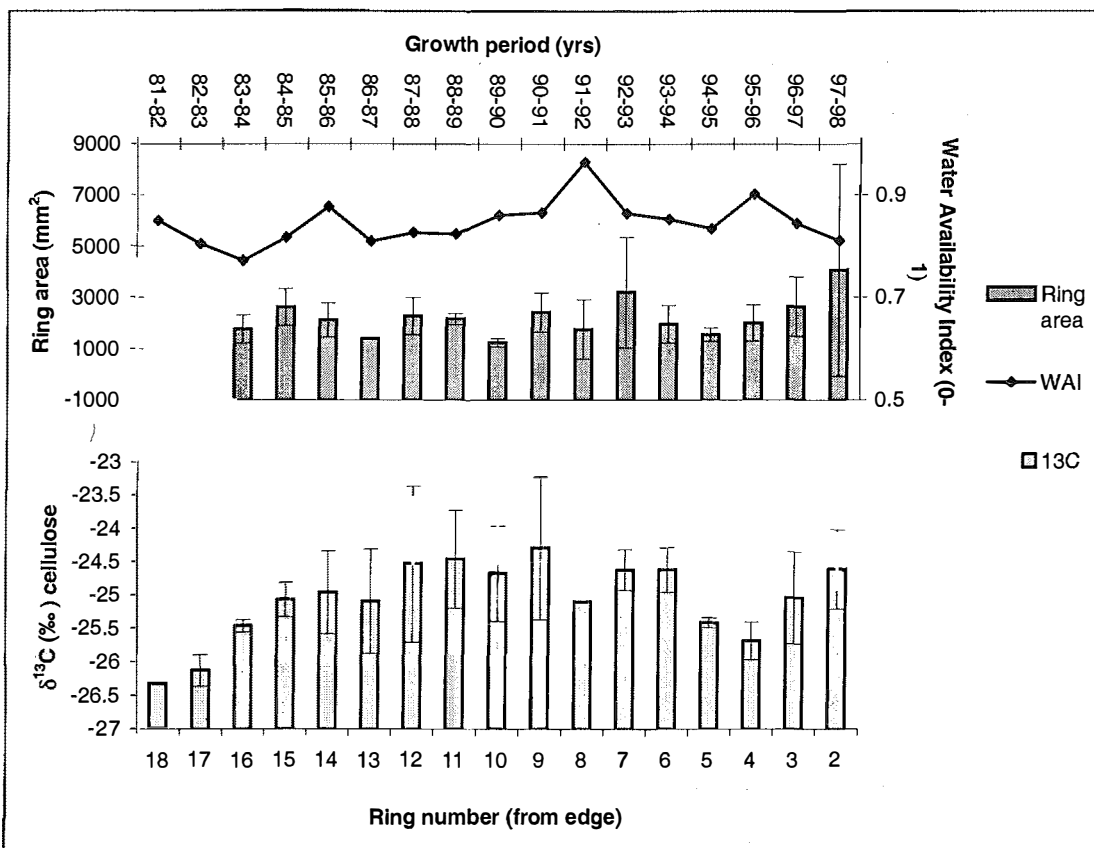
Figure 5.32: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean δ13C (lower Y-axis) of C trees at Banganup Lake. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.

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5.3.3.2.2 Thomsons Lake

The changes in water availability, mean ring area and $\delta^{13}C$ of trees from the A position at Thomsons Lake over time are presented by Figure 5.33. During the growth period of 1991-1992 when water availability peaked at Thomsons Lake, it was found that the average width of the corresponding tree ring also peaked, measuring over 2000mm^2 at breast height. This year also saw a drop in $\delta^{13}C$, indicating that the trees were low in water use efficiency. 1995-1996 was also a year of high water availability, and was similarly accompanied by low $\delta^{13}C$ formed during the same year. However, the mean width of the same tree ring was relatively low.

For the trees located in the B position at Thomsons Lake, it was found that the area of wood formed, and the $\delta^{13}C$ signatures obtained during the period of 1986-1991 were fairly constant (Figure 5.34). However, the increase in water availability in the early 1990s (particularly 1991-1992) saw a rise in ring area, and a decrease in $\delta^{13}C$.

Figure 5.35 combines water availability, ring area and ring $\delta^{13}C$ over time for the trees located on the upper slope at Thomsons Lake. A relatively high degree of standard error was found within the means of ring area and ring $\delta^{13}C$. Despite this, it was evident that as water availability decreased from the late 1980s, so did the area of wood formed annually. There was no evident trend observed in $\delta^{13}C$ during this period, however the peak in water availability in 1992 corresponded to the period in which the trees were most negative in $\delta^{13}C$, and therefore less water use efficient.
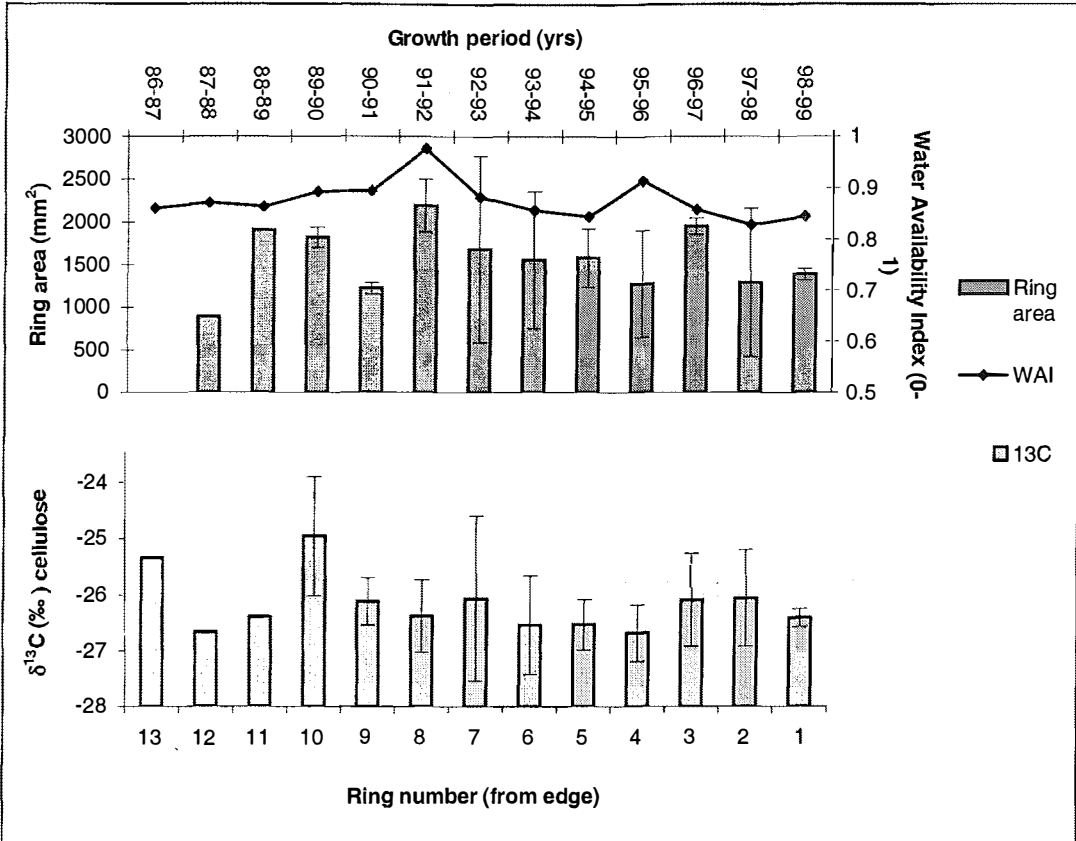
Figure 5.33: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean δ¹³C (lower Y-axis) of A trees at Thomsons Lake. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.

145
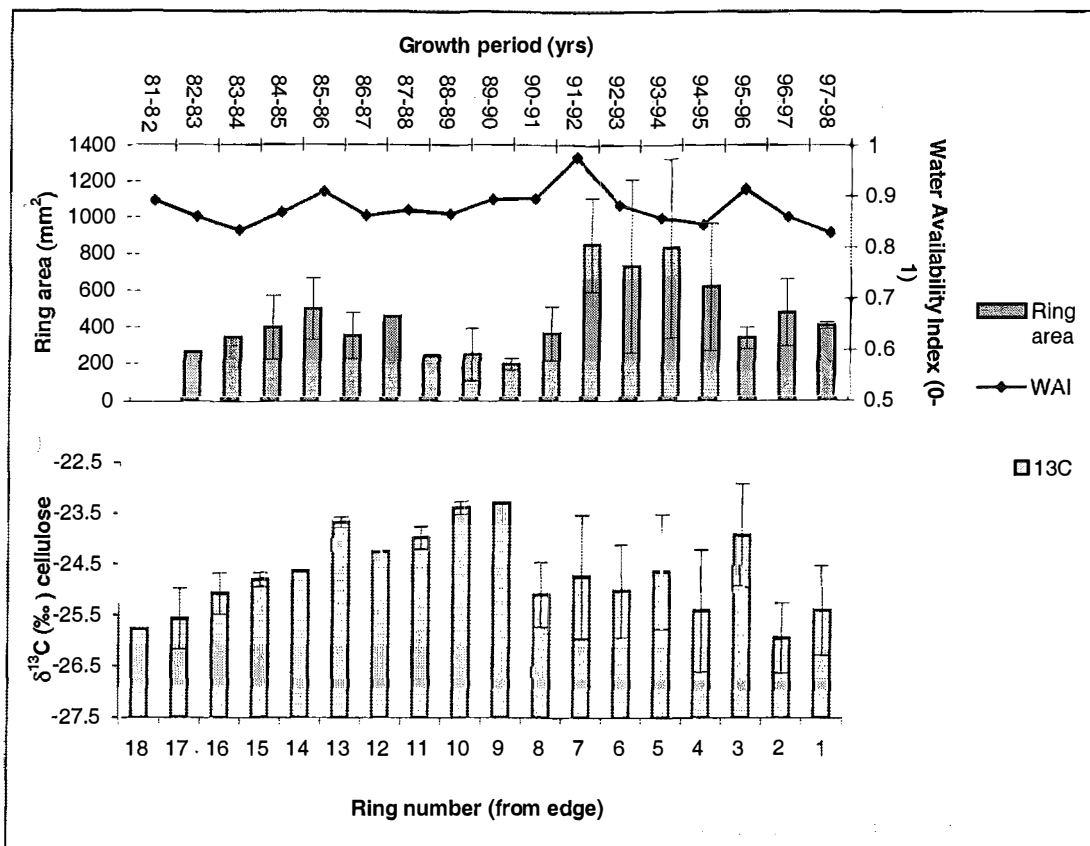
Figure 5.34: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean δ13C (lower Y-axis) of B trees at Thomsons Lake. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.

