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Avian nectarivory and pollination in *Aloe marlothii* Berger: interactions between bird communities and a winter-flowering succulent

By

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***Philosophiæ Doctor* (Zoology)**

in the

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2008

Dedicated to my parents

Eric Thomas Gilbert Symes

and

Beverley Margaret Symes (née Brown)

PREFACE

The work described in this dissertation was carried out in the Department of Zoology and Entomology, University of Pretoria, Pretoria, from March 2005 to January 2008, under the supervision of Professor Susan W. Nicolson (University of Pretoria), and co-supervision of Dr Andrew E. McKechnie (University of the Witwatersrand) and Dr Stephan Woodborne (Council for Scientific and Industrial Research). The work was submitted for examination in February 2008 and corrections completed by July 2008 when the final copy was submitted.

This study represents the original work of the author and has not otherwise been submitted in any form for any degree or diploma at any other University. Where use has been made of the work of others it is duly acknowledged in the text. The work is not written in the first person since each chapter is prepared for submission for publication in a peer reviewed journal; the contribution of co-authors is thus acknowledged where applicable. At the time of submission Chapter 2 was published as, Symes, C.T., Nicolson, S.W. and McKechnie, A.E. 2008. Response of avian nectarivores to the flowering of *Aloe marlothii*: a nectar oasis during dry South African winters. *Journal of Ornithology* 149: 13-22, and at the time of final submission, after making corrections, Chapter 3 was published online as, Symes, C.T. and Nicolson, S.W. 2008. Production of copious dilute nectar in the bird-pollinated African succulent *Aloe marlothii* (Asphodelaceae). *South African Journal of Botany*.

All animal handling procedures and sampling techniques was approved by the University of Pretoria Ethics Committee (Ref: EC 050912-019).

.....

Craig Symes

Pretoria

July, 2008

Acknowledgements

"I am part of all that I have met" - Alfred Lord Tennyson

My whole life has been moulded by the numerous people I have shared my life with. I cannot mention all individuals but would like to thank a few special people. For those whose exclusion appears obvious I apologise.

I met Tracy while studying for my M.Sc. in Pietermaritzburg. Over the years we have grown closer and it never ceases to amaze me how she tolerates my eccentricities. For her love and trust in me I am eternally grateful. Since meeting, we have shared numerous adventures together; may we continue to do so for many years to come.

My family have always supported my adventurous endeavours and for their positive reinforcement of my passions I am most grateful. My mother and father, Beverley and Eric, have always guided me with advice and love, and without this I would be a lesser person. I have dedicated this work to them, as a small measure of my appreciation for the many blessings I have in this life. Although my sisters usually have little idea of what my work entails they have always been concerned and cared for their “wayward” brother; to Caryl, Sandra, Robyn and Jane, and your now extended families, thank you for always being there.

During my formative years as a child growing up on the farm Menin, near Creighton in the KwaZulu-Natal Midlands, I spent many holidays with my grandparents. My grandfather, Thomas Reginald Symes (10 April 1910 - 1 February 1999), taught me much about the natural world and most of all, an appreciation of the outdoors. Those experiences live on in me and for that valuable part of my life I thank him dearly. I have met few people with the patience and level-headedness of my grandmother, Betty (19 May 1915 – 17 May 2008); if the role of nature and nurture are equally important in our lives then I have much to be thankful for.

My step-grandmother, Edith Rogotta, provided a second home from our noisy flat in Church Street, Pretoria, and our times with her will always be cherished. She married my maternal grandfather, Charles Rutherford Brown (7 March 1914 - 24 September 1978) who I never got to know, and is as close as any family member.

During my undergraduate years I met and worked for Olaf Wirminghaus (28 April 1964 - 10 March 1996), a Ph.D. student in the Department of Zoology and Entomology at the University of Natal. Olaf's passion and knowledge of the natural world was an immense inspiration to my enquiring mind and he taught me much. I knew Olaf for less than two years as his life was cut short by a malignant brain tumour. The short time I knew him will always be cherished. The completion of Olaf's work was overseen by his wife, Prof. Colleen Downs. Vast amounts of Olaf's meticulously kept field notes finally saw fruition in numerous publications and a monograph on the Cape Parrot *Poicephalus robustus*. Colleen's caring and compassionate nature, and her similar passion for the natural world, has continued to inspire me. If not for her support and belief in me I would not be where I am today.

In 2002 I travelled to Papua New Guinea where I spent seven months, in remote villages of the Eastern Highlands, studying the effects of home-gardens (slash-and-burn) on bird communities. I thank Dr Stuart Marsden (Manchester Metropolitan University, UK) and the principal funders of this project (Chester Zoo, UK) immensely for this opportunity. Although I was lucky to come out alive the experiences I absorbed have left a memorable print on my life.

While working as an assistant, in early-1994, at an outdoor shop during my undergraduate years I began a conversation with a client. He was a traveller from the USA and asked me a question, "What do you want to do with your life?" I was taken aback by this forward question from a complete stranger. We began talking and I learned of his adventures over the past few years. He was Tom Claytor, a pilot on a solo trip around the world. He eventually completed his adventure and shared a few of his harrowing experiences with me. I remember very little else except his provoking question. Thanks Tom for that question that I am often reminded of when trying to make a decision. I'm here to enjoy myself as much as possible, and even be happy on

the way. So until evidence suggests otherwise I'm willing to accept that there is no second chance at life.

My M.Sc. project investigated the biology of the Greyheaded Parrot *Poicephalus fuscicollis suahelicus* and was supervised by Prof. Mike Perrin. Mike allowed me the freedom to take the project in the direction I wanted. This is epitomised in advice he gave me soon after returning from the field with a mountain of data. I asked him what he thought I should do from thereon. His advice was, "It's your project". I appreciate the faith he had in me, even when I had little idea at the time that what I was doing was correct.

This project would not have been possible were it not for the support of numerous people and organisations. My project supervisor Prof. Sue Nicolson (University of Pretoria) and project co-supervisor, Dr Andrew McKechnie (University of the Witwatersrand) are thanked for their support during my project. Both have provided invaluable academic input whilst at the same time allowing me to strive ahead with my own ideas. Living in Pretoria would be near impossible on an NRF bursary alone; thank you Sue for topping up my funding so that I could concentrate all my time and efforts towards the project. Thank you both, for the opportunity to attend conferences, both local (Zoological Society of South Africa bi-annual conference, Potchefstroom, 8-13 July 2007) and international (VIII Neotropical Ornithological Congress, Maturín, Venezuela, 13-19 May 2007).

Dr Stephan Woodborne (Natural Resources and the Environment, Council for Scientific and Industrial Research) was a patient advisor whilst learning how to analyse isotope samples. Without his contribution my project would not have been a success. Stephan's insight into a field not typically his (ornithology) provided a different angle to my project, especially when I often became so focussed on a specific outcome.

It was anticipated that my samples be analysed at the Department of Archaeology, University of Cape Town. However, arrangements were made to use the stable isotope unit at the Natural Resources and the Environment, Quaternary Dating

Research Unit (QUADRU) laboratory, at the Council for Scientific and Industrial Research (CSIR) in Pretoria. This proved successful and the addition of a new elemental analyser and mass spectrometer to the unit in March 2006 was a welcome addition. Grant Hall and Caroline Duncan gave assistance during sample analysis and logistics in running the facilities. Dr Siep Talma provided thoughtful discussion and insight into aspects of my project, as well as data on stable carbon isotope values for numerous *Aloe* species collected by Dr John Vogel.

The National Research Foundation (NRF) of South Africa provided financial support for my study, and an NRF Mobility Grant financed my trip to Cape Town for the Stable Isotopes Course at the University of Cape Town (Department of Archaeology) in May 2005. This was an inspiration at the start of my project; where Professors Nikolaas van der Merwe, Thure Cerling and Judith Sealy, and staff and students on the course provided valuable input and stimulation during the development of my project. Additional funding was provided by the Sandton Bird Club for field trips in 2007.

Gauteng Provincial Government, Department of Agriculture, Conservation and Environment (GDACE) permitted access to Suikerbosrand Nature Reserve where most of my fieldwork was conducted. The relevant permits were granted to collect plant material and animal tissues (blood from birds for isotopic analysis) (Permit numbers: 1311, 1326, 1520 and 1534). Coral Birss and Dr Craig Whittington-Jones made the application process run smoothly and supported my efforts to do research in a protected area. Bird capture and ringing was conducted under authority of Safring (C.T.S. A-permit No. 364), to where all ringing data are submitted, and in Gauteng ringed under permit of GDACE (Permit numbers: 0454, 0472 and 0484).

Neville Green, Johnny Hennop, Daniel Koen and numerous Suikerbosrand Nature Reserve staff are thanked for their support during my monthly field trips to the aloe forest. The reserve provided accommodation at the Schoongezicht and Mayfair farmhouses at reduced rates, and although I did not always have hot water during cold winters, I am appreciative of the resources provided. Ajeeth Sooklal stepped in when

the farmhouses could not be used and is thanked for providing subsidised rates at Kareekloof Campsite (Protea Hotels Group).

Field work would be so much more difficult if it were not for extra assistance. Tracy helped during numerous days in the field and was always enjoyable company. Darren Pietersen stepped in when I needed help during bird capture. By removing birds trapped in the mist nets I was able to concentrate on processing birds and collecting valuable data.

Pierre van Heerden made my project known to the South African public through the preparation of a documentary (50/50, TV2, 7 January 2007), and it was a pleasure working with him. He organised scaffolding provided by David Warrender (Rapid Scaffolding, Pretoria) and with the assistance of Marius Pretorius it was erected in the aloe forest to provide an observation platform from which to view and photograph birds.

Professor Braam van Wyk in the Department of Botany (University of Pretoria) first suggested Suikerbosrand Nature Reserve as a study site and his botanical and natural history knowledge provided valuable insight throughout my project. Magda Nel in the herbarium assisted in plant identification, and Lorraine Middelton, Atte Berga and staff of the Department of Botany greenhouse are thanked for support during my seed-bank experimental trials. This series of experiments are, however, not presented in the thesis.

Members of the Pretoria Bird Club are thanked for additional help; Dirk and Karen van Styvenberg assisted in constructing spring traps for bird capture and Dr Graham Grieve is thanked for assistance during bird ringing in July 2006.

At the Department of Zoology and Entomology numerous people provided valuable assistance and input into my project; Babsie Potgieter made life a little easier with logistical support, Martin Haupt remained supportive when organising and using university vehicles and Human Buirski sorted out numerous gremlins on my personal computer and provided valuable IT support. The following provided support and

discussion during my project; Dr Hannelie Human, Dr Mike Scantlebury, Cromwell Purchase, Johnny Wilson and Carolina Leseigneur. Additional comments on manuscript drafts were welcomed from Prof. Braam van Wyk, Guillermo Pérez, Dr Hannelie Human, Dr Daryl Codron and Dr Fredrik Dalerum.

Tracy Young assisted with map construction, using data provided by GDACE. Prof. Ian Meicklejohn is thanked for additional assistance in map construction, and giving time to Tracy outside of her lecture periods in the use of ArcGIS.

Prof. Adrian Craig, Dan Parker, Ben de Boer, Mostert Kriek, Christo Botes and Sandra Strudwick are thanked for reporting additional sightings of nectar-feeding by birds on *A. marlothii*, and Dr Adrian Shrader, Daniel Koen and Mike Panagos for additional observations of animals feeding on *A. marlothii*.

Kroons Gourmet Chickens provided chicken blood for isotope standards; Sandra van Wyngaardt (Department of Biochemistry, University of Pretoria) assisted in freeze drying and preparing these samples.

Academic Information Services were always helpful in locating obscure references and material from other libraries. Tersia Perregil, librarian at the Transvaal Museum (Northern Flagship Institute), assisted further in locating references from the museum library.

None of this would ever have happened if it were not for my Class I and II teacher at Creighton Government School, Mrs Jean Smith, who started it all by teaching me how to read and write.

Summary

Avian nectarivory and pollination in *Aloe marlothii* Berger: interactions between bird communities and a winter-flowering succulent

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Aloe marlothii is a winter-flowering succulent that is widespread in the savanna biome of northern and north-eastern South Africa. Plants grow up to 8 m in height and are commonly found on rocky north-facing slopes. Nectar production occurs through a 24 h period with flowers producing copious amounts (*c.* 250 µl) of dilute nectar (*c.* 12%). This abundant nectar supply, that is available for a 5-10 week period during June-August, is utilised by numerous opportunistic avian nectarivores. At a study site in Suikerbosrand Nature Reserve, 60 km south-east of Johannesburg, at least 59% (38 species) of birds recorded during census transects fed on nectar; throughout the range of *A. marlothii* at least 85 species feed on nectar. This diversity surely far exceeds the number of species ever recorded feeding on nectar of a single plant. During the flowering period an influx of birds at the aloe forest occurred, with an overall increase in abundance and diversity. Pollinator exclusion experiments supported the hypothesis that *A. marlothii* is pollinated by generalist birds; specialist nectarivores are possibly excluded as inefficient pollinators by the nectar of low concentration and high volume. Fruit set was higher in plants that had avian visitors and very low when pollinators were absent.

Stable carbon isotope analysis of whole blood was used to quantify the importance of nectar sugars for opportunistic nectarivores. During flowering there was an enrichment in the $\delta^{13}C$ isotopic signature of whole blood of nectar-feeding birds

towards that of nectar ($\delta^{13}C$ = -12.6‰). This shift was most prominent in frugivores, insectivores and omnivores that typically fed on a diet depleted in ^{13}C when nectar was not available. The C_4 grass seed diet of granivores was similar to the isotopic signature of *A. marlothii* nectar, so we were unable to determine to what degree granivores benefitted from nectar. Stable nitrogen isotopes in whole blood may suggest that many nectar-feeding birds shift their trophic position during flowering. However, we interpret these results with caution because of insufficient knowledge on diet-tissue fractionation factors of wild birds and/or temporal changes in vegetation isotopic values. Stable carbon isotope analysis of breath samples was used to show that *A. marlothii* nectar is a readily available income energy source for nectar-feeding birds. Because *A. marlothii* nectar is so dilute we expected it to be an important water source for many opportunistic nectar-feeding bird species. There was no correlation between the enrichment of $\delta^{13}C$ of breath CO_2 (representing metabolised nectar sugars) and the $\delta^{18}O$ in breath CO_2 (representing a highly evaporated water source in nectar); for most birds the $\delta^{18}O$ in breath CO_2 was more similar to that of free-standing water sources. However, because our knowledge on the relationship between $\delta^{18}O$ of ingested water and body water, and fractionation processes when CO_2 is exhaled is limited, we were unable to quantify water obtained from nectar. The sugars of *A. marlothii* nectar are probably more important, as a food source for opportunistic nectarivores during dry winter months when insect abundance is low, than the water in nectar, because birds are able to source water from other drinking sites.

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General Conclusion: Red-eyed bulbul *Pycnonotus nigricans* feeding at an *Aloe marlothii* inflorescence.

[1] All photographs in text are taken by Craig Symes unless otherwise indicated.

GENERAL INTRODUCTION

"In all things of nature there is something of the marvellous"

Aristotle *(384-322 BC)*

Background and motivation

The conspicuous orange to red inflorescences of *Aloe* species are a distinct feature of the South African landscape during the austral winter (May-August) (Reynolds 1969; Reynolds 2004; Van Wyk and Smith 2005). Most species within this genus flower during winter months (Jeppe 1969; Reynolds 1969; Glen and Hardy 2000; Van Wyk and Smith 2005; Fig. 1) and it is suggested that they form an important food and/or water source for numerous animal species. One of the most conspicuous and charismatic species, with a widespread distribution in the eastern half of South Africa, is *Aloe marlothii* A. Berger (Reynolds 1969). Flowering is often prolific in these large aloes (up to 8 m) and occurs during the dry season when water and food resources for animals are often low, thereby providing an important nectar oasis for many opportunistic nectarivores (Oatley and Skead 1972). In addition, the flowering of other

Aloe species also provides an important nectar source for numerous bird species (Marloth 1915; Oatley 1964; Oatley and Skead 1972; Nicolson 2002; Nicolson and Fleming 2003; Nicolson and Nepi 2005).

In southern Africa, specialised nectarivores are from the family Nectariniidae (sunbirds; 12 species) and Promeropidae (sugarbirds; 2 species) (Skead 1967). However, additional opportunistic [2] nectar feeders include white-eyes (Zosteropidae), weavers (Ploceidae), bulbuls (Pycnonotidae), barbets (Lybiidae), mousebirds (Coliidae) and starlings (Sturniidae) (Marloth 1915; Oatley 1964; Skead 1967; Jacot Guillarmod et al. 1979; Craig and Hulley 1994; Oatley 2001). In southern Africa numerous insectivores are also known to occasionally utilise aloe nectar, and also likely benefit from the abundant supply of insects visiting aloes (Oatley 1964).

No detailed study has been conducted on *A. marlothii*, despite it being a common and widespread species in South Africa (Reynolds 1969; Glen and Hardy 2000; Van Wyk and Smith 2005). Broader studies have included *A. marlothii* in assessing the effects of browser impact at an ecological level (Thrash 1998; Wiseman et al. 2004), investigating chemical properties (Cavallini 1993; Viljoen et al. 1996), and resolving taxonomic issues and understanding aloe biogeography (Brandham 1969; Bayer 1972; Holland 1978; Riley and Mujamdar 1979; Smith 1991; Smith et al. 2001; Treutlein et al. 2003). A few studies of other aloe species have addressed pollination (Hoffman 1988; Ratsirarson 1995; Stokes and Yeaton 1995; Pailler et al. 2002; Johnson et al. 2006), whilst some have made reference to the relationships between aloes and birds (Oatley 1964; Oatley and Skead 1972; Hoffman 1988; Ratsirarson 1995).

Since the early works of Oatley (1964) and Oatley and Skead (1972), where the use of aloes by birds in general was described, no studies have quantified or analysed aloe-avian relationships. A number of aloe species are considered important for birds, particularly those that flower during winter months when food resources may be low (Fig. 1). More specifically, it is the larger species of aloe including *A. marlothii* and *A.*

[2] Throughout this thesis I have opted to use the term opportunistic rather than facultative nectarivore. Opportunistic also implies a reliance on nectar to a lesser degree than facultative. In some instances I have made use of occasional, which similarly implies less reliance on nectar than facultative.

ferox (see below) that provide an abundant and important food supply to birds, particularly in the eastern half of South Africa (Oatley 1964; Oatley and Skead 1972). In the drier western parts of the country large tree aloes such as *A. dichotoma, A. pillansii* and *A. ramosissima* may be important nectar sources for birds. Understanding the interactions between consumers and these valuable nectar resources will enable us to develop a better understanding of the importance of potential pollinators for aloes, and in particular *A. marlothii*.

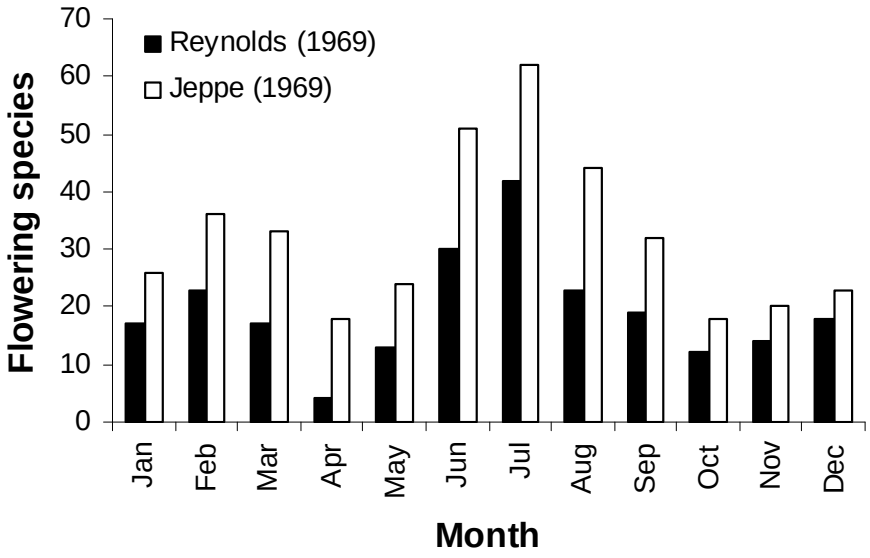

Figure 1. Number of South African (after Jeppe 1969) and southern African (after Reynolds 1969, *n* = 133) *Aloe* species flowering in different months of the year. The different references may have included each aloe species flowering in more than one month.

Aloe marlothii *A. Berger*

There is debate concerning the taxonomic status of *A. marlothii* and *A. ferox*, with recent publications recognizing *A. spectabilis* and *A. candelabrum* as synonymous with *A. marlothii* and *A. ferox* respectively (Reynolds 1969; Viljoen et al. 1996; Glen and Hardy 2000; Van Wyk and Smith 2005). Before proceeding I will attempt to resolve basic issues concerning the taxonomic status within this allied group, which are similar in a number of morphological and ecological respects.

Aloe marlothii occurs on the highveld of Limpopo, Mpumalanga and Gauteng provinces, South Africa (Reynolds 1969; Glen and Hardy 2000; Van Wyk and Smith 2005; see Fig. 2). Despite its xeromorphic growth form it is confined to predominantly the savannah biome in the mesic north and north-east of the sub-region (Fig. 2). It ranges west into Botswana, east into southern Mozambique and Swaziland, and south into northern KwaZulu-Natal and the southern hills of the Witwatersrand (Reynolds 1969; Glen and Hardy 2000; Van Wyk and Smith 2005). To the north its range terminates in the Soutpansberg, and does not extend into Zimbabwe (Reynolds 1969). It is sometimes recognised as a species distinct from *A. spectabilis* that occurs further south in the central hills of KwaZulu-Natal, in the regions of Bergville, Ladysmith, Dundee, Muden and the central Tugela River valley (Reynolds 1969; Glen and Hardy 2000; Fig. 2a).

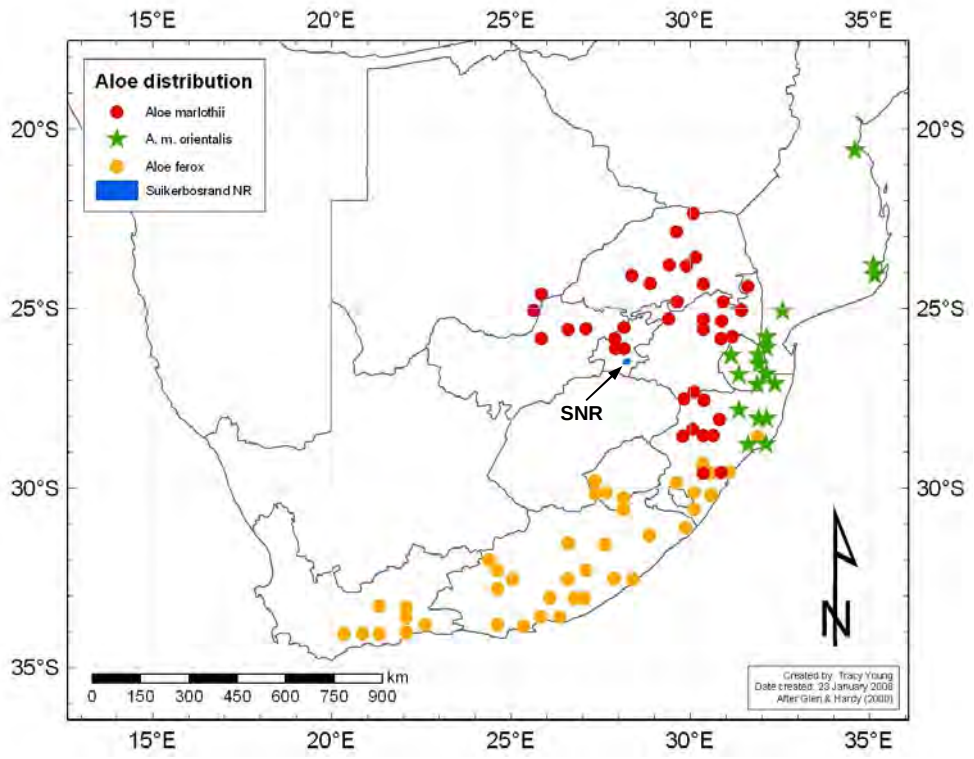

Figure 2. Distribution of *A. marlothii* and *A. ferox*. *Aloe marlothii* includes *A. marlothii* and *A. spectabilis* recognised by Reynolds (1969). Two subspecies, *A. m. marlothii* and *A. m. orientalis* are identified; *A. m. orientalis* is not synonymous with *A. spectabilis*, as recognised by Reynolds (1969). *Aloe ferox* includes *A. ferox* and *A. candelabrum* recognised by Reynolds (1969). Digitised from maps 75 and 80 in Glen and Hardy (2000).

Figure 3. **(a)** *Aloe marlothii* plants in flower showing horizontal racemes; **(b)** mature *A. marlothii* plants showing rosulate growth form and persistent dry leaf skirt characteristic of most plants; **(c)** Sub-erect growth of racemes characteristic of *spectabilis* form of *A. marlothii* (formerly known as *A. spectabilis*); **(d)** Secund flower arrangement of *A. marlothii* flowers; **(e)** flower arrangement of *A. spectabilis* from Ladysmith, KwaZulu-Natal. See Appendix for further descriptions of taxa.

The distinguishing feature of *A. marlothii* is its inflorescence, a branched panicle with up to 30 racemes (sometimes 50 have been recorded) that develops from the apex of the rosulate growth form (Fig. 3a, b, c; Reynolds 1969; Glen and Hardy 2000; Van Wyk and Smith 2005). Across the wide geographical range the flowers of *A. marlothii* vary in shape, size, colour, and raceme angle. In the north of the range the racemes of each inflorescence develop horizontally, with the KwaZulu-Natal form (previously known as *A. spectabilis*) bearing racemes at an angle, but not erect as in *A. ferox* (Fig. 3a, c; Van Wyk and Smith 2005). Bright-red to golden-yellow flowers are subtended by bracts 4-9 mm long (Fig. 3d, e; Reynolds 1969; Glen and Hardy 2000;

Van Wyk and Smith 2005). In *A. ferox* these bracts are about twice as long (Glen and Hardy 2000).

An additional subspecies recognised by Glen and Hardy (2000), *A. m. orientalis*, occurs to the east of the distribution and prefers sandier soils (Fig. 2a). This subspecies is, however, not synonymous with *A. spectabilis*. For the purposes of this study and until further evidence is furnished to the contrary I will follow the taxonomy of van Wyk and Smith (2005). Allusion to the forms *marlothii* and *spectabilis* is done with reference to the species recognised by Reynolds (1969). These forms relate to the horizontal and oblique raceme angles characteristic of the northern and southern forms respectively.

Aloe ferox (hereafter including *A. ferox* and *A. candelabrum*, according to Reynolds 1969) occurs to the south of *A. marlothii* (see Fig. 2), where it fills a similar ecological niche for birds (Skead 1967; Botes 2007). This taxonomic grouping is supported following findings by Viljoen et al. (1996) that there is no single morphological, chemical or biochemical character, or character combination, to support *A. ferox* and *A. candelabrum* as distinct species. Also, there is no distinct geographical separation between these two taxa (Reynolds 1969; Glen and Hardy 2000; see Fig. 2).

Aloe marlothii is a common plant in South African bushveld habitats and is often seen growing with a clumped distribution. It is predominant on north-facing slopes and frequently occurs at ecotones between habitat types (Reynolds 1969). Its distribution is also thought to be closely associated with early inhabitants of South Africa and it is more widespread than many other *Aloe* species (Holland 1978; Bredenkamp and Van Vuuren 1987; Van Wyk and Smith 2005). Flowers occur from May/June-August/September (austral winter), the colour varying with geographical location (Reynolds 1969; Glen and Hardy 2000; Van Wyk and Smith 2005). The red form in the Utrecht district of KwaZulu-Natal contrasts with the yellow to orange colour evident in flowering plants further north (Van Wyk and Smith 2005). Plants are thought to flower every year (Johannsmeier 1976) but this remains to be investigated.

In addition, there is debate concerning higher classification of the *Aloe* genus. Early classifications traditionally placed aloes in the family Liliaceae together with the true lilies and tulips (Marloth 1915; Holland 1978; Cavallini 1993). More recent classifications have considered *Aloe* species as members of the Asphodelaceae together with red-hot pokers and bulbines (Van Wyk et al. 1993; van Wyk and Smith 2005; Human 2006). However, a more refined classification considers *Aloe* as part of Aloeaceae, a group that includes other plants with succulent leaves i.e. haworthias and gasterias (Viljoen et al. 1996; Glen and Hardy 2000; Smith et al. 2001; Reynolds 2004). For the purposes of this study we consider *Aloe* as a member of the family Asphodelaceae.

The importance of A. marlothii *to nectar consumers*

Numerous studies have indicated the importance of nectar sources for bird communities in southern Africa (Frost and Frost 1981; Daniels 1987; Tree 1990; Craig and Hulley 1994; Symes et al. 2001). In addition to widespread (and dispersed) flowering species providing nectar over a number of months, less abundant (and clumped) stands of certain species (e.g. *Kniphofia*) may provide food over a shorter period (Skead 1967; Niven 1968; Dowsett-Lemaire 1989; Symes et al. 2001; Tree 2004). A number of strategies, on both a temporal and spatial scale, may be used by different nectarivores, and in particular sunbirds, in accessing these food resources (Tree 1990; Craig and Hulley 1994).

Aloe marlothii acts as a source of nectar and nutrients for numerous organisms (Marloth 1915; Oatley 1964; Oatley and Skead 1972; Maclean 1993). The flowers of *A. marlothii* are visited by a number of bird species for possibly different reasons. The inflorescences of *A. marlothii* suggest a bird pollinated syndrome (ornithophilous) that is supported in studies of other aloes, i.e. *A. ferox* and *A. divaricata* (Hoffman 1988; Ratsirarson 1995). However, numerous nectar-feeding species may rob aloes of nectar without benefitting the plant (Hoffman 1988). The attraction for birds visiting flowering aloes is the abundant supply of nectar, with at least 98 species in 26 families recorded probing flowers (Oatley 1964; Oatley and Skead 1972; Hoffman 1988; Maclean 1993). Seventy-three bird species (including three sunbird species) have been

recorded feeding on *A. marlothii* in particular (Oatley and Skead 1972). These include numerous species not typically classified as nectarivores; moreover such feeding occurs at a time when natural food supplies may be low (Oatley 1964; Oatley and Skead 1972; Maclean 1993). Similarly in Brazil *Erythrina dominguezii* nectar is an important food source for bird species when environmental food reserves are low (Ragusa-Netto 2002). However, in the case of Australian honeyeaters large variations in nectar production did not affect seasonal abundance of birds, indicating that honeyeater density was related more to the spacing behaviour of the birds than to food availability (Pyke et al. 1993). The importance of *A. marlothii* nectar for birds remains to be investigated.

Oatley (1964) suggested that it seems unlikely that opportunistic avian nectarivores rely on aloe nectar as a food source during winter months because of a shortage of natural food items. Feeding on aloes occurs at a time when many birds are preparing to breed, and they benefit from a high energy food source in this preparation process (Oatley 1964). However, the response of certain feeding guilds to feeding on nectar may be different. For example, food supplies for seed eaters (granivores) may reach a maximum in winter when grass seeds remain dormant on the ground, and nectar may supplement an already abundant food supply. In contrast, insect abundance may be low by the end of a dry season, with periods of low food abundance for insectivores. The appearance of insectivores at aloes may thus be a response to insects attracted to aloes, with some individuals turning to nectar as an additional source of energy (Oatley 1964). Some opportunistic nectarivores probing aloe flowers may fortuitously feed on insects, yet it is still unclear what aloe feeders target (Oatley 1964). Our understanding of bird resource availability is unclear and periods perceived as low food availability (e.g. winter) by humans may not be so for birds. For folivores that prefer the new buds of plants, flowers may act as an important food source; grey go-away *Corythaixoides concolor* birds have been observed feeding on the whole flowers of *A. ferox* and dark-capped bulbuls *Pycnonotus barbartus* have been observed feeding on whole *Kniphofia* spp. (Asphodelaceae) flowers (C.T.S. pers. obs.). The importance of aloes to non-nectarivores (species that would typically feed on fruit, seeds and insects, or a combination of these) is not apparent. Investigating these

relationships on a broad scale will enable us to develop a greater understanding of the importance of this unique food source to numerous avian consumers.

Aloe marlothii is important for a number of other organisms. It has been recorded as browsed by eland *Taurotragus oryx* and nyala *Tragelaphus angasi* (Anderson and Pooley 1977; Glen and Hardy 2000), and is also appreciated by kudu *Tragelaphus strepsiceros* (M. Panagos pers. comm.). It is heavily browsed in areas where black rhinoceros *Diceros bicornis* occurs (Wiseman et al. 2004) and is also favoured by African elephants *Loxodonta africana* (A. Shrader pers. comm.). In each of these cases it is the succulent leaves that the animals prefer. Vervet monkeys *Cercopithecus aethiops* have been recorded with pollen-covered faces whilst in pursuit of *A. ferox* nectar (Skead 1967; D. Koen pers. comm.). The nectar attracts bees, ants (Hymenoptera), flies (Diptera), small beetles (Coleoptera) and thrips (Thysanoptera) which may in turn satisfy avian insectivores (Oatley 1964). These insect visitors, as observed in *A. ferox*, may be important pollinators (Hoffman 1988). Birds have also been recorded in nests built on the horizontal naviculate leaves (Laughing Dove *Streptopelia senegalensis* and Cape Sparrow *Passer melanurus*), and Pied Barbets *Tricholaema leucomelas* have been recorded excavating nesting cavities in the stem (Johannsmeier 1976).

The importance of birds to A. marlothii

Features of flowers that are attributed to a bird-pollination syndrome (ornithophily) include: i) diurnal anthesis with nectar peak and flower opening in early morning; ii) large pollen-nectar distance; iii) vivid colours, bright reddish-orange flowers; iv) absence of odour and nectar guides; v) distinctly curved tubular flowers; vi) secund flower arrangement, and; vii) abundant nectar held in flower by capillarity (Faegri and Van der Pijl 1979; Smith 1991; Maclean 1990; Proctor et al. 1996). These features are nearly all represented in *A. marlothii*. However, it is still unclear who pollinates most aloe species.

The flower morphology of *A. ferox* and *A. candelabrum* suggests an ornithophilous syndrome (Hoffman 1988, Stokes and Yeaton 1995). Similarly, *A.*

marlothii with its horizontal to slanting racemes (Van Wyk and Smith 2005) is suggestive of a system focussed towards bird pollination. Inflorescences are robust, being able to support large bird species such as grey go-away bird and columbids (>300 g) (Proctor et al. 1996). Generalist bird pollinators are most often attracted to flowers with low concentration (*c.* 8-12%) and high volume (*c.* 40-100 µl) whilst specialist nectarivores prefer nectar of high concentration (*c.* 15-25% w/w) and low volume (*c.* 10-30 µl) (Johnson and Nicolson 2008). The long tubular flower shape of most aloes excludes certain insect species, although honeybees *Apis mellifera* are able to access the nectar of some flowers (Reynolds 1969; Hoffman 1988; Human 2006, Human and Nicolson 2008). In *A. castanea* the open perianth makes the dilute (8-10%) nectar accessible to bees that prefer pollen (Nicolson and Nepi 2005); whilst in other aloes with a fused perianth, constricted tubular corolla and tightly packed stamens (like *A. marlothii*), nectar may be more difficult for bees to access.

Aloe marlothii flowers during periods of little to no rainfall, so it is likely that copious nectar production supplies birds with both water and a sugary food source (Oatley 1964). In *A. ferox* four of five bird visitors and five of eight insects were classed as pollinators (Hoffman 1988), and for *A. divaricata* in Madagascar the Souimanga sunbird *Nectarinia souimanga* appeared to be the most effective pollinator (Ratsirarson 1995). On Mayotte Island, the endemic *A. mayottensis* was primarily pollinated by a single sunbird species, *Nectarinia coquereli*, with occasional visits from *Zosterops maderaspatana* (Zosteropidae) and an unidentified hymenopteran (Pailler et al. 2002). Numerous cases have demonstrated the importance of insect visitors for plants (see Proctor et al. 1996). In many instances a specialised pollination syndrome is indicated in which a plant taxon relies on a specific pollinator (or specialised group of pollinators), as occurs with the relationship between nemestrinid and tabanid flies and long-tubed flowers (Manning and Goldblatt 1997; Johnson and Steiner 1997). *Aloe vryheidensis* has been demonstrated to filter flower visitors with long bills (those perceived as nectar robbers) through increased concentrations of bitter tasting phenolic compounds in the nectar (Johnson et al. 2006). Despite obvious perceptions of a bird pollination syndrome, the contribution of different animals to pollination of *A. marlothii* remains to be investigated.

Stable isotopes

Stable isotopes are atoms of an element with different atomic masses i.e. they have the same number of protons (same atomic number) but different numbers of neutrons in their nuclei (Hoefs 1980). For example the natural stable isotopes of carbon are ^{13}C and ^{12}C, with relative atomic masses of 13 and 12 respectively (Hoefs 1980). Only 21 elements occur as pure elements, the rest are mixtures of at least two isotopes (Hoefs 1980). In most cases one isotope is predominant while others present in trace amounts, but they are not found in uniform ratios in nature (Hoefs 1980). It is this lack of uniformity, brought about by physical and biological processes, that is of interest to many scientists.

The ratios of isotopes are expressed relative to arbitrary element-specific standards using delta-value (δ) notation (Hoefs 1980; Ehleringer and Osmond 1989; McKechnie 2004). For numerous reasons absolute measurements are not reliable and it is more common to calculate these ratios relative to a standard reference material (Gonfiantini 1978; Ehleringer and Osmond 1989), e.g. standard mean ocean water (SMOW), or more recently Vienna standard mean ocean water (V-SMOW), for hydrogen (D/H) and oxygen ($^{18}O/^{16}O$) in water; *Belemnitella americana* from the Cretaceous Peedee formation, South Carolina (PDB or Peedee Belemnite) for carbon ($^{13}C/^{12}C$) and oxygen ($^{18}O/^{16}O$) in carbonates and organic material; atmospheric air (N_2) for nitrogen (N^{15}/N^{14}); and troilite (FeS) from the Canyon Diablo iron meteorite (Canyon Diablo meteorite, CD) for sulphur (Gonfiantini 1978; Hoefs 1980; Ehleringer and Osmond 1989; Lee-Thorp and Thalma 2000; McKechnie 2004). The isotopic compositions of natural materials can be measured with great accuracy with a mass spectrometer (Peterson and Fry 1987) and the accepted isotope ratio δ-value, expressed in parts per thousand (per mil), is calculated by:

$$\delta_{sample}\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R represents the isotopic ratio of the sample and standard respectively (Hoefs 1980; Peterson and Fry 1987). These standards, obtained from the IAEA (International Atomic Energy Agency), are often used to calibrate other laboratory

standards that are run with samples. This is because some of these original standards are long exhausted. Therefore, when expressing results the standard used is always noted. This also facilitates conversions that relate to other standards, e.g. $\delta^{18}O_{SMOW} = 1.03091.\delta^{18}O_{PDB} + 30.91$, when comparing different studies.

Research applications of stable isotopes

Stable isotopes have been used as a tool in palaeontological research for decades (Hoefs 1980; Ehleringer and Osmond 1989; Lee-Thorp and Thalma 2000). Also, botanists have long studied the patterns of ^{13}C distribution in plants resulting from climatic, altitudinal and photosynthetic factors (Park and Epstein 1960, 1961; Smith and Epstein 1971; O'Leary 1981). It is only recently that the technique has been applied in the zoological arena (Peterson and Fry 1987), with a plethora of research publications appearing in the last five to ten years (McKechnie 2004; West et al. 2006). The use of variations in unique stable isotope signatures in particular environments, and the changes in these ratios due to physical and biological processes, has gained popularity as a research tool for biologist's worldwide (Ehleringer and Osmond 1989; Lee-Thorp and Thalma 2000; McKechnie 2004).

Implicit in the application of isotope techniques for biologists is the assumption that the isotope ratio of a consumers' tissue is related to its diet (Hobson and Clark 1992). Isotope compositions change in predictable ways as elements are cycled through ecosystems (Peterson and Fry 1987). For example, differential isotopic fractionation occurs during carboxylation because lighter isotopes react faster than heavier isotopes (Bigeleisen 1965; Park and Epstein 1961; Hoefs 1980; Kelly 2000). In incomplete reactions more of the lighter isotope is used up and the reaction products become depleted (i.e. they contain more of the lighter isotope). The unreacted material thus becomes enriched (i.e. containing more of the heavier isotope). In photosynthesis, the degree of carbon isotope fractionation is established during two rate-controlling processes; the diffusion of CO_2 into the chloroplasts, and the carboxylation process itself (Hoefs 1980; Ehleringer and Monson 1993). In photosynthesis there is thus a depletion of ^{12}C in the remaining CO_2 because the light ^{12}C is concentrated in the synthesized organic material (Hoefs 1980). In C_3 plants (trees, shrubs and herbs, and

temperate or shade grasses), where the initial product of photosynthesis is a three-carbon molecule, the CO_2 fixing enzyme Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) discriminates against ^{13}C more strongly than another CO_2 fixing enzyme, PEP (phosphoenolpyruvate) carboxylase, that occurs in C_4 (mainly tropical grasses) and CAM (Crassulacean acid metabolism) plants (Park and Epstein 1960, 1961; Smith and Epstein 1971; Ehleringer 1991; Lee-Thorp and Thalma 2000). Plants exhibiting C_3 photosynthesis become more depleted in ^{13}C relative to C_4 and CAM plants. Fractionation thus occurs at significantly different levels in C_3, and C_4 and CAM plants; typical $\delta^{13}C$ values as follows (Vogel et al. 1978; Ehleringer and Osmond 1989; Ehleringer 1991; Dawson et al. 2002; Fig. 4);

-26.5‰ (-37 to -24‰) for C_3
-12.5‰ for (-16 to -9‰) for C_4
-17‰ (-19 to -9‰) for CAM

This is in turn represented in the tissue of consumers, with further fractionation along the food-chain (De Niro and Epstein 1981; Ehleringer and Osmond 1989). Typically the whole body of animal is enriched in ^{13}C relative to its diet by 1‰, although this fractionation can differ among species, diets and tissue types (De Niro and Epstein 1978; Hobson and Clark 1992). Also, diet-tissue fractionation values can increase due to nutritional stress (Hobson and Clark 1992; Hobson et al. 1993). Because of this it may be necessary to establish levels of fractionation in the laboratory under controlled conditions (Gannes et al. 1997).

Stable isotopes have been used to reconstruct the proportions of isotopically distinct diets in different animals. These dietary reconstructions rely on linear mixing models, with the proportions of two food sources in an animal's diet calculated by:

$$\delta X_{tissues} = p\delta X_A + (1 - p)\delta X_B + \Delta$$

where $\delta X_{tissues}$ is the isotope ratio in the animal's tissues, δX_A and δX_B are the isotope ratios of the respective food sources and p is the proportion of food A in the diet. The discrimination factor between the diet and the food source is represented by Δ (Hoefs 1980; McKechnie 2004).

These levels of fractionation provide the ideal opportunity to test hypotheses concerning the diets of grazing and browsing herbivores in tropical environments (Cerling et al. 2003; Sponheimer et al. 2003a). A study across species of southern African bovids using analysis of tooth enamel, bone collagen and hair supported literature on previous diet reconstructions, as determined through other techniques (Sponheimer et al. 2003a). However, isotopic analysis indicated that eland *Taurotragus oryx* and steenbok *Raphicerus campestris* eat/assimilate less grass than is widely believed, and red hartebeest *Alcephalus buselaphus* graze more than expected (Sponheimer et al. 2003a). These data are likely to vary geographically (Gagnon and Chew 2000). This is evident in a study of impala *Aepyceros melampus* in the Kruger National Park, where $\delta^{13}C$ and $\delta^{15}N$ in faecal and hair samples indicated regional sex based dietary differences based on habitat use (Sponheimer et al. 2003b). Analysis of $\delta^{13}C$ indicated that northern impala fed on 41% grass in a mopane-dominated habitat while southern impala fed on 63% grass in the marula-knobthorn and bushwillow woodlands of the south, and males grazed more than females (Sponheimer et al. 2003b; Gertenbach 1983). The lack of variation in $\delta^{15}N$ indicated that impala optimised their diet quality irrespective of plant composition (Sponheimer et al. 2003b).

