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SOUTH AFRICAN Journal of Science

SEPTEMBER/OCTOBER 2014

ISSN: 0038-2353

ASSAf
ACADEMY OF SCIENCE OF SOUTH AFRICA

volume 110
number 9/10

SEPTEMBER/OCTOBER

ISSN: 0038-2353 (Print) | ISSN: 1996-7489 (Online)

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SOUTH AFRICAN
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volume 110
number 9/10

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Published by
the Academy of Science of South Africa (www.assaf.org.za) with financial assistance from the Department of Science & Technology.

Design and layout
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Open sesame

The 1973 edition of the *Shorter Oxford English Dictionary on Historical Principles* (just 2762 pages long), which stands on a bookshelf behind my desk, has a declaration which, in the age of almost free online dictionaries and Creative Commons licences, may seem strange:

> *All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior permission of the Oxford University Press.*

Presumably, this requirement applies equally to the passage just quoted as it would, for example, to citing the definition of 'open' as being:

> *Open a. (adv.) 1. Of a door, gate, etc.: Not 'put to' not closed or shut; '-up', set up so as to allow free passage through.*

If so, reproducing a statement does not only require an appropriate citation to the source of the information but also, more stringently, it requires permission (presumably in writing) from the Oxford University Press. Thus two copyright infringements have just occurred.

Anyone who has access to the full, constantly updated, *Oxford English Dictionary* online faces no such restrictions, although, of course, the online version is hardly 'set up so as to allow free passage' unless, of course, one pays handsomely in advance. But things have changed. In 1974, I paid dearly earned postgraduate stipend funds for the *Shorter Oxford English Dictionary*, but still had to face the 'rights reserved' condition. Now one may pay and quote. The world of scholarly publishing has come a long way over the past 40 years.

From 20 October to 26 October, the world will celebrate, and advance the cause of, open access through 'Open Access Week,' an event whose location is, appropriately, posted on the Scholarly Publishing and Academic Resources Commission (SPARC) site as being 'everywhere'. SPARC

> *…believes that faster and wider sharing of the outputs of the scholarly research process increases the impact of research, fuels the advancement of knowledge, and increases the return on research investments. SPARC focuses on taking action in collaboration with stakeholders – including authors, publishers, and libraries – to build on the unprecedented opportunities created by the networked digital environment to advance the conduct of scholarship.*[1]

As a notion, movement and, now, a well-established practice (in some quarters), open access began its freedom march with the Budapest Declaration 12 years ago.[2] This declaration opens with these words:

> *An old tradition and a new technology have converged to make possible an unprecedented public good. The old tradition is the willingness of scientists and scholars to publish the fruits of their research in scholarly journals without payment, for the sake of inquiry and knowledge. The new technology is the internet. The public good they make possible is the world-wide electronic distribution of the peer-reviewed journal literature and completely free and unrestricted access to it by all scientists, scholars, teachers, students, and other curious minds.*[3]

Leaders of the movement that brought the declaration to life include Jean-Claude Guedon of the University of Montreal and Lars Bjornhauge (both of whom recently addressed the South African National Scholarly Editors' Forum in Cape Town), along with a distinguished list of people from institutions including The Wellcome and Open Society Foundations; Harvard, Minho and Southampton Universities; the Max Planck Institute and the European Union.

The Budapest Declaration was followed by the Bethesda Statement on Open Access Publishing in June 2003[4] and the Berlin Declaration on Open Access to Knowledge in the Sciences and Humanities in October 2003.

Since then, the movement has made considerable progress across publications, scholarly societies and nations, and the theoretical underpinnings of the need for open access have been clearly spelled out. Jean-Claude Guedon has explained the manner in which scientific cores and peripheries are created using geo-economic theory, while Erin McKiernan, a researcher working in experimental and theoretical neuroscience, points out that:

> *Every day we make amazing discoveries, some of which could even save lives. Then we lock that information in journals that most of the population cannot read. In many parts of the world, access to subscription journals is just too expensive. ….A lack of access to information hinders learning, stifles innovation and slows scientific progress.*[5]

There are also more radical and, at the same time, very human positions on the importance of open access. Delivering an address entitled 'The case against privatising knowledge', during a Vice Chancellor's Open Lecture at the University of Cape Town last month, Dr Rajesh Tandon, who holds the UNESCO Chair in Health Research and Social Responsibility at the University, observed that

> *…knowledge industries have workers and elites…so you have the propertied classes and the property-less masses when it comes to knowledge as a commodity. It creates the divide of the haves and the have-nots, and it creates therefore control over knowledge in ways that* [create] *not just power…but also wealth.*[6]

Yet, despite the unquestionable logic and morality of Tandon's observations and the valuable contributions made by McKiernan and by Guerdon, there is much progress to be made. Large publishing corporations make substantial profits by charging either article-processing charges or subscription fees (or both). Universities have to reduce serial subscriptions in order to cover these costs. And researchers face the damaging consequences of the remaining challenges that limit scholarly communication. I urge you to read Czernewizc and Goodier's article in this issue for more on the topic of open access.

Here's to Open Access Week.

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HOW TO CITE: Butler-Adam J. Open sesame. S Afr J Sci. 2014;110(9/10), Art. #a0082, 1 page. http://dx.doi.org/10.1590/sajs.2014/a0082

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New possibilities for research on reef fish across the continental shelf of South Africa

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Subtidal research presents numerous challenges that restrict the ability to answer fundamental ecological questions related to reef systems. These challenges are closely associated with traditional monitoring methods and include depth restrictions (e.g. safe diving depths for underwater visual census), habitat destruction (e.g. trawling), mortality of target species (e.g. controlled angling and fish traps), and high operating costs (e.g. remotely operated vehicles and large research vessels).[1] Whereas many of these challenges do not apply or are avoidable in the shallow subtidal environment, the difficulties grow as one attempts to sample deeper benthic habitats. This situation has resulted in a paucity of knowledge on the structure and ecology of deep water reef habitats around the coast of South Africa[2,3], and in most marine areas around the world[4,5]. Furthermore, the inability to effectively survey deep water benthic environments has limited the capacity of researchers to investigate connectivity between shallow and deep water habitats in a standardised and comparable fashion.[6]

With the recent advent of sophisticated and cost-effective remote sampling methods suitable for deep water research,[1] ecologists have been able to describe finer-scale patterns of reef ecosystems in both deep and shallow waters. This ability has led to the identification of ecological drivers of shallow and deep water fish community structure in a standardised and comparable manner.[7-16] The baited remote underwater stereo-video system (stereo-BRUVs; Figure 1) has been at the forefront of these developments[17-19], and has emerged as the most comprehensive, precise and cost-effective tool to measure the ecosystem effects of fisheries[14,20], and patterns in fish abundance[7,8,15,21].

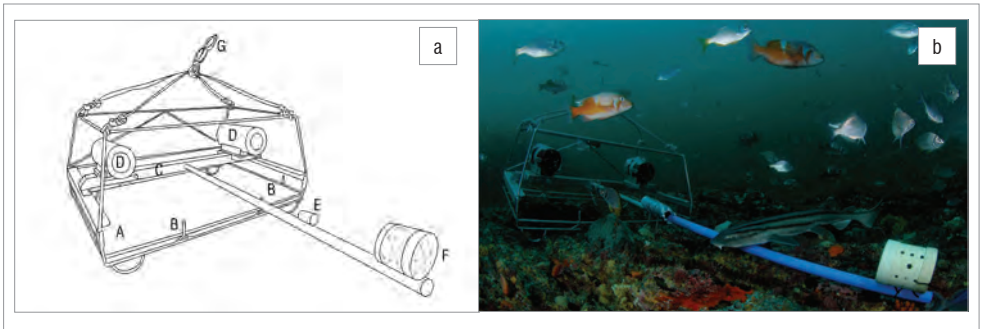

Figure 1: (a) Schematic of a baited remote underwater stereo-video system (stereo-BRUVs) showing stainless steel frame (A) with pins to mount additional weights (B) and rigid centre bar (C) that holds the housed digital high-definition cameras (D). Extending perpendicularly from the centre bar is a pole that holds the synchronising diode (E) and the bait container (F). The system is linked to the surface by a buoy and rope system that attaches to the stainless steel frame (G). (b) Stereo-BRUVs deployed at a depth of 20 m on Rheeders Reef off Storms River, Tsitsikamma National Park Marine Protected Area (photo: Steve Benjamin).

For the first time in South Africa, through a collaborative project of the Elwandle Node of the South African Environmental Observation Network (SAEON), the South African Institute for Aquatic Biodiversity (SAIAB), Rhodes University, the University of Western Australia, and Curtin University (Australia), stereo-BRUVs research on reef fish assemblages is being conducted within the Agulhas Ecoregion from the shallow subtidal area to the edge of the continental shelf. The purpose of this piece is to place in context the necessity of standardised research on the populations of reef fish across the continental shelf of South Africa, to put forward the case for employing stereo-BRUVs in this research, and to introduce the South African marine science community to the research possibilities available with stereo-camera systems.

Video sampling techniques

Although there is some variation in how different studies have approached video sampling,[22-27] the techniques can broadly be grouped as (1) unbaited remote underwater video systems (RUVs), (2) baited RUVs (BRUVs), (3) diver-operated video systems (DOVs), (4) stereo-RUVs, (5) stereo-BRUVs and (6) stereo-DOVs. In addition, remotely

KEYWORDS:
subtidal reef fish; standardised monitoring; stereo-BRUVs; underwater video techniques

HOW TO CITE:
Bernard ATF, Götz A, Parker D, Heyns ER, Halse SJ, Riddin NA, et al. New possibilities for research on reef fish across the continental shelf of South Africa. S Afr J Sci. 2014;110(9/10), Art. #a0079, 5 pages. http://dx.doi.org/10.1590/sajs.2014/a0079

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operated vehicles (ROVs) and autonomous underwater vehicles (AUVs) equipped with video cameras are being used more frequently for research. However, both ROVs and AUVs are expensive and logistically complicated to operate and have been largely restricted to oceanographic (AUVs) or exploratory (ROVs) research. Both RUVs and BRUVs are deployed from a research vessel with the system resting on the sea floor or in the water column, while tethered to a surface marker buoy or to the research vessel. The only distinction between these two approaches is that BRUVs use a food-based attractant to draw fish into the field of view of the camera. DOV is synonymous with underwater visual census (UVC), except that the survey is recorded and the researcher identifies and counts fish post sampling from the video footage, as opposed to identifying and counting the fish while underwater (as in UVC). Stereo-video sampling is a variation of mono-video camera techniques (RUVs, BRUVs and DOVs) that allow for fish lengths to be measured and the survey area to be quantified, greatly increasing the data output per sample and the value of the data for studying the effects of fishing.[14]

Since the mid-1990s there has been an exponential increase in the number of research articles published in peer-reviewed scientific journals that employed BRUVs, RUVs, DOVs or their stereo-video equivalents to collect fish assemblage data from subtidal reefs across continental shelves (Figure 2). Stereo-video sampling techniques first emerged in the early 1980s[28]; however, it was only in the mid-1990s that development of the remote stereo-video approach was initiated by researchers at Otago University and Melbourne University.[24] Only in the last 4 years has the method expanded globally, resulting in a rapid increase in the number of publications based on data collected with this approach (Figure 2). Further growth in the publication rate can be expected over the next few years as the tools become more available and awareness of the new research possibilities they provide increases.

