



NAMIBIA UNIVERSITY OF SCIENCE AND TECHNOLOGY

Faculty of Natural Resources and Spatial Sciences

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**Investigating the environmental and anthropogenic spatio-temporal
patterns of plant health of *Welwitschia mirabilis* in the central
Namib Desert**

By

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Declaration

I, Titus Shuuya, hereby declare that the work contained in the thesis, entitled Investigating the environmental and anthropogenic spatio-temporal patterns of plant health of *Welwitschia mirabilis* in the central Namib Desert, is my own original work and that I have not previously in its entirety or in part submitted it at any university or other higher education institution for the award of a degree.

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Dedication

I would like to dedicate this thesis to Gobabeb Research and Training Centre (GRTC) for the research experience acquired.

Disclaimer

The thesis is presented in four chapters, with chapter three prepared as a manuscript for publication in the Journal of Arid Environment as a research article. The style and formatting of all the chapters are according to the Journal of Arid Environment requirements. There might be some unavoidable duplication of information in the manuscript.

The relative contribution of all the authors is as follows: Mr Titus Shuuya is the student and the main author who was responsible for the overall project design, data collection, analysis, discussion and manuscript write-up. Ms Barbara Curtis was responsible for general guidance with specific attention to the English language and editing. Dr. Theo Wassenaar was responsible for the general guidance with a focus on the topic relevant to the spatial and temporal patterns of plant health in the environment, including scientific English language editing. Dr. Jacques Berner was responsible for guidance on the plant physiology, specifically refining the chlorophyll a fluorescence data collection protocols, analysis, interpretation and discussion.

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Abstract

Environmental and anthropogenic patterns are the major factors that determine plant persistence across the landscape over a long-term period. The spatio-temporal patterns of vegetation distribution in the Namib Desert biome is attributed to the climatic conditions, geomorphology and edaphic features. Landscape patterns in the environmental conditions (e.g. rainfall and fog) of the Namib Desert have been described as a complex gradient that has a major influence on vegetation over time. *Welwitschia mirabilis* Hook. fil (Gnetales: Welwitschiaceae), commonly known as Welwitschia, is one of the Namib Desert endemic species. Many surveys have reported that desert plants, such as Welwitschia are well adjusted to environmental stressors. Nevertheless, cumulative impacts (potential impacts include interference with the water supply and deposition of dust) resulting from developments in the central Namib such as uranium mines might significantly affect the Welwitschia plants' survival. These impacts and the changing baseline conditions might affect their physiological processes, thus their growth and reproduction.

In this study, we measured chlorophyll *a* fluorescence (as an indicator of photosynthetic efficiency), leaf growth rate and cone dimensions to determine whether there are spatial and temporal differences among Welwitschia plants across catchments over time. The study showed a clear effect of topography, climatic conditions and anthropogenic effects, with plants in different catchments being clearly healthier than others during some months. We observed an increase in the photosynthetic efficiency and leaf growth rate accelerated by the episodic rainfall that occurred during January and April months across all catchments. Anthropogenic effects such as browsing by domestic animals probably caused the reduction in photosynthetic efficiency of plants located at the Welwitschia Wash catchment in December. Plants that were located at Welwitschia campsite catchment had the lowest photosynthetic efficiency and leaf growth rate throughout our investigation when compared to plants in other catchments. We have established a baseline study that can be used to develop a protocol to monitor the plant physiological status of Welwitschia. In this way, the results from the study will feed into a management strategy for this Welwitschia population. Apart from that, the findings may also aid restoration as well as rehabilitation measures such as transplantation and re-introduction of this unique plant by understanding its current functional health status across the landscape and over time.

Table of Contents

Declaration	i
Retention and use of thesis.....	ii
Dedication	iii
Disclaimer	iv
Acknowledgments.....	v
Abstract	vi

Chapter one

General background	- 1 -
--------------------------	-------

Chapter two

Literature review	- 3 -
-------------------------	-------

Chapter three

Environmental and anthropogenic spatio-temporal patterns of photosynthetic efficiency and reproductive characteristics of <i>Welwitschia mirabilis</i> Hook. fil in the central Namib Desert	- 13 -
--	--------

Chapter four

References	- 45 -
------------------	--------

Chapter one

1. General background

Environmental and anthropogenic patterns are the major factors that determine plant persistence across the landscape over a long-term period. For example, Jürgens *et al.*, (1997), described the spatial variation in the environmental conditions (e.g. rainfall and fog) of the Namib Desert as a complex gradient that has a major influence on vegetation from north-south and east-west. The Namib Desert landscape varies in vegetation structure and composition, ranging from relative dense ground layer of herbs, forbs, dwarf shrubs to smaller trees and other flora including the monotypic gymnosperm *Welwitschia mirabilis* Hook. fil (*Welwitschia*). In general, the spatial and temporal patterns of vegetation distribution in the Namib Desert biome is attributed to the climatic conditions, geomorphology and edaphic features Jürgens *et al.*, (1997). However, anthropogenic effects might influence the vegetation function, structure and composition over a long-term period. This is especially problematic for endemic plants (such as *Welwitschia*) who have restricted geographic ranges. For example, Brinckmann and von Willert (1987) reported 215 *Welwitschia* plants killed within five years at the *Welwitschia* Plains due to human carelessness through road construction and the result of tourism. Anthropogenic impacts such as habitat destruction, geomorphology and edaphic features modification resulting from developments (e.g. mining) are likely to contribute to the fragmentation of unique and endemic species, thus threatening their persistence.

About a decade ago, the global plant status was that 20% of the world's plants were threatened with extinction (Brummitt & Bachman, 2010). Moreover, 36% of the world's threatened plant species belong to the gymnosperm group which includes *Welwitschia* (Brummitt & Bachman, 2010). Brummitt & Bachman (2010) estimated that anthropogenic impacts account for more than 80% of the threats as compared to natural threats. The former includes habitat destruction for agriculture and mining, while the latter are natural disasters and intrinsic factors. Meanwhile, anthropogenic and natural stressors may pose direct and indirect negative impacts that can cause plants stress. For example, the increasing threats such as interfering with the water flow and dust resulting from developing mines such as the Husab mine in the central Namib can be additional deleterious stressors on *Welwitschia*. Stressed plants tend to have a poor growth rate, be unable to reproduce and have malfunctioning physiological

processes (Meyer *et al.*, 1973), thus having implications for the conservation of unique and long-lived plants such as *Welwitschia*. Plant stress implies adverse impacts on the plant's physiology induced by a sudden change from optimum environmental conditions where homeostasis is maintained, thus disrupting the equilibrium condition (Meyer *et al.*, 1973). In this report, plant stress and plant health will be used in the sense that stress is the inverse of health (in other words, a highly stressed plant is in very poor health and *vice versa*).

Causes of stress in plants can be classified as abiotic or biotic and plants can either be resistant, susceptible or avoiders in response to a stressful condition (Meyer *et al.*, 1973). Stress in plants can be investigated in many ways such as visual observation and physiological measurements. Physiological measurements such as chlorophyll *a* fluorescence, gas exchange and water potential can provide critical information about plant stress. In recent years, chlorophyll *a* fluorescence analysis has become one of the most powerful and widely used techniques to understand the photosynthetic apparatus in situ and ex-situ (Lepeduš *et al.*, 2012; Strasser *et al.*, 2004). The technique can provide early information on stress effects and stress resistance at the physiological level (Strasser *et al.*, 2004).

The chlorophyll *a* fluorescence technique, as a signature of photosynthetic efficiency was used in combination with measurements of leaf growth and reproductive characteristics, to assess the stress levels that *Welwitschia* plants show across the landscape at different times. The study aims to contribute to the development of the protocol to monitor the plant health of *Welwitschia* across the landscape over a long-term period.

The thesis is essentially divided into four chapters. Chapter one (this chapter) is the general introduction, chapter two is the literature review with a focus on the general effects of environmental stress on plants and the importance of the fast chlorophyll *a* fluorescence, leaf growth rate and reproduction characteristics of *Welwitschia* plants. Chapter three is written in the form of a research paper, which is the application of chlorophyll *a* fluorescence to understand the spatial and temporal environmental patterns of photosynthetic efficiency, leaf growth rate and reproductive characteristics of *Welwitschia*, thus providing an overall understanding of the patterns of plant health of *Welwitschia*. Chapter four is all the references for the thesis and manuscript. References that do not appear in the manuscript will be removed from the list during the manuscript submission.

Chapter two

2. Literature review

Welwitschia is an endemic Namib Desert gymnosperm. It is distributed at scattered locations within the Namib, from the most southerly population near Gobabeb to southern Angola (Henschel & Seely, 2000). Welwitschia is well known for its longevity and there is a large population surveyed at the Welwitschia Plains, south of the developing Husab mine (Cooper-Driver, 1994; Kananjembo & Kilbourn, 2013). The species is unique in biology with an eco-physiological and anatomical adaptation that enables it to persist in the dry environment. These include, large leaves that have been shown to contribute significantly to the energy flux balance at leaf temperatures of up to about 6°C above the ambient air temperature (Schulze *et al.*, 1980), which is not common in other desert plants.

Plants respond to environmental stress in several different ways, depending on the abiotic and biotic stress. This leads to plant's responding to stress levels through acclimation, senescence or survival. While senescence may lead to death, at least plants that are able to resist stress through their unique eco-physiological structure and function, may exhibit the ability to acclimate (Hüner & Hopkins, 2008), thus be able to persist in their environment.

Many studies have reported that Welwitschia is well adapted to extreme environmental stress conditions through their morphological and physiological characteristics which are not related to other desert plants (Bornman *et al.*, 1973; Eller *et al.*, 1983; Henschel & Seely, 2000; Herppich *et al.*, 1996; Schulze & Schulze, 1976; Schulze *et al.*, 1980; Winter & Schramm, 1986). For example, the two persistent non-succulent leaves that regulates the leaf temperature and energy balance as well as the ability to use 3-carbon (C₃) photosynthesis (primarily), but also using Crassulacean Acid Metabolism (CAM). However, with cumulative anthropogenic stressors (e.g. potential impacts include interference with the water supply and deposition of dust) resulting from development, it is not known how Welwitschia will survive with such deleterious conditions. Anthropogenic stressors, together with natural stressors, are likely to cause negative impacts on Welwitschia health. This might be accelerated by climate change, which could for instance lead to increased water stress (if precipitation

decreases) or damage to the photosynthetic apparatus (if temperatures increase) (Hüner & Hopkins, 2008).

Even though *Welwitschia* is not endangered, there are concerns regarding the fungal pathogen that infests female cones and seeds (Cooper-Driver, 1994; Di Salvatore *et al.*, 2013b; Jacobson & Lester, 2003). The fungal infestation reduces seed viability, thus causing a subsequent reduction in seedling recruitment which may cause long-term problems for *Welwitschia*. A long-term study observed only three seedlings in fourteen years (Henschel & Seely, 2000), suggesting that *Welwitschia* plants have a poor recruitment.

The current study was initiated with the aim to achieve a better understanding of long-term spatial and temporal patterns of plant health of *Welwitschia* for conservation management of plants within the Namib Desert. Plants survive in relation to the optimal environmental factors that support their ability to grow and reproduce with well-functioning physiological processes. Some of the environmental conditions that affect the growth of plants include limiting factors (e.g. temperature) and toxic factors (e.g. salinity), predation and parasites (Hüner & Hopkins, 2008). Furthermore, the main natural and anthropogenic factors that are expected to affect the growth of *Welwitschia* are temperature, dust deposition, availability of water and light (Bornman *et al.*, 1973; Hirano *et al.*, 1995; Meyer *et al.*, 1973; Thompson *et al.*, 1984). This review chapter will focus on the general effects of environmental stress on plants with a specific focus on *Welwitschia* and how it has adapted to such stressors. The review will also give a description of the photosynthetic process as one of the physiological processes that can be affected by environmental stressors and how it can be quantified by using the chlorophyll *a* fluorescence technique. Lastly, the review will look at leaf growth rate and cone production to complement the physiological measurements in order to understand the spatial and temporal environmental patterns of plant health of *Welwitschia in vivo*.

2.1. Effects of environmental stress on plants

In the natural environment, plants have upper and lower extremes of climatic conditions such as temperature that can interfere with plant growth (Hüner & Hopkins, 2008; Meyer *et al.*, 1973). *Welwitschia* has been reported to tolerate stress levels of negative water potential, extreme temperatures and strong winds (Eller *et al.*, 1983). Storms have been observed to cause mechanical stress to the leaves by bending and

twisting them (Eller *et al.*, 1983), with leaves splitting into strips and changing colour from green to reddish-grey, due to damage to the subepidermis layer. Extremely high ambient temperatures can increase leaf temperature (Herppich *et al.*, 1996), which denatures protein enzymes in the thylakoid membrane, especially in C₃ plants (Hüner & Hopkins, 2008). As a result, photosynthetic efficiency, particularly that of photosystem II and its associated oxygen evolving complex or water splitting efficiency is decreased. Additionally, photoinhibition is more pronounced in older leaves (Herppich *et al.*, 1996).

Water absorption and transpiration are the main factors that control water dynamics in plants (Meyer *et al.*, 1973). For example, if the rate of transpiration exceeds water absorption over a period of time, the water volume within a plant will decrease. As a result, the rate of growth will be affected, depending on other environmental factors. Unlike other desert plants, *Welwitschia* is known to follow the C₃ metabolism, but it has been reported to switch into CAM metabolism when conditions are favourable for photosynthesis, for example during a morning fog event (Cooper-Driver, 1994; Henschel & Seely, 2000; Schulze & Schulze, 1976; van Jaarsveld & Pond, 2013; von Willert *et al.*, 2005). Water deficit can interfere with plant's carbon metabolism and *Welwitschia*'s carbon metabolism has been reported to be disrupted by the leaf water deficit, high temperature, high water vapour deficit of the air and high radiation load during the day leading to loss in carbon dioxide (Herppich *et al.*, 1996). The stomata of C₃ plants are open during the day and photosynthesis occurs throughout the leaves, whereas the CAM plant's stomata are open at night when evapotranspiration is lower (Cooper-Driver, 1994; van Jaarsveld & Pond, 2013; von Willert *et al.*, 2005; Winter & Schramm, 1986). CAM plants are known to use water efficiently, because they can close their stomata at night and during the day for some periods, while using oxygen given off during photosynthesis is used for respiration and CO₂ given off during respiration is used for photosynthesis (Herppich *et al.*, 1996; Rodin, 1958; van Jaarsveld & Pond, 2013). *Welwitschia* leaves have stomata on both adaxial and abaxial surfaces (ca 61 mm⁻¹ and 86 mm⁻¹ respectively, on average) (Rodin, 1958; Schulze *et al.*, 1980). These have been reported to open during cool weather conditions, such as in the morning, late afternoon and until fog has lifted (Bornman *et al.*, 1972).

