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# **Geohydrological characteristics of Table Mountain Group aquifer-fed seeps and the plant ecophysiological consequences**

by  
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BSc (Hons)

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Master of Science

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## General Introduction, thesis layout and background

Fynbos, the native vegetation of the Western Cape of Southern Africa experiences a mild, Mediterranean type climate with hot dry summers and cool wet winters. In terms of climate, fynbos is comparable with other Mediterranean systems found around the Mediterranean in Europe, in parts of Chile, south-western Australia and in the Chaparral in California (Aschmann, 1973). The Cape Floristic Region, of which fynbos is part, is one of the world's most botanically diverse regions, home to an estimated 9030 vascular species (Goldblatt, 1978; Goldblatt and Manning, 2002). The region has exceptionally high levels of endemism. Almost 69% of its 8920 species of flowering plants are endemic (Goldblatt and Manning, 2002), and, despite its small area, it is regarded as one of the six global plant kingdoms (Takhtajan, 1986). Ericaceae, Iridaceae, Proteaceae and the Restionaceae are well represented and there are a number of families that are endemic or nearly so (Goldblatt and Manning, 2002). The largest is the Penaeaceae, followed by Grubbiaceae, Roridulaceae and Geissolomataceae, which together contain 15 endemic genera (Goldblatt and Manning, 2002). These families are almost without exception evergreen sclerophyllous shrubs and are thought to be palaeoendemic remnants from an ancient temperate flora, when conditions were cooler and wetter (February *et al.*, 2004). As a result, many of these species are restricted to wetter areas such as wetlands and mountain seeps (February *et al.*, 2004). Many of these seeps, as well as other groundwater-fed ecosystems, are likely to be connected to the Table Mountain Group (TMG) aquifer from which the city of Cape Town may begin to abstract water.

Cape Town's population growth rate is expected to decline, nonetheless our current population of approximately 3.2 million may increase to 4.2 million by 2021 (Romanofsky, 2006). Until now, dams have been built to meet expanding water demand. The Berg River Dam is expected to be completed by December 2007, however, after that, there are no more major rivers left to dam. The need to find an alternative water supply has already been recognised. In 1994 a summary was compiled where all options open to Cape Town were considered (Carter and Little, 1994). Abstraction from the TMG aquifer was recognised as a possibility, but dismissed on the basis that groundwater from the aquifer was thought to provide some  $300 \times 10^6 \text{m}^3/\text{a}$  of baseflow to the rivers in the Cape (Carter and Little, 1994). It was thought unlikely that much water could be mined from the aquifer without a similar reduction in surface flow. The costs of aquifer exploitation were considered high relative to the cost of exploiting the same water as surface water and large scale exploitation was therefore not considered viable (Carter and Little, 1994). Recognising the need to have an integrated approach to water resource planning, incorporating demand management as well as the augmentation of supply, the City published a report in 2002 condensing the findings of a number of prior studies (City of Cape Town, 2002). This study compared the pros and cons of eight water demand management and nine supply augmentation options, including abstraction from the TMG aquifer. Aside from the promotion of private boreholes, abstraction from the TMG scored the lowest with regards to the impact that it is likely to have on the environment (City of Cape Town, 2002). However, the fact that large amounts of water could be abstracted from the aquifer relatively cheaply using existing technologies meant that it was scored 8<sup>th</sup> out of 17 overall (City of Cape Town, 2002).

The CMA Bulk Water Supply Study (2001- 2002) followed from the IWRP study and recommended investigating three options – the Voëlvlei Augmentation Scheme, desalination and the TMG aquifer - in more detail. The TMG Feasibility Study and Pilot Project started in 2002. It is a five-year project that aims to determine the feasibility of using the TMG aquifer as a water source to augment Cape Town's water supply (WRC, 2005). This thesis forms part of an associated study being carried out by the CSIR looking at the ecological impacts of abstraction from this aquifer.

### **The Table Mountain Group (TMG) aquifer**

The TMG is the lower most of the three groups of which the Cape Supergroup is comprised (Fig I.). The sediments that form the TMG accumulated over a relatively short time (over roughly 170my between the Early Ordovician and the Early Devonian (Tankard *et al.*, 1982), in a 'trough' resulting from the subsidence of the basement rock (Vos and Tankard, 1981). The TMG is mostly comprised of quartzitic sandstone which erodes to give rise to the nutrient poor soils characteristic of fynbos (Goldblatt, 1978).

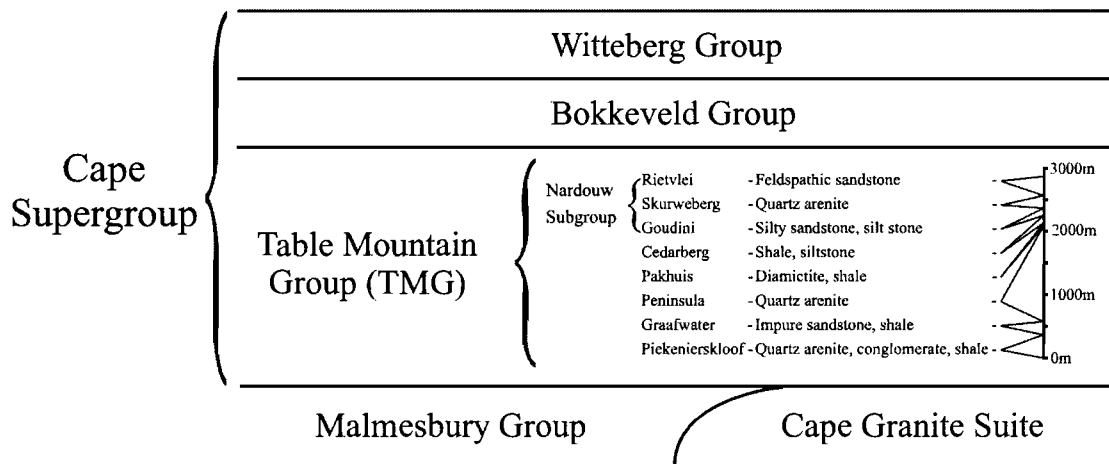


Fig I. Stratigraphic column for the TMG showing its orientation relative to other groups found in the Cape and the lithology of its different formations (Theron and Thamm, 1990; de Beer, 2002). The thickness of each of its formations is indicated.

Metamorphosed quartzite sandstone itself is relatively impervious to water, so the TMG has a very low primary porosity. The sandstones making up the TMG are, however, brittle and fracture readily (Rosewarne, 2002). Between 280 and 235mya the South American and African continents collided to form part of Pangaea, giving rise to the Cape Fold Mountains (Lock, 1980). The associated compressional forces resulted in widespread deep fracturing throughout the TMG with larger faults, or ‘hydrotects’, running for tens of kilometres (Hartnady and Hay, 2002).

An ‘aquifer’ is defined as ‘a geological formation which has structures or textures that hold water or permit appreciable water movement through them’ (National Water Act, 1998). Groundwater in the TMG aquifer resides within fractures in an otherwise impermeable sandstone matrix, and is therefore termed a ‘secondary aquifer’. An aquifer is classified as being confined or unconfined depending on whether or not there is a less permeable confining layer between the water table and the Earth’s surface (Parsons, 2004). The TMG aquifer is classified as being ‘semi-confined’ as while it is unconfined in some areas (*i.e.* the TMG is on the surface and the surface of

the aquifer is represented by the local water table) in other areas it is confined below an impermeable layer. Bulk abstraction from the TMG aquifer will likely involve drilling through a confining layer into the confined layers of the aquifer beneath. This may result in artesian flow, initially reducing the cost of pumping. Yields of 100 litres per second are proposed for successful production boreholes, which may extend as deep as 1000m (City of Cape Town, 2006).

The formations making up the TMG differ widely in their ability to conduct water. Interspersed between the Nardouw, Peninsula and Piekenierskloof formations which are comprised predominantly of sandstone and make up the bulk of the TMG are two layers of shale: the Cedarberg/Pakhuis formation and the Graafwater formation. These shale layers act, to some degree, as confining layers or aquitards and split the TMG aquifer into three: The Piekenierskloof, the Peninsula and the Nardouw aquifers. While productive wells have been sunk into the Piekenierskloof aquifer, it is relatively thin and has a limited geographic distribution (Rosewarne and Weaver, 2002). The Peninsula aquifer is thought to have a greater potential for bulk abstraction than the Nardouw aquifer (Rosewarne and Weaver, 2002). It is much thicker (see fig 1) but, more importantly, has a lower shale content and faults should therefore remain open to great depths (Rosewarne and Weaver, 2002). The Peninsula formation usually outcrops at higher altitudes and is separated from the Nardouw by the Cedarberg/Pakhuis aquitard. The Cedarberg/ Pakhuis aquitard above the Peninsula formation is an important marker horizon, with the Cedarberg shale eroding to form gentle, nutrient-rich slopes that often support visibly distinct plant communities. The aquitard is not always continuous, and the Nardouw Formation can come into contact with the Peninsula along fault lines (see example in fig 1.2 in Chapter 1). Drawdown

associated with abstraction from, for example, the Peninsula formation, may therefore occur also within the Nardouw - the two groups should not be assumed to be operating as distinct hydrological units in all locations.

There are a number of characteristics that make the TMG aquifer an attractive option to the City's developers.

- Large volumes of water could potentially be abstracted relatively cheaply from confined sections of the aquifer where boreholes may have artesian flow.
- The quartzites that form the bulk of the TMG are inert and release very low levels of dissolved ions when they weather. Water within the TMG aquifer is therefore of an exceptionally high quality, with levels of dissolved solutes amongst the lowest recorded in South Africa (Rosewarne, 2002).
- The TMG aquifer is massive, being exposed along the whole length of the Cape Fold Mountains in the high orographic rainfall areas (Fig II.) and extending to great depths (Fig I).

The nature of the aquifer makes for difficulties in predicting where the impacts of abstraction will occur. Where it is a confined aquifer, impacts are unlikely to manifest anywhere near the point where abstraction is occurring. Surficial impacts - as opposed to those on ecosystems within the aquifer itself - may occur a great distance away, where connected portions of the aquifer are no longer confined and intercept the surface. The fact that the aquifer is a secondary aquifer confounds the problem. It is not yet possible to determine the transmissivity and interconnectedness of deeper faults and fractures. It will therefore be unknown whether draw-down associated with

abstraction will be confined along a single fault for a great distance or will occur along a number of faults over a smaller distance. There is also likely to be a significant time lag between when abstraction begins and when impacts occur. It took seven years before aquifer-fed springs dried up in the Kammanasie Nature reserve following relatively small abstraction from the TMG aquifer (Cleaver *et al.*, 2003). It is therefore unlikely that the cessation of abstraction will be able to halt impacts once they begin to occur. The 'let's pump and see what happens approach' is perhaps unavoidable considering the complexity of the aquifer. Nonetheless it is cause for concern.

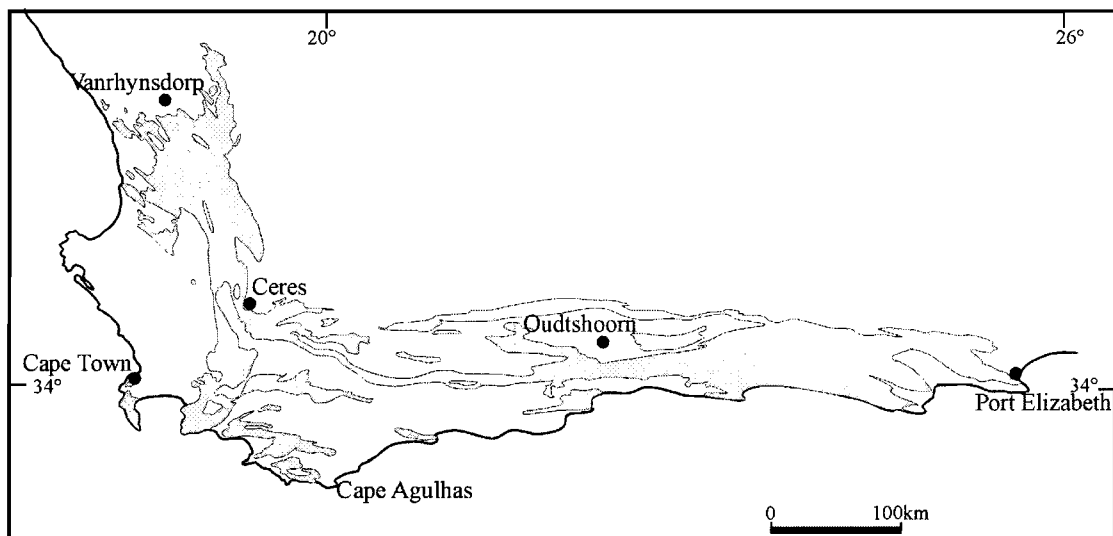


Fig II. The Distribution of exposed TMG (from de Beer (2002))

## **Ecosystems dependent on the TMG aquifer**

Any ecosystem containing organisms that utilize groundwater can be described as having some degree of groundwater dependence. Le Maitre *et al.* (2002) identified three main terrestrial ecosystem types thought to have some degree of dependence on the TMG aquifer and therefore likely to be impacted upon by the declines in water levels associated with abstraction, namely:

- Mountain seeps, springs and wetlands;
- Riparian habitats and
- Bottomland wetlands.

These areas of 'localised' groundwater dependence are usually fairly obvious - the vegetation tends to be taller and denser than the surrounds (Cronk and Fennessy, 2001). These systems occur in areas where the aquifer is unconfined and local topography brings the level of the water table (*i.e.* the surface of the aquifer) close enough to the ground surface for it to be utilisable by terrestrial ecosystems (Parsons, 2004). Over time this results in a community shift towards riparian, wetland or seep plant communities (Cronk and Fennessy, 2001). Such localised groundwater-fed ecosystems are likely to have distinct ecotones, as phreatophytic species are unable to survive the levels of water stress experienced by 'dryland' fynbos species (Cronk and Fennessy, 2001; Jacobsen *et al.*, 2007), and fynbos species are unable to survive in water-logged soils or are out-competed by species adapted to an increased supply of water in the wet environment (Cronk and Fennessy, 2001). Terrestrial aquifer-fed systems will occur on top of some subsurface heterogeneity, such as a fracture or a fault or at a contact between the TMG and a less permeable layer (Le Maitre *et al.*,

2002). Seeps, small wetlands found on the side of mountains that are not connected to a river, may occur on faults or on contacts in locations that appear to make no hydrological sense, such as on top of a mound or downhill from a very small catchment (personal observation).

Two other, less obvious TMG aquifer-fed ecosystems have been identified (Le Maitre *et al.*, 2002):

- those in caves and other underground systems and
- those occurring at zones where freshwater discharges into the ocean.

Sharrat *et al.*, (2000) carried out faunal surveys in randomly chosen caves on the Cape Peninsula. Though the overall number of cavernicolous species found (85 species in 80 caves) was low by world standards, the caves are by far the most faunistically diverse in South Africa (Sharrat *et al.*, 2000). Twenty-one of the 85 species recorded are thought to be endemic and, interestingly, the cave fauna of the Cape Peninsula was found to be more similar to South American and Australasian cave fauna than to that of other caves in Africa (Sharrat *et al.*, 2000). The authors therefore propose that Cape Peninsula cave fauna is biased towards Gondwanan relicts and Cape Peninsula Caves should be considered conservation hotspots (Sharrat *et al.*, 2000). The caves in this study are formed in the Peninsula formation of the TMG and have relatively constant high humidities. Though not all of the fauna living in these caves are directly dependent on groundwater, they are thought to be extremely sensitive to disturbance with a reduction in groundwater supply having been identified as a major threat (Sharrat *et al.*, 2000).

There may also be life living within the TMG aquifer itself; stygobiont is defined as “free living taxa which are exclusively found, or almost so, in all their developmental stages in one or more subterranean aquatic habitats” (Botosaneanu, 1986). The stygal biotope tends to be obscure, subject to smaller variations in temperature etc. that occur later than they do in neighbouring surface habitats and they tend to be impoverished with regards to trophic resources (Botosaneanu, 1986). Nonetheless, stygofauna are usually extremely diverse and aquifers have been described as “living museums” as they often house a sample of lineages that comprised the faunas from various geological periods (Humphries, 2006). Though stygofauna have been found in fractured rock aquifers in Pilbara, Australia, (Humphreys, 2006) no work has been carried out on the stygofauna associated with the TMG aquifer and any invertebrates found when abstracting water should be preserved for study.

The discharge of freshwater from the TMG aquifer into the ocean is likely to set up a number of physical gradients, which may influence marine flora and fauna. A biological survey carried out where an unconfined aquifer was discharging into the ocean found a definite relationship between biological zonation and groundwater discharge - a number of species known to only be able to survive in brackish water had replaced marine forms in the less saline waters near shore (Kohout and Kolipinski, 1967). The input of oxygenated water across sediment surfaces has also been shown to prevent them from becoming reducing where they would be home to just anaerobic or microaerophilic organisms (Riedl *et al.*, 1972). The input of nutrients into marine systems via submarine groundwater discharge can be several times as high as that from river runoff (Johannes, 1980). However, levels of nitrate and phosphate in the TMG aquifer groundwater are extremely low, with pollution

explaining their isolated occurrence (Smart and Tredoux, 2002). Groundwater discharge from unconfined aquifers into the sea usually occurs close to shore, as a salt water 'wedge' is likely to intrude beneath less dense freshwater and impede the downward mixing. Obviously, these systems are difficult to study yet warrant investigation.

## **Thesis aims and layout**

Little is known of the hydrological functioning of groundwater dependent ecosystems in fynbos. I aimed to characterise the hydrological functioning of TMG aquifer seep habitats, and examine the ecophysiological consequences on seep species. There are three data chapters in this thesis, each of which falls into a different scientific discipline.

Chapter 1 deals with geohydrology. I aimed to get a better understanding of the hydrological character of TMG aquifer-fed seeps and assess the use of stable isotopes and water level measurements as diagnostic tools in their identification. Fluctuations in groundwater levels and stable isotope composition were measured in three seeps thought to dependent on the TMG aquifer and a perched seep thought to be disconnected from the aquifer over both the long and short term.

Chapter 2 deals with ecohydrology. It aims to determine how the hydrological character of TMG aquifer-fed seeps influences the levels of plant water stress (xylem pressure potentials) of seep plants throughout a summer. Stable isotopes were also

used to determine from what depth seep plants source their water and their use as a tool to identify TMG aquifer-fed seeps is assessed.

Chapter 3 deals with ecophysiology, in particular the sensitivity of plant species to increases in xylem pressure potentials. There may be a relationship between water availability and drought tolerance in fynbos, i.e. permanently wet areas may support a suite of species that are particularly sensitive to drought stress. If particularly vulnerable seep species can be identified then their distribution may be of use in mapping the occurrence of permanently wet areas likely to be connected to the TMG aquifer. In this chapter I aimed to identify seep species that have xylem that is particularly vulnerable to water stress induced failure. I also assess the predictive capacity of more easily measured physiological correlates with plant adaptation to water stress that may have potential in screening a wider range of fynbos seep species.

# Chapter 1: Variation in water levels and the stable isotope ratios of mountain seep groundwater throughout a summer and a single rainfall event

## Abstract

In this chapter I aim to get a better understanding of the hydrological character of TMG aquifer-fed seeps and assess the use of stable isotopes and water level measurements as diagnostic tools in their identification. Rain and groundwater samples were collected for isotope analysis ( $\delta^2\text{H}$ ) and fluctuations in the water table levels were measured throughout the length of a summer for 4 seeps in the Kogelberg, Western Cape, South Africa. The aquifer-fed seeps had differing amounts of variation in the  $\delta^2\text{H}$  composition of their groundwater with some of them tracking the seasonal isotopic enrichment of rainfall in the area. Stable isotope ratios have potential to be used in hydrological studies involving flow path lengths and water residence times. The variable amounts of long term isotopic variation in aquifer-fed seep groundwater, together with the fact that when samples of groundwater could be collected from the perched seep they had a stable isotope composition within a similar range, perhaps limit the use of long term monitoring of stable isotope ratios as a diagnostic tool.

The groundwater from the perched seep and two aquifer-fed seeps were sampled before and after a rainfall event. It was anticipated that the perched seep would assume the isotopic signal of the rain falling within its catchment. However, this was not the case, suggesting that the seep was in fact connected to a large body of water.

Sampling isotopes throughout a rainfall event also appears, therefore, to be of limited use.

Monitoring water levels appeared to have potential as a tool to identify aquifer-fed systems. All four of the aquifer-fed systems remained permanently wet throughout the summer, while the perched system dried up. The water levels of aquifer-fed seeps were also found to be much less responsive to the input of water from a rain event than those of a perched system. A piece of equipment (the ASTON) was designed to sample the ascending limb of a seep's water level during a rainfall event. ASTON's could also be used over the longer term, installed in mid-summer and collected after the rainy season to determine the range in water levels in seeps over a longer time period.

## Introduction

The TMG aquifer is a secondary aquifer with water being stored within, and moving through, extensive deep fractures that have formed in the brittle, otherwise impermeable sandstone. The TMG sandstones are resistant to weathering and form the backbone of the Cape Fold Mountains. The Cape Fold Mountains form huge catchment areas draining into major river systems. Dams on these rivers supply much the south western part of South Africa with water. The Cape Fold Mountains have largely escaped development and are protected areas. The flora of these mountains is exceptionally diverse, housing much of the Cape Floral Kingdom, home to 9000 vascular plants of which 69% are endemic (Goldblatt and Manning, 2000). In the mountains of the Cape it is common to find groundwater-fed wetlands that are not connected to rivers, termed 'seeps'. Mountain seeps are potentially at risk from abstraction from the TMG aquifer (Le Maitre *et al.*, 2002). The aim of this chapter is to assess the potential of two techniques - water levels and stable isotopes - to identify seeps dependent on the TMG aquifer. Seeps were monitored over both the long and the short term to determine which sampling approach was more effective. It is hoped that this information will be useful in the identification of ecosystems that may be threatened by abstraction.

It is important to recognize that not all of subsurface water is groundwater - it is just one of the three components, along with soil-water and capillary water. The term 'soil-water' refers to the water found between the ground surface and the water table (Whitten and Brooks, 1972). Soil-water is bound by matric forces in air-filled pore spaces in the unsaturated 'vadose' zone. If partial saturation increases until field

capacity is reached, and gravity is able to overcome the matric forces soil-water will infiltrate deeper. Below the vadose zone will either be an underlying impermeable layer or the capillary zone bordering the saturated zone. The capillary zone contains 'capillary water', which is drawn upwards by capillary forces of individual soil or rock particles from the saturated zone below. The boundary between the capillary zone and the saturated zone is termed the 'water table', the level below which soil is saturated with water (Whitten and Brooks, 1972). Water in the saturated zone, below the water table, is termed 'groundwater'. Groundwater is the component of subsurface water that can be abstracted using well fields and that may also feed rivers, wetlands and seeps.

There are two broad categories of groundwater-fed seeps in the sandstone mountains of the Western Cape. Firstly, there are those that receive groundwater supply from the regional water table. In ecosystems residing on top of exposed TMG, this is referred to as the TMG aquifer. These systems are generally at a low altitude relative to the local topography and are often on top of, or running along, geological inconsistencies such as faults or contacts (Le Maitre *et al.*, 2002). Secondly there are perched systems which are at too high an altitude to be fed by the regional water table, suspended above it and totally dependent on rain falling within their catchments (Parsons, 2004). While the water in these systems may gradually run down and seep through mountains and recharge the regional water table, they are not affected by the height of the regional water table and should therefore not be at risk from draw-down associated with abstraction. In this chapter I examine variation in water levels and stable isotope ratios for perched and aquifer-fed seeps. I do this throughout a summer as well as within a single rainfall event. The aim of this chapter is to develop a better

understanding of how hydrological characteristics of these two categories of groundwater-fed ecosystems differ. It is hoped that this information will be useful in identifying, and monitoring seeps which are likely to be the most sensitive to draw-down associated with abstraction (Parsons, 2004).

### **Variation throughout a summer**

The South Western Cape experiences a typical Mediterranean climate, with cool wet winters and hot dry summers (SAWB, 1996). Perched groundwater-fed systems which are dependent solely on rainfall in their catchments may 'dry up' in the dry summer months, as they are not connected to a large enough body of water to provide them with a permanent supply of water. Systems connected to the TMG aquifer may be provided with a constant supply of water due to the large size of the body of groundwater to which they are connected, and may therefore be less variable remaining permanently wet.

Water within the TMG aquifer originates as precipitation in rainfall events that vary in their stable isotope composition (Gat, 1982; Harris *et al.*, 1999). The small amount of available data regarding the hydraulic properties of the TMG aquifer measured the hydraulic conductivity of the Peninsula and Skurweberg formations as being less than 100 m per annum (Rosewarne, 2002). Slow movement through cracks in the TMG sandstones could result in a mixing of the water within the aquifer and a 'smoothing out' of the variation associated with different events (Midgley *et al.*, 2001). If this is the case, and water in the TMG aquifer is well mixed (Midgley *et al.*, 2001), then we would expect the water discharging from systems fed exclusively by the TMG aquifer

to have small amounts of isotopic variation. In contrast, a perched system dependent solely on water derived from local precipitation moving through shorter flow paths before it discharges, is likely to be less well mixed and have greater temporal variation in the stable isotope ratios of its groundwater.

### **Variation throughout a single rainfall event**

The input of water into a hydrological system is likely to raise the level of water in groundwater-fed ecosystems at the base of catchments. Perched seeps are likely to be connected to relatively small bodies of groundwater compared to aquifer-fed seeps (Fig. 1.1). Though they have smaller catchments, the amount of rainfall that falls into a perched seeps catchment will be likely be relatively large compared to the body of groundwater. Though aquifer-fed seeps may have larger catchments, the amount of water that enters the aquifer during a rainfall event is likely to be insignificant in relation to the amount of groundwater already within it. I hypothesised that this would result in a greater rise in the water level of a perched seep than in an aquifer-fed seep throughout a rainfall event.

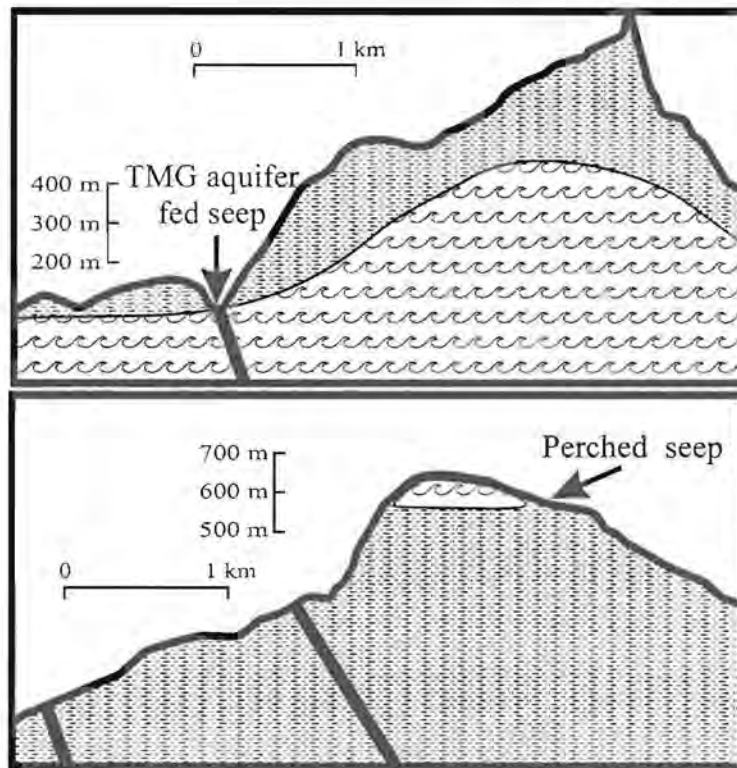


Fig. 1.1 Schematic depicting differences in the size of groundwater body to which perched and aquifer-fed seeps are connected.

Apart from differing in the degree to which water levels are affected, perched systems may also differ in the source of this water. A study using  $\delta^2\text{H}$  of rainwater conducted by Midgley and Scott (1994) found that less than 5% of stormflow in a river after a rainfall event was comprised of direct runoff. They suggested that storm flow from a single event was comprised of displaced groundwater already within the system. A rise in water levels in a system that is connected to the TMG aquifer is likely to be made up of water already within the system, lifted through an increase in hydraulic pressure. While this may also be the case in perched systems, the fact that they are connected to much smaller bodies of groundwater may result in antecedent rainfall making up a relatively large portion of the total amount of water. If this is the case, then the stable isotopic composition of groundwater in an aquifer-fed seep should

remain unchanged, while the isotopic composition of groundwater in a perched seep will move towards that of the antecedent rainfall.