Several studies have compared the benefits and shortcomings of different underwater video sampling techniques,[19,29-31] or contrasted them with more established subtidal sampling techniques such as UVC[18,32,33], controlled angling[14,34], research trawling[25,35] or trapping[35,36]. In general, results show that the stereo-BRUVs technique outperforms all other available methods with the data characterised by low levels of variability, high levels of species richness, high abundances of species targeted by fisheries and accurate information on the size structure of these populations.[14,18,19,24,30,36]

Remote video sampling techniques are flexible in that the systems can be deployed with or without bait, or with the bait type varying between units or deployments, and can be tailored to address a multitude of questions by targeting specific components of the fish community. As such, remote video sampling techniques reduce the research footprint and provide data capable of addressing research and management questions across geographical and depth gradients, within special management areas (e.g. no-take marine protected areas, MPAs) and across different habitats. Importantly, the feasibility of long-term standardised monitoring will be increased significantly by the cost-efficiency of the method.

One of the major advantages of stereo-video over mono-video techniques is that it allows for precise length measurement of fish underwater. This advantage means that the potential for observer error is avoided (compared with UVC) and the need to remove fish from the water, which frequently leads to barotrauma, is eliminated (compared with controlled angling and fish traps).[17,37,38] The size distribution of a fish community is a more sensitive measure of fishing pressure than abundance, as larger individuals of a species are typically more aggressive and caught first when fishing.[1] Research from Western Australia has demonstrated that the size data collected with stereo-BRUVs are comparable to fisheries-dependent data sources and are therefore potentially useful for informing management of stocks.[36,39] In addition, size-based indicators, such as mean length in a population or community, mean maximum length in a community, and the slope and intercept of size spectra, are particularly useful for long-term ecosystem-based fisheries management (EBFM).[40,41] Furthermore, the use of stereo-video also allows the distance from the cameras to a fish to be measured and hence the area sampled can be standardised.[42]

Over the last 4 years, single camera remote underwater video systems (RUVs and BRUVs) have been successfully employed to survey the reef fish communities in the Tsitsikamma National Park, Still Bay National Park and Table Mountain National Park MPAs in South Africa.[31,43,44] The results suggest that BRUVs are highly suitable for obtaining relative abundance data for most of the reef fish species occurring in the Agulhas Ecoregion. Furthermore, data collected in the Tsitsikamma National Park

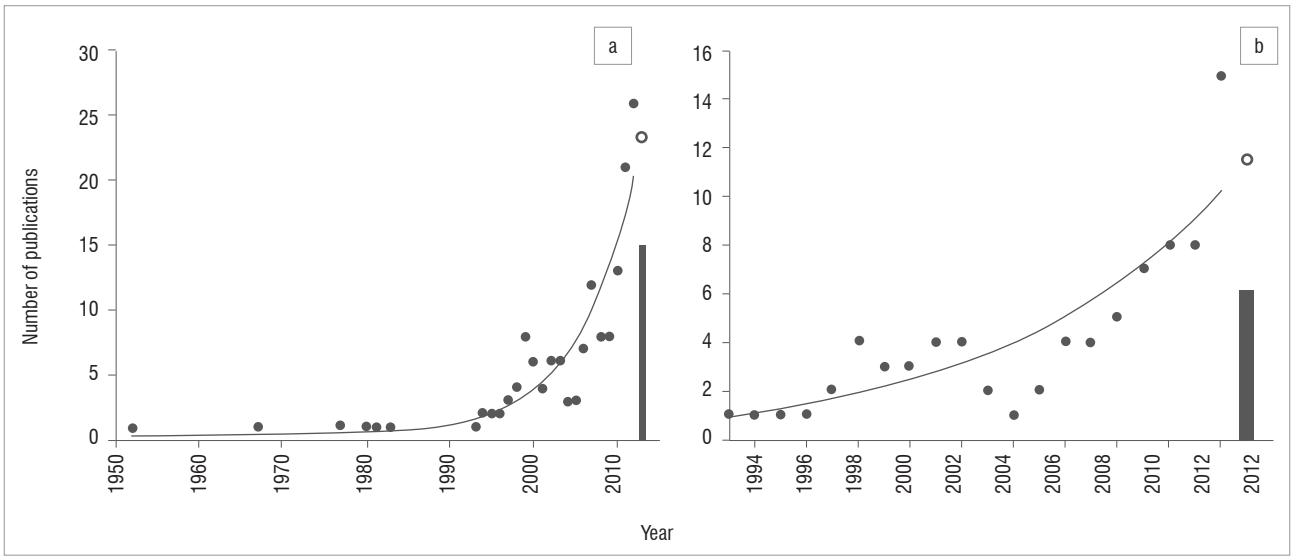

Figure 2: Number of articles published in peer-reviewed scientific journals (a) from 1952 to June 2013 that included use of video sampling techniques and (b) from 1994 that included use of stereo-video sampling techniques. The bars indicate the number of articles published by July 2013, while the open circle indicates the expected number of published articles by the end of 2013. The articles were collated by means of academic publication search engines (Academic Search Premier, Cambridge Journals, CSIRO, ESA, JSTOR, Google Scholar, Inter-Research, Science Direct, Sci-Verse Scopus, Springer Link, Web of Knowledge) and reference list reviews of key literature. Search terms entered included: BRUVs, RUVs, DOVs, remote video and fish. Studies using remotely operated vehicles and autonomous underwater vehicles and those with data collected beyond the continental shelves were excluded.

MPA, using six different techniques, show that BRUVs detected 92% of all bony fish and 71% of all cartilaginous species recorded by the six methods, compared with 68% and 43%, respectively, for the second best method – RUVs (Table 1).[45] Interestingly, in the same study UVC recorded zero cartilaginous species and only 60% of the bony fish species, even though it had three times the sampling effort compared with both RUVs and BRUVs.[45] These results further emphasised the efficiency of BRUVs, with the method requiring only 21 samples per annum to detect a 10% increase in the abundance of important commercial fisheries species, compared with 49 samples required by RUVs and 72 samples required by UVC.[45]

During 2013, stereo-BRUVs were deployed for the first time in South Africa (and Africa), to collect fish assemblage data within the Tsitsikamma National Park MPA. A total of 194 samples, equating to 388 h of video footage, was collected from inside and outside the MPA using four units during 17 sampling days. Sampling depth ranged from 6 m to 80 m, encompassing the deepest extent of the reef habitat inside the MPA and equivalent habitat outside the MPA. All sampling was conducted off an 8-m semi-rigid ski-boat fitted with a simple capstan winch to retrieve the weighted systems. Preliminary analysis of 36 samples has resulted in the detection of 41 species and a total of 3644 size measurements, showing that the stereo-BRUVs not only provide the same benefits as the mono-camera BRUVs, but also produce a considerable amount of length frequency data (±100/sample).

Importance of standardised monitoring

Over the last few decades it has become evident that both management and monitoring of fish resources have been inadequate, or inappropriate, to ensure the conservation of biodiversity and sustainable utilisation of target species.[46-49] The single or multi-species approach to traditional management is considered by many to be outdated, particularly for reef fish, and there is a drive to implement holistic EBFM.[3,47,48,50] Similarly, the assumption that managers can rely on resource users to abide by regulations has been repeatedly disproved, compelling managers to adopt approaches that are feasible to manage and enforce, such as no-take MPAs.[3,48,49]

Ecosystem-based fisheries management requires sound knowledge on the ecology of the natural systems being managed.[50,51] Because of the vulnerable nature of populations of over-exploited fish species, monitoring methods need to be non-destructive to remove the possibility of additional impacts on stocks. Furthermore, many reef fish species occupy broad depth ranges during the course of their life histories.[52-54] In South Africa, this applies for most of the important endemic reef dwelling species targeted in the line-fishery industry, with some occupying a depth range in excess of 150 m.[52,53] This is true for species such as carpenter (*Argyrozona argyrozona*), dageraad (*Chrysoblephus cristiceps*), red stumpnose (*C. gibbiceps*), roman (*C. laticeps*), black musselcracker (*Cymatoceps nasutus*), blue hottentot (*Pachymetopon aeneum*), hottentot seabream (*Pachymetopon blochii*), red steenbras (*Petrus rupestris*), scotsman (*Polysteganus praeorbitalis*) and panga (*Pterogymnus laniarius*), many of which are considered over-exploited and are listed by the South African Sustainable Seafood Initiative as species of concern or as unsustainable.[48,49,55] Adequate monitoring across these broad distributional ranges in a standardised and comparable fashion is required to provide data that promotes effective management of such species. The remote underwater stereo-video system, whether baited or unbaited, offers an opportunity to meet this need.

Conclusions

There is a growing global recognition that high-resolution, non-destructive and in-situ stereo-video techniques can provide improved understanding of fine-scale ecology on deep and shallow reef habitats, and deliver data that support effective EBFM. Preliminary work in South Africa has proved that the method can be cost-efficiently employed by small research teams working off small vessels. The stereo-video research platform developed at SAIAB and SAEON will be able to operate down to depths of 250 m, covering the entire depth range of the continental shelf of South Africa, and will open an extensive array of new research possibilities to scientists based at tertiary education facilities and research institutes in South Africa. The next step is to implement priority research projects that, for example, provide data necessary to develop an understanding of the ecology of shallow- and deep-water habitats and their connectivity, and to determine the spatial patterns of abundance and biomass distributions of vulnerable and endemic fish species. This will support the implementation of effective EBFM and form the basis of long-term monitoring programmes that inform adaptive EBFM.

Beyond the scientific value, hours of video footage will be available for educational purposes to raise awareness regarding the vulnerability of reef fishes and the role of no-take MPAs in protecting reef ecosystems. This material can be used to stimulate interest in marine biology amongst the younger generation and inform communities on the importance of MPAs and fisheries regulations.

Acknowledgements

Funding was provided by the South African Institute for Aquatic Biodiversity, the South African Environmental Observation Network, the British Ecological Society, the Western Indian Ocean Marine Science Association and the National Research Foundation of South Africa.

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Table 1: Fish species sampled by means of controlled angling (CA), fish trap (FT), remotely operated vehicle (ROV), remote underwater video systems (RUVs), baited RUVs (BRUVs) and underwater visual census (UVC) in the Tsitsikamma National Park Marine Protected Area during a simultaneous method comparison.[45] Each method was allocated two randomly selected days for data collection within a 24-day sampling period.