Water stress is the most persistent threat to plant survival, especially the desert plants. Water stress in plants can be viewed in two ways, either water deficit or an

excess of water (e.g. flooding). The latter is not common in the Namib Desert, especially in the *Welwitschia* localities. However, water deficit is the major concern to all the Namib Desert biota. Water stress is known to damage the cell membrane through desiccation of the cytoplasm and also control of stomatal opening and closure (Hüner & Hopkins, 2008). Furthermore, the physiological processes, in particularly photosynthesis are sensitive to water stress and it was shown in *Welwitschia* under drought conditions (Herppich *et al.*, 1996; Hüner & Hopkins, 2008). *Welwitschia* leaves were found to survive dehydration to water contents of 56% of the water content when tissue was fully saturated, corresponding to the relative humidity of 91% (Gaff, 1972). The main source of water in *Welwitschia* is still questionable, suggesting the need for further research work to quantify their water balance or budget. Light is another important limiting factor in all green plants, because of its primary role in photosynthesis (Meyer *et al.*, 1973). Light can have numerous effects on the physiological condition and processes such as chlorophyll synthesis, stomatal action, temperature of aerial organs and the rate of transpiration. Additionally, too much light can inhibit the photosynthesis process, due to excess light energy beyond that required for photosynthesis. However, plants have other pigments apart from chlorophyll such as carotenes and xanthophylls that play a role in protecting the photosynthetic system from prolonged photoinhibition (Hüner & Hopkins, 2008).

The global impacts of human activity on plant species is estimated to outweigh the natural threats, accounting for more than 80% of threats (Brummitt & Bachman, 2010). Human activity that results in pollution such as deposition of airborne particulates on vegetation and soil, may influence leaf and soil elemental composition (Alfani *et al.*, 1996). Such constituent elements can be taken up and accumulated by plants through the root system and the foliar surface. Particulate matter such as dust has been reported to decrease photosynthetic efficiency by shading the leaf surface in cucumber and bean plants (Hirano *et al.*, 1995). Furthermore, Hirano *et al.* (1995) found that dust can increase leaf temperature, resulting in the absorption of incident radiation, and consequently changing the photosynthetic rate, thereby increasing the transpiration rate. An increase in the transpiration rate is associated with water loss and *Welwitschia* plants were reported losing about a litre of water per day (Bornman *et al.*, 1973; Henschel & Seely, 2000), without additional stressors from human activities (e.g. dust resulting from mining activities). Furthermore, the impacts of particulate matter such

as dust may require the understanding of chemical composition, particle size, deposition rate and the dust load.

Anthropogenic stressors such as accumulating dust and interfering with the availability of water supply have been identified as the main threats to *Welwitschia* at Husab mine (Petrick, 2013). *Welwitschia* plants need to adapt to such stresses or their physiological processes might be affected. *Welwitschia* plants have been observed browsed by wild animal such as gemsbok, springbok and zebra during drought period, but plants are known to continue growing as usual. Accumulating anthropogenic stressors, in addition to the natural stressors and the changing baseline conditions may threaten the survival of *Welwitschia*. It is therefore critical to understand how *Welwitschia* will respond to extreme abiotic and biotic stressors.

2.2. Photosynthesis and environmental stress

Photosynthesis is the ultimate source of energy that sustains most of life of the earth. The process involves the conversion of sunlight energy, together with water and carbon dioxide in order to produce carbohydrates. Discovered in the 17th century, after a series of experiments photosynthesis laid the basic foundation of understanding the photosynthetic organisms (Hüner & Hopkins, 2008; Meyer *et al.*, 1973). In order to reduce carbon dioxide into carbohydrate, energy conservation through the photosynthetic electron transport chain needs to take place in the thylakoid membrane. The photosynthetic electron transport chain is composed of two large, multimolecular pigment protein complexes known as photosystem I (PSI) and photosystem II (PSII). The two photosystems operate in series and they are connected by the third multiprotein, cytochrome complex, a catalyst that is responsible for the transfer of electrons from plastoquinol to plastocyanin, which are enzymes found in the thylakoid membrane in chloroplasts of plants (Hüner & Hopkins, 2008). PSI and PSII differ in the way they absorb photons of a wavelength, the former optimally absorbs 700 nm and the latter absorbs 680 nm. Furthermore, PSII uses the light energy to oxidize two molecules of water into one molecule of molecular oxygen, while PSI uses light energy to reduce NADP⁺ (Nicotinamide Adenine Dinucleotide phosphate) to NADPH.

Photosystems are important in carrying out the primary photochemistry of photosynthesis from the absorption of light to the transfer of energy. Each

photosystem consists of a reaction centre, antenna chlorophyll and the light harvesting complexes (LHC). The light energy is gathered by the antenna and LHC, which passes it to the reaction centre. Here the electron flow is initiated by a charge separation (photooxidation) which occurs when a water molecule is split using the light energy (Caffarri *et al.*, 2014; Hüner & Hopkins, 2008). The electrons are passed through the three large multiproteins (PSII, cytochrome complex and PSI) to NADP^+ , and then carried by adenosine triphosphate (ATP) and NADPH to drive phosphorylation in order to make glucose. Glucose, a simple sugar, is the energy source for respiration, but is also used to store energy (e.g. as starch or sucrose), and is the main component of the cellulose in cell walls. As such it is the raw material for growth, repair and replacement of damaged parts.

The main limiting factors that can affect photosynthesis are the light intensity, carbon dioxide concentration and temperature. However, there are other underlying biotic and abiotic limiting factors that can affect photosynthesis such as disease, herbivory, infestations by insects, availability of water, herbicides and essential mineral elements (Hüner & Hopkins, 2008, Meyer *et al.*, 1973). In all plants, light intensity, carbon dioxide concentration and temperature are essential for the biosynthesis of ATP and NADPH, thus limiting the rate of photosynthesis (Fig. 1). Light intensity is important in photosynthesis, because the more photons of light absorbed by the leaf (depending on the availability of nitrogen), the greater the number of chlorophyll molecules that will be synthesised to generate more ATP and NADPH. However, an increase in the light intensity can inhibit photosynthesis, for example too much light will lead to light saturation and as a result ATP and NADPH will be affected due to damaged chlorophyll molecules (Fig. 1a). An increase in carbon dioxide concentration increases the rate of photosynthesis up to a maximum rate and the further increase in carbon dioxide concentration will result in a constant rate of photosynthesis (Fig. 1b). The rate of photosynthesis can be limited by temperature, because the reactions are catalysed by enzymes that can be denatured once optimum temperature is reached (Fig. 1c). Low temperature will slow down the enzymes reactions, but enzymes will be more efficient once optimum temperature is reached, thereafter, the enzymes structures will be destroyed at higher temperature.

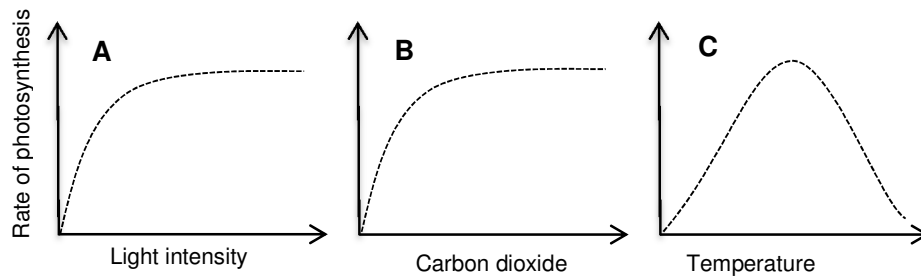


Fig. 1: Main factors that limit photosynthesis (adapted from Hüner & Hopkins, 2008)

2.3. Chlorophyll *a* fluorescence as a measure of photosynthetic efficiency

Chlorophyll *a* fluorescence is a widely used technique to quantify the photosynthetic efficiency of a plant, which is an indication of plant health. Based on the theory of energy fluxes in biomembranes, photochemical efficiency and the processes of non-photochemical de-excitation can be investigated by using chlorophyll *a* fluorescence (Li, Gao, & Strasser, 2005; Strasser *et al.*, 2004). The theory introduces and explains energy influxes and outfluxes that are rapidly equilibrated in a pigment system, thus total outflux is equal to the total influx (Strasser *et al.*, 2004). Chlorophyll *a* fluorescence transient is measured on dark-adapted leaves exposed to a 670 nm light pulse. A rapid rise of fluorescence intensity is observed that lasts for about 1 second, followed by a slow decrease of about 10 minutes until it reaches the maximum fluorescence intensity (Strasser *et al.*, 2004). Strasser *et al.* (2004) reported that the primary photochemical reactions that are fundamental for the phases of photosynthesis such as light energy absorption (ABS), light trapping (TR) and electron transfer (ET) can be quantified from the chlorophyll fluorescence kinetics.

The absorption of light energy initiates a charge separation between an electron donor and an acceptor at the pigment antenna, which can be quantified through the OJIP-transient test. The OJIP-transient test is represented by the O-step (initial fluorescence at 0.05 ms), J-step (fluorescence at 2 ms), I-step (fluorescence at 30 ms), P (time in ms taken to reach maximal fluorescence) (Strasser *et al.*, 2004). The OJIP-test can be used as a tool to understand plant health in situ through different analyses of environmental effects on photosynthetic organisms (Li *et al.*, 2005; Strasser *et al.*, 2004). The OJIP test and performance index ($PI_{\text{ABS, total}}$), with its partial potential parameters, are widely used to investigate plant health (Strasser *et al.*, 2004; Van Heerden *et al.*, 2007).

Original data from OJIP tests can be translated from the fluorescence transient to biophysical parameters that can be used to quantify PSII (Strasser *et al.*, 2004). As a result, information about the structure, conformation and function of photosynthetic efficiency at any physiological state can be provided (Strasser *et al.*, 2004). Original measurements include: maximal fluorescence intensity (F_M); fluorescence intensity at 0.05 ms (measured as F_0); fluorescence intensity at 300 ms ($F_{300\text{ ms}}$) required for calculation of the initial slope (M_0) of the relative variable fluorescence (V) kinetics; and the fluorescence intensity at 2 ms (the J step) denoted as F_J (Strasser *et al.*, 2004). The difference between F_M and F_0 called variable fluorescence (F_V), can be calculated (Strasser *et al.*, 2004). The ratio F_V/F_M can be related to the maximum quantum yield of primary PSII photochemistry in higher plants, usually with the value in the range of 0.78–0.84 (Stirbet, 2011).

Performance index (PI_{ABS}) is a multi-parametric expression that is derived from the three functional steps that regulate the initial stage of photosynthetic activity per reaction centre (RC) namely: absorption of light energy (ABS), trapping of excitation energy (TR) and conversion of excitation energy to electron transport (ET) (Stirbet, 2011; Strasser *et al.*, 2004; van Heerden *et al.*, 2007). Another more informative performance index is the PI_{total} which includes the fourth parameter which is the probability to reduce an end electron acceptor (Stirbet, 2011; van Heerden *et al.*, 2007). These multi-parametric expression can be observed in order to understand the structure and function of the photosynthetic efficiency, particularly the PSII of *Welwitschia* across the landscape over a period.

2.4. Leaf growth and cone production of *Welwitschia*

Leaf growth rate and cone production can provide additional supplementary information about the plant health of *Welwitschia*, for example an unhealthy plant may have a slow growth rate with a reduced reproductive output (Hüner & Hopkins, 2008). The spatial distribution patterns in *Welwitschia* leaf growth rate, reproduction characteristics have been investigated over 14 years, but the same study also identified the need for further studies focusing on growth and reproduction (Henschel & Seely, 2000).

Welwitschia has two broad and flat leaves (Photo 1A) that grow throughout its entire life, even though they give an impression of a multi-leaved (Photo 1B) plant

when twisted (Bornman *et al.*, 1972; Schulze *et al.*, 1980). The leaf split has been reported to be caused by stochastic events such as strong wind as well as browsing by some wild animals at the leaf base (Brinckmann & von Willert, 1987), unequal growth of the meristem (Bornman *et al.*, 1972) and grazing by micro-arthropods on the under surface of the leaves (Marsh, 1987). *Welwitschia* leaves have been shown to grow continuously, with an average monthly growth rate of 0.37 mm.day^{-1} at the *Welwitschia* Wash (Henschel & Seely, 2000). However, the growth rate can fluctuate between season and years. Henschel & Seely (2000) found that plant location can affect growth rate, for example a plant growing on the low banks or ledges of the main drainage channel grows at a higher rate than plants elsewhere in the channel. This could be due to optimum conditions of environmental drivers such as nutrients and moisture, among others (Henschel & Seely, 2000; Meyer *et al.*, 1973). Since it was first described, *Welwitschia* has been subjected to numerous scientific studies but some features are still not understood well such as root morphology, water sources and plant health.

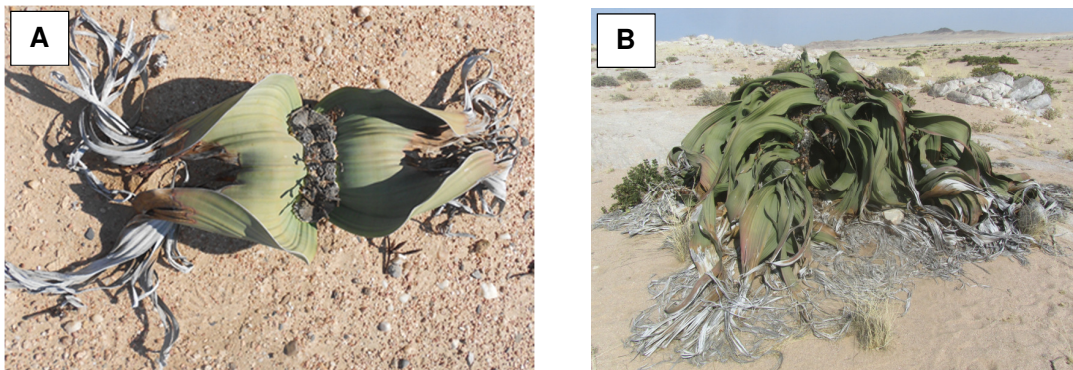


Photo 1: A young female *Welwitschia* indicating the two broad and flat leaves that grow throughout its entire life (A) and a matured female *Welwitschia* with twisted leaves, giving an impression of a multi-leaved plant (B). Photos were taken at the *Welwitschia* Plains.

Welwitschia is dioecious, with male cones being smaller than female cones (Bornman *et al.*, 1972). In general, cones ripen from December to July, but they have been observed to open at any time of the year (Bornman *et al.*, 1972; van Jaarsveld & Pond, 2013). The cones are carried on a stalked, branched inflorescence emerging from the meristem tissue at the stem base. It has been reported that continuous growth occurs in the meristem tissue below and above the leaves (Rodin, 1958; van Jaarsveld & Pond, 2013). The female plants have a micropyle structure which consists of a starch

storage fluid secreted by the ovules (Carafa *et al.*, 1992). This mechanism of micropylar drop secretion generally visible in the morning hours is related to the pollination process (Carafa *et al.*, 1992), which can be an indicator of a functioning reproduction system.

Chapter three

Environmental and anthropogenic spatio-temporal patterns of photosynthetic efficiency and reproductive characteristics of *Welwitschia mirabilis* Hook. fil in the central Namib Desert

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Key words: *Welwitschia mirabilis*, chlorophyll *a* fluorescence, OJIP transient, photosynthetic efficiency, photosystem II

Abstract

Welwitschia mirabilis (Welwitschia) is a long-lived gymnosperm endemic to the Namib Desert. It occurs from the Kuiseb River in Namibia to the Nicolau River north of Namibe in Angola, in a region where the rainfall varies from <50 mm to about 200 mm per year. It is protected by law in Namibia and is also listed as a CITES Appendix II plant. Apart from being unique in both appearance and biology, Welwitschia has been one of the main tourist attractions of the central Namib and a subject of several scientific studies.