146
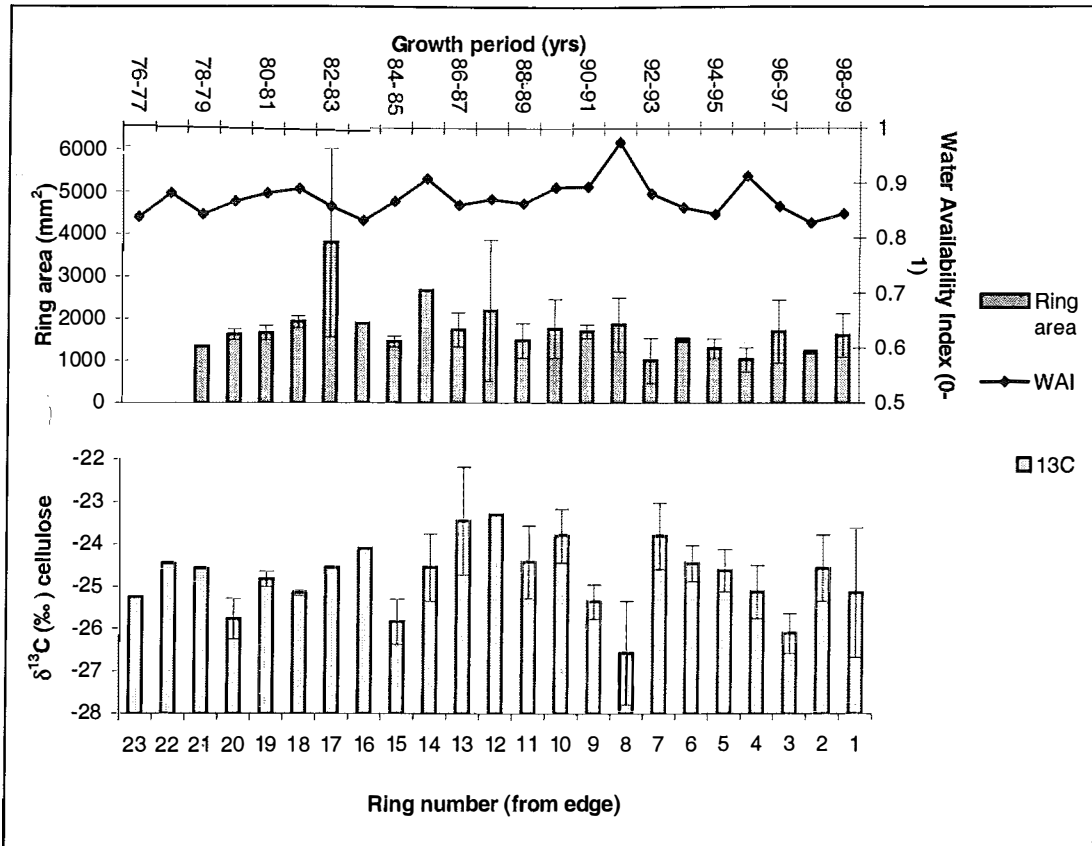
Figure 5.35: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean δ¹³C (lower Y-axis) of C trees at Thomsons Lake. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.

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5.3.3.2.3 Lake Jandabup

For the trees located in the A position at Lake Jandabup, there were no obvious trends existing between $\delta^{13}C$, water availability and the width of tree rings (Figure 5.36). In contrast to theory, the lowest ring area of trees were found to have formed during years of higher water availability. $\delta^{13}C$ showed a progressive decrease in time from the late 1980s, indicating that the trees were becoming less water use efficient. The area of wood formed during this period fluctuated largely and failed to show any trends.

Figure 5.37 presents these data for the mid slope trees at Thomsons Lake (B position). Similarly to the A trees, the trees located mid slope displayed a decrease in $\delta^{13}C$ over time from the mid 1980s. This corresponded to the overall increase in water availability (based on WAI) over time at Jandabup Lake. From the late 1980s onwards there were no distinct trends observed, with water availability fluctuating during this period. The average ring area increment was found to decrease during the 1980s, which corresponded to the period of low lake levels at Lake Jandabup. The rise in ring area was observed from 1993, and corresponded to the period when lake levels rose.

Water availability and mean $\delta^{13}C$ and ring area for the trees in the C position at Lake Jandabup are illustrated by Figure 5.38. The decrease in $\delta^{13}C$ from 1991 onwards and the subsequent increase in ring area were likely to be due to the variations that occurred by averaging the tree ring measurements from only two cores. Prior to this period, the results were biased towards the results from one core only (tree 1c). Due to this bias, it was difficult to investigate relationships existing between ring $\delta^{13}C$, ring width and water

availability accurately for *M. preissiana* in this position of the landscape. However, it was found that in 1992 when water availability was highest, wood formation was also high and $\delta^{13}C$ declined, indicating lower water use efficiency.
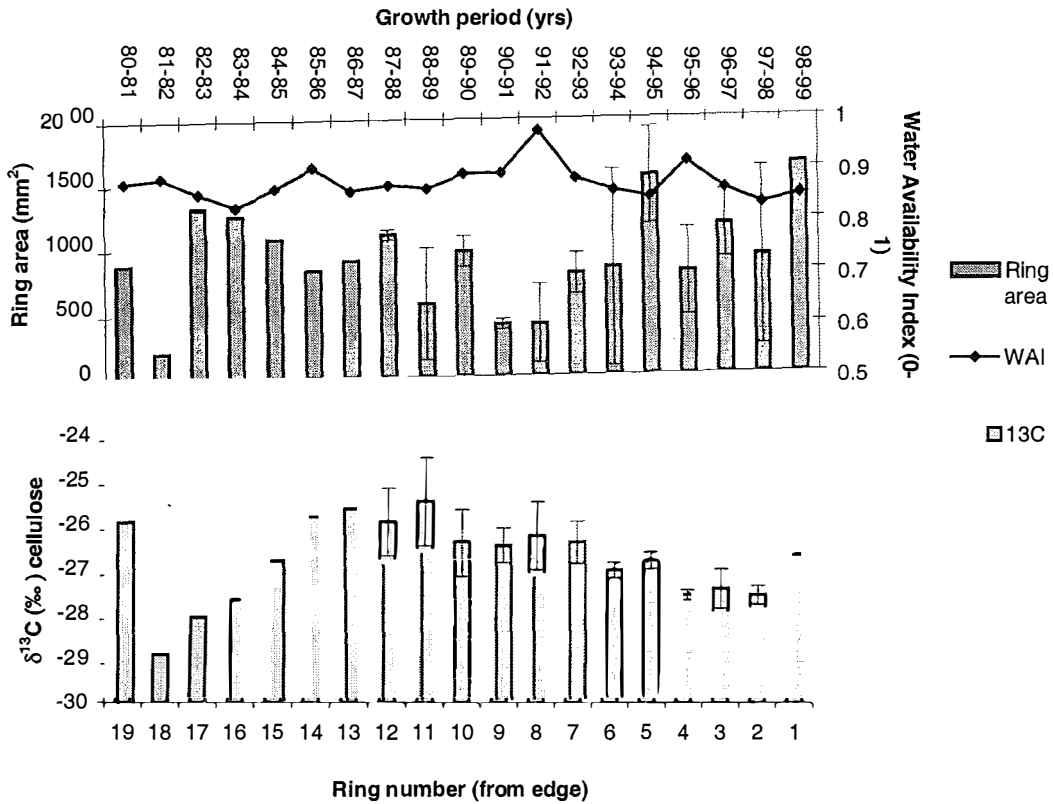
Figure 5.36: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean $\delta^{13}C$ (lower Y-axis) of A trees at Lake Jandabup. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.
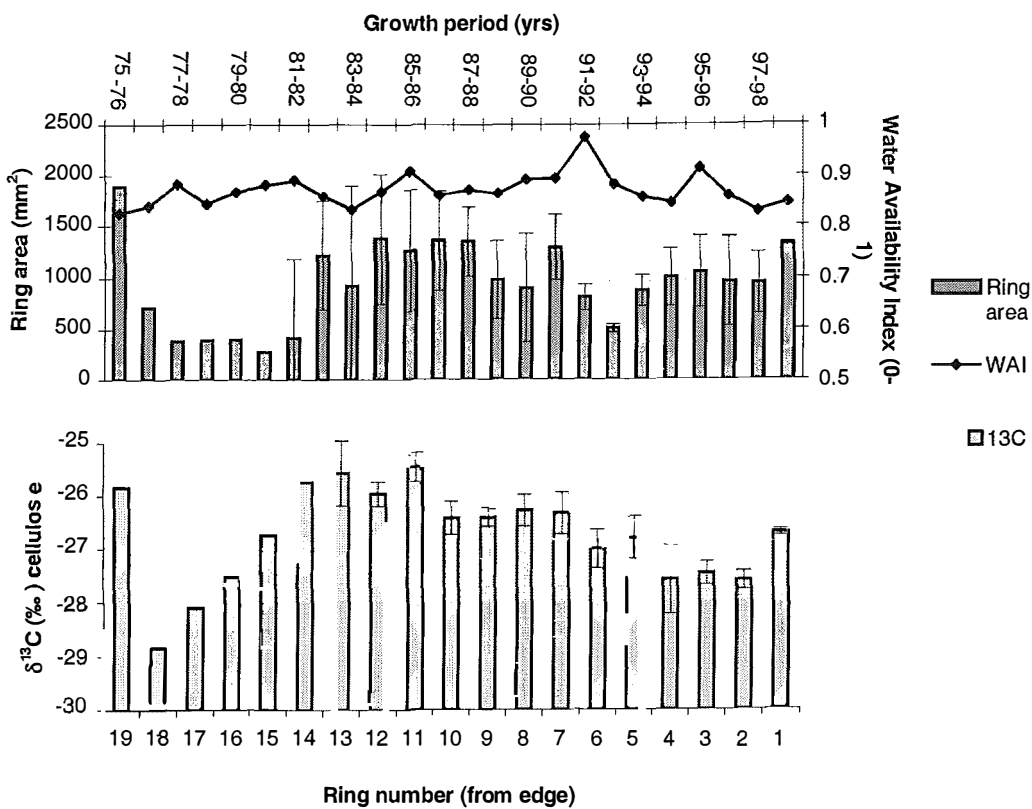
Figure 5.37: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean δ¹³C (lower Y-axis) of B trees at Lake Jandabup. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.
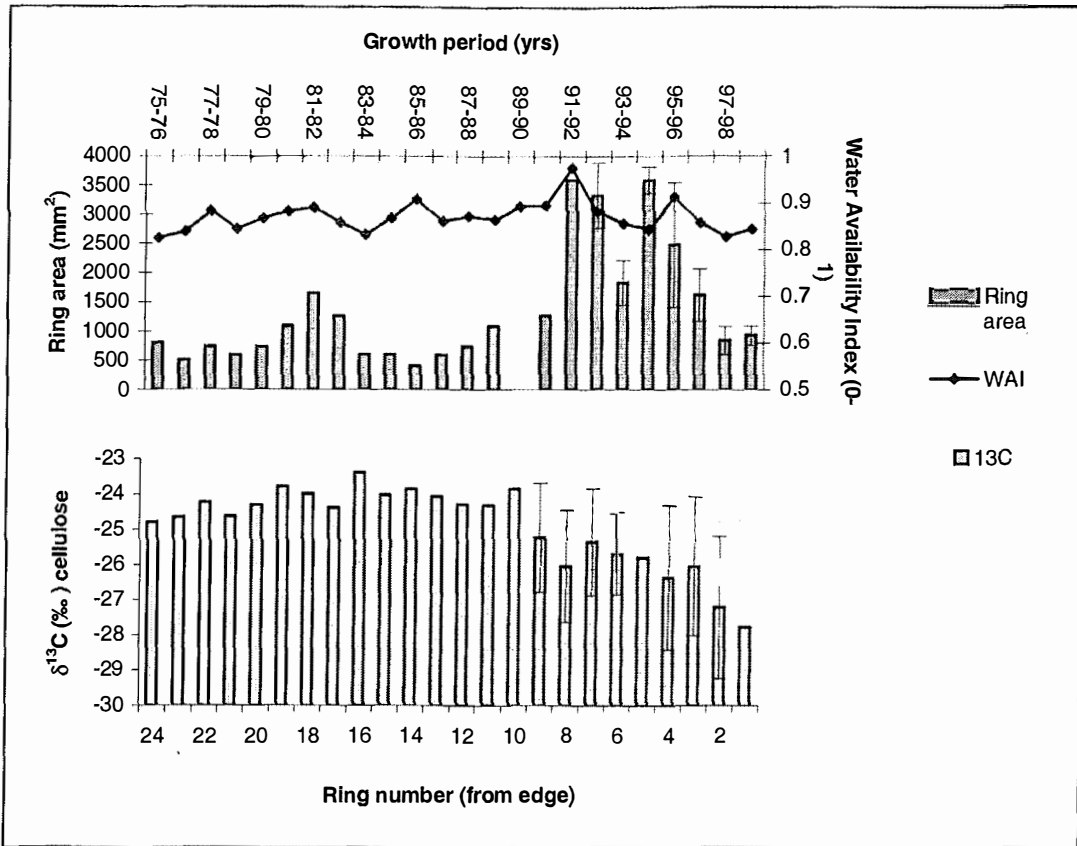
Figure 5.38: Water Availability Index (second, top Y-axis), Mean ring area (first, top Y-axis) and mean δ¹³C (lower Y-axis) of C trees at Lake Jandabup. Rings are numbered from the edge (lower X-axis) and matched to corresponding growth periods for WAI (top X-axis). Standard error bars are shown.