	Method (number of samples)					
Common name (total number of species recorded with all six methods)	**CA (16)**	**FT (25)**	**ROV (7)**	**RUVs (10)**	**BRUVs (10)**	**UVC (30)**
Jawless fishes (1 species)		1				
Cartilaginous fishes (7 species)	3	4	1	3	5	0
Bony fishes (25 species)	11	5	9	17	23	15

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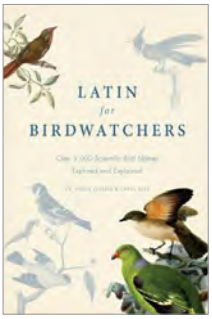
Excalfactoria and a bird and word book to keep you warm

BOOK TITLE:
Latin for birdwatchers

BOOK COVER:



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ISBN:
9781775840930

PUBLISHER:
Struik Nature, Cape Town, ZAR220

PUBLISHED:
2014

REVIEW TITLE:
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HOW TO CITE:
Mitchell D. *Excalfactoria* and a bird and word book to keep you warm. S Afr J Sci. 2014;110(9/10), Art. #a0077, 1 page. http://dx.doi.org/10.1590/sajs.2014/a0077

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Chico, California, bordered by almond orchards and home to the US Yo-Yo Museum, seems an unlikely place to find an erudite husband-and-wife team who love birds, and words, and Latin. Roger Lederer and Carol Burr both have PhDs and both are Emeritus Professors at California State University, Chico – he of Biological Sciences and she of English. On his website, Lederer says he 'knows exactly what birds you will find anywhere in the world'; he has travelled to over 100 countries. He has written five previous books on birds, and a 1984 textbook on *Ecology and Field Biology*. Burr is using her retirement from teaching English and Women's Studies to paint and draw, in oils, water colours, pen and coloured pencils (the medium that she has used in illustrating *Latin for Birdwatchers*).

Latin for Birdwatchers sets out to explore and explain 3000 scientific bird names, listed alphabetically. It does not claim to be comprehensive: there are about 20 000 bird names. Those scientific names are the Linnaean binomial names, that is, the paired names of genus and species in the system set up by Carl Linnaeus (1707–1778) for naming everything alive. Although BirdLife South Africa works hard to promote the use of these names, birdwatchers tend to show less interest in Linnaean binomials than do, for example, tree watchers, but they are the only definitive names for birds (and trees). Most Linnaean binomials for birds are Latin-derived constructs, but enough, and especially those for African bird species, are Greek-derived to have justified '*and Greek*' in the book's title: *meleagris*, as in *Numida meleagris* (helmeted guineafowl) is the Greek word for guinea fowl and *Tyto*, as in *Tyto alba* (barn owl), the Greek word for owl. Some names are derived from other languages. *Pitta* – as in *Pitta angolensis* (African pitta) – is an Indian word meaning 'little bird'. The *kori* in *Ardeotis kori* (kori bustard) comes from its Setswana name *kgôri*. For all the Linnaean binomials listed, the authors offer an easy-to-interpret pronunciation as if in post-Renaissance Latin, with the appropriate syllable emphasised: mel-ee-AH-gris and TI-to.

Most Linnaean binomials are descriptive of the bird's biology, and for each of those listed the authors explain the derivation. To cover 3000 names, their explanations necessarily have to be brief, although they have expanded a few in boxes that they have called 'Latin in action' (Picus, after whom the woodpecker family Picidae was named, was a king in Roman mythology turned into a woodpecker by the witch Circe). Wildlife author Richard Conniff, who reviewed the book, otherwise favourably, in May 2014 for the *Wall Street Journal*, complained that some explanations were too brief to be satisfactory. (The US version that he reviewed was called *Latin for Bird Lovers*; South Africans apparently are allowed to 'watch' birds but not to 'love' them.) I did not find the explanations unsatisfactorily brief; they are more comprehensive, for example, than the explanations in the 'big Roberts'[1], which, of course, admirably serves many purposes other than explaining binomials. Some Linnaean binomials are not descriptive but acknowledge a person associated with a species, and the person celebrated is identified in a phrase: Neergaard ('recruiter for Witwatersrand mines') of Neergaard's sunbird *Cinnyris neergaardi* is recognised, as is Barlow ('South African businessman') of Barlow's lark *Calendulauda barlowi* and Vigors ('Irish secretary of the Zoological Society of London') of the Karoo korhaan *Eupodotis vigorsii*, but not Stierling of Stierling's wren-warbler *Calamonastes stierlingi* or Rüppell of Rüppell's vulture *Gyps rueppellii*. Some Latin names are onomatopoeic: *Pitohui* as in *Pitohui dichrous* (hooded pitohui, a poisonous oriole-like bird from New Guinea) 'is the sound made after a human tastes and immediately rejects the poisonous bird', and *Tockus,* the hornbill genus, comes from 'a Portuguese imitation of the bird's call'. Here are some of the descriptive Latin names related to birds well known to South African birdwatchers: '*cista*, a wooden basket, and *colo*, dwell', describing the nesting habits of cisticolas; *Ispidina* 'from *hispidus*, rough, shaggy, hairy, as in *Ispidina picta'* (African pigmy kingfisher); *Mirafra* in *Mirafra africana* (rufous-naped lark, called *Mirafra mirafra* in the book) from '*miras*, wonderful, and *afra*, African'; '*Musophaga*, from *musa*, banana, and *phagus*, eater of, as in *Musophaga rossae*' (Ross's turaco).

Just the list of Linnaean binomial names would have entertained me sufficiently, but that is not where Lederer and Burr left their book. They added two-page biosketches of 11 'famous birders', including John Gould (1804–1881), after whom 24 bird species have been named (more than after anyone else), and the real James Bond (1900–1989), after whom Ian Fleming named 007. There are 20 one-page 'genus profiles', including the Laniidae, from *lanius*, Latin for 'butcher', amongst which are the fiscals, from the Afrikaans *fiskaal*, meaning, according to Lederer and Burr, 'a public official, particularly a hangman'. There are eight two-page essays on bird biology, containing interesting information that even experienced birdwatchers may not know. And then every page has one or more illustrations by Burr, attractively drawn not so much for ornithological accuracy but, as she herself said in an interview on Chico's Northstate Public Radio, to try to capture the personality and aesthetics of the bird.

The book has been produced beautifully by Quid Publishing in the UK for Struik Nature. It has a textured hard cover, is a comfortable size, and is printed on elegant *fulvus* paper. I found some repetition in the grammar and a few typographical errors, which easily can be fixed in later editions, and do not intrude in this edition. If you like birds and words, and especially if you like Latin and Greek, read *Latin for Birdwatchers* cover to cover, or explore a favourite genus or historical ornithologist, or absorb a bird's personality from an illustration, or just take a lucky dip. I do not think that the book will work well in electronic format. This is a book that you need to hold in your hands, to keep you warm. And I'll leave it to you to discover why the king quail is called *Excalfactoria chinensis*.

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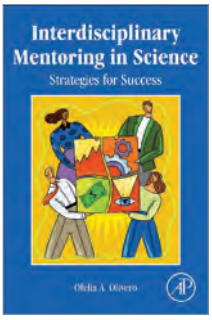
Interdisciplinary mentoring in science

BOOK TITLE:
Interdisciplinary mentoring in science : Strategies for success

BOOK COVER:



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ISBN:
9780124159624

PUBLISHER:
Academic Press, Oxford, USD34.95

PUBLISHED:
2013

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HOW TO CITE:
Wingfield B. Interdisciplinary mentoring in science. S Afr J Sci. 2014;110(9/10), Art. #a0081, 1 page. http://dx.doi.org/10.1590/sajs.2014/a0081

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Mentorship is something I am passionate about and it was possibly for this reason that I was asked to provide a review on this book. As a starting point, I should mention that this book is not so much about mentorship as about how to put in place a mentoring programme in a scientific organisation. It is thus not a book that one should just read, but a book that one should read with the idea of putting into practice the tasks suggested.

The book is interesting and challenging and I appreciated a number of aspects about it. However, as a scientist in a developing world environment I did find the strong American focus distracting at times. Perhaps this focus was to be expected but there are some strong statements about the need for mentoring in the developing world, and particularly in sub-Saharan Africa, and I would have hoped that the American view point would not be quite so overwhelming. My advice to readers is to thus try to get beyond this view as the book does provide some very useful insights which do have an international value.

The first four chapters deal overwhelmingly with minority mentoring and gender diversity. All but one of the experts who are showcased in the book are women. From a South African perspective, there is a disjunction, as our so-called previously disadvantaged communities are in the majority. Perhaps the real 'take-home message' here is the importance of diversity. In South Africa, diversity is pretty much a given – which does not mean that we necessarily always appreciate the possible advantages of our diversity. My own opinion is that there is only one thing worse than a committee of only men: a committee of only women.

My favourite chapter was without question Chapter 5, which focuses on 'Interdisciplinary Mentoring'. This chapter was for me the most important one of the book. The value of interdisciplinarity has always been obvious to me, but this chapter served to highlight some examples as well as to explain the issues in ways that I would not have thought about. In South Africa we engage with scientists from very different backgrounds, yet very little time is spent considering these backgrounds and how some of the traditional Western norms of a scientific career can be at odds with traditional expectations. This is not to say that we have to either adopt the Western norms or just accept tradition. We need to converse and find ways of doing things even better.

The book gives some details about what is needed to be a good mentor and I would hope that most scientists would reflect on this section. So often the 'lone inventor/innovator or the multifunded, independent laboratory head, is seen as the pinnacle of success'. While this can be the case, and often has been the case in the past, I think that often the nature of how we practise science today has meant that things have changed. In most disciplines we are all involved in increasingly large data sets, meaning that collaboration, particularly across many disciplines, has become the norm. Thus we all must learn how to collaborate and put in place sustainable research cultures. There is no question that mentorship will play an important role in doing so.

I enjoyed reading this book and will be recommending it to others who have an interest in managing research programmes. In many ways, the book is very timely as we all are dealing with many of the issues discussed and could learn a lot more about mentorship.

Good news from the South: Biodiversity mainstreaming – A paradigm shift in conservation?

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KEYWORDS:
biodiversity; mainstreaming; conservation; Global Environment Facility

HOW TO CITE:
Huntley BJ. Good news from the South: Biodiversity mainstreaming – A paradigm shift in conservation? S Afr J Sci. 2014;110(9/10), Art. #a0080, 4 pages. http://dx.doi.org/10.1590/sajs.2014/a0080

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'Bad governance stifles everything' said ecologist Richard Cowling, a pioneer in promoting mainstreaming approaches to conserving biodiversity. Cowling was addressing an international workshop convened in Cape Town in October 2013 to review progress in the impressive body of 327 projects in 135 countries supported since the late 1990s by the Global Environment Facility (GEF).[1] With over USD1.6 billion invested by the GEF, and USD5.6 billion in co-financing by partners, the mainstreaming agenda is one of the largest biodiversity initiatives on record. A whopping 48% of these funds went to the 10 countries that hold most of the world's biodiversity treasure troves – Brazil, India, China, Mexico, South Africa, Colombia, Russian Federation, Indonesia, Vietnam and Argentina.

The obvious reciprocal to bad governance – good governance – certainly holds true, and is demonstrated by the success of mainstreaming projects in post-apartheid South Africa and in that icon of democratic good governance, Costa Rica.[2,3] These two countries lead the world in innovative approaches to biodiversity conservation, most especially in moving from the traditional 'protected areas' model to an integrated landscape paradigm. The emerging trends and the challenges to the successful implementation of mainstreaming are considered here.

What is 'biodiversity mainstreaming'?