In the central Namib Desert, there is a reference population of about 52 000 individuals occurring south of but adjacent to the developing Husab mine, slated to become the second largest uranium mine in the world. The cumulative impacts from the developing mine (potential impacts include interference with the water supply and deposition of dust) are likely to be additional to the factors that affect plant health, yet it is not known how Welwitschia will respond to these potentially deleterious conditions. In this study we investigate the photosynthetic efficiency (by measuring chlorophyll *a* fluorescence), leaf growth rate and cones dimensions across the landscape and how these change in different months. Our investigations show a clear effect of topography, climatic conditions and anthropogenic effects, with plants in different catchment being clearly healthier (with higher photosynthetic efficiency and leaf growth rate) than others. Episodic rainfall of about 10 mm measured across all catchment increased the

photosynthetic efficiency and leaf growth rate at all sites. Spatial and temporal patterns of photosynthetic efficiency and leaf growth rate of *Welwitschia* are associated with various environmental and anthropogenic stressors. We have established a baseline study that can be used to develop a protocol to monitor the plant physiological status of *Welwitschia*. In this way, the results from the study will feed into a management strategy for this *Welwitschia* population. Besides that, the findings may also aid restoration as well as rehabilitation measures such as transplantation and re-introduction of this unique plant by understanding its current functional health status across the landscape and over time.

Introduction

The Namib Desert plant *Welwitschia mirabilis* Hook. fil (Gnetales: Welwitschiaceae), commonly known as *Welwitschia*, has been called a living fossil plant (Henschel & Seely, 2000; Rydin *et al.*, 2003). It is endemic to the central and northern Namib Desert; its range extending from the Kuiseb River in Namibia to the Nicolau River north of Namibe in Angola (Kers, 1967; Rodin, 1953). The southern part of its population occurs in isolation, ranging from 2 to > 1000 individuals near Gobabeb (Henschel & Seely, 2000), with a larger population (>52 200 individuals) surveyed at the *Welwitschia* Plains (Cooper-Driver, 1994; Kananjembo & Kilbourn, 2013). The *Welwitschia* Plains population is located south of but adjacent to the developing Husab uranium mine (Goeller & Stobart, 2010; Petrick, 2013). Husab is an open-cast mine and its ore body is considered to be the highest-grade, granite-hosted uranium deposit in Namibia (Anonymous, 2010; Goeller & Stobart, 2010). Once full production is reached, the Husab mine will become the world's second largest uranium mine.

The development of Husab mine is guided by an Environmental Management Plan (EMP) that resulted from a comprehensive Environmental Impact Assessment (EIA) (Goeller & Stobart, 2010; Petrick, 2013). The EIA studies have identified the importance of avoiding cumulative impacts on vegetation health in general, but especially on that of *Welwitschia*. In this regard, to improve the chances of successfully managing the risks to the species, one of the EIA study's most prominent recommendations was that the factors that affect plant health need to be better understood and be incorporated into a long-term monitoring programme as part of a species management plan.

The cumulative impacts from the developing mine (potential impacts include interference with water supply and deposition of dust) are likely to be additional to the factors that normally affect the photosynthetic efficiency and plant health in general of *Welwitschia*, yet it is not known how *Welwitschia* will respond to these potentially deleterious conditions (Farmer, 1993; Goeller & Stobart, 2010; Petrick, 2013). These impacts and the changing baseline conditions might affect the plants' physiological processes, thus their growth, reproduction and development (Hüner & Hopkins, 2008). The ecophysiology and ecohydrology of *Welwitschia* has been investigated in a number of studies (Eller *et al.*, 1983; Herppich *et al.*, 1996; Soderberg, 2010), however the plant health of *Welwitschia* across the landscape over time is not well understood. Moreover, understanding the patterns of plant health of *Welwitschia* will require long-term studies across the spatial and temporal drivers due to the plant longevity. *Welwitschia* has an unusual metabolic pathway: even though it displays C3 photosynthetic pathway, it also indicates CAM characteristics (Cooper-Driver, 1994; Henschel & Seely, 2000; von Willert *et al.*, 2005).

The Husab mine EIA has identified a number of significant negative environmental impacts that may accumulate. In this regard, mine-generated dust, groundwater abstraction, seepage from the tailings dam and rock waste dump are possible significant negative impacts that may cumulatively affect the health of *Welwitschia* plants (Goeller & Stobart, 2010; Petrick, 2013). For example, the drainage networks that supply water to *Welwitschia* might be blocked by the planned waste rock dump and pits. Interfering with surface runoff or pollution of groundwater (likely from the tailings dam) may lead to negative impacts on *Welwitschia*. Water is an important environmental driver for *Welwitschia*, but *Welwitschia* water sources and use is still unclear (Bornman *et al.*, 1973; Henschel & Seely, 2000; Soderberg, 2010; van Jaarsveld & Pond, 2013; von Willert & Wagner, 1994). The sources of water for *Welwitschia* at the Husab mine are thought to include water from infiltrated surface runoff or deeper groundwater sources (Goeller & Stobart, 2010; Petrick, 2013). Even though the aquifer system around the Husab mine is known to be about 60 m below surface (Goeller & Stobart, 2010), it is also not known to what extent *Welwitschia* is obtaining its water from deep groundwater (Henschel & Wassenaar, 2013), and thus it is possible that a reduction in groundwater level or groundwater pollution may lead to plant stress. Additionally, interfering with the surface hydrology (e.g. blocking water flows in the drainage networks resulting from mining infrastructure such as the waste

rock dump and open pits) might lead to less water being available in the vadose zone. Furthermore, *Welwitschia* plants occur in hyper-arid conditions with limited water available, thus interference with any minimal water available for the plants can be regarded as significant. Lack of water can interfere with the physiological processes and lead to mortality or reduced growth and reproduction rate (Meyer *et al.*, 1973).

Water stress and dust have been observed to interfere with the photosynthetic efficiency and reproductive capability of plants in general (Meyer *et al.*, 1973). Photosynthesis requires water, carbon dioxide and light, therefore a reduction in any of the factors can lead to photosynthetic inefficiency (Meyer *et al.*, 1973). For example, lack of water will influence the photosynthetic electron transport, cause more rapid response typically from shutting stomata and subsequent decrease in CO₂ availability (Stirbet, 2011), thus plants may be stressed. *Welwitschia* can be damaged through browsing by animals and strong sandstorms, but populations may more likely be decreased by human carelessness (Brinckmann & von Willert, 1987). From 1981 to 1986, 215 plants have been reported killed by road construction and the result of tourism at the *Welwitschia* Plains (Brinckmann & von Willert, 1987). Anthropogenic threats might be exacerbated by climate change (e.g. increase in temperature and a decrease in precipitation patterns), resulting in severe impacts on the *Welwitschia* persistent.

Plant health can be investigated in numerous ways, ranging from growth rate and reproductive parameters to the photosynthetic efficiency or rate of response in key physiological processes. A good indicator of photosynthetic efficiency is chlorophyll *a* fluorescence, which indirectly reflects different functional levels of photosynthesis such as processes at pigment level, primary light reaction and electron transport reaction (Lichtenthaler, 1996; Redillas *et al.*, 2011; Strasser *et al.*, 2004). The fast chlorophyll *a* fluorescence induction is a popular, reliable, non-destructive physiological indicator of plant health in response to changing environmental conditions (Redillas *et al.*, 2011; Strasser *et al.*, 2004).

The response of the photosynthesis apparatus to a pulse of light is measured as fluorescence, which is translated by a so-called JIP-test tool into several biophysical parameters that each quantifies a different aspect of the behaviour of Photosystem II (PSII) (Li *et al.*, 2005; Stirbet, 2011; Strasser *et al.*, 2004). In this way, chlorophyll *a* fluorescence is used to develop a number of perspectives on the efficiency of the

photosynthetic process. Photosynthetic efficiency can be limited by various natural and anthropogenic factors such as, extreme levels of light and temperature, water deficiency, herbivory, dust and toxic elements.

Dust, generated by vehicles travelling on unpaved roads, material handling, crushing and screening operations, drilling and blasting as the most likely sources of dust pollutants, may accumulate from the mine (Goeller & Stobart, 2010; Petrick, 2013). Open cast mining and road traffic have been shown to increase dust deposition on vegetation (Farmer, 1993). Dust has been reported to collect on the plants leaves, physically smothering them and blocking the stomata, thus affecting gas exchange and photosynthesis (Farmer, 1993). Recent studies also found that the *Welwitschia* leaves have a waxy layer, which is a good adaption to dry conditions (van Jaarsveld & Pond, 2013; von Willert *et al.*, 2005; Winter & Schramm, 1986). Dust is likely to get stuck on the waxy layer and interfere with the stomata opening and closing. Numerous studies have reported dust to interfere with stomatal function, increase leaf temperature and transpiration, reduce photosynthesis and increase the uptake of gaseous pollutants of plants (Darley, 1966; Farmer, 1993; Hirano *et al.*, 1995; Manning, 1971; Mansfield, 1998; Naidoo & Chirkoot, 2004; Sharifi *et al.*, 1997; Thompson *et al.*, 1984; van Heerden *et al.*, 2007). Thus dust generated from the developing mine is likely to affect the plant health of *Welwitschia*, requiring spatial and temporal investigation over a long-term scale.

Welwitschia is protected by law in Namibia and is also listed on Appendix II of the Convention on International Trade in Endangered Species (CITES). It is a unique plant, both in appearance and biology. It has been one of the tourist attractions of the central Namib and a subject of several scientific studies.

In this study, we measured chlorophyll *a* fluorescence (as an indicator of photosynthetic efficiency), leaf growth rate and cone dimensions to determine whether there are spatial and temporal differences among *Welwitschia* plants across catchments. Due to spatial and temporal variation in environmental conditions, we expect significant differences among catchments over time. Differences among catchments maybe be due to catchment characteristics such as the topography, shape, size, soil type, geology, climatic conditions and anthropogenic effects (Jürgens *et al.*, 1997). Furthermore, we expect an effect of topography, climatic conditions and anthropogenic effects, with plants in different catchments being clearly healthier (with

higher photosynthetic efficiency and leaf growth rate) than others. The study aimed to provide a clear mechanistic information needed for the possible management strategies for mines (e.g. avoiding construction of the mining infrastructure on the major catchments and blasting during heavy wind) and other developments that may have adverse impacts on the *Welwitschia* population. This will include developing and implementing a long-term protocol to monitor the plant health of *Welwitschia* in the central Namib Desert. Besides that, the findings may also aid restoration as well as rehabilitation measures such as transplantation and re-introduction of this unique plant by understanding its current functional health status across the landscape and over time.

Methods and Materials

Description of the study area

The study was conducted in the central Namib Desert within the Namib Naukluft Park (NNP), at *Welwitschia* Plains (Fig. 2) and near Gobabeb (Fig. 3). The *Welwitschia* Plains population is found between the Swakop River (north) and the Khan River (south), approximately 70 kilometres east of Swakopmund. The Gobabeb population occurs in tributaries of the Kuiseb River that are located about 15 km (*Welwitschia* Wash) and 30 km (Hope mine) east of Gobabeb Research and Training Centre (GRTC), which is about 120 km south-east of Swakopmund.

There is an extreme variation in moisture availability in the central Namib Desert. Rainfall patterns are sporadic with an increase from west (~10 mm along the coast) to the east (~60 mm at 100 km inland) (Shanyengana *et al.*, 2002a). Annual average rainfall recorded at Gobabeb is about 25 mm, however, there are variations between the years with a higher total rainfall of 145 mm recorded in 2011 (Eckardt *et al.*, 2012). Sporadic rainfalls are often important for seed germination, rapid growth rate and improved reproductive output for most plants including *Welwitschia* (Henschel & Seely, 2000). Conversely, fog and dew are the most important primary sources of water for many plants in the central Namib (Henschel & Seely, 2000; Shanyengana *et al.*, 2002a). Unlike rainfall, fog events and amount decreases with the distance from west (at coast) to east (inland) with about 60 – 200 days of fog events recorded at Gobabeb (Henschel & Seely, 2000; Shanyengana *et al.*, 2002a).

Even though the geology and soils of the two study areas are not the same, generally both habitats are characterised by rocky outcrops, inselbergs, rocky valleys, drainage networks and plains. These characteristics are important for surface and underground transportation of water for downstream plants such as *Welwitschia*. Apart from *Welwitschia* (dwarf shrub), other common plants from both study areas include *Acacia erioloba* (tree), *Arthroerua leubnitziae* (shrub), *Zygophyllum stapffii* (shrub), *Euphorbia virosa* (shrub), *Commiphora saxicola* (tree) and *Stipagrostis* (grass) species. Ungulates such as *Antidorcas marsupialis* (springbok), *Equus zebra hartmannae* (Hartmann's mountain zebra), *Tragelaphus strepsiceros* (greater kudu) and *Oryx gazella* (gemsbok) are also common in both study areas. They have been reported browsing on *Welwitschia* during the dry seasons, but their browsing has not been found to threaten *Welwitschia* survival (Brinckmann & von Willert, 1987). Nevertheless, the Gobabeb *Welwitschia* population has recently been experiencing near-continuous monthly browsing by horses. The leaves of *Welwitschia* plants were browsed to the meristem for about 8 months at *Welwitschia* Wash catchment near Gobabeb. Browsing stopped between January and April 2015. There are no domestic animals present on the *Welwitschia* Plains, nor have there been reports of wild animals browsing plants there.

The study occurred before any significant impacts from mining. Anthropogenic dust sources during and before the study period were small, being mostly limited to exploration activities, and no large structures had been built yet that could interfere with surface hydrology during this study. As such, the study represents an investigation of the effects of natural drivers – principally rainfall, catchment and to an extent also topography – on the physiological health of *Welwitschia*.

Experimental design

The 2011-2013 survey and database

At the *Welwitschia* Plains population, Swakop Uranium, the company operating Husab mine, conducted a *Welwitschia* census between 2011 and 2013. The census showed at least 52 200 individuals in the mine's exclusive prospecting licence and along a permanent water pipeline route (Kananjembo & Kilbourn, 2013). The survey was comprehensive but may have missed some scattered plants in small valleys around the main population. The database consists of photographs, coordinates, gender,

health condition scoring, landscape position and relative size of all plants (Kananjembo & Kilbourn, 2013). There is also a database of *Welwitschia* at the Gobabeb population (*Welwitschia* Wash and Hope mine) which has been the subject of many studies in the past (Henschel & Seely, 2000). The survey and database were used as a reference for sampling design.

Sampling design

The Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) Global Digital Elevation Model (GDEM) version 2 of the two study areas were downloaded from the United States Geological Survey's (USGS) EarthExplorer (Abrams, 2000). The overall accuracy for the GDEM 2 is approximately 17 m at the 95% confidence level, with a horizontal resolution on the order of 75 m (Abrams, 2000). The spatial analyst tool within ArcGIS 10.2 software was used to digitise the catchments and drainage networks from the GDEM 2 (Fig. 2 & Fig. 3).

The *Welwitschia* Plains and Gobabeb study sites were subdivided into five catchments. Three catchments, which we called *Welwitschia* campsite (33 km²), River channel (59 km²) and Zone 6 (83 km²), were located on the *Welwitschia* Plains (Fig. 2). The Gobabeb study site consisted of two catchments namely *Welwitschia* Wash (36 km²) and Hope mine (978 km²) (Fig. 3).