Stable isotope analysis has been instrumental in reconstructing the diets of early hominins (Sponheimer and Lee-Thorpe 1999). In addition, prehistoric foodwebs can be reconstructed. Analyses of extinct elephant tooth enamel and tusks have also shown that early elephants (e.g. *Deinotheres*) were grazers and that an evolution to grazing occurred approximately 7.5 Mya (Cerling et al. 1999). The modern elephant *Loxodonta africana* is a browser as is indicated by tooth structure; but isotope analyses of modern elephants indicate a mixed diet (75/25 to 25/75) unlike that of most other African herbivores (Cerling et al. 1999). This switch to C_3 grasses occurred approximately 100-150 kya, at the same time as when the savannah elephant underwent a genetic bottleneck (Cerling et al. 1999).

Research applications of stable isotopes for birds

The use of stable isotopes provides a powerful tool that can be used in reconstructing the importance of different food sources, reconstructing trophic level ecology and tracing the use of water in the diets of birds (Peterson and Fry 1987; Kelly 2000). By analysing stable isotope ratios (δD, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) of different tissues inferences on diet and diet switching, movements, trophic levels and water use can be made.

Large CAM aloes such as *A. marlothii* and *A. ferox* are a conspicuous feature of the South African landscape in many regions; not unlike the saguaro cactus *Carnegeia gigantea* of the arid regions of the Americas (Fleming et al. 1996). Studies using stable isotopes in the Sonoran Desert have provided insight into the use of saguaro fruit for individual species and bird communities (Wolf and Martínez del Rio 2000; Wolf et al. 2002; Wolf and Martínez del Rio 2003). The fruit of saguaro cacti have a unique stable isotope composition (Wolf et al. 2002). Using this feature, temporal analyses of $\delta^{13}C$ and δD revealed the importance of fruit as a source of nutrients and water for white-winged doves *Zenaida asiatica mearsnii* (Wolf et al. 2002). These results were confirmed by a positive correlation between the $\delta^{13}C$ in dove liver tissues and percent saguaro fruit in the crop contents (Wolf and Martínez del Rio 2000). Further investigations revealed that two different desert doves relied on saguaro fruit for different reasons: white-winged doves predominantly perched atop saguaros and fed on flowers or fruit, whilst Mourning Doves *Zenaida macroura* fed exclusively on the ground (Wolf et al. 2002). These differences in feeding mode affected the importance of saguaro fruit for these two dove species. Saguaro provided an important source of nutrients and water for White-winged Doves, whereas for Mourning doves saguaro provided nutrients and less importantly water (Wolf et al. 2002).

The provision of fruit by saguaro cacti can be compared with the provision of nectar by aloes in southern Africa. *A. marlothii* is a CAM photosynthesizer (Eller et al. 1993), as is *A. arborescens* (Kluge et al. 1979); they display typical $\delta^{13}C$ values like most other aloes (*A. marlothii* $\delta^{13}C$ = -14.93 and -12.09 ‰; *A. greatheadii* var. *davyana* = -14.41‰; *A. arborescens* = -16.92‰; J. Vogel unpubl. data.; Fig. 4).

However, the $\delta^{13}C$ values of aloes vary for aloe samples collected throughout Africa, ranging from -28.48‰ to -11.51‰ (mean ± SD = 17.26 ± 4.94‰) (Fig. 4), so the application of isotope techniques needs to be conducted with caution, or rather a sound understanding of the isotopic environment.

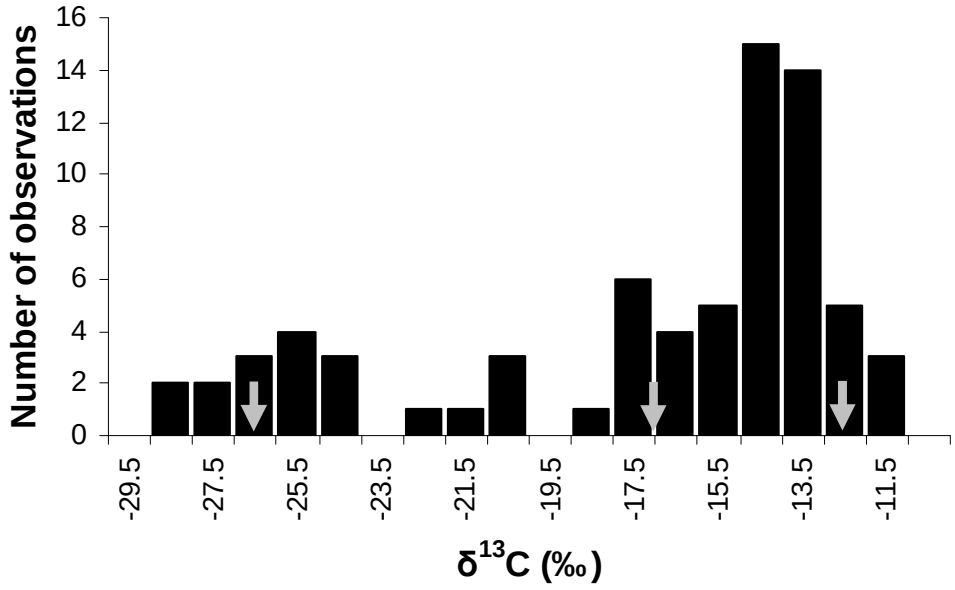

Figure 4. Frequency distribution of $\delta^{13}C$ (‰) for 64 *Aloe* species (*n* = 72) (J. Vogel unpubl. data). Arrows indicate means for C_3 (-26.5‰), CAM (-17‰) and C_4 (-12.5‰) plants (Vogel et al. 1978; Ehleringer and Osmond 1989; Ehleringer 1991; Dawson et al. 2002).

By monitoring the changes in isotope ratios of carbon in different tissues (e.g. blood, feathers) it may be possible to detect shifts in diet and the importance of aloe resources for consumers (Rubenstein and Hobson 2004). For example, it can be expected that the $\delta^{13}C$ isotope signature of a sunbird feeding on a broad range of C_3 plant nectars will be reflected in tissues utilised or grown during the period of feeding on those plants. Any shift in diet to CAM plants (e.g. aloes) will then lead to a change in isotope ratios in metabolically active tissues to reflect the new diet, as has been shown in White-winged and Mourning Doves feeding on saguaro fruit (Wolf et al. 2002). However, certain specialist nectarivores (i.e. sunbirds) may feed regularly on aloe nectar. It would therefore be difficult to detect a change in isotope composition when switching from one aloe species (e.g. *A. arborescens* which flowers before *A. marlothii*) to the next.

It is also possible, with the analysis of CO_2 in breath samples, to determine the contribution of *A. marlothii* to metabolism. This is possible because nectar sugars can be routed directly to metabolic processes instead of being assimilated into body tissues. The immediate use and importance of ingested substrates can therefore be quantified. This is different to using tissues where the turnover rate of stable isotopes (e.g. bone collagen) is low and the stable isotope signature of the diet not immediately represented in the tissue. It is thus important to acknowledge the isotopic turn-over rates for different tissue types when gaining dietary information; breath and bone collagen (or enamel in mammals) represent short- and long-term turnover rates respectively (Hobson and Clark 1992; Hobson et al. 1993; Perkins and Speakman 2001). When studying the importance of aloe nectar for non-nectarivores the confounding effects of other dietary components may be implied. The importance of aloe nectar may be over emphasized if granivores are feeding on C_4 grasses at the same time as CAM aloe nectar. Therefore, when determining dietary information and investigating trophic level reconstructions it is important to understand the isotopic ratios of the substrates involved.

Nitrogen isotopes are a useful tool in testing hypotheses concerning food web structure, energy and nutrient transfer within an ecosystem and analyses of trophic levels within an ecosystem (McKechnie 2004). Nitrogen is a useful element in determining one trophic level from the next in an ecosystem because the ^{15}N of a consumer's tissue is typically enriched relative to its diet by 3-5‰ (Minagawa and Wada 1984; Fry 1988; Mizutani et al. 1992; Hobson et al. 1994; Hobson and Wassenaar 1999). This fractionation results from the differences between nitrogen assimilation and nitrogen excretion. An animals' trophic position can thus be estimated by:

$$\textbf{Trophic level} = \lambda + [(\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}}) / \Delta_n]$$

where λ is the trophic position of the organism ($\lambda = 1$ for primary producers) used to estimate the $\delta^{15}N$ base, $\delta^{15}N_{\text{secondary consumer}}$ refers to the tissues of the consumer of interest, $\delta^{15}N_{\text{base}}$ is the corresponding value at the base of the food web, and Δ_n is the ^{15}N enrichment per trophic level.

Different bird species may visit aloe inflorescences for different reasons, some primarily for nectar (e.g. true nectarivores) and others for insects. The results of isotope analyses may assist in unravelling the complexities of trophic position and foodweb links in a bird community that utilises nectar during the short flowering period of *A. marlothii*.

Objectives of study

On a broad scale this project sought to investigate the relationships between an avian community (both nectarivores and opportunistic nectarivores) and *A. marlothii*. The importance of a seasonally abundant source of nectar for birds within an *A. marlothii* bird community, and the importance of birds as effective pollinators for *A. marlothii*, was investigated. A number of techniques were implemented to investigate these relationships, including the use of stable isotope ratio analysis. Through the use of stable isotopes, the study sought to reconstruct the representative dietary components within a nectar-feeding community, and quantitatively assess the importance of *A. marlothii* nectar within this community.

Initially the study focussed on the biology of *A. marlothii*, then incorporated the relationships between birds and *A. marlothii*. Finally, a study of stable isotopes, to investigate these interactions, was conducted.

The objectives were as follows:

1. Investigate flowering phenology and nectar production of *A. marlothii*, with an emphasis on the consequences of flowering during dry winter months.
2. Investigate the temporal use of *A. marlothii* nectar by birds in an *A. marlothii*-bushveld habitat.
3. Investigate the importance of birds and insects as pollinators of *A. marlothii*.
4. Investigate the contribution of aloe nectar to the carbon intake of bird species.

5. Establish degrees of trophic level shifting through a season of aloe flowering in a bird community feeding on *A. marlothii* nectar.
6. Determine the importance of *A. marlothii* nectar (sugar and water components) for avian consumers using stable isotopes in blood, breath and feather samples of opportunistic nectarivores.

Study site

The study was carried out at Suikerbosrand Nature Reserve (NR) 60 km south-east of Johannesburg, Gauteng Province. Recent land purchases now include additional grassland and old agricultural lands north of the park. Suikerbosrand NR covers an area of 19 779 ha and incorporates the Suikerbosrand mountain range (Fig. 7). Altitude ranges from 1 545 to 1 917 m a.s.l. and includes the highest range on the Witwatersrand[3]. Rainfall averages 650 to 700 mm p.a. (Fig. 5a). Summer and winter temperatures around the reserve range from 14 to 26^{o} C and 2 to 19^{o} C respectively (Fig. 5b). Temperatures are cooler in the reserve because of altitude effect and may often reach below freezing during winter nights.

The reserve is rich in botanical diversity and includes numerous species (+600 plant species identified to date) in the transition from true highveld to bushveld habitats. The flat hilltops support pure grasslands, characteristic of the unique Bankenveld vegetation type (Acocks 1975), whilst the warmer northern slopes and protected rocky outcrops support shrubs and wooded vegetation. *Acacia karroo* and *Acacia caffra* are common tree species within the bushveld components of the reserve. The geology of the reserve is distinct, separated into the Ventersdorp system, consisting of igneous basalt rocks, found in the west of the reserve and the sedimentary sandstones of the Witwatersrand system in the east. In the reserve the dominant force is fire so trees and aloes persist more rigorously in protected habitats such as valleys and rocky outcrops. These rocky slopes tend to occur on steeper and more fertile slopes in the west of the reserve (Fig. 7).

[3] The Witwatersrand, literally meaning the “ridge of white waters" and often shortened to Rand refers to the mining region south of Johannesburg known primarily for rich deposits of gold and other minerals.

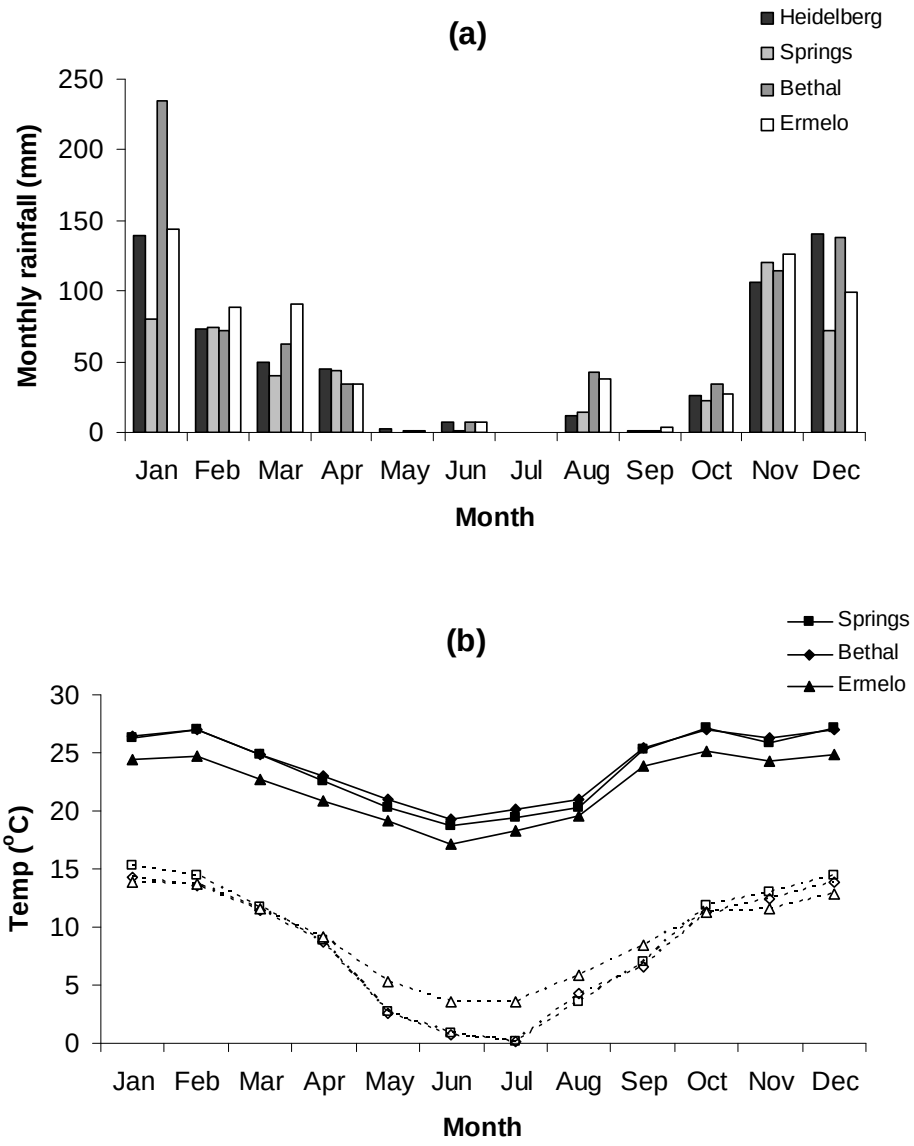

Figure 5. Mean monthly rainfall at Heidelberg (26.500°S 28.370° E, alt. 1520 m a.s.l.) and nearby localities **(a)**, and mean monthly maximum and minimum temperatures for localities surrounding Suikerbosrand NR **(b)**. (Springs, 26.200°S 28.433°E, 1592 m a.s.l.; Bethal, 26.470°S 29.450°E, 1640 m a.s.l.; Ermelo 26.497°S 29.983°E, 1769 m a.s.l.). (Data for duration of study, 2005-2007; data supplied by South African Weather Bureau: extracted 15/08/2007).

Most of the field work was conducted in the western portion (26° 31' 50" S 28° 10' 07" E, *c*. 1 600 - 1 700 m a.s.l.) of the reserve, where large stands of aloes occur (Fig. 6a, b). Tens of thousands of plants, some measuring >6m in height, occur on

north-facing slopes. Plants grow at a higher density in thick bushveld habitat in a region of transition between highveld grassland and bushveld. Individual aloe plants of all age classes are represented in most stands of *A. marlothii* in the reserve. Flowering in previous years is evident by the presence of dry inflorescences with dehisced seed pods. In addition, the remains of dry inflorescences are incorporated in the dry leaf skirts of adult plants; the space between inflorescences indicating successive flowering events.

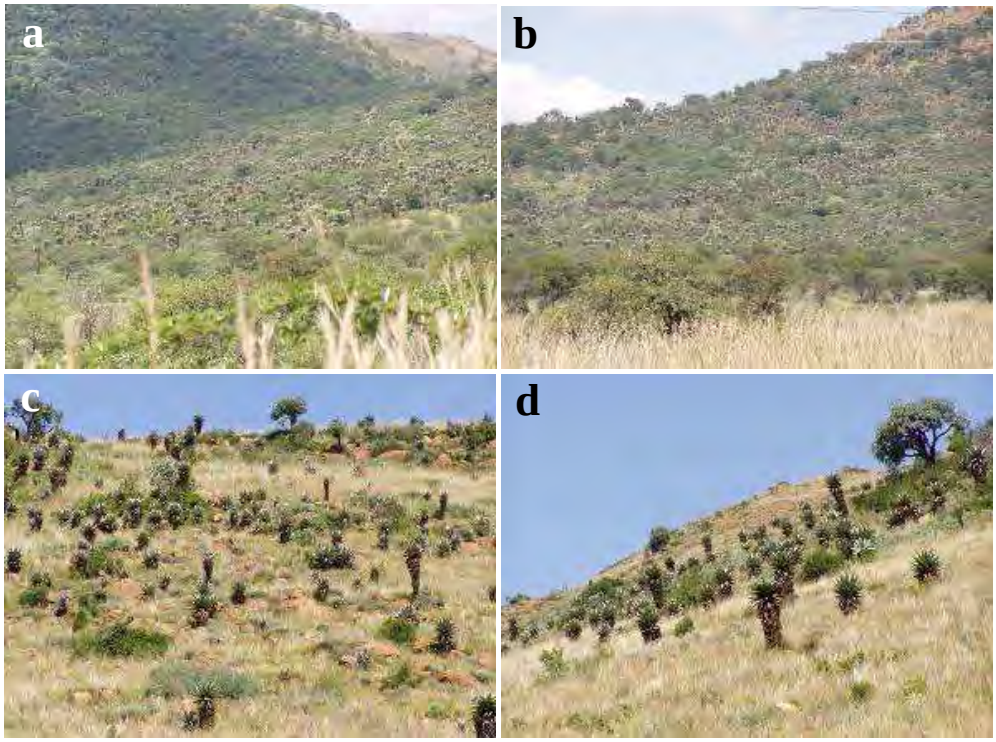

Figure 6. *Aloe marlothii* stands in Suikerbosrand Nature Reserve. **(a) & (b)** Section of *A. marlothii* forest in western part of reserve showing aloes growing on gentle north-facing slope; main area of study. **(c) & (d)** *A. marlothii* plants growing on steeper, less wooded, north-facing slope.

Aloe species in the reserve include *A. zebrina*, *A. greatheadii* var. *davyana*, *A. arborescens* and *A. marlothii*. The former two are small aloes, never exceeding 0.5m in height (Reynolds 1969). *A. zebrina* flowers during summer months whilst *A. greatheadii* var. *davyana* flowers during winter. However, *A. greatheadii* var. *davyana* is visited less by birds, and attracts honeybees that visit for nectar and pollen (Nepi et al. 2006; Human 2006). *A. marlothii* is the predominant aloe species in the reserve

where it is common on north-facing slopes, especially in the west of the reserve. It is conspicuous in abundance and size and is a characteristic of the landscape during flowering in July to September, later than other winter-flowering aloes in the sub-region. Additional sites include north-facing slopes with less woody vegetation where aloe density is less dense (Fig. 6c & d).

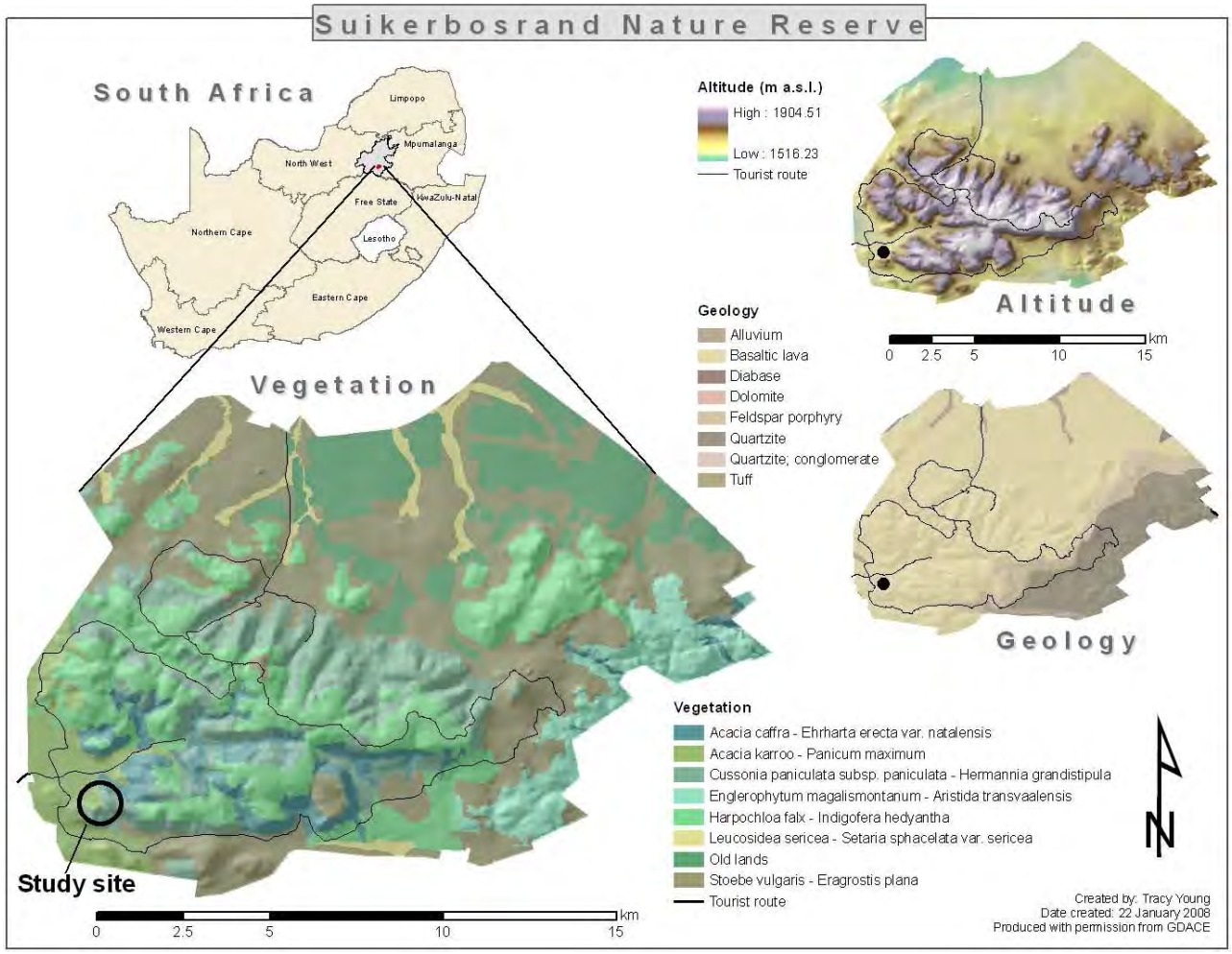

Figure 7. Map showing location of study site at Suikerbosrand Nature Reserve in Gauteng Province, 60 km south-east of Johannesburg, South Africa. Altitude (m a.s.l.) and geology of reserve shown in relation to major habitat types (Panagos 1999). Main circular tourist route (asphalt road) also indicated.

Glossary

abaxial - directed away from the stem of a plant.

acropetalous - describes the development of structures (e.g. flowers) in succession from the base towards in the direction of the apex.

adaxial - directed toward the stem of a plant.

adnate - joined to a part of a different kind, e.g. stamens joined to petals; growing closely attached.

bract - the modified leaf at the base of the pedicel, between the calyx and normal leaves.

dehisce - burst or split open.

divaricate - diverge at a wide angle; spread apart.

filiform - thread like; thin in diameter.

inflorescence - the complete flowering parts of a plant; an aggregate of flowers on an axis.

naviculate - boat-shaped.

panicle - a branched inflorescence or flower cluster; a raceme whose branches are themselves racemes.

pedicel - the short supporting stem of a single flower in an inflorescence.

peduncle - the stalk of a flower cluster or inflorescence, in the case of an aloe the portion below the first branch.

perianth - the floral envelope formed by a fused calyx and corolla.

pistillate - having pistils but no stamens; female flowers are pistillate; or referring to the mature female stage of a flower.

protandrous - relating to a flower where the anthers release their pollen before the stigma of the same flower becomes receptive.

raceme - a type of inflorescence in which cluster of flowers from a single common or lengthened axis; these pedicellate flowers are borne in acropetal succession on the stalk.

rosulate - having a rosette.

secund - flowers turned up in one direction only.

staminate - having stamens but no carpels; male flowers are staminate; or referring to the mature female stage of a flower.

xeromorphic - having special devices which protect the plant from desiccation.

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Appendix. Distinguishing characteristics of *A. marlothii* (see Fig. 3d and e) and *A. ferox*, giving past and present classifications. Descriptions after Reynolds (1969), with additions from Glen and Hardy (2000), and Van Wyk and Smith (2005). Distinct characters highlighted in bold.

Van Wyk & Smith (2005)	***A. marlothii* Berger**		***A. ferox* Miller**	
Reynolds (1969)	***A. marlothii* Berger**	***A. spectabilis* Reynolds**	***A. ferox* Miller**	***A. candelabrum* Berger**
Ht (m)	2-4, sometimes 5-7 (Fig. 3b)	2-4	2-3, sometimes 4-5	3-4
Inflorescence	single much branched panicle, *c.* 80 cm high [1]	**branched panicle (1-3)**	single branched panicle	divaricately branched panicle (usually 1)
Raceme	**horizontal to sub-oblique racemes (20-30; 50)** (Fig. 3a)	**erect or sub-erect (up to 14)** (Fig. 3c)	**erect (5-8)**	**erect and branched low (6-12), terminal raceme longer**
Raceme dimensions	30-50 cm long, 5-6 cm broad [2]	25 cm long, 9-10 cm dia.	50-80 cm long, 9-12 cm dia. at base, 6 cm dia at apex	slightly acuminate 50-80 cm long, 10 cm dia.
Flower time	April-July	June-July	May-Aug (later if colder)	June-July
Flower colour	deep gold, to red in KwaZulu-Natal	buds redder than yellow to golden yellow flowers	golden orange to bright scarlet (white form)	creamy-pink, through orange, to red
Flower position	markedly secund, orientated towards base of branch	evenly arranged around axis	buds horizontal	horizontal, or spreading slightly downwards
Pedicel	5 mm long, 3 mm diameter	3 mm long	green, 4-5 mm	6 mm
Inner segment	free, but dorsally cohering to outer for *c.* 10 mm, **deep purple near apex**	free, but dorsally adnate to outer for 1/3 of length, **margins almost white, tipped glossy purple to black**	free, but dorsally adnate to outer for 1/3 of length, **thin whitish margins, tipped dark-brown to black**	free, but dorsally adnate to outer for 1/3 of length, **thin white marginal border**
Filaments 3 inner lengthening in advance of 3 outer	flattened, pale in perianth**, exserted portion deep purple**	flattened, pale in perianth **exserted part orange**	flattened, lemon in perianth **exserted part orange to brownish-orange, then deep brown to black**	filiform flattened, included part lemon, **exserted portion deep orange**
Style	**exserted part pale brown**, lighter than exserted filaments	**exserted part paler orange** than filaments	exserted part lighter than filaments	lemon colour in perianth, exserted part yellow

Note: The KwaZulu-Natal form of *A. marlothii* is formerly referred to as *A. spectabilis*, which is not synonymous with *A. marlothii orientalis* as referred to by Glen and Hardy (2000). The KwaZulu-Natal form of *A. ferox* is previously referred to as *A. candelabrum*. [1] height of aloe increased by 30 ± 9 cm (n = 40) with inflorescence; [2] mean raceme length (± SD) = 366 ± 91 mm (n = 39).

I

CHAPTER 1

Production of copious dilute nectar in the bird-pollinated African succulent *Aloe marlothii* (Asphodelaceae)

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Abstract

Aloe marlothii is a CAM succulent plant which is widespread in the northern and north-eastern summer rainfall region of South Africa. Flowering occurs during dry winter months (June - September) and the large inflorescences attract a wide range of birds. Flowering phenology and nectar production were studied during three seasons (2005 - 2007) at a dense population of aloes in Suikerbosrand Nature Reserve. Three flower stages were recognised; 1) immature phase, 2) male phase, and 3) female phase, with extremely high nectar volumes (mean = 248 µl/flower) in stage 2, the stage to which most avian visitors are attracted. Nectar sugar concentration was very low (12 % w/w) in stage 2 when the volume was highest. Nectar volume and concentration showed little diurnal variation, despite a 24 h temperature range of up to 20^{o}C. The extremely high volume and low concentration of the nectar proved to be consistent with a generalist bird pollination syndrome. A wide range of avian visitors (42 species; 59% of the resident bird community recorded during flowering) fed on nectar throughout the day, but a decrease in nectar standing crop was only evident at mid-day. Chacma baboons *Papio hamadryas ursinus* foraged on nectar and caused significant inflorescence damage.

Key words: Aloaceae; Baboon; CAM; Mass flowering; nectarivore; sunbird

Introduction

The conspicuous yellow to red inflorescences of members of the genus *Aloe* L. (Asphodelaceae) during the austral winter months is a distinct feature of the South African landscape (Reynolds, 1969; Glen and Hardy, 2000; Van Wyk and Smith, 2005). One of the most charismatic species, with a wide distribution in the northern and north-eastern summer rainfall region, is *Aloe marlothii* A.Berger [section *Orthlophae* (Christian) Glen & D.S.Hardy] (Reynolds, 1969; Glen and Hardy, 2000). It occurs in large numbers as a dominant and conspicuous plant on especially rocky north-facing aspects (Reynolds, 1969; Glen and Hardy, 2000; Van Wyk and Smith, 2005). The inflorescence is a branched panicle of up to 30 nearly horizontal racemes, with orange or red flowers that vary in shape, size and colour across the geographical range of the species (Reynolds, 1969; Glen and Hardy, 2000; Van Wyk and Smith, 2005).

Although studies have investigated nectar production in aloes (Hoffman 1988; Nicolson and Nepi 2005; Human and Nicolson 2008), and some have addressed pollination (Hoffman 1988; Ratsirarson 1995; Stokes and Yeaton 1995; Pailler et al. 2002), few have considered flowering phenology and the consequences of visitations by a broad pollinating community during a dry winter flowering period (Oatley 1964; Oatley and Skead 1972). This study investigated flowering phenology and nectar production of *A. marlothii*, over three flowering seasons, with emphasis on the consequences of flowering during dry winter months for certain associated animal communities. The inflorescence structure of *A. marlothii* is typical of a bird (ornithophilous) pollination syndrome; sturdy axes for perching, bright orange to red flowers, long floral tube, absence of odour and nectar guides, exserted anthers and stigma, secund flower arrangement, and large pollen-nectar distance. We therefore hypothesized that nectar properties were also characteristic of a bird pollination syndrome; such traits including abundant nectar held in flowers by capillarity, low nectar sugar concentration, and diurnal anthesis with nectar volume highest in the early morning. The flowering period of *A. marlothii* is characterised by cold nights and clear warm days, with low relative humidity. We thus investigated whether nectar was affected by the large diurnal changes in temperature and relative humidity. During the flowering period bird abundance at the aloe study site increases significantly (Chapter

2). We therefore investigated whether continuous feeding by birds affected nectar availability for subsequent visitors.

Materials and Methods

Study site

The study was conducted during 2005 - 2007 at Suikerbosrand Nature Reserve, 60 km south-east of Johannesburg, South Africa, during the flowering season of *A. marlothii* (June - September). Data collection occurred predominantly within the high density site of the *A. marlothii* population (dense site; 26^{o} 31’ 50” S 28^{o} 10’ 07” E, *c.* 1 600 - 1 700 m a.s.l.) in the west of the reserve. At this site aloes occur on gradually sloping north-facing aspects and number tens of thousands of plants. An additional site within the reserve, approximately 4 km north, where stands are less dense, was included for comparative phenological studies (sparse site).

Phenology and flower development

Prior to flowering in 2005, 130 *A. marlothii* plants along an old track through the dense site were randomly marked with flagging tape for future identification. Aloes from a range of height classes were included and height was recorded using a measuring pole. In May 2006, prior to flowering, an additional 100 plants were selected and marked along a transect through the sparse site. Reproductive phenology of plants was assessed at intervals (7 - 19 days) during the flowering season by counting, i) the number of racemes on each plant, ii) racemes with open flowers, and iii) racemes with fruit. Data from these plants were recorded in 2005, 2006 and 2007 at the dense site and in 2006 at the sparse site. An index of fruit set was determined from the proportion of total racemes that developed fruit. A separate study investigating the effectiveness of different pollinator guilds for *A. marlothii* reports on actual fruit set in randomly selected plants.

The total number of flowers on 28 mature racemes from 9 plants was counted. This was done randomly at the dense site early in the flowering period (2005) when

fewer inflorescences were damaged by chacma baboons *Papio hamadryas ursinus* Kerr. Raceme length was also measured (cm).

Three racemes were collected and returned to the laboratory to test for stigma receptivity. A selection of flowers at various stages of development from each raceme were tested with hydrogen peroxide (3%) and observed under a dissecting microscope (x15). Stigma receptivity was indicated by the hydrogen peroxide reacting with peroxidases present on the stigma to form bubbles (Dafni, 1992).

Nectar measurements

Nectar volume was measured using disposable hematocrit tubes (75 µl). A hand-held refractometer (Bellingham and Stanley, Tunbridge Wells, UK) was used to measure nectar sugar concentration (% w/w). During nectar sampling, temperature and relative humidity were recorded with a portable TES 1365 thermohygrometer (Taipei, Taiwan) at the height of the flower sampled. Flowers were sampled destructively.

Nectar production (volume and concentration) was measured in three aloe plants (height = *c.* 1.8 m) over a 24 h period during the peak of flowering (23 - 24 August 2005). Prior to and during sampling, racemes were covered with fine mesh netting to exclude visitors. Three different flowers of each stage (see Results), arbitrarily selected from different racemes on each plant, were sampled every 2 h beginning at 07:00.

To compare nectar standing crop (a measure of nectar availability for consumers) at the dense site between days with different climatic conditions, three stage 2 flowers (see Results), from four different unscreened inflorescences, were sampled every 2 h during the day (07:00 - 17:00). Sampling occurred on a cool and cloudy day, with light rain during the previous night (atypical during the flowering period), and a clear warm day, both during peak flowering.

Nectar concentration and volume were compared in screened and unscreened flowers on 30 August 2006. Three stage 2 flowers on four marked aloes (two screened to determine nectar production and two unscreened to determine standing crop) were

sampled every 2 h during daylight (07:00 - 17:00). The inflorescences of screened aloes were covered in fine mesh to exclude visitors (particularly birds). During the intervals between sampling the unscreened aloes were observed to record visitors feeding on nectar.

Statistical analyses

All results are presented as mean ± SD. We used non-parametric analyses because the majority of data did not conform to normal distributions (Shapiro-Wilk W-test for normality). Comparisons of flowering between years and of heights of plants that flowered at different frequencies were made using Mann-Whitney *U*-Tests. Correlations of fruit set and plant height, and total number of flowers in relation to raceme length, were made using Spearman's rank correlations. Comparisons of fruit set success on plants in different height classes and of overall volume and concentration were made using Mann-Whitney *U*-Tests. Variation through a day in nectar volume and concentration was analysed by Kruskal-Wallis non-parametric analysis. All statistical analyses were conducted using Statistica 6.0 (1984 - 2004).

Results

Phenology

All plants that flowered produced a single inflorescence and the first inflorescences became visible in June. In 2005 the first flowers opened mid-July and by late-September flowering was complete. In 2006 and 2007 peak flowering occurred later than in 2005. Flowering was earlier at the sparse site than the dense site in 2006 (Fig. 1).

Inflorescence damage was attributed to chacma baboons that broke inflorescences to access the moisture at the base of the peduncle during the early flowering period, or to obtain nectar once flowering had begun. Baboons destroyed 31%, 33% and 79% of all inflorescences during 2005, 2006 and 2007 respectively. At the sparse site in 2006, where flowering occurred earlier, 84% of inflorescences were

completely destroyed by baboons before they could produce fruit. At the dense site, 40%, 47% and 25% of racemes produced fruit (as a percentage of all racemes produced) in 2005, 2006 and 2007 respectively; whilst at the sparse site only 4% of racemes produced fruit. There was no significant difference in the maximum number of racemes per inflorescence produced between 2005 and 2006 at the dense site (U = 3278.5, p = 0.996), although in 2007 there were fewer racemes than 2005 and 2006 (U = 1462.0, U = 1193.5 respectively, $p < 0.01$) (2005, 14.0 ± 6.7; 2006, 13.7 ± 5.0; 2007, 10.1 ± 4.1).

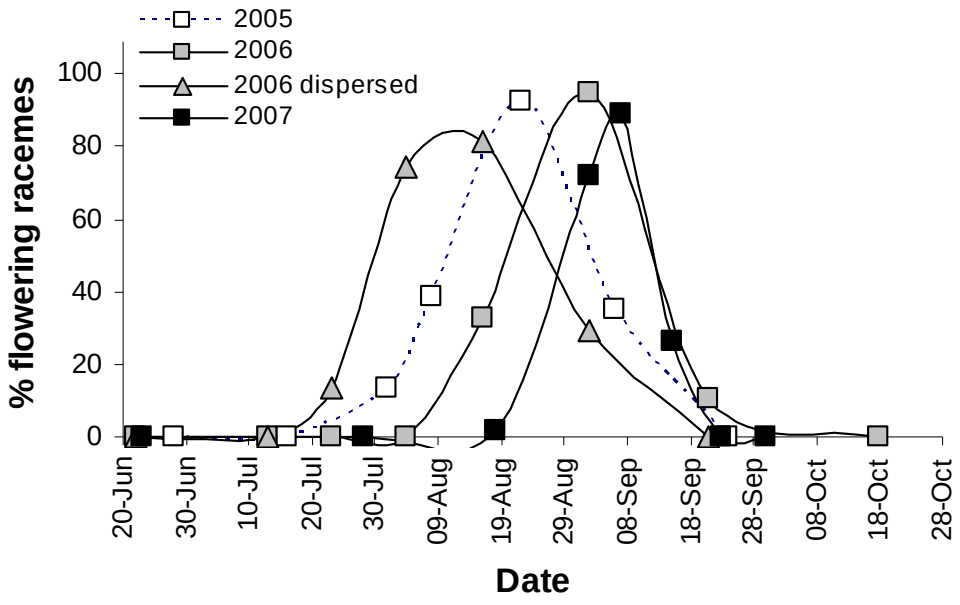

Fig. 1 Unimodal flowering curves for *Aloe marlothii* in Suikerbosrand Nature Reserve, for the dense site during 2005, 2006 and 2007 (n = 130; marked aloes), and the sparse site during 2006 (n = 100). Flowering indicated as percentage of racemes with open flowers.

In 2005 and 2006 plant height was significantly correlated with the number of racemes that bore fruit (2005, Spearman's R = 0.336, $p < 0.05$; 2006, Spearman's R = 0.367, $p < 0.05$); taller plants had a greater proportion of racemes that eventually set fruit. Shorter plants were damaged by baboons before fruit set could occur. There was no correlation in 2007 (Spearman's R = 0.254, $p > 0.05$). Taller plants also flowered more frequently. Plants that flowered in two years and three years were of similar height (U = 918.0, p = 0.269) but taller than plants that never flowered or flowered in one year only ($p < 0.01$) (Table 1).

Table 1. Percentage of *Aloe marlothii* plants that developed inflorescences in different years (2005, 2006 and 2007, *n* = 130) and height for each flowering frequency category. Data for high density site at Suikerbosrand Nature Reserve. Significant differences in height for aloes with different flowering frequencies shown by superscripts (Mann-Whitney U-test, $P < 0.05$).

Frequency of inflorescence development	**Percentage** (*n* = 130)	**Mean height** (m) ± SD
Developed in three years	45.4	3.03 ± 0.89 [a]
Developed in two years	27.7	2.85 ± 0.99 [a]
Developed in one year	9.2	2.16 ± 1.09 [b]
No inflorescence	17.7	1.46 ± 0.71 [c]

Flower development

Flowers on an aloe raceme developed in an acropetalous fashion, with those on the northern (sunny) side of each raceme opening first, like in *Aloe ferox* Mill. (Section *Pachydendron* (Haw.) Salm-Dyck) (Hoffman 1988). Flowers are protandrous with flower development similar to *Aloe castanea* Schönland (Section *Anguialoe* Reynolds) (Nicolson and Nepi, 2005). Although flower development is clearly continuous we recognised three flower stages; described as follows (Fig. 3).

Stage 1. Immature (pre-fertile) phase. Flowers were closed with the distal end of the flower swollen. The perianth was slightly open with undehisced anthers partly visible. The time from emergence of stamens to anther dehiscence was 4 - 14 h (9.2 ± 5.6 h, *n* = 5); during this period the stigma was unreceptive. Darkness retarded anthesis and took longer in the late afternoon and night.

Stage 2. Male (staminate) phase. The stamens became fully exserted and anthers dehisced with shorter abaxial stamens following longer adaxial stamens. Anthers dehiscence occurred when the stamens were at maximum length, with the stigma secluded between the anthers. Although the stigma was receptive on exsertion, it was physically not exposed to receive pollen. Flowers were a rich golden colour (Fig. 2a), and nectar production at a maximum (see below). Stage 2 duration 22 - 38 h (29.1 ± 5.5 h, *n* = 9). Higher temperatures promoted rapid senescence (desiccation) of flower parts.

Stage 3. Female (pistillate) phase. The majority of pollen dehisced and the stigma was fully exserted. Flowers bent back towards the rachis as senescence (wilting) occured. Stage 3 duration 18 - 52 h (37.6 ± 14.1 h, *n* = 5). End of stage 3 recognised when stigma became unreceptive and the style flaccid. Nectar was only available in each flower during the early phase of stage 3. Entire flowering duration was *c.*76 h.