Emerging as an effective and synergistic interface between conservation science and sustainable development, biodiversity mainstreaming has been variously defined over the past decade,[4,5] most recently at the Cape Town workshop, at which conservation practitioners and researchers from around the globe defined it thus:

> *Biodiversity mainstreaming is the process of embedding biodiversity considerations into policies, strategies and practices of key public and private actors that impact or rely on biodiversity, so that it is conserved and sustainably and equitably used both locally and globally.*

The mainstreaming approach has gained traction across major international organisations, as reflected in the strategic plans and programmes of the Convention on Biological Diversity – the United Nations Environment Programme, United Nations Development Programme and the World Bank – and, most recently, the G20's post-2015 sustainable development agenda.[6,7] Mainstreaming offers dual benefits to conserving biodiversity and to the livelihoods of people within production landscapes and seascapes – an outcome that has been difficult to achieve where traditional protected area approaches to conservation have failed to effectively embrace the interests of local peoples.[8-11]

What does the biodiversity mainstreaming record show?

Firstly, the interdependence of healthy environments and human societies has long been recognised – mainstreaming is not a new concept. In the winter of 1339, Ambrogio Lorenzetti completed a massive series of six frescoes – the Allegory of Good and Bad Government – commissioned not by the Church but by the City Council of Siena, Tuscany's picture-perfect hill city.[12] This purely secular, early renaissance masterpiece presents detailed panoramas of landscapes and cityscapes. It is the two works contrasting the effects of good and bad governance on life in the countryside that are of interest. Rolling hills, clothed by well-tended fields, woods and bountiful crops, a falconer riding off to hunt, and farmers approaching the city carrying abundant produce, presents the story of the countryside under good governance – where justice, equity, wisdom and peace prevail. In contrast, the countryside under bad governance is characterised by eroded fields, mutilated trees, burning hills, abandoned houses and roaming bands of armed bandits.

Nearly seven centuries on, the wisdom portrayed by Lorenzetti resonates with a key lesson learned at the Cape Town workshop – good governance is paramount for healthy environments and for healthy people. From medieval rules of engagement with the environment to modern international conventions, national law reforms and municipal legislation, respect for the law and its fair enforcement has been fundamental. Sound, democratically developed policy and planning are key elements. Since 1994, South Africa has seen impressive advances in environmental legislation[13] and in conservation planning[14], the pre-requisites for effective mainstreaming. That implementation has not been as energetic as planning, and that governance is slipping in several provinces, is a reality.[15]

Secondly, biophysical and socio-economic knowledge – whether traditional or modern – are also essential building blocks for mainstreaming. Again, South Africa has a robust record in using geospatial information for systematic conservation planning. The surge of science-based systematic conservation planning exercises implemented at national and regional scales during the past two decades has provided a convincing base for rational land-use planning.[16,17] Simultaneously identifying global and local sites of highest biodiversity concern, the new spatial planning tools have guided the targeting of environmentally sensitive and effective investments across landscapes. Without detailed spatial information, the trade-offs between production (agriculture, forestry, fisheries and extractive industries) and the protection, reduction of loss and restoration of biodiversity assets cannot be measured or negotiated.[18]

Thirdly, mainstreaming has focused on improving production practices across landscapes and seascapes without compromising biodiversity assets. However, supply and demand sides of trade are often geographically remote, and commodity users are often ignorant of cause and effect. It has been reported that as few as 500 companies control

70% of global trade.[19] Models of trade and threat trends demonstrate that developed nations drive biodiversity threats in developing nations. International trade has been causally linked to 30% of the vertebrate species listed by the International Union for Conservation of Nature as 'threatened'.[20] A widely tested response has been to engage the commodity sector in certification schemes for environmentally friendly coffee, cacao, timber, fish, beef, palm oil, etc. These schemes are well known in the consumer countries of the developed 'North', but have they been effective in reducing forest loss in the developing 'South'? The evidence is inconsistent[21,22] – but, it is a work in progress and should not be too hastily rejected.

The final leg of mainstreaming relates to financing mechanisms. Should biodiversity pay its way – and if so, how? While the entry points for mainstreaming interventions such as policy, planning and production systems have had measurable indicators (such as simple counts of laws enacted and implemented, hectares of land designated for production or conservation and commodities certified) and have enjoyed wide success, financial mechanisms such as the inclusion of natural capital values in national accounting frameworks, reforms in financial flows and payments for environmental services, have been criticised as more hype than hope.[24-27] But, increasingly, financial institutions are embedding environmental risks in financial models, and recognising the parallels between the systemic risks of the financial sector and the systemic risks associated with ecosystems.[28-30]

What are the challenges to mainstreaming biodiversity?

Despite the enthusiasm and tangible support from donors that biodiversity mainstreaming has enjoyed, it is not without its naysayers. Principal among these are those who question the evidence base of results reported on key mainstreaming interventions such as certification and payments for environmental services.[31-32] While there is a rapidly growing literature on spatial conservation planning and payments for environmental services, the massive investment in mainstreaming projects is not reflected in the peer-reviewed literature. Globally, mainstreaming remains on the periphery of scientific debate – because of both a lack of papers in mainstream journals (but South Africa is addressing this gap) and the absence of any unifying theory of change.

Firstly, let us consider the absence of published results. Few mainstreaming projects, designed as they are around the elaborate 'log-frames' so cherished by donors, offer the opportunity to develop and test hypotheses. Mainstreaming is a messy process.[33] It is at the soft edge of science, with diffuse methodologies that are not only highly context-dependent, but also rely on the personalities and negotiating skills of their leaders. They are high-risk initiatives, with high transaction costs and long-term investment horizons to achieve success. They are essentially both transdisciplinary and transformational, demanding a skills mix that extends well beyond the typical attributes of most researchers in the biodiversity arena.

Experimental or quasi-experimental project designs that include social as well as biophysical measures and outcomes are seldom found in mainstreaming projects. Lorenzetti's frescoes give a hint of possible approaches. South Africa, with its wide range of municipal governance capacities, offers an opportunity to use counterfactual approaches. The impact of good governance ('treatment' – i.e. mainstreaming intervention) versus poor governance ('control' – no mainstreaming intervention) could be monitored with simple biodiversity indicators. Obviously, more sophisticated counterfactual designs can be developed.

In implementing mainstreaming approaches, a critical issue is identifying mainstreaming entry points. A tension exists between project targets that require site-level interventions ('short hook') and the systemic changes required at policy and institutional level ('long hook') to achieve desired outcomes. A focus on ambitious site-level targets can lead projects away from the deeper institutional mainstreaming outcomes, and vice versa. The many successes that were reported on at the Cape Town workshop include 'long hook/upstream' interventions – policy, legislation, institutional development, planning at national levels – that seldom result in peer-reviewed papers, but have significant impact. The 'short hook/downstream' activities – locally based stewardship programmes, changes in production practices, certification in tourism and in selected food products – also fail to result in journal papers – they simply were not designed to do so.

Project outputs include a wide range of 'soft' products – most important of which are a slow, progressive, positive growth in individual and institutional capacities and behaviour (the means) – and in reaching tangible, 'hard', physically measurable outcomes such as hectares of habitat conserved or restored, stream flows maintained or stabilised, populations of threatened species increased, soil loss reduced, crop yields improved and local communities benefitted (the ends). Achieving desired ends through such transformational means requires decades, not the average 5-year project funding cycle.

Recommendations to the key funding agencies on experimental or quasi-experimental project design have not attracted much response. The operational complexity of biodiversity mainstreaming projects, usually undertaken in resource-poor countries with limited institutional capacity, makes sophisticated and costly project designs difficult to implement and to sustain. Further, the monitoring and evaluation systems of donor agencies are very coarse-grained, focusing on activities, products and compliance with administrative and financial management norms, but with few measures of biodiversity and social outcomes. The mandatory mid-term and terminal evaluations that donors require are invariably undertaken by project management consultants and seldom, if ever, lead to publications outside the grey literature. Early successes enjoy high-profile media coverage, but failed projects are seldom, if ever, reported.

A consequence of the dependency of large, complex mainstreaming projects on donor support, mostly from the three main (but discretely competing) implementing agencies of the GEF – United Nations Environment Programme, United Nations Development Programme and the World Bank – is the lack of an institutional home for the growing community of practice that has evolved during the past decade. Being at the interface between conservation science and sustainable development, mainstreaming practitioners come from a diverse and diffuse mix of backgrounds and institutional loyalties. Mainstreaming lacks the focus of professional societies and organisations such as the Society for Conservation Biology, the International Union for Conservation of Nature or the newly established Intergovernmental Platform for Biodiversity and Ecosystem Services. It needs a general theory of change – or at least a series of theories of change for each intervention type – to bring focus and inspiration to its intellectual content.

A further challenge to bringing focus to its collective strategy is that most of its technical and administrative leadership has come from the geopolitical developed North, while its field activities are in the developing countries of the South. Resource constraints have often resulted in leadership by expatriate consultants, researchers and agency officials – a situation not conducive to building long-term national or regional networks. In addition, the opportunities to build regional learning organisations have not been taken up. But there are hopeful exceptions to this over-simplification.

Across Latin America – from Mexico and Costa Rica, through Colombia, Ecuador and Brazil to Argentina – a strong body of nationally driven mainstreaming programmes has emerged. Penetrating far beyond initial projects such as the Meso-American Biological Corridor project, and the early, difficult initiatives in payments for ecosystem services and shade coffee certification, there is now an inspiring sweep of successful projects that have their roots in these early initiatives – even if they were not originally identified as mainstreaming projects. Conservation is gaining momentum across sectors, supported by new legislation, improved and detailed land-use planning, environmentally responsible production practices and impacts on the ground. The Cape Town workshop report describes many of these.[1] While the naysayers might be correct in criticising the weak evidence base of specific projects, the on-the-ground outcomes, such as the reduction of the rates of forest loss in Costa Rica, the investments in catchment protection in Ecuador and the revision of forest law in Brazil, reflect conceptual advances

beyond traditional protected area approaches. The new paradigm of mainstreaming biodiversity conservation across landscapes and seascapes has become integral to conservation thinking during the past two decades. As a workshop participant concluded: 'If last century was the century of protected areas, the 21st century must become the century of mainstreaming.'

In Africa, mainstreaming has not enjoyed as much traction as in Latin America. This situation can be ascribed to the lack of the enabling preconditions for the approach. Mainstreaming needs both prerequisites (good governance and strong institutions) and stimuli (such as political change).[34,35] Protected area establishment and expansion remains the core strategy for biodiversity conservation in Africa. But an exception to this rule is found in South Africa. Here, the transition from pariah state to rainbow nation introduced a massive law reform process which, building on the outcomes of the Rio Earth Summit, crafted new water, land-use and environmental legislation and institutions, building on frameworks such as the Convention on Biological Diversity. Today, 20 years after the dawn of the country's democracy, South Africa has one of the most robust mainstreaming programmes anywhere.

Across the world, stimuli for 'hot moments' in conservation[36] come in diverse forms. In Eastern Europe, the preconditions set by the European Union for joining the Union by countries with weak environmental policies triggered the introduction of new national legislation and investment in mainstreaming programmes. In Belarus, health problems resulting from massive air pollution from peat fires led to changes in wetland drainage developments and supporting legislation.[37] In Indonesia, region-wide air pollution from forest clearing for palm oil provoked international sanction and the subsequent investment in an international Round Table on Palm Oil to promote better land management in the industry.