5.4 DISCUSSION

The changes in $\delta^{13}C$ observed between the growth rings of the *M. preissiana* trees sampled from the three wetlands on the Swan Coastal Plain were individually examined in terms of the water availability occurring during the corresponding period of growth. This provided an understanding of how trees located at different positions along a hydrological gradient responded physiologically to changes in water availability over time.

5.4.1 Historical Water Availability

The combination of factors affecting historical water availability (total rainfall, maximum temperature, relative humidity and mean water levels) were represented by the water availability index. The changes in WAI over time showed that the three lakes experienced similar trends in water availability since 1970. The index fluctuated widely over the monitoring period, with the index being higher at Lake Jandabup than at Thomsons and Banganup Lakes. This may be due to the fact that Lake Jandabup was inundated on a permanent basis rather than seasonally.

The growth season from 1978-1979 saw the WAI drop at Banganup and Thomsons Lakes. This may be due to the decrease in water levels at the two lakes due to groundwater abstraction commencing in the area during 1979. 1983-1984 similarly saw a drop in water availability at the three wetlands. Climatic data show that rainfall and humidity were below the average expected levels during this period, which may have reduced the recharge entering the lake. Banganup Lake inundated for only one month per year during this period,

while Thomsons Lake inundated for only four months. The growth period of 1985-1986 saw a rise in WAI at the three lakes. This is possibly due to the increased lake levels and increased summer rainfall in 1986 after the four-year "dry spell".

The high water availability experienced at the three lakes in the 1990s (peaking in 1992), was likely to be due to a rise in rainfall, with particular reference to the high rainfall received in February 1992, which induced a massive increase in lake levels. Banganup Lake inundated for 10 months in 1992, while Thomsons Lake and Lake Jandabup inundated for the entire 12 months. From the early 1990s, water levels at Thomsons and Jandabup Lakes have been carefully managed. Lake Jandabup was artificially maintained from 1989 in order to prevent lake levels from drying (Froend *et al*, 1993) and CALM established criteria for the management of Thomsons Lake's levels to prevent flooding due to agricultural drainage (WAWA, 1991). 1997-1998 was a period of low water availability at the three wetlands. Rainfall was below average during both years, and lake levels fell following the influx of water during the early 1990s. Lake Jandabup dried from 1997-1999 to eradicate the mosquito fish (*Gambusia holbrooki*) from its waters (O'neill, 2000).

5.4.2   Transect position, year and $\delta^{13}C$

5.4.2.1   Banganup Lake

5.4.2.1.1 $\delta^{13}C$ variations along the hydrological gradient

At Banganup Lake, the negative relationship existing between $\delta^{13}C$ and water availability

was proven in terms of the tree ring $\delta^{13}C$ signatures of the trees located along different positions of the hydrological gradient. The average ring $\delta^{13}C$ values of trees in the A, B and C positions show that the trees located on the upper slope, where water availability was lowest, were isotopically heavier during most years of annual growth than those trees located on the mid and lower slopes. This negative relationship between water availability and $\delta^{13}C$ in *M. preissiana* at the study sites was demonstrated by the short term sampling method using nascent xylem tissue (Chapter 4). The fact that this relationship was also expressed across growth rings representing different years of annual growth supports the theory, and indicates that the spatial relationship between $\delta^{13}C$ and water availability existed during most years at Banganup Lake.

It was mentioned earlier that the reasoning behind this difference in carbon discrimination between trees of different water availabilities is because of the direct role water plays in the plant's photosynthetic capacity. When water availability is low, reduced stomatal conductance and transpiration rates cause a decline in intercelluar levels and a reduction in the $^{13}C/^{12}C$ discrimination, leading to more positive $\delta^{13}C$ (Lajtha & Marshall, 1994). This relationship has been proven between years in the growth rings of trees from environments of various water availabilities (E.g. McNulty & Swank, 1995; Saurer *et al*, 1995; Walcroft *et al*, 1997; Macfarlane & Adams, 1998). A study by Pate & Arthur (1998) compared the $\delta^{13}C$ of *Eucalyptus globulus* across growth rings in two plantation sites: one rainfed and one irrigated. It was found that the tree ring $\delta^{13}C$ of the species displayed more negative $\delta^{13}C$ at the irrigated site, as a response to the higher supply of water. The documentation of the increase in tree ring $\delta^{13}C$ in environments of lower water availability over time supports the

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findings made of *M. preissiana* at Banganup Lake.

5.4.2.1.2 $\delta^{13}C$ *variations between years*

Differences observed in $\delta^{13}C$ between rings at Banganup Lake may be attributed to episodic fluctuations in water availability associated with climate and/or lake hydrology. Average $\delta^{13}C$ values observed at the transect position level (Figure 5.18) and at the site level (Figure 5.19) show how the *M. preissiana* population responded physiologically to the environmental conditions over time. By investigating the changes in $\delta^{13}C$ at both levels, it was noted that the period of 1982 to 1985 saw tree $\delta^{13}C$ becoming more positive. This period corresponded to the "dry spell" over Perth, where rainfall was lower than the average for the four consecutive years, lake levels fell and inundation occurred for only one month per year.

The apparent drop in $\delta^{13}C$ displayed by the fifth ring in all sampled trees at Banganup Lake corresponded to the year 1995. This drop in $\delta^{13}C$ where all trees became more negative may be a response to the fire occurring in the Reserve in March 1994. The reduced WUE may be attributed to higher growth rates while the trees regenerated during the summer following the fire. This is evident by the larger ring areas in the trees from the A and B positions formed during 1995 (Figures 5.30 and 5.31). Sanquist *et al* (1993) suggested that plants with more rapid growth rates (such as those resprouting after fire) express more negative $\delta^{13}C$ signatures due to having a lower *A/E* ratio, and therefore a lower water use efficiency. As Figure 5.18a shows, trees located on the lower and mid slopes began to increase in $\delta^{13}C$ one to two years following the drop during 1995, which was also

accompanied by lower growth rates. In comparison, the trees located on the upper slope (where water availability was lowest) showed a less dramatic decrease in $\delta^{13}C$ following the fire, and their subsequent increase in $\delta^{13}C$ afterwards was less immediate. This may possibly be due to the tree's more water use efficient response to canopy damage caused by the fire. The lower growth rates of these trees during the post fire years indicated a prolonged recovery period, which may be due to the combination of the lower water availability and larger size of the trees at this position of the hydrological gradient.

This relationship between the radial growth rates of trees (using ring area as an indicator) and $\delta^{13}C$ has been investigated by many researchers (E.g. Leavitt & Long, 1986; Dupouey *et al*, 1993; McNulty & Swank, 1995; Livingston & Spittlehouse, 1996; Bert *et al*, 1997; Macfarlane & Adams, 1998). It has been found that generally, more positive $\delta^{13}C$ values are associated with narrower rings, and therefore slower growth rates. This is due to the tree's response to water availability. Periods of extreme water stress (such as a drought) can cause stomatal closure and reduced CO_2 uptake in trees. This results in slower carbon assimilation rates, slower growth, and consequently narrower tree rings (Francey & Farquar, 1982). Although no direct relationship was observed between ring area and $\delta^{13}C$ in *M. preissiana* at Banganup Lake, episodic events such as the 1994 fire induced physiological changes in the population, which supported these theories of effects of water availability on plant growth and carbon isotope discrimination.