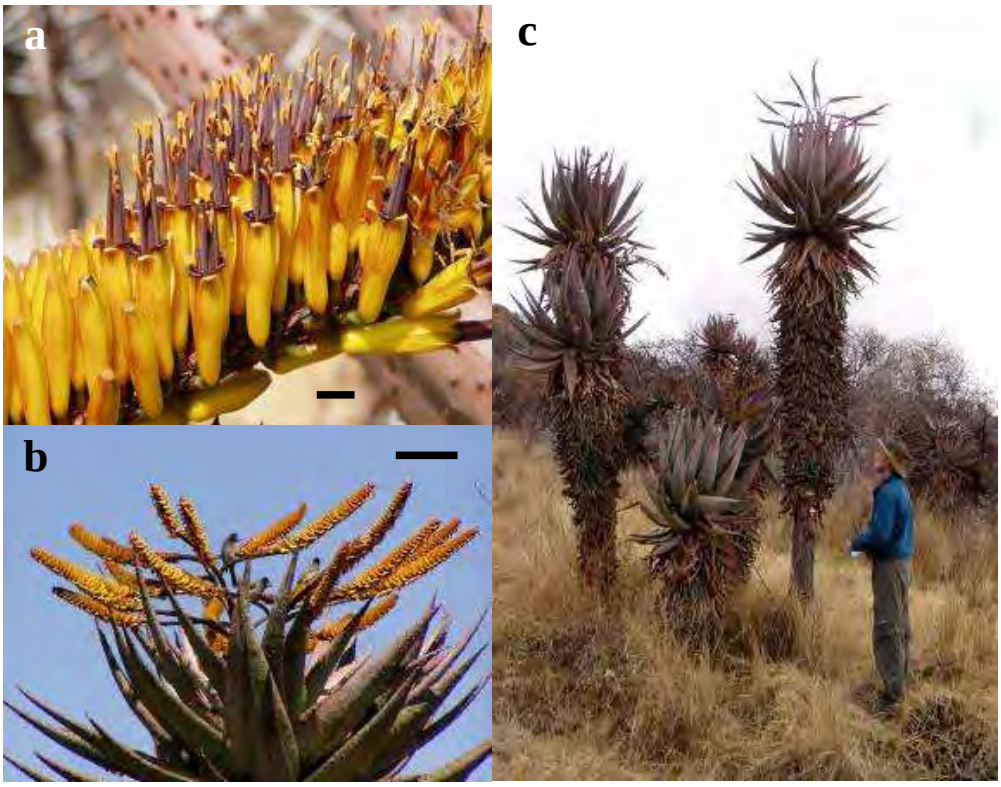

Fig. 2 *Aloe marlothii* at the high density site in Suikerbosrand Nature Reserve. a) Sequence of flowers on raceme from unopened flowers to old flowers (scale bar 10 mm; proximal end to the right), b) inflorescence with visiting African red-eyed bulbuls *Pycnonotus nigricans* (scale bar *c.* 20 cm), and, c) small clump of aloes with author (height = 1.80 m) *in situ* for perspective (Photographs: a and b, C.T. Symes; c, Tracy Young).

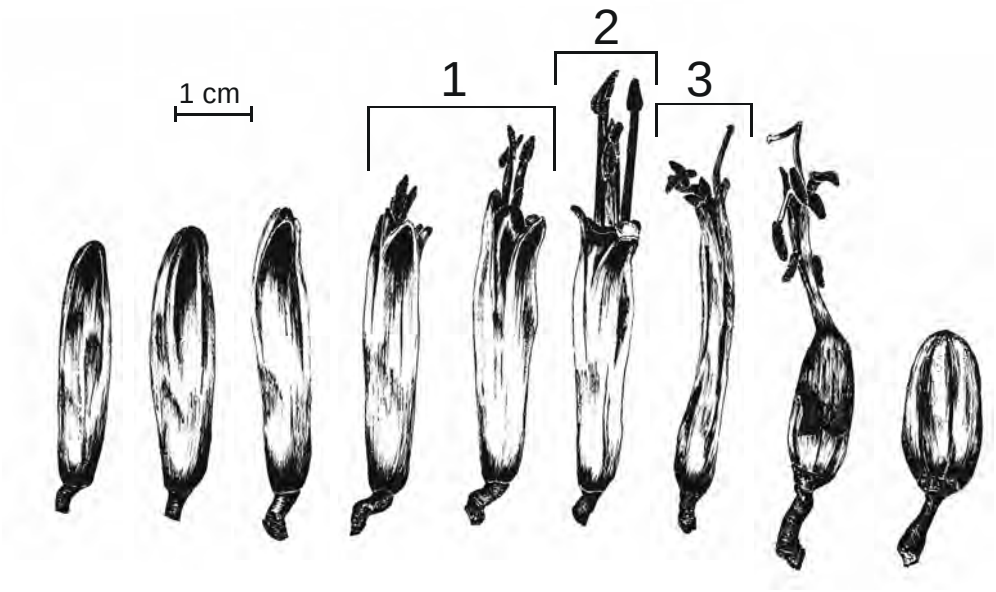

Fig. 3 Development of *Aloe marlothii* flowers indicating different stages (1 - 3) recognised during nectar sampling (see detailed descriptions in text). The sequence shown demonstrates the development from an immature flower to an immature un-dehisced fruit. (Illustrator: C.T. Symes).

Nectar analyses

Temperature and relative humidity varied through the 24 h sampling period and are shown in Fig. 4a. The nectar volume of stage 1 flowers differed through the 24 h period ($H_{11, 108} = 20.52$, $p = 0.04$). Volumes did not differ in stage 2 ($H_{11, 108} = 5.88$, $p = 0.88$), the most important for visitors, or stage 3 ($H_{11, 108} = 13.05$, $p = 0.29$) flowers (Fig. 4b). Nectar concentration of stage 1 and stage 2 flowers differed through the 24 h period ($H_{11, 108} = 31.02$, $p = 0.001$ and $H_{11, 108} = 24.25$, $p = 0.01$ respectively), but did not differ for stage 3 flowers during the 24 h period ($H_{11, 78} = 14.41$, $p = 0.21$) (Fig. 4c).

Nectar concentration increased with flower age, with wilted stage 3 flowers having the highest concentration (Fig. 4c). The mean volumes for 24 h were 28 ± 32 µl, 248 ± 109 µl, and 49 ± 64 µl for stage 1, 2 and 3 flowers respectively; nectar concentrations averaged over 24 h were 9 ± 2%, 12 ± 4% and 23 ± 8% respectively.

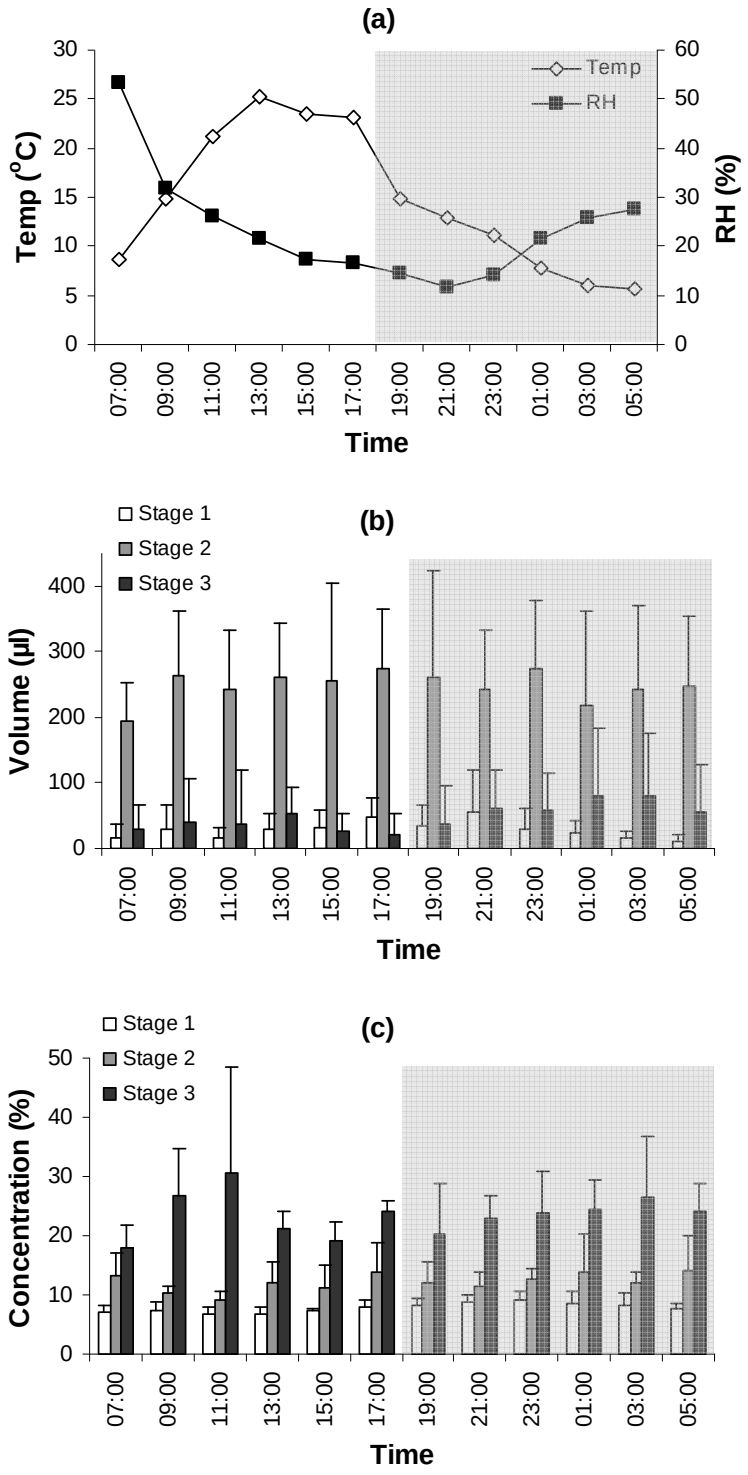

Fig. 4 Nectar volume and concentration in screened stage 2 flowers on three *Aloe marlothii* plants through 24 h. (a) Temperature (°C) and relative humidity (%) at inflorescence height where flowers were sampled (n = 3), (b) volume (µl) (n = 9), and (c) concentration (% w/w) (n = 9). Each flower was sampled once

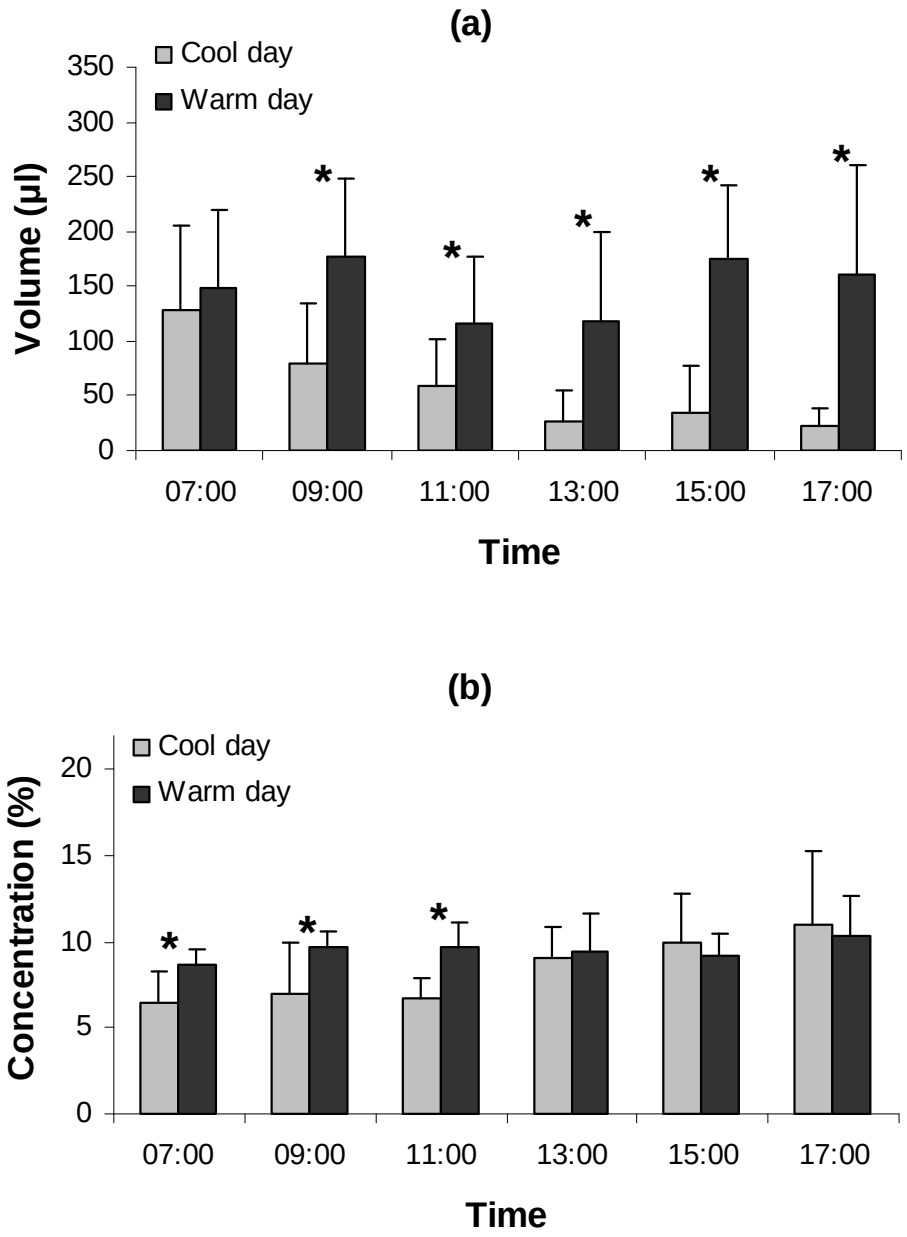

Fig. 5 Nectar volume (a) and concentration (b) for unscreened stage 2 flowers of *Aloe marlothii* on a cool and warm day: flowers sampled during peak flowering in August 2006 (n = 12). Significant differences between days for each time period indicated by ***** (Mann-Whitney U-Test, $p < 0.05$).

During the cool- and warm-day sampling, daily temperature variation was *c.* 20°C each day. Temperatures on the cool day increased from 2 - 21°C whilst warm day temperatures increased from 8 - 26°C. Relative humidity decreased from 74 - 22% on

the cool day, and from 66 - 20% on the warm day. Nectar volume and concentration varied significantly during the cool day ($H_{5, 72} = 22.84$, $p < 0.01$ and $H_{5, 72} = 25.74$, $p < 0.01$ respectively) but not during the warm day ($H_{5, 72} = 7.63$, $p = 0.18$ and $H_{5, 72} = 7.63$, $p = 0.18$ respectively) (Fig. 5).

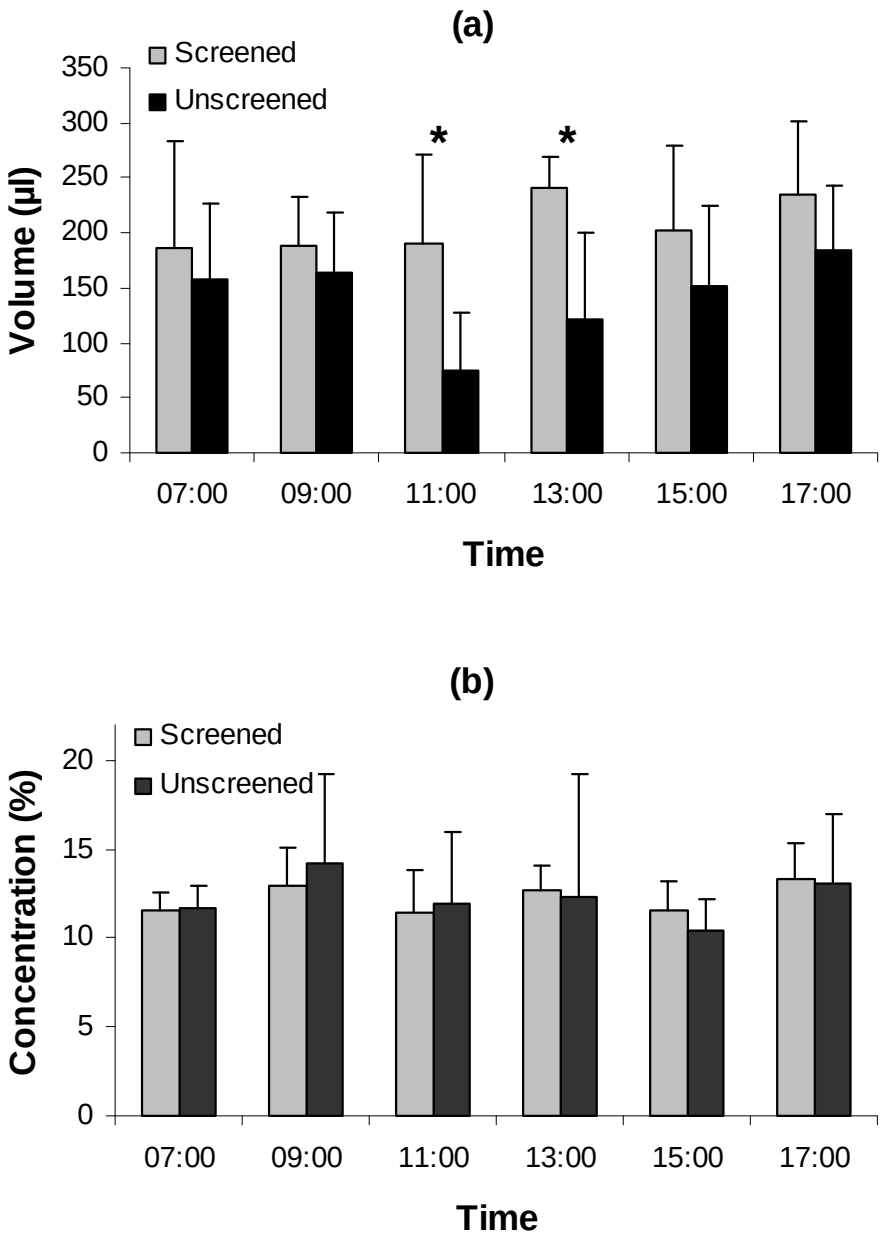

Fig. 6 Mean nectar volume (a) and concentration (b) for screened and unscreened stage 2 flowers ($n = 6$) during daylight hours for four *Aloe marlothii* plants on 30 August 2006. Significant differences between treatments for each time period are indicated by ***** (Mann-Whitney *U*-Test, $p < 0.05$).

Temperature and relative humidity on the day when screened and unscreened flowers were compared were typical of a sunny day during aloe flowering (Temp: 0 - 20^{o}C; RH: 45 - 5%). Volumes in screened and unscreened flowers were significantly different at 11:00 (U = 3.00, p = 0.02) and 13:00 (U = 4.00, p = 0.02). There were no differences in concentration during each period (p > 0.01) (Fig. 6). Birds (e.g. red-faced mousebird *Urocolius indicus* Latham, fiscal flycatcher *Sigelus silens* Shaw and Cape weaver *Ploceus capensis* L.) were observed visiting unscreened flowers and probing for nectar during the intervals between nectar sampling.

Discussion

Dry season flowering

Mass flowering of *Aloe marlothii* occurs during the dry winter period (June - September) when few other plant species flower. Many plants flowered each year (62 - 69%), like *A. ferox* (59%; Hoffman, 1988), a morphologically similar species with vertical racemes, that fills an equivalent ecological niche in the south-east of South Africa. Flowering occurred with remarkable synchrony and for individual plants of *A. marlothii* flowering duration was approximately 3 - 4 weeks. However, because not all plants began flowering at exactly the same time the entire flowering period was longer, lasting for 5 - 10 weeks. During this period nectar was available in abundance for consumers at the driest time of the year. Similarly, in monsoonal woodland of northern Australia nectar availability has been found to peak during the dry season (Franklin and Noske, 1999) and in Mexico the overall flowering peak occurs at the end of the dry season (Arizmendi and Ornelas, 1990). However, in tropical New Guinea flower resources decrease during the dry season, although there is a wide range of temporal variation within and among species in length and timing of the flowering period (Brown and Hopkins, 1996). Birds, bats and insects are attracted to the nectar of numerous *Agave* L. species in Central America and, although flowering of different species occurs during different seasons, the main attraction is abundant nectar in a predominantly arid environment (Emlen, 1973; Ornelas et al., 2002; Rocha et al., 2005). In South Africa most aloes flower during the winter months (Reynolds, 1969;

Jeppe, 1969) and in *A. marlothii* the attraction for numerous birds is an abundance of dilute nectar during the dry season when there is a shortage of free standing water (Chapter 2).

Constant nectar availability to visitors

Despite a day-night temperature range of up to 20^{o}C, no obvious peak in nectar volume through 24 h was observed. Stage 2 of flowering lasts 1-2 days and is the most important stage for visitors. The higher proportion of flowers opening at night ensures a ready supply of nectar for birds eager to feed after the nocturnal fast (Symes and Nicolson unpubl. data). Nocturnal nectar production has seldom been measured except in the context of nocturnal visitors. In five *Agave* species, where the main pollinators were bats, nectar production was measured through the night (Rocha et al., 2005). However, despite diurnal visitors contributing to pollination success, nectar volume was not measured during daylight (Rocha et al., 2005). Maximum nectar production during darkness is often correlated with visits by nocturnal pollinators (e.g. Lemke, 1984; Arizaga et al., 2000; Ibarra-Cerdeña et al., 2005)**.** Nectar production in *A. marlothii* does not suggest a clear focus towards a suite of diurnal pollinators. However, the continuous opening of flowers along the raceme ensures that stage 2 (male stage) flowers, containing large quantities of nectar, are available for diurnal visitors.

Numerous studies have demonstrated the replenishment of nectar in emptied flowers (e.g. Navarro, 1999; Castellanos et al., 2002). In flowers of *A. castanea* nectar removal stimulated nectar production until a critical amount had accumulated (Nicolson and Nepi, 2005). Because of their shape, *A. marlothii* flowers are difficult to sample repeatedly without damage, thus preventing similar experiments. On cool cloudy days, which are uncommon during the flowering period, nectar standing crop decreased. This may be explained by more bird visitors, with higher energy demands on cooler days, extracting more nectar. *Aloe marlothii* nectar remains remarkably consistent in volume and concentration, despite winter days being dry and warm to hot. Even though the nectar of *A. castanea* is more exposed than that of *A. marlothii*,

and would be expected to equilibrate faster with ambient RH, the nectar concentration also remained relatively low (6 - 12%; Nicolson and Nepi, 2005).

Avian visitors were able to reduce standing crop during the midday hours, as indicated by comparisons between screened and unscreened plants. This was likely caused by higher feeding rates by birds in the morning (Chapter 2). Although morning visitors were able to reduce the midday standing crop, nectar volumes by the afternoon had recovered and were comparable to those in the morning.

Visitors to A. marlothii *flowers*

Of particular significance in this study is the attraction of a high diversity and abundance of birds to the large volumes of dilute nectar (Chapter 2). Forty-two species (59% of the resident bird community during flowering) were recorded as nectar feeders, and stable carbon isotope evidence suggests that sugar in the nectar is important for a wide range of species, represented by frugivorous, insectivorous, granivorous and omnivorous feeding guilds (Chapter 4). Throughout the range of *A. marlothii* at least 83 bird species have been recorded feeding on nectar (Oatley, 1964; Oatley and Skead, 1972; Symes et al., 2008). This nectar is abundant and because very few inter- and intra-specific interactions are observed between birds, we suggest that these nectar resources may not be limiting, especially at large stands of *A. marlothii* like at Suikerbosrand.

Chacma baboons were observed often at both sites during the flowering season. They climbed onto aloe rosettes, undeterred by the thorny leaves. There they feed on immature inflorescence bases early in the flowering season, and later sucked open flowers for nectar. During these activities they contributed significantly to inflorescence damage and caused reduced fruit set, although this damage may be compensated for by the mass flowering (Bawa, 1983). Damage at the sparse site, where flowering occurred earlier, was greatest. This response by baboons to earlier flowering aloes indicates the importance of aloes for these animals. Small differences in flowering timing were observed during the three years of the study. In addition, more damage to inflorescences was observed in 2007 suggesting great annual variation

in aloe use by baboons. The months prior to flowering in 2007 were particularly dry and damage of inflorescences was therefore possibly exacerbated by a greater demand for alternate water sources by baboons. This variation highlights the importance of long term monitoring for ecological studies of this nature.

Aloe marlothii nectar may also be an important food and/or water source for other mammals such as striped mouse *Rhabdomys pumilio* Sparrmann, Namaqua rock rat *Michaelamys (Aethomys) namaquensis* A.Smith and slender mongoose *Galerella sanguinea* Rüppell (Chapter 3). Other aloe species are known to attract mammalian visitors; ring-tailed lemur *Lemur catta* L. fed on *Aloe divaricata* A.Berger flowers on Madagascar (Ratsirarson, 1995), and vervet monkeys *Cercopithecus aethiops* L. fed on nectar and flowers of *A. ferox* (Skead, 1967; Thomas and Grant, 2004; D. Koen pers. obs.).

During peak flowering of *A. marlothii* insect abundance is low and in the gregarious aloe stands very few honeybees *Apis mellifera* L. were observed foraging for pollen or nectar (C.T.S. pers. obs.). In *A. castanea* the main attraction for honeybees appears to be pollen, although in *Aloe greatheadii* Schönland var. *davyana* (Schönland) Glen & D.S.Hardy (Section *Pictae*) the attraction for honeybees is both pollen and more concentrated nectar than that of *A. marlothii* (Nepi et al., 2006; Human and Nicolson, 2006, 2008). In southern Africa honeybees visit, amongst others, *Aloe candelabrum* A.Berger (treated by some workers as synonymous with *A. ferox*), *A. ferox* and *A. castanea* for pollen (Hoffman, 1988; Stokes and Yeaton, 1995; Nicolson and Nepi, 2005) and in *A. divaricata* stingless bees and ants were recorded as visitors for pollen and nectar respectively (Ratsirarson, 1995).

Filtering and generalist bird visitors

The nectar of *Aloe* spp. contains low proportions of sucrose, seldom exceeding 4% of total sugar (n = 47, Van Wyk et al., 1993). Together with the production of copious and dilute nectar, *A. marlothii* is suited towards attracting a broad range of occasional avian nectarivores. Also, it may discourage insects (predominantly honeybees) that are less effective pollinators. This is of added benefit to plants because

birds are able to carry more pollen greater distances between plants which maximises cross pollination. In *A. greatheadii* var. *davyana* the main pollinators are honeybees, despite the floral features being suggestive of a bird pollination syndrome (H. Human unpubl. data); higher nectar concentrations (20 ± 7%) than in other aloes may be more important for attracting bees (Human and Nicolson 2008).

In *Aloe vryheidensis* Groenew. (Section *Anguialoe*), bitter nectar acts as a selective filter against sunbirds with long bills that do not effectively transfer pollen during visits (Johnson et al., 2006). Although *A. marlothii* nectar was not tested for phenolics, to the human taste it is sweet. It therefore seems unlikely that the deterrent is taste. Only two species of sunbirds (true nectarivores), of a possible four species within the range of the study site, were observed feeding on *A. marlothii* nectar, and in relatively low abundance (Chapter 2). It is possible that these specialist nectarivores are not attracted to nectar of such low sugar concentration. The dichotomy in nectar properties of plants pollinated by hummingbirds and passerines has been widely studied, and sugar type has often been advocated as the factor defining plant visitor type (e.g. Baker et al., 1998). However, a clearer explanation for nectar differences within the bird pollination syndrome lies not only in the type of sugar but also in the concentration and volume; flowers with low volume (*c.* 10 - 30 µl) and high sugar concentration (*c.* 15 - 25% w/w) nectars are adapted for specialist nectarivores (i.e. sunbirds and hummingbirds) whilst flowers with large nectar volumes (*c.* 40 - 100 µl) and low sugar concentrations (*c.* 8 - 12%) are adapted for generalized avian pollinators (Johnson and Nicolson 2008). Our evidence therefore supports the hypothesis that nectar characteristics − copious production of dilute nectar during dry winter days − support a bird pollination syndrome. This is similar to the nectar properties and type of associated pollinators recorded in many other species of *Aloe*, as well as in species of *Erythrina* L. (Fabaceae), namely high volumes, low concentrations and low sucrose content, adapted towards attracting a guild of generalist pollinators (Steiner, 1979; Hoffman, 1988; Cotton, 2001; Ragusa-Netto, 2002; Johnson and Nicolson 2008).

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II

CHAPTER 2

Response of avian nectarivores to the flowering of *Aloe marlothii*: a nectar oasis during dry South African winters

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Abstract

In southern Africa, *Aloe marlothii* flowers during the dry winter season and offers copious dilute nectar to a variety of birds. Avian abundance and community composition were monitored at an *A. marlothii* forest at Suikerbosrand Nature Reserve, South Africa. Sampling occurred during two summer months (February - March) when no flowers were present, and six months (May - October) that spanned the winter flowering. We hypothesized that an influx of occasional nectarivores to the *A. marlothii* forest during flowering would lead to significant changes in the avian community. Overall bird abundance increased 2-3 fold at the peak of nectar availability (August). We recorded 38 bird species, of 83 species detected during transects, feeding on *A. marlothii* nectar; this diverse assemblage of birds belonged to 19 families, including Lybiidae, Coliidae, Pycnonotidae, Sylviidae, Cisticolidae, Muscicapidae, Sturnidae, Ploceidae and Fringillidae. Surprisingly, only two species of sunbird (Nectariniidae) were observed feeding on *A. marlothii* nectar, and both occurred in low abundance. We predicted that competition for nectar resources would be high, but few aggressive inter- and intra-specific interactions occurred between birds while feeding on inflorescences. During peak flowering insect feeders (insectivores, omnivores, nectarivores) fed on nectar during the cold morning when insect activity was low, whilst non-insect feeders (frugivores and granivores) fed on nectar in the middle of the day. Our study highlights the importance of *A. marlothii* nectar as a seasonal food and water source for a diverse assemblage of occasional nectarivores.

Keywords nectarivore, sunbird, niche partitioning, seasonal flowering

Introduction

The structure and composition of avian communities change in space and time with the availability of food resources. Variation tends to be most pronounced among consumers that feed on patchy and ephemeral food resources such as nectar and fruit (Fleming 1992). The responses of nectarivores to variation in food availability are well documented, with increases in abundance and diversity being correlated with increased nectar availability (e.g. Brown and Hopkins 1996; Franklin and Noske 1999; Cotton 2006). In South Africa, sunbirds (Nectariniidae) respond to patchy nectar resources, e.g. *Protea* and *Leonotis* spp. (Skead 1967; Tree 1990; Craig and Simon 1991; Craig and Hulley 1994; Symes et al. 2001), and Gurney's sugarbirds *Promerops gurneyi* are recorded using seasonally available nectar sources (De Swardt 1991; De Swardt and Louw 1994). Cape sugarbirds *P. cafer* are also known to appear in greater numbers at flowering *Protea* spp. and movements up to 160 km within their restricted range have been recorded (Fraser et al. 1989; Fraser and McMahon 1992).

While the examples above concern specialist nectarivores, occasional nectarivory is recorded worldwide in numerous bird families, with a predominance on the southern continents (Maclean 1990). In the Neotropics, honeycreepers (Coerebidae), the New World blackbirds (Icteridae), tanagers (Thraupidae) and finches (Fringillidae) are common nectar feeders (Stiles 1981; Gryj et al. 1990). In Australasia a host of insectivores feed on nectar, with the number of species in monsoonal Australia given as 29 (from 15 families) (Paton 1986; Franklin 1999). Numerous birds have been recorded foraging on the nectar of *Erythrina* species, including generalist passerines (Steiner 1979; Raju and Rao 2004), parrots (Psittacidae) (Cotton 2001) and hummingbirds (Trochilidae) (Mendoça and dos Anjos 2006). In South Africa, at least 73 bird species in 24 families were recorded feeding on 14 *Aloe* species and eight other flowering plants and trees, although this list is far from exhaustive (Oatley and Skead 1972). Occasional nectar-feeding is therefore more common than previously believed, and the 1600 birds quoted by Peterson et al. (1968) may be no exaggeration.

In many southern African ecosystems, aloes (*Aloe* spp.) represent an important source of nectar for birds during dry winter months (Oatley 1964; Skead 1967; Oatley

and Skead 1972). *Aloe marlothii* occurs in the northern and north-eastern summer rainfall regions of South Africa where winters are dry with warm days (>20^{o}C) and cold nights (*c.*0^{o}C). This species reaches a maximum height of *c.* 8 m and produces a single inflorescence with up to 25 racemes in a flowering season, although not all plants flower each year (Chapter 1). This study was conducted at Suikerbosrand Nature Reserve during eight months that included the flowering period of *A. marlothii*. We investigated seasonal variation of the avian community, hypothesizing that bird abundance and diversity would increase with the availability of *A. marlothii* nectar. We predicted that the seasonal response would be most pronounced in true nectarivores, such as sunbirds (Nectariniidae). Occasional nectarivore species were also expected to increase in abundance during the flowering season, in proportion to their degree of nectarivory. We also expected competition for nectar during flowering, and investigated feeding behaviour of birds throughout the day in order to identify temporal niche partitioning.

Materials and methods

Our study site was an *A. marlothii* "forest" in the western part of Suikerbosrand Nature Reserve, 60 km south-east of Johannesburg, where large numbers of *A. marlothii* plants occur mainly on rocky north-facing slopes. Data were collected a during a six-month period (May - October 2006) that spanned the entire 2006 winter-flowering event, and during the summer non-flowering season (February - March 2006). Additional feeding and behavioural observations were also recorded during the 2005 flowering season (July-September).

Diversity and abundance censusing

The avian community was censused in the western portion (26^{o} 31' 50" S 28^{o} 10' 07" E, *c.* 1 600 - 1 700 m a.s.l.) of the *A. marlothii* "forest". Each census session involved a combination of point counts and transect sampling along a disused vehicle track. Following an initial 10-min point count, an observer (CTS) walked along the 300 m transect route for 20 min. Thereafter, a second 10-min point count was

conducted at the end of the transect route. After a delay of 10 min, this procedure was then repeated in the reverse direction, so that each session comprised four 10-min point counts and two 20-min transects (i.e., a total of 80 min). Sampling took place in the morning (06:00-10:00), at midday (10:00-14:00), or in the afternoon (14:00-18:00), with only one session being conducted during each period in a day. Two sessions were conducted for each period in a month, and bird census sampling was completed over 3-5 days. Total census time each month was thus 8 h.

During each census period, all birds detected visually or by sound in the aloe "forest" were recorded. Resightings during each point count and transect were not included. We also recorded all instances of feeding behaviour on aloes during the flowering season (i.e. probing flowers; feeding on buds, open flowers or seed pods). Birds feeding on nectar out of transect periods were also recorded (including pilot transects walked in 2005). Each species was classified into a broad feeding guild i.e. frugivore, granivore, insectivore, nectarivore, omnivore, and also as a seasonal migrant or not, following Maclean (1993) and Hockey et al. (2005).

Some bird species occur in greater numbers than others and we calculated an abundance index for each species that accounted for inter-species abundance variations. This was the number of individuals of a species recorded each month as a proportion of the total number of individuals recorded for that species.

Pollen loads and visitation rates

To confirm the presence of *A. marlothii* pollen on particular species, we trapped birds using mist nets at two sites, 250 m apart, along a portion of the disused vehicle track in the aloe forest. Six nets (12 x 2.4 m, 4 shelf, 16 mm mesh 210d/1 ply nets) were erected (0.6-3.0 m) on aluminium poles (3 m) along a single stretch during each trapping session. Bird capture took place throughout the day (59.6 h during nine days). Each bird was ringed and inspected visually for pollen deposited on the bill and feathers of the facial region. During flowering months, pollen swabs were collected from representatives of all species on which pollen was not immediately visible, using a piece of transparent adhesive tape that was dabbed on the bill, frons and throat region

and then placed on a slide. The slide was later scanned for pollen under a light microscope at 10x magnification.

To determine visitation rates of birds to flowering *A. marlothii*, we selected 3-8 aloes of varying height within 15 m of a randomly chosen observation point. For each visitor the time and species was recorded, whether or not it fed, and any interactions with other birds. Observations lasted for 10-45 min at each station, with sampling periods throughout the day during peak flowering in late August. For each species, we calculated the proportion of individuals that were observed feeding at different times of the day.

Results

Seasonal variation in community composition

Table 1 Number of bird species and individuals detected during transects (total and excluding migrants, $n = 9$) during February-March and May-October 2006. Peak flowering month highlighted. Totals also include unidentified species, e.g. francolin, cisticola, weaver, waxbill or canary species (See Appendix).

Month	Number of species		Number of individuals	
	Migrants included	**Migrants excluded**	**Migrants included**	**Migrants excluded**
February	52	46	954	771
March	48	44	980	802
May	48	48	892	892
June	42	42	694	694
July	45	44	1 074	1 065
August	53	52	1 746	1 746
September	47	45	745	727
October	50	46	632	579
Cumulative total:	**89**	**83**		

We observed 83 species during censuses (Appendix), with both diversity and abundance peaking in August when nectar availability was greatest (Chapter 1; Table 1, Fig. 1). Eight seasonal migrant species were present during summer and only one migrant species was present during the flowering period in August. During May - October, abundance was higher during the morning and afternoon sampling periods than at midday, whereas this pattern was not evident during February or March (Fig. 1). During flowering (August-September) the number of nectar-feeding observations for each species was correlated with the overall abundance of each species (Spearman's R = 0.836, $P < 0.05$).

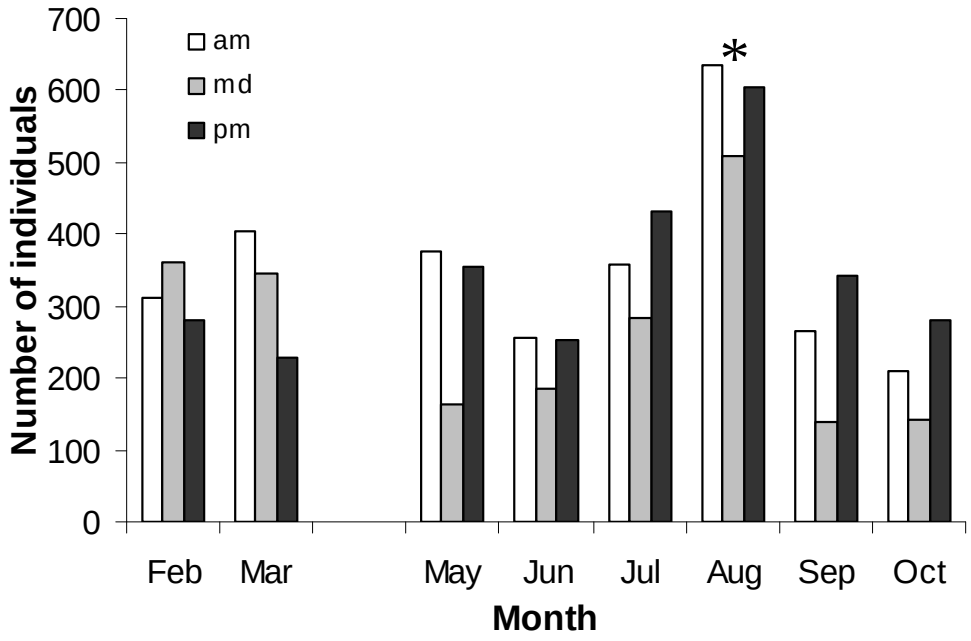

Fig. 1 Monthly bird abundance in an *Aloe marlothii* forest at Suikerbosrand Nature Reserve. Sampling took place in the morning (am; 06:00 - 10:00), midday (md; 10:00 - 14:00) or afternoon (pm; 14:00 - 18:00). An *asterisk* indicates *A. marlothii* peak flowering.

When we accounted for inter-species differences in abundance, there was no significant difference in abundance between months for all birds detected in the aloe forest during transects (Kruskal-Wallis, $P = 0.07$) (Fig. 2). However, when nectar feeders were treated separately there was a significant difference in abundance between months (Kruskal-Wallis, $P = 0.002$; Fig. 2).

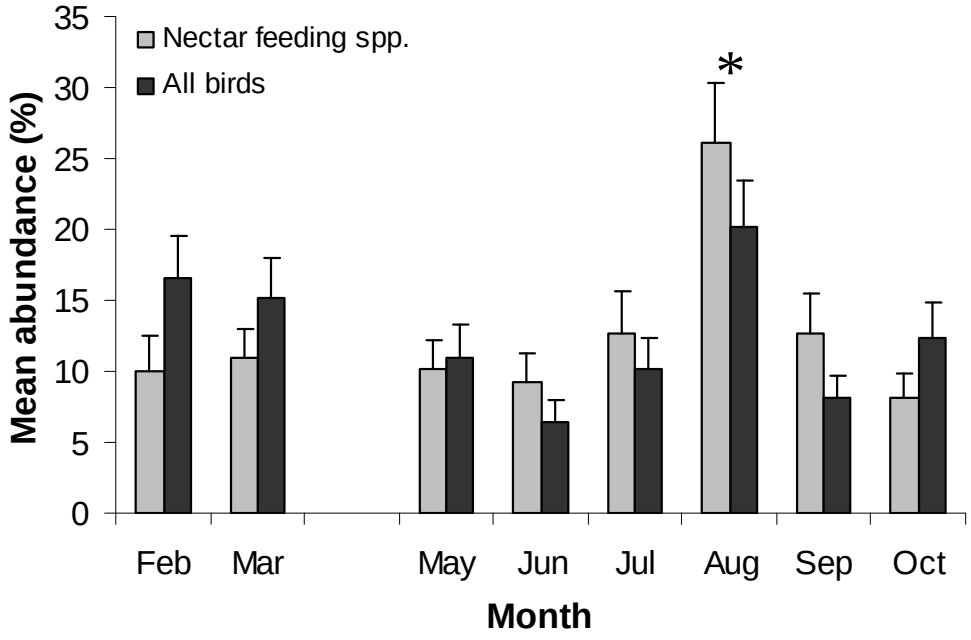

Fig. 2 Mean abundance (% ± SE) of birds recorded each month in the aloe forest at Suikerbosrand Nature Reserve. Monthly abundance for each species calculated as a percentage of total abundance for that species. All birds = all species detected during transects; Nectar feeders = only species recorded as *Aloe marlothii* nectar feeders. An *asterisk* indicates *A. marlothii* peak flowering.

Floral visitors

During transects 21 species were observed feeding on nectar. Another three species were recorded feeding on nectar out of transect periods and an additional seven species were seen feeding on nectar in 2005. Of 30 species caught, individuals of 26 species were found to have pollen on the facial area (15 with visible pollen, and 11 species with pollen detected from swabs). Seven species were classed as nectarivores from pollen swabs alone (Appendix); no pollen from other plant species was visible on the slides. A total of 38 species representing 19 families were thus recorded feeding on *A. marlothii* nectar during the study (Appendix). An additional four species known to feed on *A. marlothii* nectar, but not recorded doing so during the study, are also included in the Appendix (Oatley 1964; Oatley and Skead 1972). This gives a total of 42 occasional nectar feeders at the Suikerbosrand aloe “forest”, representing 59.2% of the community present at the site in winter (migrants and transients excluded).

The proportion of individuals of various feeding guilds (frugivores, granivores, insectivores, nectarivores and omnivores) feeding on *A. marlothii* nectar varied significantly among sampling periods (Kruskal-Wallis, $P < 0.05$; Fig. 3). Overall visitation rates to aloes were highest in the morning for insectivores, nectarivores and omnivores (all insect eaters) but higher at midday for frugivores and granivores (Fig. 3). For all species combined there was a general decrease in feeding through the day.

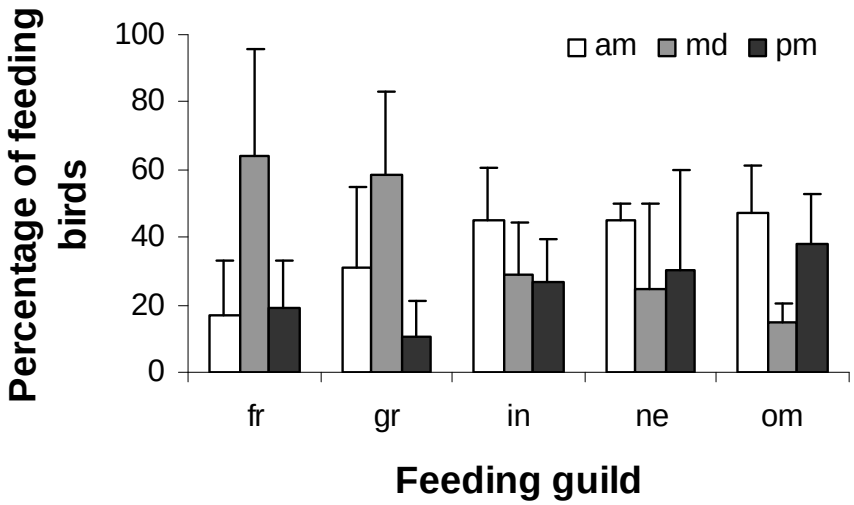

Fig. 3 Percentage of individuals belonging to various feeding guilds that were observed feeding on *Aloe marlothii* nectar at different time periods in a day at Suikerbosrand Nature Reserve (feeding guilds: fr = frugivore, gr = granivore, in = insectivore, ne = nectarivore, om = omnivore). Values given as mean ± SE. Refer to Fig. 1 for times.