South Africa and Costa Rica have provided significant leadership in the new paradigm. What accounts for two countries with such different histories, different economies and different societies achieving parallel success in mainstreaming? One must revisit mainstreaming's first principles to explain.

Firstly, democratic and transparent governance systems provide security and longevity to mainstreaming investments. Secondly, South Africa and Costa Rica have high levels of biological diversity, under high levels of threat (from deforestation and other forms of land transformation, impacts of invasive alien species, over-exploitation of threatened species, etc.) – which results in high interest and support from donors. Thirdly, both have a long history of biological research. In the case of Costa Rica, institutions such as the Organisation for Tropical Studies at La Selva and research conducted across the country by the Instituto Nacional de Biodiversidade provide a deep resource of science-based knowledge to underpin legislation and action. In South Africa, the suite of biome projects initiated in the 1970s in response to the International Biological Programme and other programmes of the International Council of Scientific Unions, addressing 'environmental problems that lend themselves to solution through multi-disciplinary, cooperative research' established a long tradition of basic and applied ecological research integrating science and society, challenging many policies of the existing regime.[38,39] In 1994, the powerful stimulus of the change to democratic governance propelled the programmes forward with major projects – such as Cape Action for People and the Environment, Succulent Karoo Ecosystem Project, the Sub-Tropical Thicket Biome Project, the Grasslands Biome Programme and the massive Working for Water Programme[40,41] – building on the strong professional community of practice developed over the preceding decades. Finally, both Costa Rica and South Africa have benefitted from the energies of national champions, in both their scientific communities and in politics – with the active personal leadership of their Ministers of Environment (Valli Moosa in South Africa and Carlos Rodriguez in Costa Rica) and of Water Affairs (Kader Asmal in South Africa) dedicated to embedding biodiversity concerns across government policy.

Turning finally to the lack of a unifying, general theory of change. An adequate collective knowledge base is now available on which to develop an overarching theory of change for biodiversity mainstreaming, building on the operational models of mainstreaming projects from across the globe. A general theory of change is needed that can effectively link hypotheses formulated around specific interventions from different entry points to desired outcomes, and to develop common indicators and measurement approaches to provide evidence to test these hypotheses. Individual projects thus become learning opportunities with exchanges at both vertical and horizontal scales. The need for a robust evidence base is clear. In South Africa, the municipality-level opportunity suggested above is available through the statistics on governance and social indicators provided by the national census, and on biodiversity indicators assembled within the many surveys included in the South African National Biodiversity Institute information system. An even bigger experiential learning opportunity lies in the GEF's information system on the 327 projects it has supported since the first GEF workshop on the topic, convened in Cape Town in 2004.[35] A thorough meta-analysis and synthesis of the GEF experience has yet to be undertaken. But the conceptual development and testing of a theory of change for mainstreaming requires the involvement of both the scientific and development communities and stretches beyond mainstreaming implementers. The next big challenge is to translate the 14th-century insights of Ambrogio Lorenzetti into an operational model for the 21st century.

Acknowledgements

The initiative taken by the GEF Scientific and Technical Advisory Panel (STAP) in convening the two Cape Town workshops (2004, 2013) is acknowledged, as is the leadership played by Richard Cowling, Nik Sekhran, Mark Zimsky, Kent Redford and Tom Hammond in the development of mainstreaming thinking and action.

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Towards SunSmart school policies in South Africa

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KEYWORDS:
policy; school; sun protection; schoolchildren; public health

HOW TO CITE:
Wright CY, Mundackal AJ, Oosthuizen MA, Albers PN. Towards SunSmart school policies in South Africa. S Afr J Sci. 2014;110(9/10), Art. #0078, 4 pages. http://dx.doi.org/10.1590/sajs.2014/a0078

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Solar ultraviolet radiation exposure is the major cause of cancers of the skin and cataracts of the eyes. Skin cancer rates in South Africa, particularly among white individuals, are among the highest in the world. Moreover, recent data have raised new concerns about increasing non-melanoma skin cancer incidence among black South Africans who are HIV positive.[1,2] Cataracts are the leading cause of blindness in South Africa.[3] Prevention of these adverse health effects requires an appropriate behavioural response in the form of sun protection. Sunscreen, clothing, hats, umbrellas and shade structures offer varying degrees of photoprotection against excess personal solar ultraviolet radiation exposure. Schools have an important part to play in supporting children to make healthy sun choices. The International Community Preventive Services Task Force released a statement that supported 'strong evidence' for the effectiveness of interventions in primary schools aimed at the prevention of skin cancer that combine education and policy approaches to increase preventive behaviours, specifically, child sun protective practices that reduce sunburn incidence and new melanocytic mole development.[4]

In South Africa, more than 10 million schoolchildren attend government schools on a daily basis. To date, sun protection has not been a top priority at schools; pressing issues such as nutrition and violence have taken precedence. However, if we wish to curtail skin cancer incidence rates, interventions in schools seem to be a reasonable place to start. Hence, a study to assess schools' sun-related efforts and schoolchildren's sun-related knowledge, attitudes and behaviours was carried out among 24 urban government primary schools in 2012. Not one school had a sun protection policy in place[5]; however, several children knew about the need for sun protection and some practised positive sun behaviours.[6] An opportunity then arose to survey children attending a government primary school in KwaZulu-Natal that implemented a sun protection policy in 2013 with the identical instrument and then compare their questionnaire results to the previous results. Here, we report on the findings of this comparison. While the results are not meant to be representative of South Africa, they raise some interesting questions and support the need for further research.

Methods

Study design

This study was designed as a cross-sectional, descriptive epidemiological study. The study took place during the third South African school term (August–October 2012) at the end of winter / beginning of spring (field research is usually not permitted in the fourth school term according to the prescribed requirements of the Provincial Departments of Education). Self-completed questionnaires were issued to Grade 7 learners at schools randomly selected in all nine South African provinces. Once the sample was defined, school principals were contacted and invited to participate. When a school chose not to participate, the next randomly selected school from the same province was contacted until the total of 24 schools was reached. Ethical clearance for this study was obtained from the Research Ethics Committee of the Council for Scientific and Industrial Research (35/2012) on 27 June 2012. Provincial approval was obtained from all nine provinces. School principals gave informed consent for the study prior to contacting the children. Children's parents/guardians/caregivers completed an informed consent form and children gave assent prior to completing the questionnaire.

Study sample and comparison school

A total of 36 schools, four from each province, were invited to participate in the study, but only 24 schools did participate. This number was determined based on the project budget and the cost of couriering questionnaires to and from schools throughout South Africa. Schools were randomly selected from the Department of Basic Education schools database. Schools were eligible for inclusion in the study if they had a Grade 7 class of children (modal age 13 years), they were public schools and they were situated in an urban setting (rural schools were excluded due to access constraints). Co-educational schools at which either English or Afrikaans was the main spoken and written language were included. Private schools, correspondence, home schools and schools for children with special needs were excluded because they require a different research methodology. Schools with Grade 7 classes with fewer than 10 children were excluded to optimise the study budget. The school with a sun policy was purposefully selected for comparison purposes based on the following criteria: it had a sun policy in place, it had Grade 7 classes with more than 10 learners, it was an urban school and English or Afrikaans was the main language of the learners.

Questionnaire

The questionnaire administered to the children was based on previously tested and validated instruments[7,8] and adapted for local cultural differences (for example, a question was added on skin bleaching). Four general sections were included: (1) attitudes towards sun exposure, suntanning and sun protection; (2) knowledge of the impacts of the sun and sun protection; (3) outdoor sun protection behaviour; and (4) demographic data. The questionnaire was piloted, translated (into Afrikaans) and posted to all schools with the information sheets and consent forms.

Statistical analyses

All questionnaire data were coded and entered into an electronic database (double data entry). These data were then prepared and imported into Stata 11.0 statistical analysis software. Descriptive statistics including observed frequencies for all variables were calculated.

Results

Table 1 provides the descriptive statistics for the two samples, i.e. children at a school ($n=1$) with a sun policy and children at schools ($n=24$) without a sun policy. At the school with a sun policy, there were equal numbers of girls and boys; most children were 12 years old, and most self-reportedly belonged to the white ethnic group and had white or light brown skin. For the children attending the 24 schools with no sun policies, there were more girls than boys; most children were 13 years old and most self-reportedly belonged to the black ethnic group with self-reported light brown or brown skin on the unexposed, inner upper arm. The age difference was likely as a result of the time of year when the Grade 7 learners were asked to complete the questionnaire. At the school with the sun policy, the learners completed the questionnaire early in the year (in February 2014), whereas the learners at the schools without sun policies completed the questionnaire in the later part of the year (in August 2012).

The frequencies of responses for each questionnaire item for children attending the school with a policy and children attending schools without policies are given in Table 2. For most questions, the percentages of responses were similar for the two groups. This was the case for behaviour questions: sunbathing regularly, not applying fake tanning or skin lightening cream; staying in the shade; wearing a hat or cap; wearing a broad-brimmed hat, bucket hat or cap with flaps; and wearing clothing. It was also the case for the following attitude questions: feeling healthier with a suntan; agreeing that friends think a suntan is a good thing; agreeing that family think a suntan is a good thing; agreeing that tanned or dark skin protects against skin cancer; agreeing that a suntan is less fashionable now than it used to be; agreeing that clothing that covers most of the arms and legs is not fashionable; and agreeing that there is little chance that I will get skin cancer. Similarly, the percentage of responses was the same for both samples for knowledge questions, including agreeing that it is safe to get sunburnt once or twice a year.

In terms of sun-related behaviours, more learners attending the school with a sun policy reportedly got a suntan last summer, did not use oils or lotions to get a suntan, used sunscreen and were sunburnt last summer than learners attending schools without sun policies. More children at the school with a policy also tended to know about the things one can do to try and avoid skin cancer, including avoiding getting sunburnt, staying out of the summer sun, covering up with clothing and using sunscreen. Compared with learners attending schools without sun policies, more children at the school with a policy knew that melanoma was a form of skin cancer, had seen or heard about the ultraviolet index and had heard about the Cancer Association of South Africa.