Overall, 100 *Welwitschia* plants were sampled with 20 plants randomly (random selection was only done at the *Welwitschia* Plains study site) selected from each catchment. The lack of an existing spatial database from which points (representing coordinates of the location of specific plants) could be selected randomly meant that the sampling design in the Hope catchment was only semi-random. Here we walked around and selected plants at random from around us. The plants in the *Welwitschia* Wash catchment were all selected previously as part of a long-term study on leaf growth (Henschel & Seely, 2000).

Because of intensive browsing that removed almost all leaf material from *Welwitschia* Wash and Hope mine catchments, and a limitation in time available, there was unequal replication in December 2014 and January 2015. We randomly selected plants but aimed overall to have a spread of plant sizes from small to large. We also separately selected the male and female plants to ensure a 1:1 sex ratio. Chlorophyll *a* fluorescence was measured within three months in December 2014, January 2015 and April 2015, while the leaf growth rate was measured every month from January

2015 to May 2015. Numbers of cones produced were counted twice (December 2014 and April 2015), while the cone dimensions were measured once in April 2015.

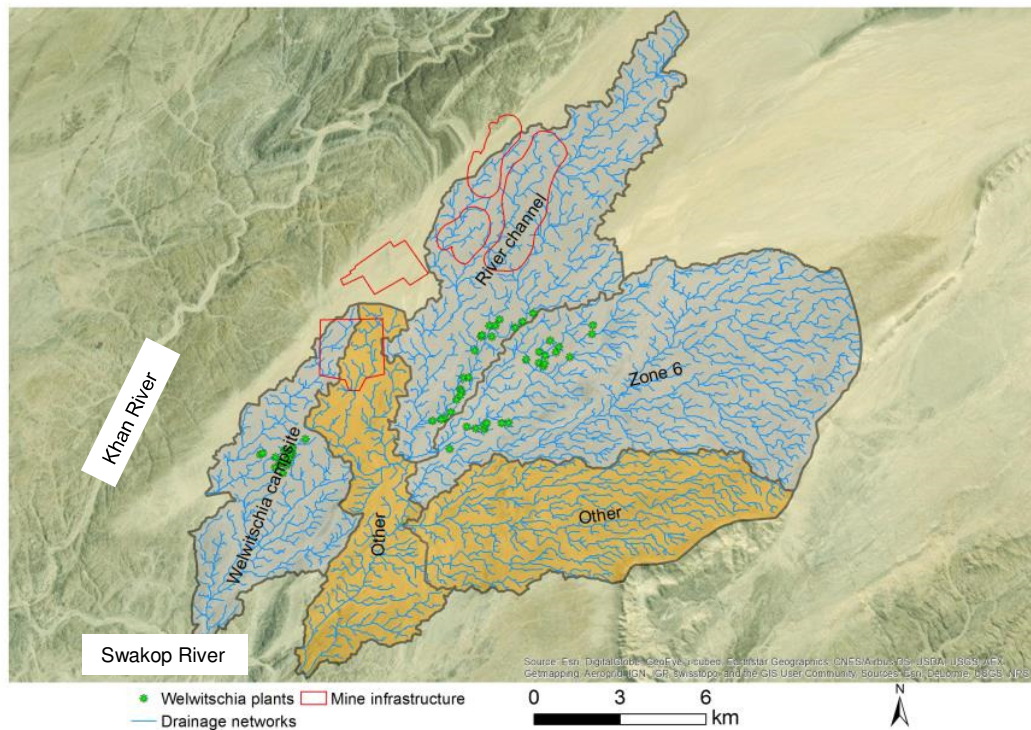


Fig. 2: Outlines of the three catchments (Welwitschia campsite, Zone 6 and River channel) showing their boundaries and surface drainage networks at the Welwitschia Plains population. The green dots indicate Welwitschia plants that were sampled. The two catchments indicated as other were not included in the study. The mine infrastructure includes (from bottom to the top) the tailing dam, office, two pits and waste rock dump on the (long polygon far east).

Chlorophyll *a* fluorescence measurements

Chlorophyll *a* fluorescence kinetics was measured *in situ* during the night (>1 hour after sunset to ensure maximum dark adaptation) with a Handy Photosynthetic Efficiency Analyser (PEA) instrument (Hansatech, UK). A one second light pulse of 650 nm at a light intensity of 3500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was given. Measurements were done on two separate leaves from each plant identified. On each leaf, five measurements were made within 15 cm from the base of a leaf, at different spots. Measurements were repeated three times; 3 – 11 December 2014 (after a pro-longed dry spell), 28 – 30 January 2015 after a light sporadic rainfall (ca 10 mm) that occurred on both study areas and 21 – 30 April 2015, after another sporadic rainfall (ca 2.5 mm). The

Chlorophyll a fluorescence measurements in January 2015 were done between three to six days after the rain events and five to ten days in April 2015.

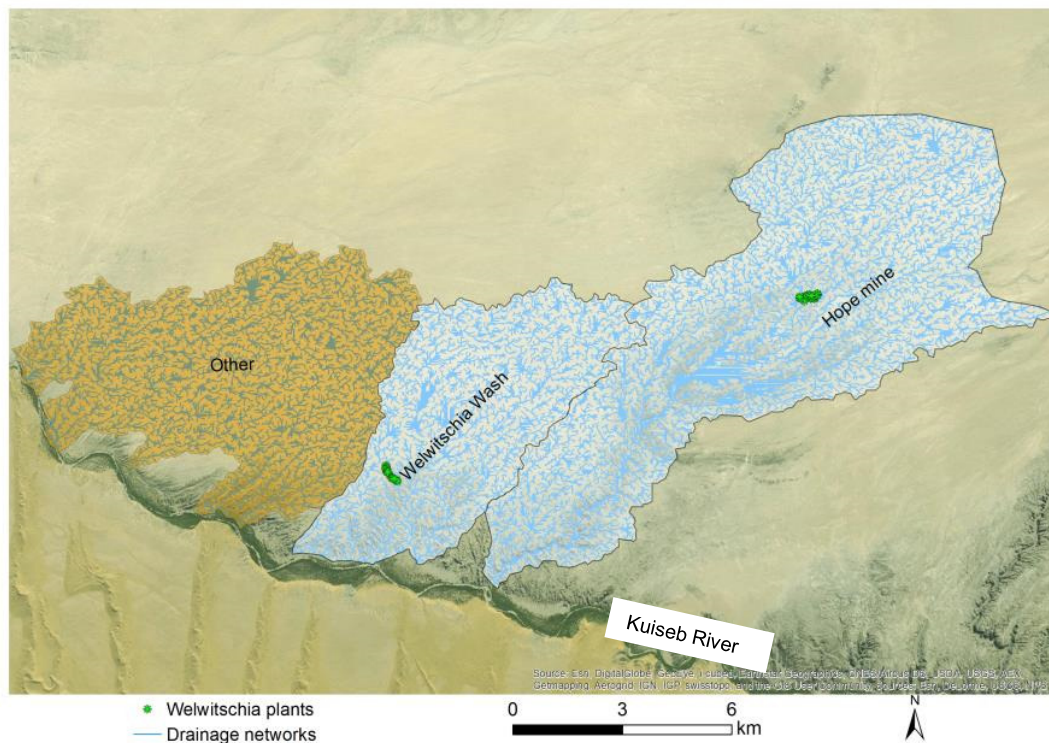


Fig. 3: Outlines of the two catchments (Welwitschia Wash and Hope mine) showing their boundaries and surface drainage networks at the Gobabeb population. The green dots indicate Welwitschia plants that were sampled. One catchment indicated as other was not included in the study.

Leaf growth rate and cone production

A light scratch (≤ 1 cm long) was made at the base of both leaves of each plant by using a fine syringe needle at the beginning of each month. Thereafter, the distance from the meristem to the scratch mark was measured every month (for five months) with an electronic calliper. Leaf growth rate was calculated in millimetre per day. The number of male cones were estimated individually in categories, because the numbers are usually high, while female cones were counted. Both male and female cones were only counted in December 2014 and April 2015. Measurement of cone dimensions (length and thickness) were done in April 2015 by using an electronic calliper. Five cones were haphazardly selected from each plant, their length measured from base-to-tip, and thickness measured at the widest part.

Calculation of fluorescence variables and statistical analysis

Fluorescence variables were downloaded and calculated with the Handy PEA Plus 1.10 software, thereafter data were transferred to SigmaPlot 12.0 software for analysis. Fluorescence data essentially form a four-stepped curve called a transient. The steps are labelled O, J, I and P, but each curve can be further unpacked into several “bands”, each of which provides information on a specific component of PSII (Srivastava *et al.*, 1997; Strasser *et al.*, 2010). In addition to the OJIP steps, in this paper we focus specifically on three of those bands, labelled K, J and I (there is some overlap in labels with those of the steps). For example, changes in the rise of the transient at 0.3 ms (K-band) are specifically associated with the oxygen evolving complex (OEC) or change in the antenna size (Strasser *et al.*, 2010). We furthermore calculated biophysical parameters such as specific energy fluxes and yields or flux ratios (Strasser *et al.*, 2004).

The O-step is initial fluorescence at 0.05 ms, the J-step is fluorescence at 2 ms, the I-step is fluorescence at 30 ms, and the P-step is the time in ms taken to reach maximal fluorescence (Strasser *et al.*, 2004). The J-step provides information on photosynthetic electron transport beyond the primary quinone (Q_A^-), which enables a charge separation on the acceptor side of the PSII reaction centre. Fluorescence intensity at the I-step provides information about the reduction of the the plastoquinone pool (which reflects electron transport in photosystem I (PS I). The performance index is a multi-parametric expression that can be used to assess the overall plant vitality, in response to environmental stress. The PI_{abs} involves energy conversion from photons absorbed by the PSII reaction centre, to the reduction of secondary quinone (Strasser *et al.*, 2004). On the other hand, the PI_{total} is more advanced than the PI_{abs} because it involves energy conservation from photons absorbed by the PSII antenna, until the reduction of PSI acceptors (Strasser *et al.*, 2004). In addition to the three parameters of the PI_{abs} (the density of the reaction centres, the quantum yield of primary photochemistry and the ability to transport the electron into the electron chain), the PI_{total} includes the fourth parameter which is the electron transport flux to the PSI acceptors.

Additional perspectives on the response across the fluorescent transient are possible by selecting one transient as a reference (we used Zone 6 for this) and subtracting each of the others from it. We calculated relative variable fluorescence (V_{ij}) for three steps: K (normalised between the O and J steps), J (normalised between J

and P), and I (normalised between O and P). Thus, $V_{ij} = (F_t - F_i)/(F_j - F_i)$, where F_t = fluorescence at time t, F_i = fluorescence at the i -th step, F_j = fluorescence at the j -th step. We then subtracted each V_{ij} for the specific catchment from that of the reference at the same time step and plotted this new value, which we call ΔV_{ij} , as a function of $\log(t)$. We qualitatively evaluated the relative performance of plants from different catchments on these plots: where the line deviated above the reference, the plants in that catchment are performing relatively poorly when compared to the reference and vice versa.

One-way ANOVA was used to test for significant differences of photosynthetic efficiency and leaf growth rate of *Welwitschia* among catchments. The Holm-Sidak method for multiple means comparison was used for post hoc analysis.

Results

Spatial and temporal patterns of photosynthetic efficiency

OJIP transient test across catchments

Differences in chlorophyll a fluorescence patterns were observed across catchments for December, January and April (Fig. 4). The maximum obtainable fluorescence (F_m) of the OJIP-transients were generally higher during April and tended to be lower during December for all the catchments (Fig. 4). We observed a dramatic increase in the OJIP-transients of the *Welwitschia* Wash catchment from December to April (Fig. 4). The *Welwitschia* campsite catchment had the lowest chlorophyll a fluorescence signal during all three months investigated (Fig. 4).

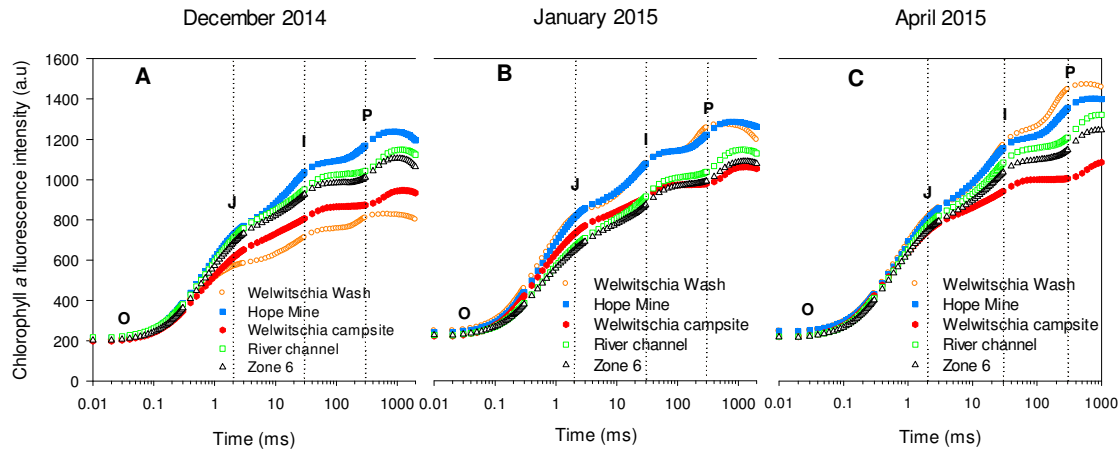


Fig. 4: The average chlorophyll *a* fluorescence transient plotted against logarithm time scale across catchments in three measurement periods (December: $n = 18$ for Welwitschia Wash and $n = 20$ for other catchments; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ for Zone 6; April: $n = 20$ for all catchments).

Chlorophyll *a* fluorescence intensity at 0.05 ms (O-step)

In general December had the lowest mean chlorophyll *a* fluorescence intensity at O-step across all catchments, while mean fluorescence intensity in the other two measurement periods were similar (Fig. 5). All three measuring periods showed significant differences among catchments (December: $F_4 = 6.366$, $P < 0.001$; January: $F_4 = 20.591$, $P < 0.001$; April: $F_4 = 22.986$, $P < 0.001$). Within measuring periods, Welwitschia campsite plants had a significantly lower ($P < 0.001$) chlorophyll *a* fluorescence intensity at O-step for the December period when compared to plants in other catchments, except those that were found in Zone 6 catchment (Fig. 5a). For the January measurement, the plants in the Welwitschia Wash and Hope Mine catchment had a significantly higher ($P < 0.001$) chlorophyll *a* fluorescence intensity at the O-step as compared to other catchments (Fig. 5b). The same trend was observed in April and the fluorescence signal at the O-step was significantly ($P < 0.001$) higher at the Welwitschia Wash and Hope Mine, when compared to the other catchments (Fig. 5c).