The following seven species accounted for 86.4% of individuals seen feeding on nectar: African red-eyed bulbul *Pycnonotus nigricans* (36.6%), Cape weaver *Ploceus capensis* (18.6%), Cape white-eye *Zosterops capensis* (9.0%), red-faced mousebird *Urocolius indicus* (7.9%), southern masked weaver *Ploceus velatus* (5.7%), fiscal flycatcher *Sigelus silens* (4.3%) and streaky-headed seedeater *Serinus gularis* (4.3%). Three species (African red-eyed bulbul, chestnut-vented tit-babbler *Parisoma subcaeruleum* and streaky-headed seedeater) were observed feeding on *A. marlothii* fruit, and ten species were observed drinking water collected in the cups of *A. marlothii* leaves (laughing dove *Streptopelia senegalensis*, fiscal flycatcher, black-

throated canary *Serinus atrogularis*, black-collared barbet *Lybius torquatus*, common scimitarbill *Rhinopomastus cyanomelas*, ashy tit *Parus cinerascens*, African red-eyed bulbul, streaky-headed seedeater, Cape bunting *Emberiza capensis*, black-faced waxbill *Estrilda erythronotos*). A further two species were observed probing unopened flowers (unidentified canary species and black-chested prinia *Prinia flavicans*).

We did not observe any aggressive inter- or intra-specific interactions during sampling periods, but did observe male malachite sunbirds *Nectarinia famosa* chasing a female sunbird, an ashy tit, a Cape white-eye and a laughing dove at the study site during 2005 and 2006. Several bird species were often observed feeding on the same inflorescence, with larger species tending to displace smaller species.

Discussion

Nectar-feeding at a mass flowering event

The occurrence of numerous bird species, with half observed feeding on nectar at the mass flowering of *A. marlothii*, indicates that nectar is an important source of food and/or water for many occasional nectarivores during dry winters. The abundance of nectar-feeding bird species increased significantly when aloes flowered, with some species being present only during flowering months; e.g. wattled starling *Creatophora cinerea*, red-winged starling *Onychognathus morio* and malachite sunbird, which are known to migrate locally while tracking food resources (Skead 1967; Craig 1996; Fraser 1997; Symes et al. 2001). Also, the presence of fairy flycatcher *Stenostira scita*, red-billed oxpecker *Buphagus erythrorhynchus*, cut-throat finch *Amadina fasciata* and golden-breasted bunting *Emberiza flaviventris* only when aloes were in flower suggests that their arrival in the area was as occasional nectarivores.

Surprisingly, true nectarivores (sunbirds) did not respond significantly to the seasonal availability of *A. marlothii* nectar, possibly because the nectar concentration is too low to be attractive to specialist nectar feeders (Johnson and Nicolson 2008). Moreover, populations of sunbirds may be equally distributed throughout the range of

flowering *A. marlothii*, resulting in more dispersed populations. Despite this, the occurrence of sunbirds in high densities at other patchy and transitory flowering events in South Africa is well recorded (see Harrison et al. 1997).

Fig. 4 Avian nectar feeders in *Aloe marlothii* forest, Suikerbosrand Nature Reserve. **(a)** Malachite sunbird *Nectarinia famosa* (immature male) and African red-eyed bulbul *Pycnonotus nigricans*, **(b)** *A. marlothii* inflorescence, **(c)** Cape white-eye *Zosterops capensis*, **(d)** Malachite sunbird (immature male) probing flowers on raceme, **(e)** Wattled starling *Creatophora cinerea*. Note liberally dusted orange pollen on facial area of bulbul (a) and starling (e) (not in original publication).

Other aloe species are likely to drive seasonal changes in avian communities like those we observed in *A. marlothii*. *Aloe ferox*, for instance, occupies a similar ecological niche and also offers large amounts of nectar to visitors during dry winter months when few other plants are flowering (Reynolds 1969; Van Wyk and Smith

2003). The vertical racemes of *A. ferox* and the horizontal to slanting racemes of *A. marlothii* both offer copious volumes of dilute nectar (180 µl, 12.5% w/w and 250 µl, 12 % w/w) (Hoffman 1988; Chapter 1). Oatley and Skead (1972) recorded 28 and 26 bird species visiting *A. marlothii* and *A. ferox* respectively. In both plants the inflorescences are robust enough to support birds as large as grey go-away-birds *Corythaixoides concolor* and columbids (>250 g) (Oatley and Skead 1972; Chapter 1). At least 360 *Aloe* species occur throughout the Afrotropical region (Jeppe 1969; Reynolds 1969; Glen and Hardy 2000). Flowering occurs at different times of the year, although in southern Africa there is a dominance of winter-flowering aloes (Jeppe 1969; Reynolds 1969). Aloe nectar provided during seasonal mass flowering events is an important food and water source for a wide range of bird species (Oatley 1964; Skead 1967; Oatley and Skead 1972; Botes 2007).

A distinct bird community during flowering

In addition to an increase in avian diversity during the flowering period (up to 20%), there was a 2-3 fold increase in overall bird abundance. This occurred despite the absence of seasonal migrants, which were only present at the aloe forest in summer months when there was no nectar. Our data thus support the hypothesis that the availability of nectar in the *A. marlothii* forest is associated with significant changes in avian community diversity and abundance.

Species like African red-eyed bulbul, Cape weaver, Cape white-eye, red-faced mousebird, southern masked weaver, fiscal flycatcher and streaky-headed seedeater that accounted for most feeding observations were the species that increased in abundance during flowering. Chestnut-vented titbabbler and black-chested prinia also increased in abundance during flowering, but because they were less common, were seen feeding less often. The importance of nectar for these species is supported by stable carbon isotope analysis which indicates that nectar contributes up to 15% of their dietary carbon intake during the aloe flowering period (Chapter 4).

The seasonal variation in avian diversity and abundance associated with nectar availability is similar to that observed in other systems. Woinarski and Tidemann

(1991), for instance, identified distinct bird communities between the dry season, transitional months and wet season months in a Northern Australian woodland bird community. They also found that nectarivore density was strongly correlated with flower availability (Woinarski and Tidemann 1991). In monsoonal Australia, opportunistic nectarivory amongst birds occurs predictably at mass flowering events and during the cool dry season (Franklin 1999). In Perth (western Australia) a guild of honeyeaters (five species) responded to peaks in nectar production of a single plant species, *Banksia menziesii* (Proteaceae), with significantly more birds being observed during winter flowering (Ramsey 1989), and in a lowland Amazonian rainforest seasonal fluctuations in resource abundance were closely correlated with variation in hummingbird species richness (~17 species) and abundance (Cotton 2006).

Niche partitioning and defending nectar resources

Insectivores are more likely than other guilds to practice occasional nectarivory (Paton 1986). A greater proportion of insect eating birds (i.e. insectivores, omnivores, nectarivores) fed on nectar of *A. marlothii* in the morning. Frugivores and granivores (non-insect eaters) fed later, and although for each guild the differences are not statistically significant, the trend indicates some degree of temporal niche partitioning between insect and non-insect eaters in a day. Timewell and MacNally (2004) showed similar patterns for a guild of honeyeaters (10 species) feeding on winter-flowering *Eucalyptus tricarpa*; the birds progressed from an almost exclusive nectar diet in the morning to increased insectivory later in the day. During cold mornings nectar is abundant but birds relying on insects are likely to experience difficulty finding food. Peak flowering of *A. marlothii* occurs at a time of low insect numbers, which possibly accentuates this separation (Chapter 5). Different temporal feeding patterns between insect and non-insect eating guilds in a day, together with an abundant supply of nectar, lead to reduced competition for nectar and possible inter and intra-species aggressiveness.

There were more flowers available in the aloe forest than could be probed by birds; a flowering aloe produced, on average, at least 860 ml of nectar (not accounting for destroyed flowers or replenished nectar in emptied flowers) (Chapter 1). This mass

flowering event is similar to the Jarrah forest site in Western Australia where the production of nectar of six plant species (e.g. Jarrah *Eucalyptus marginata*) between October and December could have supported more honeyeaters than were actually present (Collins and Newland 1986). In the aloe forest few inter- and intra-specific aggressive interactions were observed; in contrast to those at other nectar sources frequented by sunbirds, honeyeaters and hummingbirds (e.g. Gill and Wolf 1975; Frost and Frost 1980; Ford 1981; Armstrong 1991; Cotton 1998). Temporal resource partitioning in nectarivores feeding at an *Agave marmorata* nectar oasis has been interpreted as a way to reduce risk of injury (Ornelas et al 2002). At our study site temporal feeding patterns in a day were probably caused by responses to daily insect activity, rather than competition for nectar resources.

Concluding remarks

Community changes at our study site contrast with other studies in that the birds that arrived in the area in response to increased nectar availability were almost all occasional or generalist nectarivores rather than specialist nectarivores such as sunbirds. This observation may be related to nectar preferences: whereas specialist avian nectarivores tend to prefer nectars of relatively low volume (*c.* 10 - 30 µl) and high concentration (*c.* 15 - 25% w/w), generalist nectarivores prefer nectar of large volumes (*c.* 40 - 100 µl) and low concentrations (*c.* 8 - 12%) (Johnson and Nicolson 2008). A second, and not mutually exclusive, possibility is that these patterns reflect variation in avian nectarivore species richness at a global scale. Whereas the Neotropical (324 hummingbird species) and Australasian (159 honeyeaters) regions have large, speciose nectarivore radiations, the Afrotropical (78 sunbirds and 2 sugarbirds) and Indomalayan (39 sunbirds and 10 honeyeaters) regions do not (Maclean 1990).

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Appendix. Bird species (*n* = 85) recorded during transects (February - March, May - October) in the *Aloe marlothii* forest, western Suikerbosrand Nature Reserve. **Nectar:** feeding on nectar during transects (t), pollen recorded on captured birds from swabs of facial area (s), pollen observed on facial area of birds in aloe forest (p), birds observed feeding on nectar out of transect times (o), and species known to feed on aloe nectar, 1 = Oatley (1964), 2 = Oatley and Skead (1972). Birds with pollen visible on the face were not swabbed. **Status:** all birds were observed perched in the aloe forest unless indicated as transients flying over (tr), or seasonal migrants (mig). Abundance (total number) of birds indicated, 0 indicates species was heard only. August highlighted as peak flowering time. Taxonomy follows Hockey et al. (2005).

Species	Nectar	Status	Feb	Mar	May	Jun	Jul	Aug	Sep	Oct	Total
PHASIANIDAE											
Swainson's Spurfowl *Pternistis swainsonii*	-	-	4	3	17	5	23	0	1	1	54
NUMIDIDAE											
Helmeted Guineafowl *Numida meleagris*	-	-	0	1	0	-	-	-	-	1	2
ANATIDAE											
Egyptian Goose *Alopochen aegyptiaca*	-	tr	-	-	-	-	2	-	-	-	2
Spur-winged Goose *Plectropterus gambensis*	-	tr	-	-	-	-	-	-	-	1	1
INDICATORIDAE											
Greater Honeyguide *Indicator indicator*	-	-	-	-	-	-	-	-	-	0	0
Lesser Honeyguide *Indicator minor*	s,2	-	-	1	-	-	-	1	-	-	2
PICIDAE											
Red-throated Wryneck *Jynx ruficollis*	-	-	1	2	0	0	0	1	0	2	6
Cardinal Woodpecker *Dendropicos fuscescens*	s	-	0	0	2	0	4	0	0	4	10
LYBIIDAE											
Acacia Pied Barbet *Tricholaema leucomelas*	t,p,1,2	-	11	12	7	6	4	9	2	12	63
Black-collared Barbet *Lybius torquatus*	p,1,2	-	0	3	0	1	7	0	1	0	12
Crested Barbet *Trachyphonus vaillantii*	t,2	-	2	16	4	4	0	6	5	1	38
UPUPIDAE											
African Hoopoe *Upupa africana*	-	mig	-	1	-	-	-	-	-	-	1
PHOENICULIDAE											
Green Wood-Hoopoe *Phoeniculus purpureus*	-	-	-	-	-	-	-	-	-	1	1
RHINOPOMASTIDAE											

Species	Nectar	Status	Feb	Mar	May	Jun	Jul	Aug	Sep	Oct	Total
Common Scimitarbill *Rhinopomastus cyanomelas*	t,1,2	-	4	3	2	6	2	6	9	2	34
COLIIDAE											
Speckled Mousebird *Colius striatus*	t,p,1,2	-	0	-	-	1	0	6	-	-	7
Red-faced Mousebird *Urocolius indicus*	t,p,1,2	-	0	31	96	0	5	166	43	13	354
CUCULIDAE											
Diderick Cuckoo *Chrysococcyx caprius*	-	mig	1	-	-	-	-	-	-	-	1
APODIDAE											
Little Swift *Apus affinis*	-	tr	2	-	-	-	-	-	10	6	18
White-rumped Swift *Apus caffer*	-	mig	29	-	-	-	9	-	3	2	43
COLUMBIDAE											
Rock Dove *Columba livia*	-	tr	-	-	-	-	7	1	-	-	8
Speckled Pigeon *Columba guinea*	-	tr	59	1	2	2	5	-	-	1	70
Laughing Dove *Streptopelia senegalensis*	s,1,2	-	327	323	380	424	736	561	310	191	3252
Cape Turtle-Dove *Streptopelia capicola*	1	-	-	-	0	4	0	1	0	-	5
Red-eyed Dove *Streptopelia semitorquata*	-	-	-	-	1	-	-	-	-	-	1
CHARADRIIDAE											
Crowned Lapwing *Vanellus coronatus*	-	tr	-	-	-	-	-	-	0	-	0
LARIDAE											
Grey-headed Gull *Larus cirrocephalus*	-	tr	-	-	2	-	-	-	-	-	2
ACCIPITRIDAE											
Black-shouldered Kite *Elanus caeruleus*	-	-	3	2	-	-	-	1	-	2	8
Pallid Harrier *Circus macrourus*	-	tr	-	4	-	-	-	-	-	-	4
Steppe Buzzard *Buteo vulpinus*	-	mig	2	-	-	-	-	-	-	1	3
Jackal Buzzard *Buteo rufofuscus*	-	-	4	-	1	1	1	-	-	-	7
FALCONIDAE											
Amur Falcon *Falco amurensis*	-	mig	3	83	-	-	-	-	-	-	86
THRESKIORNITHIDAE											
Hadeda Ibis *Bostrychia hagedash*	-	-	-	-	-	-	-	0	0	1	1
MALACONOTIDAE											
Brubru *Nilaus afer*	-	-	-	0	0	0	-	0	0	1	1
Brown-crowned Tchagra *Tchagra australis*	s	-	10	8	3	1	6	1	12	3	44

Species	Nectar	Status	Feb	Mar	May	Jun	Jul	Aug	Sep	Oct	Total
Bokmakierie *Telophorus zeylonus*	-	-	0	0	4	1	2	1	5	4	17
Chinspot Batis *Batis molitor*	-	-	-	-	1	0	0	0	0	0	1
CORVIDAE											
Pied Crow *Corvus albus*	2	-	-	-	-	-	-	5	0	1	6
LANIIDAE											
Common Fiscal *Lanius collaris*	o	-	-	1	0	2	1	3	4	5	16
PARIDAE											
Ashy Tit *Parus cinerascens*	-	-	5	1	4	3	6	1	3	4	27
HIRUDINIDAE											
Barn Swallow *Hirundo rustica*	-	mig	143	92	-	-	-	-	-	21	256
Greater Striped Swallow *Hirundo cucullata*	-	mig	5	-	-	-	-	-	15	29	49
PYCNONOTIDAE											
African Red-eyed Bulbul *Pycnonotus nigricans*	t,p,2	-	51	73	87	50	59	403	65	96	884
SYLVIIDAE											
Fairy Flycatcher *Stenostira scita*	-	mig	-	-	-	-	-	2	-	-	2
Cape Grassbird *Sphenoeacus afer*	s	-	-	-	-	-	-	-	-	-	-
Long-billed Crombec *Sylvietta rufescens*	s,1	-	0	-	-	-	-	-	1	0	1
Willow Warbler *Phylloscopus trochilus*	-	mig	-	2	-	-	-	-	-	-	2
Chestnut-vented Tit-Babbler *Parisoma subcaeruleum*	t,p,2	-	3	6	1	6	3	14	7	8	48
ZOSTEROPIDAE											
Cape White-eye *Zosterops capensis*	t,p,1,2	-	10	2	18	6	21	63	14	25	159
CISTICOLIDAE											
Rattling Cisticola *Cisticola chiniana*	o,1	-	7	9	2	1	-	-	4	1	24
Neddicky *Cisticola fulvicapilla*	s,1	-	-	-	5	6	3	4	4	8	30
Black-chested Prinia *Prinia flavicans*	t,p	-	23	22	17	6	9	19	17	8	121
Bar-throated Apalis *Apalis thoracica*	t,p	-	0	2	5	1	2	6	2	0	18
MUSCICAPIDAE											
Cape Rock-Thrush *Monticola rupestris*	t	-	4	-	-	-	1	2	4	0	11
Karoo Thrush *Turdus smithi*	-	-	1	-	-	-	-	-	-	0	0
Marico Flycatcher *Bradornis mariquensis*	-	-	-	1	-	-	-	-	-	-	1
Fiscal Flycatcher *Sigelus silens*	t,p,1,2	-	27	74	59	29	32	93	68	71	453

Species	Nectar	Status	Feb	Mar	May	Jun	Jul	Aug	Sep	Oct	Total
Cape Robin-Chat *Cossypha caffra*	tp	-	4	16	5	7	6	6	5	18	67
Kalahari Scrub-Robin *Cercotrichas paena*	-	-	-	-	1	-	1	-	-	-	2
Familiar Chat *Cercomela familiaris*	-	-	-	1	2	-	-	-	1	-	4
STURNIDAE											
Red-winged Starling *Onychognathus morio*	1,2	-	-	-	-	-	-	2	-	-	2
Cape Glossy Starling *Lamprotornis nitens*	o,2	-	-	-	-	-	-	-	-	-	-
Wattled Starling *Creatophora cinerea*	t,p	-	-	-	-	-	-	3	-	-	3
Red-billed Oxpecker *Buphagus erythrorhynchus*	-	-	-	-	-	-	-	1	-	-	1
NECTARINIIDAE											
Malachite Sunbird *Nectarinia famosa*	t	-	-	-	-	-	-	18	5	-	23
White-bellied Sunbird *Cinnyris talatala*	t	-	-	-	-	-	3	8	-	0	11
PLOCEIDAE *											
Cape Weaver *Ploceus capensis*	t,p,2	-	-	-	-	-	1	76	38	0	115
Southern Masked-Weaver *Ploceus velatus*	t,p,1	-	12	1	33	25	18	55	6	6	156
Southern Red Bishop *Euplectes orix*	-	-	2	-	-	-	-	-	-	-	2
Red-collared Widowbird *Euplectes ardens*	t	-	22	2	1	-	-	3	-	-	28
ESTRILDIDAE											
Cut-throat Finch *Amadina fasciata*	-	-	-	-	-	-	-	2	-	-	2
Black-faced Waxbill *Estrilda erythronotos*	t,p	-	5	2	2	21	14	16	-	-	60
Common Waxbill *Estrilda astrild*	2	-	4	-	-	3	-	-	-	-	7
Violet-eared Waxbill *Granatina granatina*	s	-	-	1	1	8	-	2	1	-	13
Green-winged Pytilia *Pytilia melba*	s	-	8	3	1	10	3	1	0	3	29
Jameson's Firefinch *Lagonosticta rhodopareia*	s	-	-	-	4	0	1	1	-	-	6
VIDUIDAE *											
Dusky Indigobird *Vidua funerea*	-	-	3	-	-	-	-	-	-	-	3
Pin-tailed Whydah *Vidua macroura*	-	-	11	17	2	-	-	-	-	-	30
Long-tailed Paradise-Whydah *Vidua paradisaea*	-	-	8	12	2	-	-	-	-	-	22
PASSERIDAE											
Southern Grey-headed Sparrow *Passer diffusus*	s,2	-	4	5	10	2	12	4	2	7	46
FRINGILLIDAE											
Black-throated Canary *Serinus atrogularis*	t	-	22	15	4	3	19	4	2	6	75

Species	Nectar	Status	Feb	Mar	May	Jun	Jul	Aug	Sep	Oct	Total
Yellow Canary *Serinus flaviventris*	-	-	5	9	4	2	-	4	4	-	28
Streaky-headed Seedeater *Serinus gularis*	t,1,2	-	-	3	5	1	1	10	13	17	50
Cinnamon-breasted Bunting *Emberiza tahapisi*	-	-	-	-	-	-	0	-	-	-	0
Cape Bunting *Emberiza capensis*	t	-	15	16	11	13	12	10	17	4	98
Golden-breasted Bunting *Emberiza flaviventris*	-	-	-	-	-	-	-	1	-	-	1
Total species 85	42	17	48	45	45	39	42	51	44	48	83

Fairy flycatcher *Stenostira scita* is recorded as a migrant, but included in calculations since it arrives in the region (aloe forest) during the winter months.

* Decreases in abundance of certain Ploceidae and Viduidae are likely due to certain species being less conspicuous in non-breeding eclipse plumages during the non-breeding season.

III

CHAPTER 3

Generalist bird pollination of a winter-flowering African succulent, *Aloe marlothii*

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Abstract

Aloe marlothii is a CAM succulent that flowers during dry winter months in the summer rainfall regions of northern and north-eastern South Africa. Plants grow up to 8 m in height and produce striking inflorescences with bright yellow-orange flowers. Pollinator exclusion experiments were conducted during two successive years (2006 and 2007) on racemes of *A. marlothii* at Suikerbosrand Nature Reserve, to identify the importance of bird and insect pollinators. Fruit set was greatest in control treatments (14-20%) and lowest (0-2%) in treatments that excluded all pollinators. Generalist bird pollinators contributed most significantly (12-16%) to fruit set whilst insects, which were largely absent during the flowering period, contributed very little (3-4%) to fruit set. Fruit set was similar for tall (>2.5 m) and short plants (<2.5 m) despite taller plants being visited more often by birds. Pollinator type was an important determinant of seed production; flowers pollinated by birds produced more seeds than those with insect visitors or no visitors. Overall seed production was greater in taller plants that produced more racemes more often, indicating that plant height confers advantages for reproductive fitness. Pollen transfer occurred via the beak and head region of birds, as well as other body parts, e.g. feet and belly. Small mammals and chacma baboons also possibly contribute to pollination, but at Suikerbosrand baboons were responsible for significant levels of inflorescence damage. These experiments confirm that observed floral characters of *A. marlothii* support a generalist avian pollination syndrome.

Keywords: ornithophily, nectarivore, sunbird, *Nectarinia*, *Cinnyris*, exclusion experiment

Introduction

Pollination syndromes are typically defined by flower characteristics, with those attributed to ornithophily as bright orange to red flowers, a long floral tube, an absence of odour and nectar guides, exserted anthers and stigma, large pollen-nectar distance, and nectars of low concentration and high volume (Faegri and Van der Pijl 1979; Proctor et al. 1996). However, the reliability of pollination syndromes for predicting pollinators has recently been questioned; many plant species may be visited by multiple pollinators although not all visitors may be effective pollinators (Waser et al. 1996; Ollerton 1998; Johnson and Steiner 2000). Two southern African examples indicate this clearly. *Protea roupelliae*, which has flower characteristics reflecting specialisation for bird pollination, is visited by malachite sunbirds *Nectarinia famosa* that successfully pollinate flowers (Hargreaves et al. 2004). Flowers also attract insects (beetles and honeybees) but they are less efficient pollinators (Hargreaves et al. 2004). On the other hand, *Aloe greatheadii* var. *davyana* displays floral features typical of a bird pollinated syndrome yet is pollinated primarily by honeybees *Apis mellifera* (Human 2006). In this instance nectar characteristics are an important factor in pollinator selection (Human 2006). Although birds do visit for nectar they are filtered against because *A. greatheadii* var. *davyana* produces nectar of high concentration (*c.* 21%) and low volume (*c.* 34µl) (Human 2006, Human and Nicolson 2008; Johnson and Nicolson 2008). Flowers may also be difficult to access because they have long pedicels and are less clustered on the raceme than other aloe species typically pollinated by birds (Human 2006; Botes 2007; Human and Nicolson 2008; Johnson and Nicolson 2008).

Aloe marlothii is abundant on rocky hill-slopes in northern and north-eastern South Africa (Reynolds 1969; Glen and Hardy 2000; Van Wyk and Smith 2005). The most conspicuous visitors to the spectacular flowers of *A. marlothii* are a wide range of opportunistic nectarivore bird species, although insects and mammals also visit for nectar and pollen (Oatley 1964; Skead 1967; Oatley and Skead 1972; Chapter 2). Because birds are the most common visitors to *A. marlothii* flowers, we hypothesized that they would contribute most to pollination success. Insects are infrequent visitors and were predicted to play a minor role in pollination. Exclusion experiments were

therefore conducted to investigate the primary pollination agents (birds or insects) of *A. marlothii.* The effectiveness of different avian pollinators of *A. marlothii* was investigated by measuring the beak lengths of bird species and monitoring bird behaviour during visits to flowers. In addition, the effect of plant height on reproductive fitness was investigated by studying fruit set in plants of different sizes in relation to bird visitation rates.

Materials and methods

Study site

This study was conducted in the Suikerbosrand Nature Reserve, 60 km south-east of Johannesburg, during 2006 and 2007. Large numbers of *A. marlothii* dominate bushveld habitat on north-facing slopes in the west of the reserve. Precipitation is seasonal and falls predominantly during warm summer months. Flowering of *A. marlothii* is during winter (June-September).

Exclusion experiments and compatibility

Three treatments, on 20 plants, were conducted as follows: i) control treatment - no exclusion cage, allowing unrestricted access by all visitors; ii) insects only - cage constructed of a rigid plastic mesh (aperture *c.* 20 mm diameter; see Johnson et al. 2006), allowing unrestricted access by bees and other insects, but excluding all birds and small mammals; and iii) no visitors - exclusion of all potential visitors (i.e. birds, insects and mammals) using a fine mesh cloth around a supporting wire frame (Fig. 1a). Plants of different heights, selected randomly in the aloe forest each year (2006 and 2007), included 10 short aloes (< 2.5 m) and 10 tall aloes (> 2.5 m). We chose the division between tall and short aloes as 2.5 m because the mean height of aloes recorded during a phenology study (Chapter 1) was 2.61 ± 1.07 m (n = 130) at the start of the study and 2.75 ± 1.07 m (n = 119) at the end of the study 24 months later. Racemes were bagged at the bud stage to exclude visitors prior to flower opening. At the end of flowering fruit set was determined by calculating the proportion of flowers

on each raceme that developed into fruit. Each plant contained a set of three treatments so the contribution of bird pollinators could be determined by subtracting fruit set on the bird exclusion treatment from the control treatment. The number of seeds in five randomly selected fruits from each experimental raceme was counted to determine seed production. Because we did not have an exclusion technique that filtered bird visitors we could not determine seed set directly for the bird pollinator guild.

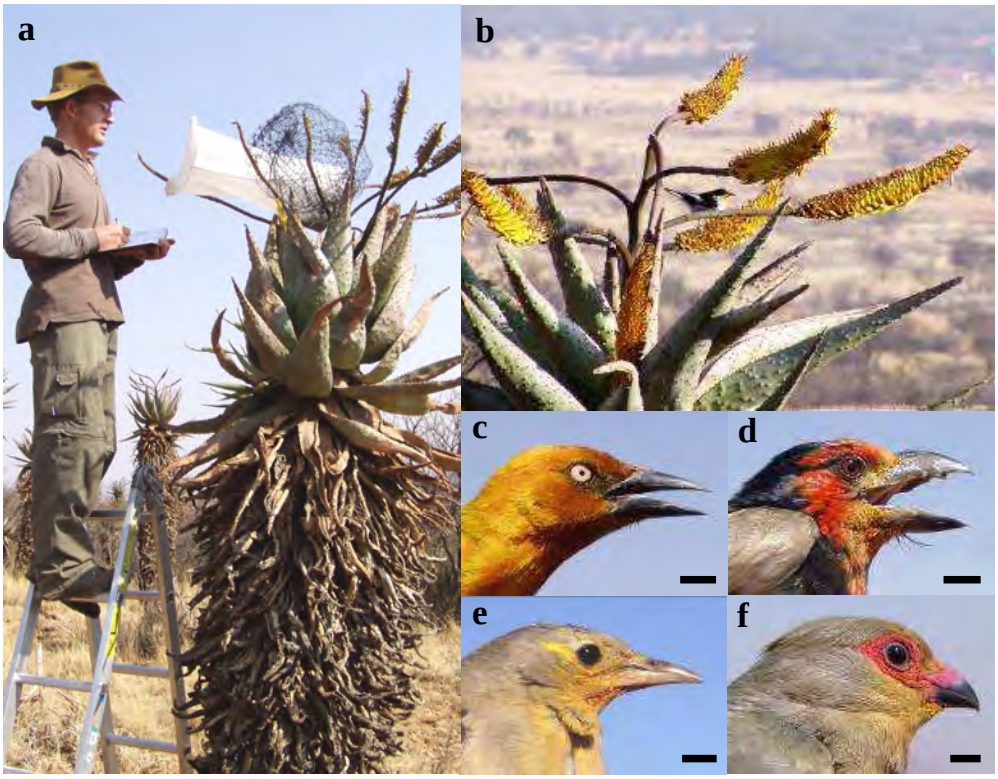

Figure 1. Exclusion cages on *A. marlothii* (3.14 m plant) during exclusion experiments (a), inflorescence with fiscal flycatcher *Sigelus silens* perched on open flowers (b), and common avian pollinators showing pollen dusted on facial area from feeding on nectar, Cape weaver *Ploceus capensis* (male) (c), black-collared barbet *Lybius torquatus* (d), wattled starling *Creatophora cinerea* (female) (e), and red-faced mousebird *Urocolius indicus* (f) (see Appendix for bill measurements; scale bar = 10 mm; Photograph credits: a, Darren Pietersen; b-f, Craig Symes).

Self-pollination was tested in five racemes that were removed from separate plants and placed in water in the laboratory. Fruit development has been observed in racemes removed from *A. greatheadii* var. *davyana*, *A. ferox* and *A. marlothii* prior to fruit set (C.T.S. pers. obs.). Also, this isolation controlled for effects of other

pollination events on each raceme. Eight flowers on each raceme were tagged before opening; four flowers were then pollinated with pollen from another plant and four with pollen from the same plant. Pollen was dusted from an anther of an untagged flower onto the stigma at different times during the female receptive phase (Chapter 1). Fruit development was then recorded for each tagged flower.

Fruit set and plant height

In order to compare fruit set on aloes of different heights, we determined the percentage of flowers that developed into fruit on a single raceme from 40 aloe plants of varying height (henceforth referred to as the height experiment). To control for effects of damaged inflorescences and aborted flowers, we selected undamaged inflorescences with high levels of fruit set. Fruit set was determined as the proportion of flowers on each raceme that developed into fruit. On each raceme five fruits were randomly selected and the number of seeds in each was counted.

Floral visitors and pollen loads

Point counts were conducted to determine visitation rates of birds to flowering aloes. Three to eight flowering plants of varying height, within 15 m of an observation point, were selected and all aloe visitors recorded. The following data concerning each visitor were collected: i) visiting species, ii) time of visit, iii) perch position on aloe (buds, open flowers, panicle, rosette leaves, dry leaves), iv) number of probes, v) destination on completion of visit to aloe. Observations occurred for 10-45 min at each randomly selected observation point in the aloe forest. Sampling occurred in late-August 2006 during peak flowering. In addition, the height and number of racemes of each monitored aloe were recorded. Sampled aloes included individuals from a range of heights.

Mistnetting and ringing of birds occurred as part of a larger study involving the collection of blood for stable isotope analysis. Each bird captured was inspected visually for pollen deposited on the bill and feathers of the facial region. During flowering months in 2006, a pollen swab was collected from representatives of all

species where pollen was not visible, using a piece of transparent adhesive tape (after Wolf and Martínez del Rio 2000). The tape was dabbed on the bill, frons and throat region and then placed on a slide. The slide was later scanned for pollen under a light microscope (10x). The method proposed by Beattie (1971), and used successfully by Hargreaves et al. (2004) for sampling malachite sunbirds *Nectarinia famosa* and Gurney's sugarbirds *Promerops gurneyi*, was not used here since ploceids (and numerous other species) commonly bite and prevent successful swabbing of the facial area with a fuschin stained gel block.

Bird measurements and flower size

Bill length of all captured birds was measured using digital Vernier calipers (nearest 0.1 mm). The length of the beak was measured from the tip of the bill to the feathers on the frons, or in the case of certain birds (e.g. ploceids), to the end of the bill. Mass was measured with a Pesola 100 g balance to the nearest estimated 0.1 g (for smaller birds) and with a Pesola 600 g balance to the nearest estimated 1 g (for larger birds). Additional mass and bill length data on birds caught outside the study area are also included (C.T.S. bird ringing database, unpubl. data).

During peak flowering small mammals were captured using Sherman small mammal traps (n = 10) baited with an oats and peanut-butter mix. Trapping occurred during peak flowering for two days and a night (31 Aug - 1 Sept 2006) by placing traps on the ground and on the rosettes of flowering and non-flowering aloes. Traps were checked in the morning and late-afternoon.

Flower length (base of corolla to flower tip) of 30 mature flowers was measured (Stage 2 flowers, Chapter 1) for comparison with the bill length of avian pollinators.

Statistical analysis

Comparisons of plant height between years were made using student t-tests. Where data were not normally distributed (Shapiro-Wilk W-test) non-parametric

statistical analyses were conducted. Because each of three different treatments were applied to a single plant we used a Friedman's ANOVA to test for differences between pollinator guilds for fruit set and seed production (i.e. pollination success). No seeds were produced in 2006 in the treatment that excluded all pollinators so we used a Wilcoxon signed rank test to compare the number of seeds produced in the insect pollinator and all pollinator treatment. We sampled different plants between years so for comparisons of fruit set and seed production between years for each treatment we used a Mann-Whitney U-test. For comparisons between reproductive characteristics of different height plants we used a Mann-Whitney U-test. All tests were conducted using Statistica 6.0 (1984-2004).

Results

Fruit and seed set

The mean height of the aloes used in exclusion cage experiments was not different between years (t-test, $P = 0.91$; 2006, 2.43 ± 0.42 m; 2007, 2.41 ± 0.48 m).

There was a significant difference in fruit set between the different pollinator guilds during both years (Friedman's ANOVA; 2006, $\chi^2 = 34.86$, $n = 18$, df = 3; 2007, $\chi^2 = 45.21$, $n = 18$, df = 3, $P < 0.01$) as well as for both years combined ($\chi^2 = 79.74$, $n = 36$, df = 3, $P < 0.01$; Fig. 2). In 2006, no fruit set occurred in the total exclusion cages due to a frost event that damaged many inflorescences, so for each treatment (pollinator guild) there was a significant difference in percentage fruit set between years (Mann-Whitney *U*-test, No pollinators $U = 120.0$, $P = 0.030$, Insect pollinators, $U = 119.5$, $P = 0.048$; Bird pollinators, $U = 99.0$, $P = 0.046$), except for the all pollinator treatment where fruit set was similar between years ($U = 111.0$, $P = 0.068$; 2006, 14.3 ± 18.6%; 2007, 19.5 ± 12.8%; Fig. 2). Also, in the exclusion experiment control treatments there was no difference in the number of flowers on each raceme between years ($U = 115.0$, $P = 0.06$; 2006, 237 ± 73; 2007, 279 ± 55).

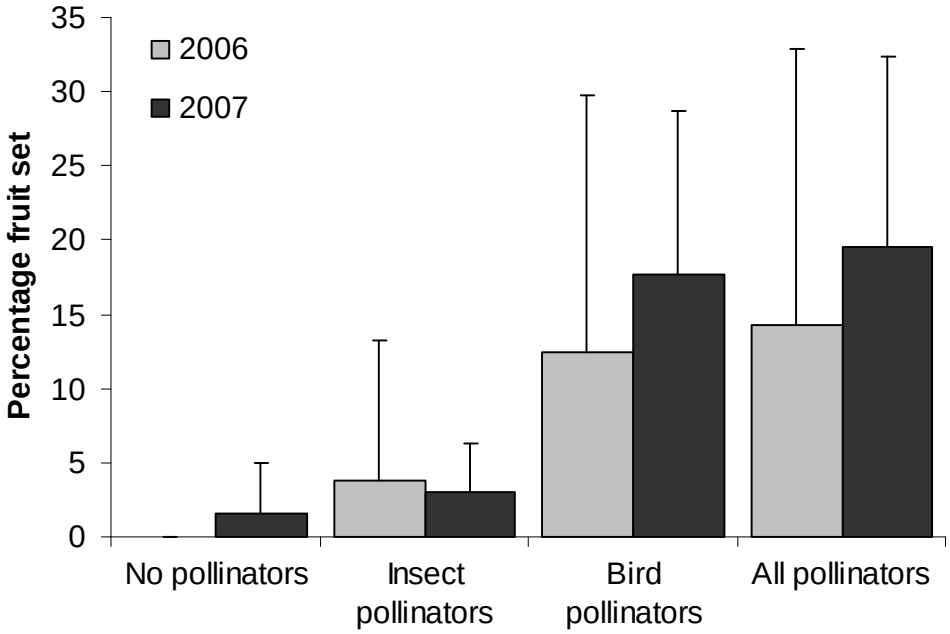

Figure 2. Percentage fruit set (± SD) of *Aloe marlothii* calculated from the number of flowers on each raceme that set fruit for different classes of pollinators (*n* = 20 plants each year).

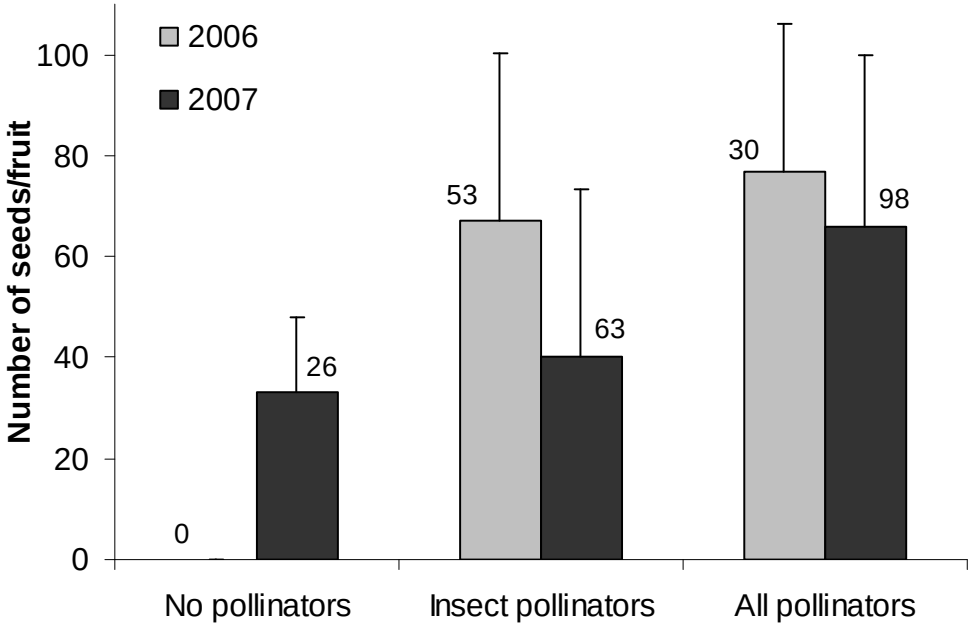

Figure 3. Seed production per fruit (± SD) for different classes of pollinators for *Aloe marlothii* during 2006 and 2007. Sample size for each treatment given. (No fruit produced in total exclusion during 2006).

There was a significant difference in seed production between no pollinators, insect pollinators and all pollinators treatments for each year (2006, Wilcoxon test [no seeds produced during 2006], Z = 4.57, $P < 0.01$; 2007, Friedman's ANOVA, $\chi^2 = 9.31$, $n = 26$, df = 2, $P = 0.01$). Overall seed production for the different treatments were significantly different (i.e. no pollinators, insect pollinators only, all pollinators; Friedman's ANOVA; $\chi^2 = 9.31$, $n = 26$, df = 2, $P = 0.01$; Fig. 3). Overall there were more seeds per fruit in 2006 (73 ± 31, $n = 83$) than 2007 (53 ± 35, $n = 187$) (Mann-Whitney, $U = 5050.0$, $P < 0.01$). Between years there was a significant difference between the number of seeds per fruit for insect pollinators ($U = 515.0$, $P < 0.001$), but not for all pollinators ($U = 2109.5$, $P = 0.057$).

Fruit set in cross-pollinated plants (25%, $n = 20$) was greater than in self-pollinated plants (5%, $n = 20$), showing *A. marlothii* to be self-incompatible.

Fruit set and plant height

Although the number of flowers on each raceme and percentage fruit set in the height experiment was similar between tall and short aloes, taller plants had more racemes per inflorescence, bore more fruit on each raceme and produced more seeds per fruit (Table 1). Fruit and seed set on the height experiment aloes was significantly greater than that on control treatments of the exclusion experiments (Fruit set in control treatments, 2006: 14.3 ± 18.6%, 2007: 19.5 ± 12.8%, $U = 99.0$, $P < 0.001$; seeds per fruit in control treatments = 70 ± 33; $U = 9597.5$, $P < 0.001$).

Table 1. Mean values of flowering characteristics for aloes from which seed set was determined (height experiment), showing differences for two height classes ($n = 20$ per height class; Mann-Whitney U-test, * *U*-value significant, $P < 0.05$).

	<2.5 m	**>2.5 m**	***U***	**All aloes**
Height (m)	2.09 ± 0.24	3.26 ± 0.50	-	2.67 ± 0.70
Number of racemes on aloe	9 ± 5	12 ± 4	101.5 *	10.3 ± 4.6
Number of fruit on raceme	125 ± 52	151 ± 45	127.0 *	138 ± 50
Number of flowers on raceme	260 ± 76	300 ± 59	144.5	280 ± 70
Number of seeds per fruit	84 ± 23	98 ± 23	103.0 *	91 ± 24
% fruit set	49 ± 16	50 ± 10	188.0	50 ± 13

In the exclusion experiment during 2006 fruit set was similar between tall and short plants (U = 25.0, P = 0.17), although in 2007 taller plants had higher fruit set than shorter plants (U = 17.0, P = 0.02). However, for both years combined there was no difference in fruit set for plants of different height (U = 168.0, P = 0.927).

Floral visitors

Birds

The number of individual birds visiting each aloe was greatest in the morning and decreased though the day, peaking again in the afternoon (Fig. 4). There appeared to be an increase in visitors to shorter aloes (< 2.5 m) as the number of visitors to taller aloes (> 2.5 m) decreased; however, this correlation was not significant (Spearman's R = -0.43, P > 0.05). The overall number of visitors to tall aloes was much greater (3.7 ± 2.0 visitors.h^{-1}) than to short aloes (0.3 ± 0.3 visitors.h^{-1}).