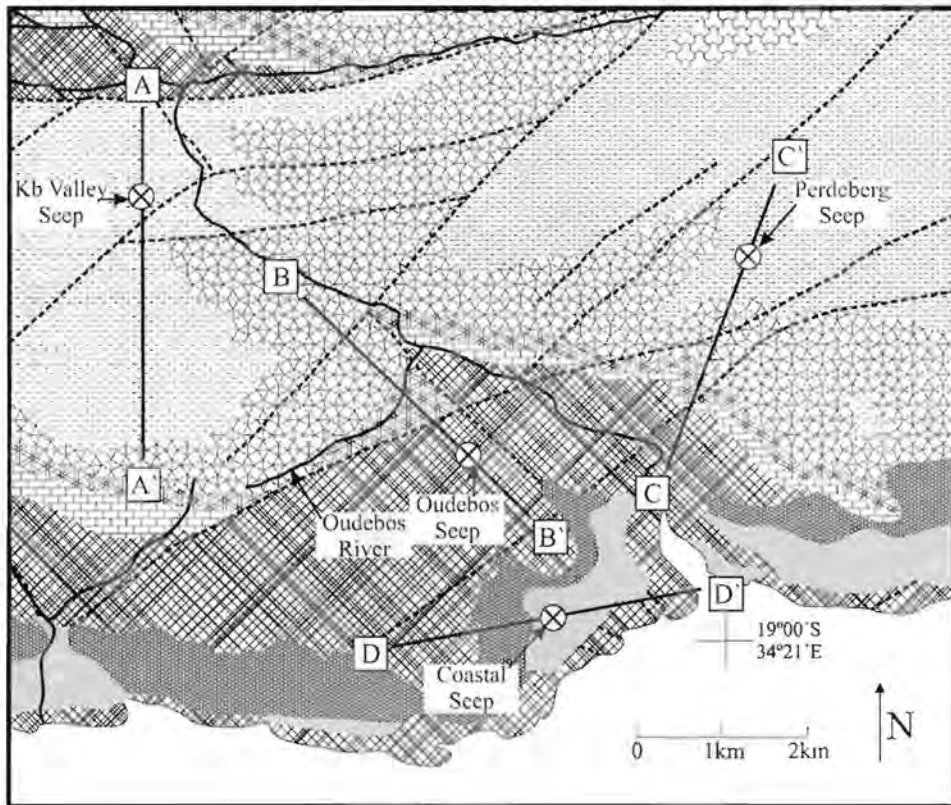
Ecosystems connected to the TMG aquifer are connected to a much larger body of groundwater than those connected to smaller perched groundwater bodies. I hypothesised that this would result in perched and aquifer-fed systems having differing fluctuations in both their water levels and stable isotope ratios of their groundwater over the long term and throughout a single rainfall event.

## Methods

### Study Area

The seeps involved in this study were found within, or near to, the Kogelberg Biosphere Reserve (34° 17' S; 18° 56' E), the so called 'Heart of the Fynbos Kingdom' on the Cape south coast approximately 100km east of Cape Town. Much of the southern Kogelberg is made up of exposed TMG sandstones which are extensively faulted, with some of the larger fault structures running inland for tens of kilometres. There is also extensive unmapped 'small-scale' fracturing that is likely to determine the position of the many low altitude seeps dotted across the landscape. As the area is very mountainous, with the higher slopes receiving large amounts of rainfall (the nearby Nuweberg at an altitude of 560m receives 1,485mm pa (Cape Nature Conservation, 1992)), there are also likely to be many perched seeps. The area therefore provides one with an excellent opportunity to study the differences between these systems. Four seeps were chosen that, on the basis of their topographic setting - in particular their altitude and proximity to fault lines - had varying levels of dependence on discharge from the TMG aquifer. Three of the seeps were at accessible, relatively low altitudes and were thought to be aquifer-fed (the Kogelberg Valley seep, the Oudebos seep and the Coastal seep). Another seep at too high an altitude for it to be aquifer-fed was chosen as an example of a perched seep (Perdeberg seep). Ideally more perched seeps would have been sampled but they tend to be inaccessible and manpower was limited. Fluctuations in water levels and groundwater stable isotope composition of these seeps were monitored throughout the length of a summer to determine if seeps fed by the TMG aquifer had a more reliable

supply of well-mixed water. Three of the seeps were monitored and sampled throughout a rainfall event to determine if water levels of the perched seep were more responsive than those connected to the TMG aquifer and whether the rise in groundwater was made up of antecedent rainfall.



- |  |                      |                               |
|--|----------------------|-------------------------------|
|  | Rietvlei formation   | } Nardouw (secondary aquifer) |
|  | Skurweberg formation |                               |
|  | Goudini formation    |                               |
|  | Cedarberg formation  | } aquitards                   |
|  | Pakhuis formation    |                               |
|  | Peninsula formation  | - (secondary aquifer)         |
|  | Sand                 |                               |
|  | Scree                |                               |

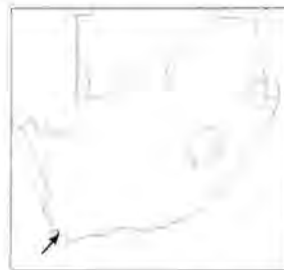


Fig 1.2 The geological context of the four studied groundwater-fed seeps and the Oudebos river. Solid lines labelled with capital letters are the lines along which the cross sections depicted in Fig. 1.3 are taken. Dashed lines represent large scale fault structures and solid lines rivers.

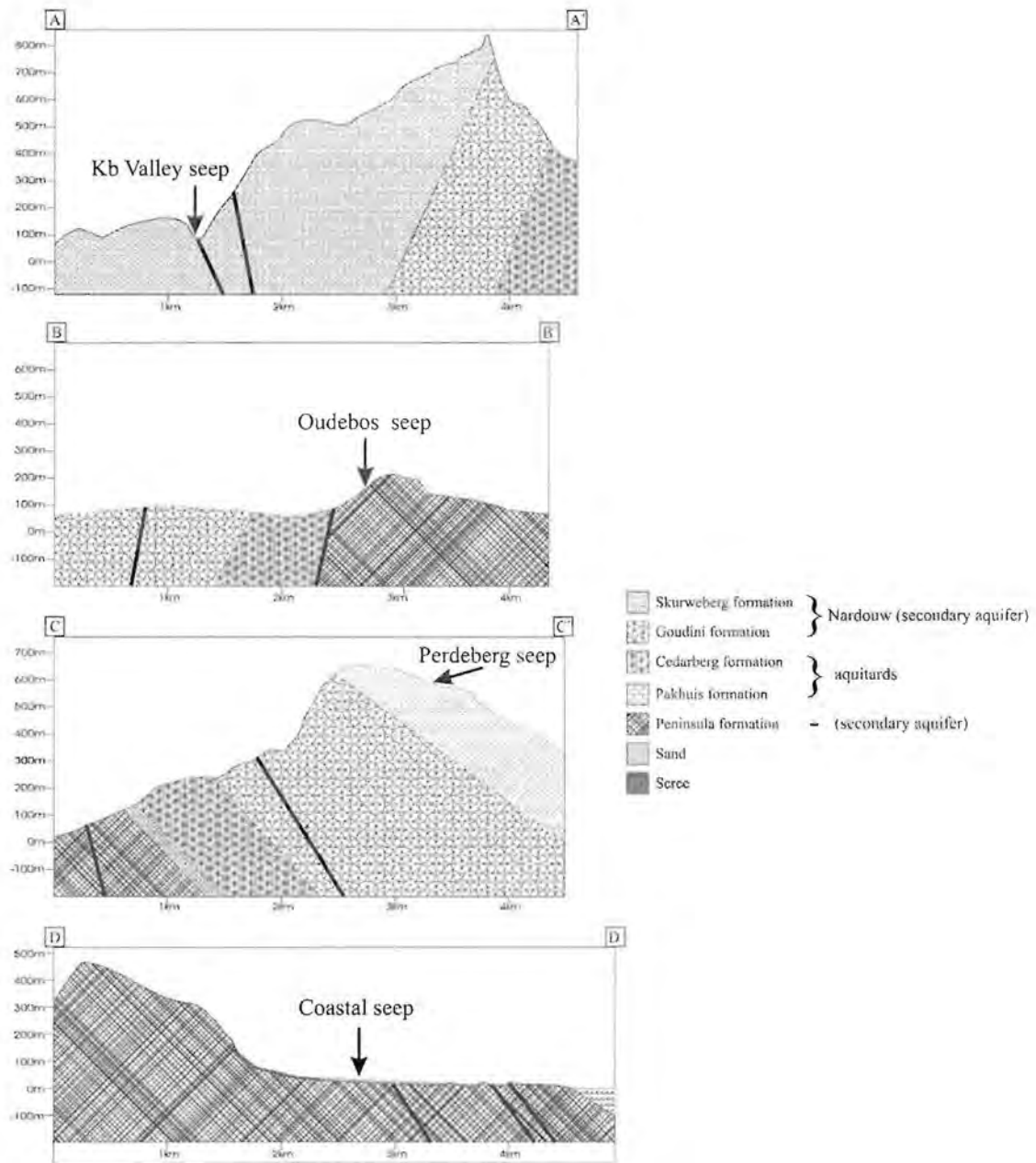


Fig 1.3 Schematic cross sections along lines labelled in Fig.1.2. The positions of the four seeps are depicted with arrows. Solid lines indicate mapped faults.

Kogelberg valley seep (34°18.203'S, 18°56.185'E) (altitude of approximately 180m). The Kb Valley seep sits on a steep southern slope of a river valley at the base of a fault- controlled gorge cutting through a mountain comprised of TMG sandstones in the Skurweberg formation. The seep is situated in such a way that appears to make little hydrological sense (sitting on a mound on a steep slope), is located very close to a large fault structure and is at a relatively low altitude which suggests that the system is dependent on the TMG aquifer.

Oudebos seeps (34°20.010"S, 18°58.321"E) (approx 220m). The 'Oud seep' is on the northern slope of a mountain comprised of TMG sandstones in the Peninsula formation. Although this seep is not near to the major fault lines that have been mapped in the area, it appears to be sitting on top of a smaller fracture (John Weaver, CSIR, personal communication). The seep is also at a relatively low altitude and has a strange hydro-geomorphological setting with almost no catchment which suggests that the system may be dependent on the TMG aquifer.

Coastal seep (34°20.846'S, 18°58.729'E approx 40m). The 'Co seep' sits on a sandy coastal plain lying on top of TMG sandstones in the Peninsula formation, just south of the Kogelberg Biosphere Reserve. The depth to bedrock across the sand plain is variable, ranging from 4.12m for a hole augured off the seep to 1.53m on the seep. The seep is part of a chain of wetlands that run parallel to the coast just below a sand shelf, where the surface drops closer to the water table. Faults mapped in the sandstone outcrops near the ocean (Fig. 1.2) will extend through the bedrock beneath the layer of sand on the coastal plain. This close proximity to fault structures together with the sites low altitude, suggests that the seep is dependent upon the TMG aquifer.

Perdeberg seep (34°18.512'S, 18°59.957'E) (approx 600m). The 'Perdeberg seep' is on a northern slope near the top of the Perdeberg, a mountain comprised of exposed TMG in the Skurweberg formation. While it is likely that rainfall at this site will contribute to aquifer recharge, the site is at too high an altitude in relation to the local topography to be connected to the TMG aquifer, and is therefore an example of a perched system unlikely to be affected by abstraction.

### **Rainfall sampling**

Longer term integrated sampling of rainfall was carried out by leaving 10l rainfall collectors out close to all of the seeps from 14/09/04 to the 05/08/05. A folded section of nylon mesh was placed in the funnel to prevent insects and leaves from falling into the collector, and a layer of silicon oil at least a centimetre thick minimised evaporation of the collected rainfall (Weaver, 1992). A syringe was used to extract water samples for stable isotope analysis from underneath the layer of silicon oil in the collectors, with a new syringe being used for each sample. A separating flask was used to remove the water from beneath the silicone oil and the amount of water in each rain collector was measured using a graduated beaker. The rainfall collectors were replaced four times, every two and half months, to determine if there was any seasonal variation in the  $\delta^2\text{H}$  of rainfall. The amount of rainfall increases (Midgley, 1994) and becomes isotopically lighter with altitude (Dansgaard, 1964). I assumed therefore that the rainfall collected in the cumulative rainfall collectors would be isotopically heavier than that falling higher in their catchments.

A correction needed to be made in order to compare the  $\delta^2\text{H}$  groundwater signal of the different systems to that of the rain falling within their catchments. To make this correction, altitudinal variations in both the amount and the ratios of stable isotopes of rainfall were determined for a single rainfall event (08/05 that was used for the event sampling). Rainfall collectors were placed at altitudinal intervals of 200 m, up each of the seeps catchments. A topographic map was used in conjunction with aerial photos viewed with a stereoscope to delineate the catchment above the seeps. ArcView GIS (v3.3) was used to break each catchment into 200 m contour bands and determine where the rain collectors needed to be placed. A GPS (Garmin Geko 2001, USA) was used to locate the sampling points. Rainfall collectors were placed in the middle of each of the contour bands prior to the rain event and collected afterwards. Coloured ribbon was tied to a nearby bush to ensure they could be found again.

The area of each 200 m contour bands was determined using 'polyarea', a free application that can be downloaded from the internet. Water for stable isotope analysis was extracted from each collector and the amount of rain measured. By dividing the amount of rain caught by the collectors by the area of the funnel openings, an estimate of the amount of rain that fell per square metre in each of the contour bands was obtained. The amount of rain that fell in each contour band was calculated by multiplying the amount that fell per square metre by the area of the contour band. The total amount of rain that fell in each catchment was calculated by adding up the amount that fell in each of its contour bands.

The mean isotopic composition of rain falling into each catchment was estimated using a mass balance equation incorporating the measured amount and isotopic composition of rainfall in each 200m band, with the area of each band:

$$\text{Corrected } \delta^2\text{H} = (\sum \delta_a \cdot \text{amnt}_a \cdot \text{area}_a) / (\sum \text{amnt}_a \cdot \text{area}_a) \quad (1)$$

where  $\delta_a$  represents the  $\delta^2\text{H}$  value of the rain falling within polygon a,  $\text{amnt}_a$  the amount of rain falling in polygon a and  $\text{area}_a$  the area of polygon a.

The offsets measured were then used to correct the values of the rain collected in the cumulative samples to make estimates of the mean signal of rain falling in the catchments above each of the four seeps.

The average isotopic ratio of the rain falling into each of the four catchments over the course of the measurement was calculated, taking into account the amount of rain that fell during each of the four sampling intervals. The following mass balance equation was used:

$$\text{Mean } \delta^2\text{H value} = \sum (\sum \text{corrected } \delta^2\text{H}_b \cdot \text{amnt}_b) / (\sum \text{amnt}_b) \quad (2)$$

where corrected  $\delta^2\text{H}_b$  is the altitude corrected value of rainfall derived for a sampling period using equation 1 and  $\text{amnt}_b$  is the amount of rain that fell during that period.

## **Water level measurements and groundwater sampling**

Samples of groundwater were collected and water level measurements made once a month for seven months from the beginning of summer (November) till early winter (May). A 12cm wide piezometer with a screened lower section with slits 0.5 mm wide was sunk into a hole augered down to bedrock in July 2004. Piezometers were sealed with a removable cap. Water level measurements were carried out throughout the summer of 2004-2005 using a tape measure with the aid of a torch. Groundwater was removed from the piezometers using a PVC water bailer and samples were collected in 13.5ml glass Vacutainers (a rubber-stoppered glass vial), which were further sealed with Parafilm. Water in a piezometer is in direct contact with air and is therefore likely to be fractionated from evaporation. To collect samples with  $\delta^2\text{H}$  values representative of the *in situ* groundwater, three times the volume of water originally in the piezometer was purged using the water bailer prior to the collection of a sample (Weaver, 1992).

Sampling the rise in seep water levels for stable isotope analysis is difficult. Removing water to take a sample will lower the water level within the piezometer making it difficult to determine when the water levels are rising. To resolve this, two piezometers could be sunk close together, with one used for measuring water levels and the other one used for taking samples at the appropriate time. Seeps, however, can have a great degree of spatial heterogeneity in the behaviour of their water tables - piezometers a few meters apart can behave very differently and could differ in their responsiveness to the pulse of water moving through a system after a rainfall event. Having the sampling hole very close to the monitoring hole would not work, as the

cone of depression associated with sampling will affect the water level in the monitoring hole. A piece of equipment was needed, and designed, to sample rising water levels in piezometers. The ASTON (Ascending Seep Tester Of Note – see Appendix 1) is made out of loops of plastic tubing connected to vacutainers bound onto a metal frame. It collects water at desired height intervals as it ascends up a piezometer and operates under the same principal as Van Wyk's rising water level sampler (Van Wyk, 1983). ASTONs were installed into the Kb Valley, Coastal and Perdeberg seeps prior to the rainfall event and the sampled water collected 24hrs afterwards. The Oudebos seep was not included in the event sampling as manpower was limited. A correction was made to account for the water in the influent tube (see Appendix 1)

### **Soil-water sampling and extraction**

Soil-water was sampled and  $\delta^2\text{H}$  values measured to be used in conjunction with stem-water  $\delta^2\text{H}$  values to determine plant rooting depth (Chapter 2) and to validate the  $\delta^2\text{H}$  values of groundwater samples. Soil samples were collected within 2 m of the piezometers in the hours before and approximately 24 hrs after, the rainfall event at the Kb Valley, Coastal and Perdeberg seeps. Samples were collected using a soil auger; 10cm soil sections were taken from the surface and in 25 cm intervals down the soil profile up to and including the saturated zone. Samples were placed in a Ziploc bag, sealed with masking tape, placed into another Ziploc bag and sealed again with masking tape. Samples were then quickly placed into a deep freeze in the ranger station in the Kogelberg. The mass of soil that needed to be distilled to yield approximately 2 ml of water was determined by measuring the soil moisture content

of the different samples by oven drying at 105°C for 24 hrs (Rowell, 1994). The approximate required mass of soil was removed and placed in a Kimax tube. Samples were collected from the centre of the soil pack, where any evaporative fractionation effects should be minimised. Soil-water was extracted from the samples on a vacuum distillation line with cryogenic collection (West *et al.* 2006).

### **Measurement of water stable isotope composition**

The methods of Harris *et al.* (1999), a variation of Coleman *et al.*'s (1982) closed tube reduction method was used to reduce water samples to H<sub>2</sub>. A microcapillary tube containing approximately 2 mg of water was dropped into a pyrex tube containing between 100 and 105 mg of "Indiana" Zn. The tube was then connected to a vacuum line, frozen in liquid N<sub>2</sub> for at least 2 minutes, evacuated and sealed using a oxygen-butane torch. After enough samples had been collected they were placed into a block furnace set at 450°C to reduce the water to H<sub>2</sub>. Isotope ratios were measured using a Finnegan MAT252 isotope ratio mass spectrometer. The standards V-SMOW and SLAP were analysed to correct for compression in the raw data, and the equations of Coplen (1993) were used to convert raw data to the SMOW scale. An independently analysed internal standard (CTMP) was run with each batch to calibrate my results relative to standard mean ocean water (V-SMOW) and correct for drift in the reference gas. The results are expressed as parts per thousand (‰) through the equation

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where  $R$  is the ratio of  $^2\text{H}/\text{H}$  in the sample and standard. CTMP was analysed regularly throughout the sampling run to correct for isotopic variation in the reference gas between different runs and to check the precision. The average difference between duplicates of CTMP and the mean of the different runs was 0.44‰ which corresponds to a  $2\sigma$  value of 0.94‰ ( $n=50$ ).

# Results

## Long term sampling

The rainfall in the cumulative rainfall collectors was isotopically the least negative in summer (Fig. 1.4).

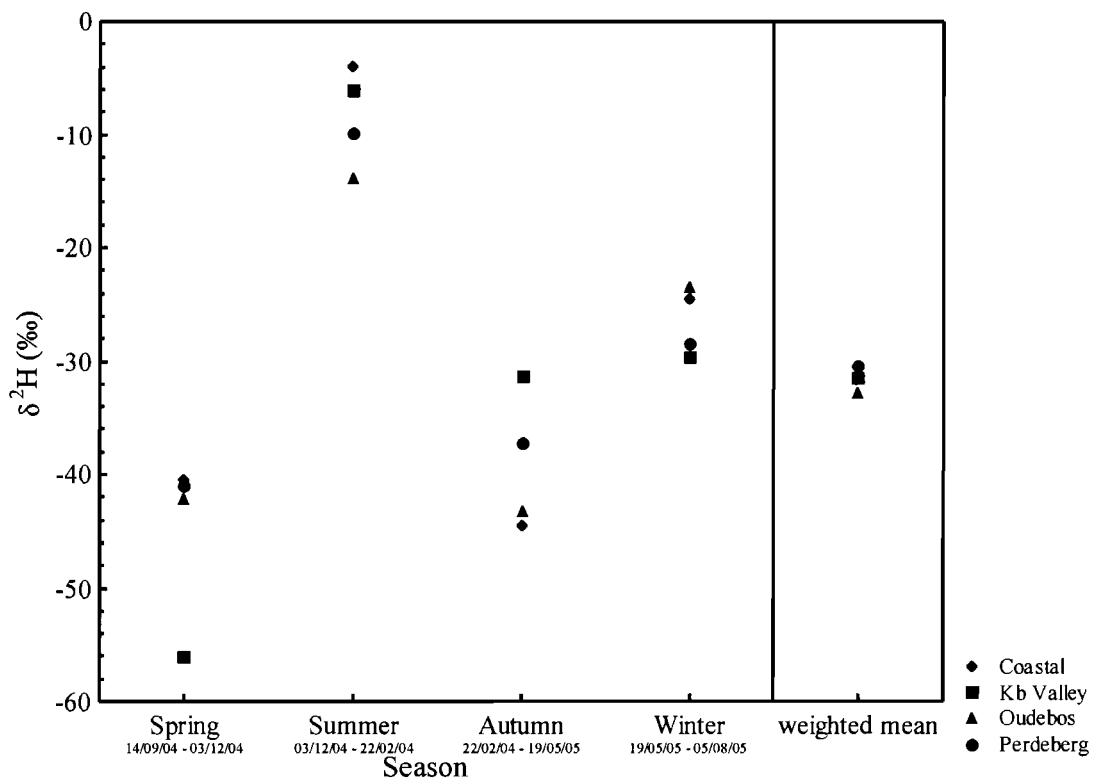


Fig. 1.4. The  $\delta^2\text{H}$  of rainfall falling into the catchments of the four seeps throughout the sampling period. Values depicted have been corrected for altitudinal effects on both the amount and ratio of stable isotopes. The weighted mean  $\delta^2\text{H}$  values are also depicted.

The groundwater from the Kb valley seep had the smallest amount of isotopic variation (3.22‰, Fig. 1.5). The ratio of stable isotopes of groundwater from the Oudebos and Coastal seeps was more variable, with the most isotopically light groundwater samples being collected in January and February (Fig. 1.5)- the period with the most isotopically light rain (Fig. 1.4). Fewer groundwater samples were collected at the Perdeberg seep (n=4) than at the other sites (n=7), as the water levels dropped too low to be sampled throughout the summer.

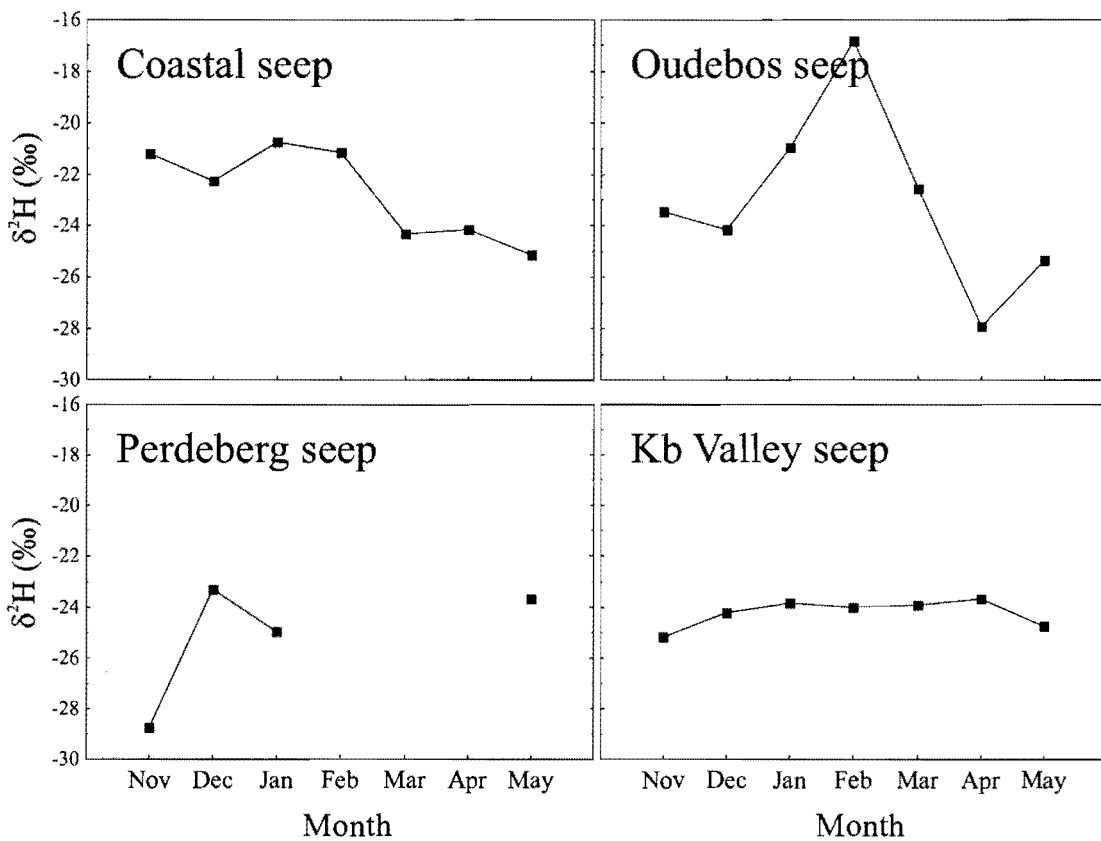


Fig 1.5. Variation in the  $\delta^2\text{H}$  of groundwater at the four seeps throughout the sampling period. Samples could not be collected from the Perdeberg seep in February, March and April.

The sites differed markedly in the amount that their water levels varied throughout the sampling period (Fig. 1.6). The Perdeberg seep's water levels were the most variable, with levels dropping right down to bedrock in January, February and March, before rising after the first heavy rain in April. The depth to the water table in the Coastal

and Oudebos sites varied, dropping in the dry summer months, but never dropping down to bedrock – their soil remained permanently saturated. Water levels at the Kogelberg Valley seep remained essentially unchanged throughout the summer, fluctuating within a range of 0.6cm.

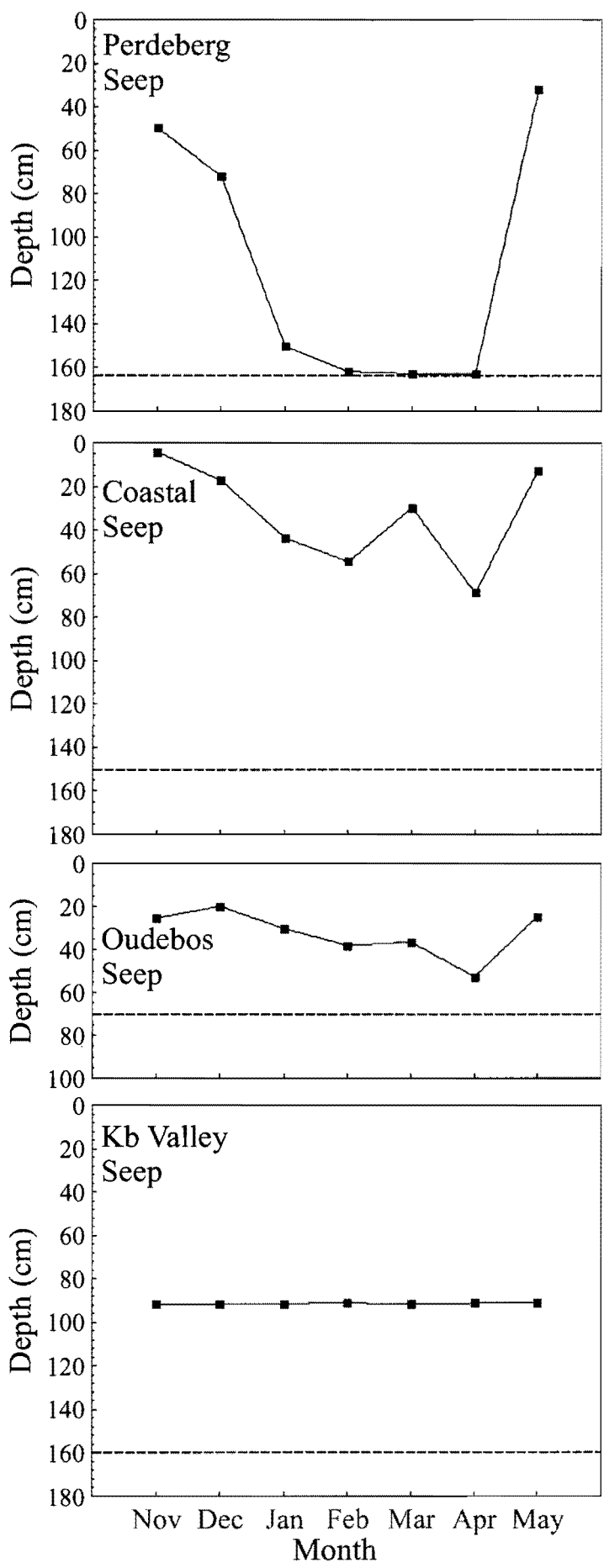


Fig.1.6. Monthly variation in the depth to the water table in piezometers sunk into the four seeps throughout the summer of 2004/05. The dashed line represents the bottom of the piezometer which was sitting on bedrock

## Short term sampling

The groundwater samples collected by the ASTON show that water levels rose by between 6 and 8cm at the Perdeberg seep throughout the rainfall event (Fig. 1.7). 24hrs later the water levels had dropped down to just 1.2cm above their initial height, although the soil above the initial height was still saturated. The Kb Valley and Coastal seeps had larger catchments (1.70 km<sup>2</sup> and 2.51 km<sup>2</sup> respectively) than the Perdeberg seep (0.06km<sup>2</sup>), and therefore received much larger amounts of rainfall during the rainfall event (an estimated 38,200 kl and 40,800 kl compared to 580 kl). Despite this, the water levels in the Kb Valley and Coastal seeps rose by less than 2cm (Figs. 1.8 and 1.9).