Discussion

In general, children attending the school that had a sun policy tended to know more about sun protection and skin cancer, to practise safe sun behaviours more often and to have healthier sun-related attitudes compared with children attending schools without sun policies in place. This finding is positive; however, we did not test our findings for statistically significant differences, mainly because of the skewed sample sizes (i.e. 82 versus 707) and different ethnic composition of the two study groups, and the results need to be verified with data from additional government primary schools that do have a sun policy in

Table 1: Sample demographics

	Children attending a school with a sun policy % (*n*)	Children attending the 24 schools without a sun policy % (*n*)
Number of learners	(82)	(707)
Gender		
Male	50.0 (41)	38.0 (269)
Female	50.0 (41)	61.3 (434)
Not disclosed	0 (0)	0.5 (4)
Age		
11 years old	6.1 (5)	0.8 (6)
12 years old	62.2 (51)	24.4 (173)
13 years old	31.7 (26)	60.6 (429)
Other	0 (0)	13.7 (97)
Not disclosed	0 (0)	0.2 (2)
Ethnic group (self-reported, children could belong to more than one group)		
Black	14.1 (12)	39.7 (312)
Indian/Asian	14.1 (12)	7.52 (59)
White	68.2 (58)	26.6 (209)
Coloured	2.35 (2)	22.5 (177)
Other	1.17 (1)	1.7 (14)
Don't know	0 (0)	1.6 (13)
Skin colour (self-reported)		
White	39.0 (32)	21.5 (152)
Light brown	48.7 (40)	54.3 (384)
Brown	10.9 (9)	15.5 (107)
Dark brown	0 (0)	5.9 (42)
Black	1.2 (1)	2.6 (19)
Not disclosed	0 (0)	0.4 (3)

Table 2: Comparison of frequencies of responses to all questionnaire items between children at a school with a sun policy and children at schools without sun policies

Questionnaire item	Children at a school with a sun policy % (*n*)	Children at schools without a sun policy % (*n*)
Got a suntan last summer	75.6 (62)	59.4 (420)
Sunbathed regularly to get a suntan	18.2 (15)	21.7 (154)
Did not use oils or lotions to get a suntan	87.8 (72)	68.7 (486)
Did not apply a fake tanning lotion	100.0 (82)	90.1 (637)
Never used a sunbed	98.7 (81)	93.7 (663)
Got sunburnt last summer	82.9 (68)	56.0 (396)
To protect yourself from getting sunburnt, did you ever:		
Stay inside	86.5 (71)	72.4 (512)
Stay in the shade	89.0 (73)	85.9 (608)
Wear a hat or cap	78.0 (64)	72.0 (509)
Wear a broad-brimmed hat, bucket hat or cap with flaps	45.1 (37)	49.3 (349)
Wear clothing (not a hat)	60.9 (50)	53.7 (380)
Use sunscreen	90.2 (74)	65.7 (465)
Applied a skin lightening cream	3.6 (3)	18.5 (131)
Feel healthier with a suntan	4.8 (4)	8.4 (60)
Feel more attractive with a suntan	28.0 (23)	17.1 (121)
Friends think a suntan is a good thing	18.2 (15)	15.9 (113)
Family think a suntan is a good thing	18.2 (15)	14.1 (100)
Tanned or dark skin protects you against skin cancer	3.6 (3)	9.7 (69)
Having a tan is less fashionable now than it used to be	18.2 (15)	19.9 (141)
Clothing which covers most of the arms and legs is not fashionable	21.9 (18)	23.0 (163)
There is little chance that I will get skin cancer	25.6 (21)	27.8 (197)
It is safe to get sunburnt once or twice a year	30.4 (25)	22.3 (158)
Things you can do to not get skin cancer†:		
Avoid getting sunburnt	91.4 (75)	64.4 (456)
Stay out of the summer sun	73.1 (60)	48.0 (340)
Cover up with clothing	68.2 (56)	45.5 (322)
Use sunscreen	96.3 (79)	65.4 (463)
Get a suntan	3.6 (3)	5.2 (37)
Go to a sunbed clinic	2.4 (2)	14.9 (106)
Eat the rights foods	32.9 (27)	38.0 (269)
Melanoma is a form of skin cancer	25.6 (21)	13.4 (95)
Seen or heard about the ultraviolet index	64.6 (53)	28.5 (202)
Heard about CANSA	86.5 (71)	74.4 (526)

†Note: children could choose more than one response.

place. This verification is currently difficult to achieve because very few government primary schools have adopted sun protection policies, but we expect that this situation will change in the next 5 to 10 years.

The most striking result was found in responses from children at the school with a sun policy compared with children at schools without sun policies: more of the former reported getting sunburnt in the last summer than the latter. At first, one would consider this finding surprising and deem it a failure of the school sun policy and implemented activities. But, upon reflection, this result probably shows that more children at the school with a sun policy are aware of the dangers of the sun and of the risk of sunburn as they have been educated about it at school. Therefore, it is possible that they would better remember any occurrence of sunburn, however mild or severe, than would the children at schools without sun policies. It might also reflect the skin photosensitivity of the children at the school with a sun policy (being mostly fair-skinned individuals) and their increased susceptibility to sunburn compared with children with darker skin. There may also be a component of the Hawthorne effect, whereby the children answered in the way that they thought they were expected to answer, an extremely difficult effect to resolve. There may also have been under-reporting by learners at schools without sun policies when asked about experiencing sunburn because learners did not understand what sunburn is, despite perhaps having experienced it. The timing of the questionnaire survey may also have influenced the results (i.e. end of summer at the school with a policy versus end of winter at the schools without sun policies), causing the recall period to be much longer for the schools without a sun policy compared to the school with a sun policy. An attempt must be made to resolve all of these issues in future studies.

Few studies have considered whether having a school sun policy results in children with greater knowledge, more positive attitudes and healthy behaviours in terms of sun protection. One recent Australian, ecological study considered whether being a SunSmart school (a school with a sun policy) influenced hat-wearing compliance. They found that SunSmart status was not consistently associated with better hat-wearing behaviour (in terms of type of hat and frequency of hat wearing).[9] Their sample size was in excess of 30 000 children and teachers, highlighting the importance of increasing our sample size for future research to augment our findings.

Conclusions

This first glimpse at the possible impact that a school sun protection policy may have on schoolchildren suggests that schools may play an important role in increasing learner knowledge about sun protection and skin cancer. However, there are many other important factors, besides school support, that will need to be considered to change learners' behaviours and attitudes in relation to sun exposure. A holistic approach to sun awareness and safe sun practices – involving school, family, government and community – is most likely needed to ensure safe sun behaviour and reduce the adverse health impacts of excess sun exposure.

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Research governance and scientific knowledge production in The Gambia

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DATES:
Received: 15 June 2013
Revised: 13 Dec. 2013
Accepted: 19 Dec. 2013

KEYWORDS:
research; governance; scientific; performance; practices

HOW TO CITE:
Ozor FU. Research governance and scientific knowledge production in The Gambia. S Afr J Sci. 2014;110(9/10), Art. #2013-0185, 9 pages. http://dx.doi.org/10.1590/sajs.2014/20130185

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Public research institutions and scientists are principal actors in the production and transfer of scientific knowledge, technologies and innovations for application in industry as well for social and economic development. Based on the relevance of science and technology actors, the aim of this study was to identify and explain factors in research governance that influence scientific knowledge production and to contribute to empirical discussions on the impact levels of different governance models and structures. These discussions appear limited and mixed in the literature, although still are ongoing. No previous study has examined the possible contribution of the scientific committee model of research governance to scientific performance at the individual level of the scientist. In this context, this study contributes to these discussions, firstly, by suggesting that scientific committee structures with significant research steering autonomy could contribute not only directly to scientific output but also indirectly through moderating effects on research practices. Secondly, it is argued that autonomous scientific committee structures tend to play a better steering role than do management-centric models and structures of research governance.

Introduction

Scientific knowledge production, technology and innovation all contribute immensely to a nation's technology-based economy. Public research institutions and scientists are principal actors in the production and transfer of scientific knowledge, technologies and innovations for application in industry as well for social and economic development. The aim of this study was to identify and explain factors in research governance that influence scientific knowledge production and to generate knowledge, which could inform public science and research policy particularly in The Gambia. However, empirical discussions on the impact levels of different governance models and structures are inconclusive in the literature. There are implicit doubts regarding the benefits of both internal hierarchical self-control/management-centric structures and academic self-management (which measures the degree to which research chairs can decide autonomously). Although the benefits or positive impacts of management-centric governance models and structures are sometimes doubted 'because it is argued that research is not a routine task and the most empowering setting is that of academic freedom'[1], it also has not been shown that the scientific committee model actually positively influences research output. Schubert identified and discussed these governance models and suggested that strengthening internal hierarchy (i.e. increasing management grip on research and decision competences of Deans and Chancellors/Presidents of research institutions) contributes positively to research efficiency. In The Gambia, research and science appear to be in a rudimentary state of development and there is no clearly articulated state science and technology policy. According to a report by the Educational Research Network for West and Central Africa[2], The Gambia evidently lacks capacity for research. The country possesses limited science and technology infrastructure and resources and no proper incentives and partnerships with the private sector (which in itself is underdeveloped) to engage in a more strategic and long-term development of the human resource base. The Gambia further makes very little investment in research and development,[3] and primary data are unavailable for concrete policy decisions. A number of authors[4,5] have argued that factors hindering scientific knowledge production in The Gambia, as in most other developing countries, include lack of legal and strategic frameworks for research and credible governance structures for research. Other factors are a lack of coordination of research activities; inadequate participation of stakeholders in research, policy and implementation processes; lack of demand for research; low accessibility and use of research findings; and inadequate financial and human capacity. However, no empirical work appears to have focused on the effects of research governance factors on scientific performance in The Gambia.

The focus of this study, however, is on the contributions of governance models to research effectiveness defined in terms of quantity of scientific production, not necessarily efficiency. Further, in addressing the lingering question in the literature – that is, what are the impacts or benefits of different governance models and structures in terms of scientific performance/output of scientists – this study identified the scientific committee model of research governance and examined its contribution to knowledge production. In doing so, an attempt was made to identify and explain individual and organisational determinants that influence the research process at the individual level of the scientist, similarly to Horta and Lacy[6]. This postmodern, mixed-methods study was structured in two phases. The first, qualitative, phase, which was a grounded theory study, was used to discover from the participants their own perspectives on those factors or variables that contribute to scientific knowledge production. In the second – quantitative – phase, a positivist approach was used to test hypotheses developed around themes and issues considered important to the research experiences of participants. However, a search for a singular solution later resulted in the integration of the findings from both phases at the point of interpretation and discussion of results.

Methodology

The overall approach to the study was a postmodern, mixed-methods approach, which is a sequential exploratory strategy[7] involving a combination of qualitative analyses in the first phase (Study 1) and a quantitative analysis in the second phase (Study 2). Specifically, the ontological position of Study 1 is essentially constructionist, its focus exploratory, descriptive and interpretative of the varied and complex research experiences of participants.

Consequently, the design of the qualitative phase is emergent in order to discover meanings as revealed by informants. Further, Study 1 takes the epistemological position of the constructionist paradigm assuming that data are contained within the perspectives of participants involved in scientific search processes as individual researchers, research teams, research governors/managers and research policymakers. Thus, the method for qualitative data collection and analysis is grounded theory, which is appropriate for describing and interpreting research routines and processes under study. As the study objective was also to generate new knowledge to inform public research policy in The Gambia, grounded theory methodology was an appropriate and effective strategy for theory building, which is a crucial basic step in an unexplored area.[8] Glaser's classic version of grounded theory was used, based on its focus on the emergent nature of theory grounded on data, to select and remain consistent in the application of one approach, thereby avoiding 'methodological muddling'.[9,10] In providing an explanation for the selection of Glaser's version of grounded theory,[11] this approach enabled the inquiry to provide a fresh slant on existing knowledge about public science policy implications for production of scientific knowledge in The Gambia. However, the overall knowledge claim of the study is pragmatic, which means that the investigation is result or problem oriented. The study design is therefore both qualitative and quantitative in thrust, and the overall strategy of inquiry involved the sequential collection of data in order to best describe, interpret and explain the research problem (see Figure 1, which represents the study plan indicating stages of development of the study). By approaching the phenomena under investigation in different ways, from different perspectives, the triangulation of data was possible. Triangulation therefore produced data which otherwise could not have been easily obtained from one source alone.