Birds fed on nectar by probing flowers, and in doing so became liberally coated in pollen on the facial area. Pollen counts of bird species where pollen was not visible to the naked eye are given in the Appendix. Usually the most pollen appeared on the throat region (C.T.S. pers. obs.). Some individuals did not have pollen recorded from swabs although the species may have been recorded as a nectar feeder (Appendix). Of the two species with the longest bills, only malachite sunbird (n = 1) did not have pollen visible; common scimitarbill *Rhinopomastus cyanomelas* (n = 2) had pollen visible on the facial area. Birds that visited inflorescences mostly perched on the inflorescence peduncle (59.9%); 45.0% perched on open flowers (see Fig. 1b), 24.3% perched on unopened flowers and 20.3% perched on aloe leaves, whilst 1.0% perched on dry leaves (see Fig. 1a). Birds collected pollen on other body parts (e.g. feet, belly) whilst perching on flowers. Most birds flew out of sight (>15 m; 67.3%, n = 159) when leaving aloes, whilst 14.5% flew to a nearby tree, 12.6% flew to another inflorescence, 4.4% to another aloe, 0.6% to the ground and only 0.6% remained on the aloe at the end of the observation session.

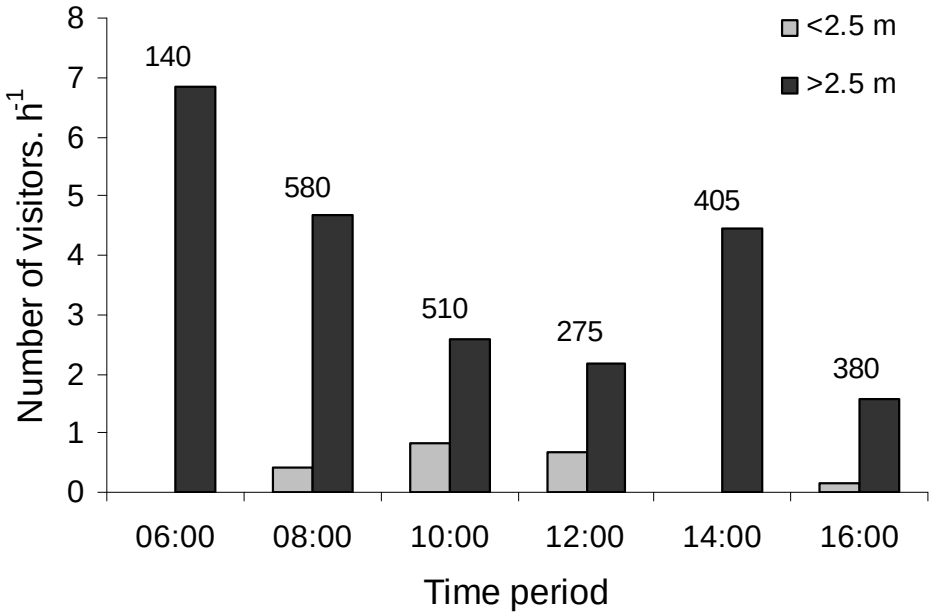

Figure 4. Bird visitation rates during two-hourly time periods (06:00 indicates 06:00-07:59, *etc.*) to aloes of different sizes during peak *Aloe marlothii* flowering. Visitation rate was calculated as the overall number of visitors for all observation periods as a proportion of total observation time during each time period. Total observation time (aloe min) for each period given on figure.

The mean number of racemes (± SD) on each aloe visited by each visitor was 2.2 ± 2.6, whilst the mean number of racemes on each aloe was 12.4 ± 5.5. Each aloe had 2.7 ± 2.0 visitors per hour and during a visit each bird probed 5.5 ± 8.8 times per inflorescence.

Other visitors

Chacma baboons *Papio hamadryas ursinus* were regular visitors to inflorescences. Before flowering they fed on emerging inflorescences, often removing the inflorescence and feeding on the base. During flowering they fed on nectar and were observed with pollen dusted around the mouth. Many inflorescences were damaged as they climbed plants to feed (Chapter 1). Slender mongooses *Galerella sanguinea* were also observed climbing inflorescences and feeding on nectar (C.T.S. pers.obs.). Striped mouse *Rhabdomys pumilio* (n = 5) was captured in traps placed on

the ground and Namaqua rock rat *Michaelamys (Aethomys) namaquensis* (*n* = 1) was captured in traps placed in aloe rosettes; pollen was detected on pollen swabs from both species.

Insects observed feeding on nectar included the following species; Xylocopinae (1 sp.), Formicidae (2 spp.), Bombyliidae (1 sp.), Muscidae (2 spp.) and Mordellidae (1 sp.). A species of thrip (Thripidae) occurred in abundance on many flowers, often inside the corolla of newly opening flowers.

Bird measurements and flower size

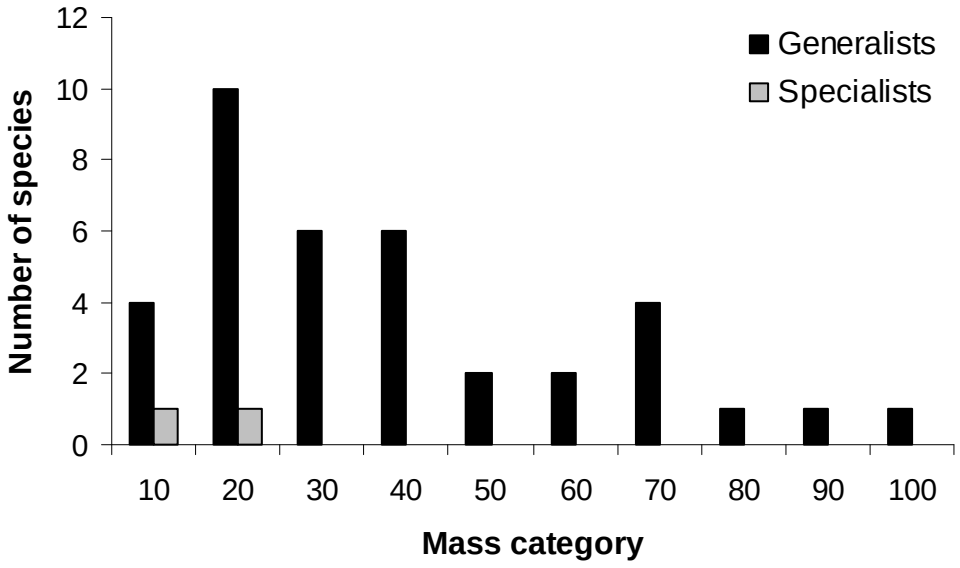

Figure 5. Mass frequency distribution of nectar-feeding birds at *Aloe marlothii* forest, Suikerbosrand Nature Reserve. Specialist nectarivores include malachite sunbird *Nectarinia famosa* and white-bellied sunbird *Cinnyris talatala.* X-axis mass category 10 indicates birds with mass 0-10 g, 20 indicates 10.1-20.0 g, *etc.*

The length of most bird bills was significantly shorter than flower length (mean flower length ± SD = 32.7 ± 2.3 mm, *n* = 35). Bill size ranged in length from 8.7 mm in black-throated canary *Serinus atrogularis* to 43.4 mm in common scimitarbill, and included a wide variety of different bill shapes (Fig. 1c-f; Appendix). Two species had bills greater in length than aloe flowers (malachite sunbird and common scimitarbill; t-

test, $P < 0.01$; Appendix). Pollen grain counts of birds that were swabbed are noted in the Appendix.

Bird masses of nectar-feeding birds at the study site ranged from 8.3 g (white-bellied sunbird *Cinnyris talatala*) to 96.0 g (laughing dove *Streptopelia senegalensis*). A frequency distribution of bird masses as are shown in Fig. 5.

Discussion

A generalist bird pollination syndrome

Flower and nectar characteristics of *A. marlothii* support a generalist bird pollination syndrome, which is confirmed by experiments excluding different classes of pollinator. At Suikerbosrand a large proportion of the bird community was recorded feeding on nectar during a 5-10 week flowering period in August and September (Chapter 1). This included at least 42 bird species (59% of the resident bird community), made up of mostly residents with some nomadic species such as large flocks of wattled starling *Creatophora cinerea* (Chapter 2). The diverse assemblage of opportunistic nectar-feeding birds included frugivores, granivores, insectivores and omnivores (Chapter 2, 4).

Little is known of the pollinators for at least 450 *Aloe* species that occur in the Afrotropical region (Reynolds 1969; Reynolds 2004). Aloes occur in a range of habitats with some species being localised and endemic, whilst others are common and widespread (Reynolds 1969; Glen and Hardy 2000; Van Wyk and Smith 2005). The pollination syndromes are therefore likely to vary and a comprehensive review of pollination in this genus is warranted. However, there does appear to be a relationship between flowering period and pollinator type. In an assessment of 125 *Aloe* species, those that flower predominantly during summer months are insect pollinated, whilst those that flower during winter are pollinated by occasional nectarivores, and those pollinated by true nectarivores (sunbirds) flower throughout the year (Botes 2007). In addition, winter-flowering *A. marlothii* has rigid racemes, like those of *A. ferox*, that

are able to support the weight of large generalist avian pollinators (Chapter 2). This is probably an important feature of large generalist bird pollinated aloes with conspicuous, gaudy and robust inflorescences. In other aloes with less rigid inflorescence stalks the pollinators may be more specialist i.e. sunbirds (Botes 2007).

A few aloes have been studied in detail. For example, *A. ferox*, which occupies a similar ecological niche in the south-east of South Africa, produces similar nectar (180 µl; 12.5% w/w) to *A. marlothii* but is pollinated by both specialist (sunbirds) and generalist avian visitors as well as honeybees *Apis mellifera* (Reynolds 1969; Hoffman 1988; Van Wyk and Smith 2005; Botes 2007). Although pollinator exclusion experiments were not conducted on *A. ferox* (Hoffman 1988) they have been conducted on *A. candelabrum* (synonymous with *A. ferox*); here birds were identified as the most important pollinators, with the role of insects being insignificant (Stokes and Yeaton 1995). Without experimental evidence, Tribe and Johannsmeier (1996) considered both sunbirds and bees the major pollinators of three species of tree aloe, *A. dichotoma, A. pillansii* and *A. ramosissima*, that flower in late-winter and spring. However, exclusion experiments are needed to confirm the true pollinators of each species; because bees visit plants does not necessarily mean they are important pollinators. Two smaller island endemic species (*A. divaricata* and *A. mayottensis*), similar in size to *A. greatheadii* var. *davyana*, are each pollinated by a sunbird species, with a minor pollination role attributed to bees and other insects (nectar characteristics were not investigated) (Ratsirarson 1995; Pailler et al. 2002). *Aloe vryheidensis*, a winter-flowering aloe with conspicuous inflorescences similar to *A. ferox*, produces dilute nectar (6-17%) that attracts generalist birds; specialist sunbird pollinators that are inefficient pollinators are excluded by the bitter tasting nectar (Johnson et al. 2006). *Aloe greatheadii* var. *davyana*, although suggestive of bird pollination, is pollinated by honeybees (Human 2006).

Pollen transfer and fruit set

It is suggested that a large and dense food source to attract pollinators has the potential to reduce inter-plant movements and result in low percentage fruit set for self-incompatible species (e.g. *A. ferox* and *A. marlothii*) (Janzen 1967; Augspurger

1983). However, pollen transfer in *A. marlothii* is ensured by an active movement of birds between plants; most birds recorded probing flowers flew out of sight after feeding (67% of observations) or flew to a nearby inflorescence (12%), whilst only 0.6% of birds remained at aloes at the end of the observation period. Pollen from other plant species was not detected in pollen swabs, so visits for nectar were mostly (if not all) to *A. marlothii* plants. Larger pollinators like birds are more reliable pollinators and move pollen greater distances (Brown et al. 1978). However, to attract larger pollinators, plants must secrete more nectar (Brown et al. 1978); in *A. marlothii* this is achieved by the continuous production of copious nectar (*c.* 250 µl; Chapter 1).

Because *A. marlothii* is self-incompatible, like most aloes, effective cross pollination is required for successful seed set (Brandham 1969; Riley and Mujamdar 1979; Hoffman 1988; Pailler et al. 2002; Botes 2007; Human 2006). In *A. marlothii* more seeds were produced from flowers with more pollinators; and cross pollinated flowers produced four-fold more fruit than self-pollinated flowers. However, this was not as prominent as in *A. vryheidensis* where seed production in cross-pollinated plants was 10-fold greater than self pollinated plants (Johnson et al. 2006). The probing of flowers by short billed birds to remove nectar ensures that pollen is dusted onto the entire facial area of many bird species, and transferred between plants. This appears to be most effective on aloes that attract generalist avian pollinators where the relatively short flowers are densely packed on the inflorescence (Botes 2007; Human and Nicolson 2008). Movement of birds along the raceme is over the densely packed flowers, so pollen is also collected on the feet and belly of visiting birds (see Fig 1b). Feeding birds make contact with adjacent male and female phase flowers, and successful pollination is ensured. The dense packing of flowers is reminiscent of *Banksia* inflorescences that are important nectar sources for honeyeaters. Honeyeaters probe a substantial proportion of the circumference of *Banksia menziesii* inflorescences, making contact with pollen presenters and stigmata of many florets (Ramsey 1989). In *B. menziesii*, although there were more visits by bees, fruit set was 10 times greater on inflorescences visited by birds (Ramsey 1988). In a *Banksia* woodland near Perth, where the flowering seasons of five *Banksia* species were sequential with slight overlapping, honeyeaters appeared to be the most important pollinators (Whelan and Burbidge 1980), and in a study of several autumn-flowering

plants in Western Australia honeyeaters carried greater pollen loads of four species (two *Banksia*) than honey possums (Hopper 1980). We recorded at least three small mammal species that may contribute to pollination, but because they do not move as great distances as birds their role as pollinators may be less important.

Table 2. Fruit set in different *Aloe* species, giving percentage fruit set and major pollinator type. Values for our study include control treatment results for exclusion experiments for two years (n = 20 aloes/year) and seed set on selected racemes during one year (n = 40 racemes).

Species	**Fruit set (%)**	**Major pollinator type**	**Reference**
A. marlothii	14-20; 50	generalist birds	This study
A. greatheadii var. *davyana*	45-55	bee	Human 2006
A. mayottensis	30	single sunbird pollinator	Pailler et al. 2002
A. ferox	27	generalist birds	Hoffman 1988
A. divaricata	15	single sunbird pollinator	Ratsirarson 1995

Note: Stokes and Yeaton (1995) only report fruit set success of *A. candelabrum*, pollinated by generalist birds, as the number of fruit per cm of raceme, which is not comparable to these studies.

We found conflicting results for fruit set in *A. marlothii* with the percentage fruit set on the height experiment (50%) greater than that during exclusion experiments (14-20%). This was likely an artefact of sampling where the most successfully fruiting plants were selected. Fruit set measured in the exclusion experiments, where plants were selected randomly before flowering, is likely a true reflection of fruiting success. This is compared to *Aloe* species from other areas where variation between species was high (Table 2). This variation may be linked to methodology and temporal and/or spatial variation between these studies.

At our study site only two sunbird species were recorded feeding on nectar (Chapter 2). They did not increase in abundance during flowering, like many other opportunistic nectar feeders (Chapter 2). Pollen was also not observed on the facial area of malachite sunbirds, but only on the bill tip, suggesting they play little role in pollination (Fig. 6a,b,d). However, common scimitarbill, which had the longest bill of all nectar feeders, and is not a specialist nectarivore, was observed covered in pollen on the facial area (Fig. 6c). Feeding method, rather than bill length, may therefore be

an important factor for ensuring that pollen is transferred by birds between plants. In five sympatric winter-flowering *Aloe* species (*pluridens, lineata, africana, speciosa* and *ferox*) in the Eastern Cape, South Africa, the specialist-generalist bird pollination community is structured according to nectar properties, floral architecture and pollen deposition sites (Botes 2007). Depending on the *Aloe* and bird species involved, pollen is deposited on the frons, bill, throat and/or chest and belly (Botes 2007). This is determined by the arrangement of flowers on the raceme and the position of the feeding bird, with densely packed flowers attracting generalists (Botes 2007). In most *Aloe* species the flowers are more widely spaced than in *A. marlothii* with the orientation changing during flower development.

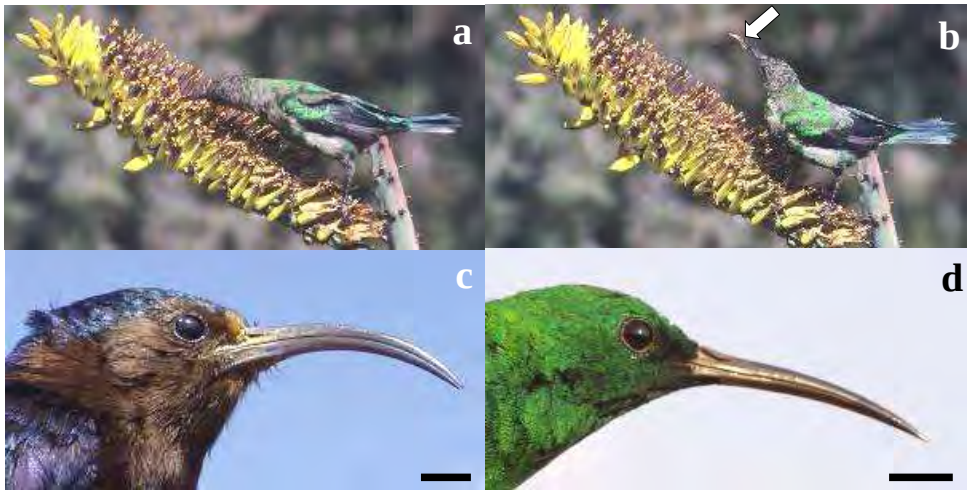

Figure 6. Malachite sunbird *Nectarinia famosa* (juvenile male) feeding on *A. marlothii* flowers showing delicate probing of flowers with tip of bill whilst standing on old flowers **(a)**, and pollen collected on tip of bill (shown by arrow) **(b)**. Common scimitarbill *Rhinopomastus cyanomelas* (juvenile) with pollen collected on face and culmen **(c),** and malachite sunbird (adult male) with clean bill **(d)** (see Appendix for bill measurements; scale bar = 10 mm; Photographs: Craig Symes).

Height benefits for plants

In some areas *A. marlothii* is known to reach heights in excess of 8 m (Reynolds 1969) with the tallest plant recorded at our site measuring 6.30 m. At our study site shorter plants flowered less often and were also damaged more by baboons (Chapter 1). Together with mass flowering and spiny leaves that reduce predation by

baboons, taller plants benefit by avoiding predators. Taller plants also produced more seeds and more racemes, thus exhibiting greater reproductive fitness through higher fruit and seed production. Although there was a similar proportion of seed in both height categories, the resulting seed crop of taller plants may also have greater genetic diversity because of a greater number of pollinators (Donnelly et al. 1998). Plant height of *A. ferox* was not correlated with percentage fruit set, but was correlated positively with the number of racemes on inflorescences and the number of fruits on each plant (Hoffman 1988).

Conclusion

Within the aloe family (Asphodelaceae) there appears to be a wide range of pollination syndromes defined by different floral characteristics and plant size. Recently, nectar characteristics have been identified as an important factor determining pollinator type (Johnson and Nicolson 2008). Nectars of high volume (40-100 µl/flower) and low concentration (8-12% w/w), as in *A. marlothii* (250 µl/flower; 12% w/w), typically attract generalist pollinators whilst those of low volume (10-30 µl/flower) and high concentration (15-25% w/w) attract specialist nectarivores e.g. sunbirds and hummingbirds (Johnson and Nicolson 2008). Most features of *A. marlothii* are thus typical of a bird pollinated plant (Table 3), with the most prominent and characteristic being the orange to red flowers on conspicuous inflorescences whose abundant and dilute nectar attracts a wide range of generalist pollinators (Chapter 1).

There are a number of other characteristics of *A. marlothii* that contribute to its reproductive success. *Aloe marlothii* is a CAM photosynthesizer (Denius and Homan 1972; Kluge et al. 1979; Eller et al. 1993) that flowers during periods of little or no rainfall; therefore water conserved prior to flowering can be used for flowering and nectar production during dry winter months, with water replenishment in the succulent leaves occurring soon after rains in early-spring (September-October). Flowering also occurs at a time of the year when few other plants flower, thereby reducing competition with other plant species. The synchronous and *en masse* flowering in dense populations of aloes also ensures that more nectar is produced than can be

consumed, thereby attracting a wide range of visitors (Chapter 2). However, the transfer pollen between plants is ensured by the active movement of birds in the aloe forest; most birds observed flew out of sight from the aloe where they fed.

Table 3. Summary of bird pollination syndrome characters (Proctor et al. 1996); related to *Aloe marlothii*. [1] Symes and Nicolson unpubl. data., [2] Chapter 1.

Ornithophilous syndrome characteristic	***Aloe marlothii***
Vivid colours; bright reddish-orange flowers	YES, bright orange to red flowers (Fig. 1b)
Longer floral tube	YES, flower length = 32.7 ± 2.3 mm
Exserted anthers and stigma	YES, anthers exserted before stigma
Inclined flower	YES, flowers face upwards on horizontal raceme
Absence of odour and nectar guides (less pronounced landing platform)	YES, no landing platform
Distinctly curved tubular flowers	YES, although curvature not distinct
Secund flower arrangement	YES, pedicel length = *c.* 5 mm
Large pollen-nectar distance	YES, *c.* 10-30 mm, nectar fills entire corolla tube in stage 2 flowers [1]
Diurnal anthesis with nectar peak and flower opening in early morning	NO, flowers continuously opening and nectar always available
Abundant nectar held in flower by capillarity	YES, 250 µl/flower (stage 2 flowers [2])
Low concentration of nectar	YES, 12% (stage 2 flowers [2])

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Appendix. Bird species (n = 39) recorded feeding on *Aloe marlothii* nectar at Suikerbosrand Nature Reserve (Chapter 2, C.T.S. pers. obs.). Nectar feeders were recorded feeding on nectar during transects (t), birds observed feeding on nectar out of transect times (o), pollen observed from swabs of facial area (s), and pollen observed on facial area of birds in aloe forest (p). Pollen counts (mean ± SD) given for species not seen feeding on nectar or without pollen observed on facial area, sample size in parentheses, * indicates all netted birds observed with pollen. Bill length significantly different to flower length for all species (# bill longer than flower; t-test, $P < 0.05$). Taxonomy follows Hockey et al. (2005).

Species	Nectar feeder	Pollen count	Mass	Bill
INDICATORIDAE				
Lesser Honeyguide *Indicator minor*	s	2.5 (2)	28.8 ± 2.1 (12)	10.0 ± 0.8 (12)
PICIDAE				
Cardinal Woodpecker *Dendropicos fuscescens*	s	3.5 (2)	32.4 ± 3.0 (10)	17.9 ± 1.4 (9)
LYBIIDAE				
Acacia Pied Barbet *Tricholaema leucomelas*	tp	*	36.5 ± 2.6 (37)	19.1 ± 1.2 (37)
Black-collared Barbet *Lybius torquatus*	p	*	58.6 ± 7.8 (36)	22.6 ± 1.6 (36)
Crested Barbet *Trachyphonus vaillantii*	t	*	72.4 ± 11.1 (29)	23.5 ± 1.1 (29)
RHINOPOMASTIDAE				
Common Scimitarbill *Rhinopomastus cyanomelas*	t	*	34.2 ± 5.9 (3)	43.4 ± 4.1 (3) #
COLIIDAE				
Speckled Mousebird *Colius striatus*	tp	*	52.7 ± 8.5 (35)	13.0 ± 1.1 (35)
Red-faced Mousebird *Urocolius indicus*	tp	0 (1)	61.8 ± 7.6 (32)	13.5 ± 0.5 (33)
COLUMBIDAE				
Laughing Dove *Streptopelia senegalensis*	s	1.6 ± 2.1 (7)	96.0 ± 15.9 (119)	15.3 ± 1.4 (119)
MALACONOTIDAE				
Brown-crowned Tchagra *Tchagra australis*	s	1.0 (2)	33.1 ± 4.2 (10)	17.7 ± 1.1 (12)
Bokmakierie *Telophorus zeylonus*	o	*	65.8 ± 5.4 (8)	22.5 ± 2.4 (8)
LANIIDAE				
Common Fiscal *Lanius collaris*	o	0 (2)	42.8 ± 4.2 (60)	16.6 ± 1.3 (58)
PYCNONOTIDAE				
African Red-eyed Bulbul *Pycnonotus nigricans*	tp	*	37.1 ± 2.8 (138)	17.0 ± 0.9 (138)
SYLVIIDAE				
Cape Grassbird *Sphenoeacus afer*	s	6.0 (1)	32.2 ± 4.0 (3)	17.0 ± 1.4 (3)
Long-billed Crombec *Sylvietta rufescens*	s	18.0 (1)	11.2 ± 1.8 (10)	13.8 ± 1.5 (13)

Species	Nectar feeder	Pollen count	Mass	Bill
Chestnut-vented Tit-Babbler *Parisoma subcaeruleum*	tp	8.0 ± 11.3 (3)	15.0 ± 1.2 (36)	10.6 ± 0.9 (34)
ZOSTEROPIDAE				
Cape White-eye *Zosterops capensis*	tp	44.3 ± 75.1 (3)	12.1 ± 3.7 (379)	11.5 ± 0.9 (377)
CISTICOLIDAE				
Rattling Cisticola *Cisticola chiniana*	o	*	13.9 ± 3.1 (13)	12.7 ± 1.6 (13)
Neddicky *Cisticola fulvicapilla*	s	0.7 ± 1.1 (3)	9.6 ± 0.9 (25)	10.4 ± 0.8 (25)
Black-chested Prinia *Prinia flavicans*	tp	0 (3)	9.8 ± 0.9 (46)	10.1 ± 0.8 (33)
Bar-throated Apalis *Apalis thoracica*	tp	55 (1)	10.6 ± 1.5 (32)	13.0 ± 1.3 (27)
MUSCICAPIDAE				
Cape Rock-Thrush *Monticola rupestris*	t	*	63.6 ± 2.9 (6)	21.9 ± 1.5 (6)
Fiscal Flycatcher *Sigelus silens*	tp	18.8 ± 26.5 (6)	28.0 ± 5.2 (85)	14.0 ± 0.9 (78)
Cape Robin-Chat *Cossypha caffra*	tp	*	29.0 ± 3.3 (165)	15.3 ± 1.3 (156)
STURNIDAE				
Cape Glossy Starling *Lamprotornis nitens*	o	*	83.2 ± 13.0 (7)	22.1 ± 2.7 (7)
Wattled Starling *Creatophora cinerea*	tp	*	60.3 ± 25.8 (6)	21.0 ± 1.8 (6)
NECTARINIIDAE				
Malachite Sunbird *Nectarinia famosa*	t	*	18.6 ± 3.3 (7)	36.1 ± 3.7 (7) #
White-bellied Sunbird *Cinnyris talatala*	t	*	8.3 ± 1.3 (33)	20.9 ± 1.2 (35)
PLOCEIDAE				
Cape Weaver *Ploceus capensis*	tp	*	44.9 ± 4.8 (46)	21.5 ± 1.1 (46)
Southern Masked-Weaver *Ploceus velatus*	tp	*	27.5 ± 4.4 (190)	16.1 ± 1.2 (192)
Red-collared Widowbird *Euplectes ardens*	t	*	19.0 ± 2.0 (14)	13.7 ± 0.7 (13)
ESTRILDIDAE				
Black-faced Waxbill *Estrilda erythronotos*	tp	129 (1)	9.2 ± 0.8 (9)	9.4 ± 0.7 (8)
Violet-eared Waxbill *Granatina granatina*	s	217.0 ± 176.0 (5)	10.9 ± 1.0 (30)	10.2 ± 0.5 (26)
Green-winged Pytilia *Pytilia melba*	s	1.9 ± 3.2 (13)	15.7 ± 7.1 (185)	12.5 ± 0.6 (169)
Jameson's Firefinch *Lagonosticta rhodopareia*	s	0.5 ± 0.6 (4)	9.9 ± 1.7 (39)	10.0 ± 0.7 (46)
PASSERIDAE				
Southern Grey-headed Sparrow *Passer diffusus*	s	3.0 (2)	24.6 ± 2.7 (31)	12.0 ± 0.7 (30)
FRINGILLIDAE				
Black-throated Canary *Serinus atrogularis*	t	*	13.7 (1)	8.7 (1)
Streaky-headed Seedeater *Serinus gularis*	t	*	18.1 ± 4.5 (5)	12.2 ± 0.8 (5)
Cape Bunting *Emberiza capensis*	t	0 (2)	23.9 ± 2.1 (8)	11.3 ± 0.5 (8)

IV

CHAPTER 4

The importance of *Aloe marlothii* nectar for an avian community: evidence from stable carbon isotopes

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Abstract

Numerous bird species feed on the nectar of *Aloe marlothii*, a CAM plant that flowers during dry winter months in the summer rainfall savannah biome of South Africa. Carbon isotopic signatures were measured in whole blood samples from birds mist-netted over a six-month period before, during and after flowering (May-October) at an *A. marlothii* nectar oasis. The nectar of *A. marlothii* had an isotopic signature of $\delta^{13}C$ = -12.6 ± 0.5‰ VPDB (mean ± SD), whilst C_3 plants and C_4 grasses measured -27.2 ± 1.5‰ and -14.7 ± 2.5‰ respectively. When *A. marlothii* nectar was available, most insectivores, frugivores and omnivores, that typically relied on a C_3 based diet, showed significantly enriched whole blood $\delta^{13}C$ values compared to during the non-flowering period. All the bird species that exhibited shifts in blood $\delta^{13}C$ towards enriched values were regularly observed feeding on *A. marlothii* nectar during the flowering season, and it is highly likely that the enrichment of their tissues reflects the incorporation of carbon from nectar. We avoided the use of mixing models to quantify the nectar carbon contribution for different species because of limitations in our understanding of diet-tissue discrimination factors for different species and diets, and routing of different food types. Because sugar carbohydrates are likely immediately metabolised and not routed to tissue for storage, a shift in the blood $\delta^{13}C$ towards nectar during flowering may depict a gross underestimation of the importance of nectar carbon in the birds' diet. Our ability to accurately quantify the contribution of *A. marlothii* nectar to the birds' blood carbon pool, particularly in granivores, is also limited by the small isotopic difference between *A. marlothii* nectar and C_4 grasses. Despite nectar of *A. marlothii* being dilute (*c.* 12% w/w) compared to plants pollinated by specialist pollinators, it is probably a more important food source for many bird species during dry South African winters than is realised.

Keywords $\delta^{13}C$ · nectarivores · sunbird · winter-flowering · South Africa

Introduction

Major avian nectarivore families have evolved independently in the Neotropical, Afrotropical and Australasian regions where they are represented by hummingbirds, sunbirds and honeyeaters respectively (Maclean 1990). Studies on nectar-feeding in birds have focused primarily on these true nectarivores, with the diversity of opportunistic nectarivores largely ignored, particularly in the Afrotropics. In Africa the number of occasional nectarivores is comparatively high compared to other zoogeographical regions (Maclean 1990; Chapter 2). They feed on a range of plant nectars, with aloes (Asphodelaceae) forming an important nectar source for many species, particularly during the dry winter period when most aloes flower (Oatley 1964; Skead 1967; Reynolds 1969; Oatley and Skead 1972; Pettet 1977; Chapter 2).

Aloe marlothii A.Berger is one of *c*. 450 *Aloe* spp. found in Africa and occurs in the summer rainfall savannah regions of northern and north-eastern South Africa (Reynolds 1969; Glen and Hardy 2000; Reynolds 2004; Van Wyk and Smith 2005). Plants grow in excess of 6 m high and produce large inflorescences with up to 39 racemes (*c.* 250 flowers/raceme) (Chapter 1). The flowers produce large quantities (*c.* 250 µl) of dilute nectar (*c*. 12% w/w) that attracts a wide range of consumers (Chapter 1). At least 82 bird species (25 families), including granivores, frugivores, insectivores and omnivores, have been recorded feeding opportunistically on the nectar of *A. marlothii* (Oatley 1964; Oatley and Skead 1972; Botes et al. 2008; Chapter 2). However, the nutritional importance of this nectar to birds is not quantitatively known.

Naturally-occurring variation in stable carbon isotope ratios provides a powerful tool for reconstructing the importance of different dietary items for organisms (Peterson and Fry 1987; Kelly 2000). Implicit in such applications is the assumption that the stable isotope ratio of a consumers' tissue is related to its diet in a predictable manner (Kelly 2000; Hobson and Clark 1992a; Hobson and Clark 1993). Stable carbon isotopes in plants vary predictably; C_3 plants and C_4 grasses typically show significant differences in $\delta^{13}C$ values because of contrasting use of CO_2 in different photosynthetic pathways (Smith and Epstein 1971; Vogel et al. 1978; Hobson 1999). Therefore, by monitoring the temporal changes in carbon isotope ratios in

different tissues it is often possible to detect dietary shifts with regards to carbon resources obtained from C_3 and C_4 components of the ecosystem (Rubenstein and Hobson 2004; Dalerum and Angerbjörn 2005). These changes can be detected in a non-destructive manner over a relatively short period if tissues with a short turnover period, such as blood (5-30 day half-life depending on bird size), are analysed (Hobson and Clark 1992b; Bearhop et al. 2002; Carleton and Martínez del Rio 2005).

Aloe marlothii is a CAM photosynthesiser (Denius and Homan 1972; Kluge et al. 1979; Eller et al. 1993) and $\delta^{13}C$ values of plant tissues are similar to those of C_4 grasses (Vogel et al. 1978). This study was conducted to determine the importance of *A. marlothii* nectar sugars for an avian community using stable carbon isotopes. Bird community abundance and diversity increase significantly during the *A. marlothii* flowering period (August-September) with >50% of species recorded during transects seen feeding on nectar (Chapter 2). We therefore hypothesized that a shift in blood $\delta^{13}C$ towards more enriched values, in avian species that feed on nectar, would coincide with the onset of flowering in *A. marlothii*. Species that fed most often on nectar were expected to show the greatest enrichment in $\delta^{13}C$ values. Because nectar consists predominantly of rapidly metabolizable hexose sugars (Van Wyk et al. 1993) we predicted that the isotopic signature of nectar would rapidly appear in whole blood of nectar-feeding birds once flowering began, and would disappear after the flowering period.

Materials and Methods

Study site

This study was conducted during 2005 and 2006 at Suikerbosrand Nature Reserve (SNR), a 19,779 ha reserve 60 km south-east of Johannesburg, with sampling spanning months before (May-July), during (August-September) and after (October) flowering in *A. marlothii*. Vegetation in SNR is dominated by grassland and savannah biomes with *A. marlothii* growing predominantly on rocky north-facing slopes. Rainfall is seasonal falling mainly during summer (October-March) months and

winters are dry with contrasting night-day temperatures of *c.* -5 to 25°C. *Aloe greatheadii* var. *davyana* also occurs in the reserve and flowers prior to *A. marlothii* but is visited less often by birds, possibly because it offers nectar of lower volume (*c.* 34 µl) (Human 2006; Human and Nicolson 2008).

Sample collection

Mist-netting was conducted during May-October each year (2005 and 2006) along a disused vehicle track through a large stand of *A. marlothii* in the western portion of the reserve (26°31'50"S 28°10'07"E, *c.* 1,600-1,700 m a.s.l) (henceforth referred to as the aloe forest). All birds were ringed (Safring, Cape Town) so recapture and sampling of the same individual could be monitored. Blood samples (10-50 µl) were collected from the brachial vein of each bird, using a 25-gauge needle to prick the vein and a 75 µl hematocrit tube to collect blood. Blood samples were then transported to a laboratory and dried to constant mass at 50°C in a drying oven.

During each month of sampling, representative C_3 and C_4 vegetation samples were collected, as well as insects associated with each vegetation type. At five grass sites in the aloe forest ten sweeps with an insect net were used to collect invertebrate samples; grass (C_4) samples gathered during sweeps were also collected. Leaf (C_3) samples were collected from five common tree species (*Acacia karroo*, *Ziziphus mucronata*, *Tarconanthus camphoratus*, *Gymnosporia heterophylla*, *Rhus leptodictya*) and an insect net (diameter = 42cm) placed beneath each tree was used to catch invertebrates shaken from the tree (10 shakes). Sample collection occurred during mid-afternoon (14:00-16:00). The invertebrates collected (henceforth referred to as C_3 and C_4 insects) were stored in alcohol (75%) and plant samples placed in labelled envelopes. Samples were returned to the laboratory where they were dried and fine ground using a mortar and pestle in preparation for isotope analysis.

Three *A. marlothii* flowers were collected from different plants during July (*n* = 3, unopen flowers), August (*n* = 9) and September (*n* = 7). During peak flowering in August 2006, *c.* 2 ml of nectar was collected from each of nine individuals, using disposable hematocrit tubes (75 µl) and placed in glass bottles. In the laboratory the

flowers and nectar samples were oven dried to constant mass and ground for isotope analysis. In 2005 nectar samples were collected from two aloe plants.

Isotope analyses

Isotopic analysis was conducted at the Natural Resources and the Environment isotope laboratory, CSIR, Pretoria. Representative samples (*c.* 0.15-0.30 mg) were weighed in tin cups (cleansed in toluene) and combusted at 1020^{o}C to CO_2 in an elemental analyser (Flash EA, 1112 Series, Thermo Electron Corporation). The $^{13}C/^{12}C$ isotope ratio was determined using a continuous-flow isotope ratio mass spectrometer (Thermo Delta V Plus, Thermo Electron Corporation) plumbed inline with the elemental analyser. During analyses, every six samples were followed with two laboratory standard aliquots (homogenized dried chicken blood; mean $\delta^{13}C \pm SD = -17.87 \pm 0.15‰$; $n = 331$), in order to correct for equipment drift. All samples were analysed in duplicate. Isotope ratios are expressed in δ notation in permil (‰) relative to the standard Vienna Pee Dee Belemnite (VPDB). The laboratory chicken blood standard was standardised against C652 ANU (Australian National University) sucrose, 1577b bovine liver (National Institute of Standards and Technology) and SRM (Standard Reference Material) 1547 peach leaves (NIST).

Data analysis

One key limitation in many isotopic studies of consumer communities, including ours, is a lack of tissue-diet fractionation factors for each species, and variation in the metabolic routing of different diet components (Voigt et al. 2008; Chapter 6). Because this study dealt with a wide range of species, and because of the compounding effects of varying diet-tissue discrimination factors on different diets, we did not attempt to apply a mixing model to calculate the proportion of nectar carbon assimilated by birds (Hoefs 1980; Phillips and Gregg 2003). For species where sufficient samples were collected during each month we used a Friedman's ANOVA to compare isotopic values between months. However, for some species sufficient samples were collected to make statistical comparison between the pre-flowering and flowering period; here we used a Wilcoxon signed rank test. To compare between

months for each vegetation/insect category we used a Friedman's ANOVA. Because data were not normally distributed (Shapiro-Wilk W-test for normality), and because sample sizes were relatively small we used a Mann-Whitney *U*-test to compare overall isotopic values between vegetation types and their associated insect assemblages. All statistical analyses were conducted using Statistica 6.0 (1984-2004).

Results

Isotopic ratios of dietary items

During our six-month sampling period the $\delta^{13}C$ values of C_3 trees and C_4 grasses varied significantly between months (Friedman's ANOVA; $\chi^2 = 14.14$, $n = 5$, df = 5, $P = 0.01$; $\chi^2 = 17.00$, $n = 5$, df = 5, $P = 0.004$ respectively). The combined $\delta^{13}C$ values of C_3 trees and C_4 grasses at our study site were significantly different (Mann-Whitney *U*-test: $P < 0.001$; Table 1). The $\delta^{13}C$ values of *A. marlothii* nectar were similar across years (2005, -12.8 ± 0.1‰, $n = 2$; Table 1).

Table 1 Monthly $\delta^{13}C$ (‰ VPDB, mean ± SD) of vegetation and insects in the aloe forest at Suikerbosrand Nature Reserve during 2006. Monthly sample $n = 5$, unless otherwise indicated in parentheses. * flowers unopen and no nectar available.

Month	C_3 plant	C_4 grass	C_3 insects	C_4 insects	Flowers	Nectar
May	-27.5 ± 1.6	-13.5 ± 0.4	-23.8 ± 1.7	-17.7 ± 4.4	-	-
June	-27.9 ± 1.2	-14.9 ± 2.5	-22.5 ± 3.7	-18.2 ± 5.9	-	-
July	-28.1 ± 1.0	-18.8 ± 1.9	-22.5 ± 4.8 (3)	-15.1 ± 3.0	-13.1 ± 0.3 (3) *	-
August	-26.7 ± 1.6	-12.6 ± 0.5	-18.9 ± 3.8 (4)	-15.8 ± 3.7	-13.7 ± 0.5 (9)	-12.6 ± 0.5 (9)
September	-27.0 ± 1.6	-14.3 ± 1.1	-20.1 ± 4.3 (4)	-19.2 ± 5.8	-13.8 ± 0.2 (7)	-
October	-26.1 ± 1.2	-14.3 ± 1.9	-19.2 ± 6.1 (4)	-17.4 ± 2.1	-	-
Total	-27.2 ± 1.4	-14.7 ± 2.5	-21.3 ± 4.2	-17.2 ± 4.2	-13.7 ± 0.5 (19)	-12.6 ± 0.5 (9)

In neither the C_4 insects nor C_3 insects was there significant temporal variation in $\delta^{13}C$ values (Friedman's ANOVA; $\chi^2 = 8.43$, $n = 5$, df = 5, $P = 0.13$; $\chi^2 = 8.52$, $n =$

5, df = 5, $P = 0.13$ respectively; Table 1). Insects were very seldom observed feeding on nectar. The $\delta^{13}C$ values of the C_3 insect samples were significantly enriched compared to the C_3 plant samples (Mann-Whitney *U*-test; U = 14.0, $P < 0.001$; Table 1), whilst the C_4 insect samples were depleted compared to the C_4 grass samples (Mann-Whitney *U*-test; U = 262.0, $P = 0.005$; Table 1). Thus, whereas $\delta^{13}C$ values differed significantly between insects associated with each habitat type (Mann-Whitney *U*-test; U = 184.0, $P = 0.001$; Table 1), in neither case did insect $\delta^{13}C$ values represent the habitat in which they were sampled.

Isotopic ratios of avian blood

During the 2006 sampling seasons, 402 blood samples were collected from 381 individual birds (41 species) in the aloe forest. Our sampling included 32 of the 38 species known to feed on *A. marlothii* nectar at this site (Chapter 2). The six nectar-feeding species that we did not obtain blood samples from included two sunbird species. An additional 56 blood samples from 19 species were collected during 2005 and although not included in the comparisons are summarised as combined data in the Appendix. Avian community composition and the timing of nectar availability were similar in both years (Chapter 2).

Changes in $\delta^{13}C$ values between flowering and pre-flowering months varied within guilds and ranged from -3.5 to 3.0‰ for granivores, -0.7 to 7.3‰ for insectivores, 1.9 to 4.4‰ for omnivores and 1.8 to 3.2‰ for frugivores (Fig. 1). Three feeding guilds, namely frugivores, insectivores and omnivores, tended to exhibit an enrichment in blood $\delta^{13}C$ values during the flowering season compared to the pre-flowering season, whereas granivore values tended to become depleted in ^{13}C (Fig. 1). Only one granivorous species (laughing doves *Streptopelia senegalensis*) showed enrichment in blood $\delta^{13}C$ during the flowering season (Fig. 1). For most species $\delta^{13}C$ after flowering became depleted again, returning to values similar to prior to flowering (Appendix).

In at least 15 bird species, blood $\delta^{13}C$ exhibited a distinct shift towards enriched values around the time that *A. marlothii* nectar became available (see Fig. 1

and Appendix). In many species this enrichment between the non-flowering and flowering period was significant (Wilcoxon test, $P < 0.05$ Fig. 1).