Soil-water became isotopically depleted in the heavier isotope with increasing depth at the Perdeberg seep (Fig. 1.7). The soil-water in the uppermost layer of soil was isotopically enriched before the rainfall event, after which it assumed a  $\delta^2\text{H}$  value very similar to that of the rain that fell next to the seep (not depicted  $\delta^2\text{H} = -19.08 \text{ ‰}$ ). Both the soil-water and the groundwater at the Perdeberg seep became isotopically depleted after the rainfall event, 'moving away' from the rain signal (Fig 1.7). The rainfall in the two and a half months prior to the event had a  $\delta^2\text{H}$  value of 28.42 ‰ (winter value in Fig. 1.4). The ascending water sampled by the ASTON had a more negative value than the groundwater sampled after the event, and became isotopically heavier as the water rose above 4cm.

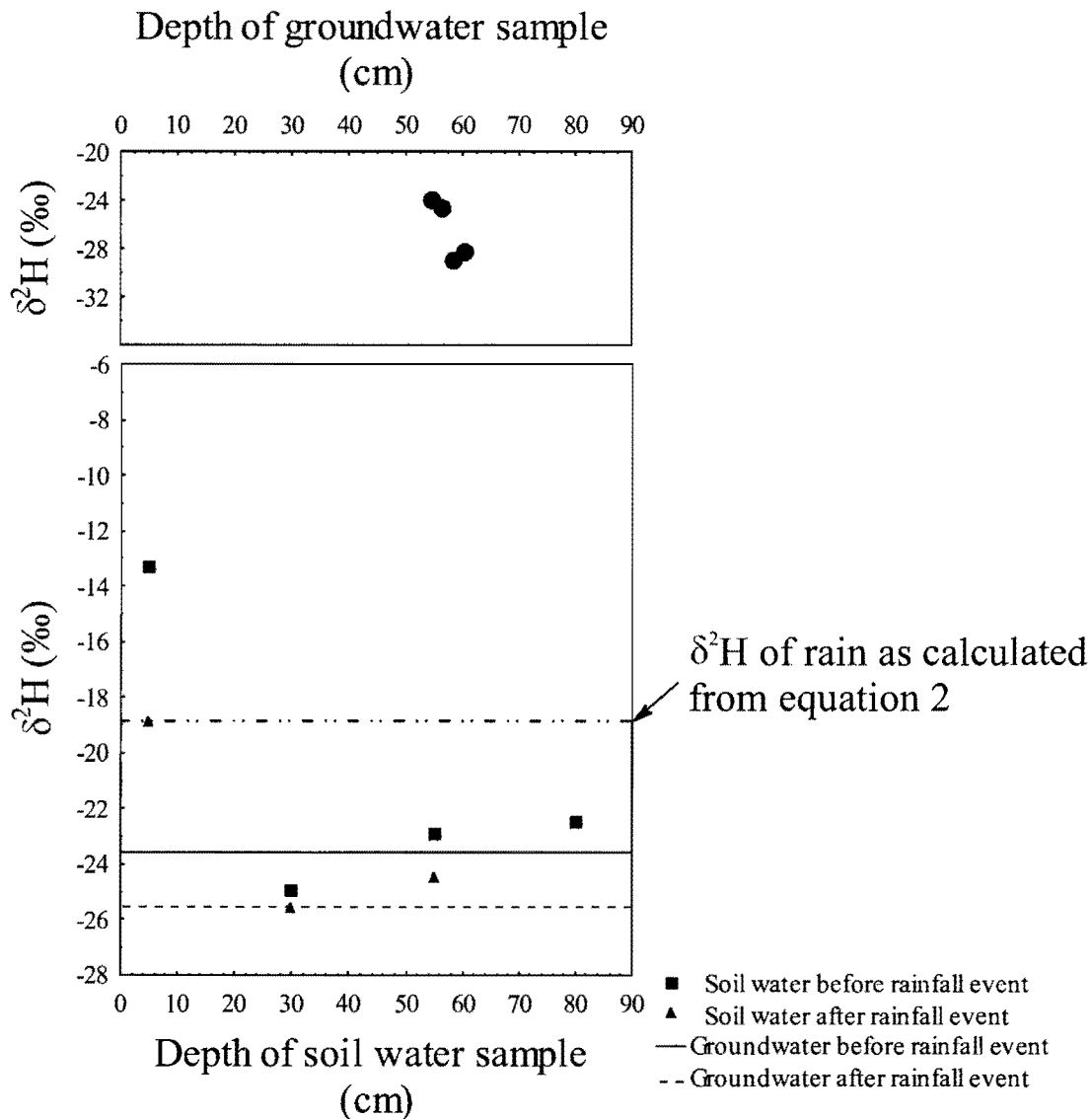


Fig 1.7. Variation in soil-water and groundwater throughout a rainfall event at the **Perdeberg seep**. The lower frame depicts the change in  $\delta^2\text{H}$  of soil-water with depth up to and including the saturated zone before and 24 hours after a rainfall event. The  $\delta^2\text{H}$  values of groundwater before and after the event are depicted, as well as the estimated mean  $\delta^2\text{H}$  value of rain falling into the seep's catchment. The upper panel depicts the  $\delta^2\text{H}$  value of groundwater sampled 50cm from bedrock by the ASTON as the water levels rose in 2cm intervals.

The soil-water became isotopically heavier with depth at the Kb Valley seep (Fig. 1.8). Apart from the anomalous value for the uppermost layer, the soil-water became isotopically depleted after the rainfall event, moving towards the  $\delta^2\text{H}$  value of the rain that fell next to the seep ( $\delta^2\text{H} = -16.83$  ‰). The groundwater became isotopically depleted after the rain event, 'moving away' from the rain signal towards the value of the rain that fell in the seep's catchment in the two and a half months prior to the

event ( $\delta^2\text{H} = -29.69 \text{ ‰}$ ). The ascending water sampled by the ASTON had a similar  $\delta^2\text{H}$  value to that of the groundwater sampled in the seep prior to the rainfall event.

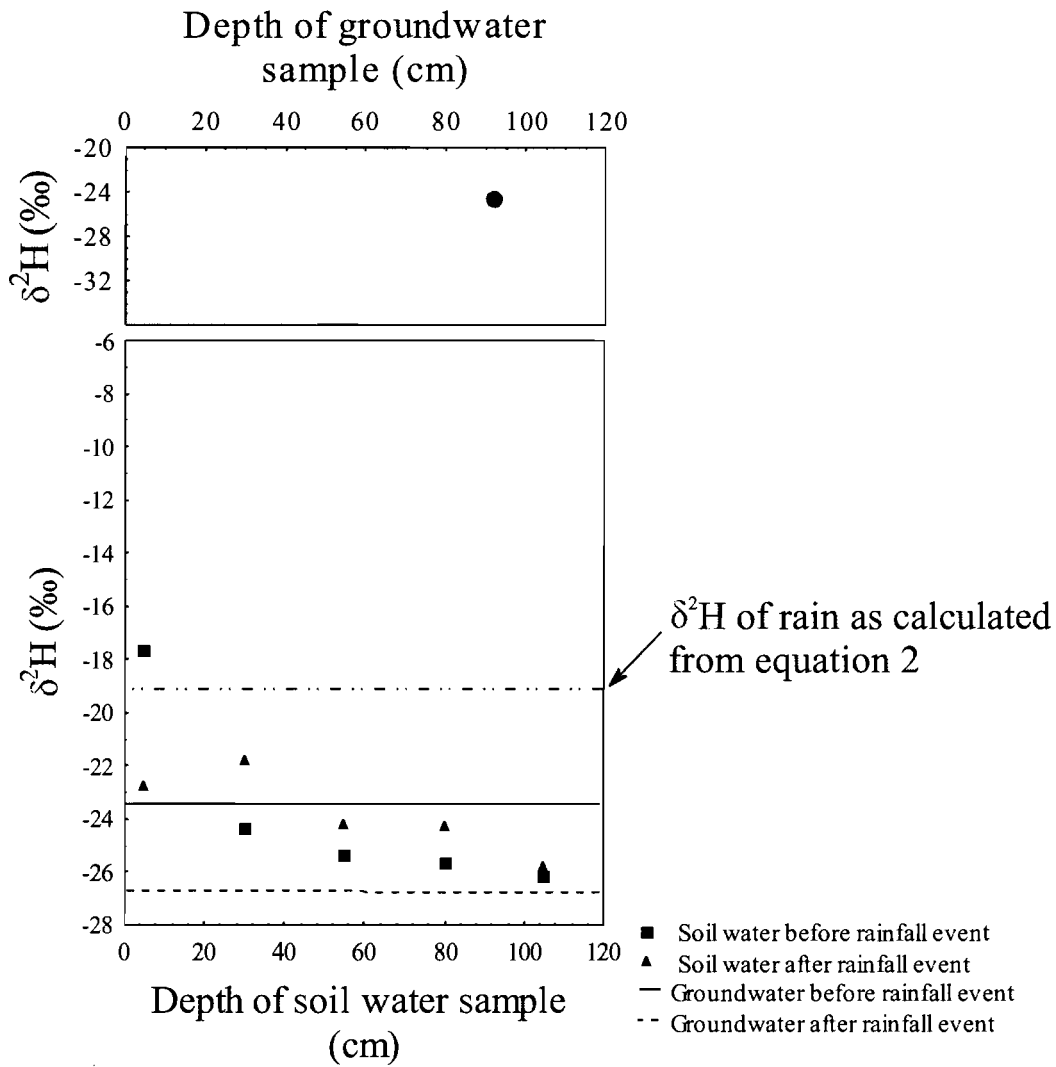


Fig. 1.8. Variation in soil-water and groundwater throughout a rainfall event at the **Kb Valley seep**. The lower frame depicts the change in  $\delta^2\text{H}$  of soil-water with depth up to and including the saturated zone before and 24 hours after a rainfall event. The  $\delta^2\text{H}$  values of groundwater before and after the event are depicted as well as the estimated mean  $\delta^2\text{H}$  value of rain falling into the seeps catchment. The upper panel depicts the  $\delta^2\text{H}$  value of groundwater sampled 50cm from bedrock by the ASTON as the water levels rose in 2cm intervals.

Again, soil-water became isotopically heavier with depth at the Coastal seep (Fig.1.9). The soil-water in the uppermost layer of soil became isotopically lighter after the rainfall event and assumed the  $\delta^2\text{H}$  value of rain that fell next to the seep ( $\delta^2\text{H} = -13.07 \text{ ‰}$ ). The groundwater moved towards the isotopic ratio of the rain that fell within the site's catchment, moving away from the value of the rain that fell in the

two months prior to the rain event ( $\delta^2\text{H} = -24.56 \text{ ‰}$ ). Water levels rose by less than 2cm, and again the water sampled by the ASTON had a similar  $\delta^2\text{H}$  value to that of the groundwater in the seep prior to the rainfall event.

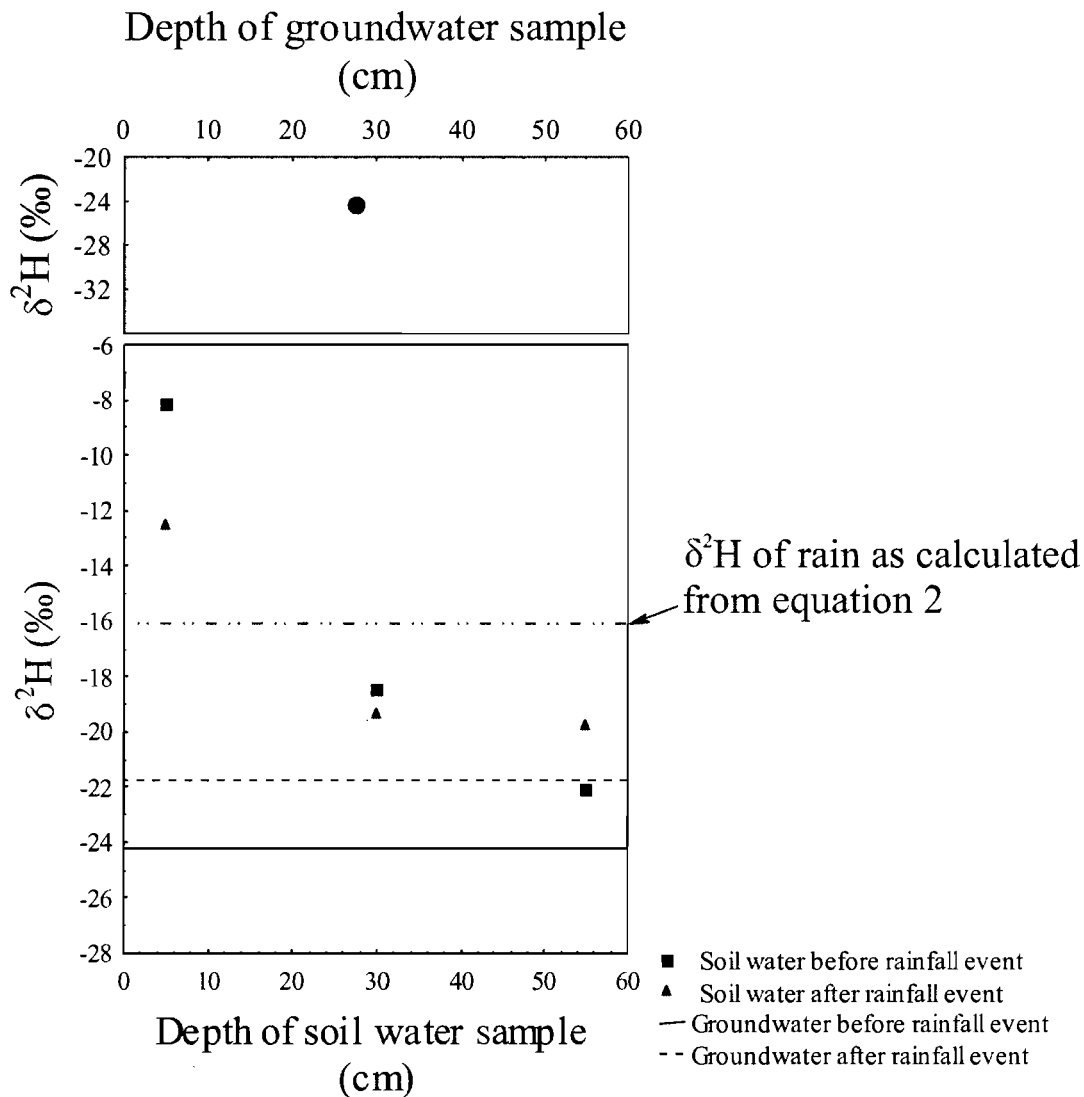


Fig. 1.9. Variation in soil-water and groundwater throughout a rainfall event at the **Coastal seep**. The lower frame depicts the change in  $\delta^2\text{H}$  of soil-water with depth up to and including the saturated zone before and 24 hours after a rainfall event. The  $\delta^2\text{H}$  values of groundwater before and after the event are depicted as well as the estimated mean  $\delta^2\text{H}$  value of rain falling into the seeps catchment. The upper panel depicts the  $\delta^2\text{H}$  value of groundwater sampled 50cm from bedrock by the ASTON as the water levels rose in 2cm intervals.

## Discussion

The aim of this chapter was to assess the potential of two different hydrological techniques - water levels and stable isotopes - to identify seeps dependent on the TMG aquifer. Seeps were monitored over both the long-term as well as throughout a single event to determine which approach was more effective.

### **Variation throughout a summer**

#### Water levels

The seeps differed in the way that their water levels fluctuated throughout the summer months as a result of their different hydrological setting. The perched Perdeberg seep is not connected to a large enough body of water to maintain its supply throughout the dry summer months when the seep 'dried up'. The water that wetted the end of the tape measure in January, February and March is likely to be that trapped in the plastic end cap at the base of the piezometer. The seeps connected to the TMG aquifer were connected to a large enough body of groundwater to provide them permanently with discharge, keeping them permanently wet. The water level on the Kb Valley seep remained essentially unchanged and was unaffected by the onset of the winter rains. The water levels on the Coastal and Oudebos seeps dropped in the drier months and rose after the onset of winter. This suggests that they are more dependent on local rainfall travelling through shorter flow paths, than the Kb Valley seep. The fact that all of the seeps that were dependent on the TMG aquifer remained permanently

throughout the year, suggests that the measurement of water levels throughout summer could be a simple, yet effective, way of determining if seeps are aquifer-fed.

### Stable isotopes

The isotopic evidence supports the hypothesis that water discharging from the TMG aquifer may be well mixed. The water level data suggest that the Kb Valley seep is the least connected to rain falling in its catchment, with it rather receiving a constant supply from the TMG aquifer, this seep had the smallest variation in  $\delta^2\text{H}$  of its groundwater throughout the sampling period.

The rainfall in the Kogelberg was isotopically heavier in early summer, probably as a result of a combination of the amount and temperature effects (Dansgaard, 1964; Gat 1982; Harris *et al.*, 1999). This trend was reflected in the  $\delta^2\text{H}$  value of groundwater of the Coastal and Oudebos seeps, which were isotopically heaviest during this period. The groundwater at these sites did not become enriched to the same degree as the rainfall, suggesting that rain that fell earlier in the year was still discharging at these seeps. The fact that the  $\delta^2\text{H}$  values of these sites is more variable than those of the Kb Valley seep and appears to track seasonal fluctuation in rainfall, suggests that the water discharging from these seeps is moving through shorter flow paths - their discharge is more closely coupled with antecedent rainfall.

The groundwater in the perched Perdeberg seep dropped below a point at which it could be sampled in the summer months, making it impossible to compare the isotopic variation it experienced with the other seeps. The stable isotope ratios of the

samples that were collected, fell within the isotopic range of groundwater samples collected from the aquifer-fed seeps. This is not surprising, as the large exposures of fractured TMG in the Kogelberg will result in local rainfall recharging the aquifer, and it is therefore likely that perched and aquifer-fed systems would have groundwater with similar ratios of stable isotopes.

## **Variation throughout a rainfall event**

### Water levels

As anticipated, despite its relatively small catchment that received a fraction of the rainfall, the water levels of the perched Perdeberg seep rose a great deal more than the levels of the two aquifer-fed seeps.

### Stable isotopes

At all three of the sites the  $\delta^2\text{H}$  of soil-water tended to become more positive nearer the soil surface as a result of evaporative enrichment. This means that extracting soil-water from the uppermost layer of soil is not going to yield a representative groundwater sample. In addition, water from the upper soil layers is likely to be more reflective of the  $\delta^2\text{H}$  value of recent rainfall events.

The Coastal and Kb valley seeps differed in the way that they responded to the rainfall event. The hypothesis was that the groundwater rise in the aquifer-fed seeps would be made up of groundwater already in the system being lifted. This appeared to

be the case in the Kb Valley seep. While the soil-water in this site became more isotopically heavy, presumably as a result of the permeation of relatively isotopically heavy rainfall, the groundwater became more isotopically light, moving towards the value of rain that had fallen into the site's catchment in the two months prior to the event. The groundwater in the coastal site however became more isotopically heavy, moving along with its soil-water away from the  $\delta^2\text{H}$  value of longer term rainfall towards that of the antecedent rainfall during the event. This different response could have to do with the fact that the water table at the Coastal site is much closer to the surface, making it easier for rain falling on the seep to permeate through the soil and influence the isotopic ratio of the groundwater.

The Perdeberg seep had an interesting and unexpected response to the rainfall event. It was expected that, because this perched system is fed by a relatively small groundwater body, the groundwater in the seep would move towards the isotope value of the antecedent rainfall. It didn't. Despite an estimated 580kl of relatively isotopically heavy rain entering the site's catchment, its groundwater became isotopically lighter. This suggests that the rain water that had not yet reached the seep groundwater and the pulse of water through the seep during the event is made up of water that was already within the system. The fact that the initial 4cm of rising groundwater sampled by the ASTON is lighter than the initial groundwater and the subsequent 4cm of rising levels, may be interpreted as suggesting that the groundwater feeding this seep is not very well mixed. The subsequent 4cm of rising water was isotopically heavier, perhaps as a result of water derived from the rain event beginning to enter the base of the piezometer. 24 hrs after the rain event, groundwater sampled was isotopically light, perhaps because this sample represents

an integrated value of the very isotopically depleted groundwater that initially moved into the piezometer sampled by the ASTON mixed with the subsequent percolation of isotopically enriched rainwater.

## Conclusion

Aquifer-fed seeps were found to differ in the amount that their stable isotope ratios varied throughout the summer, likely as a result of differences in the length of the flow paths of water discharging into the seeps. At a point in time, aquifer-fed seeps across a landscape are likely to have groundwater with different stable isotope compositions as a result of recent rainfall, making up varying proportions of the total water discharging from a seep. A single 'sampling sweep' is, unfortunately, therefore likely to be of little use in identifying aquifer-fed systems. The seasonal variation in the stable isotope composition of the Cape's rainfall does however appear to have potential, when used in conjunction with groundwater sampling, to ascertain the length of geohydraulic flow paths. Perched systems often dry up in summer, have similar values to that of the aquifer-fed seeps in the wetter parts of the year and can be connected to large enough bodies of groundwater to prevent them from assuming the  $\delta^2\text{H}$  value of antecedent rainfall during events. All three of these factors reduce the potential of stable isotope ratios to be used as a diagnostic tool in identifying perched and aquifer-fed seeps. A limitation of this study was that only one perched seep was monitored.

Monitoring variation in water levels, on the other hand, appears to have a great deal more potential as a tool in differentiating between perched and aquifer-fed seeps. It is possible that systems that dry up in summer may still be dependent on aquifer discharge to become saturated in the wetter parts of the year, and that perched systems remain permanently wet. Nevertheless, determining whether seeps do remain permanently wet is a quick, easy and relatively cheap way of determining which seeps in an area are *likely* to be aquifer-fed. Sinking piezometers and installing ASTONs in mid summer (January to March) with sampling intervals set at, say, 10cm and collecting them after the first heavy rains (they usually come in late March or April) is an easy technique that can be used to determine the range in water level fluctuations that a seep experiences. Water levels in perched seeps are likely to be low in midsummer and increase dramatically, whereas aquifer-fed seeps are likely to have higher water levels and experience a less significant increase. This technique is relatively easy and cheap, and should result in minimal trampling, to which seep plant species are extremely sensitive.

## Chapter 2 - Ecohydrology of TMG aquifer-fed seeps in the Kogelberg

### Abstract

Mountain seeps have been identified as ecosystems that may be impacted upon by bulk water abstraction from the TMG aquifer. In this study I assess the potential of two techniques, the monitoring of the progression of xylem pressure potentials throughout a summer and the measurement of stem-water stable isotope composition at the end of summer, to identify aquifer-fed seeps and distinguish them from perched seeps that are unlikely to be impacted.

As a result of their being connected to smaller bodies of groundwater, perched seeps are more likely to dry up in summer months than aquifer-fed seeps. Plants living on a perched seep in the Kogelberg that dried up in summer did however not experience significantly greater levels of midday xylem pressure potentials than those on a permanently wet, aquifer-fed seep. This may be as a result of the perched seep being on top of a mountain where it is likely to be cooler and wetter. Evidence for this is that plants living adjacent to the perched seep had significantly lower levels of xylem pressure potential than those living adjacent to the aquifer-fed seep at 420m lower altitude. Perched seeps are often likely to be found at higher altitudes which may detract from the use of the monitoring of xylem pressure potentials as a tool to identify aquifer-fed seeps.

As a result of their being connected to smaller bodies of groundwater, plants living on perched seeps may have stem-water that is isotopically heavier at the end of summer. Though the data followed the expected trend, the difference between the stem-water from plants at the two sites was insignificant. This may have been as a result of the rainfall event preceding the sampling not being as isotopically distinct as it may have been.

Aspects of the water use of seep-plants at the community scale were examined using measurements of xylem pressure potential and stem water stable isotope composition of species with different rooting depths. Deep-rooted species tended to have lower xylem pressure potentials than more shallow rooted species which, from my isotope data, appear to be sourcing the bulk of their water from the top 10cm of soil. Both methods have potential for identifying shallower rooted seep species likely to be the first impacted upon by declining water levels associated with bulk abstraction.

An unexpected result from the monitoring of xylem pressure potential throughout the summer, was that *Proteas (P. cynaroides)* growing adjacent to the TMG aquifer-fed seep were no more stressed than individuals growing on the permanently saturated seep. This suggests that they may also have access to a permanent supply of water, water that may be connected to the TMG aquifer. Should this be the case then the impacts of bulk abstraction from the aquifer may be greater than has been anticipated, extending from beyond localised river and wetland systems out into 'dryland' fynbos.

## Introduction

Cape fynbos ecosystems and plant species within them may have a diversity of water sources. This diversity in water use exists at both the landscape and local scale. Different communities may have access to different sources of water depending on the areas geohydrological context. Within communities, plants with different rooting depths have access to different soil-water 'pools'. In this Chapter I aim to get a better understanding of the water sources available to fynbos plants in the sandstone mountains of the Western Cape. I aimed to do this at these two scales, using the stable isotopes of hydrogen in water as well as the monitoring of xylem pressure potentials using a Scholander Pressure Chamber (Scholander *et al.*, 1965).

Interspersed across Fynbos landscapes are seeps, which have a greater supply of water. These groundwater-fed plant communities differ hydrologically from typical 'dryland' fynbos, as well as from one another, with different systems having different timing of groundwater supply depending on their hydrological context (see Chapter 1). Some seeps are linked to the TMG aquifer; such systems typically stay saturated throughout the dry summer (see Chapter 1). Alternatively, some seeps are at too high an altitude to be connected to the TMG aquifer and are geohydrologically isolated, 'perched' above it. Perched seeps are dependent solely on the rain falling within their catchments for recharge. Because perched seeps are not buffered by the same massive body of water below the water table, they have hydrological characteristics distinct from those fed by the TMG aquifer (Chapter 1). Water levels on perched seeps tend to be more variable than aquifer-fed seeps and are more likely to dry up in the summer months (Chapter 1). Based on these observations I hypothesise that plants living on

perched seeps will experience higher xylem pressure potentials at the end of summer than those plants living on an aquifer-fed seeps.

The Western Cape Province is a winter rainfall area: groundwater recharge is therefore likely to occur predominantly in winter months. In the first chapter I observed that winter rainfall in the Kogelberg is isotopically heavier than the relatively small amount of rain that falls in summer (Fig 1.4). Variation in the isotopic composition of stem water has been used as a tool in a number of studies to determine from where plants are sourcing their water (White *et al.*, 1985; Dawson and Ehleringer, 1991; Thorburn and Walker, 1993; Dawson and Pate, 1995; Zencich *et al.*, 2002; February *et al.*, 2004; February *et al.*, 2007). It may be possible to utilise the seasonal variation in the isotopic composition of rain water in the Western Cape to determine which areas are connected to the TMG aquifer. Differences in the stable isotope composition of groundwater between perched and aquifer-fed seeps are likely to be the most pronounced in summer. If water in the TMG aquifer is fairly well mixed, groundwater discharge from seeps in summer should have a stable isotope composition reflecting an integration of all rainfall inputs. Most of TMG aquifer recharge will occur in the wetter winter months. Summer rainfall in the Western Cape tends to be isotopically heavy relative to winter rainfall (Chapter 1; Diamond and Harris, 1991; Harris *et al.*, 1999) so in summer aquifer-fed seeps were expected to have groundwater that is isotopically light relative to the recent rainfall. Perched systems on the other hand, are connected to much smaller bodies of groundwater. Groundwater discharge from these seeps has moved through shorter paths than those within the TMG aquifer and is therefore likely to be 'younger' and have a stable isotope composition more similar to that of recent rainfall. Direct sampling of

groundwater was not possible at the end of summer as the perched seep that I studied dried up (Chapter 1). However, the fundamental observation that there is no fractionation of either hydrogen or oxygen during water uptake by roots or by its subsequent passage up the length of a plant (White *et al.*, 1985; Ehleringer and Dawson, 1992, Thorburn and Walker, 1993) means that the stable isotope ratio of plant water can be used as an integrated measure of overall water uptake, reflecting the stable isotope ratios of the various sources being exploited by the plant (Dawson *et al.*, 2002). Unlike groundwater, plant stem-water can still be sampled in summer.

The first two aims in this chapter relate to aspects of plant water use at the wider, landscape scale. My first aim in this chapter was to determine whether plants living on a perched seep that dries up in summer have higher xylem pressure potentials than those living on a permanently wet, TMG aquifer-fed seep. To this end I monitored the seasonal progression in pre-dawn and midday xylem pressure potentials. Secondly, I aimed to determine whether plants living on a perched seep have stem-water that is isotopically heavier at the end of summer than those living on an aquifer-fed seep, stem-water that more closely reflects the stable isotope composition of recent rainfall. To test this hypothesis I measured the stable isotope composition of stem water of two relatively shallow rooted species living on a perched and an aquifer-fed seep. If large enough discrepancies exist in these two measures, i.e. the seasonal progression in seep plant xylem pressure potentials and the stem water stable isotope composition of seep plants at the end of summer, then they may be of use as a tool in determining which plants across a landscape are connected to the TMG aquifer.