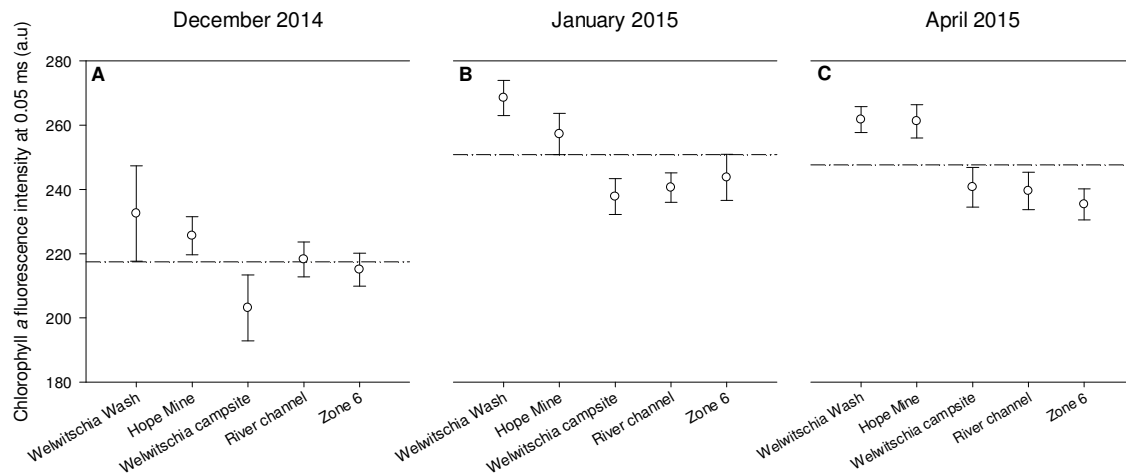


Fig. 5: Chlorophyll a fluorescence at 0.05 ms (O-step) across catchments. The dotted line represents the mean value across all the catchments (December: $n = 18$ for Welwitschia Wash and $n = 20$ for other catchments, $F = 6.366$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ for Zone 6, $F = 20.591$; April: $n = 20$ for all catchments, $F = 22.986$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

Chlorophyll a fluorescence intensity at 0.3 ms (K-step) and difference in relative variable fluorescence (ΔK -band)

All three measuring periods showed significant differences among catchments (December: $F_4 = 6.509$, $P < 0.01$; January: $F_4 = 28.813$, $P < 0.01$; April: $F_4 = 3.222$, $P < 0.01$). In December, plants in all catchments tended to have a lower K-step compared to plants in January and April (Fig. 6). Welwitschia campsite plants had a significantly lower ($P < 0.05$) K-step value than plants elsewhere, except plants that were located in the Zone 6 (Fig. 6a). Moreover, the K-band was more pronounced in plants that were located at the Welwitschia campsite during the study period (Fig. 7), suggesting that these plants were experiencing a lower water splitting efficiency or OEC in relation to plants that were located in the River channel as well as to the other catchments (Fig. 7a). However, the water splitting efficiency of plants at Welwitschia Wash was observed to be higher in April (Fig. 7c). This was also observed in the K-step of plants at Welwitschia Wash catchment which was significantly higher ($P < 0.001$) in January when compared to other catchments (Fig. 6b). The only significant difference ($P = 0.02$) observed in the K-step in April was between Welwitschia Wash and Zone 6 catchments (Fig. 6c).

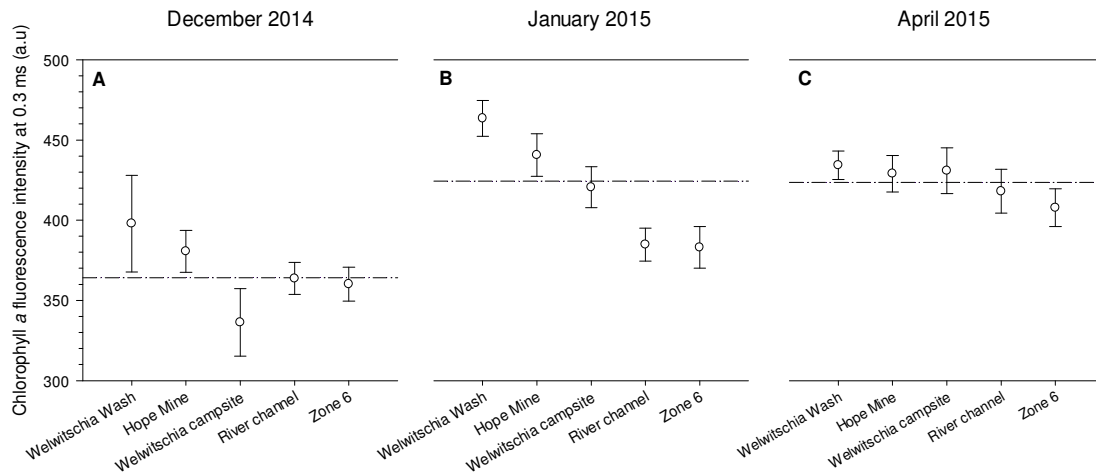


Fig. 6: Chlorophyll *a* fluorescence at 0.3 ms (K-step) across catchments. The dotted line represents the mean value across all the catchments (December: $n = 18$ for Welwitschia Wash and $n = 20$ for other catchments, $F = 6.509$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ for Zone 6, $F = 28.813$; April: $n = 20$ for all catchments, $F = 3.222$). Degree of freedom: 4; and $P < 0.01$ for all three measuring periods. Error bars indicate 95% confidence interval.

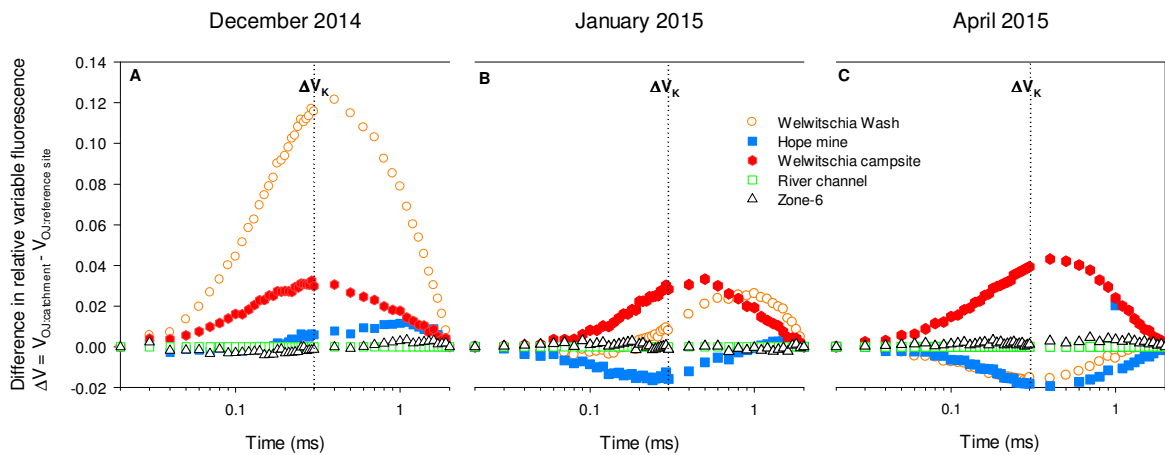


Fig. 7: Difference in relative variable fluorescence double normalised 0.02 ms and 2 ms to reveal the K-band at 0.3 ms across the catchments. River channel was used as a reference site.

Chlorophyll *a* fluorescence intensity at 2 ms (J-step) and difference in relative variable fluorescence (ΔJ -band)

All three measuring periods showed significant differences among catchments (December: $F_4 = 11.230$, $P < 0.001$; January: $F_4 = 48.669$, $P < 0.001$; April: $F_4 = 4.460$, $P < 0.001$). In January and April, plants in all catchments were generally observed to have higher fluorescence intensity at 2 ms when compared to December (Fig. 8b, c), but, plants within Welwitschia Wash and Hope Mine catchments had a significantly

higher ($P < 0.001$) fluorescence intensity at the J-step in January when compared to other catchments. In January, the J-band appeared in all plants that were located Welwitschia Wash, Hope mine, Welwitschia campsite and Zone 6 catchments, in relation to plants that were found in the River channel (Fig. 9b). However, the J-band was pronounced in plants located at Welwitschia campsite, suggesting that these plants were less able to produce electron flow beyond Q_A^- (Fig. 9b, c). In December, the fluorescence intensity at 2 ms of the Welwitschia campsite plants was significantly lower ($P < 0.001$) as compared to other catchments (Fig. 8a).

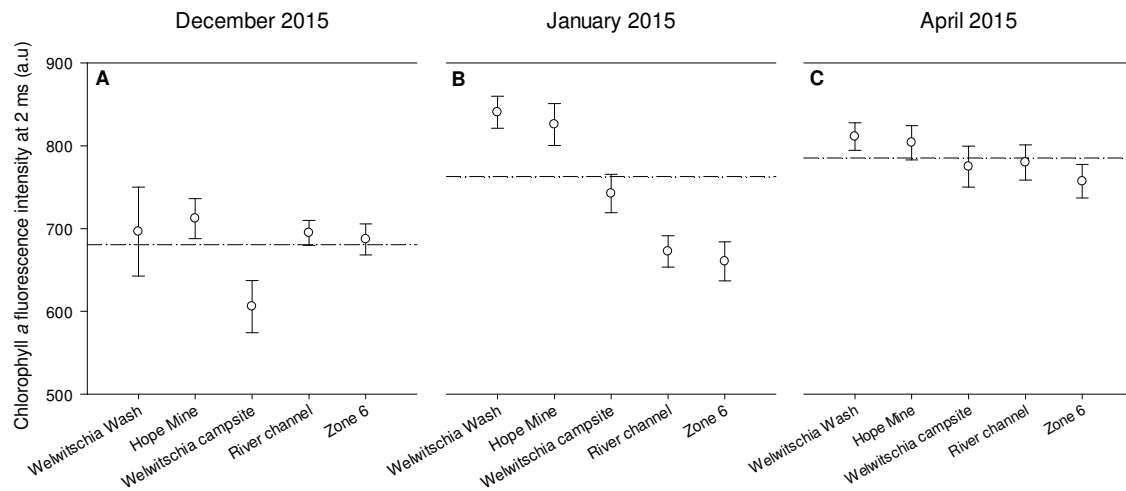


Fig. 8: Chlorophyll *a* fluorescence at 2 ms (J-step). The dotted line represents the mean value across all the catchments (December: $n = 18$ for Welwitschia Wash and $n = 20$ for other catchments, $F = 11.230$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ for Zone 6, $F = 48.669$; April: $n = 20$ for all catchments, $F = 4.460$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

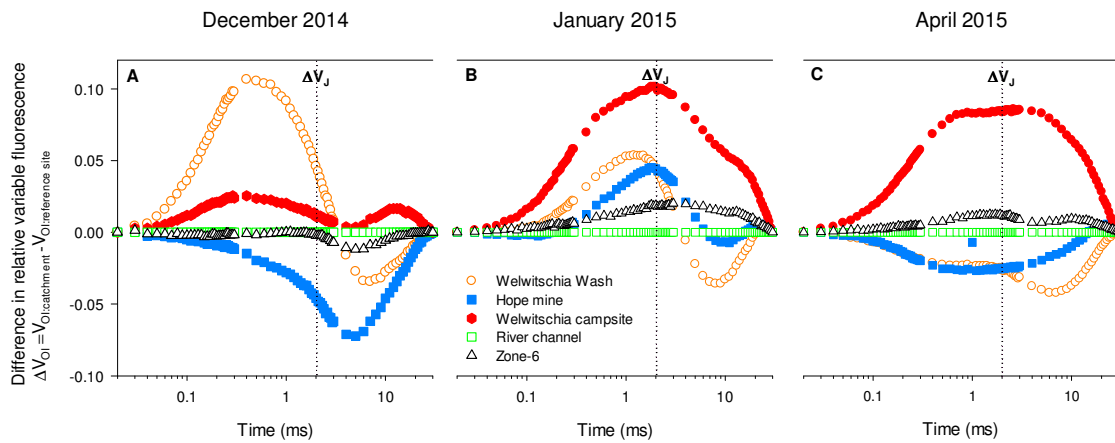


Fig. 9: Difference in relative variable fluorescence double normalised at 0.03 ms and 30 ms to reveal the J-band at 2 ms across catchments. River channel was used as a reference site.

Chlorophyll *a* fluorescence intensity at 30 ms (I-step) and difference in relative variable fluorescence (ΔI -band)

All three measuring periods showed significant differences among catchments (December: $F_4 = 19.220$, $P < 0.001$; January: $F_4 = 46.642$, $P < 0.001$; April: $F_4 = 20.988$, $P < 0.001$). In December, the I-step was significantly higher ($P < 0.001$) in plants located at the Hope Mine and significantly lower ($P < 0.001$) in plants located at Welwitschia campsite when compared to other catchments (Fig. 10a). In April, a negative ΔI -band was observed in plants at Welwitschia Wash and Hope Mine catchments indicating a more effective flow of electrons between PSII and PSI when compared to River channel plants (Fig. 11c).

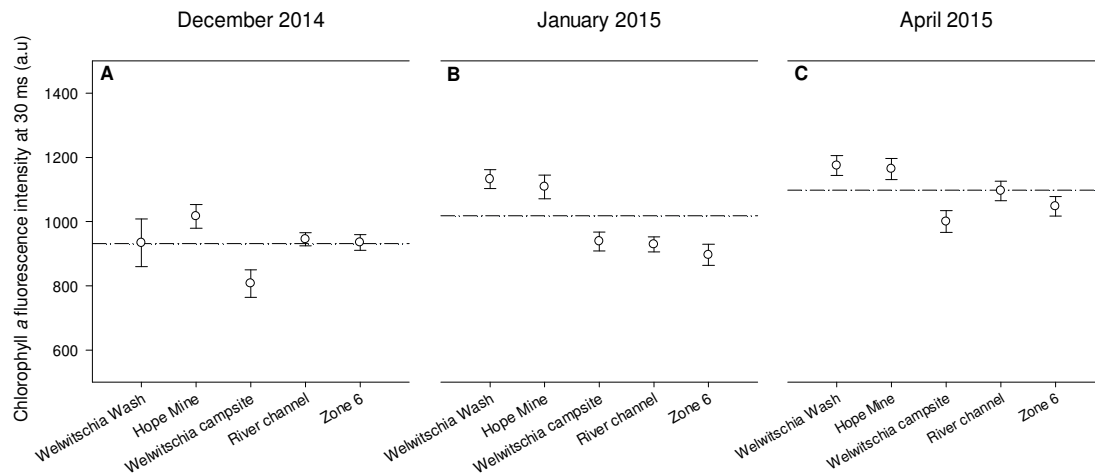


Fig. 10: Chlorophyll a fluorescence at 30 ms (I-step). The dotted line represents the mean value across all the catchments (December: $n = 18$ for Welwitschia Wash and $n = 20$ for other catchments, $F = 19.220$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ for Zone 6, $F = 46.642$; April: $n = 20$ for all catchments, $F = 20.988$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

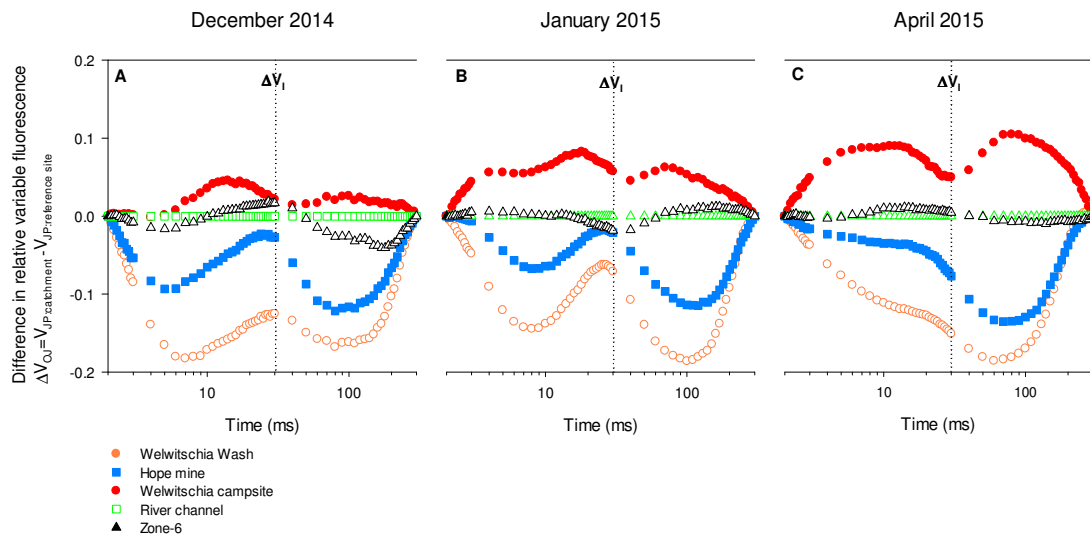


Fig. 11: Difference in relative variable fluorescence double normalised at between 2 ms and 300 ms to reveal the I-band at 30 ms across the catchments. River channel was used as a reference site.