Research methods and data design

The study area is The Gambia public research system consisting of public research institutions. Data were collected and subsequently analysed from The University of The Gambia, the Educational Research Network for West and Central Africa (both of which are engaged in basic research), the National Agricultural Research Institute, Medical Research Council, The Gambia Unit and the International Trypanotolerant Centre, which focuses on science and technology activities. The Ministry of Health Units (Malaria and Reproductive Health Units) and the Department of State for Higher Education, Research, Science and Technology, the key player in the research policy arena, were further study points. The need to enhance understanding of a variety of contextual factors that affect research practices and scientific output informed the choice of the study area. The strategies and tactics that individual 'researchers'/actors and the organisations as a whole apply in order to handle the complex organisational processes were also relevant considerations. The study area further permitted in-depth examination of the significance of collaborative research exchanges and their implications for public sector research. In addition, the ease of access to research colleagues who readily responded to the study questionnaires provided further justifications for the selection of the study area. The research design (Figure 1) outlines the use of postmodern, mixed-methods research involving both qualitative and quantitative methods.

The sequential exploratory mixed-methods[7] began with a qualitative and inductive phase and ended with a quantitative and deductive phase. The deductive part of the research was designed to explain and expand findings from the qualitative phase. Primary data for both phases of the study came mainly from survey questionnaires (structured), semi-structured interviews, three focus group discussions, interview protocols and personal observations. Secondary data were obtained from already published works on research policy as well as institutional data from research institutions in The Gambia.

In the quantitative study, structured survey questionnaires were developed around the initial predictor variables labelled research governance models (i.e. management-centric and scientific committee models of research governance). Scientific committee models anchor on professional self-steering of research by research chairs and scientific committees at different hierarchical levels with a significant degree of autonomy to decide on key research issues. There is limited control over research by the state, external stakeholders and management as well as limited state and research-chair holder competences. Although professionals have critical competence in research, they also play a supportive role in achieving the institutional strategic research agenda. The research chair at the apex hierarchy may deal mainly with strategic research issues by providing the necessary institutional research leadership and coordination of research activities. The explanatory variables emerged from the participants' point of view as the first qualitative phase

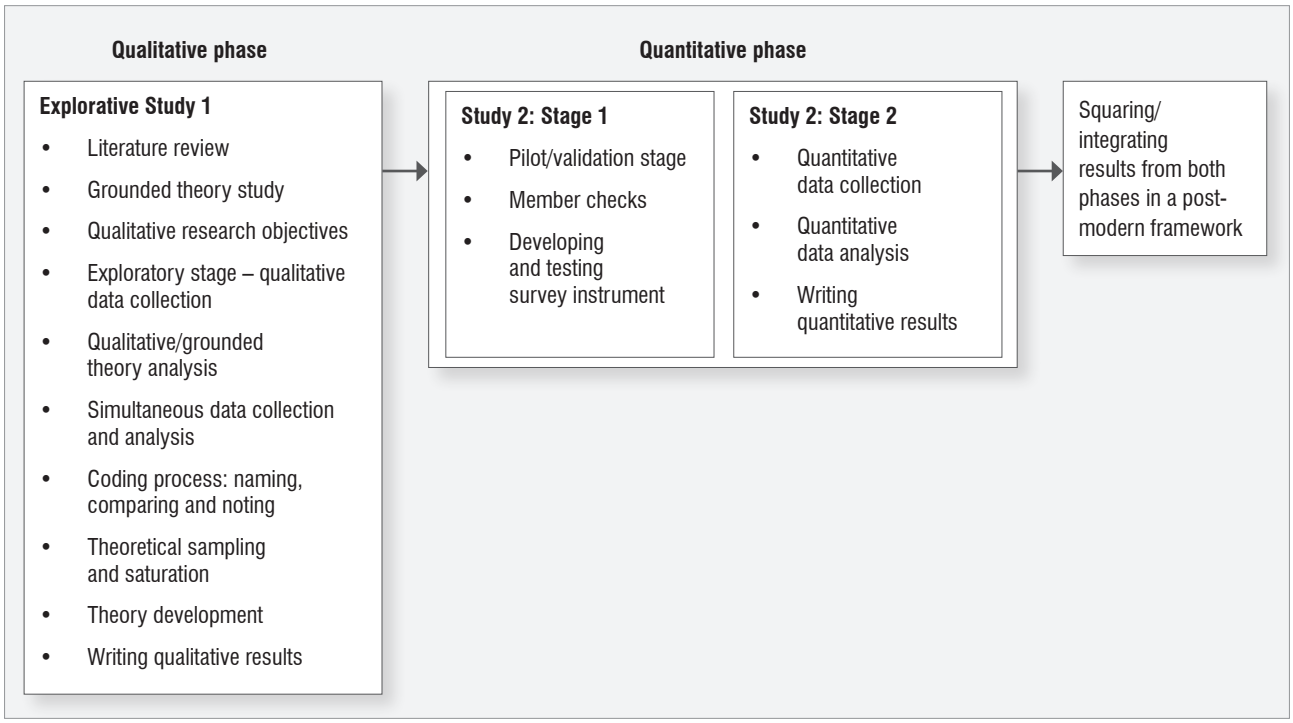

Figure 1: Research design: stages of development.

progressed. The dependent variable was scientific performance/output, which had specific variable indicators and was measured in terms of the number of scientific publications (or articles in peer-reviewed journals), editorships in journals and book series, conferred doctoral degrees (or successful PhD dissertations), research prizes/awards and advisory services to companies/consultancies. A relevant staff list of all public research organisations in The Gambia (including the University of The Gambia) constituted the quantitative study population. The quantitative phase set the sample size at 650 drawn from seven stratified sampling frames obtained from updated computerised files maintained by the University of The Gambia administration and those of the other public research organisations in The Gambia. Table 1 shows the percentage distribution of classes and the sample size.

Table 1: Percentage distribution of classes and sample size

Sample class	Study sample	Population sampling elements (no. in class sampling frame)	% of population
The University of The Gambia	135	250	20.8
Medical Research Council, The Gambia	100	185	15.4
National Agricultural Research Institute	119	220	18.3
Ministry of Health Units (Malaria, Reproductive Health, etc.)	86	160	13.3
The International Trypanotolerance Centre, The Gambia	76	140	11.7
The Educational Research Network for West and Central Africa	109	200	16.7
The Department of State for Higher Education, Research, Science and Technology	25	45	3.8
Total	650	1200	100%

The quantitative survey adopted both non-probability (convenience sampling) and random systematic sampling techniques. However, the data collection procedure in respect of Study 1 involved joint systematic data collection, coding, and analysis with theoretical sampling to develop a grounded theory of scientific knowledge production. The process involved coding and summarising data, reassembling emerging variables and making propositions about them, and, through a selective coding process (by which core variables are identified), establishing the basis for formal theory. On the other hand, quantitative data collection and analysis involved the use of simple statistical tools (frequency distributions, means or modes, standard deviation/standard error of a sampling distribution, percentage tables and a five-point Likert scale) to test hypotheses developed around themes and issues considered important to research experiences of participants. Integration of research findings from both phases of the study occurred at the point of interpretation/discussions of results in a postmodern framework to produce deeper insights and a more comprehensive analysis of public research phenomenon in The Gambia.

Qualitative and quantitative data analysis

The mixed-methods strategy for data collection and analysis was sequential exploratory,[7,12] meaning that qualitative data collection and analysis was undertaken first, followed by collection and analysis of quantitative data. Both phases were given equal priority or weight in the process. Integration of qualitative and quantitative data and findings, which occurred at the point of data interpretation and discussion of results, involved comparing and collating data and findings from the qualitative phase with data, findings and conclusions from the quantitative phase. This strategy enabled the exploration of not only the phenomenon under study, but also the elaboration and clarification of the qualitative results with results from the quantitative method.[7,12,13] It became possible to test some elements of the emerging theory of scientific knowledge production from the qualitative phase and generalise results to the study population.

Describing qualitative results

Based on Glaser's version of grounded theory method[14], the following techniques for concept coding were employed: (1) open, axial and selective coding of key themes; (2) writing memos for every interview summarising key themes; and (3) recording a 'researcher's journal' that puts together key concepts across all the interview protocols. Each data set was separately open coded when collected, and data collection continued until saturation was achieved by the 15th interview. Axial and selective coding were performed after open coding. Axial coding consisted of relating categories to one another and transforming the initial categories to their subcategories (Table 2). Selective coding permitted the identification of conceptual ideas that integrated the existing categories by making relational statements using memos created during the continuous process of data collection and analysis. As categories, which emerged and evolved during data collection, became structured and saturated, relationships between categories were examined by means of systematic comparison. Memos created as the research progressed directed the creation of a 'researcher's journal', in which all key concepts were listed and relational statements between concepts formed during axial coding were indicated. Codes collapsed these memos as they began to resemble each other during organisation and memo sorting. Consistent with the rigor of implementing methodological procedures of grounded theory, discipline in the methodology and the need to properly explain the process by which the theory was generated,[10,15] a resultant theory of knowledge production emerged as categories became saturated and concepts and relational statements connecting them became fully defined and clarified.

All the reported qualitative results were derived inductively from qualitative data, which were generated from semi-structured interviews, focus group discussions and two interview protocols. By undertaking a grounded theory approach, it was found that research practices and behaviour not only impact on the scientific output of the scientist but are also a function of governance categories. For the purpose of this study, the term 'research practice' is used to describe how scientists organise their work, the structure of their research process, and the 'doing of' research. It is about the scientist's decisions regarding research topics, priorities and agendas. It is also about research funding decisions, decisions on how much time is devoted for teaching and research (in respect of academics), decisions about research output orientation in terms of quality versus quantity, publication strategy and collaborative research efforts.[16] Research practice is about all the research-related decisions and behaviours of the scientist. The expectations of scientific committees, demands from professional competitions, recognition among peers, and pressures arising from peer evaluations affect these decisions and behaviours. Through similar mediating influences by means of institutional research policies, the management-centric governance category impacts on research practices. Funding decisions of scientists remained largely constrained, and choices and decisions about research interests and priorities depended on limited funding options in a stifling research context defined by state and institutional policies. Participant 13 limited his research to 'things I can fund myself…so the areas I am hoping to work on, or areas I am not working on are basically things I feel that I can't fund by myself'.