My third aim in this chapter is to examine aspects of seep-plant water use at the community scale. Plant communities are often made up of species with varying rooting depths. Shallow-rooted species are likely to be the first to be impacted upon by a lowering of water levels whereas deeper rooted species may still have access to groundwater further down the soil profile. Seeps have the peculiar hydrological setting where the water table is often just centimetres below the surface. Shallow-rooted species may therefore have the same access to groundwater as deeper rooted species with no difference in xylem pressure potential. I aimed to use xylem pressure potential measurements to determine whether shallower rooted seep species get more stressed in summer than their deeper rooted counterparts and to use stable isotopes of plant water and soil-water to determine from what depth different species source their water and how this may change after a rainfall event.

## Methods

### Study Site

The Kogelberg biosphere reserve is a mountainous area on the Cape south coast with geology dominated by TMG sandstones. The KB Valley seep (34°18.203'S, 18°56.185'E) appears to be fed by the TMG aquifer (see Chapter 1). It is in the Palmiet River valley on the steep northern slope of a fault controlled gorge that bisects the lower reaches the Palmiet River. The Perdeberg seep (34°18.512'S, 18°59.957'E) appears to be an example of a perched system. It is on a northern slope

just off the top of the Perdeberg, almost directly east of the TMG aquifer-fed seep (see Fig. 1.1). The perched seep is at an altitude of approximately 600m and the TMG aquifer-fed seep at an altitude of approximately 180m (see Fig 1.2).

Restioid and ericoid species tend to be shallower rooted than proteoid shrubs (Higgins *et al.*, 1987; Stock *et al.*, 1992; February *et al.*, 2004). Plant species were selected on the basis of their having perceived differences in rooting depths. A shallow-rooted species of Restionaceae, *Restio purpurascens* was sampled on both seeps. *Osmotopsis asteriscoides*, the marsh daisy in the Asteraceae, is a sparsely branched, aromatic shrub and was perceived to have deeper roots than *Restio purpurascens*. It was sampled on both seeps as was the deep-rooted *Protea cynaroides* from the Proteaceae. Both seeps had related (in the same genera) off-seep analogous species nearby. *Chondropetalum tectorum* was chosen as the off-seep Restionaceae pair of *R. purpurascens* and the sclerophyllous, woody *Phaenocoma prolifera* as the Asteraceae pair to *O. asteriscoides*. *Protea cynaroides* is unusual in that it occurs both on and off seeps and was sampled adjacent to the TMG aquifer-fed seep while *Leucadendron laureolum* was used as the off-seep pair to *P. cynaroides* at the perched seep.

## **Measurement of xylem pressure potentials**

Plant water stress was measured by determining the xylem pressure potential (XPP) of plants using a Scholander pressure chamber (Model 1000, PMS instrument co., Corvallis, Oregon; Scholander *et al.*, 1965). Plant samples were sampled predawn (03:00 – 05:30) as by this time plants have typically recovered from diurnal water

stress and have xylem pressure potentials in equilibrium with the water potential of the soil-water to which they have access (Klepper, 1968). Plants were also sampled on clear days at midday (12:00 -14:00) by which point xylem pressure potentials are at their highest (Klepper, 1968). Effort was taken to load excised leafy twigs as quickly as possible (i.e. less than a minute) and a 10x magnifying glass was used to increase accuracy while watching for the appearance of stem-water at the cut surface.

## **Stable isotopes**

Stem samples for water extraction were collected pre-dawn at the end of summer, on the same morning that xylem pressure potentials were measured in March. On- and off-seep Restionaceae and Asteraceae analogues were chosen as these more shallow-rooted wetland species may be more likely to have seasonal variation in their stem water stable isotope composition (Busch *et al.*, 1992; Smith *et al.*, 1998). Fractionation of stable isotopes occurs during transpiration, with leaf-water becoming enriched as lighter isotopes evaporate more readily (Gonfiantini *et al.*, 1965; Wershaw *et al.*, 1966). Care was taken to not sample photosynthesizing parts of the plants that may contain fractionated stem water, with the 'woody' corms of the restio species being sampled. Samples were immediately sealed in Kimax Tubes using Parafilm and were frozen upon return from the laboratory. Water was extracted from the stems while they were still within the Kimax Tubes by vacuum distillation, as described for soil-water extraction in Chapter 1. All of the water in the stem was collected to avoid any fractionation effects, a process that regularly took over 10

hours to complete. Ratios of the heavier stable isotope deuterium ( $^2\text{H}$ ) relative to hydrogen in the  $\text{H}_2$  of the water samples was determined using a Finnegan MAT252 isotope mass spectrometer as described in Chapter 1. Values were reported in the familiar  $\delta$  notation where

$$\delta = ((R_{\text{sample}}/R_{\text{standard}})-1)*1000$$

and  $R=^2\text{H}/\text{H}$ . The average difference between duplicates of the standard and the mean of the different runs was 0.44 ‰ which corresponds to a  $2\sigma$  value of 0.94‰ (n=50). Samples were reported relative to V-SMOW.

Student t-tests were carried out (Statistica v.7, Statsoft) to compare differences in the stable isotope ratio of stem-water between the different species living on and adjacent to the two seeps.

To determine the mean  $\delta^2\text{H}$  of antecedent rainfall, bulk rain samples were collected over approximately 6 week intervals in large 10 L rain collectors placed next to the seeps. Rainfall isotope values were corrected for altitudinal effects on both amount of rain and its stable isotope ratio (see methods in Chapter 1). A single rainfall sampler, placed out at the ranger station (115 m north of the Oudebos seep - Fig. 1.2), was used to collect water from the most recent rainfall event prior to the end of summer plant water sampling. The  $\delta^2\text{H}$  of the rainfall at the TMG aquifer-fed seep was assumed to be the same as that sampled at the ranger station, which is less than 3.5km away and at approximately the same altitude (80m). The perched seep was a similar (3.8km) distance away but at a much higher altitude (600m). The effects of altitude on the  $\delta^2\text{H}$  of rainfall were measured in the Kogelberg in a separate event. The effect was

assumed to be constant and used to 'correct' the  $\delta^2\text{H}$  value of the measured sample to derive an estimate of the  $\delta^2\text{H}$  value of rainfall at the top of the Perdeberg. Only 2.3mm of rain had fallen in the 13 days preceding this event (see Fig 2.2)

To determine the depth from which plants are sourcing their water and how this differs before and 24 hours after a rainfall event, samples of stems from species with varying rooting depths were collected, the stem water extracted and its  $\delta^2\text{H}$  value determined. This was compared to the  $\delta^2\text{H}$  of soil-water extracted from soil sampled at increasing depths down the soil profile. Water and organic content was determined by first weighing the soil sample and then re-weighing it after oven drying at 105°C for 24 hrs and burning off the organics in a furnace at 300°C for 24 hrs (Rowell, 1994). The rain that fell during the event was sampled intensively using a network of rainfall collectors placed up the catchments at both seeps (this was the same event as discussed in Chapter 1).

## Results

November through March was the driest time of the 04/05 summer in the Kogelberg (Fig. 2.1). November and March were the two driest months with totals of 13.7mm and 18.0mm having fallen respectively. From Fig 2.2 it can be seen that it rained fairly regularly throughout the summer with some wet days (for example the 33.8mm that fell on 22 December) in the middle of summer. The heavy winter rains started in early April with 141.5mm of rain falling within six hours on the morning of 11 April.

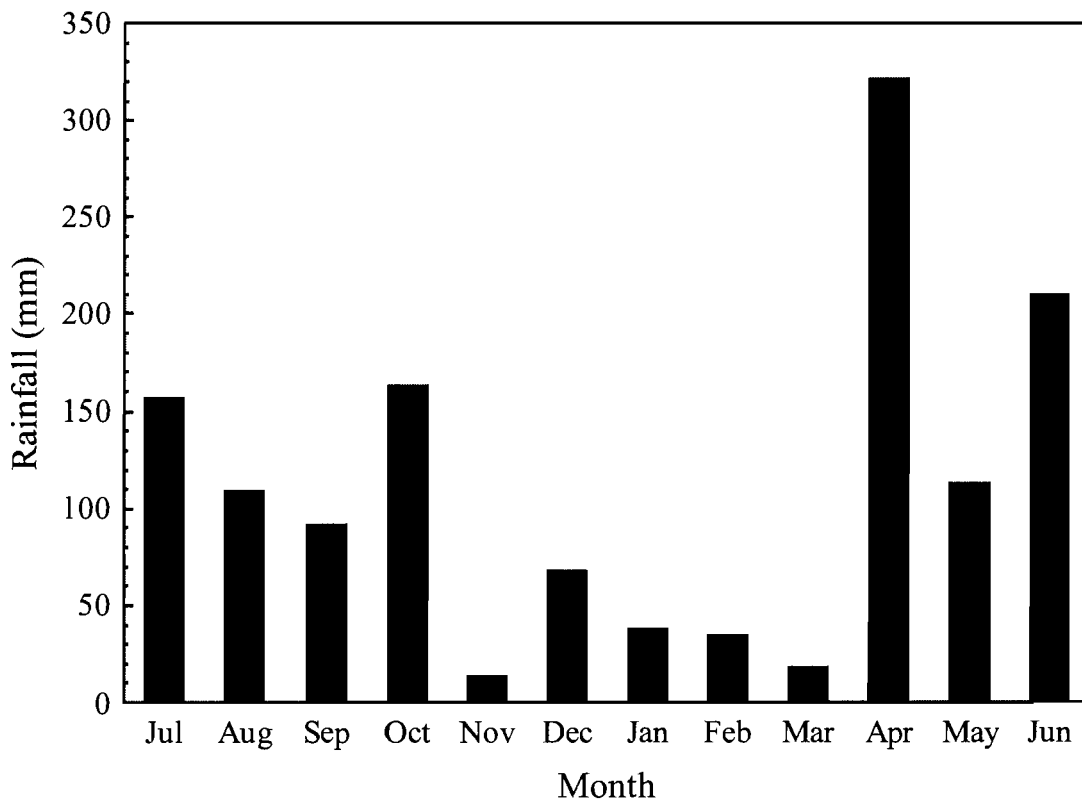


Fig 2.1. The amount of monthly rainfall that fell at the weather station in the Kogelberg Valley from July 2004 to June 2005

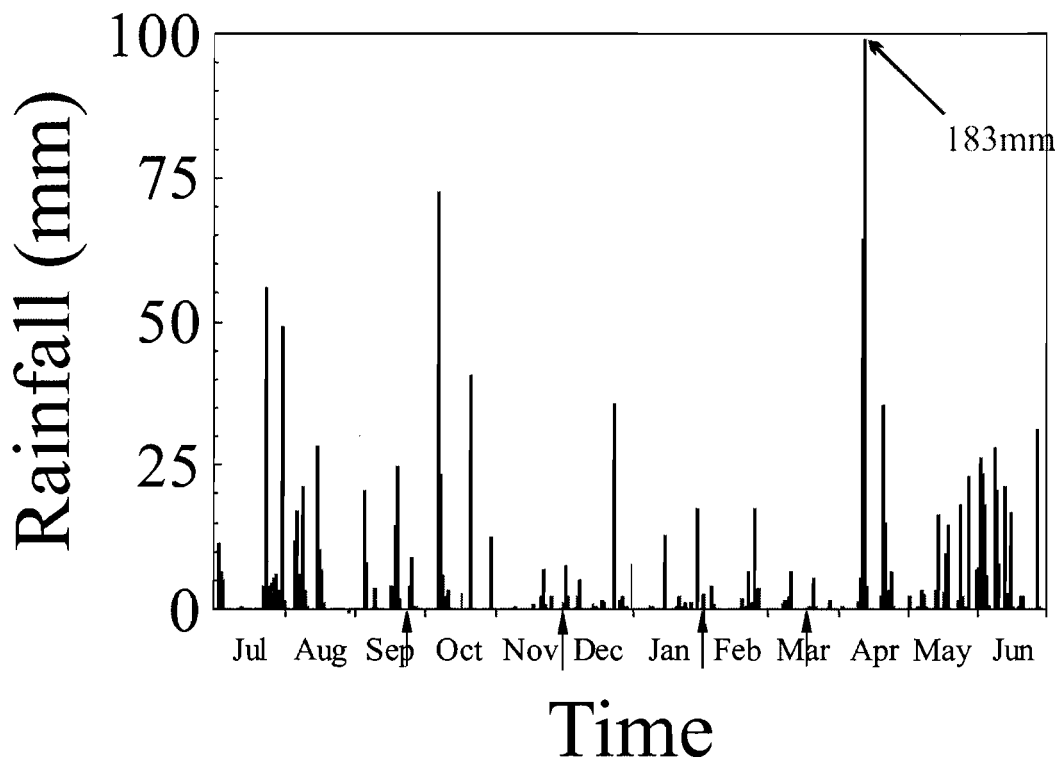


Fig 2.2. The daily amounts of rain that fell in the Kogelberg valley, from July 2004 until June 2005. Rainfall on the 11<sup>th</sup> of April fell off the scale. The arrows on the x-axis indicate the timing of the four sets of xylem pressure potential measurements

My first aim in this chapter was to determine whether plants living on a perched seep that dries up in summer get more stressed than those living on a permanently wet TMG aquifer-fed seep. The levels of both pre-dawn and midday xylem pressure potentials of plants living on the perched seep did not differ significantly ( $t = -0.31$ ,  $p = 0.75$ ;  $t = -0.65$ ,  $p = 0.52$  respectively) from stress levels experienced by plants living on the TMG aquifer-fed seep (Table 1). Plants living adjacent to the two seeps experienced similar levels of xylem pressure potential predawn ( $t = 0.32$ ,  $p = 0.75$ ) but differed significantly in the values experienced at midday ( $t = -7.06$ ,  $p = 0.001$ ), with plants adjacent to the TMG aquifer-fed seep experiencing an average of 0.37MPa higher xylem pressure potentials than those living adjacent to the perched seep.

Table 1: Mean levels of predawn and midday XPP (MPa) across all species (n=21) on and adjacent to the two seeps

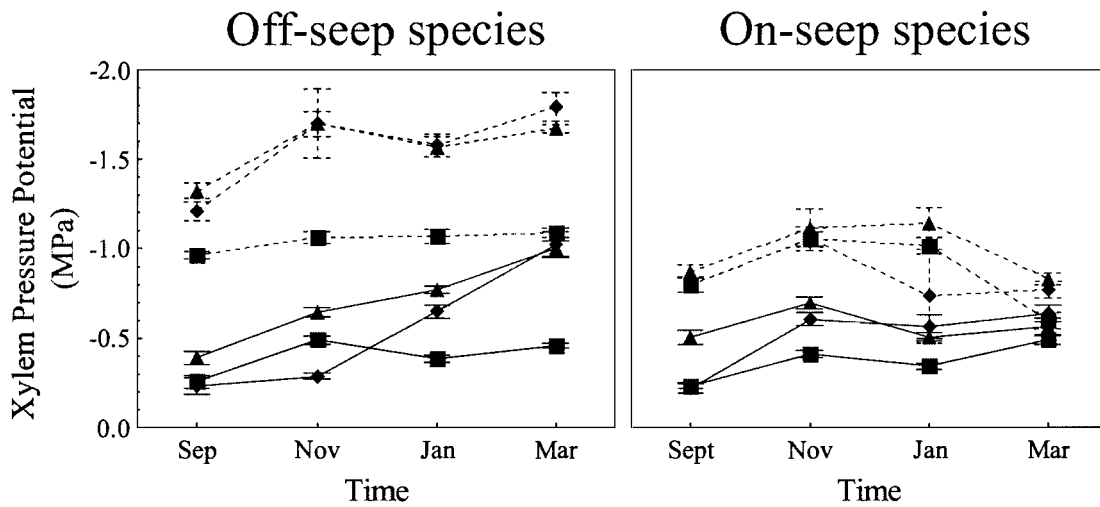
	TMG aquifer-fed seep		Perched seep	
	Predawn	Midday	Predawn	Midday
On-seep	-0.48	-0.90	-0.47	-0.87
Adjacent	-0.55	-1.39	-0.56	-1.02

None of the species on the TMG aquifer-fed seep experienced a seasonal increase in xylem pressure potential throughout the summer (Fig. 2.3). Only the Restionaceae sampled on the perched seep experienced a seasonal increase in xylem pressure potential, and had apparently ‘shut down’ by the end of summer.

Deeper-rooted Protea species, experienced the lowest xylem pressure potentials (Fig. 2.3). Analogues living adjacent to the TMG aquifer-fed seep were on average no more stressed predawn than their on-seep analogues ( $t = 0.88$ ,  $p = 0.38$ ). In fact on average Proteas living adjacent to the perched seep had xylem pressure potential significantly lower ( $t = 3.42$ ,  $p = 0.001$ ) than their on-seep analogues. At midday Proteaceae living on the TMG aquifer-fed seep had significantly lower xylem pressure potentials than their off-seep analogues ( $t = -3.88$ ,  $p = 0.001$ ) while there was no significant difference between individuals living on, and adjacent to, the perched seep ( $t = -0.48$ ,  $p = 0.63$ ) (Fig. 2.3).

Plants living at the two sites differed in how their xylem pressure potentials changed throughout the summer. Plants adjacent to the TMG aquifer-fed seep experienced a gradual increase in predawn and midday xylem pressure potentials throughout the summer, while plants living adjacent to the perched seep experienced their highest values in November (Fig 2.3)

## Aquifer-fed site



## Perched Site

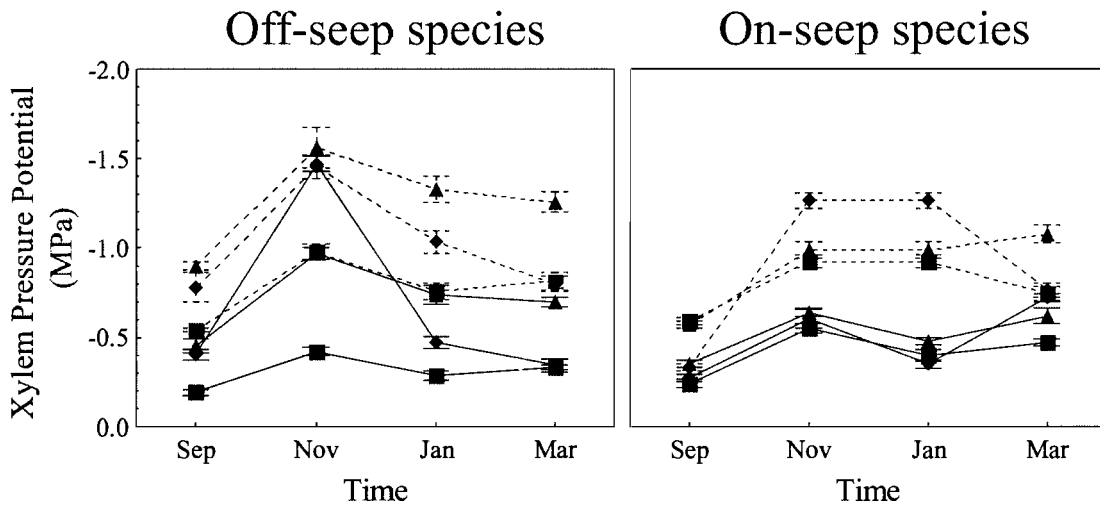


Fig 2.3. Variation in Midday (dashed lines) and pre-dawn (solid lines) xylem water potentials of species living on, and adjacent to, the TMG aquifer-fed Seep and the perched seep throughout the 2004 / 05 summer. Means ( $n = 7$ ) are depicted as well as the SE. Restionaceae are represented by diamonds, Proteaceae by squares and Asteraceous species by triangles

The second aim of this chapter was to determine whether plants living on a perched seep have heavier stem-water at the end of summer than those living on an TMG aquifer-fed seep.

There was no significant difference in xylem pressure potentials between the Restionaceae ( $p = 0.21$ ) or the Asteraceae ( $p = 0.31$ ) sampled on the either the TMG

aquifer-fed seep or the perched seep (Fig 2.5). Both the Restionaceae and the Asteraceae living adjacent to the TMG aquifer-fed seep have significantly higher xylem pressure potentials than those living adjacent to the perched seep ( $p < 0.0001$ ). Restionaceae and Asteraceae living adjacent to the TMG aquifer-fed both had significantly higher xylem pressure potentials than their on-seep analogues ( $p < 0.001$ ). Restionaceae living adjacent to the perched seep had lower xylem pressure potentials than their on-seep analogue ( $p < 0.0001$ ) while the difference between the Asteraceae on the two sites was insignificant ( $p = 0.19$ ).

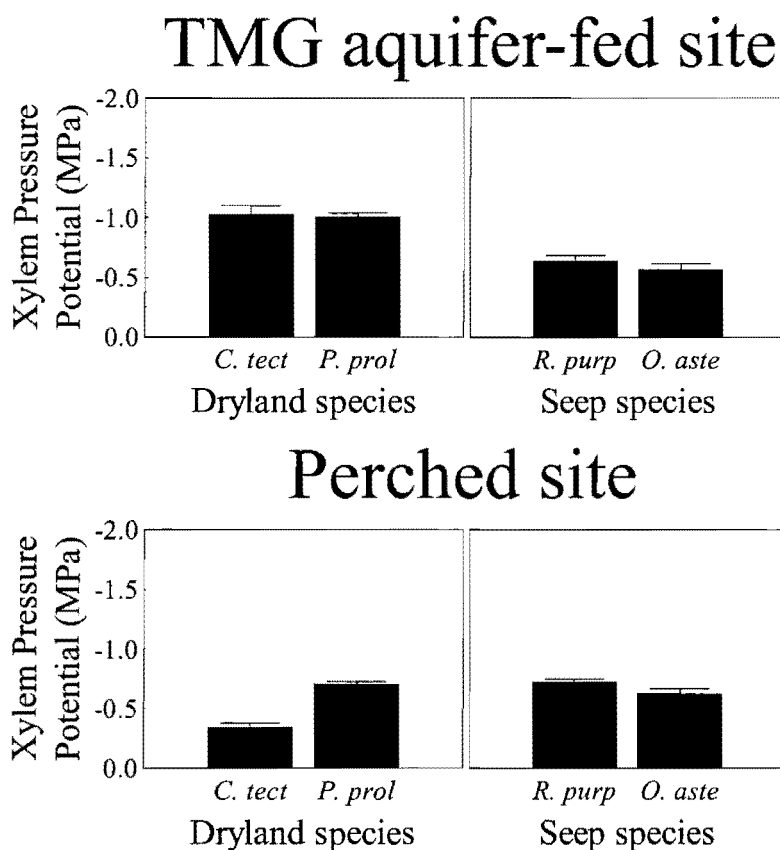


Fig. 2.5 . Levels of predawn XPP at the end of summer of plants sampled on and adjacent to two mountain seeps in the Kogelberg. Means ( $n = 7$ ) are depicted as well as the SE

Though the difference was insignificant ( $p = 0.09$ ), shallow-rooted restios living on the perched seep had stem water that was on average isotopically heavier than those living on the TMG aquifer-fed seep (Fig 2.6). Isotope ratios were more variable between species living off the seeps with stem-water of *C. tectorum* tending to be more fractionated than that of *P. prolifera*, though the differences were insignificant

( $p= 0.04$  and  $0.18$  for the TMG aquifer-fed and perched seeps respectively). *C. tectorum* and *P. prolifera* living adjacent to the perched seep both had stem-water that was more enriched than that of those living adjacent to the TMG aquifer-fed seep, though only *P. prolifera* was significantly so ( $p= 0.0005$ ). The Restionaceae (*C. tectorum*) living adjacent to the TMG aquifer-fed seep had the isotopically heaviest stem-water, with three of the individuals sampled having stem-water isotopically indistinct from that of the preceding - relatively isotopically heavy - rainfall event. However, some of the individuals sampled had stem-water that was isotopically indistinct from that of *P. prolifera*.

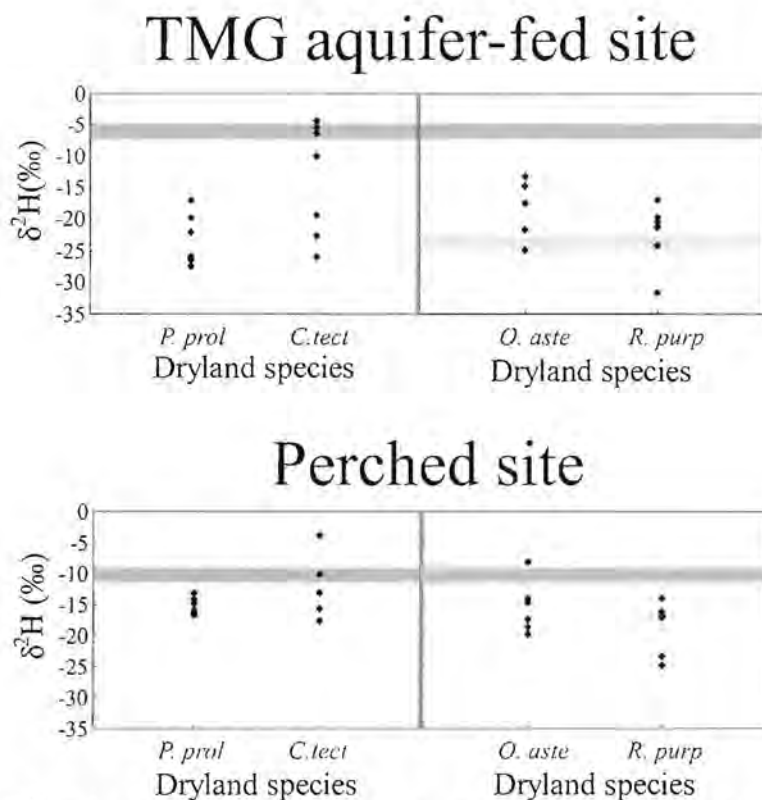


Fig. 2.6.  $\delta^2\text{H}$  values of stem-water of Restionaceae and Asteraceae living on and adjacent to the TMG aquifer-fed seep and the perched seep on the 13/03/06. An estimate of the  $\delta^2\text{H}$  of rain that fell on the 09/03/06 is depicted with the darker bar. The  $\delta^2\text{H}$  of groundwater at the TMG aquifer-fed seep is depicted by the lighter bar.

## Seep-plant water use at the community scale

My third aim in this chapter was to examine aspects of seep-plant water use at the local scale, specifically to determine from what depth they source their water and how this may change after a rainfall event. In all four cores sampled before and 24hrs after a rainfall event, soil near the surface had the highest water fraction and the highest organic fraction (fig 2.7).

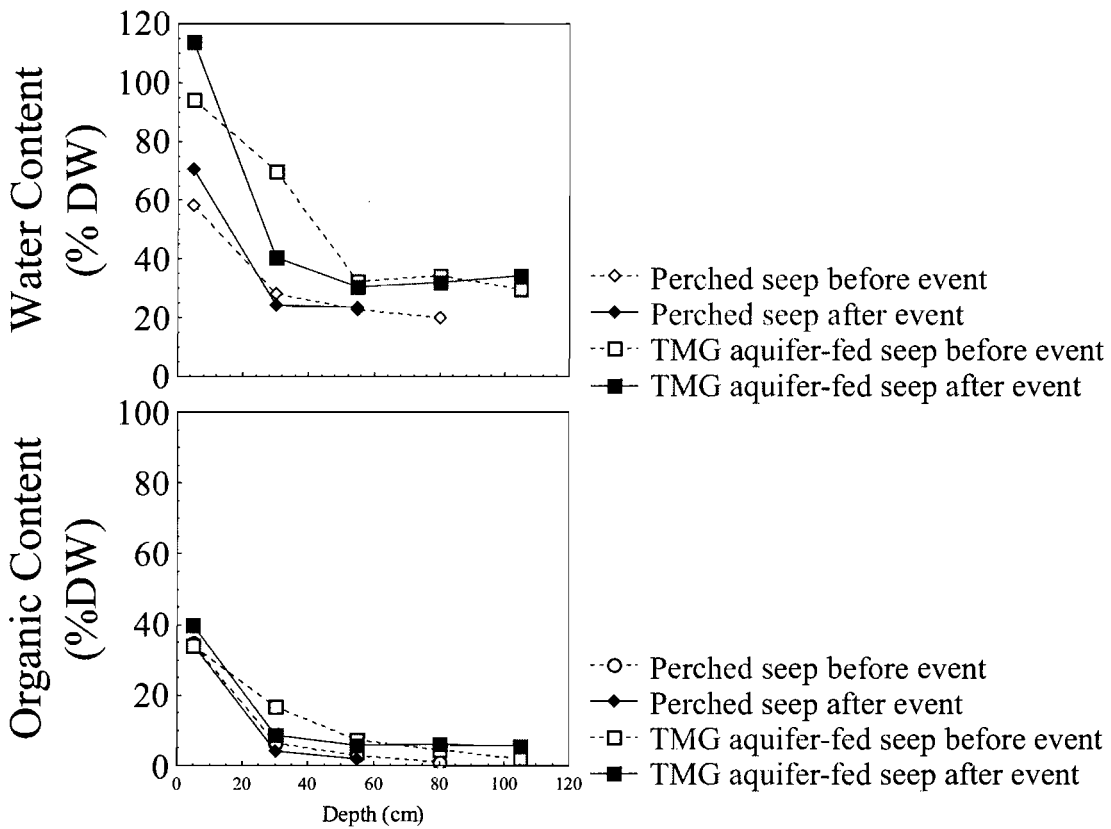


Fig 2.7. Variation in water and organic content of soil with depth at the perched and aquifer-fed seeps before and after a rainfall event. Contents are expressed as a percentage of the dry mass. A sample was taken from each soil layer up to and including the saturated zone.