<u>5.4.2.2   Thomsons Lake</u>

5.4.2.2.1 $\delta^{13}C$ variations along the hydrological gradient

The differences observed in $\delta^{13}C$ between the trees along the hydrological gradient at Thomsons Lake reflected those found at Banganup Lake. As Figure 5.23 shows, the $\delta^{13}C$ values of trees located on the lower slope, where water availability was higher, were more negative than those displayed by trees located on the mid and upper slopes where water availability declined. The trees located on the lower slope (A position) of each transect experienced seasonal inundation from the 1990s, which may have been a factor affecting the lower $\delta^{13}C$ of trees in this position.

5.4.2.2.2 $\delta^{13}C$ variations between years

The variability between ring $\delta^{13}C$ within the *M. preissiana* population at Thomsons Lake may represent a species response to the temporal variations in water availability. The decreasing trend in $\delta^{13}C$ over time at Thomsons Lake was interrupted during the mid to late 1980s, where $\delta^{13}C$ of the *M. preissiana* population decreased. This time frame represented a period of successively lower lake levels and seasonal inundation. The trees in the B position also displayed lower annual growth increments during this period. The drop in $\delta^{13}C$ displayed in all trees during the early 1990s corresponded to the large rise in water availability during 1992 at Thomsons Lake. The influx of water in 1992 induced higher growth rates of trees in the A and B positions in terms of ring area. These findings suggest that the radial growth rate and $^{13}C/^{12}C$ discrimination of the population was affected by water availability. Similarly to the findings made at Banganup Lake, it was only episodic

events such as the 1992 rise in water availability, that induced *obvious* responses in tree growth and $\delta^{13}C$, rather than prolonged trends over time. This was evident by the failure of tree ring $\delta^{13}C$ to correlate significantly with the water availability parameters over time.

5.4.2.3 Lake Jandabup

5.4.2.3.1 *$\delta^{13}C$ variations along the hydrological gradient*

Variations observed in the tree ring $\delta^{13}C$ values of the *M. preissiana* population at Lake Jandabup were less conforming to the current theories relating $\delta^{13}C$ and WUE than those observed at Banganup and Thomsons Lakes. For example, in transects 2 and 3, the $\delta^{13}C$ of trees located on the upper slope were more negative in most years than the trees on the mid and upper slopes where water availability was higher. The reasoning for this may be a result of tree age or size. Although the trees were generally shorter than those sampled at Thomsons and Banganup Lakes, variations in diameter between the A, B and C positions of the hydrological gradient were less pronounced. Therefore, the age or size effects that affect $\delta^{13}C$, as described by Francey & Farquar (1982), Donovan & Ehleringer (1992) and Sanquist *et al* (1993) were removed. However, the examination of *mean* differences in $\delta^{13}C$ between the trees of different transect positions (Figure 5.28) revealed the expected trends, with areas of higher water availability (A and B positions) supporting trees with more negative $\delta^{13}C$ values during most years of growth.

5.4.2.3.2 *$\delta^{13}C$ variations between years*

From the mid 1980s, it was evident that the fluctuations in $\delta^{13}C$ and ring area over time by

trees from different transect positions were less prominent than those displayed by trees at Banganup and Thomsons Lakes. Prior to the mid 1980s, $\delta^{13}C$ was biased towards the results of only one or two cores due to the different aging sequences contained by the cores from differently aged trees. Following this period, it was evident that $\delta^{13}C$ of trees in the A and C positions progressively decreased in time, while the $\delta^{13}C$ of trees in the B position remained fairly constant. Hydrographs show that water levels at Lake Jandabup fluctuated during this period, with high levels experienced in 1986 and 1991-1993 and low levels occurring during the late 1980s and late 1990s. The declining trend in $\delta^{13}C$ suggested a regional increase in water availability during this period. This was indicative of the higher water availability index calculated for Lake Jandabup.

5.4.3   Future Applications for Time-Integrated, Long Term $\delta^{13}C$ Measurements

Due to the difficulties found with linking the variations in $\delta^{13}C$ of annual wood tissue of *M. preissiana* with the environmental parameters of the lakes, it was important to investigate possible causes of $\delta^{13}C$ variations over time. McNulty & Swank (1995) identified that part of the difficulty in using wood tissue $\delta^{13}C$ as a tool for measuring historic ecosystem characteristics involves factoring out the potential influences on wood tissue other than water stress. In this study, there were a number of factors which needed to be accounted for before assessing the applicability of using time-integrated, long term measures of $\delta^{13}C$ to determine the WUE of *M. preissiana* populations.

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5.4.3.1   Within ring variations in $\delta^{13}C$

5.4.3.1.1   Seasonal $\delta^{13}C$ variability

One factor that needed to be accounted for when analysing the $\delta^{13}C$ of annual ring tissue in trees is the variation that occurs *within* individual rings. Seasonal variations in water availability have been found to induce variations in $\delta^{13}C$ between the early and latewood contained within annual ring tissue. Complying with the theory that $\delta^{13}C$ and water availability are inversely related, the early wood formed during the growth season (spring/summer) is generally isotopically heavier than the late wood formed during winter (Wilson & Grinsted, 1977; Francey & Farquar, 1982; Leavitt, 1992; Walcroft *et al*, 1997; Mcfarlane & Adams, 1998). Due to the higher proportion of early wood vessels contained in growth rings, it is possible that the $\delta^{13}C$ signatures obtained from integrated, one-year ring tissue in this study were biased towards the $\delta^{13}C$ formed during the growth season (i.e. contained in the early wood). Due to the limited scope of this study, it was impossible to analyse within-ring variations in $\delta^{13}C$, however, annual $\delta^{13}C$ values were correlated with not only *annual* water availability parameters, but also with *seasonal* water availability (e.g. during summer and winter), in order to account for potential variations in seasonal $\delta^{13}C$ of ring tissue. Additionally, the water availability index incorporated the variations occurring during the summer and winter of each year to also account for possible variations in $\delta^{13}C$. The growth ring $\delta^{13}C$ was not found to correlate significantly with these seasonal water availability parameters.

5.4.3.1.2 Longitudinal $\delta^{13}C$ variability

$\delta^{13}C$ of wood tissue has also been found to vary longitudinally along the length of the stem. This is due to differences existing in the $\delta^{13}C$ of the cellulose laid down in different branches of the same individual. Sunlight and shading are two factors that may cause these differences, with $\delta^{13}C$ increasing towards the canopy due to increasing light intensity (Leavitt & Long, 1986). In this study, coring occurred at the same height for all trees in order to eliminate possible variations in $\delta^{13}C$ associated with stem height. Some trees sampled at Jandabup and Thomsons Lake were multiple stemmed, and in these cases, cores were taken from the largest stem. However, unequal conditions of soil moisture, ambient air temperature and light in the microenvironment may have induced differences in $\delta^{13}C$ between stems, and therefore the $\delta^{13}C$ values obtained from these trees may not have been indicative of the WUE of the individual.

5.4.3.1.3 Circumferential $\delta^{13}C$ variability

Another source of variation in tree ring $\delta^{13}C$ is circumferential differences occurring within rings along the circumference of the stem. Leavitt & Long (1986) recognise that such variations in $\delta^{13}C$ are a reflection of variations in isotopic fractionation *where* the carbon is fixed (i.e. the leaves). Therefore, circumferential differences in the $\delta^{13}C$ of leaves within a tree's canopy (due light conditions) are often reflected in the $\delta^{13}C$ of the wood taken from a similar position around the stem's circumference. Leavitt & Long (1986) found a 0.5-2.5‰ range in isotopic values for leaves within a single canopy, which may have also been reflected in the $\delta^{13}C$ of the stem wood. For this reason, it was recommended that rings from four orthogonal cores be pooled from individual trees for $\delta^{13}C$ analysis, in order to ensure

accurate representation of the WUE of the tree (Leavitt & Long, 1986; Leavitt, 1992; Saurer *et al*, 1995; Macfarlane & Adams, 1998). Such variability occurring within the circumference of tree rings was reduced in this study by averaging $\delta^{13}C$ values of three replicate trees at each transect position. However, subsamples were not taken from individual trees due to financial and time limitations. Taking a number of subsamples of growth rings from individual trees may be a direction for future carbon isotope studies based on this species.

5.4.3.2   Other $\delta^{13}C$ variations

5.4.3.2.1   Age effects and $\delta^{13}C$ variations

The differences observed in $\delta^{13}C$ between trees from different positions along the hydrological gradient may be attributed to possible age effects. This was addressed in Chapter 4, where it was found that the younger trees exhibited more negative $\delta^{13}C$ values than older trees, possibly due to their lower water use efficiency (Sanquist *et al*, 1993). This tree age factor could have similarly influenced carbon discrimination between individuals by sampling tree ring wood. However, temporal fluctuations in the $\delta^{13}C$ of *M. preissiana* (such as the seasonal variations found by measuring short term $\delta^{13}C$, and the annual variations found by measuring long term $\delta^{13}C$) suggested that $\delta^{13}C$ was more responsive to water availability than differences in tree age.

The greatest detriment that tree age played in this study was the fact that the regular sizing of cores encapsulated different years of growth for different trees. The typical 40mm length

of the cores sampled contained between six to over forty rings, depending on the age and size of the individual. This created difficulty when making comparisons between differently aged trees, and created biases when averaging the $\delta^{13}C$ between corresponding annual rings of several individuals.

5.4.3.2.2 Tree aging techniques

A final consideration to be made in assessing the applicability of $\delta^{13}C$ analysis in understanding the long term trends in water use efficiency of *M. preissiana* is the reliability of the tree aging and division techniques used. Microscopic detail ensured that growth ring visibility in cores was sufficient to make distinctions between annual growth rings. However, the development of "false rings", where abnormal conditions in growth cause a defect in ring formation, can create difficulties in accurately aging and dividing tree rings (Mitchell Beazley Publishers Ltd, 1976). The possible development of "false rings" in the *M. preissiana* individuals sampled might have affected the results obtained of $\delta^{13}C$ changes across growth rings. Additionally, the $\delta^{13}C$ value formed during one year can ultimately be affected by the environmental conditions of the site during the previous year, rather than the current growth period. Dupouey *et al* (1993) recognise that the climatic conditions of one year can pre-condition the physiological functioning of the tree during the following growth season. Characteristics such as the root system, the size of xylem vessels and the carbon reserves are determined by the climate experienced during the preceding year, and can ultimately affect the WUE of the tree during the following year. It is important to acknowledge factors such as these that affect the $\delta^{13}C$ values in tree rings, however, accounting quantitatively for these factors were beyond the scope of this study.