Chlorophyll a fluorescence intensity at 300 ms (P-step)

All three measuring periods showed significant differences among catchments (December: $F_4 = 25.562$, $P < 0.001$; January: $F_4 = 62.368$, $P < 0.001$; April: $F_4 = 68.544$, $P < 0.001$). The Welwitschia campsite plants had a significantly lower ($P < 0.001$) maximum chlorophyll a fluorescence intensity at 300 ms in December and April

when compared to plants in other catchments (Fig. 12a, c). During April the Welwitschia campsite and Hope Mine had a significantly higher ($P < 0.001$) fluorescence intensity at 300 ms, when compared to other catchments (Fig. 12c).

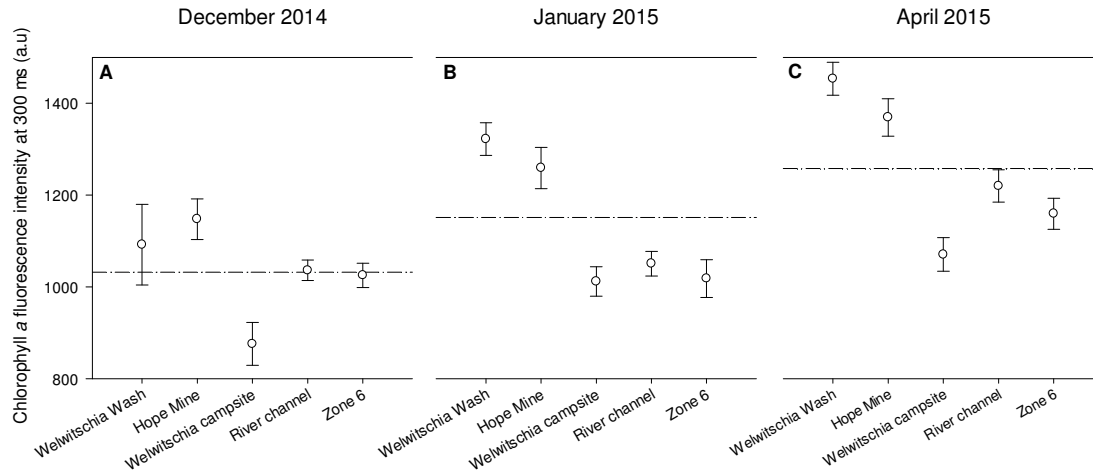


Fig. 12: Chlorophyll *a* fluorescence at 300 ms (P-step). The dotted line represents the mean value across all the catchments (December: $n = 18$ for Welwitschia Wash and $n = 20$ for other catchments, $F = 25.562$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ for Zone 6, $F = 62.368$; April: $n = 20$ for all catchments, $F = 68.544$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

Performance indexes (PI_{abs}) and (PI_{total})

Variations in the overall plant vitality (PI_{abs} and PI_{total}) were observed across catchments and over the months (Fig. 13). All three measuring periods showed significant differences among catchments (December: F_4 , $PI_{abs} = 19.727$, $PI_{total} = 15.053$, $P < 0.001$; January: F_4 , $PI_{abs} = 44.223$, $PI_{total} = 43.865$, $P < 0.001$; April: F_4 , $PI_{abs} = 68.681$, $PI_{total} = 68.786$, $P < 0.001$) (Fig.13). Nevertheless, across all catchments, the PI_{total} of all plants tended to increase from December to April, except that of plants from Welwitschia campsite (Fig. 13), probably due to the episodic rainfall received in January and April. Welwitschia Wash plants had a remarkable increase in vitality from December to April. The decline in the PI_{abs} of plants at the Welwitschia Wash catchment in January was probably due to the inhibition of the electron transport flux into the PSI antenna when compared to the PI_{total} during the same period (Fig. 13b, e). Plants that are located at the Welwitschia campsite had significantly lower

PI_{abs} and PI_{total} ($P < 0.001$) in January and April as compared to plants in other catchment areas (Fig. 13b, c).

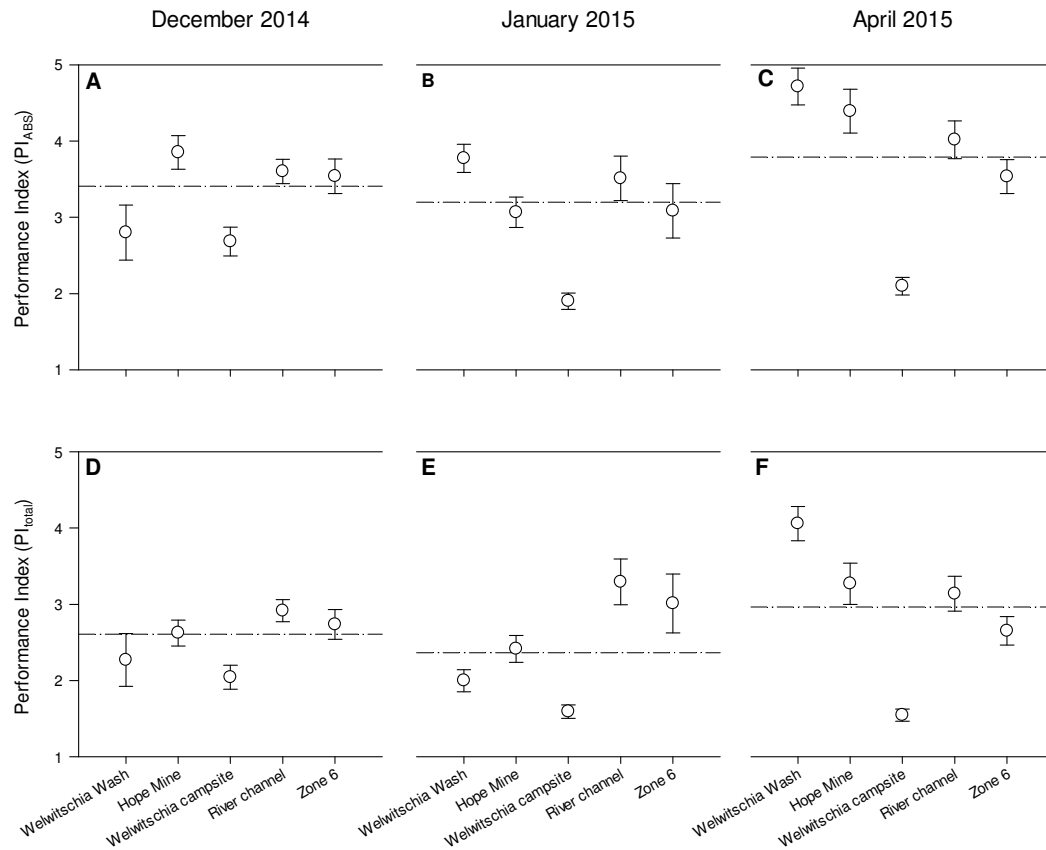


Fig. 13: Performance Index (PI_{abs} : A, B and C; and PI_{total} : D, E F) across catchments. The dotted line represents the mean value across all the catchments for that period (December: $n = 18$ for Welwitschia Wash and $n = 20$ for other catchments, (PI_{abs} : $F = 19.727$ and PI_{total} : $F = 15.053$); January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ for Zone 6, (PI_{abs} : $F = 44.223$ and PI_{total} : $F = 43.865$); April: $n = 20$ for all catchments, (PI_{abs} : $F = 68.681$ and PI_{total} : $F = 68.786$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

Absorption of light energy ($Y_{RC}/(1-Y_{RC})$)

All three measuring periods showed significant differences in absorption of light energy across catchments (December: $F_4 = 17.831$, $P < 0.001$; January: $F_4 = 17.741$, $P < 0.001$; April: $F_4 = 34.068$, $P < 0.001$) (Fig. 14). In December, the ability to absorb sunlight of the plants located in the River channel catchments were significantly

healthier ($P < 0.001$) when compared to other catchments, except those at the Zone 6 catchment (Fig. 14a). During the same month, plants within Welwitschia Wash catchment had a significantly lower ($P < 0.001$) ability to absorb light energy as compared to other catchments (Fig. 14a), probably due to browsing by domestic animals. However, absorption of light energy was significantly higher ($P < 0.001$) at the Welwitschia Wash in April as compared to other catchments, except for the Hope Mine catchment (Fig. 14c). The episodic rainfall that occurred at all study sites in January and April might have stimulated the increased variation in absorption and trapping of light energy at some catchment areas during this period (Fig. 14 and Fig. 15). In January, the plants at the Hope mine catchment area had significant a better light absorbing efficiency ($P < 0.001$), when compared to all plants in other catchments (Fig. 14b). Plants growing at the Welwitschia campsite catchment had the lowest ($P < 0.001$) efficiency of light absorption during the January and April (Fig. 14b, c).

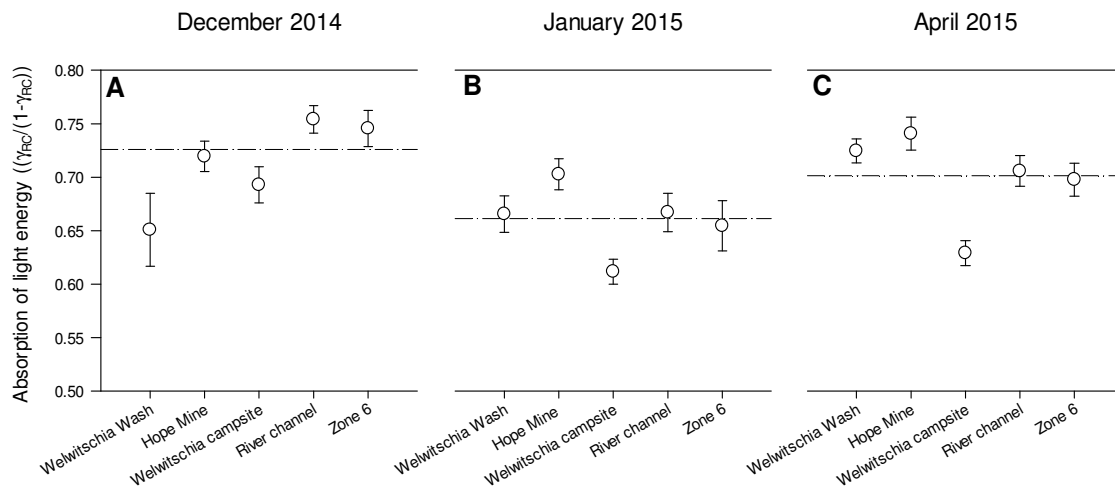


Fig. 14: Absorption of light energy ($(Y_{RC} / (1 - Y_{RC}))$) across catchment. The dotted line represents the mean value across all the catchments for that period (December: $n = 18$ for Welwitschia Wash, and $n = 20$ for other catchments, $F = 17.831$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ from Zone 6, $F = 17.741$; April: $n = 20$ for all catchments, $F = 34.068$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

Trapping of light energy ($\phi_{P0} / (1 - \phi_{P0})$)

All three measuring periods showed significant differences in the ability to trap light energy across catchments (December: $F_4 = 32.408$, $P < 0.001$; January: $F_4 = 16.799$,

$P < 0.001$; April: $F_4 = 24.056$, $P < 0.001$) (Fig. 15). The ability to trap light energy was observed to be significantly higher ($P = 0.01$) in plants that were located at Hope Mine and Welwitschia Wash in January (Fig. 15b). Welwitschia campsite catchment had the lowest significant ($P < 0.001$) efficiency in trapping of light energy in January and April months (Fig. 15b, c).

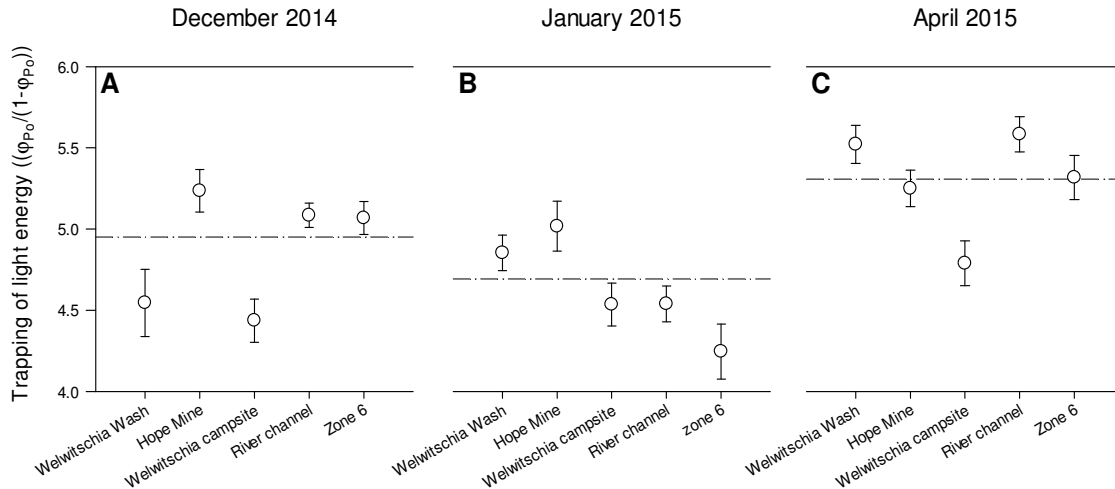


Fig. 15: Trapping of light energy ($\phi_{P0} / (1 - \phi_{P0})$) across the catchment. The dotted line represents the mean value across all the catchments for that period (December: $n = 18$ for Welwitschia Wash, and $n = 20$ for other catchments, $F = 32.408$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ from Zone 6, $F = 16.799$; April: $n = 20$ for all catchments, $F = 24.056$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

Probability to move electrons ($\Psi_{E0} (1 - \Psi_{E0})$)

All three measuring periods showed significant differences in the ability to move electrons further than Q_A^- across catchments (December: $F_4 = 12.890$, $P < 0.001$; January: $F_4 = 58.005$, $P < 0.001$; April: $F_4 = 93.242$, $P < 0.001$) (Fig. 16). The ability to move electrons further than Q_A^- was the best ($P < 0.001$) at Hope Mine during December (Fig. 16a). During April, Welwitschia plants in the River channel and Zone 6 had a significantly higher ($P < 0.001$) electron transport efficiency (Fig. 16c). During April (Fig. 16c) all the catchments differed statistically ($P < 0.001$) from each other. Plants in the Welwitschia Wash had the highest electron transport efficiency, while the Welwitschia campsite plants had the lowest electron transport efficiency (Fig. 16c).