Soil-water from near to the surface was always isotopically enriched relative to the underlying groundwater (see frames on the right in Figs 2.8 and 2.9). Prior to the rainfall event the soil-water at the perched seep tended to be isotopically enriched in the uppermost 10cm, below which it had a  $\delta^2\text{H}$  value similar to that of the underlying groundwater (Fig. 2.8). After the rainfall event, the soil-water in the uppermost 10cm became depleted and assumed the  $\delta^2\text{H}$  value of rain that fell on the seep during the event (Fig. 2.8). The stable isotope ratio of 10cm of soil-water sampled at a depth of 30cm remained essentially unchanged, while that at 55cm, which was now closest to the groundwater, became depleted to a similar degree to that of the groundwater (Fig. 2.8).

Before and after the rainfall event, *R. purpurascens* on the perched seep had the most isotopically enriched stem-water, and *P. cynaroides* the most depleted (Fig. 2.8). After the rainfall event all three species became isotopically enriched, with none of the plants sampled having stem-water more enriched than that of the antecedent rainfall (Fig. 2.8).

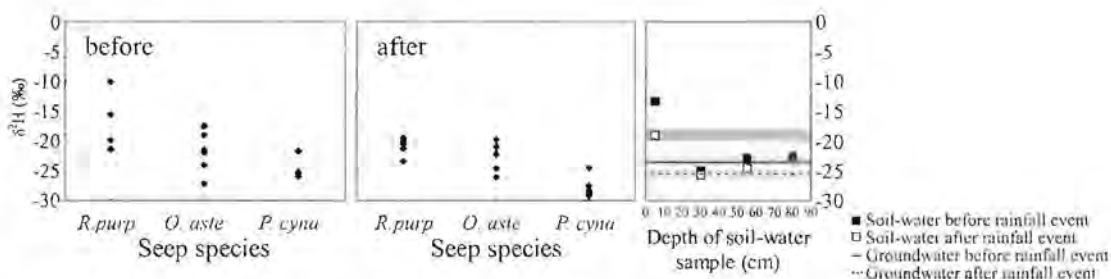


Fig 2.8. Variation in the  $\delta^2\text{H}$  of stem, soil- and ground water at the perched seep before and after a rainfall event. The frame on the right depicts the variation in the  $\delta^2\text{H}$  of soil-water before and after the same event. 10cm zones of soil were sampled at 25cm intervals down the soil profile up to and including the saturated zone. The  $\delta^2\text{H}$  value of rainfall is depicted by the darker bar.

After the rainfall event the soil-water in the uppermost 10cm of the TMG aquifer-fed seep became depleted, more so than that of the antecedent rainfall (Fig 2.9). The soil-water down the rest of the profile became enriched, moving towards the  $\delta^2\text{H}$  value of the antecedent rainfall except for that sampled within the saturated zone at 105cm which remained essentially unchanged (Fig 2.9).

Once again, more shallow-rooted species tended to have the most isotopically enriched and most variable stem-water (Fig 2.9). There was little change in the ratios of stable isotopes of the plants' stem-water (Fig 2.9). Before and after the rainfall all of the plants sampled had stem-water enriched relative to the underlying groundwater and even soil-water at as shallow as 30cm (Fig 2.9).

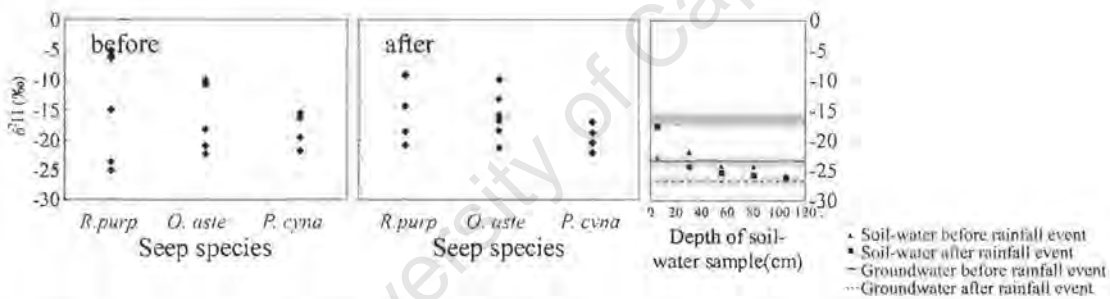


Fig 2.9 Variation in the  $\delta^2\text{H}$  of stem, soil- and ground water at the TMG aquifer-fed seep before and after a rainfall event. The frame on the right depicts the variation in the  $\delta^2\text{H}$  of soil-water before and after the same event. 10cm zones of soil were sampled at 25cm intervals down the soil profile up to and including the saturated zone. The  $\delta^2\text{H}$  value of rainfall is depicted by the darker bar.

## Discussion

My first two aims in this chapter were to examine aspects of plant water use at the landscape scale. If discrepancies in seasonal water potential and stem water stable isotope values exist then these tools may be of use in mapping the occurrence of aquifer-fed and perched systems across a landscape. There was no significant difference between the levels of predawn or midday water potential that were experienced by plants living on the two seeps, despite the fact that by the end of summer the water table at the perched seep had dropped below that of bedrock. Plants in the control, living adjacent to the perched seep had significantly lower xylem pressure potentials at midday than those living adjacent to the TMG aquifer-fed seep, presumably because it is cooler and wetter on top of the Perdeberg. Differences in microclimate are likely to be a factor reducing the amount of xylem pressure potentials experienced by plants living on the 'dry' perched seep i.e. if TMG aquifer-fed seep in the hotter, drier valley bottom were to dry up it is likely that the plants would have higher xylem pressure potentials. My results suggest that measurements of xylem pressure potential may have limited use in the identification of aquifer-fed seeps across a landscape.

Plants living adjacent to the two seeps generally experienced higher xylem pressure potentials than their on-seep counterparts. Aside from the amounts of xylem pressure potential experienced, off-seep plants also differed in the timing of when they were at their most stressed. Plants adjacent to the TMG aquifer-fed seep had the highest xylem pressure potentials in March, at the end of summer. Interestingly xylem pressure potentials in plants growing adjacent to the perched seep 'peaked' in

November. November was the driest month, with the longest dry periods. Perhaps even shallow-rooted plants living adjacent to the Kb valley seep still had access to groundwater derived from winter precipitation in November - water that was not available to them in March or adjacent to the perched seep. Direct precipitation of cloud-water is likely to be an important source of water to plants living on top of the Perdeberg. I tried to sample cloud-water but three versions of collectors were not able to withstand the powerful wind on top of the Perdeberg. Perhaps water stress of plants living adjacent to the perched seep was relieved by a supply of cloud-water in March.

Because of its extreme topography and close proximity to the ocean, the Kogelberg is relatively wet compared with much of Western Cape and receives fairly regular rainfall throughout summer and often has cloud covering the top of its mountains year-round. This could be one of the reasons, off-seep plants experienced relatively low xylem pressure potentials compared to values reported from studies in more arid areas (Miller *et. al.* 1983, Moll and Summerville 1985; Jacobsen *et. al.* 2007). The most negative of all the pressure potentials was the rather unimpressive -1.79 MPa reached by *C. tectorum* living adjacent to the Kb Valley seep in March. A number of other species sampled in the Kogelberg also had low (i.e. less than -2MPa) xylem pressure potentials (see Appendix 3). The fact that the Kogelberg landscape is so “unstressed” is interesting, especially considering the high plant diversity.

Perhaps the most interesting and pertinent result was that species of Proteaceae living adjacent to the two seeps experienced no increase in xylem pressure potentials throughout the summer. Pre-dawn levels remained constant at around -0.4MPa. A similar results, i.e. that deep-rooted Proteaceae did not experience any seasonal

increases in xylem pressure potential, have been reported in a number of other studies (Moll and Summerville, 1985; Richardson and Kruger, 1990; Miller *et al.*, 1983). My results suggest that the species of Proteaceae living adjacent to the seeps have a permanent supply of water throughout the summer. Their constant xylem pressure potentials are not due to them having to shut their stomata to avoid dangerously high levels water stress, i.e. they are not 'tolerating' heavy drought condition. The difference between pre-dawn and midday levels of xylem pressure potential remained constant at around 1MPa. Plants generated xylem pressure potentials throughout the day, they had not 'shut down', they were losing water. Should this be the case and these deep-rooted species have access to groundwater, it is likely to be *the same* water as that discharging closer to the surface on the nearby seeps. That deeper-rooted off seep species may also be dependent on the TMG aquifer has not been considered and dryland fynbos has not been included in the list of ecosystems threatened by abstraction from the TMG aquifer (Le Maitre *et al.*, 2002). Furthermore, should the active roots of deeper rooted fynbos species span a gradient in soil water potentials, there is no reason why 'hydraulic lift' should not occur (Dawson, 1993; Caldwell *et al.*, 1998) creating indirect dependencies from more shallow-rooted species on water in the TMG aquifer.

The plants at the end of summer with the highest xylem pressure potentials were the Restionaceae living adjacent to the Kb Valley seep. These shallow-rooted plants have less access to soil-water, only being able to reach water in drier, surficial soil-layers. A number of the sampled Restionaceae had isotopically enriched stem water with a stable isotope composition similar to that of the 8.6mm of rain that fell in the preceding rainfall event (Fig. 2.6), suggesting that they had taken this water up. There

was large variation in the stable isotope composition of restio stem water, from the values as positive as the prior rainfall event to values as low as that sourced by the deeper rooted asteraceous species (Fig. 2.6). This is perhaps as a result of high levels of small-scale spatial heterogeneity in the  $\delta^2\text{H}$  of soil-water in the upper soil layer, perhaps in part due to the hydraulic lifting of more isotopically heavy water from deeper down the soil profile.

The deeper rooted *P. prolifera* had higher xylem pressure potentials than its on-seep analogue, yet it did not appear to be utilising recent rainfall. Its stem-water was more isotopically depleted, presumably sourced from greater depths, and more closely approximated the value of winter rainfall. The smaller difference between isotopic ratio of the stem water of the shallower rooted Restionaceae and Ericaceae species living on the seep suggests that they utilise soil-water from similar depths. This is not surprising since the fact that the bedrock on the seeps is close to the surface (less than 1.7m on both seeps) constraining rooting depth.

Though the difference is statistically insignificant, plants on the perched seep had stem water that was isotopically enriched compared to plants living on the TMG aquifer-fed seep. Samples of groundwater could not be collected from the perched seep throughout the summer, as the water level had dropped to bedrock. However, all of the species sampled - both on and adjacent to the seep - had stem water enriched relative to the same species living near the Kb valley seep, more similar to that of recent rainfall. In winter, when rainfall is isotopically light, *O. asteriscoides* sampled on the perched seep had stem-water that was depleted in  $^2\text{H}$  (compare  $\delta^2\text{H}$  values in Fig. 2.6 to those in Fig. 2.9). This increased variability in the ratio of plant stem-water

is likely a result of the perched seep being connected to a relatively small groundwater body, and therefore not as well ‘buffered’ against recent rainfall inputs as the TMG aquifer-fed seep (see Chapter 1). Nonetheless, at the end of summer when the seeps water level had dropped to bedrock there is still an apparent abundance of water at the top of the Perdeberg, judging by how “un-stressed” the plants are. Even though isotope data from the end of summer plant stem water sampling was insignificant the data followed the expected trend, i.e. plants on the perched seep having isotopically heavier stem-water than those living on the TMG aquifer-fed seep. This sampling approach may have potential in identifying seeps dependent on the TMG aquifer across a landscape, especially if the ‘sampling sweep’ is preceded by isotopically heavier rainfall than that which fell in the event prior to my plant sampling.

Changes in the isotopic ratio of soil water with depth on the seeps in winter were much less obvious than those reported in other studies (Busch *et al.*, 1992; Meinzer *et al.*, 1999), which is likely to be as a result of the water table being too close to the surface to allow for the establishment of a large soil-water fractionation gradient. It was only the uppermost fraction of the soil that was ever significantly fractionated relative to that of the underlying groundwater. As expected, deeper-rooted species tended to have isotopically depleted stem-water with stem-water from *P. cynaroides* the most isotopically similar to that of the underlying groundwater. However, nearly all of the plants sampled had stem-water that was more enriched than soil-water deeper than 30cm. This suggests that, while deeper rooted plants may use mainly ‘deep’ water, the vast majority of a seep community utilizes at least some water from very close to the surface. The large amounts of organic material in the uppermost soil layer of the seeps is likely to contribute to the layer’s high water content – a lot of this

soil-water was not stored in a soil matrix, but rather within damp organic material. Plants absorb water from roots as close to their stem as possible – my data suggests that they are doing just that, utilising water from right at the surface despite there being a saturated zone less than a metre further down the soil profile. The more fractionated surficial soil-water at the perched seep appeared to no longer be utilised after the rainfall event. None of the plants sampled had stem water that was more fractionated than that of the rainfall which fell during the event. After the rainfall event the plants on the Kogelberg had stem water that was more fractionated than that of the rain that fell. This may be as a result of the fact that it is a lot hotter and drier in the base of the Palmiet River valley than it is on the top of the Perdeberg. Surficial soil-water may therefore become fractionated by evaporation more quickly, resulting in shallow-plants having stem water enriched in deuterium.

## Conclusion

Plants living on a seep fed by the TMG aquifer experienced no increase in xylem pressure potentials throughout a summer. These permanently wet habitats may have been a feature of the sandstone mountains of the Cape for tens of millions of years. These unique localised habitats, as well as the apparent more widespread water supply, may have played a significant role in the evolution of the Fynbos Kingdom by allowing seep species sensitive to water stress to survive periods of drought.

The fact that plants living on the perched seep were no more stressed than those living on the permanently wet seep fed by the TMG aquifer limits the potential of water

potential measurements as a tool to differentiate between perched and aquifer-fed seeps, at least in the Kogelberg. It may be useful as a tool in more arid areas or across areas with smaller variations in microclimate.

Though differences were insignificant, plants living on the perched seep did appear to track the seasonal variation in the  $\delta^2\text{H}$  of rainfall more than those plants living on the aquifer-fed seep. Stable isotopes therefore appear to have potential as a tool to determine which seeps across a landscape are perched and connected to relatively small bodies of groundwater.

At the local scale it was interesting that all of the plants on a seep appear to utilise water from very close to the surface. *Protea* species living on the seeps had stem water that was less fractionated than the other species, suggesting that they source the bulk of their water from greater depths, despite bedrock being so close to the surface. The sandstone in the Kogelberg, and over much of the Western Cape, is extensively fractured. It is likely that bedrock is therefore often likely to be penetrable to plant roots. The xylem pressure potential data can be interpreted as suggesting that the Asteraceae and Proteaceae species sampled on the perched seep have roots extending deeper than the surface of the bedrock. Even though the water level at the seep dropped to below bedrock these plants did not experience an increase in xylem pressure potential. Deeper rooted species with their roots growing down these cracks are likely to be less sensitive to drops in the water level of the seep than more shallow rooted species.

Perhaps the most unexpected and interesting result from this study was that some

deep-rooted plants in the Kogelberg appeared to have similar amounts of access to groundwater, despite living in 'dryland' parts of the landscape. Apparent permanent access to water has been reported in Proteas in a number of other studies ((Moll and Summerville, 1985; Richardson and Kruger, 1990; Miller *et al.*, 1990)) conducted in more arid parts of the Western Cape. It may be possible that at low altitudes in the fractured sandstone mountains in the Cape, Proteas may be able to get their roots deep enough down cracks to reach the regional water table, representing the surface of the TMG aquifer in areas where the aquifer is unconfined. Should this be the case, then the ecological impacts associated with abstraction may be more significant than has been anticipated, extending beyond localised river and wetland systems out into dryland Fynbos. If this is the case then the impacts of abstraction of water from the TMG aquifer may have wider than anticipated consequences for fynbos ecosystems and landscape ecology.

# Chapter three –Xylem anatomy and water stress tolerance of seven seep- and four dryland species from the fynbos

## Abstract

The primary aim of this chapter was to identify seep plant species that are particularly sensitive to drought stress, species that may be restricted to permanently wet habitats fed by the TMG aquifer. Eleven species were studied, of which seven were seep species (*Leucadendron salicifolium*, *Mimetes hirtus*, *Erica perspicua*, *Witsenia maura*, *Osmotopsis asteriscoides*, *Brunia alopecuroides* and *Psoralea pinnata*) - and four were dryland species (*Leucadendron argenteum*, *Leucadendron xanthoconas*, *Mimetes cucullatus* and *Erica baccans*). Vulnerability curves were compiled for each of the species to determine the susceptibility of their xylem to water stress induced failure. The second aim was to assess the use of more easily measured physiological correlates with vulnerability that could be used to screen a wider range of vulnerable seep species. Minimum seasonal pressure potential ( $P_{\min}$ ), mean hydraulic vessel diameter ( $d_h$ ), xylem specific conductivity ( $K_s$ ), xylem density and theoretical implosion pressure ( $((t/b)_h)^2$ ) were measured.

*Mimetes hirtus* appeared to be the most drought sensitive species. It had the steepest vulnerability curve, losing a high percentage of its conductivity under relatively low

levels of xylem pressure potential. It also had the lowest  $((t/b)_h^2)$  suggesting that it is also the most sensitive to water stress induced vessel implosion. The fact that this species appears to be so sensitive to drought stress may well result in it being confined to permanently wet habitats, such as those supported by the TMG aquifer.

Contrary to my expectation, there was little apparent difference in vulnerability curves between seep- and related dryland species. Each of the three genera sampled had vulnerability curves with a distinctive shape, suggesting that the vulnerability of a species xylem to water stress induced failure is phylogenetically constrained. If so, this may limit the potential of vulnerability curves used in isolation to compare interspecific differences in drought sensitivity.

Correlates between all of the measured variables were small, probably as a result of the small range in vulnerability of the sampled species. When plotted alongside data from other studies it becomes apparent that even the dryland fynbos species in this study appear to be relatively sensitive to drought stress. This prompted a comparison between fynbos and chaparral in terms of the one correlate with drought stress, ( $P_{min}$ ), for which there is a large data set. On average Fynbos was significantly ( $p < 0.01$ ) more sensitive to drought stress than chaparral and may therefore be more severely impacted upon by climate change.

## Introduction

A Mediterranean climate, characteristic of the Fynbos biome, is characterised by a degree of summer drought, which can result in a seasonal increase in plant water stress in shallow rooted species (Moll and Sommerville, 1985). Fynbos plants have to cope with this stress and withstand the negative pressures that develop in their xylem in order to continue to provide water to their evergreen leaves. However, within the Fynbos are small mountain wetlands or seeps, which receive a permanent supply of water (Chapter 1), with seep species experiencing no seasonal increase in xylem pressure potentials (Chapter 2). Coping with water stress has a cost to the plant, in terms of both the carbon cost per unit conductive tissue (Hacke *et al.*, 2001) as well as a decline in conductive efficiency when soil moisture is high (Zimmermann, 1983; Tyree *et al.*, 1994; Alder *et al.*, 1996). Plants with access to a permanent supply of water may have evolved extremely vulnerable, but very efficient hydraulic architectures. Many of the permanently wet habitats in the Western Cape are likely to be connected to the TMG aquifer from which the City of Cape Town is considering abstracting water. The ultimate aim of this chapter was to identify plant species that are extremely vulnerable to drought stress. These species may be restricted to permanently wet habitats, likely to be fed by the TMG aquifer. Their distribution could be used to assist in the mapping of permanently wet habitats. Species relatively vulnerable to water stress are likely to be the first impacted by declining water levels (Froend and Drake, 2006). More vulnerable species should therefore be the ones most closely monitored during abstraction. The second aim of this chapter was to assess the use of physiological correlates with drought tolerance that could be used to screen a wider range of vulnerable seep species.

## **Plant hydraulic theory**

The need to exchange gasses during photosynthesis means that the loss of water as plants fix carbon is unavoidable. The soil-plant-atmosphere continuum is the pathway through which transpired water leaves ecosystems. Pressure potentials generated by the loss of water from leaves are transmitted through a water column under tension to the roots. If the water potential in the root decreases to below that of ambient soil water absorption will take place (Lambers *et al.*, 1998). The water that is under tension in xylem is susceptible to cavitation - a rapid phase change to vapour which can severely limit plant water transport (Zimmermann, 1983; Sperry *et al.*, 1988; Lambers *et al.*, 1998). The amount of tension, or 'negative pressure', that a plant can withstand before its vessels begin to cavitate is dependent on the physical structure of the conduits conducting the water to the leaf. In angiosperms water is conducted along vessels set in a network of fibres. A vessel is comprised of a catena of vessel elements stacked end to end (Zimmermann, 1983). Vessels are connected via pits in their cell walls which are covered with a mesh known as a pit membrane (Zimmermann, 1983). When intact, pit membranes are permeable to water but impermeable to air and serve to compartmentalise the plant water transport system, isolating emboli resulting from cavitation away from functioning vessels (Sperry and Saliendra, 1994; Tyree *et al.*, 1994). Should a vessel become cavitated the air-water meniscus will be drawn onto its pit membranes and broken up into smaller menisci, the size of which is dependent on the size of pores in the pit membrane. Smaller pores result in smaller menisci, which are able to withstand greater pressure gradients (i.e. more negative pressures in plants) before they fail. Should a membrane fail, air will be drawn into the adjacent vessels where the air bubble will be rapidly expanded. The 'air-seeding

hypothesis' (Zimmermann, 1983) has been extensively tested (see Cochard *et al.*, 1992; Sperry and Saliendra, 1994; Holbrook *et al.*, 1995 and Sperry *et al.*, 1996) and is generally accepted as the mechanism by which decreasing xylem water pressures bring about xylem failure. The size of the pores in pit membranes determine the amount of negative pressure that a species xylem can sustain and directly set the limit of soil dryness (i.e. soil water potential) from which a species is able to extract water.

The lower the transport resistance, the greater the volume of water that can flow up a plant under a given pressure gradient and the greater the capacity for rapid carbon fixation. There are two components to transport resistance; resistance from the pit membranes and resistance from the sides of the vessels. Vessels range in diameter from a maximum of around 0.5 mm (Tyree and Zimmermann, 2002) to a minimum of around 20  $\mu\text{m}$  (Sperry *et al.*, 2006). Pores in pit membranes are much smaller and range from around 50 nm to sizes too small to be visible even when using SEM (Choat *et al.*, 2006). Resistance to flow through a pore is much greater than through a vessel but the distance travelled through a pore makes up a tiny fraction of the total distance water travels in its passage through the plant. Sperry *et al.* (2005) found that end wall resistance accounted for an average of 54% of the total resistance, a result confirmed across a wide range of angiosperm species (Wheeler *et al.*, 2005; Hacke *et al.*, 2006 ). Another recent study where pit membrane resistance was measured directly, suggests that the pit membrane component may be higher, accounting for more than 80% of the total resistance for two species (Choat *et al.*, 2006). The relative contribution that pit and vessel resistance have to total transport resistance and how this may vary between species is unclear.

Though the air seeding hypothesis does not provide a mechanistic reason why a larger (i.e. longer and wider), less resistive vessel should be more vulnerable to cavitation and there is a weak, but significant relationship between vulnerability to cavitation and vessel diameter, vessel size is still an important component of a plants vulnerability to water stress. This is because that having lots of small vessels provides a plant with a greater degree of redundancy, the loss of a vessel through cavitation will be more severe and result in a greater decline in conductance for a species with few large vessels (Zimmermann, 1983).

### **Measuring plant water stress tolerance**

The fact that pit membranes are so tiny means that their structure can only be studied directly using scanning electron microscopy (e.g. Jarbeau *et al.*, 1995; Choat *et al.*, 2006) which is time consuming and makes it difficult to measure meaningful sample sizes. Vessel width, which correlates with vessel length (though  $p > 0.46$  - Sperry *et al.*, 2006), is easy to measure though there are complications with the reporting of a hydraulically meaningful mean (Tyree and Zimmermann, 2002). Vessel size is independent of pit membrane pore size but a larger vessel is likely to have a larger area of pit membranes. As the area of pit membrane increases so does the likelihood of a vessel having a pit membrane that has slightly larger pore size. This is known as the 'pit area' hypothesis and has been put forward as the explanation as to why larger vessels tend to be more vulnerable to cavitation (Sperry *et al.* 2006). While correlations of vessel size and water stress tolerance across 60 taxa may be significant, the relationship is still weak (Tyree *et al.*, 1994) which limits the predictive capacity of vessel size measurements.

An integrated measure of water stress tolerance can be obtained by determining the reduction in xylem conductance resulting from an increase in water stress. A 'vulnerability curve' is a plot of percentage loss of conductance (PLC) against the Xylem Pressure Potential ( $\psi_{xp}$ ) that induced the loss of conductivity (Tyree *et al.*, 1994). Species adapted to an abundant water supply may be expected to maximise conductance by having few wide vessels with relatively wide pores. Maximising conductivity comes at the expense of safety from cavitation. Such species would have steep vulnerability curves shifted towards less negative  $\psi_{xp}$ 's. The position of a vulnerability curve on the  $\psi_{xp}$  axis is often reported as  $P_{50}$ , the pressure corresponding to a 50% loss in conductivity. More vulnerable species will have a vulnerability curve shifted towards less negative  $\psi_{xp}$ 's and a higher, less negative  $P_{50}$ .

### **Physiological correlates with vulnerability curves**

A number of more easily measured physiological variables have been found to correlate with  $P_{50}$ . Minimum seasonal water potentials ( $P_{min}$  - midday  $\psi_{xp}$ 's during the time of year when plants are most stressed) have been found to correlate fairly well with plant water stress tolerance ( $P_{50}$ ) across a wide range of values (Hacke *et al.*, 2000, Sperry and Hacke 2002).

In addition to having pit membranes able to withstand significant levels of negative pressures, vessels also need to have walls reinforced enough to prevent vessel implosion. There is no point in a species having small pored, resistive pit membranes if vessels implode before they cavitate. Conversely there is no point in having 'over built', very well reinforced vessels, which cost more carbon to construct, if a species'

pit membranes are unable to withstand any significant levels of negative pressure. An estimate of vessel implosion resistance is the thickness to span ratio  $((t/b)_h)^2$  - Hacke *et al.*, 2001) which is the square of intervessel wall thickness ( $t$ ) divided by the lumen diameter ( $b$ ).  $(t/b)_h^2$  has been found to correlate with  $P_{50}$  across a wide range of water stress tolerance (Hacke *et al.*, 2001).

A species  $(t/b)_h^2$  appears to be uncoupled from the  $(t/b)_h^2$  of its fibres which often make up the vast majority of the volume of its xylem (Jacobsen *et al.*, 2005), whether this is as a result of the development of xylem or has a mechanistic cause as the authors suggest is unclear. However, as a result xylem density (wood density after removal of the bark and pith) correlates with  $(t/b)_h^2$  (Hacke *et al.*, 2001) and therefore  $P_{50}$ . Xylem density has the advantage that it is very easy to measure, and unlike  $P_{min}$ , measurements can be carried out at any time of the year.

These variables ( $P_{min}$ ,  $(t/b)_h^2$  and xylem density) were measured for the same stems used to compile vulnerability curves to determine their potential for screening species for susceptibility to drought stress.

## Materials and methods

Xylem traits were measured in 11 Fynbos species. Measurements were made of three sets of paired seep and dryland species (*Leucadendron argenteum*, *Leucadendron xanthoconus* and the seep species *Leucadendron salicifolium*, *Mimetes cucullatus* and the seep species *Mimetes hirtus* and *Erica baccans* and the seep species *Erica perspicua*. Four other seep species were also measured - *Witsenia maura* and *Psoralea pinnata* were thought to be confined to wetter areas while *Osmotopsis asteriscoides* and *Brunia alopecuroides* are more widespread. With the exception of *W. maura* and *E. baccans*, which were collected from Silvermine (34°8' 15.63" S, 18°19' 47.3" E) and the slopes above UCT, respectively, all species were collected from within, and nearby, the Kogelberg biosphere reserve (34°28.553'S, 18°50.119' E).

## Vulnerability curves

Plants have evolved in such a way that their more expendable parts are the most sensitive to water stress induced hydraulic failure (Tyree and Zimmermann, 2002). Roots, which are relatively 'cheap' and easily regenerated are typically the most sensitive to water stress (i.e. they tend to have shallower vulnerability curves with higher, less negative  $P_{50}$  values) (Alder *et al.*, 1996). They will therefore be the first to cavitate should a plant experience increasing levels of drought stress. This adaptation has the advantage of effectively insulating the plant from lower water potentials in dryer soil horizons. Shoot tips are also more vulnerable than the main stem of a plant. This is important to bear in mind considering that for practical reasons the vast

majority of vulnerability curves in the literature, and in the ones in this study, are constructed from measurements taken from relatively thin (typically less than a cm diameter) more apical parts of a plant. Vulnerability curves are therefore likely to overestimate the levels of water stress that plants can tolerate. Nonetheless, the loss of branches would be catastrophic if the plant did not have sufficient carbon stored to replace them. Therefore, while curves drawn up from branches may overestimate a plant's absolute water stress tolerance, they are still of use in comparing intra-specific vulnerability to drought stress (Hacke *et al.*, 2000).