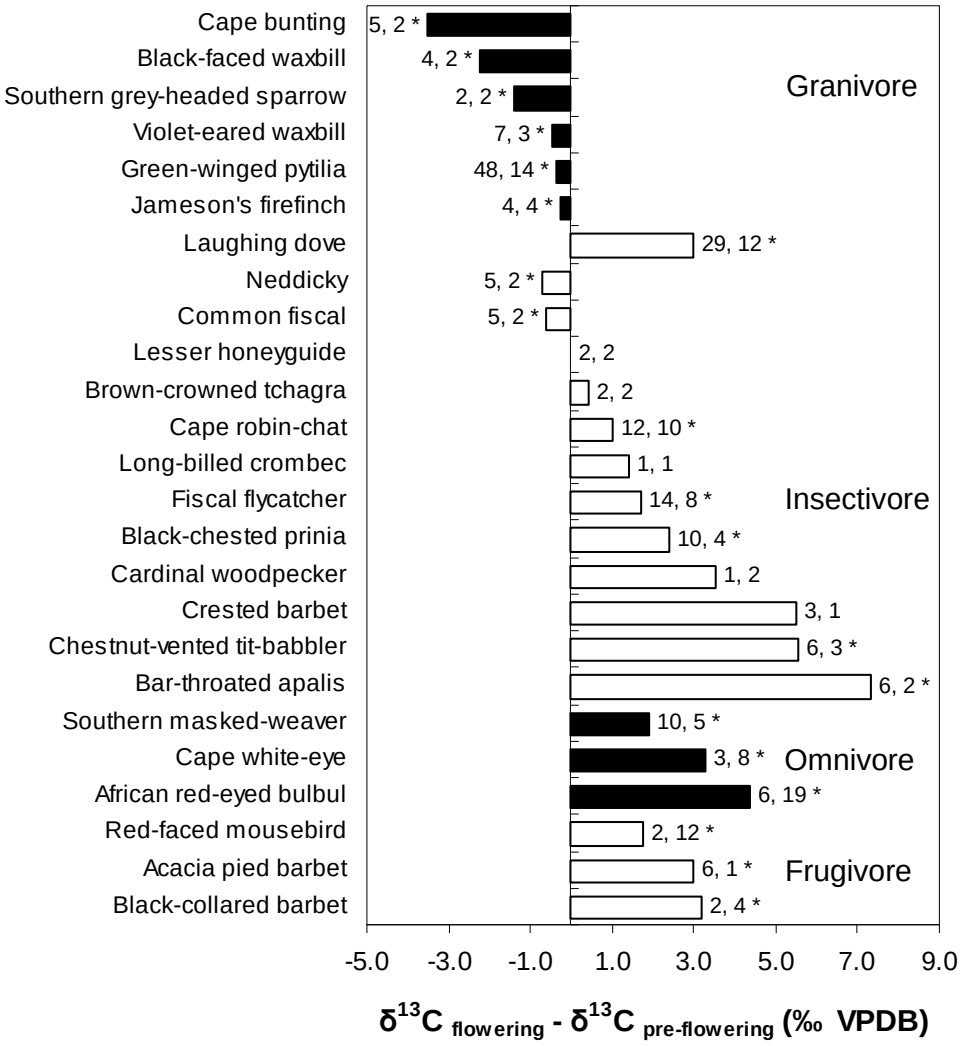

Fig. 1 Changes in blood $\delta^{13}C$ associated with the onset of flowering in *Aloe marlothii* at Suikerbosrand Nature Reserve Only species where samples were collected during pre-flowering (May-July) and flowering (August-September) periods are shown. Positive values indicate enrichment in ^{13}C and shift towards nectar isotopic signature. Sample size showing pre-flowering and flowering totals, * indicating significant difference between seasons (Wilcoxon test, $P < 0.05$), no analysis where $n < 3$. All species shown were recorded as nectar feeders (Chapter 2; Appendix).

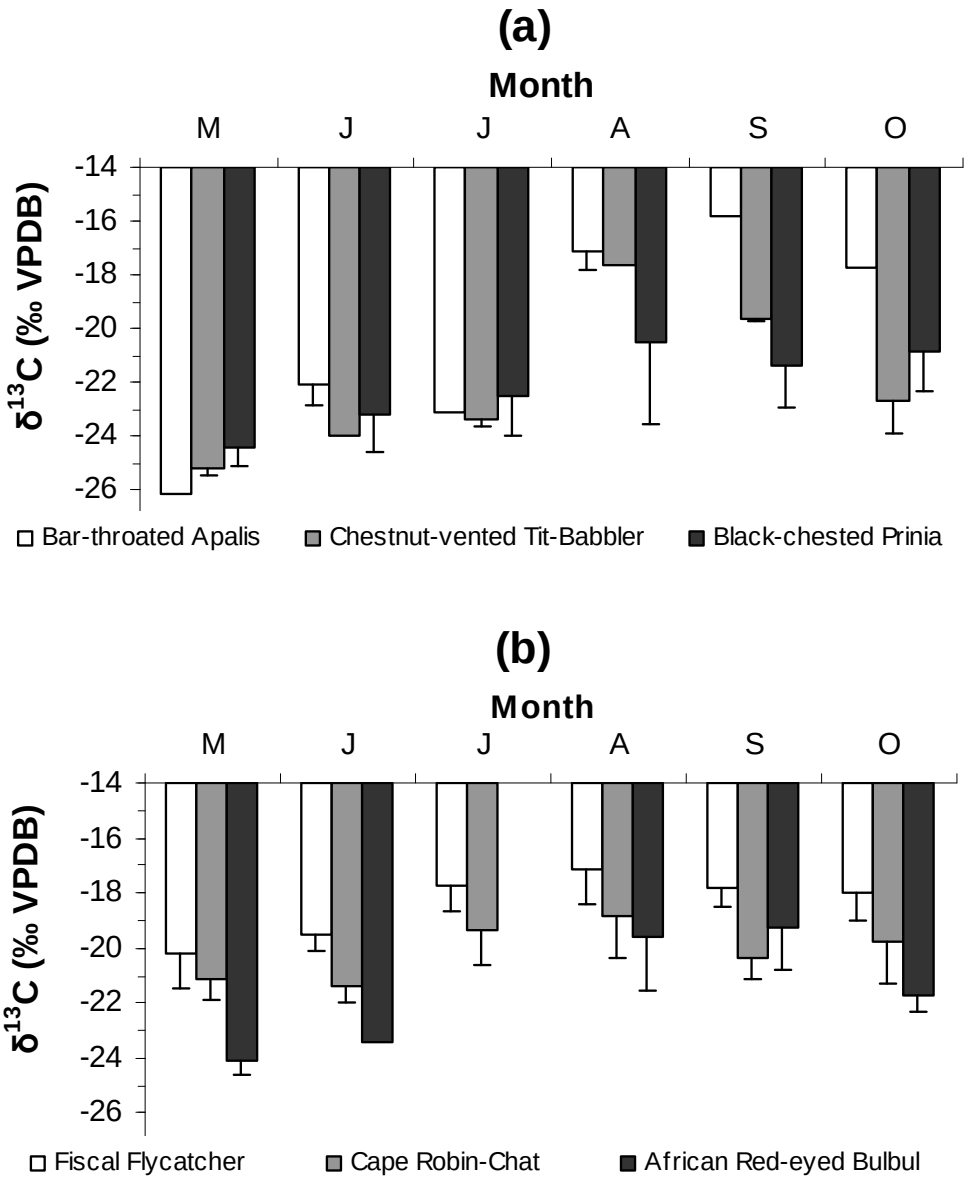

Fig. 2 Mean monthly changes in $\delta^{13}C$ (± SD) for a subsample of avian species recorded feeding on *Aloe marlothii* nectar in Suikerbosrand Nature Reserve, showing enrichment of ^{13}C during the flowering period (August-September). See Fig. 1 for species' sample sizes.

Few species were captured in all months, either because of seasonal changes in abundance (Chapter 2) or low capture rates for species. For species regularly observed feeding on nectar (Fig. 2; Chapter 2), where there were sufficient samples for analysis, there was a significant difference in blood $\delta^{13}C$ values between months for two species

(Fig. 2; Friedman's ANOVA, Fiscal flycatcher, $\chi^2 = 14.23$, $P = 0.01$; Cape robin-chat, $\chi^2 = 13.48$, $P = 0.02$) and no detectable change in one species (Black-chested prinia, $\chi^2 = 9.43$, $P = 0.09$). For non-nectar feeding species there was a monthly difference in blood $\delta^{13}C$ values between months for one species (Green-winged pytilia, $\chi^2 = 11.14$, $P = 0.03$) and no change in another species (Laughing dove, $\chi^2 = 19.57$, $P = 0.002$).

Table 2 General summary showing changes in blood $\delta^{13}C$ for different avian feeding guilds between pre-flowering (May-July) and flowering (August-September) periods at the *Aloe marlothii* forest, Suikerbosrand. Typical diet for each guild for different periods given (see Table 1 for $\delta^{13}C$ values of diets). To eliminate weighting by more common species each value was calculated as mean of species averages in each guild. Before flowering and during flowering values of whole blood (mean $\delta^{13}C \pm SD$) given.

Guild	Period	Diet	Mean $\delta^{13}C$ of whole blood (‰ VPDB)
Granivore	Pre-flowering	C_4 grasses, minor C_3 contribution	-13.2 ± 2.1 (*S. senegalensis* excluded; -12.4 ± 1.0)
	Flowering	nectar?, C_4 grass, some C_3 (*S. senegalensis* includes C_4 maize)	-12.9 ± 1.8 (*S. senegalensis* excluded; -13.0 ± 1.9)
Insectivore	Pre-flowering	C_3 & C_4 insects	-20.9 ± 2.6
	Flowering	nectar, C_3 & C_4 insects	-18.4 ± 2.1
Omnivore	Pre-flowering	C_3 & C_4 insects and fruit	-20.6 ± 4.3
	Flowering	nectar, C_3 & C_4 insects and fruit	-17.8 ± 3.6
Frugivore	Pre-flowering	C_3 & C_4 plants, some insects	-23.0 ± 0.8
	Flowering	nectar, C_3 & C_4 plants, some insects	-21.4 ± 1.7

To investigate whether enrichment of blood $\delta^{13}C$ was related to observed rates of nectar-feeding, we compared feeding rates from Chapter 2 to the mean $\delta^{13}C$ change for each species, but found no significant correlation (Spearman's R = - 0.126, $P < 0.05$, $n = 9$).

The origin of blood carbon varied among avian feeding guilds (Table 2). With the exception of granivores, all feeding guilds exhibited blood $\delta^{13}C$ values

intermediate between those of C_3 and C_4 photosynthetic pathways, whether derived from plant or insect diets (Table 2). We suspect that grass seeds were still available for granivores, possibly in abundance, during the *A. marlothii* flowering period (C.T.S. pers. obs.).

Discussion

Flowering in *A. marlothii* coincides with an isotopic shift towards CAM/C_4 values in the resident bird community. Since all the bird species that exhibited enriched blood $\delta^{13}C$ values during this period were regularly observed feeding on *A. marlothii* nectar (Chapter 2), it is highly likely that the enrichment of their tissues reflects the incorporation of carbon from this source. Although we cannot rule out an isotopic dietary shift caused by an increased reliance on C_4 plants, this explanation is not consistent with behavioural observations (Chapter 2). The enrichment of ^{13}C was evident in most nectar-feeding species during peak flowering, although in some species (e.g. bar-throated apalis *Apalis thoracica*) it was only evident in September, and in others (e.g. fiscal flycatcher *Sigelus silens*, Cape robin-chat *Cossypha caffra*) it was evident early in the flowering season when few flowers were open (Chapter 1).

Our study highlights a key limitation of community-level isotopic diet reconstructions: uncertainty regarding tissue-diet fractionation factors. Whereas "educated guesses" regarding fractionation factors for particular species can be made on the basis of published data (Hobson and Clark 1992b; Hobson and Bairlein 2003; McCutchan et al. 2003; Pearson el al. 2003; Carleton and Martínez del Rio 2005; Herrera et al. 2006), diet-tissue fractionation factors depend on animal body condition and several other factors (McCutchan et al. 2003; Pearson et al. 2003; Vanderklift and Ponsard 2003; Podlesak and McWilliams 2006; Voigt et al. 2008). Uncertainty in fractionation factors becomes particularly problematic when isotopic differences between dietary items of interest are small, since small errors in fractionation factors will significantly affect estimated contributions of dietary items. Determining this for all species was beyond the scope of the study, and until a clearer understanding of diet-

tissue fractionation is understood, certain assumptions, particularly in field studies, will need to be made if actual dietary contributions are to be calculated.

Aloe marlothii *and avian nectarivory*

Only two true nectarivores (Nectariniidae), white-bellied sunbird *Cinnyris talatala* and malachite sunbird *Nectarinia famosa*, were recorded feeding on the copious amounts of *A. marlothii* nectar, and they occurred in lower numbers than expected (Chapter 1, 2). The low numbers of sunbirds recorded at the study site is in contrast to large numbers of sunbirds that feed on *A. ferox* nectar in the Eastern Cape, South Africa (A.Craig pers. comm.). Although this may reflect regional patterns of sunbird diversity it is more likely due to nectar characteristics. At our study site it is possible that sunbirds do not feed on nectar of *A. marlothii* because it is too dilute. Nectars of low volume (*c.* 10-30 µl) and high concentration (*c.* 15-25% w/w) are utilised by specialist nectarivores (i.e. sunbirds and hummingbirds) whilst flowers with large nectar volumes (*c.* 40-100 µl) and low concentrations (*c.* 8-12%) are utilised by generalist pollinators (Johnson and Nicolson 2008). However, despite the apparent low sugar rewards offered, the nectar is an important resource for numerous generalist pollinators, particularly during dry South African winters (Chapter 2).

Oatley (1964) suggested that birds feeding on *A. marlothii* nectar in northern KwaZulu-Natal, South Africa, were not food stressed and used the winter flowering period in preparation for accumulation of reserves prior to breeding. At a time of little rain and possible reduced food availability we suggest that the strategy of feeding on nectar is used efficiently by many birds to supplement normal diets. Other aloe species, such as *A. ferox*, *A. speciosa*, *A. africana*, *A. barberae*, *A. vryheidensis* and *A. greatheadii* var. *davyana* are also visited by generalist birds for their nectar (Oatley 1964; Oatley and Skead 1972; Johnson et al. 2006; Botes 2007; Botes et al. 2008; C.T.S. pers. obs.) and it is suggested that throughout southern Africa the importance of aloes for birds may be under-estimated. In South Africa, at least 73 bird species in 24 families were recorded feeding on 14 *Aloe* species and eight other flowering plants and trees (Oatley and Skead 1972); for *A. marlothii* the number of species possibly exceeds

86 (Oatley 1964; Oatley and Skead 1972; Chapter 2; General Conclusion; C.T.S. pers. obs.).

Succulent plants and avian consumers

Our study provides further evidence that succulent plants that occur in moderate to high densities can significantly alter landscape-level energy and water fluxes if they produce nectar and/or fruit during the dry season. In terms of the importance of nectar as a resource for a diverse assemblage of avian consumers, striking similarities exist between *A. marlothii* in southern Africa and the saguaro cactus (*Carnegiea gigantea*), a CAM succulent in the Sonoran Desert of North America (Wolf and Martínez del Rio 2000; Wolf et al. 2002; Wolf and Martínez del Rio 2003). In both these systems, a seasonal pulse of food resources by a succulent species results in broad-scale diet switching in avian communities (Wolf and Martínez del Rio 2003). However, the timing of these resource pulses is different; flowering and fruiting of the saguaro occurs during hot summer months whilst flowering in *A. marlothii* occurs during winter (Wolf and Martínez del Rio 2003). However, in both systems, resources become available when conditions are dry and water resources are possibly limiting (Wolf and Martínez del Rio 2003).

Although the major resource offered to avian consumers by saguaros is fruit, whereas in *A. marlothii* it is nectar (Wolf and Martínez del Rio 2000), in both cases these resources result in significant shifts in the $\delta^{13}C$ value of consumers' tissue. However, when white-winged doves *Zenaidia asiatica* fed on saguaro nectar there was little, if any, shift in $\delta^{13}C$ values – the isotopic shift towards a CAM signature only occurred after fruit became available (Wolf and Martínez del Rio 2000). Nectar is consumed in significant quantities by white-winged doves and it is surprising, in view of the isotopic shifts associated with opportunistic nectarivory we observed in our study, that a similar carbon signal was not detected in white-winged dove tissues. This may be because the carbon of nectar was routed directly into metabolism and not tissue synthesis (Wolf and Martínez del Rio 2000; Voigt et al. 2008). In *A. marlothii* the fruit offers very little to consumers and only one species, streaky-headed canary *Serinus gularis* was very occasionally observed feeding on fruit.

Although an increase in bird abundance and diversity was observed at the aloe forest during flowering, the vast majority of species that utilize aloe nectar are non-migrant, year-round residents (Chapter 2). However, some species like starlings (Sturnidae) were common nectar feeders and they appeared in the area in large flocks. Sturnids are unable to digest sucrose (Martínez del Rio et al. 1988; Martínez del Rio and Stevens 1989; Martínez del Rio et al. 1992; Nicolson and Fleming 2003); however, aloes contain predominantly hexose sugars in the nectar (Van Wyk et al. 1993). Wattled starlings (*Creatophora cinerea*) are absent from our study site when aloes are not flowering so their arrival in the area suggests that nectar is of greater importance to them than previously suspected (Chapter 2). Whereas *A. marlothii* nectar is predominantly utilised by year-round resident species, saguaro nectar and fruit are utilized by both Sonoran Desert residents and long-distance migrants moving between Central and North America (Wolf and Martínez del Rio 2000, 2003).

Concluding remarks

The ability of animals to utilise seasonal pulses in food resources is particularly important when the availability of food varies spatially and temporally. The use of stable isotopes allows ecologists to quantify the use of isotopically distinct food types in the diets of organisms, and is particularly well-suited to quantifying diet switching in consumers during seasonal fluctuations in the availability of a particular dietary component. For example, isotopic analysis of blood samples demonstrated that salmon carcasses comprised a large proportion of the autumn diets of American martens *Martes americana* during years of low rodent abundance (Ben-David et al. 1997), and the annual variation in summer and winter diet of arctic foxes *Alopex lagopus* was correlated with lemming (*Dicrostonyx* and *Lemmus* spp.) abundance (Roth 2002). Stable isotopes have also been used successfully to demonstrate the relative importance of various dietary sources for wild birds; some have considered whole communities (Wolf and Martínez del Rio 2003; Herrera et al. 2003, 2006) whilst most have considered single or a few species (e.g. Hobson and Clark 1992b; Mizutani et al. 1992; Pain et al. 2004; Podlesak et al. 2005; Yohannes et al. 2005). In glaucous gulls *Larus hyperboreus* there was significant temporal and geographic variation in

terrestrial dietary components (Schmutz and Hobson 1998) and in light-bellied Brent geese *Branta bernicla* isotope analysis of blood demonstrated a temporal change in diet during the overwintering period (Inger et al. 2006).

By analysing blood, a tissue with a rapid turnover period, we were able to detect changes in diet for numerous bird species (Hobson and Clark 1992b; Dalerum and Angerbjörn 2005). We could not control for sampling the same species in all months so analysed the overall patterns within certain species and major feeding guilds, thus gaining a broad perspective of the temporal use of *A. marlothii* nectar by the avian community. However, the variation in the apparent importance of nectar, as reflected by enrichment in blood ^{13}C, may give a gross underestimation of nectar importance because nectar sugars may be immediately metabolised and not represented in bird blood (Chapter 6). This may be a broad reflection of individual species' responses to nectar and overall food availability at the time of the study.

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Appendix. Mean monthly isotope values ($\delta^{13}C \pm SD‰$ VPDB) of birds sampled in aloe forest during 2005 and 2006. Number of samples in parentheses; one unless indicated otherwise. * indicates recorded feeding on *Aloe marlothii* nectar (33 species; Chapter 2; C.T.S. pers. obs.). Major feeding guild; gr = granivore, in = insectivore, fr = frugivore, om = omnivore, with reference to Maclean (1993). Avian nomenclature follows Hockey et al. (2005).

Species	Guild	May	June	July	August	September	October
PHASIANIDAE							
Swainson's Spurfowl *Pternistis swainsonii*	gr	-	-15.8	-	-	-	-
INDICATORIDAE							
Lesser Honeyguide *Indicator minor* *	in	-	-22.2	-20.7 ± 0.5 (2)	-	-21.5 (2)	-
PICIDAE							
Cardinal Woodpecker *Dendropicos fuscescens* *	in	-19.7	-	-	-16.2 (2)	-	-14.8
LYBIIDAE							
Acacia Pied Barbet *Tricholaema leucomelas* *	fr	-22.8 (2)	-22.5 (2)	-22.7 ± 0.3 (2)	-19.5	-19.9	-22.6
Black-collared Barbet *Lybius torquatus* *	fr	-22.4 (2)	-	-	-19.3 ± 1.7 (4)	-	-
Crested Barbet *Trachyphonus vaillantii* *	in	-21.8	-21.7	-17.1 ± 1.6 (2)	-14.7	-	-
RHINOPOMASTIDAE							
Common Scimitarbill *Rhinopomastus cyanomelas* *	in	-	-	-23.6 (1)	-	-	-
COLIIDAE							
Speckled Mousebird *Colius striatus* *	fr	-	-	-	-21.3 (2)	-	-
Red-faced Mousebird *Urocolius indicus* *	fr	-	-	-24.4 ± 0.4 (2)	-22.4 ± 0.7 (12)	-22.8	-
COLUMBIDAE							
Laughing Dove *Streptopelia senegalensis* *	gr	-16.8 ± 2.5 (8)	-16.4 ± 2.2 (7)	-13.6 ± 2.2 (15)	-12.3 ± 1.3 (4)	-12.9 ± 1.7 (9)	-13.9 ± 1.7 (5)
Cape Turtle-Dove *Streptopelia capicola*	gr	-	-	-	-12.9	-	-
MALACONOTIDAE							
Brown-crowned Tchagra *Tchagra australis* *	in	-22.1	-19.7	-	-20.1	-20.9	-20.8
Bokmakierie *Telophorus zeylonus* *	in	-23.1 (2)	-20.3 (2)	-	-	-	-19.4
Chinspot Batis *Batis molitor*	in	-23.8	-	-	-	-	-
LANIIDAE							
Common Fiscal *Lanius collaris* *	in	-17.7 (2)	-18.8 (2)	-18.1 (1)	-	-18.8 ± 1.3 (3)	-
PARIDAE							
Ashy Tit *Parus cinerascens*	in	-24.9	-	-14.5 (1)	-	-	-
PYCNONOTIDAE							

Species	Guild	May	June	July	August	September	October
African Red-eyed Bulbul *Pycnonotus nigricans* *	om	-24.1 ± 0.5 (5)	-23.4	-	-19.6 ± 2.0 (26)	-19.3 ± 1.5 (5)	-21.7 (2)
SYLVIIDAE							
Fairy Flycatcher *Stenostira scita*	in	-	-	-21.9	-	-	-
Cape Grassbird *Sphenoeacus afer* *	in	-	-	-	-20.6	-	-
Long-billed Crombec *Sylvietta rufescens* *	in	-	-	-18.3	-14.8 (2)	-19.0	-
Chestnut-vented Tit-Babbler *Parisoma subcaeruleum* *	in	-25.2 ± 0.3 (3)	-24.0	-23.4 (2)	-17.6	-19.7 (2)	-22.7 ± 1.2 (6)
ZOSTEROPIDAE							
Cape White-eye *Zosterops capensis* *	om	-25.2 ± 0.5 (3)	-	-	-21.0 ± 1.4 (11)	-22.8 (2)	-22.5
CISTICOLIDAE							
Rattling Cisticola *Cisticola chiniana* *	in	-20.6	-	-	-15.7 (2)	-	-
Neddicky *Cisticola fulvicapilla* *	in	-	-17.2	-16.2 ± 0.2 (4)	-15.5	-19.3 (2)	-18.8 (2)
Black-chested Prinia *Prinia flavicans* *	in	-24.4 ± 0.7 (6)	-23.2 (2)	-22.5 (2)	-20.5 (2)	-21.4 (2)	-20.8 ± 1.5 (5)
Bar-throated Apalis *Apalis thoracica* *	in	-26.1	-22.1 ± 0.8 (4)	-23.1	-17.1 ± 0.7 (4)	-15.8	-17.7
ALAUDIDAE							
Sabota Lark *Calendulauda sabota*	gr	-	-15.8	-	-	-	-
MUSCICAPIDAE							
Cape Rock-Thrush *Monticola rupestris* *	in	-20.3	-	-	-	-	-
Fiscal Flycatcher *Sigelus silens* *	in	-20.2 ± 1.3 (6)	-19.5 ± 0.5 (6)	-17.7 ± 0.9 (3)	-17.1 ± 1.3 (11)	-17.8 ± 0.7 (3)	-18.0 ± 1.1 (5)
Cape Robin-Chat *Cossypha caffra* *	in	-21.2 ± 0.7 (5)	-21.4 ± 0.6 (4)	-19.3 ± 1.3 (4)	-18.8 ± 1.5 (8)	-20.4 ± 0.8 (7)	-19.8 ± 1.6 (3)
Kalahari Scrub-Robin *Cercotrichas paena*	in	-18.9	-	-	-	-	-
STURNIDAE							
Wattled Starling *Creatophora cinerea* *	in	-	-	-	-18.7 ± 2.3 (5)	-	-
PLOCEIDAE							
Cape Weaver *Ploceus capensis* *	om	-	-	-	-13.1 ± 0.9 (11)	-12.9 ± 1.2 (4)	-
Southern Masked-Weaver *Ploceus velatus* *	om	-19.3 ± 2.3 (6)	-14.9	-15.4 ± 3.8 (4)	-14.6 ± 2.7 (8)	-	-17.9 (2)
Red-billed Quelea *Quelea quelea*	gr	-	-	-	-	-	-13.0
ESTRILDIDAE							
Black-faced Waxbill *Estrilda erythronotos* *	gr	-13.0 ± 0.5 (4)	-	-	-15.3 ± 2.7 (3)	-	-
Violet-eared Waxbill *Granatina granatina* *	gr	-12.4 ± 0.1 (3)	-12.4 ± 0.6 (3)	-11.9	-12.7 ± 0.7 (3)	-	-13.0 (2)
Green-winged Pytilia *Pytilia melba* *	gr	-12.3 ± 0.3 (13)	-12.1 ± 0.3 (18)	-11.8 ± 0.2 (17)	-12.0 ± 0.6 (12)	-12.9 ± 1.9 (5)	-13.2 ± 0.7 (4)
Jameson's Firefinch *Lagonosticta rhodopareia* *	gr	-11.5 (2)	-11.4	-11.3	-11.8 (2)	-11.5 (2)	-

Species	Guild	May	June	July	August	September	October
PASSERIDAE							
Southern Grey-headed Sparrow *Passer diffusus* *	gr	-12.3 (2)	-	-	-	-13.7 ± 0.9 (3)	-
FRINGILLIDAE							
Cape Bunting *Emberiza capensis* *	gr	-	-	-14.3 ± 1.1 (5)	-	-17.9 (2)	-15.5
Number of samples	**458**	**83**	**60**	**72**	**141**	**58**	**44**
Number of species	**41**	**26**	**20**	**21**	**27**	**20**	**18**

V

CHAPTER 5

Interpreting an *Aloe marlothii* nectar diet shift using stable nitrogen isotopes

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Abstract

Aloe marlothii, a CAM succulent with a wide distribution in the savannah biome of northern and north-eastern South Africa, flowers during dry winter months and offers copious amounts (*c.* 250 µl/flower) of dilute nectar (*c.* 12% w/w) to numerous occasional avian nectarivores. The $\delta^{15}N$ and $\delta^{13}C$ of avian whole blood was measured during pre-flowering (May-July) and flowering months (August-September) of *A. marlothii* to determine possible temporal dietary and trophic level shifts that accompanied an increase in feeding on nectar. We expected that an increase in the dietary importance of nectar would result in a decrease in more depleted tissue $\delta^{15}N$ values (i.e. lower trophic position.) caused by a diet where the C:N ratio increased. During flowering the blood of most species that fed on nectar became enriched in ^{13}C suggesting that aloe nectar was assimilated as an important food source. The increased reliance on nectar was, however, not accompanied by the expected downward shift in $\delta^{15}N$ values. We did not apply mixing models nor attempt to estimate trophic levels, since we detected monthly changes in the plant and insect $\delta^{15}N$ (food base) values and our knowledge of diet-tissue discrimination factors for different species, diets and tissues is limited. Overall, birds that fed more often on insects (insectivores and to a lesser degree omnivores and frugivores) had higher $\delta^{15}N$ values and decreased more in body mass during the winter, when insect abundance (and N intake) decreased, than those that did not (i.e. granivores). Our results emphasize the need for a better understanding of changes in diet-tissue discrimination factors associated with changes in dietary C:N ratios, and temporal variation in the $\delta^{15}N$ of vegetation forming the base of food chains. For occasional avian nectarivores, nitrogen contributions from nectar are probably insignificant compared to that obtained from insects. Never-the-less, *A. marlothii* nectar represents an important food/carbon source for many bird species. We hypothesize that the unexpected increase in $\delta^{15}N$ values reflects an increase in the C:N ratios of diets resulting from decreases in N intake associated with opportunistic nectarivory

Keywords: nectarivore, winter-flowering, dilute nectar, Suikerbosrand, Asphodelaceae, Aloaceae

Introduction

Morphological, physiological, behavioural and ecological adaptations to nectarivory are most pronounced in the sunbirds (Nectariniidae), hummingbirds (Trochilidae) and honeyeaters (Meliphagidae) (Maclean 1990; Gartrell 2000; Nicolson and Fleming 2003). However, not all rely solely on a nectar diet and for many species insects are incorporated into the diet as an additional source of protein (Skead 1967; Daniels 1987; Maclean 1990; Markman et al. 1999, 2004; Gartrell 2000). For example, during breeding adult orange-tufted sunbirds *Nectarinia osea* may collect a greater number of insects to fulfil the protein requirements of nestlings (Markman et al. 1999, 2004). Similarly, for many non-nectarivorous bird species, the incorporation of nectar into the diet may supplement typical diets of insects, fruit or seeds when normal (staple) food resources are low (Chapter 4). For example, the common fiscal *Lanius collaris* (Laniidae), a rapacious insectivore/carnivore that was previously not known to feed on nectar, was recorded feeding on aloe nectar at Suikerbosrand, South Africa (Chapter 2). For other birds, diet may vary temporally because of changes in the availability of specific food items, different nutritional requirements during breeding and/or changes in diet when migrating (e.g. Arroyo 1997; Knoff et al. 2002). Therefore, for different reasons, birds may switch diet in their annual cycle.

Aloe marlothii is a Crassulacean acid metabolism (CAM) succulent that flowers during June-September in the savannah biome of South Africa (Reynolds 1969; Glen and Hardy 2000; van Wyk and Smith 2005). At least 86 bird species have been recorded feeding on the dilute nectar (*c.* 12% w/w) produced in abundance during a period when little rain occurs (Oatley and Skead 1972; Chapter 2; General Conclusion). *Aloe marlothii* nectar has been shown to contribute significantly to carbon intake of many bird species during the 5-10 week flowering period (Chapter 2, 4). However, it is not known whether this dietary shift affects trophic position. Many bird species appear to spend significant amounts of time to feeding on nectar and shifts in trophic levels during a time of corresponding low food availability are suspected.

Isotopic enrichment along a food chain is typically greater for ^{15}N than for ^{13}C, making nitrogen a more reliable element for analysing changes in trophic levels of

consumers (De Niro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987; Mizutani et al. 1992; Hobson et al. 1994; Hobson and Wassenaar 1999). Typically, the $\delta^{15}N$ values of a consumer's tissues are enriched by 3-5‰ relative to its diet (De Niro and Epstein 1981; Mizutani et al. 1992; McCutchan et al. 2003). If tissues that turn over rapidly are sampled, it is possible to detect trophic level changes over short time scales. For example, the isotopic signature of blood typically reflects the diet of an organism over a time scale of days to weeks (Hobson and Clark 1992a; Wolf and Martínez del Rio 2003; Carleton and Martínez del Rio 2005; Tsahar et al. 2008). Therefore, shifts in $\delta^{15}N$ values related to dietary changes may reflect trophic shifts.

We hypothesized that feeding on *A. marlothii* nectar by occasional nectarivores results in significant dietary shifts during the flowering season; this shift being manifested as depletion in blood $\delta^{15}N$ values because of a diet shift incorporating less animal matter. We predicted that this shift would be a response to a change in diet when nectar became available and nitrogen rich food sources (i.e. insects) became reduced in the dry winter period. An animal's trophic position is typically estimated by using a mixing model of the form,

$$\text{Trophic level} = \lambda + \left[\left(\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}}\right)/\Delta_n\right],$$

where λ is the trophic position of the organism ($\lambda = 1$ for primary producers), $\delta^{15}N_{\text{secondary consumer}}$ refers to the tissues of the consumer of interest, $\delta^{15}N_{\text{base}}$ is the corresponding value at the base of the food web, and Δ_n is the ^{15}N enrichment per trophic level (Ehleringer and Osmond 1989; McKechnie 2004). However, we did not apply mixing models that calculate trophic level position because of a lack of available knowledge on species-specific discrimination factors, diet quality, body condition and routing. Also, there is variation in $\Delta^{15}N$ between different consumers (e.g. herbivores and carnivores; Vander Zanden & Rasmussen 2001) so when the wrong discrimination factor is estimated there are large errors in the estimation of trophic position. Rather, we assessed overall temporal changes in species that related to an increase in nectar consumption during the dry winter period.

Materials and Methods

Study site

The study was conducted during 2006 at Suikerbosrand Nature Reserve, 60 km south-east of Johannesburg, South Africa, with sampling covering pre-flowering (May-July) and flowering periods (August-September) for *A. marlothii*. Vegetation at the study site is dominated by open savannah and highveld grassland habitats with large populations of *A. marlothii* dominating rocky north-facing slopes (Panagos 1999). Rainfall is seasonal, falling mostly in summer (October-March) months, and winter temperatures are warm during the day (*c.* 25^{o}C) and cold at night (*c.* 0^{o}C).

Sample collection

Mist-netting was conducted along a disused vehicle track through a large stand of *A. marlothii* in the western portion of Suikerbosrand Nature Reserve. All birds caught were ringed (Safring, Cape Town) so that recapture and sampling of the same individual could be monitored. A blood sample (10-50 µl) was collected from the brachial vein of each bird, labelled, returned to the laboratory and dried to constant mass in a drying oven at 50^{o}C (Chapter 4). δ^{15}N and δ^{13}C values were then simultaneously determined on a select number of samples.

During each month representative C_3 and C_4 vegetation samples were collected, as well as insects [4] associated with each vegetation type. Ten sweeps were conducted at each of five grass sites in the aloe forest; with no attempted bias to collect either flying or crawling insects. Insects collected were stored in alcohol (75%) and returned to the laboratory where they were identified and dried. Grass samples collected at each sweep site were enveloped and returned to the laboratory where they were dried (Chapter 4). Leaf samples were collected from each of five common tree species (*Acacia karoo*, *Ziziphus mucronata*, *Tarconanthus camphoratus*, *Gymnosporia heterophylla*, *Rhus leptodictya*) and an insect net placed beneath each plant was used

[4] Throughout the text we use the term insects even though sampling included other invertebrates (e.g. spiders) that insectivores may feed on.

to catch insects shaken from the tree (10 shakes). All insects were counted and identified and then dried before being fine ground in a mortar and pestle for isotopic analysis. All insect sampling occurred during mid-afternoon (14:00-16:00).

Isotope analyses

Isotopic analysis was conducted at the Natural Resources and the Environment isotope laboratory at the Council for Scientific and Industrial Research (CSIR), Pretoria. Representative samples (0.15-0.30 µg) were weighed in tin cups (pre-cleaned in toluene) and combusted at 1,020^{o}C in an Elememental Analyser (Flash EA, 1112 Series, Thermo ™). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios were then determined using a continuous-flow isotope ratio mass spectrometer (CFIRMS) (Thermo Finnigan, Delta V Plus) coupled to the EA. Two aliquots of a laboratory standard (dried chicken blood; mean $\delta^{13}C \pm SD = -17.87 \pm 0.09‰$; $\delta^{15}N \pm SD = 2.89 \pm 0.29‰$; $n = 104$) were used for every six unknowns in sequence, with duplicates run for each sample. The laboratory standard was standardised against C652 ANU (Australian National University) sucrose, 1577b bovine liver (National Institute of Standards and Technology) and SRM (Standard Reference Material) 1547 peach leaves (NIST). Isotope ratios are expressed in δ notation in parts per thousand (‰) relative to Vienna Pee Dee Belemnite for carbon and atmospheric air for nitrogen.

For determining plant (C_3 and C_4) and insect (C_3 and C_4) isotope values all samples ($n = 5$ per month) were run to determine $\delta^{13}C$ values for each month, whilst three samples were run to determine $\delta^{15}N$ values for each month (see Table 1). For plant samples $\delta^{13}C$ and $\delta^{15}N$ values were determined separately.

Data analysis

To compare isotopic values over the five-month period for each plant and insect category we used a Friedman's ANOVA. The combined vegetation samples for each plant/insect category were each compared using a Mann-Whitney *U*-test. To compare between the pre-flowering and flowering period within each plant/insect category we used Mann-Whitney *U*-tests. All bird species were not captured in all

months so we were unable to make comparisons between the pre-flowering and flowering period for all species. For species where we had sufficient samples to compare the $\delta^{15}N$ value of the pre-flowering period and the flowering period we used a Wilcoxon signed rank test. Samples collected during May-July represented pre-flowering months and August-September represented flowering months. We did not attempt to use a mixing model to calculate trophic positions for bird species because we were unable to account for different discrimination factors that were diet-, tissue or species-specific. All values are given as mean ± SD. All statistical analyses were conducted using Statistica 6.0 (1984 - 2004).

Results

Blood samples were obtained from 178 birds, representing 32 species (18 families), during five months that covered the pre-flowering and flowering periods (Appendix; Chapter 4 for further $\delta^{13}C$ values for birds over six months).

Plant and insect isotopic values

For each vegetation type the $\delta^{15}N$ values did not differ significantly among months (Friedman's ANOVA; C_3 plants, $\chi^2 = 5.33$, $n = 3$, df = 4, $P = 0.25$; C_4 grass, $\chi^2 = 6.13$, $n = 3$, df = 4, $P = 0.19$; Table 1). There was no overall difference in $\delta^{15}N$ values between the vegetation types (Mann-Whitney, $U = 69.0$, $P = 0.07$) nor the monthly overall plant $\delta^{15}N$ values among months (Friedman's ANOVA; $\chi^2 = 8.67$, $n = 6$, df = 4, $P = 0.07$; Table 1). For insects associated with each vegetation type the $\delta^{15}N$ values differed significantly among months (Friedman's ANOVA; C_3 insects, $\chi^2 = 9.87$, $n = 3$, df = 4, $P = 0.04$; C_4 insects $\chi^2 = 7.60$, $n = 2$, df = 4, $P = 0.11$; Table 1). However, overall the insect $\delta^{15}N$ values did not differ significantly among months (Friedman's ANOVA; $\chi^2 = 5.44$, $n = 5$, df = 4, $P = 0.25$; Table 1). Overall, C_3 insects were more enriched in ^{15}N than C_4 insects although this difference was not significant (Mann-Whitney, $U = 57.0$, $P = 0.06$).

Table 1. Mean (± SD) of $\delta^{15}N$ for C_3 tree, C_4 grass, C_3 insect and C_4 insect samples during the study period (May-September) at Suikerbosrand Nature Reserve (*n* = 3 for each category each month). C_4 trees measured each month included *A. karoo*, *Z. mucronata* and *R. leptodictya*.

Period	Month	C_3 tree (‰ Air)	C_4 grass (‰ Air)	Plant mean (‰ Air)	C_3 insect (‰ Air)	C_4 insect (‰ Air)	Insect mean (‰ Air)
Pre-flowering	May	3.7 ± 0.6	4.4 ± 2.4	2.3 ± 1.8	6.7 ± 0.3	5.4 ± 0.5	5.8 ± 2.3
	June	0.6 ± 0.5	2.4 ± 0.1		9.5 ± 1.2	3.0 ± 0.0	
	July	0.2 ± 1.0	2.4 ± 0.7		5.3 ± 0.9	2.9 ± 0.1	
Flowering	August	0.1 ± 1.7	0.9 ± 1.6	1.0 ± 1.7	5.1 ± 0.5	8.0 ± 1.4	5.6 ± 1.8
	September	1.6 ± 2.8	1.5 ± 0.9		4.6 ± 0.6	3.9 ± 0.2	
Mean		1.2 ± 1.9	2.3 ± 1.7	1.8 ± 1.9	6.2 ± 1.9	4.9 ± 2.1	5.6 ± 2.1

The $\delta^{15}N$ of nectar samples were not measured since nectar contains very little nitrogen; any nitrogen in amino acids occurs in very low quantities and although it may be of importance to bird diets would be difficult to detect using stable isotope analysis (Nicolson 2007; see below).

Bird isotopic values

Not all species were captured in all months so we were unable to make comparisons between the pre-flowering and flowering periods for all the species we obtained data from; changes in those species with sufficient samples are depicted in Figure 1, whilst all $\delta^{15}N$ values are shown in the Appendix. The change in $\delta^{15}N$ varied between species with a depletion in ^{15}N was most prominent in an omnivore (African red-eyed bulbul) and a granivore (violet-eared waxbill, change not significant though) and an enrichment most prominent in a frugivore (Acacia pied barbet; Fig. 1). All frugivores for which we obtained sufficient data to compare across seasons (*n* = 3) became enriched in ^{15}N while most insectivores became depleted in ^{15}N.

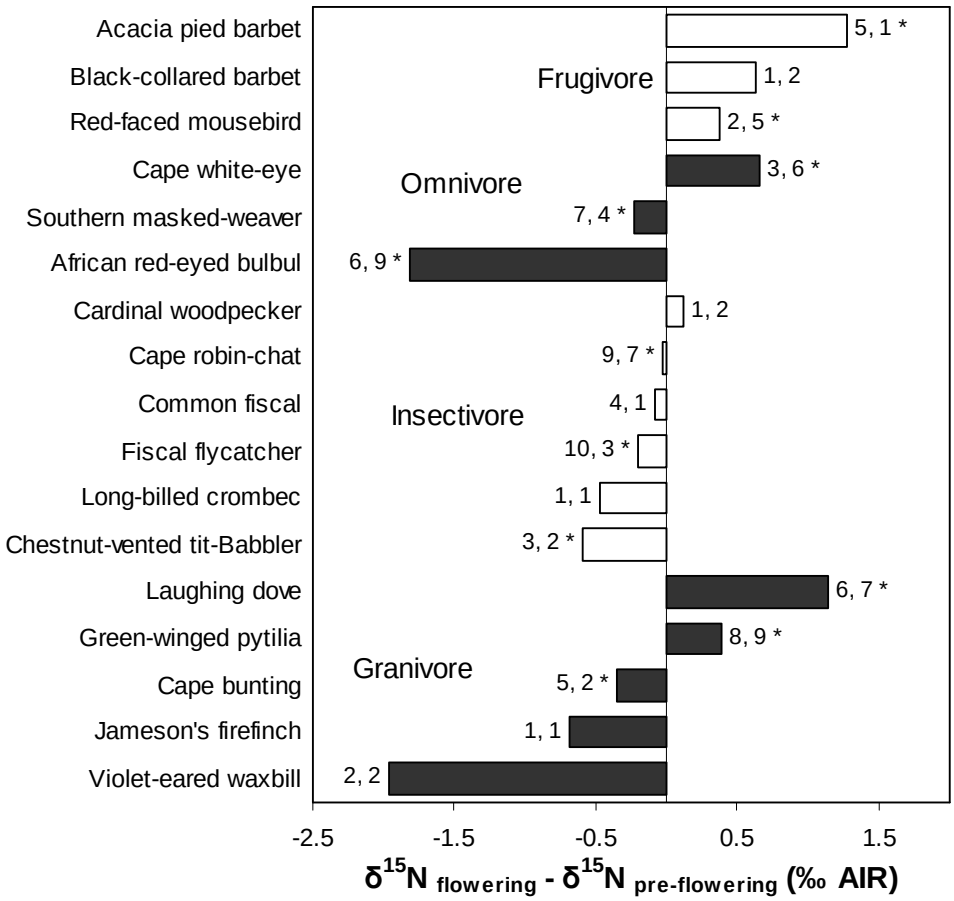

Figure 1. Isotopic depiction of an avian community at the *Aloe marlothii* forest, Suikerbosrand Nature Reserve, showing changes in blood $\delta^{15}N$ between pre-flowering (May-July) and flowering (August-September) period. Only species where samples were sampled in both periods are shown. Positive values indicate enrichment in ^{15}N. Sample size showing pre-flowering and flowering totals, * indicates significant difference between seasons (Wilcoxon test, $P < 0.05$), no analysis where $n < 3$ (Appendix).