CHAPTER 6: CONCLUSIONS AND MANAGEMENT IMPLICATIONS

6.1   CONCLUSIONS

The heterogeneity displayed within and between wetland ecosystems on the Swan Coastal Plain in terms of spatial and temporal water availability was likely to be a principle factor determining the physiological responses of fringing tree populations in their use of water. The dynamic nature of wetland hydrology, now largely driven by anthropogenic influences such as land clearance, groundwater abstraction and artificial drainage, provides reason for understanding the complex interactions between plant physiology and water availability. The results from this study supported the application of $\delta^{13}C$ analysis as a measure of the water use efficiency of *M. preissiana* in response to spatial and temporal variations in water availability. However, the findings identified that uncertainties associated with the sampling techniques did exist. These uncertainties must be addressed and accounted for in order to obtain a detailed representation of the role water availability plays in the $^{13}C/^{12}C$ discrimination process of plant populations in natural, heterogeneous environments.

The relationship between $\delta^{13}C$, instantaneous water use efficiency and water availability of *M. preissiana* in a controlled environment conformed to the findings documented for other C_3 species (e.g. Farquar & Richards, 1984; Ehleringer & Cooper, 1988; Leavitt, 1992; Dupouey, 1993; Saurer *et al*, 1995; Stewart *et al*, 1995; Zhang & Marshall, 1995; Walcroft *et al*, 1997; Macfarlane & Adams, 1998; Warren & Adams, 2000). *M. preissiana* seedlings undergoing experimental drought conditions responded by becoming more water use

efficient and exhibiting more positive $\delta^{13}C$ values. The inverse relationship between water availability and $\delta^{13}C$ in plants is due to their increasing water stress and water use efficiency inducing a reduced discrimination against ^{13}C during carbon assimilation (Boutton, 1991b). However, the direct relationship that light plays in carbon assimilation during photosynthesis means that light intensity is also a factor influencing the $^{12}C/^{12}C$ discrimination in leaves (Farquar *et al*, 1989). Unfortunately, light intensity was not accounted for in the glasshouse experiment, and the resulting $\delta^{13}C$ measurements were not anticipated. In order to improve the certainty of the relationship existing between $\delta^{13}C$ and water availability, future applications of the sampling methods would require the elimination of possible variations caused by factors such as light intensity.

The applicability of time-integrated $\delta^{13}C$ measurements in comparison to instantaneous WUE measurements in *M. preissiana* was of particular interest. Traditional measures of WUE using infrared gas analysis are used to understand short term gas exchange. However long term measures of WUE using this method are difficult to make, as stomatal conductance and other gas exchange variables change over the lifetime of the plant (Lajtha & Marshall, 1994). $\delta^{13}C$ measurements can examine long term WUE by integrating carbon assimilation over all periods of growth for the lifetime of the tissue sampled (Leffler & Evans, 1999). These comparisons between the two methods were reflected in the glasshouse experiment. The controlled environment induced an instantaneous response in the WUE in the treated seedlings to watering "pulses" occurring over a drying period. However, the $\delta^{13}C$ values measured from the recently developed plant tissue did not reflect these occasional watering events, and instead provided an indication of the overall water

stress of the seedling. In the natural environment, the failure of the short term $\delta^{13}C$ values of nascent xylem tissue to indicate instantaneous WUE in *M. preissiana* was likely to be due to the variable nature of instantaneous measures and the difficulties associated with comparing with time integrated measures such as $\delta^{13}C$ in the natural environment. The choice of method for measuring plant water use efficiency is largely determined by the purposes behind data collection, however, Le Roux *et al* (1996) acknowledge that $\delta^{13}C$ of plant tissue is best applied in conjunction with a variety of other techniques to screen for overall WUE in natural tree populations.

The application of $\delta^{13}C$ analysis to measure the spatial and temporal WUE of natural populations of *M. preissiana* was reliable, however it did incorporate some uncertainties which were impossible to avoid in the natural environment. On a short term, one-month basis, nascent xylem tissue $\delta^{13}C$ measurements indicated that a spatial gradient existed, with *M. preissiana* individuals within a population increasing in water use efficiency with increasing distance from the lake margin. On a temporal scale, the $\delta^{13}C$ values within *M. preissiana* populations were found to decrease from early autumn to late winter in correspondence to the influx of water associated with winter rainfall. These findings conform to popular theory and represent the inverse relationship existing between $\delta^{13}C$ and water availability. However, an identification of the other possible factors affecting this relationship is necessary in order to evaluate the applicability of this approach. At the organism scale, the morphological attributes of individual trees are potential sources of variation in $^{13}C/^{12}C$ discrimination. Tree age (Sanquist *et al*, 1993), tree height (Francey & Farquar, 1982), canopy structure (Leavitt & Long, 1986), root structure (Donovan &

Ehleringer, 1992), and branch length (Warren & Adams, 2000), have been identified as possible morphological characteristics which influence the $\delta^{13}C$ signatures expressed in plant tissue. At the population and community scales, competition for resources such as sunlight, water and nutrients affect the partitioning of carbon throughout the tree and subsequently influence $^{13}C/^{12}C$ discrimination. These factors were not quantitatively measured in this study and could have possibly been a source of variation in the $\delta^{13}C$ values of *M. preissiana*.

On a longer-term basis, $\delta^{13}C$ signatures contained within older plant tissue (e.g. growth ring tissue) were subject to greater variation than those measured from younger tissue in *M. preissiana*. Difficulties arising between the links in growth ring $\delta^{13}C$ and environmental parameters such as water availability are often difficult to make due to ecosystem complexity and plant size, and detailed measurements of historical ecological parameters are required (McNulty & Swank, 1995). The scope of this study was limited in the fact that detailed measurements such as plant water demand and soil stratigraphy were not made over time. However, it was found that the historical trends in $\delta^{13}C$ displayed by the *M. preissiana* populations spatially, were reflective of the water regime gradient. Additionally, episodic events in time such as fire, prolonged dry spells and high summer rainfall were found to be reflected in the time series of $\delta^{13}C$ contained within the ring tissue of *M. preissiana*.

From the findings of this study, it can be concluded that the time-integrated short and long term methods of measuring the $\delta^{13}C$ in natural *M. preissiana* populations were relatively

reliable in detecting trends between tree WUE and water availability. The selected methods identified spatial and temporal variations in $\delta^{13}C$ of trees occurring along a natural hydrological gradient, and recognised how the populations responded physiologically to fluctuations in water availability. The degree of applicability in using this approach to understand the water use of *M. preissiana* is affected by the consideration of "outside" factors other than water availability that affect the $^{13}C/^{12}C$ discrimination in plants. By accounting for these external factors, the application of $\delta^{13}C$ analysis in understanding the complex interactions between tree physiology and wetland hydrology can be improved and applied as an integrated approach to wetland management.

6.2   MANAGEMENT IMPLICATIONS

Of most importance, this study applied the technique of $\delta^{13}C$ analysis to natural populations in an environment where water limitations are of extreme significance to the health and survival of the plant community. These findings can be applied in a management context to understand the complex relationship existing between plant physiology and water availability.

The spatial and temporal variations in $\delta^{13}C$ observed within populations of *M. preissiana* suggest that there is much environmental heterogeneity occurring in the fringing area of wetland ecosystems. As a response to water availability, some individuals were more water use efficient than others. Those trees located on the upper slope, where water availability was lowest had the highest water use efficiency, and possibly recruited during a period of

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higher water levels. Their persistence is likely to be due to the plasticity of the individual and ability to adapt to fluctuating water levels (Froend *et al*, 1993). Therefore, rapid or prolonged decreases in groundwater levels may increase water stress to the point of local extinction at that position of the landscape (Froend *et al*, 1993). Conversely, prolonged periods of flooding may also be detrimental to the survival of local populations of *M. preissiana*. $\delta^{13}C$ values can be applied to understand the physiological responses of trees to both extremes of the water availability scale (drought and flooding). However, this study focused mostly on the effects of drought.

The wetlands located on the Gnangara and Jandakot groundwater mounds on the Swan Coastal Plain are currently undergoing hydrological changes associated with surrounding landuse. For example, the establishment of pine plantations on the Gnangara Mound have induced a regional drawdown in groundwater levels (WAWA, 1995). The future harvesting of plantations will deliver a regional rise in water levels (CALM, 1999), and therefore higher regional water availability. Conversely, the proposed Stage 2 of the Jandakot Public Water Supply Scheme where public groundwater abstraction is estimated to increase from 4 to 8 million cubic metres per year (EPA, 1991) is expected to see a 0.2m drawdown in groundwater levels at Banganup Lake (Wilkins, 1992). Knowledge of $\delta^{13}C$ discrimination in *M. preissiana* may improve the understanding of how the species will respond physiologically to expected, localised changes in hydrology such as these.

The $\delta^{13}C$ signatures expressed by *M. preissiana* individuals in the natural wetland ecosystems ranged from -30‰ to -22‰. With additional studies, perhaps a scale of $\delta^{13}C$

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signatures could be established to indicate relative water stress in individuals of this species. This could assist in wetland management by identifying fringing tree populations at risk of degradation due to reduced water availability (such groundwater drawdown). With the increased pressure placed on wetland water regimes by surrounding anthropogenic activities on the SCP, this information would be of use in understanding the physiological tolerance range of the species to fluctuations in water availability.

Additionally, long term sampling of $\delta^{13}C$ (using growth ring tissue) provides a historical record of the water use efficiency of the plant. By investigating the range of $\delta^{13}C$ values in populations of *M. preissiana* (which have survived the past fluctuations in water availability), an understanding can be obtained regarding the species' tolerance levels (in terms of $\delta^{13}C$) to various environmental parameters from a historical perspective. For example, an understanding of how individuals within a population responded physiologically to a previous fire may assist in the future planning of fire management strategies. The $\delta^{13}C$ signatures contained within plant tissue formed during periods of prolonged drought can be applied in the understanding of how the population will respond physiologically to similar variations in water availability in the future. Additionally, a range of "allowable levels" in water availability could be identified based on historical $\delta^{13}C$ signatures of plant populations, to which hydrological monitoring of wetlands can be compared (R. Froend, pers. Comm., 2000).