Stems, 142 mm long with a diameter of between 6-12mm, with at least 600 mm of plant material on either side were collected from the field, tightly wrapped in cling wrap and transported back to the lab in a cooler box. To avoid air from being pulled into the xylem, sections were cut back under water to slightly longer than the required length. The ends were then trimmed with a fresh razor blade to obtain a straight, unbranched, 142mm stem section. Vulnerability curves are plots of stress induced reduction in conductivity from a maximum. To obtain this maximum conductivity, stems were flushed with a deionised, degassed (20min), filtered (1.2 $\mu$ m) weak HCl solution (adjusted to a pH of less than 2 to prevent microbial growth) at 150 kPa for an hour to remove all embolisms.

Vulnerability curves were compiled using the centrifuge technique (Alder *et al.*, 1997) with six stems spun per species. Stems were spun in a centrifuge around their central axis in a custom built rotor with cups designed to keep the ends of the stem immersed in HCl solution. The rotation of a stem creates a water potential gradient, with pressures at the midpoint being dependent on the length of the stem as well as

the speed at which it is spun. Flushed stems were loaded into the rotor and spun for four minutes at a speed corresponding to the establishment of specific  $\psi_{xp}$  (for a detailed description of the wetlab see Appendix 3). Stems were then connected to a high resolution flow-meter with a low pressure head (<1 kPa) to prevent the flushing out of embolisms (Sperry *et al.*, 1988). Flow ( $\text{g s}^{-1}$ ) through the stem was measured as mass increments of solution every ten seconds (0.01mg resolution). These readings were converted into a volumetric measure ( $\text{cm}^2 \text{s}^{-1}$ ) taking into account the temperature. Measurements taken immediately after stems were removed from the centrifuge were found to be inconsistent. Stems were therefore placed in a degassed filtered HCl solution for at least 4 minutes to allow water potentials to equilibrate. Stems were spun at increasingly higher speeds corresponding to 0.5MPa increments, until conductance dropped to zero. Stems were then reflushed at 150 kPa and stained with safranin under low pressure (<1kPa) to determine the active xylem area. Active xylem area ( $\text{mm}^2$ ) was used to express the conductance on the active area basis ( $\text{Ks, mm}^2 \text{kPa}^{-1} \text{s}^{-1}$ ) commonly reported in the literature (Tyree *et al.*, 1994).

Data were then fitted to an exponential sigmoidal equation:

$$\text{PLC} = 100 / ( 1 + \exp (a(\psi_{xp} - b))),$$

where PLC is the percent loss in conductivity from a flushed maximum,  $\psi_{xp}$  xylem pressure potential and  $a$  and  $b$ , two constants (Pammenter and vander Willigen, 1998). Coefficient  $a$  reflects the slope of the vulnerability curve (i.e. the range over which vessels cavitate) and  $b$  represents its position on the water potential axis and corresponds to  $P_{50}$  (Pammenter and vander Willigen, 1998).

## **Minimum seasonal xylem pressure potentials ( $P_{\min}$ )**

Minimum seasonal  $\psi_{xp}$  ( $P_{\min}$ ) were determined at midday in February and March of 2006 using a Scholander pressure chamber (PMS Instrument Company, Corvallis, Oregon, USA; Scholander *et al.*, 1965). Xylem pressure potentials were measured on leafy twigs of seven plants per species. Samples were loaded into the pressure chamber as quickly as possible and a 10X magnifying glass was used to watch for the wetting of the stem surface.

## **Xylem density**

The xylem density of the stems used in the drawing up of vulnerability curves was determined as the dry mass per fresh volume of wood with the pith and bark removed. Portions of the stems used in the plotting of vulnerability curves (n=6) were saturated in a degassed, filtered HCl solution and the volume of xylem determined using water displacement in a 5ml graduated cylinder. Stems were then dried at 105°C for 36hrs and their dry mass determined (Jacobsen *et al.*, 2007).

## **Hydraulic vessel diameter ( $d_h$ ) and vessel implosion resistance $(t/b)_h^2$**

$(t/b)_h^2$  and  $d_h$  were determined for the same stem sections used in the drawing up of vulnerability curves. 20 $\mu$ m cross sections were taken from the stems used in the plotting of vulnerability curves (n=6) using a base sledge microtome (Reichert-jung,

Austria) using a blade sharpened at 35° and set at 15°. Sections were left immersed in a 0.25% safranin solution for at least 20min, carefully placed on a slide then rinsed with a glycerine and glycerol solution. The slide was then mounted with a cover slip, and a wedge shaped section from pith to bark large enough to include at least 100 vessels demarcated using a fine tipped pen. All of the vessels within this wedge were then photographed at 400X magnification (Leitz, laborlux k, Germany) with a digital camera (4 mega pixel Nikon Coolpix 4500, Japan). A blue filter was used to increase definition. Photographs of a graticule were taken prior to each series of photos to be used in their calibration. Measurements were made using digital image analysis software (SigmaScan Pro, version 5.0.0). The images had a large amount of contrast between the red stained vessel walls and their opaque lumen, making it possible to use the 'paint bucket' tool to quickly and accurately measure their lumen. The ferrit diameter (the diameter of the vessel were it a perfect circle) of all the vessels in the wedge were measured. According to the Hagen Pousellie equation, flow rate is proportional to the fourth power of the radius of a capillary (Zimmermann, 1983). Large vessels are therefore are of a great deal more conductive significance than smaller vessels. Larger vessels were weighted by expressing vessel diameter as the mean hydraulic diameter ( $d_h$ )

$$(d_h) = \frac{\sum d^5}{\sum d^4}$$

where  $d$  represents the lumen diameter. All of the vessels within the sampled wedge shaped section were measured with six stems sampled per species (n=6).

The vessel implosion resistance  $((t/b)_h^2)$  was determined for 10 randomly chosen vessels within the wedge that were a member of a vessel pair and within  $5\mu\text{m}$  of the calculated  $d_h$ .  $t$ , the combined wall thickness of adjoining vessels and  $b$  the lumen diameter were measured. Vessel pairs can be rare in diffuse porous species. For example, despite sampling an average of 293 vessels in each wedge shaped sector, it was not possible to find 10 vessels within  $5\mu\text{m}$  of  $d_h$  that were a member of a pair for *E. baccans*. For this species the thickness of the combined wall thickness between vessels and the lumen of adjacent fibres was reported.

# Results

## **Vulnerability curves**

Species in the same genera had similar shaped curves, regardless as to whether or not they are seep species (Fig. 3.1). Genera appeared to have distinctly shaped curves. *Mimetes* species had steep, short curves with conductance dropping from 30 percent loss at -0.5 MPa to nearly 100 % loss by -2.5 MPa (Fig. 3.1). *Leucadendron* species had flatter, wider curves with PLC gradually increasing over a broad range of XPP's, with total loss of conductance occurring at XPP's of around -0.4 MPa. *Erica* species had flat, wide curves and had lost more than 50 % of their conductance at XPP's of 0.5 MPa, After this rapid initial decline, their conductance gradually decreased until they had reached total loss of conductance by 4 MPa. The similarities in the shapes of seep and dryland species in the same genera translated into related species having similar  $P_{50}$  values (Fig. 3.4)

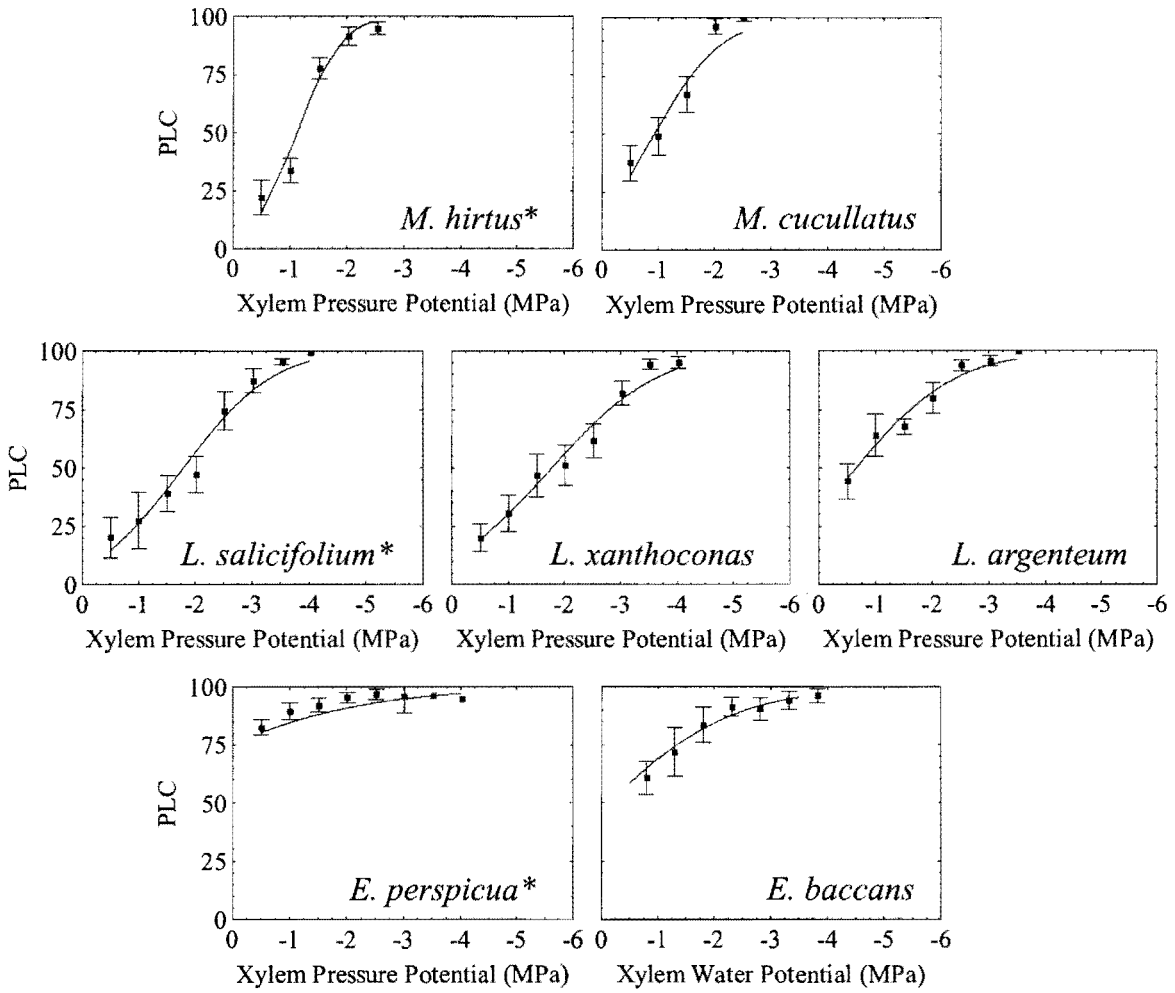


Fig. 3.1. Vulnerability curves for fynbos seep species (marked with an asterisk) and analogous dryland species (mean  $\pm$  SE,  $n=6$ )

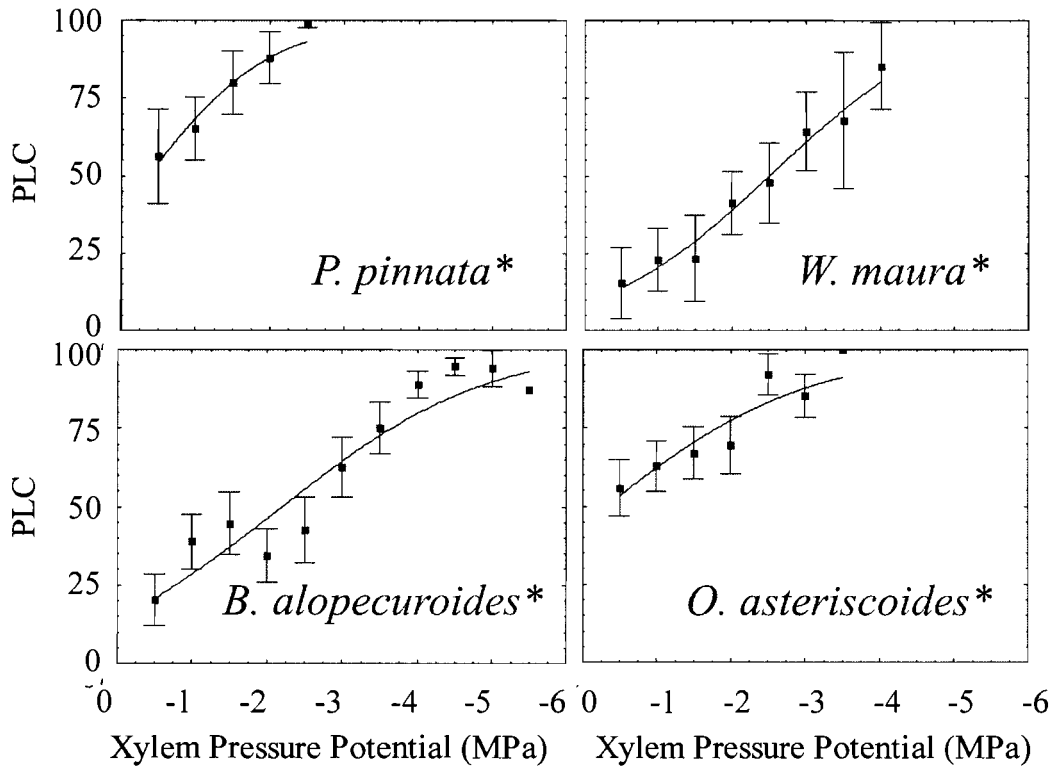


Fig. 3.2. Vulnerability curves showing the percentage loss of conductance (PLC) vs. xylem water potential for four fynbos seep species (mean  $\pm$  1SE,  $n=6$ )

There is variability in the shape of the vulnerability curves of seep fynbos species (Figs 3.1 and 3.2). Some species, such as *M. hirtus* and *P. pinnata*, have very steep, short curves and experience large declines in conductivity at relatively low XPP's. *B. alopecuroides* had the flattest, widest curve and had relatively high levels of conductivity at high XPP's. At -3 MPa approximately only 62% of *B. alopecuroides*' xylem had cavitated whereas *M. hirtus* and *P. pinnata* had experienced total xylem failure i.e. 100% loss of conductance. The wider error bars associated with the curves for *W. maura* are as a result of its low flows (see  $K_s$  graph in Fig 3.4) typical of monocots.

## Minimum seasonal xylem pressure potentials ( $P_{\min}$ )

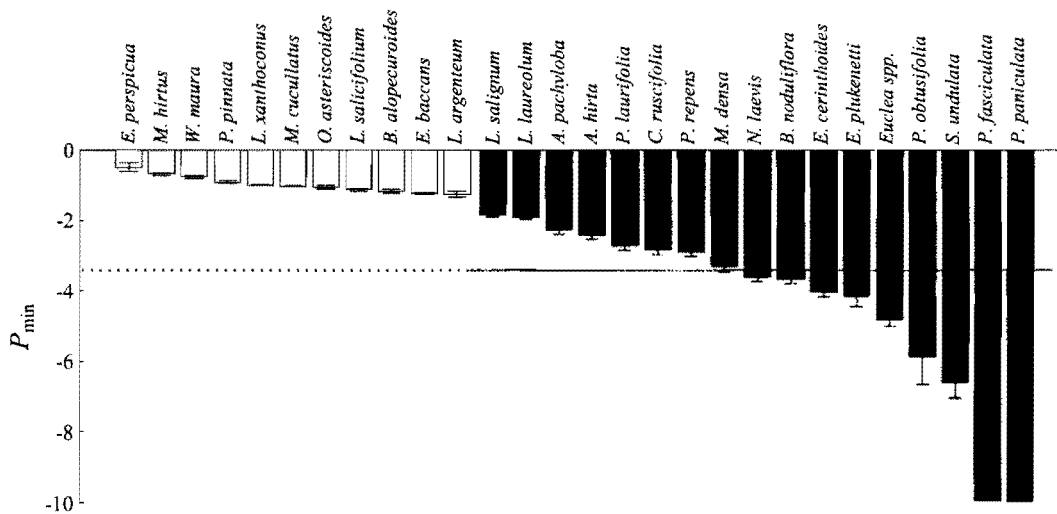


Fig. 3.3. Minimum seasonal pressure potentials ( $P_{\min}$ ) measured from this study (open bars) and those reported in Jacobsen *et al.*, 2007 (solid bars) ( $\pm 1SE$ ;  $n = 7$  for open bars and 5-12 for solid bars). Pressure potentials for *P. fasciculata* and *P. paniculata* were greater than could be measured and therefore error bars are missing. The line represents the mean  $P_{\min}$  for species (excluding *P. fasciculata* and *P. paniculata*) reported in Jacobsen *et al.*, 2007).

Minimum seasonal water potentials ( $P_{\min}$ ) were low for all species, regardless of whether or not they were seep species (Fig.3.3), ranging from a high of -0.5MPa for *E. perspicua* to a low of -1.25MPa for *L. argenteum*. Seep species tended to be less stressed than their dryland analogues. However this was not always the case with *L. xanthoconus* being significantly ( $t=3.24$ ,  $p=0.009$ ) less stressed than *L. salicifolium*.  $P_{\min}$  values were a great deal higher than those reported from the arid end of the fynbos spectrum in Jacobsen *et al.* (2007) (compare my data with the mean reported in Fig 3.3).

**Xylem density, Hydraulic vessel diameter ( $d_h$ ) and vessel implosion resistance  $((t/b)_h)^2$**

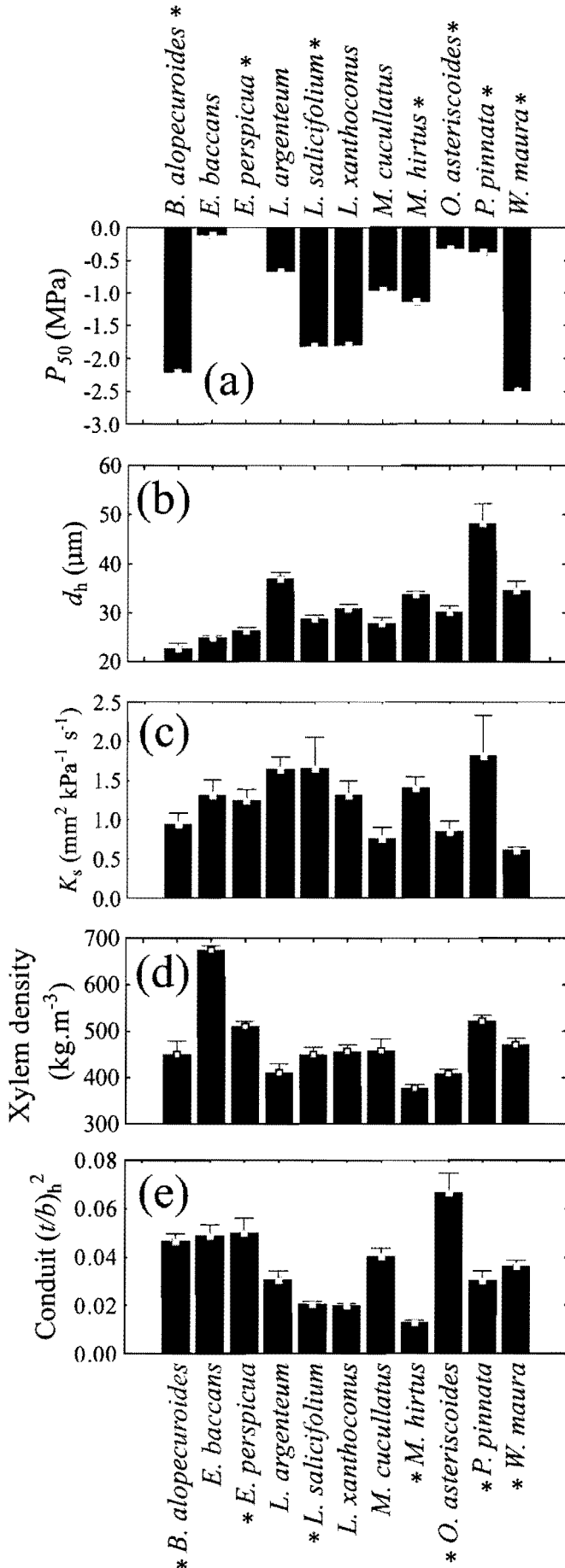


Fig. 3.4. Summary of mean ( $\pm$ SE)  $P_{50}$ ,  $d_h$ ,  $K_s$ , xylem density and  $((t/b)_h)^2$  data for 11 Fynbos species. Seep species are marked with an asterisk. (a) The xylem water potential that results in a 50% loss of stem conductivity. The vulnerability curve of *E. perspicua* was too steep for  $P_{50}$  to be measured (see fig 3.1). (b) Mean hydraulic diameter. (c) maximum xylem specific conductivity. (d) Xylem density. (e) an estimate of vessel implosion resistance  $((t/b)_h)^2$ .

$P_{\min}$	$P_{50}$	xylem density	$d_h$	$(t/b)_h^2$	
$r^2=0.12$ $p=0.71$	$r^2=0.13$ $p=0.30$	$r^2=0.01$ $p=0.84$	$r^2=0.24$ $p=0.13$	$r^2=0.28$ $p=0.10$	$K_s$
	$r^2=0.08$ $p=0.44$	$r^2=0.02$ $p=0.67$	$r^2=0.02$ $p=0.64$	$r^2=0.01$ $p=0.73$	$P_{\min}$
		$r^2=0.14$ $p=0.29$	$r^2=0.07$ $p=0.45$	$r^2=0.11$ $p=0.35$	$P_{50}$
			$r^2=0.03$ $p=0.63$	$r^2=0.13$ $p=0.28$	xylem density
				$r^2=0.15$ $p=0.24$	$d_h$

Table 3.1. The coefficients of determination ( $r^2$ ) and probability values (p) for regressions between different traits

Mean hydraulic vessel diameters,  $d_h$ , varied greatly amongst the 11 species (Fig 3.4) from a high of 48.2 $\mu\text{m}$  for *P. pinnata* to a low of 22.7 $\mu\text{m}$  for *B. alopecuroides*. Seep species tended to have higher  $d_h$  values aside from *L. salicifolium* which had narrower vessels than both of its dryland analogues.

Xylem specific conductivities varied from a high of 1.82  $\text{mm}^2 \text{kPa}^{-1} \text{s}^{-1}$  for *P. pinnata* to a low of 0.60  $\text{mm}^2 \text{kPa}^{-1} \text{s}^{-1}$  for *W. maura*, a monocot. There was no statistical difference between seep and dryland species of Erica ( $t=-1.00$ ,  $p=0.34$  or between analogous Leucadendron species ( $t=-1.57$ ,  $p=0.15$  – between *L. xanthoconas* and *L. salicifolium*). *L. salicifolium* had higher  $K_s$  values than *L. xanthoconas* ( $t=1.25$ ,  $p=0.24$ ) and were similar to those of *L. argenteum* (Fig 3.4). The mean  $K_s$  value of *M. hirtus* was almost double that of its dryland analogue *M. cucullatus*, differences were however statistically insignificant ( $t=-1.80$ ,  $p=0.1$ ).

Xylem density varied amongst species from a high of 674  $\text{kg m}^{-3}$  for *E. baccans* to a low of 378  $\text{kg m}^{-3}$  for *M. hirtus*. Seep species tended to have significantly ( $t=10.28$ ,  $p<0.001$  for the Ericas;  $t=2.94$ ,  $p=0.01$  for Mimetes) less dense wood than their dryland analogues. However, while *L. salicifolium* had slightly less dense wood than

*L. xanthoconus* ( $t=0.25$ ,  $p=0.81$ ), it was more dense than *L. argenteum* ( $t=1.44$ ,  $p=0.18$ ).

$(t/b)_h^2$  varied approximately seven fold between species, from a high of 0.07 for *O. asteriscoides* to a low of 0.01 for *M. hirtus*. While *M. hirtus* had a  $(t/b)_h^2$  ratio a great deal lower than *M. cucullatus* ( $t=7.10$ ,  $p<0.001$ ) the other seep species were statistically indistinct from their dryland analogues with the exception of *L. argenteum* that had a  $(t/b)_h^2$  ratio significantly higher than both *L. xanthoconus* ( $t=2.83$ ,  $p=0.02$ ) and *L. salicifolium* ( $t=2.37$ ,  $p=0.05$ ).

There were no significant correlations between any of the measured variables (Table 3.1).

Xylem density did not correlate with  $P_{\min}$ , however when my data were plotted with data from other Fynbos species reported in Jacobsen *et al.* (2007) a trend becomes apparent with species with less dense xylem tending to have higher  $P_{\min}$  values (Fig. 3.5).

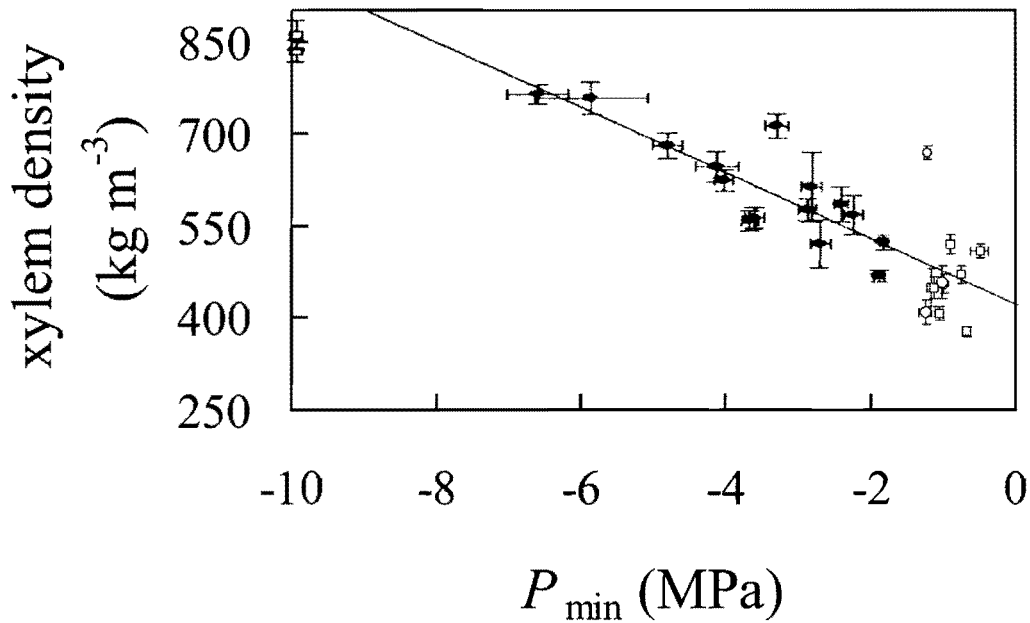


Fig. 3.5. Xylem density as a function of minimum seasonal pressure potential ( $P_{\min}$ ) for fynbos seep species (*open squares*) and dryland species (*open circles*) from this study. Data from fynbos (*closed circles*) and succulent Karoo (*open squares*) species reported in Jacobsen, 2007 are also displayed. Data points are means  $\pm$ SE.

There was no correlation in my data between  $P_{50}$  and xylem density ( $r^2=0.14$ ,  $p=0.63$ ), however when plotted alongside data reported from 14 angiosperm across a range of habitats, my data fits the trend reported in Hacke *et al.*, (2001), with less dense wood corresponding to higher  $P_{50}$  values.

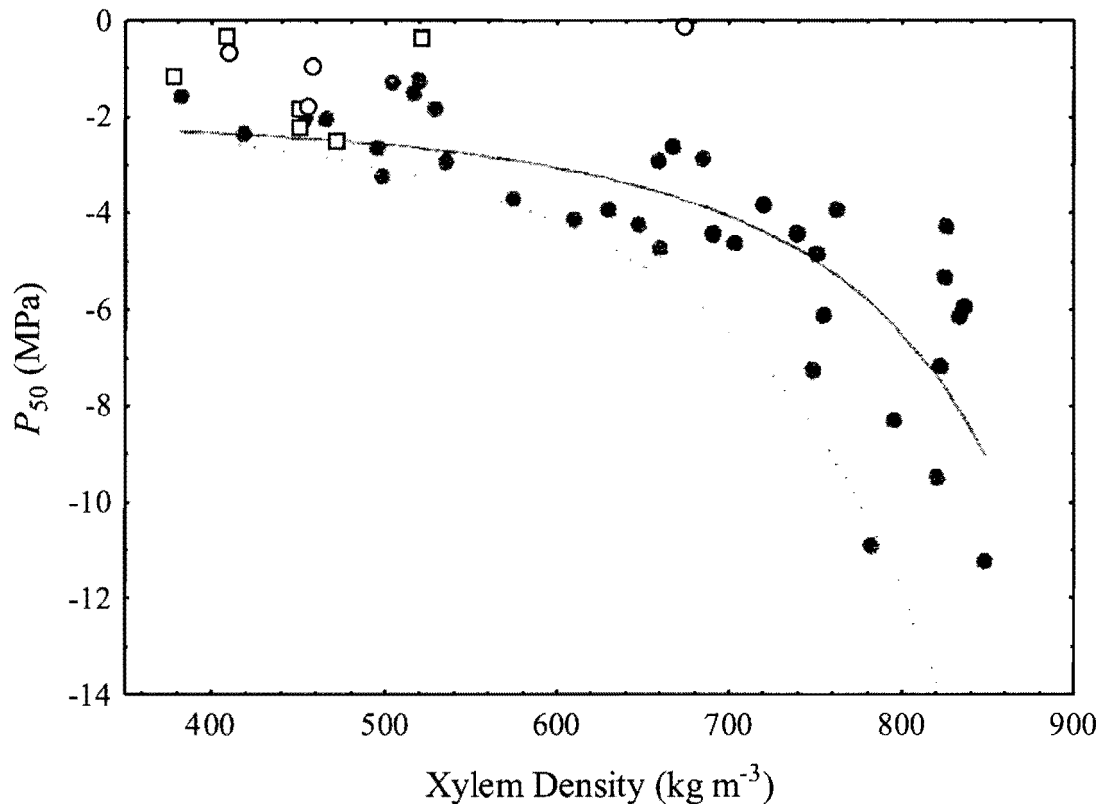


Fig. 3.6. Cavitation resistance ( $P_{50}$ ) versus xylem density for species in this study (*open symbols*) and those Angiosperm species reported in Hacke *et al.*, 2001 (*closed circles*). In my data *squares* represent seep species and *circles* dryland species. *E. perspicua* is excluded as it has too steep a vulnerability curve to measure  $P_{50}$ . The *solid line* represents the best fit through the Hacke (2001) data, and the *dotted line* represents a suggested physical boundary beyond which vessels are unable to operate (Hacke *et al.*, 2001).