Bird body mass and insect abundance

Body mass data were collected to monitor changes in bird condition related to changes in food availability (i.e. predominantly insects). There was a general decrease in the percentage body mass loss of birds in different guilds between the pre-flowering and flowering months (Table 2). Most individual species decreased in mass during the flowering period, although these decreases were not significant (Wilcoxon test, $P > 0.05$).

Table 2. Summary of mean $\delta^{15}N$ (± SD) values of birds in different feeding guilds during *Aloe marlothii* pre-flowering (May-July) and flowering months (August-September), and percentage mass decrease (± SD) of birds between pre-flowering and flowering periods. Sample size in parentheses indicates number of species.

Feeding guild	$\delta^{15}N$ (‰)		Mass decrease
	Pre-flowering	Flowering	
Frugivore	6.1 ± 0.97 (5)	6.5 ± 1.3 (4)	0.8 ± 7.2 (2)
Granivore	5.1 ± 0.69 (8)	5.4 ± 1.2 (9)	2.2 ± 7.3 (7)
Insectivore	7.0 ± 0.9 (29)	6.9 ± 0.7 (12)	4.7 ± 6.6 (9)
Omnivore	6.2 ± 1.0 (5)	6.1 ± 1.7 (7)	2.5 ± 10.5 (3)
Total	6.5 ± 1.1 (47)	6.3 ± 1.3 (32)	3.2 ± 7.0 (21)

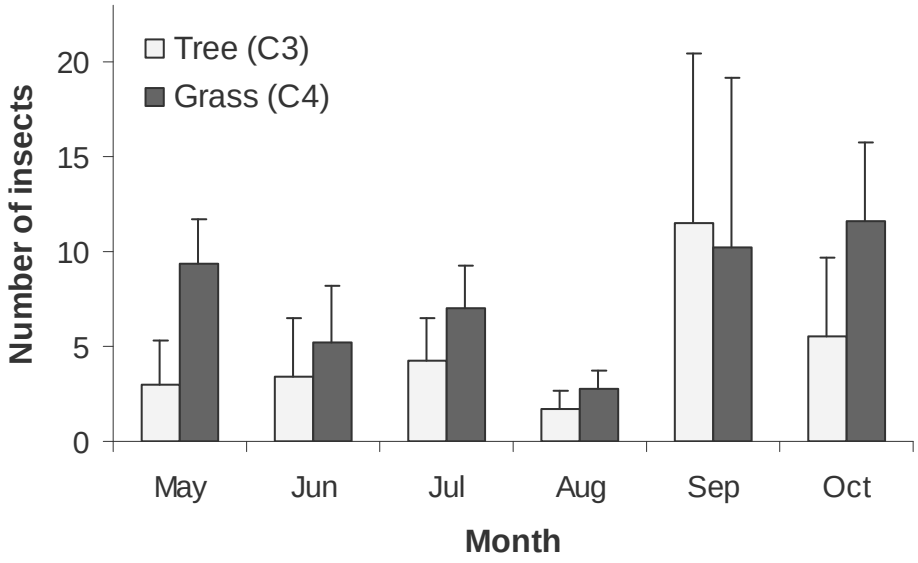

Figure 2. Mean number (± SD) of insects (including other invertebrates) collected during sweep (C_4 grass) and shake (C_3 tree) sampling in the *Aloe marlothii* forest at Suikerbosrand Nature Reserve.

The mean number of insects collected each month differed significantly (Kruskal-Wallis test, $H_{5, 54} = 13.98$, $P = 0.02$) with the lowest number of insects captured during peak flowering in August (Fig. 2).

Discussion

Tropic level shifts and a low concentration nectar diet

When birds fed opportunistically on *A. marlothii* nectar there was no change in the $\delta^{15}N$ of whole blood; the expected change being one that would represent a downward trophic level shift. This finding contradicts our prediction that whole blood of birds that fed on *A. marlothii* nectar would become depleted in ^{15}N because of a diet shift to nectar. However, if a monthly depletion in the $\delta^{15}N$ of food available (i.e. C_3 and C_4 plants) for nectar feeders were accounted for then an upward shift in trophic position would become apparent in most birds (assuming discrimination factors remain constant). This upward trophic shift would suggest that feeding on nectar by opportunistic nectarivores constitutes a significant dietary change, during a period when normal food resources are possibly low.

However, in our study the interpretation of temporal changes in $\delta^{15}N$ values, and associated trophic level shifts, is complicated by insufficient knowledge on diet-tissue discrimination factors, dietary changes in different species and temporal changes in $\delta^{15}N$ of food sources (i.e. vegetation and insects). Numerous other factors can affect diet-tissue fractionation and studies to date have addressed these issues for a range of animal species, mostly under controlled conditions in captivity (see Post 2002; Vanderklift and Ponsard 2003; Robbins et al. 2005). Interpreting trophic shifts in a diverse community of consumers, as in our study, is complicated by a number of factors. For example, discrimination factors may be affected by, i) prey type, ii) prey quality, iii) temperature, iv) form of nitrogen excretion, v) habitat type, vi) water stress, and vii) nutritional status (Ambrose 1991; Hobson and Clark 1992a,b; Hobson et al. 1993; Hobson et al. 1994.; Pinnegar and Polunin 1999; Perkins and Speakman 2001; Bearhop et al. 2002; Vanderklift and Ponsard 2003; Pearson et al. 2003; Evans Ogden et al. 2004; Cherel et al. 2005; Robbins et al. 2005; Podlesak and McWilliams 2006). Because we do not know more about discrimination factors related to these factors we are unable to make clear interpretations, concerning trophic positions, within the community of nectar-feeding birds.

Also, we do not know much about changes in fractionation factors of ^{15}N caused by changes in dietary C:N. This is a further factor that may confound our interpretations of trophic levels during the pre-flowering and flowering periods for different feeding guilds. The dilute nectar of *A. marlothii* contains amino acids (that contribute to the nitrogen content of nectar) in concentrations greater than most other aloes (Nicolson 2007). However, the overall C:N ratio in *A. marlothii* nectar is very high (*c.* 95; van Wyk et al. 1993 for mean of sugar type proportion; Nicolson 2007 for amino acid data; Chapter 1 for concentration data), so there is likely very little nitrogen contribution to the diet of birds from aloe nectar. Feeding on nectar thus increases the overall C:N ratio of ingested food. Unfed animals have been found to show higher $\delta^{15}N$ values due to recycling of endogenous nitrogen as body mass is lost without replacement of preferentially excreted ^{14}N (Hobson et al. 1993). In studies where animals have high C:N ratio diets (nitrogen becomes limiting at higher C:N ratios) the diet-tissue discrimination factor is therefore shown to be higher (Adams and Sterner 2000; McCutchan et al. 2003; Vanderklift and Ponsard 2003; Robbins et al. 2005; Tsahar et al. 2008), although Hobson and Bairlein (2003) found no significant difference in discrimination factor for different C:N diets for garden warblers. Thus we hypothesize that the increased $\delta^{15}N$ in many species reflects a change in dietary C:N, rather than a trophic shift. If the discrimination factor were in fact to be increased (e.g. by *c.* 0.8‰) to account for a reduction in nitrogen intake, and we were to account for the seasonal downward shift in vegetation values between flowering and non-flowering months, then the trophic position prior to flowering for all birds would, on average, be the same as that prior to flowering.

The importance of insects

Aloe marlothii nectar has low sugar concentrations and we suggest that nectar is used as a carbohydrate food resource that is rapidly metabolized during periods of low food availability (Voigt et al. 2008; Chapter 6). The increase in nectar intake and decrease in insect abundance, during the dry winter months, coincides with body mass loss in many species that feed on nectar. Similarly, in Australia where insect abundance is lowest during the dry season, and many birds tend to lose mass, nectar may be an important alternate food for insectivores (Keast 1985). Seasonal changes in

the use of insects by birds have also been demonstrated, using stable nitrogen isotopes, in a tropical dry forest in Mexico where an increase in insect consumption in the rainy season matched the phenology of food resources (Herrera et al. 2006).

During our six-month study period there was little change in the $\delta^{15}N$ signature of insects, or any correlation with that of the vegetation type (C_3 or C_4) in which they were found. This implies that although plants may have become nitrogen-stressed during periods of water stress (indicated by a decrease in $\delta^{15}N$ values during June, July and August for C_3 trees and August for C_4 grasses; Table 1), insects did not. Insect values more likely represent nitrogen incorporated over a relatively long period of time, covering both the pre-flowering and flowering seasons (see Table 1). The most reasonable calculation would therefore be to use monthly insect values, a more accurate representation for food nitrogen base for each month, to calculate trophic positions for birds (Post 2002). This uniform value may be more representative of diet for determining trophic position. However, the $\delta^{15}N$ values of insects were similar to those of the birds that fed on them, and certainly no more than the *c.* 2.5‰ fractionation factor we would expect with a single trophic level shift. Either the insects we sampled are not representative of what the birds were feeding on or the insect-bird tissue fractionation factors are not as high as the literature suggests. We can only speculate so far and, without a clear understanding of $\delta^{15}N$ values at the base of the food chain, there is no way to determine whether $\delta^{15}N$ of organisms reflect changes in the trophic position or just variation in $\delta^{15}N$ at the base (Post 2002).

Insect eating guilds (insectivores and omnivores) decreased most in mass, although not significantly, suggesting they were more food stressed during the flowering period. To compensate for a decrease in food availability (i.e. insects) they probably increased their nectar intake, thereby decreasing the proportion of nitrogen ingested. If the diet-tissue fractionation factor decreases with increased C:N intake, as suggested by Pearson et al. (2003) who found a general decrease in discrimination factors with a reduction in insects for yellow-rumped warblers (i.e. reduced nitrogen intake and increased C:N in diet) (see also Caut et al. 2008), then this could explain a decrease in $\delta^{15}N$ of whole blood for insect eating guilds (i.e. insectivores and omnivores) only. For non-insect eaters (frugivores and granivores) the insect reduction

may have affected the C:N of their diet less because they decreased less in mass. However, the overall diet of non-insect eaters may still have been reduced in nitrogen by an increase in nectar intake; although they may be better adapted at coping on diets of lower nitrogen concentrations. Any increase in $\delta^{15}N$ values for non-insect eaters could only be explained by an increase in fractionation factors between pre-flowering and flowering. However, both these scenarios are assuming that the base remains the same, which did not. These results may, therefore, be misinterpreted due to our lack of understanding of $\delta^{15}N$ in the environment.

Concluding remarks

The use of stable isotopes has provided insight into the use of food resources by animals not otherwise possible (Ehleringer and Osmond 1989; Kelly 2000; Dalerum and Angerbjörn 2005). Whereas diet analyses have usually considered ingested material, gut contents or excreted matter to infer diets for animals, stable isotopes are able to provide an interpretation of assimilated matter using non-destructive means (Hobson and Clark 1993; Phillips and Gregg 2003). Although a greater understanding of diet-tissue fractionation factors is required before more convincing conclusions can be reached, our results have demonstrated the complications faced in elucidating the complexities of trophic levels in a diversity of opportunistic nectar feeders using nitrogen stable isotopes. For a clearer understanding of trophic positions more reliable estimates of $\delta^{15}N$ at the base, and changing diet-tissue fractionation factors related to diet switches, particularly over a long period of time, are required (Post 2002).

The early publication of Oatley (1964) questioned whether the attraction of opportunistic nectarivores to *A. marlothii* was for nectar sugars, water in the dilute nectar, or the insects attracted to the nectar. It has previously been demonstrated that during flowering of *A. marlothii* an enrichment of ^{13}C in the whole blood of many bird species that fed on nectar was only detected with a large sample size (Chapter 4). For nitrogen the situation is more complex, and any apparent dietary shift is complicated by temporal changes in isotope ratios of food resources (food base) and changing fractionation factors under different environmental and physiological conditions.

These factors may even differ between species so inter-species comparisons using generic diet-tissue discrimination factors should be treated with caution. In recent years the use of stable isotopes has provided valuable insight into the complexities of plant-bird interactions (Wolf and Martínez del Rio 2000; Wolf et al. 2002; Herrera et al. 2003; Wolf and Martínez del Rio 2003; Herrera et al. 2006) and further research may be required to understand trophic level and isotope partitioning in a diverse bird community that does not usually feed on nectar.

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Appendix. Mean (± SD ‰ Air) of $\delta^{15}N$ values for bird species at Suikerbosrand during May-September. Sample size in parentheses for each month, *n* = 1 unless indicated otherwise. No SD where *n* = 2. * Nectar feeders. Guilds indicated by ins = insectivore, fr = frugivores, gr = granivore, om = omnivore, with reference to Maclean (1993). Taxonomy follows Hockey et al. (2005).

Species	Guild	May	Jun	Jul	Aug	Sep	Pre-flower	Flower	Tot
INDICATORIDAE									
Lesser Honeyguide *Indicator minor*	ins	-	6.1	10.2	-	-	8.1	-	8.1
PICIDAE									
Cardinal Woodpecker *Dendropicos fuscescens*	ins	5.6	-	-	5.7 (2)	-	5.6	5.7	5.7 ± 0.1
LYBIIDAE									
Acacia Pied Barbet *Tricholaema leucomelas*	fr	6.2 (2)	5.9 (2)	5.0	7.1	-	5.7 ± 0.7	7.1	6.0 ± 0.7
Black-collared Barbet *Lybius torquatus*	fr	5.6	-	-	6.2 (2)	-	5.6	6.2	6.0 ± 1.3
Crested Barbet *Trachyphonus vaillantii*	ins	6.7	7.0	7.1 (2)	-	-	6.9 ± 0.2	-	7.0 ± 0.2
RHINOPOMASTIDAE									
Common Scimitarbill *Rhinopomastus cyanomelas*	ins	-	-	8.7	-	-	8.7	-	8.7
COLIIDAE									
Speckled Mousebird *Colius striatus*	fr	-	-	-	4.9 (2)	-	-	4.9	4.9
Red-faced Mousebird *Urocolius indicus*	fr	-	-	7.6 (2)	8.0 ± 1.5 (5)	-	7.6	8.0	7.8 ± 1.3
COLUMBIDAE									
Laughing Dove *Streptopelia senegalensis*	gr	-	4.4 (2)	5.1 ± 0.7 (4)	5.8 ± 0.4 (3)	6.1 ± 1.7 (4)	4.7 ± 0.5	6.0 ± 0.2	5.4 ± 1.1
Cape Turtle-Dove *Streptopelia capicola*	gr	-	-	-	7.7	-	-	7.7	7.7
MALACONOTIDAE									
Brown-crowned Tchagra *Tchagra australis*	ins	-	-	-	7.3	7.5	-	7.4	7.4
Bokmakierie *Telophorus zeylonus*	ins	-	6.6	-	-	-	6.6	-	6.6
LANIIDAE									
Common Fiscal *Lanius collaris*	ins	7.2	7.2 (2)	7.0	-	7.1	7.1 ± 0.1	7.1	7.1 ± 0.1
PARIDAE									
Ashy Tit *Parus cinerascens*	ins	-	-	6.5	-	-	6.5	-	6.5
PYCNONOTIDAE									
African Red-eyed Bulbul *Pycnonotus nigricans*	om	6.8 ± 0.3 (5)	5.5	-	4.9 ± 1.0 (8)	4.5	6.2 ± 1.0	4.7 ± 0.3	5.5 ± 1.2
SYLVIIDAE									
Cape Grassbird *Sphenoeacus afer*	ins	-	-	-	8.0	-	-	8.0	8.0
Long-billed Crombec *Sylvietta rufescens*	ins	-	-	6.1	-	5.6	6.1	5.6	5.9 ± 0.3
Chestnut-vented Tit-Babbler *Parisoma subcaeruleum*	ins	7.5	6.9	8.0	6.5	7.2	7.5 ± 0.6	6.9	7.2 ± 0.6

Species	Guild	May	Jun	Jul	Aug	Sep	Pre-flower	Flower	Tot
ZOSTEROPIDAE									
Cape White-eye *Zosterops capensis*	om	7.5 ± 0.4 (3)	-	-	7.6 ± 0.9 (4)	9.3 (2)	7.5	8.4 ± 1.2	7.9 ± 1.0
CISTICOLIDAE									
Neddicky *Cisticola fulvicapilla*	ins	-	-	6.9	-	-	6.9	-	6.9
Black-chested Prinia *Prinia flavicans*	ins	6.4	6.5	6.5 ± 0.4 (3)	-	-	6.5 ± 0.0	-	6.5 ± 0.3
Bar-throated Apalis *Apalis thoracica*	ins	6.8	6.9 ± 0.8 (4)	6.7	-	-	6.8 ± 0.1	-	6.9 ± 0.6
MUSCICAPIDAE									
Fiscal Flycatcher *Sigelus silens*	ins	6.2 ± 2.0 (4)	7.2 ± 0.3 (4)	6.6 (2)	6.5 ± 1.8 (3)	-	6.7 ± 0.5	6.5	6.6 ± 1.3
Cape Robin-Chat *Cossypha caffra*	ins	7.2 ± 1.0 (4)	6.5 ± 0.3 (3)	6.8 (2)	7.8	6.7 ± 0.5 (6)	6.8 ± 0.4	7.2 ± 0.8	6.9 ± 0.7
STURNIDAE									
Wattled Starling *Creatophora cinerea*	ins	-	-	-	6.7 ± 1.9 (3)	-	-	6.9	6.7 ± 1.9
PLOCEIDAE									
Cape Weaver *Ploceus capensis*	om	-	-	-	5.8 ± 0.4 (6)	5.7 ± 0.4 (3)	-	5.7 ± 0.0	5.7 ± 0.4
Southern Masked-Weaver *Ploceus velatus*	om	5.2 ± 1.6 (5)	-	6.0 (2)	5.2 ± 0.8 (4)	-	5.6 ± 0.5	5.2	5.4 ± 1.1
ESTRILDIDAE									
Black-faced Waxbill *Estrilda erythronotos*	gr	-	-	-	5.5	-	-	5.5	5.5
Violet-eared Waxbill *Granatina granatina*	gr	-	4.8	6.7	3.8 (2)	-	5.7	3.8	4.8 ± 2.0
Green-winged Pytilia *Pytilia melba*	gr	-	4.7 ± 0.4 (5)	5.1 ± 0.5 (3)	5.2 ± 1.0 (7)	5.3 (2)	4.9 ± 0.3	5.3 ± 0.1	5.1 ± 0.8
Jameson's Firefinch *Lagonosticta rhodopareia*	gr	-	-	5.0	4.3	-	5.0	4.3	4.6
FRINGILLIDAE									
Cape Bunting *Emberiza capensis*	gr	-	-	5.0 ± 0.3 (5)	-	4.7 (2)	5.0	4.7	4.9 ± 0.4
Grand Total		6.5 ± 1.2 (30)	6.1 ± 1.1 (29)	6.3 ± 1.3 (36)	6.0 ± 1.5 (59)	6.3 ± 1.5 (24)	6.3 ± 0.2	6.2 ± 0.2	6.2 ± 1.3

VI

CHAPTER 6

Isotopic routing and its implications for the utilisation of sugars and water in *Aloe marlothii* nectar by opportunistic avian nectarivores

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Abstract

Aloe marlothii is a CAM succulent that flowers during the dry winter period in the savannah biome of northern and north-eastern South Africa. Flowers produce copious amounts (*c.* 250µl/flower) of dilute (*c.* 12% w/w) nectar with a distinctive isotopic signature ($\delta^{13}C$ = -12.6‰) that is utilised by numerous bird species for a 5-10 week flowering period. Stable isotope ratios ($\delta^{13}C$, $\delta^{18}O$) were measured in feather, blood and breath samples in eight opportunistic avian nectarivore species, and in nectar and water samples (δD, $\delta^{18}O$), during peak flowering. Granivores (*n* = 2 species) had a predominantly C_4 grass seed diet during feather growth (summer), whilst omnivores (*n* = 2 species), insectivores (*n* = 3 species) and nectarivores (*n* = 1 species) sourced carbon from a C_3 diet during feather growth. During the flowering season, most species showed a greater enrichment of ^{13}C in breath than in blood; this enrichment indicating more routing of income energy nectar sugars directly to metabolism. Species that showed less enrichment of ^{13}C in exhaled CO_2 likely obtain a significant proportion of energy from endogenous reserves assimilated prior to flowering, or routed nectar sugars to stored energy. The higher variation of $\delta^{13}C$ in exhaled CO_2 than in blood suggests that in different species there is heterogeneity in the routing of nectar carbohydrates (i.e. directly to metabolism or to stored energy reserves).

Aloe marlothii nectar was significantly more enriched in $\delta^{18}O$ (and δD) and than two other water sources near the aloe forest. Enrichment in the $\delta^{13}C$ of exhaled CO_2, related to an increased reliance on nectar sugars, was not correlated with enrichment in $\delta^{18}O$ of exhaled CO_2; also the $\delta^{18}O$ value of *A. marlothii* nectar water was significantly more enriched in ^{18}O than that detected in CO_2 of exhaled breath, suggesting that the intake of nectar is primarily for carbohydrates. However, because we do not know enough about the proportion of drinking water that contributes to body water, and changes in ^{18}O between nectar water (source) and breath (tissue), we cannot quantify with any confidence the contribution of nectar water to body water sources. During peak flowering birds were observed in great numbers drinking at a water trough. This suggests that other water sources, besides dilute nectar, are important for water balance during the dry winter period.

Keywords: *Aloe marlothii*, nectarivore, *Nectarinia famosa*, stable isotope, $\delta^{18}O$, $\delta^{13}C$

Introduction

Temporal changes in the diets of animals can be traced by measuring the ratios of stable isotopes in different tissues (Kelly 2000; Phillips and Gregg 2003; Podlesak et al. 2005). This is because different tissues have different turnover rates; for example isotopic signatures of breath[5] will reflect immediately metabolized nutrients, blood will reflect dietary contributions over a time scale of days to a few weeks and muscle will reflect a longer period of dietary incorporation of up to several months (Hobson and Clark 1992a; Carleton and Martínez del Rio 2005; Podlesak et al. 2005). Feathers, on the other hand, remain metabolically inert after synthesis, and thus reflect dietary contributions during the period of feather growth (Mizutani et al. 1992; Kelly 2000). Comparisons of the isotopic signatures of different tissues can therefore be used to infer past dietary patterns on different temporal scales, which in turn can reflect spatial patterns of dietary intake (Perkins and Speakman 2001; Hatch et al. 2002; Podlesak et al. 2005; Dalerum and Angerbjörn 2005; Carleton et al. 2006).

Aloe marlothii, a CAM succulent, grows predominantly on north-facing slopes in the savannah biome of north and north-eastern South Africa (Reynolds 1969; Glen and Hardy 2000; van Wyk and Smith 2005). During flowering in dry winter months its copious (*c.* 250 µl) dilute (*c.* 12% w/w) nectar is available to numerous bird species and at Suikerbosrand Nature Reserve, 60 km south-east of Johannesburg, South Africa, a large proportion (59%) of birds recorded during the flowering period fed on nectar (Chapter 1, 2; Fig. 1). When nectar ($\delta^{13}C$ = -12.6‰) became available there was an enrichment in ^{13}C in blood, suggesting that a wide range of species that do not typically feed on nectar benefit from this seasonally available resource (Chapter 4). This isotopic enrichment reflects the fact that most species at this site (excluding granivores) feed on diets with a predominantly C_3 isotopic signature ($\delta^{13}C$ = *c.* -27 ‰; Chapter 4). Although the carbon of nectar sugars is represented in blood during the flowering period, it is not clear how these sugars are used as an energy source by birds. Some dietary items, for example carbohydrates, may not enter tissue synthesis pathways, but rather enter metabolic pathways directly (Perkins and Speakman 2001;

[5] Although breath is not a tissue, for ease of comparison with true tissue, we refer to breath as "tissue" throughout the article (following Podlesak et al. 2005).

Voigt et al. 2008). Particularly for food stressed animals, nectar diets that are routed directly to metabolic processes may thus be under-represented in tissues important for storing food reserves, e.g. muscle and blood (Perkins and Speakman 2001; Carleton et al. 2006). Therefore, if the isotopic signature of blood is measured, the importance of nectar for nectar- feeding birds may be under-represented if it is routed directly into catabolic pathways rather that being assimilated into tissues.

Figure 1. *Aloe marlothii* inflorescence **(a)**, flowers on raceme showing acropetalous flower opening **(b)**, African red-eyed bulbuls *Pycnonotus nigricans* on *A. marlothii* inflorescence **(c)**, malachite sunbird *Nectarinia famosa* (immature male) about to probe flower for nectar **(d)**, and Cape white-eye *Zosterops capensis* feeding on nectar, the only species recorded feeding on racemes by hanging underneath **(e)**.

In this study we investigated the utilisation of nectar by a number of bird species in an occasional nectarivore community. It was hypothesized that, as found in broad-tailed hummingbirds *Selaphorus platycercus*, a large proportion of daily energy expenditure would be fuelled by immediately-metabolised sugars (Carleton et al. 2004, 2006). Immediately metabolised energy for an animal can be a mixture of dietary items assimilated during numerous past feeding events (Perkins and Speakman 2001). Because many bird species lose body mass during the *A. marlothii* flowering period we predicted that a greater proportion of metabolised energy would be supplied by income resources (i.e. nectar). This is because if ingested material consists of simple carbohydrates it is less likely to be represented in body stores, but rather utilized immediately (Perkins and Speakman 2001; Hatch et al. 2002; Podlesak et al. 2005; Carleton et al. 2006). The carbon in exhaled CO_2 represents immediately metabolised energy resources so we expected to find greater variation in breath carbon isotopic values compared to blood isotopic values among individuals during the *A. marlothii* flowering period. This variation would reflect the variable use of C_3 and C_4/CAM resources; such variation also reflecting the time since the last nectar meal. This greater variation would in turn also represent the exploitation of a sporadic and locally abundant food resource (Perkins and Speakman 2001).

Because *A. marlothii* flowers produce copious amounts of dilute nectar during the dry season (winter) we hypothesized that nectar would represent an important water resource for many bird species. Oxygen isotope ratios in body water are directly related to those of expired carbon dioxide, so the $\delta^{18}O$ values of exhaled CO_2 reveal $\delta^{18}O$ values in body water (Lifson et al. 1949; Lifson and McClintock 1966; Lifson et al. 1975). However, only a proportion of body water is sourced from drinking water; additional contributors to the $\delta^{18}O$ of body water being atmospheric O_2 and food (Podlesak et al. 2008). Also, body water is not in isotopic equilibrium with exhaled water vapour and breath CO_2 because of the equilibrium fractionation that occurs when water changes phase from liquid to gas across the surfaces of the lungs (water vapour is more depleted than water) and ^{18}O fractionation/equilibration between water and CO_2 respectively (Lifson et al. 1955; Schoeller 1988). Therefore, although we predicted an enrichment in ^{13}C (sugars) of blood to be correlated with an enrichment in ^{18}O (water) (related to the assimilation of nectar sugars and water in a highly

evaporated nectar source respectively), we did expect that fractionation processes between source (water) and tissue (breath CO_2) would make quantifying the proportion of body water from nectar more difficult.

Methods

Study site

The study was conducted at the *A. marlothii* forest in the west of Suikerbosrand Nature Reserve. During the winter flowering period there is little rainfall, with day temperatures reaching *c.* 25°C and night temperatures *c.* 0°C. Vegetation at the site is dominated by mixed *Acacia* savanna with tens of thousands of mature *A. marlothii* plants growing on rocky north-facing slopes. Grassveld habitats dominate higher altitudes.

Sample collection

Birds were mistnetted along an abandoned vehicle track through the *A. marlothii* forest over four days during peak flowering of *A. marlothii* in August 2007. All birds captured were ringed so that recapture and sampling of the same individual could be monitored. Representative individuals of nectar-feeding species and species not recorded feeding on nectar were sampled. Blood samples (10-50 µl) were collected from the brachial vein of birds by pricking the vein with a 25-gauge needle and collecting blood in a 75 µl non-heparinised capillary tube. Labelled samples were returned to the laboratory where they were allowed to dry in a drying oven at 50°C. Dried blood was then weighed (0.10-0.35 µg) into tin cups (pre-cleaned in toluene) for isotope analysis. For each bird a section of the tip of the first rectrix feather was cut, rinsed in a mix of 2:1 chloroform:methanol [6] and dried for isotopic analysis. Sections of feather were then cut with stainless steel scissors and weighed (0.10-0.35 µg) into tin cups for isotopic analysis.

[6] Feather oils have been shown not to affect isotope ratios of feathers (Mizutani et al. 1992); cleaning was done to remove other possible contaminants like human body oils from handling.

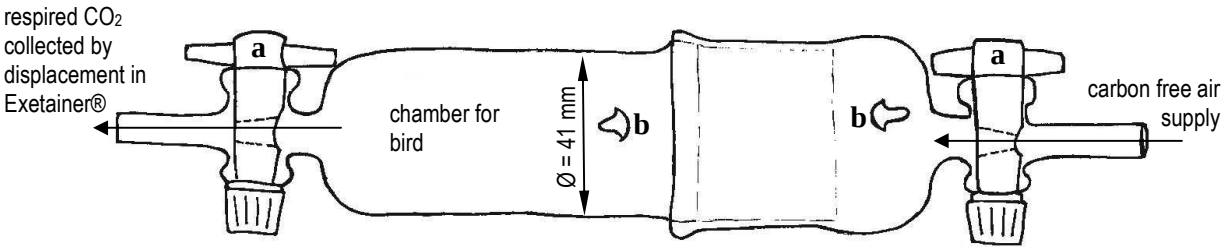

Figure 2. Glass chamber for collecting breath samples from birds. **(a)** indicates teflon stopcocks for controlling gas flow and sealing the chamber during accumulation of respired CO_2; **(b)** indicates hooks for elastic band to hold cone and socket parts of chamber together. Illustrator: C.T. Symes.

Breath samples were collected from captured birds using a sealed chamber (empty volume = 205 ml). The empty volume of the sealed chamber was significantly reduced (up to 60%) by the presence of the bird in the chamber during breath collection, thereby justifying the flow rate implemented (see below). The chamber was constructed using two modified ground pyrex glass cone and socket parts (B45) with a 25 mm teflon stopcock to control gas flow at each end (Fig. 2). One end was connected to a carbon-free gas supply (Afrox® Instrument Grade Zero, Johannesburg; Fig. 2). The bird was placed in the chamber, as soon after capture as possible, for *c.* 60 s with a steady flow (*c.* 100-200 $ml.min^{-1}$) of gas through the chamber, to clear it of any residual atmospheric carbon gases (i.e. CO and CO_2). The chamber was then sealed for *c.* 40-60 s to allow for an accumulation of respired CO_2 from the contained bird. The time allowed for exhaled CO_2 build-up in the chamber was also sufficient to minimize the effect of any possible atmospheric CO_2 contamination (C. Martínez del Rio pers. comm.). The bird's condition was continually monitored through the glass chamber. A breath sample was then collected by displacement in a 10 ml biosilicate gas-tight glass Exetainer® vial (Labco Ltd, High Wycombe), by continuing the gas supply flow for another 50-60 s. The gas (bird breath) entered the Exetainer® via a needle pierced through the airtight seal, displacing gas (CO_2-free) that escaped through a second needle open to the atmosphere. The breath samples were labelled and returned to the isotope laboratory where they were analysed within two days of sampling. This allowed for minimum fractionation with atmospheric gases. However, we did not

remove respiratory water in breath to prevent $\delta^{18}O$ in the breath equilibrating with $\delta^{18}O$ in CO_2 in the collection vial.

Isotope analysis

Representative C_3 (tree) and C_4 (grass) vegetation samples were collected during peak flowering (August) the previous year because there was little variation in samples between six months (Chapter 4). Ten sweeps were conducted at five grass sites in the aloe forest for C_4 samples. A leaf sample (C_3 samples) was collected from five tree species (*Acacia karroo, Ziziphus mucronata, Tarconanthus camphoratus, Gymnosporia heterophylla, Rhus leptodictya*), common at the study site. All samples were placed in separate labelled envelopes and returned to the laboratory where they were oven dried at 50^{o}C to constant mass. Samples were then finely ground using a mortar and pestle before being weighed in preparation for isotope analysis.

Nectar samples were collected from three flowering *A. marlothii* plants and during peak flowering three water samples were collected at the same time from two surface water sources, a stream and animal drinking trough both near the aloe forest. Approximately 2 ml of nectar/water were collected and placed in sealed glass vials, and returned to the isotope laboratory for $\delta^{18}O$ (and δD) analysis. $\delta^{13}C$ values for dried nectar were obtained from nectar samples collected the previous year (Chapter 4). $\delta^{13}C$ values of nectar between 2005 and 2006 did not differ (Chapter 4).

Isotopic analysis was conducted at the Natural Resources and the Environment isotope laboratory, at the Council for Scientific and Industrial Research (CSIR), Pretoria. Blood and feather samples (*c.* 0.15-0.30mg) were weighed in tin cups (cleansed in toluene) and combusted at 1,020^{o}C to CO_2 in an elemental analyser (Flash EA, 1112 Series, Thermo Electron Corporation). The $^{13}C/^{12}C$ ratios were then determined using a continuous-flow isotope ratio mass spectrometer (CFIRMS) (Thermo Delta V Plus, Thermo Electron Corporation) coupled to the elemental analyser. All samples were analysed in duplicate with every six samples being followed by two laboratory standard aliquots (homogenized dried chicken blood; mean $\delta^{13}C \pm SD = -17.87 \pm 0.13‰$, $n = 104$) to correct for equipment drift. The solid

laboratory standard was standardised against C652 ANU sucrose, 1577b (NIST) bovine liver and 1547 peach leaves (NIST). Isotope ratios are expressed in δ notation in parts per thousand (‰) with Vienna Pee Dee Belemnite (VPDB) as a standard for carbon, and Vienna Standard Mean Ocean Water (VSMOW) for oxygen and hydrogen.

The breath (gas) samples were placed on a GC PAL gas bench connected to the CFIRMS where the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios were determined. A laboratory gas standard (CO_2; mean $\delta^{13}C \pm SD = -31.41 \pm 0.22$‰; mean $\delta^{18}O \pm SD = -22.25 \pm 0.36$‰; $n = 8$) was used for every five unknowns in sequence. Water was extracted from breath samples in-line so only the $\delta^{18}O$ values of CO_2 were measured.

Liquid samples were processed in a Thermo Finnigan thermo chemical elemental analyser (TC/EA) and gases transferred to the CFIRMS via the Thermo Finnigan open split Interface Conflo III. A liquid autosampler automatically loaded samples. Dual measurements of hydrogen (D/H) and oxygen ($^{18}O/^{16}O$) from a single sample (water or nectar) were performed using the TC/EA, Conflo III and IRMS system, and corrected against international laboratory standards (SLAP 603, GISP 542) and a laboratory standard.

Results

Feather ($n = 51$), blood ($n = 47$) and breath ($n = 51$) samples were collected from 51 individuals representing eight species over four days during peak *A. marlothii* flowering (late-August).

Isotopic environment

Nectar water was significantly enriched in ^{18}O compared to water collected from the water trough (Mann-Whitney *U*-test; $U = 0.00$, $P < 0.05$) which in turn was more enriched than the water from the nearest stream ($U = 0.00$, $P < 0.05$) (Table 1).

Although we did not measure δD values in birds sampled, these values for the trough and stream are given to demonstrate and emphasize the differences in isotope values of water sources available to birds. The $\delta^{18}O$ and δD values of the nectar samples (n = 3) fitted well to an evaporative water line, extrapolating onto the Global Meteoric Water Line (GMWL; Craig 1961) to suggest that water in aloes was obtained from light rain showers. The trough water samples (n = 3) were also typical of a water source subject to evaporation (i.e. enriched $\delta^{18}O$ and δD values) whilst those for the stream were typical of rain for the region obtained from light showers (Craig 1961).

Plant $\delta^{13}C$ values collected during August the previous year (2006) are given in Table 1 since there was little temporal variation over six months during that period (Chapter 4).

Table 1. Isotope ratios of C_3 plants, C_4 grasses, water and nectar samples at and near the *Aloe marlothii* forest during flowering (mean ‰ ± SD, sample size in parentheses). * Chapter 4.

	C_3 (trees)	**C_4 (grass)**	**Nectar**	**Water (trough)**	**Water (stream)**
$\delta^{13}C$ (‰ VPDB) *	-26.7 ± 1.6 (5)	-12.6 ± 0.5 (5)	-12.6 ± 0.5 (9)	-	-
$\delta^{18}O$ (‰ VSMOW)	-	-	17.6 ± 3.5 (3)	6.2 ± 0.4 (3)	3.7 ± 1.9 (3)
δD (‰VSMOW)			54.3 ± 11.1 (3)	24.5 ± 2.1 (3)	19.7 ± 9.5 (3)

Dietary shifts

All species, except two weavers, had diets that were C_3-dominated during moult in non-flowering months, as indicated by the $\delta^{13}C$ values of feather samples (Fig. 3). A dietary shift towards a C_4/CAM diet during *A. marlothii* flowering was detected, to varying degrees, in different species as indicated by enriched $\delta^{13}C$ values for blood and breath samples. All species, except Kalahari scrub-robin *Cercotrichas paena*, are recorded nectar feeders. However, not all nectar feeders showed enrichment in ^{13}C values in blood and breath (Fig. 3).

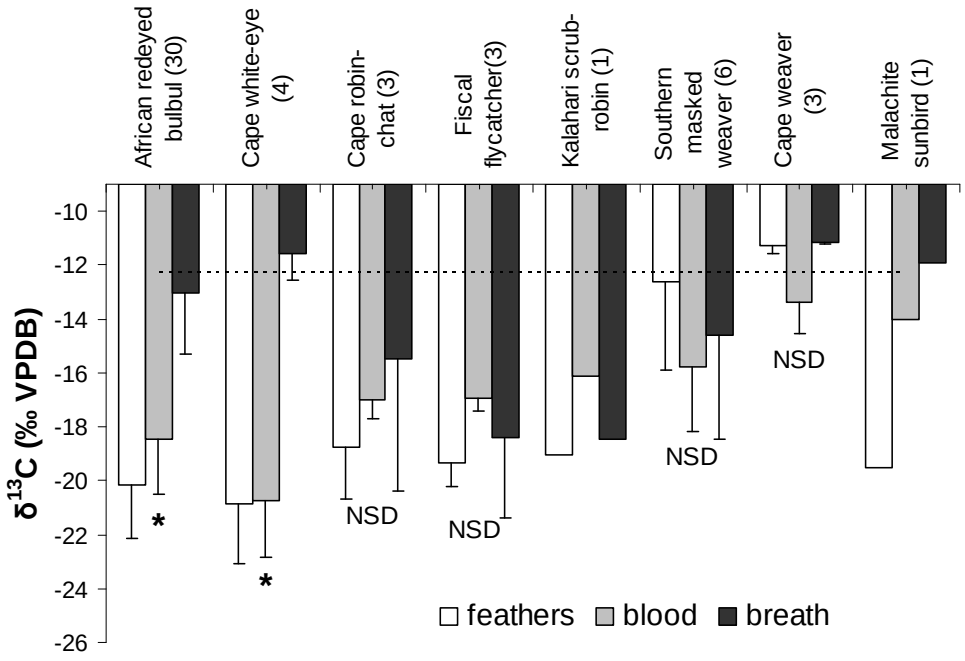

Figure 3. Mean (± SD) of $\delta^{13}C$ values for feathers (rectrix), whole blood and breath, * indicates significant difference and NSD indicates no significant difference between tissue types (Friedman's ANOVA, $P < 0.05$) from bird species captured in the *Aloe marlothii* forest at Suikerbosrand NR during peak flowering. Nectar $\delta^{13}C$ isotopic signature indicated by dotted line at -12.6‰. Sample size in parentheses.

Use of income resources

Figure 4 indicates the changes in $\delta^{13}C$ for breath, blood and feather samples through a day for African red-eyed bulbuls *Pycnonotus nigricans* which were sampled first at 06:30, approximately 30 min after first light. The $\delta^{13}C$ values of neither tissue type varied through time (Spearman's R, $P > 0.05$), so we compared overall values for each tissue. The breath, blood and feather $\delta^{13}C$ values differed significantly (RM-ANOVA, $F = 1098.5$, df = 2, $n = 24$, $P < 0.001$). A Tukey's HSD post-hoc test indicated breath samples, which were most similar to the nectar signature, were significantly more enriched in ^{13}C than blood samples ($P < 0.001$) and feather samples ($P < 0.001$), whilst blood and feather samples were similar ($P = 0.287$).

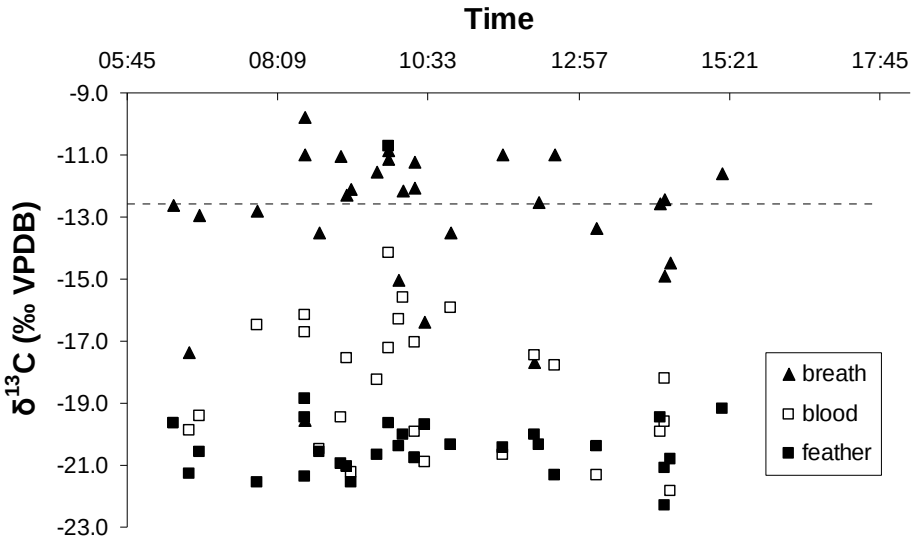

Figure 4. $\delta^{13}C$ values of breath, blood and feather samples collected in a day during peak flowering of *Aloe marlothii* for African red-eyed bulbul *Pycnonotus nigricans* (*n* = 30). The $\delta^{13}C$ of nectar (-12.6‰ VPDB) is indicated by a dashed line.

The use of Aloe marlothii *nectar water determined from* $\delta^{18}O$

The isotopic relationship between $\delta^{13}C$ and $\delta^{18}O$ in exhaled CO_2 for birds captured at the *A. marlothii* forest (not all nectar-feeding species) is shown in Figure 5. We expected that when the $\delta^{13}C$ in the CO_2 of nectar feeders became more enriched because of feeding on nectar, so the $\delta^{18}O$ of breath CO_2 (in equilibrium with $\delta^{18}O$ of body water) would also become more enriched. The more enriched $\delta^{18}O$ value represents a highly evaporated water source, possibly that from nectar (Table 1). However, there was no positive correlation between the $\delta^{13}C$ and $\delta^{18}O$ values of breath samples for African red-eyed bulbuls (Fig. 5; Spearman's $R = 0.346$, $P > 0.05$).