6.3   DIRECTIONS FOR FUTURE RESEARCH

An important direction for future studies in $\delta^{13}C$ analysis of natural plant populations according to spatial and temporal variations in water availability is to eliminate the confounding effects of external factors (other than water availability). Light is one factor that was likely to have influenced the degree of $^{13}C/^{12}C$ discrimination in *M. preissiana* seedlings in this study. To obtain a *direct* relationship between water availability and $\delta^{13}C$, it is necessary to exclude the effects light intensity (and other environmental parameters) in the experimental design. Plant morphological attributes also affect the degree to which the individual discriminates against ^{13}C during carbon assimilation. Future studies investigating the relationship between $\delta^{13}C$ and water availability in *M. preissiana* would require variations in tree morphology to be minimised by sampling from similarly aged and sized individuals. Greater replication and sampling sizes (e.g. sampling several cores per tree) would also remove the potential of variation in $\delta^{13}C$ signatures due factors other than water availability.

An interesting direction for future research is the application of $\delta^{13}C$ analysis to dead individuals of *M. preissiana*. In areas of localised species extinction, tree ring analysis of $\delta^{13}C$ could be used to identify the cause of death of individuals. Historical $\delta^{13}C$ signatures may be interpreted to determine the time period during which trees were stressed before mortality. Relating $\delta^{13}C$ to environmental parameters may subsequently suggest the possible cause/s of death of affected populations, and therefore identify the conditions that the population could not tolerate.

There is a diverse range of $\delta^{13}C$ applications in terrestrial plant studies, many of which are increasing in importance due to the need to understand complex ecological relationships. The ability of stable carbon isotope measures to detect the physiological functioning of plants in both the present and the past indicates the true potential of this method in linking plant survival mechanisms with environmental parameters. With ongoing technological advancements, it is certain that the future application of isotopic techniques such as $\delta^{13}C$ analysis will become widespread in the scientific community.

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APPENDIX 1: SIZE DISTRIBUTION OF *M. PREISSIANA* POPULATION AT BANGANUP LAKE.
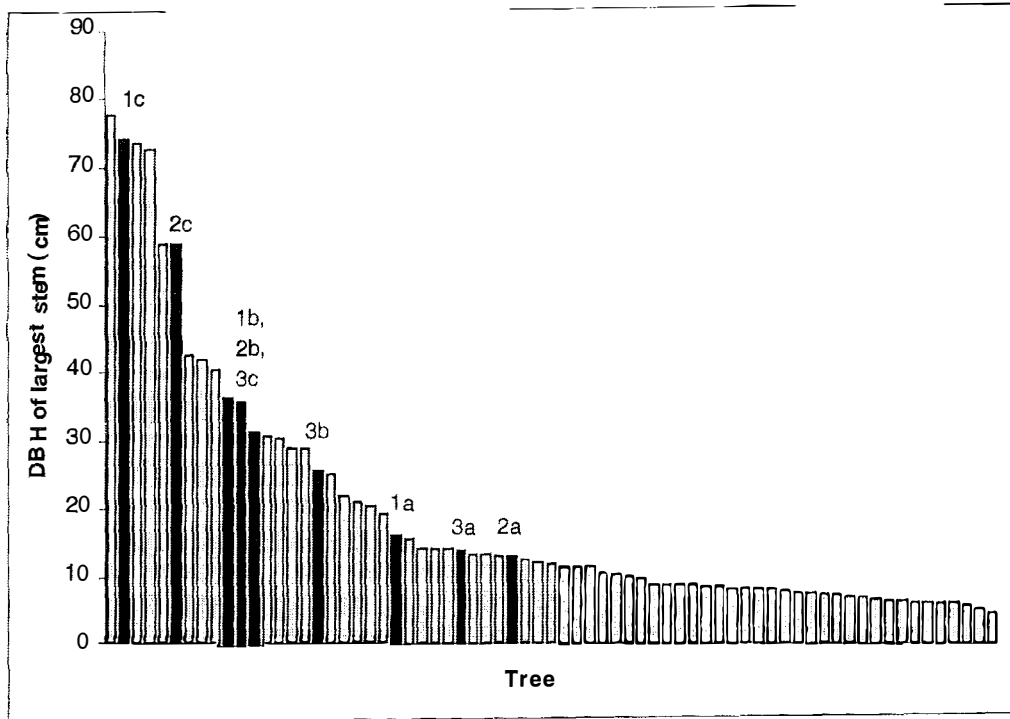
Size distribution (Diameter at Breast Height) of *M. preissiana* population at Banganup Lake. Measurements were taken from a randomly placed transect 100m x 10m in area. *M. preissiana* individuals falling within the transect were measured for DBH. Trees selected for this study are shaded in black and labelled.

APPENDIX 2: DETAILS OF HYDROLOGICAL MONITORING BORES AT BANGANUP, THOMSONS AND JANDABUP LAKES

Table listing bore details used for this study at Banganup, Thomsons and Jandabup Lake

Site	Bore #	Aquifer	Location	Elevation (mAHD)	Monitoring period
Banganup Lake	G61419614 LB1	Groundwater	Transect 1	14.344	1993-current
	G61419605 LB5	Groundwater	Transect 2	16.607	1992-1994
	G61419611 LB11	Groundwater	Transect 3	16.484	1963-current
	Q6142516	Surfacewater	All transects	14.345	1985-current
Thomsons Lake	G61611111 TM4C	Groundwater		14.636	1985-current
	G61611108 TM10C	Groundwater	Transect 2	14.227	1985-1999
	G61611116 TM11C	Groundwater	Transects 1 & 2	14.87	1985-1993
	Q6142517	Surfacewater	All transects	14.31	1952-current
Lake Jandabup	G61610763 JB12A	Groundwater	All transects	50.41	1977-current
	Q6162578	Surface water	All transects	54.457	1954-current

APPENDIX 3: ANNUAL GROWTH RING WIDTHS TAKEN FROM *M. PREISSIANA* TREE 2. RINGS MEASURED FROM 1 CROSS SECTION AND 2 CORES.
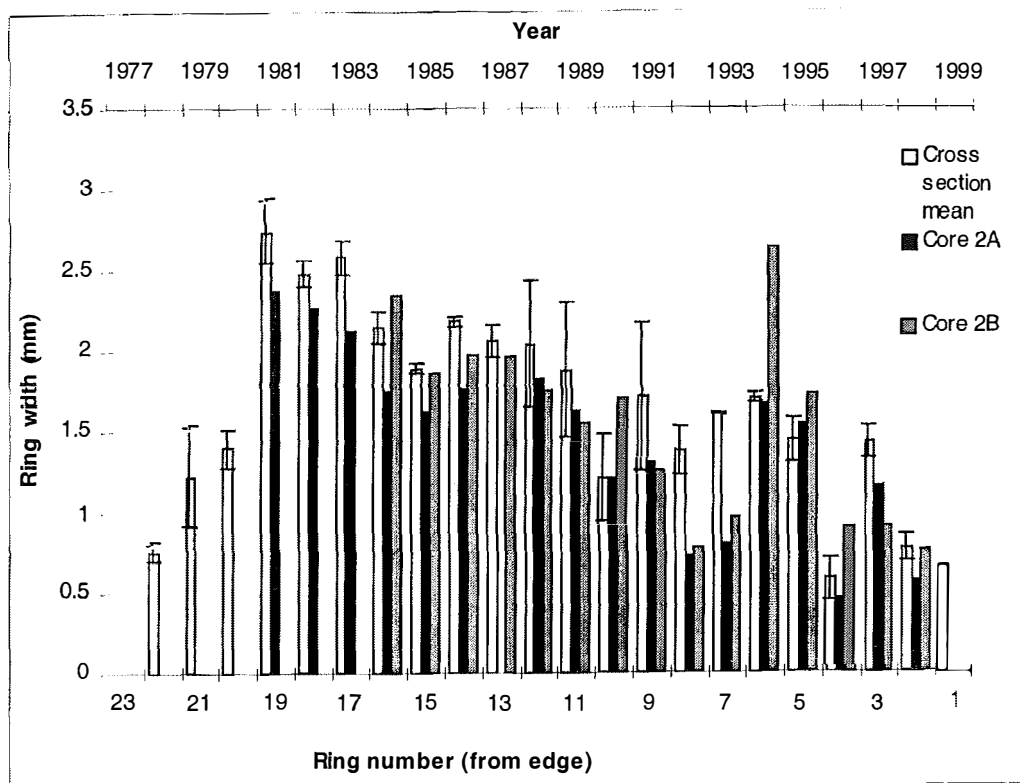
Width of tree rings taken from cross section 2. Mean refers to the average width of the tree ring taken from 3 radii across the cross section along with standard error bars. Cores 2a and 2b show the widths of corresponding rings taken from the two cores. Tree rings numbered from the edge of stem (bottom X-axis) and matched to corresponding years (top X-axis).