There is no correlation in my data between  $P_{50}$  and conduit  $(t/b)_h^2$  ( $r^2=0.11$ ,  $p=0.35$ ). When plotted alongside data reported in Hacke *et al.* (2001) my data falls off the trendline, with species further away from the theoretical implosion limit, being more vulnerable to cavitation than their  $(t/b)_h^2$  ratio would suggest

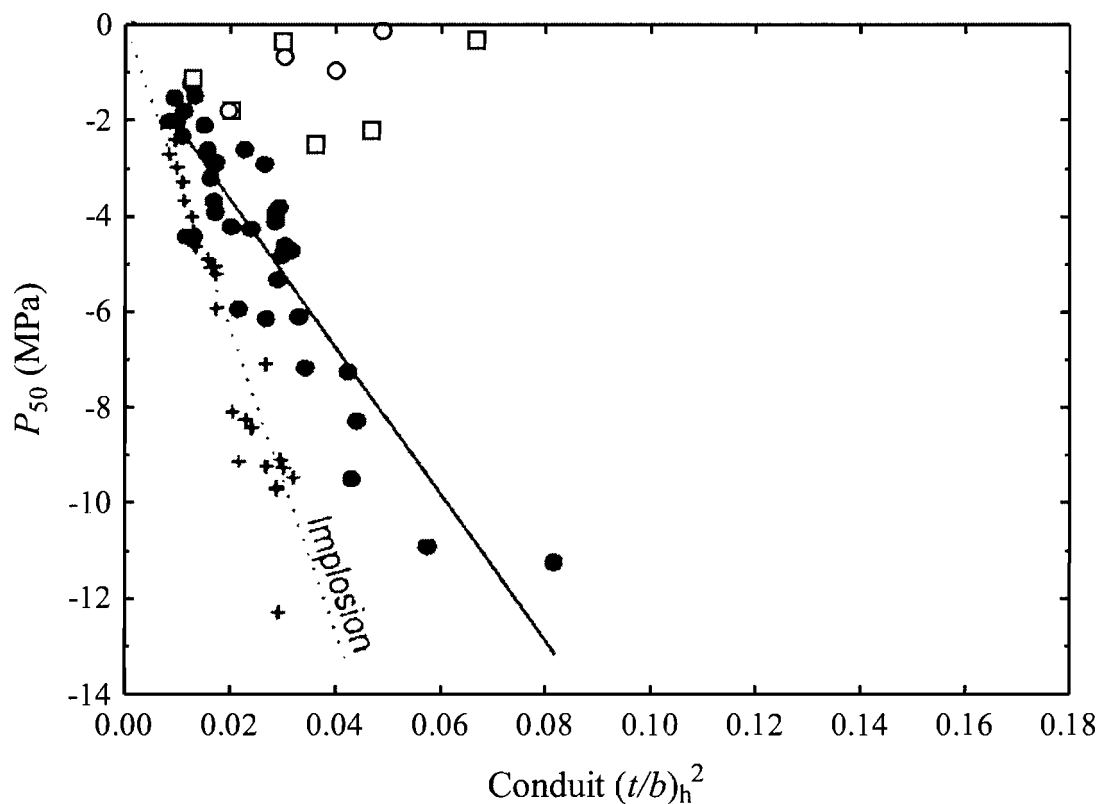


Fig. 3.7. Cavitation resistance ( $P_{50}$ ) versus  $(t/b)_h^2$  for species in this study (*open symbols, squares represent seep and circles dryland species*) and those reported in Hacke (2001) (*closed circles*). *E. perspicua* is excluded as it has too steep a vulnerability curve to measure  $P_{50}$ . The *solid line* is a regression through the Hacke data and the *crosses and dashed line* represent their theoretical implosion limit.

## Discussion

The ultimate aim of this chapter was to identify seep species that are extremely vulnerable to drought stress, which may restrict their distribution to permanently wet habitats. *M. hirtus* appeared to have the most vulnerable xylem of the species in this study. It had the ‘steepest’ vulnerability curve suggesting that it is relatively sensitive to drought induced embolism. It also had the lowest  $(t/b)_h^2$ , which suggests that it is the most sensitive to water stress induced vessel implosion. *M. hirtus* is usually found in low altitude, wet coastal seeps (Burman and Bean, 1985) that are likely to often be fed by the TMG aquifer (Chapter 1; February *et al.*, 2004). The species extreme sensitivity to drought stress may limit its distribution to permanently wet habitats. If so, then its distribution may have great potential to assist in the mapping of these permanently wet habitats, many of which are likely to be connected to the TMG aquifer.

Contrary to my expectation, there was little apparent difference between the water stress tolerance of seep species- and dryland species. The absolute stress tolerance of a species, as defined by the ability of its xylem to withstand negative pressures, appears to be phylogenetically constrained, i.e. related species had similar shaped curves regardless of whether or not they are seep species (see Fig 3.1). Presumably variations in Fynbos species vulnerability curves occurs during large scale evolutionary events (i.e. the evolution of new genera). From the shape of their vulnerability curves it appears as if *Mimetes* evolved in wet habitats or during wet periods in the past. Adaptation to drier habitats such as that undergone by the ‘vulnerable’ *M. cucullatus* may have occurred either by it developing deeper rooting

systems or at the leaf level. Interspecific variation in drought vulnerability as inferred from vulnerability curves has been found to correlate with species susceptibility to drought (Froend and Drake, 2006) but my results suggest that the use of vulnerability curves alone as a diagnostic tool is somewhat limited in Fynbos, i.e. the vulnerability curves of *L. xanthoconas* and *L. salicifolium* are essentially identical despite *L. salicifolium* being a seep species.

Two seep species, *M. hirtus* and *P. prolifera* have extremely vulnerable xylem, having experienced total xylem failure by a xylem pressure potential of -2.5MPa. The individuals that were sampled were from a permanently wet coastal seep just outside of the Kogelberg. Whether these are species that have their distribution limited to permanently wet areas is unknown and warrants investigation. Aside from *B. alopecuroides*, which is apparently a relatively drought tolerant species, all seep species had experienced total xylem failure by around -3.5MPa. They will therefore be unable to survive in soils with water potentials permanently below -3.5MPa.

Correlations between all of the measured variables were small (Table 3.1). This is probably as a result of the fact that there was little variation amongst the species measured i.e. seep species were not that different from the included dryland species. When viewed alongside data collected from more drought resistant species living in arid Fynbos at the edge of the Karoo, my xylem density and  $P_{\min}$  data fit well with recorded patterns (Fig. 3.5)(Jacobsen *et al.*, 2007). Similarly  $P_{50}$  and xylem density data fit the trend reported for a wide range of North American angiosperm species (Fig 3.6) (Hacke *et al.*, 2001). Species in this study were further away from the theoretical implosion limit than reported in other studies (Fig 3.7), i.e. they have

vessels that are reinforced enough to withstand negative pressures much greater than their pit membranes are able to. In other words, many fynbos species appear to be ‘overbuilt’.

The majority of species were sampled from the Kogelberg Biosphere Reserve which falls at the wetter end of the Fynbos spectrum, with an average of 1540 mm of rain in the recorded period between 1996-2006. Nonetheless the apparent drought sensitivity of typical dryland species was unexpected and could have serious implications for the sensitivity of Fynbos to predicted reductions in precipitation associated with climate change. Fynbos is comparable to Californian chaparral, the Mediterranean system for which plant water relations are the best researched. The average  $P_{\min}$  for Chaparral species is -4.2 MPa (n=45) (see Appendix 2). A study of fynbos species sampled at the edge of the Karoo (Jacobsen *et al.*, 2007) reported an average of -3.4MPa, less than chaparral. However, on average Fynbos species are more significantly more vulnerable ( $p < 0.01$ ), with an average  $P_{\min}$  of -2.6MPa, (n=33). This difference may be a result of water stress tolerant Chaparral species having received more research attention than less tolerant species. However, from the authors own experience having somewhat randomly measured  $P_{\min}$  from a fairly wide range of Fynbos species (once again see appendix x), it is extremely rare to encounter  $P_{\min}$  values less negative than -2 MPa in Fynbos species. The apparent plethora of presumably drought sensitive species in Fynbos may be as a result of it’s relatively stable climate throughout the quaternary (Cowling *et al.*, 2004). Drought sensitive species may have already gone extinct during prehistoric drier periods in other Mediterranean systems. The stability of the Cape’s climate, coupled with the refuge provided by cooler, wetter higher altitudes may have allowed the survival of drought sensitive species, contributing to

the Kingdoms high biodiversity. The fact that fynbos appears to be more sensitive to drought stress than chaparral, may result in it being more severely impacted by increases in drought associated with climate change.

## Synthesis

The study arose from the need to enhance our understanding of the links between water in the TMG aquifer and dependent ecosystems, in light of the aquifer being identified as a possible future water source. The first step in assessing these impacts is to determine which groundwater-fed ecosystems are dependent on the TMG aquifer. In this study I have evaluated the use of a number of different techniques to distinguish aquifer-fed seeps from perched seeps which are unlikely to be affected by abstraction.

In Chapter one I used a hydrological approach and tested the potential of stable isotope measurement and water level monitoring over both the long and short term to determine if a seep is aquifer-fed. The long-term monitoring of seep groundwater stable isotope composition revealed apparent variation in the flow path length of aquifer-fed seeps, a single isotope sampling sweep across a landscape is therefore likely to be of little use in determining which systems are dependent on the TMG aquifer as recent rainfall may make up varying proportions of groundwater discharge. Here, short term sampling appeared to also be of limited use, as the groundwater body to which a perched system was connected was either too large or too poorly mixed to prevent the groundwater assuming a stable isotope composition similar to that of antecedent rainfall. Therefore, while the long term monitoring of fluctuations in the stable isotope composition of seep groundwater may be useful in developing a detailed understanding of the hydrology of an area, they appear to have limited use in assessing TMG aquifer dependence at a broader scale. In contrast, the monitoring of

variation in seep groundwater level appears to have promise; water levels of a perched seep were more variable throughout a summer as well as throughout a single rainfall event, than those of aquifer-fed seeps. The ASTON was designed as a piece of equipment that can measure how much water levels rise in a piezometer. They could be installed in seeps across a landscape in the middle of summer, and retrieved after winter to determine the annual variation in a seep's water level. Where their use has even more promise is by measuring the rise in groundwater in a seep after a rainfall event. Despite the perched system receiving a much lower volume of rainfall into its catchment during the sampled event, its water levels were raised a great deal more than they were in the aquifer-fed seeps. This suggests that the site is connected to a body of groundwater with a smaller aerial extent.

Chapter 2 used an ecohydrological approach, in which I tested the potential of the monitoring plant water stress and the monitoring of plant water stable isotope composition to determine if a seep is aquifer-fed. Throughout summer, plants living on the perched seep did not experience an increase in water stress relative to those living on a permanently wet aquifer-fed seep. This surprising result was possibly as a result of the cooler wetter climate on top of the mountain where the perched seep was found. Perched seeps are often at higher altitudes which may limit plant moisture stress, therefore limiting the potential of plant water stress measurements as a means to distinguish them from aquifer-fed seeps, at least in areas with climatic gradients as steep as in the Kogelberg. An unexpected and interesting result from the water stress monitoring was that the deep-rooted proteas off seep appeared to have similar amounts of access to groundwater. It may be possible that they are able to get their roots deep enough down cracks to reach the regional water table. Should this be the

case, then they may also be impacted upon by the drawdown in water levels associated with abstraction and the ecological impacts may be greater than has been anticipated. Plant stem-water stable isotope composition analysis does appear to have potential in identifying aquifer-fed seeps. The expected trend was observed, yet the differences were not significant. Were the rainfall event that occurred prior to the plant sampling more isotopically distinct from the long term mean, then the results may have been more conclusive.

In Chapter 3 I looked at the ecophysiological implications of the existence of habitats that remain permanently wet. Within it I assess whether the permanently wet habitats connected with the TMG aquifer, on which plant communities experience no seasonal increase in xylem pressure potentials, may have allowed the survival of species that are very sensitive to drought. I thought that vulnerability curves would be a way of answering this question. Some of the species, in particular *M. hirtus*, did have very vulnerable xylem and whether they are restricted to permanently wet areas remains to be seen. A key finding was that there was very little difference in the vulnerability of related on- and off-seep species, suggesting that the vulnerability of xylem is phylogenetically constrained. This suggests that vulnerability curves cannot be used in isolation as a measure of drought tolerance, but rather need to be coupled with some leaf level measurement, such as stomatal response curves. In this chapter I also determined the potential of a range of more easily measured physiological variables, for determining susceptibility to drought stress. All of my species, including dryland species were however vulnerable to drought stress and the small amount of variation resulted in none of the correlations being significant. My data fits well when plotted alongside data reported in other studies, with my species sitting at the sensitive end of

the spectrum. The apparent sensitivity of the dryland species sampled was surprising and prompted a comparison in drought sensitivity between fynbos and Californian Chaparral. On average fynbos plant species are significantly more sensitive to drought stress than chaparral and may therefore potentially be at greater risk from climate change induced drought stress.

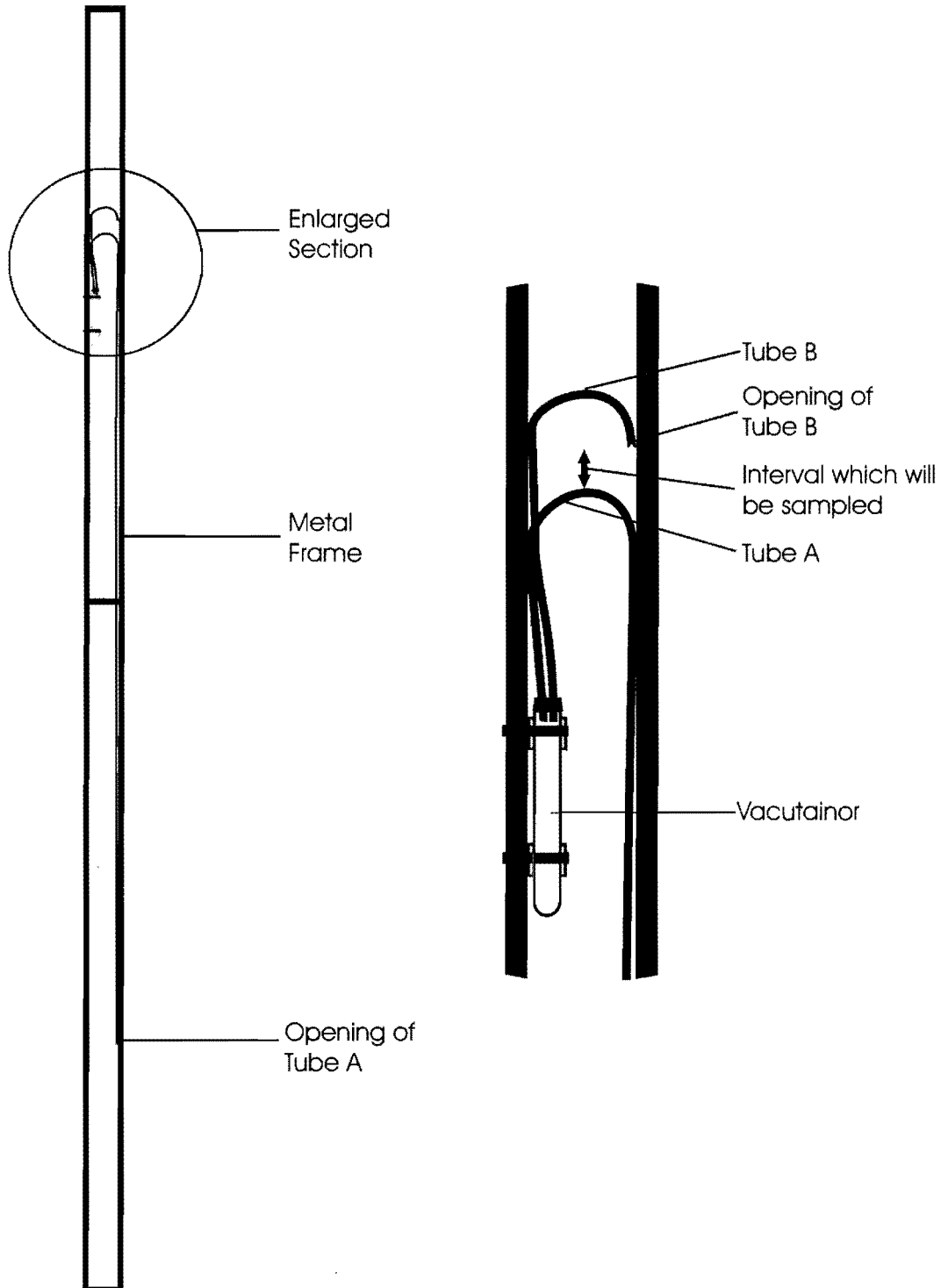
In terms of future research, a good place to begin would be to establish a detailed, long-term monitoring of seep water levels across an area containing aquifer-fed as well as perched systems. Once a good understanding of an areas eco-geohydrology has been developed the site could be used to ‘calibrate’ remote sensing imagery from different times of the year, which potentially could be used to map the occurrence of aquifer-fed seeps across a wider area. In terms of broader fynbos ecohydrology, research attention needs to be focused on the importance of cloud water and groundwater to fynbos ecosystems. Systems dependent on these sources may be decoupled to some extent from rainfall, the only hydrological parameter that has been incorporated into models looking at future impacts of climate change on biodiversity.

In conclusion, the most distinct hydrological feature of TMG aquifer-fed seeps, that may assist in their mapping, is that they appear to often remain permanently wet. The existence of these habitats in the Cape has allowed the survival of a suite of drought sensitive plant species, that otherwise may have gone extinct during drier periods in the past. The palaeoendemic species and genera living in these habitats, add tremendous diversity to the fynbos as they tend to be very distantly related to current extant species. The number of people living in Cape Town is set to increase and new water sources do need to be found. However, in light of the drought sensitivity of the

unique flora associated with the TMG aquifer, careful consideration must be made to ensure that Cape Town's future water supply is ecologically sustainable.

# Appendix 1 – The ASTON

A device was designed to sample the ascending limb of a seeps water level. It has no moving parts, is easy to and cheap to build and is quick to install.



The ASTON

The ASTON (Ascending Seep Tester of Note) collects water at desired height intervals as it ascends up a piezometer sunk into a seep. It operates under the same principal as Van Wyks rising water level sampler, designed to sample rising water levels in a river (Van Wyk, 1983). A steel frame was built 1 cm thinner than the width of the piezometer and galvanised to prevent it from rusting. vacutainers were attached to the inside of the frame with cable ties, they were set at a height that would sit below that of the water within the piezometer. Sections of 2x2x2mm rigid nylon tubing (that used in the hydraulic braking systems in trucks) were inserted into the vacutainers through 1mm holes drilled through the vacutainor's rubber bung. The height of the loops in the tubing was carefully measured to sit just above the height of the water in the piezometer prior to the rainfall event. As water levels rose above the top of loop of tube A (see figure), water began to move into the vacutainor while the air left the vacutainor through the opening of tube B. Water stopped moving into the vacutainor when the water level reached the opening of tube B because the air within the loop of tube B will act as a seal. The distance between the top of the loop of tube A and the opening of loop B therefore determines what interval of rising water the ASTON will sample.

The section of water which the ASTON will sample will depend on the position of the opening of tube A. The base of piezometers is generally screened, and is likely to be the area where the water is best mixed with that of groundwater supply. The influent tube connected to loop A was therefore extended down to the midpoint of the screened section 50cm above bedrock. A mass balance was used to correct the volume of water that was sitting in the tubing connected to loop A prior to the rainfall event. This water would be a mix of the water throughout the piezometer that moved into the

ASTON as it was lowered into the piezometer, and is assumed to have the same  $\delta D$  value as the groundwater before the ASTON was installed.

The volume of water in the vacutainor that was made up of water initially sitting in the piezometer was calculated according to:

$$V_{\text{init}} \text{ (ml)} = \text{length (mm)} \cdot \pi \cdot r^2$$

where 'length' is the length of tube A between water surface and 50cm above bedrock and r is the radius of tube A

The  $\delta D$  value of the groundwater as the water levels began to rise ( $\delta D_{\text{rise}}$ ) was calculated according to:

$$\delta D_{\text{rise}} = (V_{\text{total}} \cdot \delta D_{\text{total}} - V_{\text{init}} \cdot \delta D_{\text{init}}) / V_r \quad 3$$

where  $V_{\text{total}}$  is the total volume of the vacutainor (13.5mls),  $\delta D_{\text{total}}$  is the  $\delta D$  value of the water collected in the vacutainor,  $V_{\text{init}}$  is the volume of the water moving into the vacutainor that was already sitting in tube A and  $\delta D_{\text{init}}$  is the  $\delta D$  value of this water which was assumed to be the same as the  $\delta D$  value of the groundwater in the seep prior to the rainfall event.

## Appendix 2 - P<sub>min</sub> data

Minimum water potentials reported in the literature. Most values are estimates read off published graphs. Note these are not implicit P<sub>min</sub> values, though all studies included report P<sub>x</sub> in the driest part of the year. Species that exceeded the limits of the pressure chamber are indicated in bold and were excluded from the estimation of the mean.

### Californian Chapparal

Author	Species	Pmin
Poole and Miller, 1975	<i>Adenostoma fasciculatum</i>	-6.0 MPa
Burk, 1977	<i>Ceanothus crassifolius</i>	-5.3 MPa
	<i>Quercus dumosa</i>	-3.4 MPa
	<i>Adenostoma fasciculatum</i>	-3.0 MPa
Poole and Miller, 1975	<i>Rhus ovata</i>	-2.8MPa
	<i>Adenostoma fasciculatum</i> =	-6.8 MPa
	<i>Arcostaphylos grandulosa</i>	<b>-6.5MPa</b>
	<i>Ceanothus greggi</i>	<b>-6.5MPa</b>
	<i>Quercus dumosa</i>	-6.0MPa
	<i>Arcostaphylos pungens</i>	<b>-6.5MPa</b>

Author	Species	Pmin
Lo Gullo and Salleo, 1988	<i>Olea oleaster</i>	-3.5MPa
	<i>Ceratonia siliqua</i>	-1.85MPa
	<i>Laurus nobilis</i>	-2.3MPa
Hart and Radosevich, 1987	<i>Adenostoma fasciculatum</i>	-4.0MPa
	<i>Arctostaphylos stanfordiana</i>	-5.5MPa
Davis <i>et al.</i> , 1999	<i>Cerastes megacarpus</i>	-8.4 MPa
	<i>Cerastes crassifolius</i>	-6.0 MPa
	<i>Cerastes cuneatus</i>	-7.3 MPa
	<i>Ceanothus leucodermis</i>	-2.6 MPa
	<i>Ceanothus. oliganthus</i>	-4.3 MPa
	<i>Ceanothus spinosa</i>	-6.2 MPa
Davis <i>et al.</i> , 2002	<i>Caenothus crassifolius</i>	-11.2MPa
Ackerly, 2004	<i>Adenostoma fasciculatum</i>	-3.7MPa
	<i>Arbutus menziesii</i>	-2.3MPa
	<i>Arctostaphylos tomentosa</i>	-3.4MPa
	<i>Baccharis pilularis</i>	-3.4MPa
	<i>Caenothus cuneatus</i>	-5.1MPa
	<i>Caenothus oliganthus</i>	-3.7MPa
	<i>Cercocarpus betuloides</i>	-3.8MPa
	<i>Dirca occidentalis</i>	-3.6MPa
	<i>Eriodictyon californicum</i>	-1.5MPa

Author	Species	Pmin
	<i>Heteromeles arbutifolia</i>	-2.3MPa
	<i>Holodiscus discolor</i>	-2.2MPa
	<i>Lepechinia calycina</i>	-3.2MPa
	<i>Lotus scoparius</i>	-2.4MPa
	<i>Mimulus aurantiacus</i>	-4.2MPa
	<i>Prunus ilicifolia</i>	-2.1MPa
	<i>Rhamnus californica</i>	-1.8MPa
	<i>Rhamnus crocea</i>	-4.4MPa
	<i>Ribes californicum</i>	-1.6MPa
	<i>Sambucus mexicana</i>	-1.2MPa
	<i>Toxicodendron diversiloba</i>	-0.64MPa
Jacobsen <i>et al.</i> , 2005	<i>Adenostoma fasciculatum</i>	-8.13MPa
	<i>Adenostoma sparsifolium</i>	-4.5MPa
	<i>Ceanothus megacarpus</i>	-10.1MPa
	<i>Malosma laurina</i>	-2.5MPa
	<i>Rhus Ovata</i>	-2.9MPa
	<i>Caenothus spinosus</i>	-10.1 MPa

## Fynbos

Author	Species	Pmin
Miller <i>et al.</i> , 1983	<i>Protea laurifolia</i>	-1.75MPa
	<i>Leucadendron pubescens</i> (female)	-1.75MPa
	<i>Elytropappus gnaphaloides</i>	-2.3 MPa
	<i>Diosma hirsuta</i>	- 3.9MPa
	<i>Cannomois acuminata</i>	-1.7MPa
	<i>Protea nerifolia</i>	-1.8MPa
	<i>Erica plukenetti</i>	-3.2MPa
	<i>Erica sphaeroides</i>	-3.2MPa
	<i>Tetraria ustulata</i>	-2.4MPa
	<i>Merxmuellera stricta</i>	-3.9MPa
Richardson and Kruger	<i>Brabejum stellatifolium</i>	-2.5 Mpa

Author	Species	Pmin
	<i>Brachylaena neriifolia</i>	-2.5 MPa
	<i>Cunonia capensis</i>	-2.6 MPa
	<i>Protea nitida</i>	-2.7 MPa
	<i>Protea repens</i>	-2.5 MPa
Moll and Sommerville, 1985	<i>Thamnochortus punctatus</i> =	-4.0MPa*
	<i>Leucospermum parile</i>	-0.9MPa
Jacobsen <i>et al.</i> , 2007	<i>Brunia noduliflora</i>	-2.8 MPa
	<i>Nebelia laevis</i>	-3.7 MPa
	<i>Aspalathus pachylob</i>	-2.2 MPa
	<i>Cliffortia ruscifolia</i>	-2.5 MPa
	<i>Erica cerinthoides</i>	-4.1 MPa
	<i>Leucadendron laureolum</i>	-2.0 MPa
	<i>Leucadendron salignum</i>	-2.0 MPa
	<i>Metalasia densa</i>	-3.8 MPa
	<i>Protea laurifolia</i>	-3.0 MPa
	<i>Protea repens</i>	-3.0 MPa
	<i>Aspalathus hirta</i>	-2.4 MPa
	<i>Erica plukenetti</i>	-4.3 MPa
	<i>Euclea spp</i>	-5.0 MPa

Author	Species	Pmin
Aston, 2007	<i>Brunia albiflora</i>	-1.8 MPa
	<i>Pteridium aquilinum</i>	-1.7 MPa
	<i>Asteraceae compacta</i>	-1.6 MPa
	<i>Empleurium unicapsula</i>	-1.3 MPa
	<i>Cliffortia grami</i>	- 1.3MPa
	<i>Protea compacta</i>	- 1.2 MPa
	<i>Brunia alopecaroides</i>	-1.2MPa
	<i>Leucadendron xanthoconas</i>	-1.0MPa
	<i>Osmotopsis asteriscoides</i>	-1.0MPa
	<i>Erica perspicua</i>	- 0.9MPa
	<i>Leucadendron salicifolium</i>	-0.9 MPa
	<i>Psoralea aphylla</i>	-0.8 MPa
	<i>Serruria rubricaulis</i>	-0.7 MPa
	<i>Mimetes hirtus</i>	-0.7 MPa

## Appendix 3 - Wetlab Manual

# Wetlab Manual

Tim Aston

University of Cape Town

July 2006

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## The Wetlab

The layout of the wetlab is depicted in Fig. 1, the lab has two main components; a *flow metre* and *flushing system*. It is constructed out of 11mm and 18mm clear plastic tubing. Originally, expensive autoclavable Nalgene tubing was used but it melted in an autoclave. Nylon plastic tubing is a fraction of the price and works just as well. Sections of tubing were connected with hose clamps (parts 6832-04 and 6832-14 from [www.coleparmer.com](http://www.coleparmer.com)) to three-way stopcocks (Nalgene, [www.coleandparmer.com](http://www.coleandparmer.com) or part # LNAL6470-0004 from Lasec) and linked to a 4 gallon captive airtank ([www.sears.com](http://www.sears.com).) which contains a bladder and converts regulated air pressure into water pressure. Degassed solution is forced under pressure through a 1µm filter (nylon, calyx capsule [www.osmolabstore.com](http://www.osmolabstore.com)) after which it moves into either the flow metre or the flushing system.