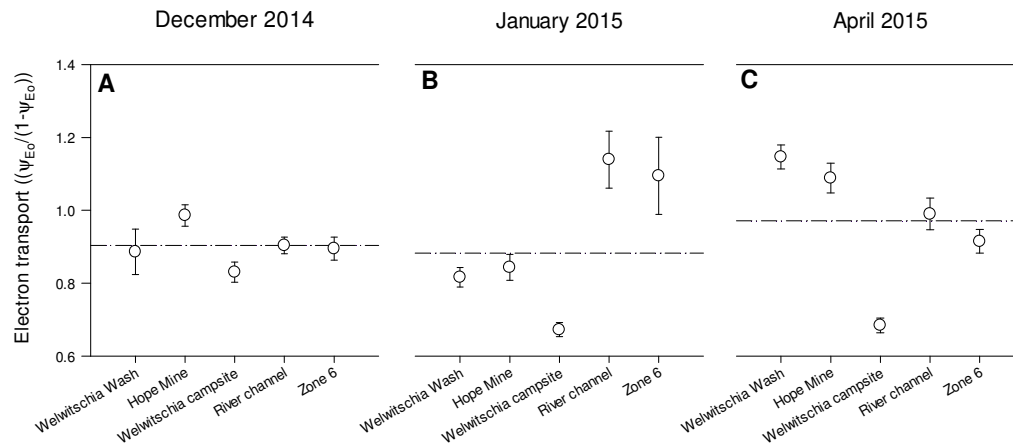


Fig. 16: Electron transport ($\Psi_{E0}/(1-\Psi_{E0})$) across catchments. The dotted line represents the mean value across all the catchments for that period (December: $n = 18$ for Welwitschia Wash, and $n = 20$ for other catchments, $F = 12.890$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ from Zone 6, $F = 58.005$; April: $n = 20$ for all catchments, $F = 93.242$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

Reduction of end electron acceptors ($\delta_{Ro}/(1-\delta_{Ro})$)

All three measuring periods showed significant differences in the reduction of end electron acceptors across catchments (December: $F_4 = 40.345$, $P < 0.001$; January: $F_4 = 72.318$, $P < 0.001$; April: $F_4 = 37.142$, $P < 0.001$) (Fig. 17). In December, significantly lower ($P < 0.001$) reduction of the end electron acceptors was observed in plants that were located at the Hope Mine catchment when compared to other catchments (Fig.17a). During January, significant differences ($P < 0.001$) were observed among plants in all catchments, with Zone 6 being more efficient in the reduction of end electron acceptors and Welwitschia Wash the least efficient (Fig.17).

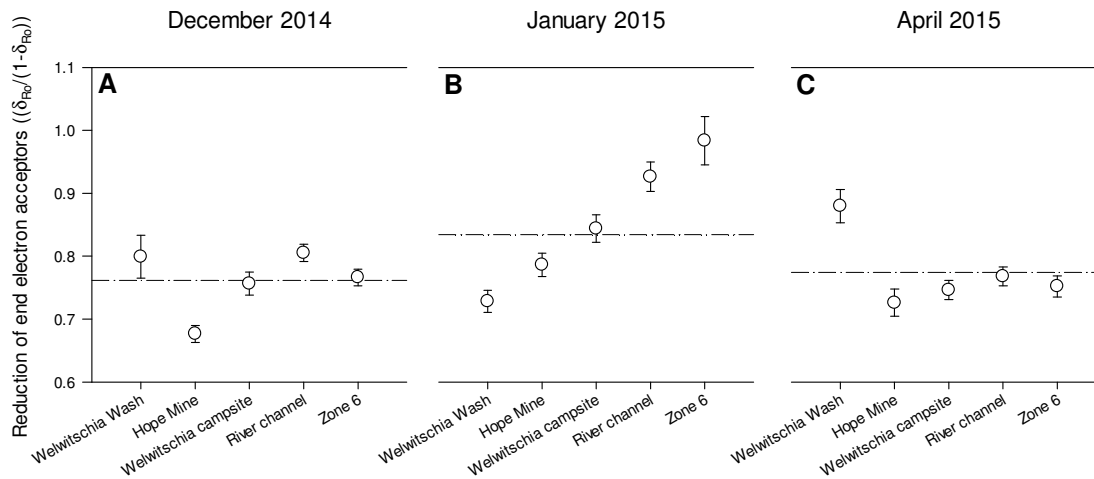


Fig. 17: Reduction of end electron acceptor ($\delta_{R0}/(1-\delta_{R0})$). The dotted line represents the mean value across all the catchments for that period (December: $n = 18$ for Welwitschia Wash, and $n = 20$ for other catchments, $F = 40.345$; January: $n = 20$ for other catchments, $n = 14$ for River channel and $n = 9$ from Zone 6, $F = 72.318$; April: $n = 20$ for all catchments, $F = 37.142$). Degree of freedom: 4; and $P < 0.001$ for all three measuring periods. Error bars indicate 95% confidence interval.

Leaf growth rate and cone production

Leaf growth rate across catchments

All three measuring periods showed significant differences in leaf growth rate across catchments (January: $F_4 = 9.042$, $P < 0.001$; February: $F_4 = 24.278$, $P < 0.001$; March: $F_4 = 24.278$, $P < 0.001$; April: $F_4 = 45.332$, $P < 0.001$; May: $F_4 = 25.726$, $P < 0.001$) (Fig. 18). Differences in leaf growth rate were observed, generally decreasing from January to May (Fig. 18). Plants that were found in the Welwitschia campsite catchment had a significantly lower ($P < 0.001$) leaf growth rate as compared to all other catchments over time, but not significantly different from plants that are located in Zone 6 in April (Fig. 18). In general, plants located in the Welwitschia Wash catchment grew faster than plants at other catchments during the whole period (Fig. 18). Moreover, plants at Welwitschia Wash grew significantly faster ($P < 0.001$) as compared to plants in other catchments, except those that are found in the Hope Mine catchment area during March until April (Fig. 18c, d, e).

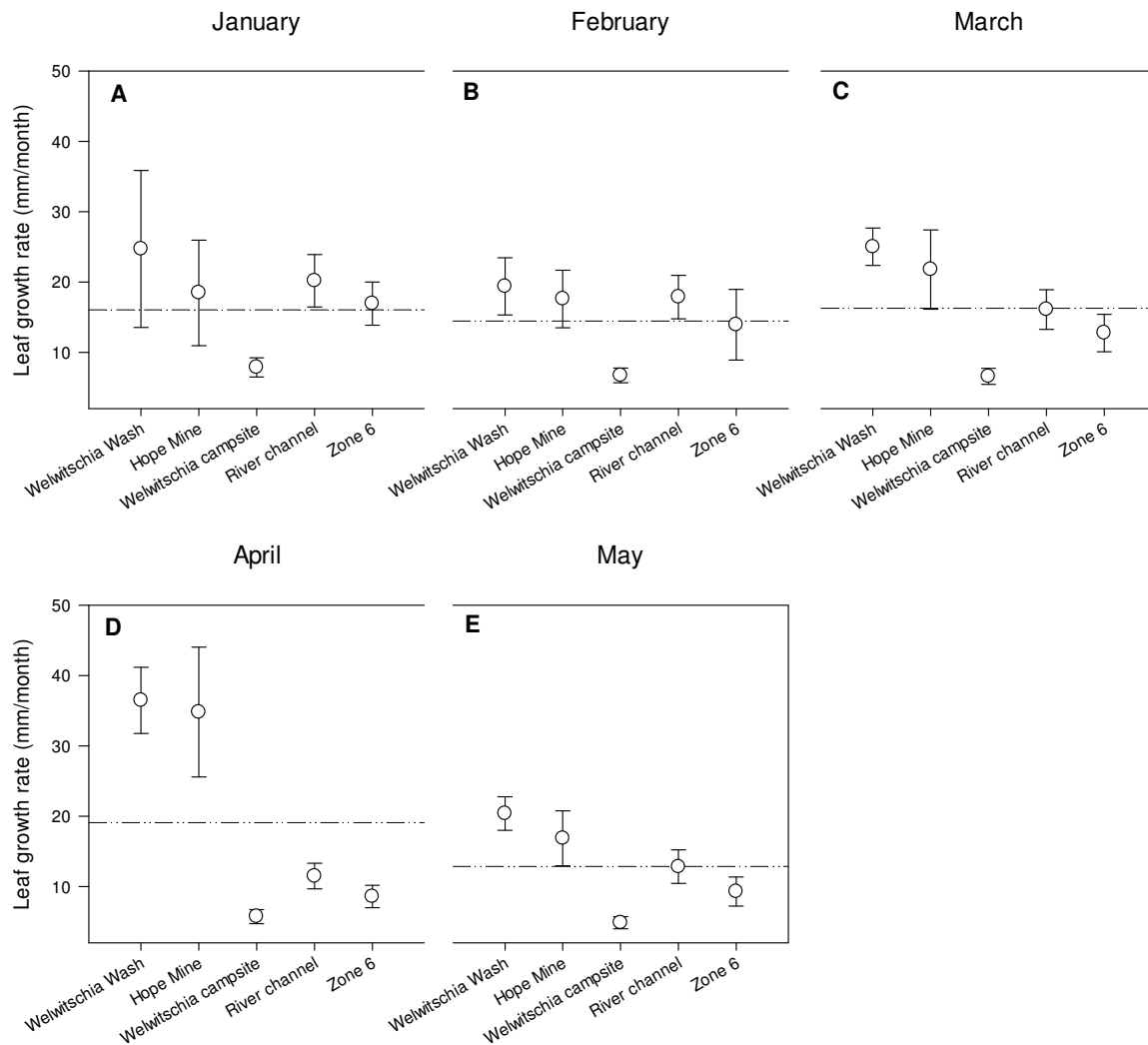


Fig. 18: Leaf growth rate (mm/month) across catchments over five months ($n = 20$ for all catchments; $F = 9.042$ (January), 8.527 (February), 24.278 (March), 45.332 (April) and 25.726 (May); degree of freedom: 4; and $P < 0.001$ for all five months. Error bars indicate 95% confidence interval.

Cone production across catchments

Variations in the number of female cones produced at different catchments were observed in December 2014 and April 2015 (Fig. 19). No female cones were observed in December 2014 and April 2015 at Welwitschia Wash catchment (Fig. 19). Plants that were located at Hope Mine and Zone 6 tended to produce more cones during December 2014, as compared to April 2015 (Fig. 19). Welwitschia plants that were located at Welwitschia campsite and River channel produced more cones in April 2015 than in December 2014 (Fig. 19). In December, there was no significant difference ($P = 0.08$) in the mean number of female cones observed between catchments (Fig. 19). However, in April plants found at Hope Mine had significantly fewer ($P = 0.004$)

mean number of female cones produced when compared to River channel catchment (Fig. 19).

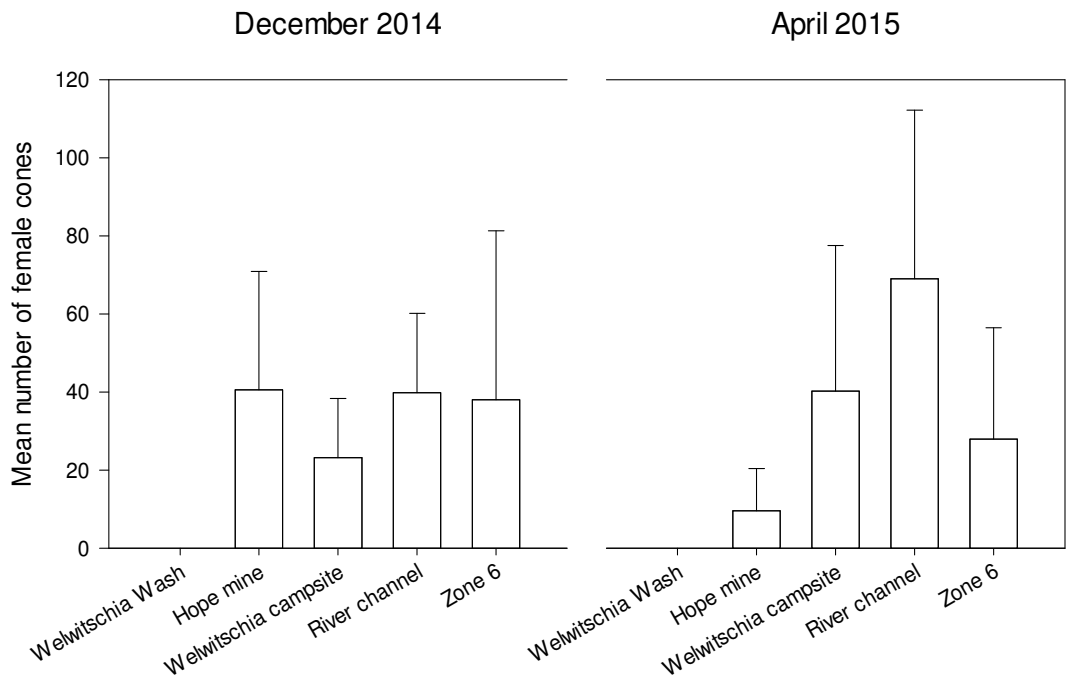


Fig. 19: Female cone production across catchments in December 2014 and April 2015 (n = 20). Degree of freedom: 4; P = 0.08 for December 2014 and P = 0.004 for April 2015. Error bars indicate 95% confidence interval.

Plants that were located in the River channel catchment produced more than 200 male cones in December and April (Fig. 20, Fig. 21). Male plants at Hope mine catchment produced more than 200 cones in December (Fig. 20). No male plants produced any cones at Welwitschia Wash catchment in December, but one plant produced 55 cones in April 2015 (Fig. 21).

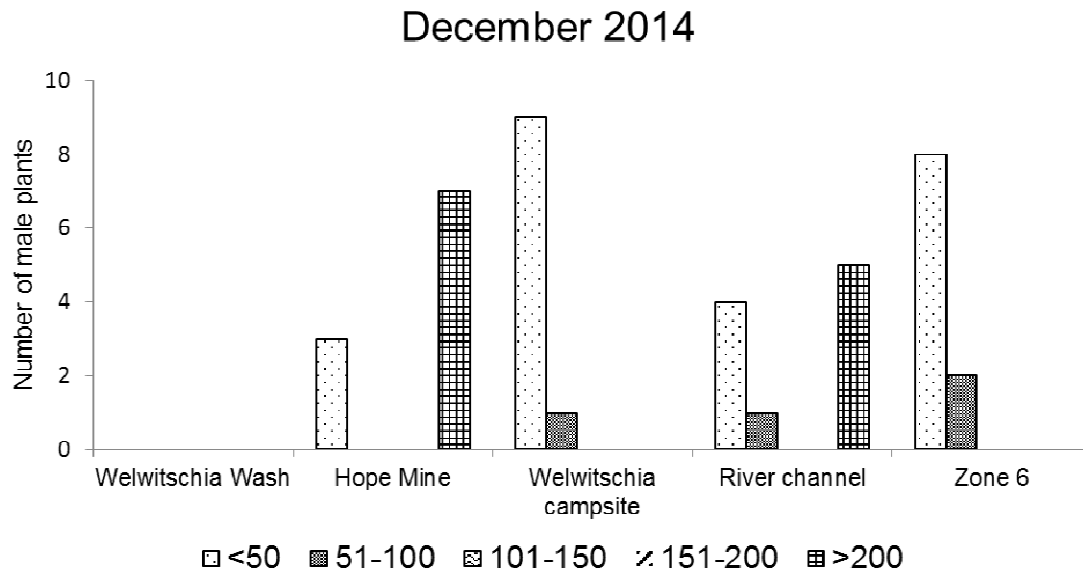


Fig. 20: Number of male plants with cones placed in categories (as indicated in the legend below the graph) across catchments in December 2014 (n = 20).