APPENDIX 4: ANNUAL GROWTH RING WIDTHS MEASURED FROM CORES FROM *M. PREISSIANA* TREES AT BANGANUP, THOMSONS AND JANDABUP LAKES
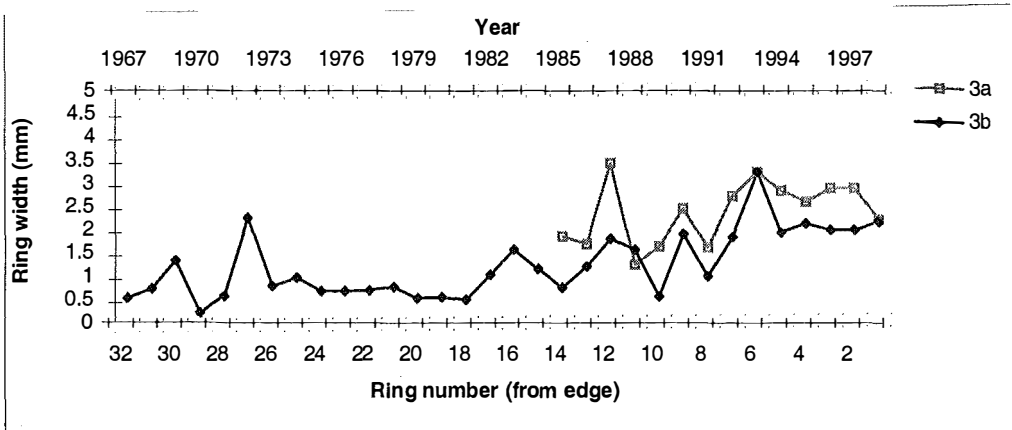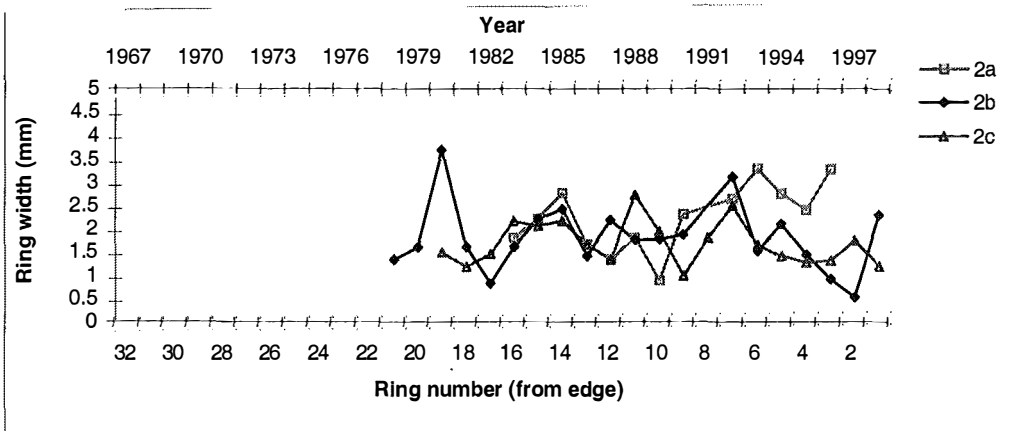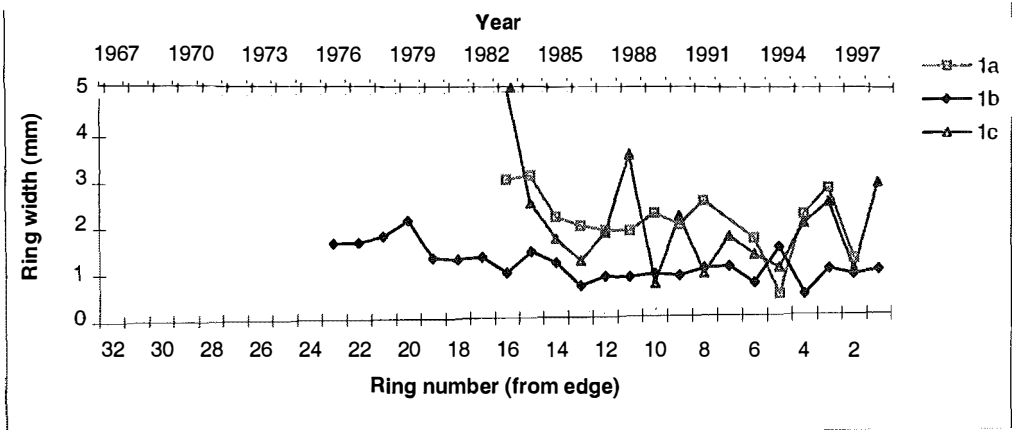
Transect 1, Banganup Lake

Transect 2, Banganup Lake

Transect 3, Banganup Lake

Graphs showing width (mm) measured of the rings contained in each core taken from *M. preissiana*

trees at Banganup Lake. Rings are numbered from the edge of the stem (lower X axis), and corresponding years matched (upper X-axis)
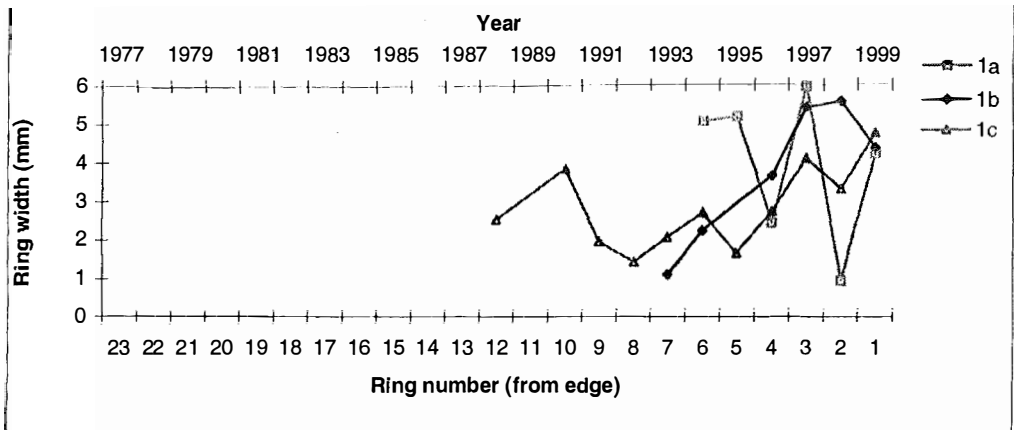
Transect 1, Thomsons Lake
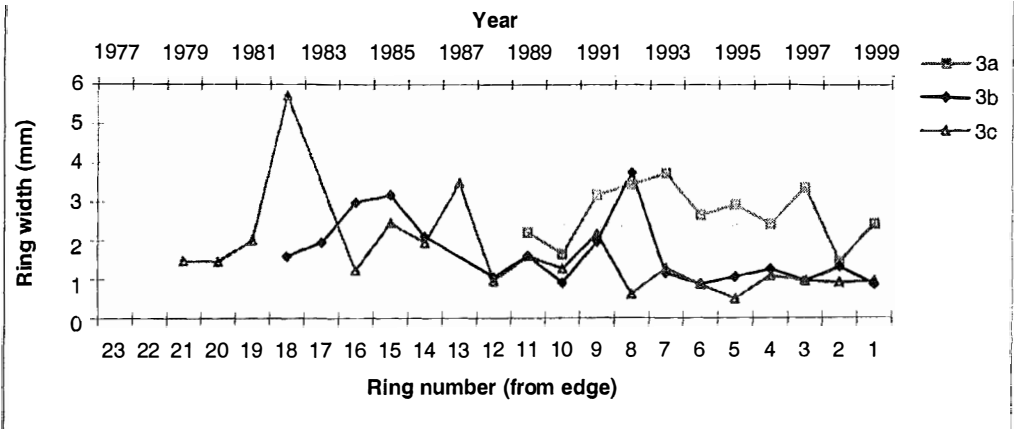
Transect 2, Thomsons Lake
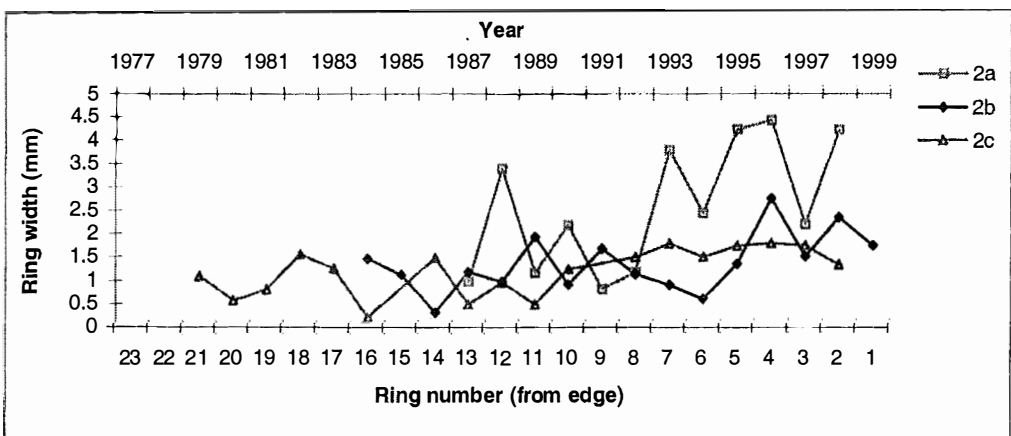
Transect 3, Thomsons Lake

Graphs showing width (mm) measured of the rings contained in each core taken from *M. preissiana* trees at Thomsons Lake. Rings are numbered from the edge of the stem (lower X-axis), and corresponding years matched (upper X-axis)
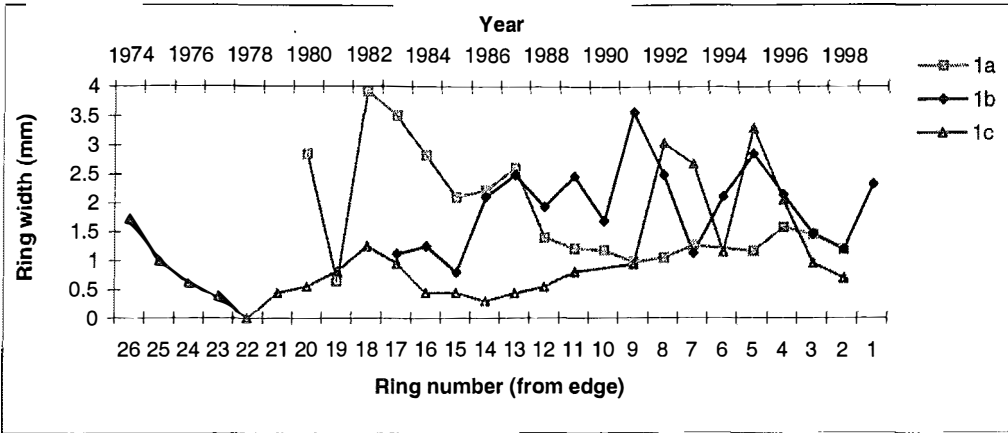
Transect 1, Lake Jandabup
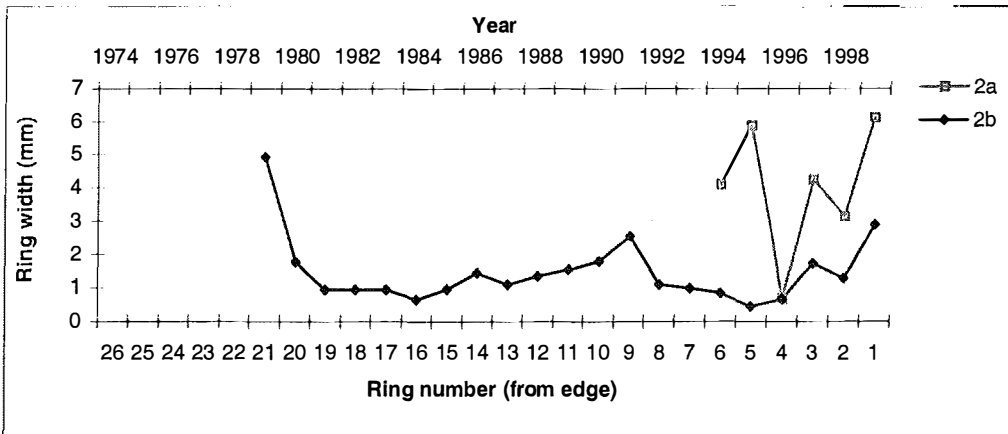
Transect 2, Lake Jandabup
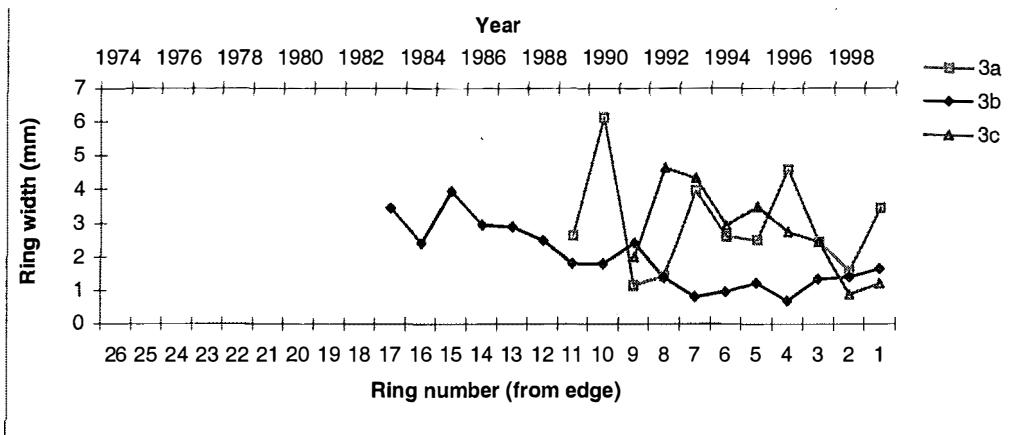
Transect 3, Lake Jandabup