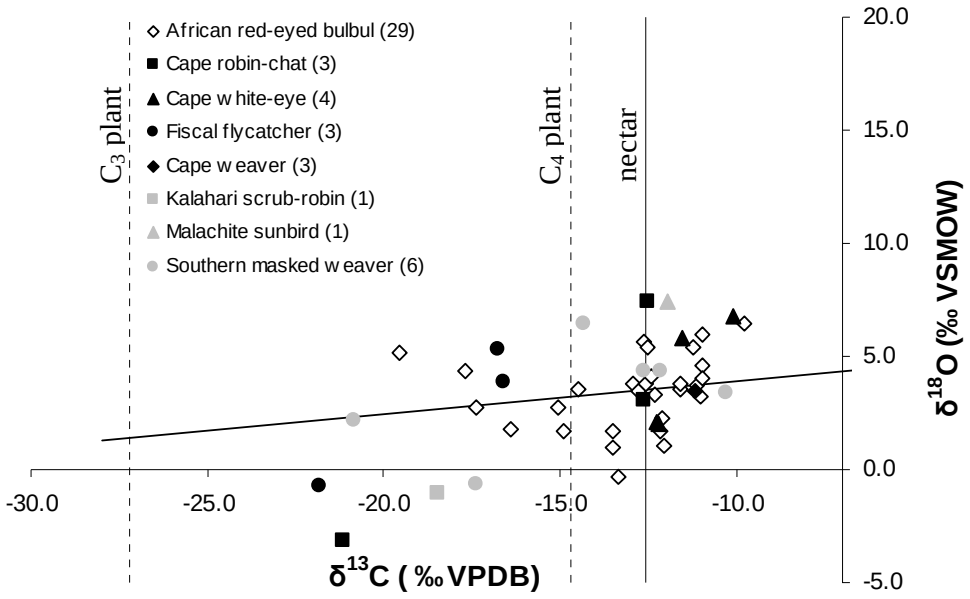

Figure 5. Relationship between $\delta^{13}C$ and $\delta^{18}O$ in exhaled CO_2 of eight bird species (n = 48) captured during peak flowering of *Aloe marlothii*. $\delta^{13}C$ of nectar sugars and C_3 and C_4 plants at the study site are also shown. (Correlation of African red-eyed bulbul samples not significant, y = 0.1452x + 5.3327, Spearman's R = 0.346, P > 0.05; See Table 1 for $\delta^{18}O$ values of water and nectar water samples. Samples sizes in parentheses).

Discussion

A significant dietary shift

The use of nectar as an income resource for opportunistic nectarivores is emphasized by the differences in ^{13}C of breath, blood and feather samples of nectar-feeding species (most evident in bulbuls; Fig. 3, 4). However, this dietary shift was not evident in all species analysed. Tissues with the highest turnover rates provide information on the most recent dietary history (Perkins and Speakman 2001), and in our study there was a greater enrichment in $\delta^{13}C$ of breath than for blood. This isotopic response was only detectable because most (non-granivore) bird species have a typically C_3 based diet during summer months (Chapter 4). It is particularly interesting to note that the shift in breath isotopic values was most prominent in both omnivore species (i.e. Cape white-eye *Zosterops capensis* and African red-eyed bulbul) and the

single nectarivore (i.e. malachite sunbird), suggesting that their ability to utilise a sugar diet is greater than that of insectivores. However, in two insectivore species (i.e. Cape robin-chat and fiscal flycatcher) that were regular nectar feeders (Chapter 2) there was no difference between tissues suggesting little reliance on nectar sugars for energy. Although the two omnivorous weaver species (i.e. southern masked-weaver *Ploceus velatus* and Cape weaver *Ploceus capensis*) were regular nectar feeders, we cannot comment with confidence on the contribution of nectar to their diet because feather and blood isotopic values were strongly influenced by a C_4 contribution to the diet (i.e. C_4 grass seeds). Both weaver species were regular nectar feeders, arriving at the aloe forest in abundance during flowering (Chapter 2).

The change in isotopic signatures of animal tissues with a corresponding change in diet is typical of many studies that have used stable isotopes to investigate the diets of birds. For example, Podlesak et al. (2005) used carbon isotope analysis of breath, blood, faeces and feathers to infer changes, and the timing of changes, in diets of wild birds and Alexander et al. (1996) showed that diets of migrating shorebirds, as indicated by stable isotopes, were different to those indicated by gut content analysis. However, few terrestrial studies have analysed tissues for changes in diet with contrasting isotopic signatures (but see Wolf and Martínez del Rio 2000; Wolf et al. 2002; Wolf and Martínez del Rio 2003). More common are studies of birds that investigate marine and freshwater/terrestrial inputs where $\delta^{13}C$ signatures are unique to each system (Hobson 1987, 1990; Hobson and Sealy 1991; Hebert et al. 1999; Bearhop et al. 1999; Sabat and Martínez del Rio 2002; Sabat et al. 2006). Understanding the importance of a unique food such as nectar is therefore made possible due to its unique isotopic signature.

Nectar - an income energy resource

Insect abundance has been shown to decrease during the winter period (Chapter 5) so for insect-reliant birds income resources may be more important during periods of low food availability. However, at our study site we can only speculate whether this decrease causes insectivores to become food-stressed. Broad-tailed hummingbirds *Selaphorus platycercus* have been shown to fuel their metabolism largely (*c.* 90%)

from assimilated sugars, although when birds were losing mass they fuelled their metabolism from endogenous reserves (Carleton et al. 2004, 2006). Similarly, nectar-feeding bats (*Glossophaga soricina*) use recently ingested sugars to fuel a large proportion of metabolism (Voigt and Speakman 2007; Welch et al. 2008). In both the African red-eyed bulbul and Cape white-eye breath samples were more depleted than blood and feather in ^{13}C, indicating they fuelled their energy with income nectar reserves. Contrary to expectations, Cape robin-chat and fiscal flycatchers had breath samples that were similar to blood and feather samples indicating that they did not need to catabolize nectar sugars for immediate energy requirements. They most likely fuelled there metabolism from stored energy reserves, i.e. lipids.

We did not calculate the proportion of metabolism fuelled by ingested *A. marlothii* nectar because tissue breath fractionation factors are not well understood. For yellow-rumped warblers *Dendroica coronata* mean discrimination factors for breath were 1.4‰ and 0.4‰ on a C_3 and C_4 bulk diet respectively, although values varied greatly for carbohydrates, lipids and proteins in red blood cells and plasma (Podlesak et al. 2005). For rock doves *Columba livia* the mean $\delta^{13}C$ of breath was 3.0‰ less than a corn diet; on a wheat diet it was 1.3‰ more than that of wheat (Hatch et al. 2002). Mice fed an isotopically constant diet since weaning had $\delta^{13}C$ values of breath CO_2 depleted in relation to diet by 5.7‰, with differences between individuals being significant (Perkins and Speakman 2001). Because the breath of some birds (particularly Cape white-eye, African red-eyed bulbul and malachite sunbird) shifted significantly from a pre-flowering season diet of C_3 to that of aloe nectar (within 1.0‰) we can confidently say that ingested nectar fuelled a significant proportion, possibly >90%, of their metabolism (Fig. 3, 4).

Nectar - a water source for birds?

The clear distinction of the $\delta^{18}O$ (and δD) values between nectar water and two accessible water sources near the aloe forest may provide valuable insight into the source of water for nectar-feeding birds. The $\delta^{18}O$ in expired CO_2 was most similar to the water sources near the aloe forest (Fig. 4). One of these sources provided a consistent supply of water during the flowering period when conditions became dry,

with an increase in the number of avian visitors drinking water during flowering (C.T.S. pers. obs.). However, the faces of many nectar-feeding birds were dusted in pollen, evidence of numerous floral probes, supporting the idea that they consume significant volumes of nectar (see Fig. 1c; Chapter 2). The $\delta^{18}O$ in expired CO_2 was markedly depleted compared to $\delta^{18}O$ in nectar water; the isotopic signature of water in *A. marlothii* nectar being typical of a highly evaporated water source. An immediate interpretation of these results is that an insignificant amount of water from nectar was absorbed by nectar-feeding birds (McWhorter et al. 2003). To meet their daily energetic requirements from *A. marlothii* nectar (12%) alone African red-eyed bulbuls (mass = 37.2 ± 2.8 g, *n* = 139; C.T.S. unpubl. data) would need to ingest almost double their body mass (62 g) in nectar (assuming 100% assimilation efficiency and a field metabolic rate of 123.2 $kJ.day^{-1}$predicted from Nagy et al. 1999 equation for passerines). However, under laboratory conditions, yellow-vented bulbuls *Pycnonotus xanthopygos* (35-37 g) will consume approximately 105 g of low protein nectar (12%) during 12 h (Tsahar et al. 2005) implying that a significant proportion of nectar is not assimilated as energy. If bulbuls are fuelling their energetic requirements with a significant proportion of nectar then we can assume that they are ingesting sufficient water to meet daily water requirements (90% of energy requirements equates to 56-95 g nectar sugar i.e. 50-84 g water). This quantity is sufficient for the daily water requirements of bulbuls (Williams 1996) so we can only speculate on why the $\delta^{18}O$ in expired CO_2 did not represent the nectar water consumed by bulbuls (and other birds). During handling many birds that defaecated had faeces with a watery consistency; however, the faecal constituents were not determined. Therefore, it is quite possible that a large proportion of body water requirements are fulfilled by consuming free-standing water, that nectar consumed is inefficiently assimilated, and only a fraction of sugars ingested are assimilated.

Our study attempted to use $\delta^{18}O$ in exhaled CO_2 to determine the origin of water for birds. McKechnie et al. (2004) examined stable hydrogen isotopes in rock doves under controlled conditions, to determine discrimination factors between the environment and body water. The main source of error in tracing the importance of various water resources is that fractionated evaporative water losses result in isotopic enrichment of the body water pool. These authors concluded that using body water δD

to accurately trace water sources in natural system was only feasible when large differences in δD exist between water sources (McKechnie et al. 2004). The same constraint applies to reconstructing water origins using $\delta^{18}O$, where the $\delta^{18}O$ of body water is higher than the $\delta^{18}O$ of imbibed water because of respiratory evaporative water loss and cutaneous evaporation (Bryant and Froelich 1995; Schoeller et al. 1986; Speakman 1997; McKechnie et al. 2004). In addition there is fractionation in CO_2 relative to oxygen in water (Lifson et al. 1955; Speakman 1997). A mass balance model has also demonstrated that drinking water is responsible for an estimated 56% and 71% of the oxygen and hydrogen in the body water of woodrats *Neotoma* spp. respectively (Podlesak et al. 2008). This varying relationship between $\delta^{18}O$ of imbibed water, body water and exhaled CO_2, together with variation in the routing of isotopes explains why the highly evaporated signature of nectar is not directly represented in the breath of birds feeding on nectar, and hence why it is difficult for us to quantify the contribution of nectar water to body water.

Concluding remarks

Without an understanding of the metabolic routing of different food resources, and isotopic discrimination between source (i.e. nectar) and tissue (i.e. breath), it is difficult to accurately quantify the contribution of nectar water to birds. Therefore, until the proportion of ^{18}O in body water that is obtained from drinking water (Ayliffe et al. 2004) and fractionation factors between source and breath are determined for each species under different conditions, we cannot accurately quantify the water input from nectar. Although the $\delta^{18}O$ of nectar was not directly represented in the CO_2 of exhaled breath the $\delta^{13}C$ of nectar was, with enrichment in $\delta^{13}C$ towards that of nectar during the flowering period. This supports earlier findings of a longer study (6 months) analyzing $\delta^{13}C$ in whole blood alone - that nectar sugars are an important assimilated food (carbon) source for many opportunistic nectarivores (Chapter 4). The analysis of breath further indicates that nectar sugars (carbohydrates) provide an important income energy source for birds (Chapter 4); in the case of an opportunistic nectarivore community, an atypical nectar diet during dry winters ensures that energy deficits are met.

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GENERAL CONCLUSION

Understanding *Aloe marlothii* - bird interactions

This thesis attempts to provide perspective on the relationship between the winter-flowering succulent *Aloe marlothii* and the large diversity of bird species that feed on its nectar. The approach has been holistic, in that it has involved studying the symbiotic relationship from the perspectives of both *A. marlothii* and the birds. In studying this association we combined extensive field observations with cutting-edge technology in the form of stable isotope analysis. Part one of the study considered flowering phenology, nectar production, pollination and the effect of a copious and dilute nectar supply on the bird community during dry winter months. This involved an investigation of the phenology of flowering *A. marlothii* and nectar production in flowers (Chapter 1). At an ecological level we studied the effect that a nectar oasis has on a diverse bird community (Chapter 2), and using exclusion cages, that restricted visitors of different pollinator guilds, investigated pollination and the contribution of the numerous avian visitors to successful reproduction in *A. marlothii* plants (Chapter 3). Part two used biological patterns of naturally-occurring stable isotopes (δD, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$) to quantify nectar importance for avian consumers. Firstly, we studied the contribution of nectar sugars to assimilated carbon in nectar-feeding birds by measuring $\delta^{13}C$ values in whole blood before, during and after flowering (Chapter 4).

We also measured $\delta^{15}N$ in whole blood during the same period to investigate whether the reliance and shift to feeding on nectar was accompanied by a trophic level shift (Chapter 5). Finally, we measured more isotopes (δD, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$) in more tissues (breath, blood and feathers) to elucidate more clearly the link between birds and the food and water sources they rely on (Chapter 6).

An *Aloe marlothii* nectar oasis for birds

The nectar produced by *A. marlothii* during the dry winter period attracts a host of opportunistic nectarivores. Nectar production occurred through a 24 h period with no peak in nectar production or focus towards a distinct suite of pollinators (e.g. diurnal or nocturnal). However, copious amounts (*c.* 250 µl) of dilute (*c.* 12%) nectar suggest that pollination is geared towards generalist avian pollinators (Johnson and Nicolson 2008). The importance of birds as pollinators for *A. marlothii* was confirmed by exclusion experiments. Nectar standing crop is reduced by a greater number of birds in the morning period although continually opening flowers (and possibly nectar replacement) ensure that sufficient nectar remains available for nectar feeders. Bird diversity and abundance increased significantly during flowering, suggesting that many birds move into the aloe forest for additional food sources of nectar. Most of the species observed are residents, although certain species only appeared when nectar was available.

The list of birds recorded feeding on aloes has increased from that previously known and includes 105 species in 27 families; with an additional six species suspected as nectar feeders (Appendix). Of these 105 species, 86 (including three sunbirds) are recorded feeding on *A. marlothii* nectar. This phenomenal diversity of opportunistic nectarivores is possibly the highest number of bird species recorded feeding on nectar of a single plant species worldwide.

Avian nectarivory is most broadly dominated by three families: the New World hummingbirds (Trochilidae), the Old World sunbirds (Nectariniidae) and the Australo-Pacific honeyeaters (Meliphagidae) (Maclean 1990; Table 1). However, nectar-feeding

is not confined to specialist taxa and occurs in a wide range of other families. In some regions where these major nectarivore families have not colonised diversification within other families has occurred (Nicolson and Fleming 2003). In the Old World these include the Hawaiian honeycreepers (Drepanidinae) (Pratt 2005), the asities and sunbird-asities (Philepittidae; 4 species) of Madagascar (Prum 1993; Irestedt et al. 2001), the Irenidae (fairy-bluebirds; 2 species) and Chloropseidae (leafbirds; 8 species) of the Indomalayan Region (Wells et al. 2003a; Dickinson 2003), and the Dicaeidae (flowerpeckers, 42 species) of Australasia (Beecher 1953; Delacour 1944; Sibley and Ahlquist, 1991; Ericson and Johansson 2003). The Lorinae (Psittacidae) of Australasia are uniquely adapted for nectarivory with a brush-tip tongue (Forshaw 2006) as is a single member of the Timalidae (babblers), the fire-tailed myzornis *Myzornis pyrrhoura* (Wells et al. 2003b). However, the Melanocharitidae (berrypeckers, 12 species) of New Guinea lack the specialized structure of the tongue for feeding on nectar (Beehler et al. 1986). The stitchbird **(**Notiomystidae), recently relegated to a monotypic family (from Meliphagidae), is a nectar feeder endemic to New Zealand (Driskel et al. 2007). The Aegithinidae (ioras; 4 species) occurs in Indomalaya and are also specialist nectar feeders (Fuchs et al. 2006). In the Neotropics the number of nectar-feeding specialists, besides hummingbirds, is low and includes members of the Dacnini and Coerebini, tribes of the Thraupidae and Parulidae respectively, that are specialist nectarivores (Burns et al. 2003; Dickinson 2003).

Table 1. Number of extant species in dominant nectarivore families, including sunbirds (Nectariniidae), sugarbirds (Promeropidae), honeyeaters (Meliphagidae) and hummingbirds (Trochilidae), in each of the world's zoogeographic regions (after Maclean 1990).

	Neotropics	Nearctic	Palaearctic	Afrotropics	Indomalaya	Australasia	Total
Trochilidae	324	13	0	0	0	0	337
Nectariniidae	0	0	2	78	39	2	121
Promeropidae*	0	0	0	2	0	0	2
Meliphagidae	0	0	0	0	10	159	169
	324	13	2	80	49	161	629

* the monotypic genus *Promerops* endemic to Africa and a *Protea* specialist, is sometimes included in the Nectariniidae (Sibley and Ahlquist 1991); a recent study suggests additions to the Promeropidae (i.e. spot-throats *Arcanator* spp. and dapple-throats *Modulatrix* spp.) that are not nectar feeders (Beresford et al. 2005).

In the Afrotropics the overall diversity of opportunistic nectar-feeding birds is not known, although this study has highlighted a high diversity of opportunistic nectar feeders for a single aloe species in southern Africa. It is likely that the diversity of opportunistic nectarivores is greater than other zoogeographic regions (Chapter 2), and is probably explained by the different feeding roles birds play in different regions. In the Australo-Pacific region the equivalent role of opportunistic nectarivores may be filled by honeyeaters whilst in the Afrotropics the role of opportunistic nectarivores may be filled by several families (Keast 1985; Chapter 2; Appendix). Furthermore, competition of opportunistic nectarivores with specialist nectarivores (i.e. sunbirds) in the Afrotropics may be high, thereby explaining the low number of sunbird species in the Afrotropics compared to the number of hummingbird and honeyeater species in the Neotropics and Australasia respectively (Table 1).

Insight from stable isotopes

Because many aloes have a $\delta^{13}C$ isotopic signature distinct from that of the C_3 environment in which they grow, it is possible to quantify their contribution to assimilated carbon in animal consumers. In this study the blood of most nectar-feeding birds became enriched in ^{13}C at the same time as they fed on nectar. Because of a limited knowledge on diet-tissue discrimination factors for a wide range of species, a wide range of reported values for different tissues and different diets, and the compounding effects of routing of different dietary items we avoided the use of a two end-point mixing model and Isosource (Phillips and Gregg 2003; Chapter 4). At the study site enrichment in blood $\delta^{13}C$ values occurred at the same time that aloe nectar became available, indicating that a proportion of sugar carbon was assimilated by species that do not regularly feed on nectar. This change was not observed in many granivorous species because most grasses at our study site were C_4, with a similar $\delta^{13}C$ signature to *A. marlothii* (Vogel et al. 1978; Chapter 4). A downward shift in $\delta^{15}N$ values was expected to accompany the increased reliance on nectar and decrease in insect food available during flowering; however, no shift was detected. However, accounting for a seasonal shift in vegetation values and increase in diet-tissue discrimination factors between the pre-flowering and flowering period may indicate

that there is no apparent shift in trophic position for all feeding guilds (Vanderklift and Ponsard 2003; Pearson et al. 2003; Robbins et al. 2005; Podlesak and McWilliams 2006; Chapter 5). A greater understanding of diet-tissue discrimination factors for many of the nectar-feeding species when they incorporate nectar into the diet would be required to better understand these changes (Post 2002). Many nectar feeders also drink water from other sources when the dilute nectar of *A. marlothii* becomes available. The presence of nectar was accompanied by a shift in $\delta^{13}C$ of breath CO_2 that represented nectar; this shift was not correlated with an enrichment in $\delta^{18}O$ of breath CO_2, representing the assimilation of water from a more evaporated water source (Chapter 6). Nectar water was highly evaporated but because we are not sure of the proportional contribution of ^{18}O in imbibed water to the ^{18}O in body water, and fractionation between source and tissue, it was difficult to accurately quantify the proportion of water sourced from nectar. Because birds were able to source water elsewhere besides nectar (Fig. 1), nectar water may not be as important for them as nectar sugars; nectar sugars were probably more important as an income (and capital to a degree) food resource during a period of low insect abundance.

Other interactions involving aloes

The importance of *A. marlothii* nectar to numerous bird species has been highlighted (Chapter 2; Appendix), with few other aloe-animal interactions considered in detail. The use of nectar may be an important determinant of local movements and use of resources in, and outside, the reserve, for chacma baboons *Papio hamadryas ursinus*. Observations suggest that troops with ranges overlapping that of *A. marlothii* may make greater use of the aloe forest during flowering, utilizing nectar and succulent leaves as a food and water source (Chapter 2; C.T.S. pers. obs.). The contribution of *A. marlothii* leaves as browse for eland *Taurotragus oryx* is also important; using stable carbon isotopes Wallington et al. (2007) speculated that assimilated carbon from aloes at Suikerbosrand was *c.* 3%, although they were unable to distinguish this from contribution of C_4 grasses. The succulent leaves, despite the extremely bitter taste to humans, may also be an important water source for other animal species. Further work on the importance of aloes for larger consumers is, therefore, required.

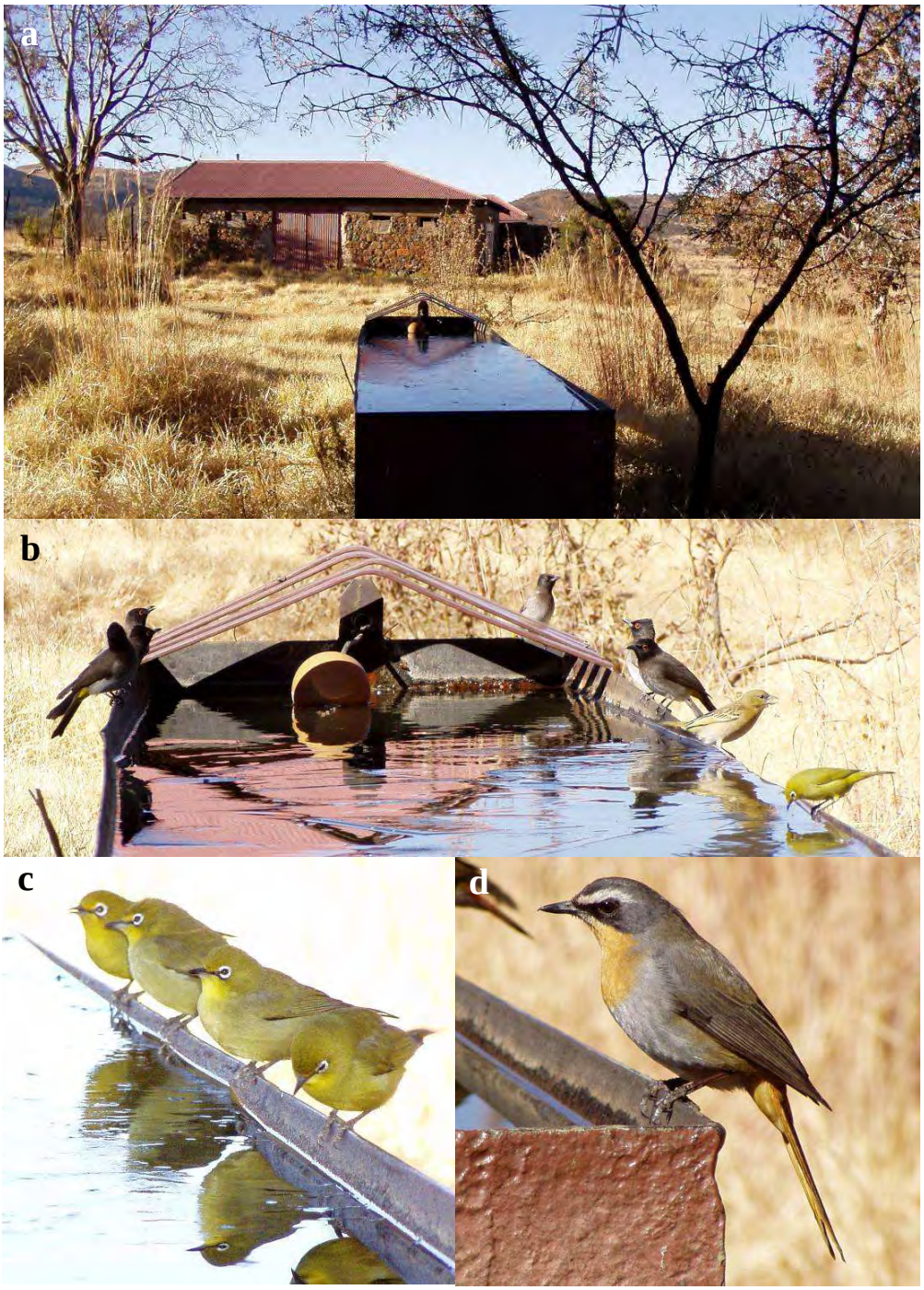

Figure 1. Alternate water source to *Aloe marlothii* nectar, drinking trough at Schoongezicht farmhouse, Suikerbosrand Nature Reserve (see General Introduction; Chapter 6) **(a)**; birds drinking at water trough, African red-eyed bulbuls *Pycnonotus nigricans*, southern masked-weaver *Ploceus velatus* (non-breeding plumage) and Cape white-eye *Zosterops capensis* **(b)**; Cape white-eyes **(c)**; and Cape robin-chat *Cossypha capensis* **(d)**.

Aloe marlothii is also important to birds for many other reasons. The most abundant bird species recorded during censuses was the laughing dove *Streptopelia senegalensis* a species that seldom (if ever) fed on nectar (Chapter 2). It was observed nesting during drier months (August-September) on horizontal leaves of tall aloes. Although the spines of aloes afford protection from predators, baboons are able to ascend plants to access nectar or rob nests. Also, avian predators may still be a threat; in one instance a pied crow *Corvus albus* was recorded robbing a nest with eggs (C.T.S. pers. obs.). Aloes also benefited birds in other ways besides providing nectar; water collected after rains in the horizontal leaves was used as a drinking and bathing source for birds, barbets excavated nesting cavities in trunks of tall aloes (Fig. 2a) and the dry leaf skirt was used as a site for birds to construct nests (e.g. fiscal flycatcher) (Fig. 2b).

Figure 2. Dead main stem of *Aloe marlothii* with excavated barbet nest cavity **(a)**, and view of the *A. marlothii* forest at Suikerbosrand Nature Reserve (during rainy season), showing dense stands of plants in the west of the reserve, a single plant visible in the foreground showing protective spines for nesting birds, and dry leaf skirt encompassing main stem **(b)**.

Future studies

Aloes are widespread in the Afrotropical region with a concentration of diversity in southern Africa (Holland 1978; Reynolds, 1969; Glen and Hardy, 2000; Van Wyk and

Smith, 2005). Considering the few studies to date on animal-aloe relationships it is not surprising the paucity of information on the relationship between birds and aloes. It therefore remains to be investigated how important many other aloe species are for birds. Is it possible that aloes occurring in xeric habitats are more important for birds as a source of water; unlike in this study where birds were able to source water elsewhere? It is the use of stable isotopes that make quantifying such phenomena a reality in the field.

The dichotomy between generalist and specialist pollinated plants has been suggested to be defined by nectar traits, with flowers adapted to generalized bird pollinators characterized by nectar of high volumes (*c.* 40-100 µl), dilute concentration (*c.* 8-12% w/w) and low sucrose content (*c.* 0-5%), and flowers adapted for specialized passerine pollinators (i.e. sunbirds) having nectar of lower volume (*c.* 10-30 µl), higher concentration (c. 15-25% w/w) and higher sucrose content (*c.* 40-60%) (Johnson and Nicolson 2008). Most aloes produce nectar of high glucose and fructose content, with low concentrations of sucrose (Van Wyk et al. 1993) suggesting that most aloes fit the generalist pollination syndrome. However, some aloes like *A. greatheadii* var. *davyana* produce nectars of low volume and high concentration suggesting pollination by specialist pollinators i.e. sunbirds (Human 2006). Most aloes are also orange to red in colour, typical of many bird pollinated plants (Faegri and Van der Pijl 1979), but *A. greatheadii* var. *davyana* is pollinated predominantly by honeybees *Apis mellifera* (Human 2006). Aloes occur in a variety of growth forms, from small grass aloes that are almost indistinguishable in the habitat in which they grow, to large tree-like plants that dominate the landscape (Reynolds, 1969; Glen and Hardy, 2000; Van Wyk and Smith, 2005). Flowering occurs throughout the year and further work is required to investigate the importance of different pollinators for a diversity of aloe species (Botes 2007).

Advances in stable isotope research have grown rapidly in the past decade, with the publishing of numerous studies, covering a wide range of ornithological topics. However, some topics remain clearly unresolved, and considering the ambiguity in published literature, may not be clarified in the near future. Possibly the most perplexing aspect of this project has been the interpretation of $\delta^{15}N$ in elucidating

trophic positions in the nectar-feeding community. Understanding the numerous factors affecting fractionation factors for ^{15}N will require numerous laboratory experiments under controlled conditions (Gannes et al. 1997). For example, particular species that are offered diets with different C:N ratios, may assist in a greater understanding of ^{15}N routing and fractionation under variable environmental conditions, for different levels of food stress and between different organisms (Pearson et al. 2003). Whether these finding can be applied to the ecological level remains to be tested. Of particular interest in this study are the findings that nectar sugar resources provide for an important income energy resource for birds that do not usually feed on nectar. Offering sugar diets to some of these species, particularly those that often take nectar besides aloe nectar (e.g. African red-eyed bulbul, weaver spp.), in controlled laboratory conditions, may provide further insight into the importance of sugar diets for birds under different conditions, e.g. when facing food and/or water stress. In the field, experiments may involve the "starving" of birds that have typically C_3 diets before flowering. At regular time periods, from the time of capture, a breath sample could be collected. Measuring the $\delta^{13}C$ isotopic ratio would then allow the origin of fuelled metabolism through a day to be assessed more accurately.

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Appendix. Bird species recorded feeding on nectar of aloe flowers, also specifically showing those recorded feeding on *Aloe marlothii*. Guild: In - insectivore; Fr - frugivore; Ne - nectarivore; Gr - granivore; Fo - folivore; Ca - carnivore (guilds indicated with dominant guild first; Maclean 1993; Hockey et al. 2005). Level of nectarivory: 0 = non-feeder but present, therefore suspected; 1 = occasional; 2 = casual; 3 = regular; 4 = "addict"; 5 = true nectarivore (Oatley 1964). Suikerbosrand Nature Reserve (SNR) nectar feeders are indicated as recorded feeding on nectar during transects (t), pollen observed on swabs of facial area (s), observations of birds with pollen (p), birds observed feeding on nectar out of transect times (o) (Chapter 2; Oatley 1964; Oatley and Skead 1972) *. References: 1 = Oatley 1964; 2 = Skead 1967; 3 = Oatley and Skead 1972; 4 = Hoffman 1988 (records attributed to *A. ferox*); 5 = Maclean 1993; 6 = Oatley 2001; 7 = C.T.S. pers. obs. 8 = Chapter 2; 9 = A. Craig pers. comm., 10 = B. de Boer pers. comm., 11 = M. Kriek pers. comm., 12 = C. Botes 2007, 13 = S. Strudwick pers. comm. Taxonomy follows Hockey et al. (2005). # exotic species.

Species	Guild	Level	*A. marlothii*	SNR	Reference
INDICATORIDAE (Honeyguides)					
Lesser Honeyguide *Indicator minor*	In	1	1	s	3
PICIDAE (Woodpeckers)					
Cardinal Woodpecker *Dendropicos fuscescens*	In	1	1	s	8
LYBIIDAE (African Barbets and Tinkerbirds)					
White-eared Barbet *Stactolaema leucotis*	Fr/In	2	1	-	1
Yellow-rumped Tinkerbird *Pogoniulus bilineatus*	Fr/In	1	1	-	3
Yellow-fronted Tinkerbird *Pogoniulus chrysoconus*	Fr/In	2	1	-	10
Red-fronted Tinkerbird *Pogoniulus pusillus*	Fr/In	2	1	-	1
Acacia Pied Barbet *Tricholaema leucomelas*	Fr	2	1	tp	1, 3, 8
Black-collared Barbet *Lybius torquatus*	Fr/In	2	1	p	1, 3, 8
Crested Barbet *Trachyphonus vaillantii*	In/Fr	1	1	t	3, 8
PHOENICULIDAE (Woodhoepoes)					
Green Wood-Hoopoe *Phoeniculus purpureus*	In	1	-	-	3
RHINOPOMASTIDAE (Scimitarbills)					
Common Scimitarbill *Rhinopomastus cyanomelas*	In	1	1	t	1, 3, 8,11
COLIIDAE (Mousebirds)					

Species	Guild	Level	*A. marlothii*	SNR	Reference
White-backed Mousebird *Colius colius*	Fr/Fo	2	-	-	3
Speckled Mousebird *Colius striatus*	Fr/Fo	4	1	tp	1, 2, 3, 8,12
Red-faced Mousebird *Urocolius indicus*	Fr/Fo	4	1	tp	1, 3, 8
PSITTACIDAE (Parrots and Lovebirds)					
Brown-headed Parrot *Poicephalus cryptoxanthus*	Fr	4	1	-	1
MUSOPHAGIDAE (Turacos)					
Grey Go-away-bird *Corythaixoides concolor*	Fr/Fo	3	1	-	3, 7
COLUMBIDAE (Pigeons and Doves)					
Laughing Dove *Streptopelia senegalensis*	Gr/In	1	1	s	1, 3, 8
Cape Turtle-Dove *Streptopelia capicola*	Gr/In	1	1	*	1, 3
Emerald-spotted Wood-Dove *Turtur chalcospilos*	Gr/Fr	0	1	-	1
ORIOLIDAE (Old World Orioles)					
Black-headed Oriole *Oriolus larvatus*	In/Fr	3	1	-	1, 2, 3, 9,10
DICRURIDAE (Drongos)					
Square-tailed Drongo *Dicrurus ludwigii*	In	0	1	-	1
Fork-tailed Drongo *Dicrurus adsimilis*	In/Ca	3	1	-	1, 2, 3, 9,12
MALACONOTIDAE (Bush-shrikes, Puffbacks, Tchagras, Boubous, Helmet-Shrikes, Batises and Wattle-eyes)					
Brown-crowned Tchagra *Tchagra australis*	In	1	1	s	8
Southern Boubou *Laniarius ferrugineus*	In/Fr	3	1	p	1, 3, 8
Bokmakierie *Telophorus zeylonus*	In	2	1	p	7, 8
Chinspot Batis *Batis molitor*	In	0	1	-	1
CORVIDAE (Crows and Ravens)					
Pied Crow *Corvus albus*	Ca/Gr/Fr	1	1	*	3
White-necked Raven *Corvus albicollis*	Ca	3	1	-	Garland in 1, 3
LANIIDAE (Shrikes)					
Common fiscal *Lanius collaris*	In	1	1	o	8
PARIDAE (Tits and Penduline-Tits)					

Species	Guild	Level	*A. marlothii*	SNR	Reference
Grey Penduline-Tit *Anthoscopus caroli*	In	3	1	-	1
Southern Black Tit *Parus niger*	In	2	1	-	1, 3
PYCNONOTIDAE (Bulbuls and Nicators)					
Dark-capped Bulbul *Pycnonotus tricolor*	Fr/In	4	1	p	3,11
African Red-eyed Bulbul *Pycnonotus nigricans*	Fr/In	4	1	p	3, 8
Cape Bulbul *Pycnonotus capensis*	Fr	3	-	-	5
Sombre Greenbul *Andropadus importunus*	In/Fr	4	1	-	1, 3,12
Yellow-bellied Greenbul *Chlorocichla flaviventris*	Fr/In	4	1	-	1
Terrestrial Brownbul *Phyllastrephus terrestris*	In/Fr	4	1	-	1
SYLVIIDAE (Leaf-Warblers, Babblers and Warblers)					
Cape Grassbird *Sphenoeacus afer*	In	1	1	s	3, 8
Yellow-bellied Eremomela *Eremomela icteropygialis*	In	1	-	-	3
Burnt-necked Eremomela *Eremomela usticollis*	In	2	1	-	1
Long-billed Crombec *Sylvietta rufescens*	In	2	1	s	1, 3, 8
Arrow-marked Babbler *Turdoides jardineii*	In/Fr	2	1	-	1, 3
Chestnut-vented Tit-Babbler *Parisoma subcaeruleum*	In/Fr	1	1	tp	3, 8
ZOSTEROPIDAE (White-eyes)					
African Yellow White-eye *Zosterops senegalensis*	In/Fr	3	1	-	1
Cape White-eye *Zosterops capensis*	In/Fr	4	1	tp	2, 3, 8, 9,10,12
CISTICOLIDAE (African Warblers)					
Rattling Cisticola *Cisticola chiniana*	In	4	1	o	1, 3, 8
Neddicky *Cisticola fulvicapilla*	In	4	1	s	1, 3, 8
Tawny-flanked Prinia *Prinia subflava*	In	3	1	-	1, 3
Black-chested Prinia *Prinia flavicans*	In	3	1	tp	3, 8
Karoo Prinia *Prinia maculosa*	In	1	-	-	3
Bar-throated Apalis *Apalis thoracica*	In	2	1	tp	1, 8
Yellow-breasted Apalis *Apalis flavida*	In/Fr	1	1	-	3
Rudd's Apalis *Apalis ruddi*	In	3	1	-	3

Species	Guild	Level	*A. marlothii*	SNR	Reference
Green-backed Camaroptera *Camaroptera brachyura*	In	0	1	-	1
MUSCICAPIDAE (Thrushes, Robins, Chats, Old World Flycatchers)					
Cape Rock-Thrush *Monticola rupestris*	In/Fr	1	1	t	3, 8,12
Pale Flycatcher *Bradornis pallidus*	In/Fr	2	1	-	1
Southern Black Flycatcher *Melaenornis pammelaina*	In/Fr	2	1	-	1
Fiscal Flycatcher *Sigelus silens*	In/Fr	3	1	tp	1, 3, 8
African Dusky Flycatcher *Muscicapa adusta*	In/Fr	2	-	-	12
Cape Robin-Chat *Cossypha caffra*	In/Fr	1	1	tp	8
White-throated Robin-Chat *Cossypha humeralis*	In/Fr	0	1	-	1
White-browed Scrub-Robin *Cercotrichas leucophrys*	In/Fr	2	1	-	1
Buff-streaked Chat *Oenanthe bifasciata*	In	3	-	-	3
Mocking Cliff-Chat *Thamnolaea cinnamomeiventris*	In/Fr	1	-	-	3
STURNIDAE (Starlings, Mynas and Oxpeckers)					
Red-winged Starling *Onychognathus morio*	Fr/In	3	1	*	3, 4, 7, 9,12
Black-bellied Starling *Lamprotornis corruscus*	Fr/In	3	1	-	1, 3
Cape Glossy Starling *Lamprotornis nitens*	In/Fr	3	1	o	1, 3, 7, 8, 9
Pied Starling *Spreo bicolor*	In/Fr	3	-	-	1, 3, 9
Wattled Starling *Creatophora cinerea*	In/Fr	1	1	tp	8
Common Myna *Acridotheres tristis* [#]	In/Fr	1	-	-	13
Red-billed Oxpecker *Buphagus erythrorhynchus*	In	1	1	-	8
NECTARINIDAE (Sunbirds)					
Olive Sunbird *Cyanomitra olivacea*	Ne/In	5	-	-	-
Grey Sunbird *Cyanomitra veroxii*	Ne/In	5	-	-	-
Amethyst Sunbird *Chalcomitra amethystina*	Ne/In	5	-	-	2, 4, 7, 9,12
Scarlet-chested Sunbird *Chalcomitra senegalensis*	Ne/In	5	-	-	2, 7
Bronzy Sunbird *Nectarinia kilimensis*	Ne/In	5	-	-	2
Malachite Sunbird *Nectarinia famosa*	Ne/In	5	1	t	2, 7, 8, 9,12
Collared Sunbird *Hedydipna collaris*	Ne/In	5	-	-	7,12

Species	Guild	Level	*A. marlothii*	SNR	Reference
Southern Double-collared Sunbird *Cinnyris chalybeus*	Ne/In	5	-	-	2, 4, 7, 9
Greater Double-collared Sunbird *Cinnyris afer*	Ne/In	5	-	-	2, 7, 9,12
White-bellied Sunbird *Cinnyris talatala*	Ne/In	5	1	t	2, 7, 8
Dusky Sunbird *Cinnyris fuscus*	Ne/In	5	1	-	2, 6, 9
Marico Sunbird *Cinnyris mariquensis*	Ne/In	5	-	-	2, 6
PROMEROPIDAE (Sugarbirds)					
Gurney's Sugarbird *Promerops gurneyi*	Ne/In	5	-	-	2
Cape Sugarbird *Promerops cafer*	Ne/In	5	-	-	2
PLOCEIDAE (Weavers, Queleas and Widowbirds)					
Lesser Masked-Weaver *Ploceus intermedius*	In/Gr	3	-	-	5
Spectacled Weaver *Ploceus ocularis*	In/Fr/Gr	4	1	-	1
Cape Weaver *Ploceus capensis*	In/Gr	3	1	tp	2, 3, 4, 7, 8, 9
Yellow Weaver *Ploceus subaureus*	In/Gr/Fr	4	1	-	1, 3
Southern Masked-Weaver *Ploceus velatus*	In/Gr	4	1	tp	1, 3, 8
Village Weaver *Ploceus cucullatus*	In/Gr	4	1	-	1, 2, 3
Dark-backed Weaver *Ploceus bicolor*	In/Fr	2	1	-	1
White-winged Widowbird *Euplectes albonotatus*	Gr/In	1	-	-	3
Red-collared Widowbird *Euplectes ardens*	Gr/In	1	1	t	3, 8
Thick-billed Weaver *Amblyospiza albifrons*	Gr/In	0	1	-	1
ESTRILDIDAE (Waxbills, Firefinches and Twinspots)					
Black-faced Waxbill *Estrilda erythronotos*	Gr/In	1	1	tp	3, 8
Common Waxbill *Estrilda astrild*	Gr/Fr	1	1	*	3
Violet-eared Waxbill *Granatina granatina*	Gr	1	1	s	8
Green-winged Pytilia *Pytilia melba*	Gr	1	1	s	8
Jameson's Firefinch *Lagonosticta rhodopareia*	Gr	1	1	s	8
PASSERIDAE (Sparrows and Petronias)					
House Sparrow *Passer domesticus* [#]	Gr/Fr/In	1	1	-	3
Cape Sparrow *Passer melanurus*	Gr/In/Fr	1	-	-	3

Species	Guild	Level	*A. marlothii*	SNR	Reference
Southern Grey-headed Sparrow *Passer diffusus*	Gr/In	2	1	s	3, 8
Yellow-throated Petronia Petronia superciliaris	In/Gr	2	1	-	2, 3
FRINGILLIGAE (Chaffinches, Canaries and Buntings)					
Black-throated Canary *Serinus atrogularis*	Gr/In	1	1	t	3, 8
Yellow-fronted Canary *Serinus mozambicus*	Gr/In/Fo	3	1	-	2, 3
Yellow Canary *Serinus flaviventris*	Gr/In/Fo	2	-	-	3
Brimstone Canary *Serinus sulphuratus*	Gr/Fr/Fo	3	1	-	2, 3
Streaky-headed Seedeater *Serinus gularis*	Gr/Fo	3	1	t	1, 3, 4, 8,12
Cape Bunting *Emberiza capensis*	Gr	1	1	t	8
TOTAL		**110**	**85**	**45**	