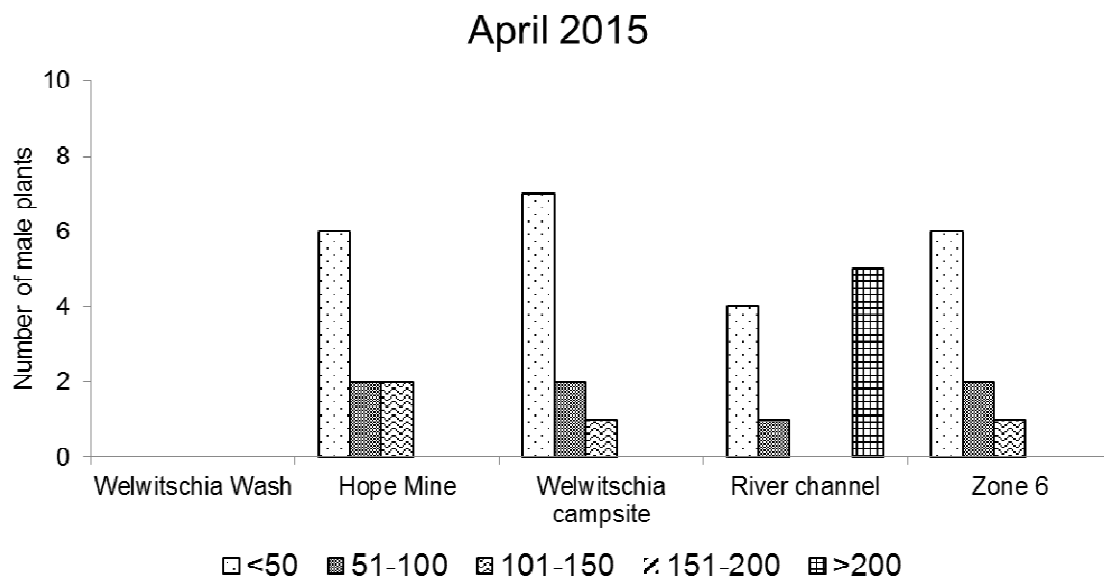


Fig. 21: Number of male plants with cones placed in categories (as indicated in the legend below the graph) across catchments in April 2015 (n = 20).

Cone dimensions across catchments

Female cones were observed to be longer and thicker in dimensions when compared to male cones (Fig. 22). No significant differences were observed in the female cone dimensions among the catchments, except Welwitschia Wash that had no cones

during this period (Fig. 22). *Welwitschia* Wash male cones were significantly shorter ($P = 0.002$) when compared to River channel and Zone 6 catchments (Fig. 22).

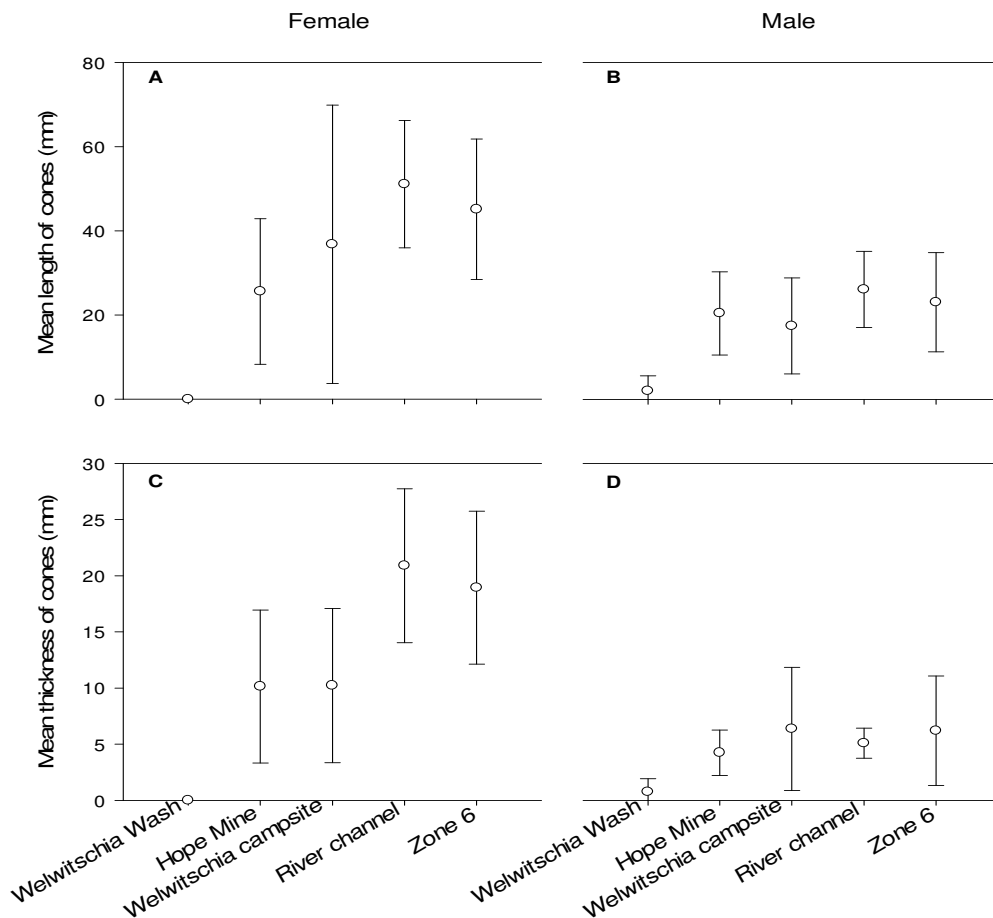


Fig. 22: Mean female and male cone dimension (length: A and B; thickness: C and D) across catchments in April 2015. ($n = 20$ for all catchments; $F_{\text{female length}} = 5.468$, $F_{\text{male length}} = 4.915$, $F_{\text{female thickness}} = 9.523$, $F_{\text{male thickness}} = 2.173$; degree of freedom = 4 for all catchments; $P < 0.001$ for all catchments. Error bars indicate 95% confidence interval.

Discussion

In the natural environment, *Welwitschia* plants tend to experience diverse natural and anthropogenic stressors which vary over space and time. Landscape characteristics such as geological formations, topography and soil type as well as climatic conditions and anthropogenic effects have been described and studied as important determinants of vegetation patterns in the central Namibia Desert (Jürgens *et al.*, 1997). In this study we found spatial and temporal differences in the photosynthetic efficiency, leaf growth rate and cone production in *Welwitschia* plants. We observed an effect of topography, episodic rainfall and anthropogenic effects (browsing by

domestic animals), with plants in different catchment being clearly healthier (with higher photosynthetic efficiency and leaf growth rate) than others during the three measurement periods. Jürgens *et al.* (1997), reported the spatial and temporal patterns in the environmental conditions (e.g. rainfall and fog) of the Namib Desert as a complex gradient that has a major influence on vegetation from north-south and east-west. Topographic features such as elevation and slope may change vegetation exposure to wind and solar incidences. Such exposure can indirectly change the soil moisture and other favourable climatic conditions, thus contributing to the patterns of environmental stress to plants. Our digital elevation models showed a higher elevation (data not shown) around most of the plants at *Welwitschia* campsite catchment which has resulted in the low water flow accumulation (data not shown) when compared to plants in other catchments. The flow accumulation implies water accumulates along the flow paths which can be influenced the topography.

Climatic conditions such as extreme temperature and availability of water can inhibit the flow of electrons between the two photosystems, which can ultimately lead to photooxidative damage (Herppich *et al.*, 1996; Strasser *et al.*, 2004; van Heerden *et al.*, 2007). Photooxidative damage to the photosystems will result in the malfunction of the proteins that contain the functional cofactors, oxidation of water and reduction of plastoquinone pool, thus affecting the overall photosynthetic efficiency (Strasser *et al.*, 2004). We observed an increase in the photosynthetic efficiency and leaf growth rate, which was accelerated by the episodic rainfall that occurred during January and April months across all catchments. Episodic rainfall in the Namib Desert is known to play a critical role in the plants persistence (Eckardt *et al.*, 2012; Henschel & Seely, 2008, 2000; Shanyengana *et al.*, 2002b). Apart from breaking seed dormancy, increasing recruitment and stimulating other growth patterns, Henschel & Seely (2000), showed that the *Welwitschia* leaf growth rate quadrupled from 0.37 mm per day in three years following episodic rainfall of about 11 mm during the mid-summer. The current study also confirmed a remarkable increase in leaf growth rate following the episodic rainfall in January and December of plants in some of the catchment areas. Availability of moisture is one of the limiting factors to *Welwitschia* plants and plants can utilise every moisture available for growth, reproduction and development (Di Salvatore *et al.*, 2013; Henschel & Seely, 2000).

Many studies have indicated the impact of natural stresses such as drought, high light exposure, nutrient deficiency and salinity on photosynthetic efficiency (Goltsev *et*

al., 2012; Gómez *et al.*, 1998; López-Climent *et al.*, 2008; Srivastava *et al.*, 1997; van Heerden *et al.*, 2007). The ability of the plants to split water increased from December to April probably due to the episodic rainfall. The ΔK -band can be used as an indicator of water splitting efficiency of PSII (Srivastava *et al.*, 1997). However, the appearance of the ΔK -band can also be an indication of an increase in the antenna size (van Heerden *et al.*, 2007). Water splitting efficiency can influence the electron transfer of the reduction of quinone (Srivastava *et al.*, 1997), thus inhibiting electron movement within the photosystem. Our study found a positive ΔK -band more pronounced in plants that were located at the Welwitschia campsite throughout the study period and plants located at Welwitschia Wash during December. This suggest that the plant's ability to split water is less effective than other catchments during those measurement periods. Due to the dynamic nature of the natural environment, changes in the water splitting efficiency is unlikely to appear in healthy or unstressed plants (Srivastava *et al.*, 1997). Meanwhile, if a positive ΔK -band is observed it could imply that the plant is under stress (especially heat stress) and that damage occurred on the electron donor side (Stirbet, 2011), which might interfere with the water splitting efficiency (Srivastava *et al.*, 1997). In December the weather was dry and hot, while in January and April the weather was moderate with two episodic rainfall events (personal observations). Climatic conditions such as heat and drought have been reported to influence the ΔK -band by effecting the water splitting process of electron transport and regulation (Srivastava *et al.*, 1997, Stirbet, 2011).

Similar trends were observed with the appearance of the ΔI -band and ΔJ -band with plants at the Welwitschia campsite. These bands indicate a less effective flow of electrons between PSII and PSI, thus negatively influencing the photosynthetic efficiency. The occurrence of the episodic rainfall probably increased the vitality of the plants, thus plants are able to use this opportunity to grow faster in January and April. This most likely increase the leaf growth rate, which requires energy from photosynthesis and hence the improved flow of electrons beyond the Q_A^- across all catchments after the episodic rainfall. In December, the plants at Welwitschia campsite catchment had a reduced ability to move electrons beyond Q_A^- , probably due to the accumulation of electrons downstream (before Q_A^-) (Strasser *et al.*, 2004). As a result there was a reduction in the efficiency of producing a charge separation on the acceptor side of the PSII reaction centre, thus inhibiting the overall photosynthetic efficiency (Meyer *et al.*, 1973; Strasser *et al.*, 2004). The observed increase in the

reduction of the plastoquinone pool of the plants in December which implies that the electrons move more efficiently between PSI and PSII (Meyer *et al.*, 1973). As a result plants had a higher photosynthetic efficiency due to a larger pool of PQ which is available to accept electrons from PSI and to produce plastoquinol during that period.

Anthropogenic effects such as browsing by domestic animals probably caused the reduction in photosynthetic efficiency of plants located at the Welwitschia Wash in December. Plants were observed to be browsed up to the meristem at Welwitschia Wash catchment, however, when browsing stopped in December, the plants recovered remarkably from January to April. Damage to Welwitschia plants, including browsing by wild animals, has been reported in several studies (Brinckmann & von Willert, 1987; Cooper-Driver, 1994). However, there are no evidence of adverse impacts on their survival. Nevertheless, continuous intensive browsing by domestic animals might threaten their persistence in the long run.

Welwitschia plants have probably compensated for the browsing damage by increasing the leaf growth rate (Belsky, 1986; Belsky *et al.*, 1993; Paige & Whitham, 1987). Compensatory growth implies the positive rapid growth in response to browsing exceeding that found in the unbrowsed plants (Meyer *et al.*, 1973), however, this may require more scientific evidence through comparative studies. Furthermore, variation in the photosynthetic efficiency of plants located at Welwitschia Wash could be due to the carbohydrate sink-source dynamic mechanism that plants develop (Belsky *et al.*, 1993). Compensatory growth can trigger plants to absorb more light energy while freeing the reserved carbohydrates during the sink-source dynamics, which probably contribute to the increase in the photosynthetic efficiency and leaf growth rate (Belsky *et al.*, 1993; Belsky, 1986). Recent studies indicated that damaged plants are likely to have a higher photosynthetic efficiency and an increased proportion of light energy absorption which can be utilised in photosynthesis (Retuerto *et al.*, 2006; Thomson *et al.*, 2003).

The study provided the first look at how stress affects this unique plant species at a physiological level and suggests a number of questions about the levels of stress it can endure. Since Welwitschia probably relies on groundwater of some sort (Henschel & Wassenaar, 2013; Soderberg, 2010), we expected to find consistent differences among catchments as this would reflect different hydrological regimes and environmental stressors. This was evident especially in the low photosynthetic

efficiency and leaf growth rate response of the plants located at Welwitschia campsite and at the Welwitschia Wash. Episodic rainfall does increase the rate of photosynthesis and leaf growth rate. The former implies that chlorophyll *a* fluorescence is a sensitive indicator of the overall plant's health from a physiological prospective. However, the swift increase in photosynthesis after rain also implies that a weak photosynthesis signal is probably a natural but rapidly-reversible response to episodic and other environmental stressors. A similar and somewhat more dramatic increase in photosynthesis and leaf growth in browsed plants after rain showed that the plants can also integrate an anthropogenic stressor without becoming fatally compromised, at least not in the short period we observed them.

As a tool to be used in long-term monitoring, the chlorophyll *a* fluorescence technique method has high potential to investigate plant health in situ. However, a fuller understanding of how Welwitschia reacts to different stressors will require long-term spatial and temporal integration and across a number of other potential drivers. Our study was a preliminary study conducted in only a short part of the year. Considering the longevity of Welwitschia, longer-term studies should now be conducted to understand the spatial and temporal patterns of plant health of Welwitschia.

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Chapter four

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