Graphs showing width (mm) measured of the rings contained in each core taken from *M. preissiana* trees at Lake Jandabup. Rings are numbered from the edge of the stem (lower X-axis), and corresponding years matched (upper X-axis)

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APPENDIX 5: CORRELATION COEFFICIENTS BETWEEN TREE RING δ¹³C OF *M. PREISSIANA* TREES AND WATER AVAILABILITY PARAMETERS AT BANGANUP, THOMSONS AND JANDABUP LAKES.

Table showing results from Pearson's correlation coefficient (r), testing relationship between water availability variables at Banganup Lake, and tree ring $\delta^{13}C$ from corresponding years of trees sampled. Annual and summer (Dec-Feb) values tested. Bold print indicates a significant correlation ($p<0.05$).

ANNUAL

|	BL1a	BL1b	BL1c	BL2a	BL2b	BL2c	BL3a	BL3b
Mean GW	0.451	**0.465**	**0.595**	0.174	0.152	-0.232	0.399	0.257
Min GW	**0.575**	**0.516**	0.399	0.260	0.282	0.044	**0.499**	0.291
Max GW	0.000	**0.473**	**0.859**	0.254	0.130	-0.308	0.435	0.300
Mean SW	0.113	-0.143	0.232	0.129	0.152	0.414	0.495	0.129
Min SW	-0.148	-0.364	0.174	0.023	0.022	0.193	0.289	0.118
Max SW	0.345	0.205	0.377	0.300	0.175	0.303	**0.553**	-0.120
Total rainfall	-0.068	-0.023	-0.122	-0.342	-0.232	0.290	-0.234	-0.048
Number of raindays	0.004	0.059	-0.184	-0.137	-0.198	0.121	-0.176	-0.195
Mean max temperature	0.061	-0.280	0.255	0.243	-0.054	-0.213	-0.088	0.047
Mean humidity (3pm)	-0.332	-0.369	0.304	-0.147	-0.391	-0.056	-0.047	-0.333
Water Availability Index	0.035	-0.022	0.266	0.121	-0.066	**0.495**	0.053	0.252

SUMMER

	BL1a	BL1b	BL1c	BL2a	BL2b	BL2c	BL3a	BL3b
Mean GW	0.261	-0.123	**0.614**	**0.442**	-0.017	-0.417	0.408	0.255
Min GW	0.274	-0.053	**0.668**	**0.528**	0.038	-0.268	**0.506**	**0.371**
Max GW	-0.299	-0.323	**0.510**	0.279	-0.126	**-0.586**	0.233	0.095
Mean SW	-0.191	-0.322	0.115	-0.081	0.128	0.202	0.364	0.251
Min SW	-0.260	-0.304	0.160	-0.102	0.091	0.119	0.311	0.083
Max SW	-0.138	-0.128	0.099	-0.054	0.150	0.233	0.364	0.095
Total rainfall	-0.100	0.220	-0.418	-0.118	0.042	0.068	0.197	0.073
Number of raindays	-0.108	0.183	-0.427	-0.136	-0.038	-0.179	0.045	0.018
Water Availability Index	0.223	-0.003	0.193	0.215	-0.048	**0.499**	0.037	0.263

Table showing results from Pearson's correlation coefficient (r), testing relationship between water availability variables at Thomsons Lake, and tree ring $\delta^{13}C$ from corresponding years of trees sampled. Annual and summer (Dec-Feb) values tested. Bold print indicates a significant correlation ($p<0.05$).

ANNUAL

	TL1a	TL1b	TL1c	TL2a	TL2b	TL2c	TL3a	TL3b	TL3c
Mean GW	-0.557	0.065	0.282	-0.264	0.000	-0.305	-0.285	0.222	0.195
Min GW	-0.532	0.149	0.406	-0.204	0.069	-0.159	-0.237	0.330	0.283
Max GW	-0.560	0.147	0.480	-0.089	0.067	-0.068	-0.128	**0.492**	0.377
Mean SW	-0.524	0.187	0.369	-0.102	0.191	-0.167	-0.190	0.465	0.403
Min SW	-0.731	-0.022	0.464	0.150	0.344	-0.013	-0.076	0.479	0.404
Max SW	-0.443	0.188	0.271	-0.245	-0.055	-0.242	-0.233	0.355	0.293
Total rainfall	**0.838**	-0.041	-0.250	**-0.582**	-0.018	-0.254	-0.079	-0.167	-0.095
Number of raindays	0.664	-0.361	0.075	**-0.629**	-0.104	-0.288	0.200	-0.200	-0.135
Mean max temperature	-0.254	0.044	-0.571	0.349	-0.056	0.275	-0.401	-0.413	-0.258
Mean humidity (3pm)	0.150	0.390	0.039	-0.440	0.310	-0.300	0.401	0.368	0.107
Water Availability Index	-0.681	-0.039	0.214	-0.304	0.235	**-0.463**	-0.022	0.189	0.054

SUMMER

	TL1a	TL1b	TL1c	TL2a	TL2b	TL2c	TL3a	TL3b	TL3c
Mean GW	-0.484	0.227	0.468	-0.026	0.215	0.150	-0.260	0.209	0.260
Min GW	-0.558	0.198	0.490	0.009	0.372	0.288	-0.240	0.402	0.295
Max GW	-0.416	0.270	0.458	0.055	0.145	0.129	-0.189	0.385	0.373
Mean SW	-0.642	0.184	0.489	0.184	0.357	0.005	-0.152	**0.510**	0.413
Min SW	-0.728	0.103	0.453	0.233	0.376	-0.001	-0.138	**0.521**	**0.458**
Max SW	-0.553	0.259	0.513	0.132	0.368	0.028	-0.169	0.476	0.322
Total rainfall	0.566	0.287	0.082	-0.291	-0.379	**-0.696**	0.323	0.282	0.059
Number of raindays	0.483	0.068	0.214	-0.342	-0.359	-0.404	**0.694**	0.268	0.043
Water Availability Index	**-0.892**	-0.129	0.250	-0.233	0.091	**-0.561**	0.246	0.391	0.156

Table showing results from Pearson's correlation coefficient (r), testing relationship between water availability variables at Lake Jandabup, and tree ring $\delta^{13}C$ from corresponding years of trees sampled. Annual and summer (Dec-Feb) values tested. Bold print indicates a significant correlation (p<0.05).

ANNUAL

	JL1a	JL1b	JL1c	JL2a	JL2b	JL3a	JL3b	JL3c
Mean GW	-0.076	0.462	0.245	-0.272	-0.178	-0.397	-0.400	**-0.709**
Min GW	-0.005	0.398	0.224	-0.376	-0.126	-0.497	**-0.571**	**-0.737**
Max GW	0.021	**0.553**	0.308	-0.034	-0.113	-0.220	-0.311	-0.658
Mean SW	0.218	0.344	-0.005	-0.320	-0.154	-0.255	-0.247	**-0.708**
Min SW	0.111	0.434	-0.137	-0.754	-0.060	-0.263	-0.353	-0.673
Max SW	0.230	0.300	0.052	-0.056	-0.130	-0.143	-0.152	-0.644
Total rainfall	0.148	-0.176	0.127	0.453	0.044	0.037	0.272	-0.167
Number of raindays	0.097	-0.271	0.086	0.055	0.025	-0.341	0.377	-0.314
Mean max temperature	-0.389	-0.115	**-0.435**	0.078	-0.323	**0.623**	0.286	0.635
Mean humidity (3pm)	0.320	-0.190	**0.160**	0.060	-0.020	**-0.660**	0.260	-0.590
Water Availability Index	0.410	-0.099	0.080	-0.292	0.145	**-0.797**	0.139	-0.357

SUMMER

	JL1a	JL1b	JL1c	JL2a	JL2b	JL3a	JL3b	JL3c
Mean GW	**-0.542**	**0.555**	0.222	-0.541	-0.329	-0.454	**-0.582**	0.276
Min GW	**-0.528**	**0.612**	0.240	-0.472	-0.273	-0.381	**-0.602**	0.352
Max GW	**-0.553**	**0.516**	0.185	-0.579	**-0.403**	-0.494	**-0.537**	0.232
Mean SW	0.104	0.098	-0.123	-0.608	-0.226	-0.284	-0.344	-0.602
Min SW	0.040	0.249	-0.095	-0.654	-0.170	-0.278	-0.353	-0.568
Max SW	0.123	0.008	-0.173	-0.687	-0.250	-0.296	-0.269	-0.573
Total rainfall	0.149	-0.004	0.155	**0.819**	0.070	-0.393	-0.007	-0.605
Number of raindays	-0.044	0.071	0.341	0.471	0.103	-0.277	-0.013	-0.096
Water Availability Index	0.346	-0.340	0.140	0.754	0.100	-0.014	0.324	-0.042