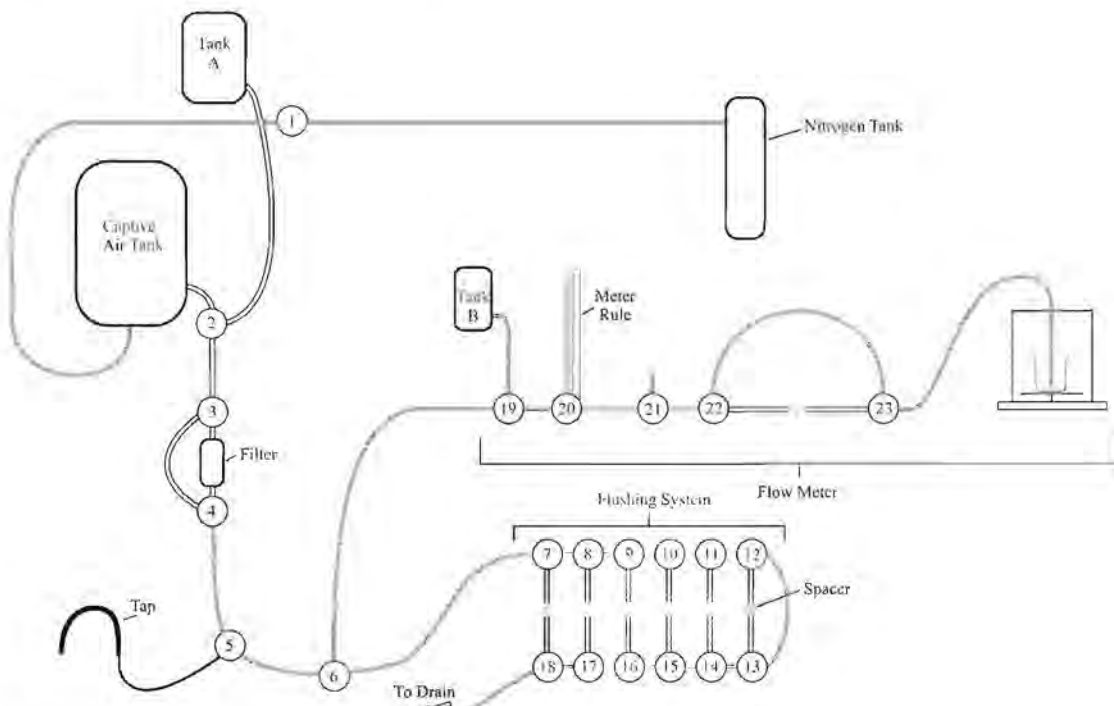


Fig. 1. The layout of UCT Botany's wetlab. Three-way stopcocks, or 'taps', are numbered.

## 1) Preparing the flow meter and flushing system

Flows are measured with filtered (0.2 $\mu$ m), degassed HCl solution.

- 1) Fill the large glass tank to the black line with de-ionised water from Ed's lab (the blue tap in the sink).
- 2) Add acid (or other solute). I used 100mls of 32% chemically pure HCl (obtained from the chemistry store in PD Hahn) mixed with 10l of de-ionised water. The solution has a pH of less than 2, to limit microbial growth.
- 3) Place the glass tank on the magnetic stirring plate in the fume cupboard and slide it around until the white magnet sits in the middle of the base of the tank.
- 4) Attach the bung connected to the vacuum pump to the top of the glass tank.
- 5) To dry the air being drawn into the vacuum pump, draw it through a tank of silica gel. Check the gel looks alright, it should be orange. If it isn't, dry it in the ovens on the ground floor.
- 6) Switch on the stirrer; the white magnet should spin in the middle of the glass tank. If the speed is turned up too high, the magnet will fall off the centre axis.
- 7) Switch on the vacuum pump. The solution should start to 'bubble' as the dissolved gas is drawn out. Let it run for 20 minutes.

While waiting, rinse out the system. It is easier to use tap water but the system **must** be flushed with filtered solution before you begin the measurements.

*Note- I find that the easiest way of working with this system is to work out where you want to send solution and work backwards. For example, say you wanted to fill tank A with tap water, 'create a pathway' by turning taps 2 then taps 3, 4 and 5.*

Rinsing the captive air tank:

- 8) Turn tap 2 to close off the tube leading to tank A, creating a path from the captive air tank to the filter.
- 9) Tap 3 is used to hold the pressure from the captive air tank by being turned into the position illustrated in Fig. 9. Turn tap 4 to bypass the filter and taps 5 through 18 to create a path from the captive air tank to the drain.
- 10) Open the valve on top of the nitrogen tank and check that air isn't leaking out of tap 1 but that the air pressure (150kPa) is being transferred to the captive air tank.
- 11) Turn tap 3 to release the pressure from the captive air tank around the filter.
- 12) When the captive air tank is empty 'close' the captive air tank using tap 3 and use tap 1 to release the air pressure- be careful that tap isn't in position to allow air to leak out of the nitrogen tank.
- 13) Turn tap 5 so that water is directed from the tap, around the filter and into the captive air tank.
- 14) Turn on the tap and open tap three so that water can flow into the captive air tank; air should escape from tap 1. Let it fill for 10-15secs.
- 15) Turn off the tap, bypass it with tap five and turn tap 1 to re-apply pressure into the captive air tank.
- 16) Refill the captive air tank with tap water and rinse it again.

- 17) 'Fill' the captive air tank again and hold back the pressure close it off with tap 3.
- 18) Turn tap 2 and rinse the attached tube that is later used to connect tank A

Rinsing the filter:

Turn tap 4 to introduce the filter and tap 5 so that a path is created to the drain.

- 19) Release the pressure from the captive air tank and direct it through the filter by turning tap 3.
- 20) Release the air from both sides of the filter by opening the red knobs and waiting for water to flow out – you may need to use the small pliers. **Be careful that the knobs don't shoot off and go down the drain.**

Rinsing the flushing system:

- 21) Turn taps 7 and 18 so that a path through the first loop is created, turn tap 5 so that water is sent through towards the flushing system, through the loop and turn on the tap. Let it rinse for a few seconds. You can tell when the bleach has been washed out because the water will no longer feel 'soapy' between your fingers.
- 22) Turn taps 7 and 18 to bypass the first loop and turn 8 and 17 to open the second loop etc. etc. until all of the loops have been rinsed.

Rinsing the flow meter:

- 23) Place the end of flow meter that would sit normally sit in the beaker on the balance in the basin, turn taps 19 through 23 so that all of the sections have been bypassed.

- 24) Turn tap 6 to introduce the flow meter and tap 5 to direct the water towards it, turn on the tap.
- 25) Turn tap 19 and thoroughly rinse tank B.
- 26) Turn tap 20 and rinse out the height meter
- 27) Turn tap 21 and rinse the *zero balance tube*
- 28) Turn taps 22 and 23 to rinse the loop in the Sperry Apparatus.
- 29) Check that the captive air tank is empty

Filling the system with solution:

- 30) Lift 'tank A' onto the shelf above the captive air tank.
- 31) Pour any water remaining in the tube attached to tap 2 down the drain.
- 32) Fill this tube with solution from tank A
- 33) Tap out any air-bubbles that are in the tube and attach it to the tap from tank A while the tanks tap is open and the tap is open (to prevent any air-bubbles entering the system).
- 34) Release any pressure that remains in the captive air tank and fill it with contents of tank A by turning tap 2 (make sure that air is able to escape from the tank out of tap 1). When tank A is empty repeat steps 1-7.
- 35) Now rinse the entire system with solution (steps 8-29). After rinsing tank B, fill it to the line.
- 36) Refill the captive air tank with at least 5l of solution

## 2) Measuring the percent loss of conductivity (PLC)

A stems PLC is determined by comparing flows to those after the stem is flushed.

### *2a) Preparing Stems*

When collecting stems from the field bear in mind that you will need a straight, unbranched section 14.2cm long (assuming you are doing vulnerability curves). **You need at least an additional 40cm of plant material on either side of this section.** Leave all leaves attached. Wrap it tightly in cling wrap and put it in a cooler box and run them as soon as possible.

- 1) I normally run six stems in a session. Unwrap them, label them and draw an arrow pointing towards the leaves (i.e. in the direction of water flow) in the middle of the straight, unbranched 14.2 cm section.
- 2) Place the water bath on the floor and fill it with water using one of the hoses on the taps in the sink.
- 3) Chop the stems to slightly longer than the length required underwater, preventing air from being drawn into the xylem.
- 4) Trim the ends with a fresh razorblade underwater.
- 5) Find a grommet that is roughly the same width as the stem.
- 6) Wrap plumbing tape around the ends of the stem to ensure a tight fit with the grommet, ensure that it doesn't obstruct the ends of the stem.

- 7) Put the grommet on- it's easiest to 'screw' it on in the same direction as the plumbing tape is wrapped, leave the stems in a beaker filled with filtered, degassed solution.
- 8) Put the distal plant material in a labelled bag for later leaf area measurements.

## ***2b) Measuring Conductivity***

Flow ( $\text{g t}^{-1}$ ) through a stem resulting from the application of a known pressure is measured using a stopwatch and a four point balance. In order to avoid the flushing out of existing embolisms this applied pressure needs to be very small. It is generated by raising the height of the solution in tank B higher than that in the beaker on top of the balance. Height of the solution tank B is measured using a metre rule. A ruler is attached to the side of the beaker on the balance and used to measure the height of the solution. I kept the height difference between solution in tank B and that in the beaker on the balance at around 10cm, which generates a pressure of approximately 1kPa.

Once the stem is connected, water will begin to move out from living cells in the stem down an osmotic gradient, causing slow mass increments to occur even when no pressure is applied. In order to negate these effects, *zero measurements* are taken using the Sperry Apparatus before and after a flow measurement. If the mass registered on the balance drops, then there is a leak somewhere in the system which needs to be fixed.

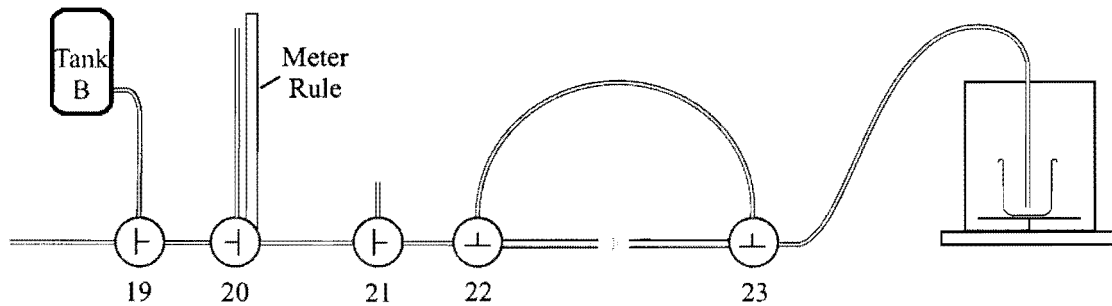


Fig. 2. The high resolution flow meter with taps positioned to hold back the pressure from tank A. The solution in the zero balance tube connected to tap 21 is at the same height as the water in the beaker on the balance.

- 1) All of the air-bubbles need to be removed from the system. Place the end of the tube that would go in the balance into a large beaker. Turn taps 2, 3, 4, 5, and 6 so that solution from the captive air tank moves through the filter. While the solution is flowing under pressure through the system dislodge all air-bubbles by tapping the sides of the tubing. Use the taps to direct the flow through all of the flow meter components, removing all air-bubbles. The flow metre is extremely accurate, a single one will interfere with flow measurements. Hold back the water under 'high' pressure using tap 3.
- 2) Place the beaker with the attached section of ruler on top of the balance. Turn taps 23 to ensure that no solution can enter the beaker and 21 to bypass the short 'zero balance tube'. Place the end of the tube into the beaker on the balance. Turn tap 19 to allow the solution under 'low' pressure to flow from tank B into the beaker. Fill the beaker to the appropriate height and use tap 23 to turn off the flow. Add a layer of silicone oil to the surface of the solution in the beaker to prevent evaporation.
- 3) Put the bung with the glass tube set through it firmly into the top of tank B. Turn tap 20 so that solution can flow up the tube attached to meter rule. Turn tap 21 to allow solution to flow out of the 'zero balance tube' until air starts being drawn

through the tube through the bung in tank B. Turn tap 20 to prevent the pressure from tank B entering the flow meter but still allowing it to hold solution up the tube attached to the flow meter.

- 4) Now to set up the *zero balance tube*. Set the top of the short tube attached to tap 21 to the same height as that of the solution in the beaker using a retort stand. Turn taps 21 and 23 so that they are in the positions illustrated in Fig. 2. Raise or lower the zero balance tube so that its meniscus forms at the top the tube. The height of the water in the beaker on the balance can be adjusted by changing the height of this tube. If need be, introduce more solution into the beaker on the balance using taps 20 and 21. You are now ready to load your stems.

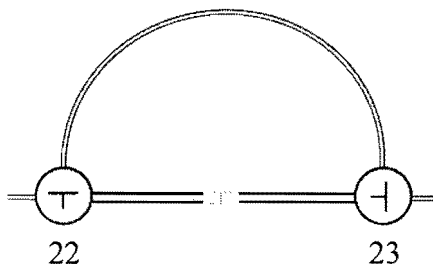


Fig. 3. The loop in the Sperry Apparatus. Stems are loaded in the position held by the spacer.

- 5) Turn tap 23 90° counter clockwise to prevent flooding of the balance. Introduce pressure into the loop by turning taps 20 and 21 by 90°. Turn taps 22 and 23 into positions illustrated in Fig. 3. Unplug the spacer and load proximal end of the stem into the tubing connected to tap 22. It is important that no air-bubbles enter the system so load the stem while solution is flowing out of the tubing. Secure it with a hose clamp. Turn tap 22 90° and load the distal end of the stem.

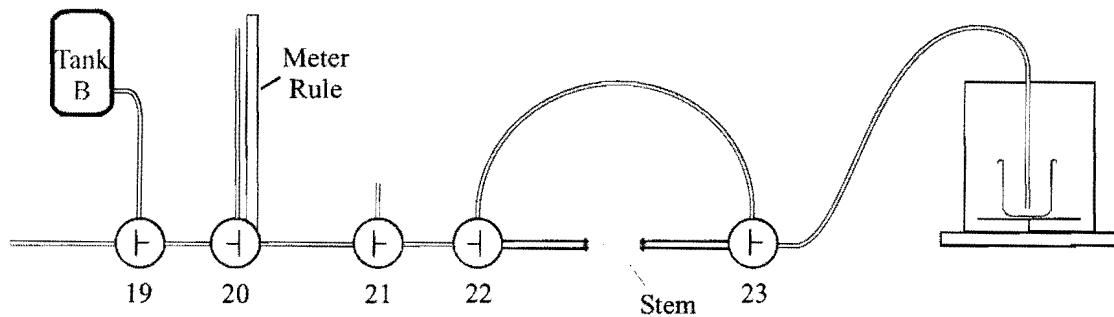


Fig. 4. The high resolution flow meter with tap positions indicated

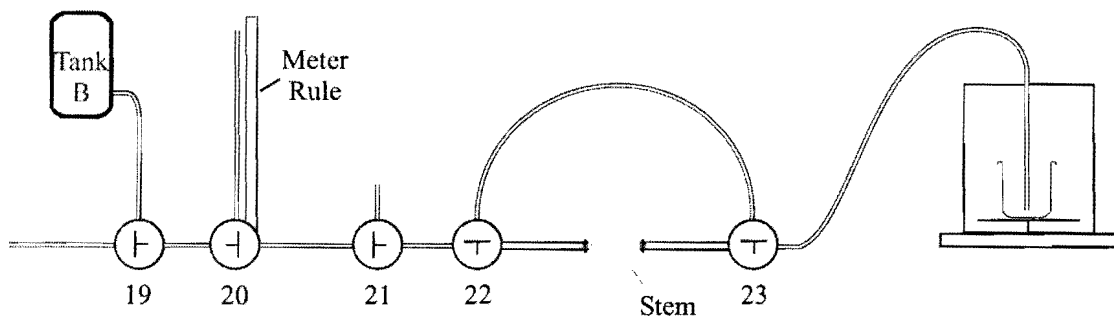


Fig. 5. The high resolution flow meter with taps adjusted into position for the measuring of osmotic effects. The meniscus at the top of the *zero balance tube* is at the same height as that in the beaker.

- 6) Before taking zero flow measurements check that there are no leaks or air-bubbles in the system. This is done by turning taps 22 and 23 into the positions illustrated in Fig. 4. The mass on the balance should stabilise, if it slowly increases you have an air-bubble in the system, if it decreases you have a leak.
- 7) Once all air-bubbles and leaks have been eliminated, turn the taps into the positions illustrated in Fig. 5. Wait for the system to stabilise.
- 8) Use a stopwatch and record the last three digits on the balance every 10s. It is important that the system is not disturbed while taking measurements so don't press on the bench whilst writing and make sure that the door to the lab is closed. Take at least 7 readings.

- 9) Measure the height of the solution on the balance using the ruler attached to the beaker and the 'effective' height of the solution in tank using the meniscus in the tube attached to tap 20 and the metre rule.
- 10) Record the temperature.

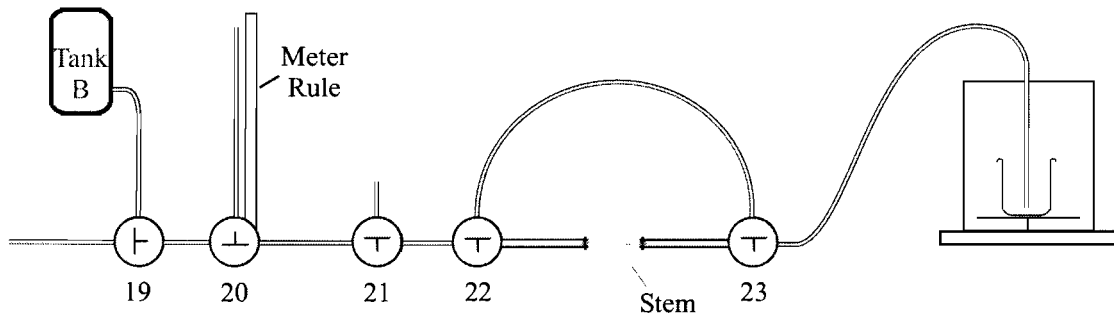


Fig. 6. The high resolution flow meter with taps adjusted into position for the measuring of flows ( $\text{g s}^{-1}$ )

- 11) Turn taps 20 and 21 into the positions illustrated in Fig. 6. Wait a few seconds for the flow to stabilise and take another seven recordings.
- 12) During these measurements solution will have moved into the beaker on the balance, drain this solution back through the system and out of the zero balance tube by turning the taps back to the position in Fig. 4.
- 13) Take the second set of zero measurements by turning the taps back into the arrangement in Fig. 5.
- 14) Remove the stem and return it to the water bath.
- 15) Load the next stem...

## 2c) Flushing

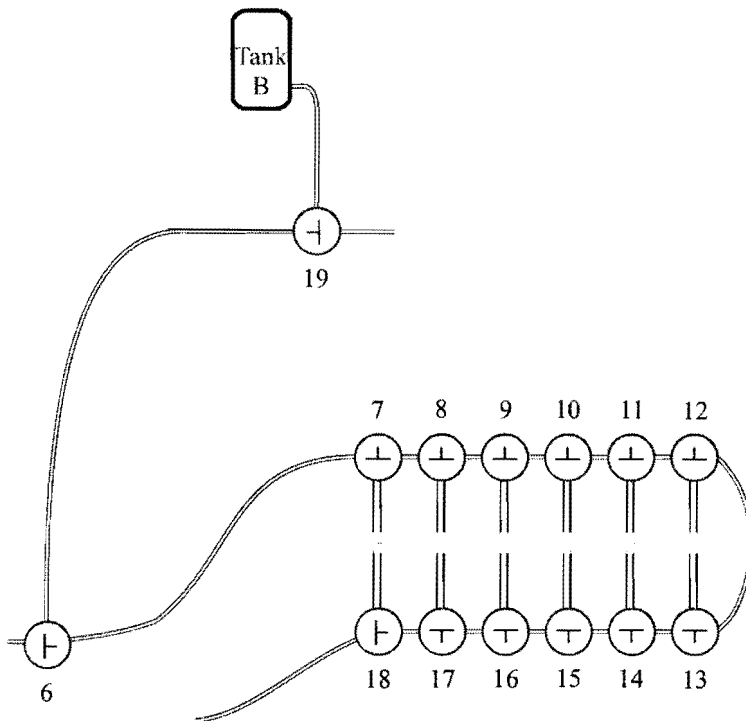


Fig. 7. The flushing system under low pressure

- 1) Turn tap 6 so that the captive air tank is connected to the flushing system, via the filter. Remove any air-bubbles with high pressure from the captive air tank.  
Turn tap 3 90° to hold back the pressure from the captive air tank.
- 2) The stems are loaded in the same way as done when taking conductivity measurements. Redirect the 'low' pressure from tank B by turning the taps to the arrangement illustrated in Fig. 7; the pressure can be increased by raising the height of tank B. Turn tap 7 270°, remove the spacer and load the lower end of the first stem. Again, it is very important that no air-bubbles are introduced. Turn tap 7 another 90° to direct the solution around the flushing

system and load the proximal end of the twig. Load the distal ends of the twigs in the same way by turning taps 13-18 270°.

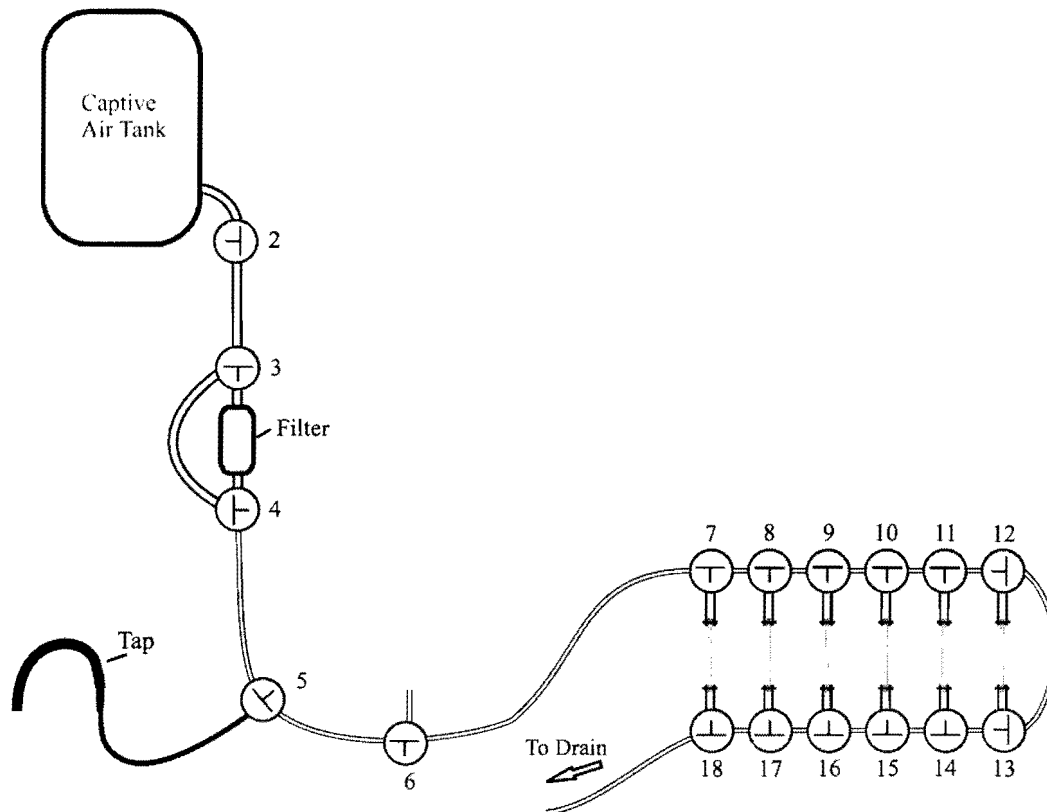


Fig. 8. The flushing system with taps adjusted into position for flushing. Pressure is applied by turning tap 3 90° counter clockwise.

- 3) Arrange the taps to configuration illustrated in Fig. 9. Turn tap 3 90° counter clockwise.
- 4) Flush the stems for an hour. Solution should slowly drip out of the pipe in the drain and air-bubbles will be observed in tubing attached to the distal ends of the twigs as embolisms are flushed out.

## ***2d) Staining and measuring leaf area***

### **Measuring leaf area**

1. Remove the leaves off distal branches.
2. Measure leaf area using the leaf area meter in the instrument room. Keys are available from Darwood, Gonzalo or Sandy.

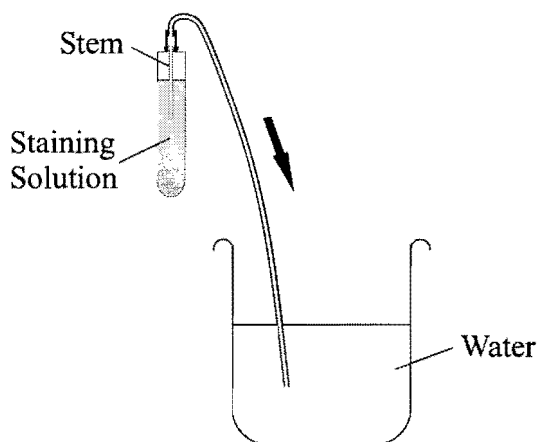


Fig. 9. The set up for the staining of active xylem under low pressure.

### Staining active xylem

1. The aim is to create a closed system with no air-bubbles through which gravity will then draw the staining solution into the stem (see Fig. 9). A low pressure gradient, similar to that used in the measurement of flows, is created by raising the height of the staining solution. Low pressure is used to avoid staining inactive xylem.
2. Fill the plastic tubing with water hold, gently blow through the small end and load the distal end of the stem into the large end as the water is slowly pouring out.
3. Fill the tube with water and flick out any air-bubbles.
4. Hold your thumb over the small end of the tube and place it in the beaker.
5. Check for air-bubbles.
6. Place the proximal end of the stem into the staining solution.
7. Let the stem stain for 20 minutes, rinse it.

8. Use a razor blade to take as thin a section as possible and use superglue it to a piece of graph paper.
9. Use a stereoscopic microscope to take a digital photo and use image analysis software to work out the area of the stained section, calibrating the image using the graph paper.

### **3) Plotting Vulnerability Curves**

‘Vulnerability curves’ are plots of xylem water potential (MPa) against the stems PLC from a flushed maximum. Negative xylem water potentials are established by spinning stems in a centrifuge around their central axis, Stems are secured in a rotor designed to keep the ends of the stem immersed while spun (Alder,x).

1. Flush stems and measure maximum conductivity
2. Fill the cups in the rotor with 3.8ml of HCl solution using a burette. It is important to do this accurately as even slight mass imbalances can cause the centrifuge to become unbalanced.
3. Keep the rotor cups, plates, screws and washers in the correct positions. They have numbers lightly engraved.
4. Remove the grommets and load a 142mm stem with both its ends in a rotor cup. Secure the stem with a plate. The screws need only be ‘finger tight’ as they have a reverse thread and will tighten as the rotor is spun
5. Close the lid of the centrifuge, it has an electronic lock and therefore needs to be ‘on’ in order to open.
6. Check the brake is on maximum.
7. Turn the timing knob into the hold position and press the start button.

8. Increase the speed (rpm) until the speed displayed on the display is that which is required. In order to check the accuracy of this display a 'centrifuge velocity sensor' was custom made. Aaron Wetzler built the sensor. A reflective object sensor transmitted a smaller current every time it was passed by a black strip painted onto the centrifuge motor flywheel. While the speeds on the 'knob' were found to underestimate the centrifuge speed, speeds displayed on the dial were found to be accurate.
9. Start the stopwatch (I spun the stems for *four minutes*).
10. Press stop, wait for the rotor to stop spinning and remove the stem(s).
11. Let them equilibrate by leaving them immersed in solution for at least four minutes.
12. Measure the flow through the stem
13. Load the stem(s) back into the rotor and spin them at increasingly higher speeds (I spun at speed intervals corresponding to 0.5MPa decreases in xylem water potentials) until their conductance drops to zero.
14. Use recorded temperatures, height differences, flows and the area of active xylem to calculate stem conductivity  $K_s$  ( $\text{m}^2 \text{MPa}^{-1} \text{s}^{-1}$ ).
15. Plot curves as the percent loss from a maximum, flushed conductivity (PLC) against water potential (MPa)

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