On the other hand, by means of intervening conditions defined by the expectations of peer evaluators for quality, ethical standards, procedural norms and values of scientific research, the scientific

Table 2: Axial coding sheet

Phenomenon	Scientific committee governance	Management-centred governance
Causal conditions	Significant autonomy over research issues by scientific committees; state and institutional research policy; external stakeholder factors	Dominant control over research by institutional management; state and institutional research policy; external stakeholder factors
Context	Autonomy for researchers and supportive feedback from research governors/scientific committees	Overbearing management; some support from professionals and scientific committees; often stifling research situation
Intervening conditions	Mediating influences of procedural values, norms and rules; professional ethics (regulating scientific enterprise) on research practices/behaviours	Defining factors of state and institutional science and research policies
Action/interaction	Conduct of research; interaction with scientific committees; wide and extensive collaborations and networks	Conduct of research; interaction with management and external stakeholders; very limited internal research collaborations
Consequences/inferences	Better positioned than other governance models and structures to impact on research practices and scientific performance	Could positively contribute to effective scientific performance provided supported by scientific teams

committee category impacts on research practices, such as deciding how much time to spend on research, and how to structure the work. Participant 3 thought that 'quality has to be given high priority in organising your research'. Most respondents preferred to publish their work in foreign scientific journals, and, because of limited publication options available, they believed that this publication strategy encouraged them to focus on the quality of the work. Participant 13 thought that by focusing on quality, there is a focus on doing it [research work] rightly to be accepted for publication'. Offshore publication, and thus publication behaviour, exposed respondents to foreign professional research expertise, which shaped their overall research behaviour and consequently positively influenced the quality of their research. In brief, the qualitative study inductively established that the scientific committee category impacts on research practices by means of support for quality of research, peer-based evaluations and supportive feedbacks as well as fostering professional competition for recognition among peers and collaborative exchanges. Participant 1 thought that 'publishing encourages competition…and publishing offshore…ensures that your work meets international standards or quality'. Participant 2 added 'you go for foreign journals so that the more of these you publish the more recognised the researcher becomes'. Apparently, the scientist learns through doing and exposure to the expertise of 'other professional colleagues'. Thus far, the description of results underpins the process of theoretical integration, and, drawing from Glaser's[17,18] ideas on theoretical coding during the advanced coding stage, as well as employing existing theories, it is inductively evident that a fluid interface between the scientist and scientific committee governance structures is a necessary condition for improving research practices and behaviour and scientific performance. Through support provided by professional colleagues in terms of opportunities for collaborative networking and supportive feedbacks, and through doing, exposure and experience were enhanced and research practices and behaviour improved. The scientific committee category thus impacts on communication behaviour and other research-related behaviours of scientists. Qualitative evidence shows that, although limited research is currently taking place in The Gambia and public and private sectors, linkages and support for research are still expanding; institutional science policy nevertheless encourages collaboration, particularly external exchanges. Apparently, external networks and contacts with external educational and scientific institutions are growing rapidly. Qualitative evidence further indicates a high degree of preference among academics and scientists to interact with colleagues from external universities and research organisations. There is also evidence of internal co-authorship networks. Publication data and information obtained from institutional databases show that all publications by respondents interviewed were 'offshore', that is, papers were published in international scientific journals. In this context, most informants agreed that collaborative exchanges 'enhanced human capacity' and produced 'quality [research] and added value'. Collaborative research exchanges positively affected the 'capacity' of the scientist to conduct research. Participant 7 believed that collaboration with international research institutions can 'give you insight into other research activities that are taking place elsewhere in the world' and can also help to 'build relationships between researchers in the international arena'. Participant 5 stated that collaboration expanded the scope of his research interests. Participant 13, a medical scientist, added:

> *…those kind of collaborations, whether with funders, industry, or even among colleagues, you know… brings out the best in research because definitely surgeons have an expertise in certain areas and if we have people who are good statisticians for example, we would have made excellent combination to collaborate with such kind of people* [sic].

Moreover, Participant 10 thought that 'collaborative research reduced costs and time [spent on research]', expanded funding sources and provided opportunities for dissemination of research results. Collaborative research exchanges defined how scientists organised their work. These exchanges or collaborations are also about decisions to link up with colleagues, and share and benefit from the resources and expertise of others involved in research. Thus, collaborative research exchanges are categorised as communication behaviour, which is one of several research-related behaviours.

Theory of scientific knowledge production

Theoretical integration of these concepts led to the following theoretical postulations. Firstly, a fluid interface between the scientist and scientific committee governance structures is a necessary condition for improving research practices and scientific performance. Research practice, through experience, becomes productive as scientists deepen their professional interaction with colleagues. In addition, the steering of research by scientific committees is not only a prerequisite for productive research practices, but also enhances research competences. In this context, the analysis of qualitative data inductively established that the scientific committee category impacts on research practices by means of support for quality of research, peer-based evaluations and supportive feedbacks as well as by fostering professional competition for recognition among peers and collaborative exchanges. Hence, research practice/behaviour is a function of governance categories. Secondly, scientific committee structures with significant research steering autonomy tend to be better at steering roles than management-centric models and structures of research governance. Further, management-centred governance structures are characterised by tight control and coordination of research processes; overbearing and unchallenging, non-competitive

and de-motivating research environments impair scientific performance. In brief, key findings from this phase are:

1. Research practices/behaviour is a function of governance categories, and influences the scientific output of the scientist.
2. By means of intervening conditions defined by the expectations of peer evaluators for quality, ethical standards, procedural norms and values of scientific research as well as fostering professional competition for recognition among peers and collaborative exchanges, the scientific committee category impacts on research practices.
3. A fluid interface between the scientist and scientific committee governance structures is a necessary condition for improving research practices and scientific performance.
4. Scientific committee structures with significant research steering autonomy tend to be better at steering roles than management-centric models and structures.
5. Management-centric structures characterised by tight management control and coordination of research process, and overbearing, unchallenging, non-competitive and de-motivating research environments impair scientific performance.

A validation stage preceded the quantitative data collection and analysis in order to ensure that relevant survey questions were asked and directed towards the quantitative research objectives and to enhance the validity of the findings. Validation measures consisted of a member check, development of a survey instrument based on specific themes and views of respondents in the qualitative phase for generating quantitative data, pre-test of the survey instrument, and triangulation of data sources.

Quantitative data analysis involved testing the relationships among scientific committee governance structures, management-centric structures and scientific performance. The analysis further involved the use of descriptive and inferential statistics, employing the chi-squared technique in testing the following hypotheses:

- Hypothesis 1 – the effect of scientific committee structures and management-centric structures on research output/scientific performance is insignificant.
- Hypothesis 2 – the effect of scientific committee structures and management-centric structures on research output/scientific is significant.

Summary of key quantitative findings

Two governance categories – scientific committee structures and management-centric structures – were used in a Likert-type scale to measure and analyse the degree of their impact on research output or scientific performance defined in terms of the number of published scientific works, conference papers, and supervision of graduate theses. The quantitative responses to a five-point itemised rating are shown in Table 3. A total of 15 respondents strongly agreed and 45 agreed that scientific committee structures enhanced scientific performance; this finding is further supported by the mean score of 3.62 on the Likert scale (Table 3). Table 4 summarises the sample means. The measure of dispersion of responses used was the standard deviation. The lowest standard deviation (0.94) was for scientific committee structures, which showed that respondents did not differ much in their responses in respect of these factors. The highest standard deviation (1.10) was for management-centric structures, indicating that respondents varied in their responses towards these factors. However, about 84 of the respondents agreed and 254 disagreed (while 141 strongly disagreed) that management-centric structures enhanced research performance (Table 3). This result (a mean score of 2.56, i.e. less than 3, on the Likert scale) therefore indicates that management-centred research steering structures do not support scientific performance. From all indications, the results of analysis of the quantitative data show that scientific committee structures positively influenced research output (scientific performance). Overall, key quantitative findings are:

1. A management-centric structure of research governance limits scientific performance.
2. A significant correlation exists between research output or scientific performance (as a dependent variable) and scientific committee structures (as a predictor).

Discussion

The major contributions of the study to the ongoing, although limited and mixed, empirical discussions in the literature concerning the benefits and impact levels of different governance models, will be elaborated on. The analysis fills a gap in the literature by addressing the complex intervening and moderating influence of governance structures on research behaviour and practices of researchers and their scientific output. The multidimensional nature of research-related behaviours and their mediated contribution to scientific performance also are discussed.

Conclusion 1: *Management-centric models and structures of research governance can positively contribute to scientific performance, provided they are supported by advisory groups of mainstream scientists or academics at key hierarchical levels of governance.*

Table 3: Influence of governance structures

Governance structure	X	Frequency	Cumulative frequency	Mean	Standard deviation	%
Scientific committee structures	5	97	485			15
	4	292	1168			45
	3	195	585	3.62	0.94	30
	2	48	96			7
	1	16	16			3
Management-centric structures	5	28	140			4
	4	84	336			13
	3	254	762	2.56	1.10	39
	2	141	282			22
	1	141	141			22

Response levels of agreement on predictor impact on scientific performance: 1, no view; 2, strongly disagree; 3, disagree; 4, agree; 5, strongly agree.

Table 4: Summary of means

Predictors/explanatory variables	*N*	Mean	Standard deviation	Minimum	Maximum
Scientific committee structures	648	3.62	0.94	1	5
Management-centred structures	648	2.56	1.10	1	5

Scientific committee structures with significant research steering autonomy tend to be better at steering roles than management-centric model structures. With appropriate competences and steering autonomy, scientific committees could take and implement more informed research decisions than institutional management or even research chairs who, when acting alone, may either ignore or overrule professional advice and inputs.

A common finding from both phases of the study was that the scientific committee model of research governance positively influenced scientific performance across research institutions in The Gambia. A mean score of 3.62 (close to 4) on the impact of scientific committee structures indicates a significant effect. A majority of respondents in the qualitative study believed that effective research performance was possible if scientists and academics and their professional committees played a significant role in steering research. However, the findings of this study indicate that management-centric structures could in fact inhibit performance where overbearing management-controlled research structures create unchallenging research environments. From the quantitative analysis of data, the mean score of 2.56 (below 3) on the impact of management-centred structures indicates an insignificant effect. These results suggest that, across research institutions in The Gambia, the insignificant contributions of management-centric models and structures of research governance to scientific performance were a result of ineffective management systems, poor management of staff and resources and non-inclusion of inputs and support from mainstream scholars. Management ineptitude and non-prioritisation of research, ineffective research governance structures and weak coordination of research activities are other plausible explanations for the insignificant contribution of management-centric governance structures, which is corroborated by earlier reports.[4,5] Another contributing factor could be very limited investment in research and development in The Gambia.[3] Overall, it is not surprising that, under these conditions, management-centric structures of governance limited scientific output. Nonetheless, this finding does not suggest that management-centred structures of research governance do not produce important research outputs. On the contrary, Schubert[1] found positive impacts on research steering by management authorities. According to the European Commission[19], executive leadership in research governance could promote higher quality education and more relevant research output, if a number of hierarchical levels of research decision competences are in place and external stakeholders provide a supportive role in driving research. These hierarchical structures and support from scientific teams are found at the University of Melbourne, where there are several levels of research hierarchies with the Deputy Vice Chancellor (Research) at the apex providing academic leadership in research and delivery of the university's research agenda. The Pro Vice Chancellor (Research Collaboration) and Pro Vice Chancellor (Graduate Research) support the Pro Vice Chancellor (Research) who is responsible for research performance and research ethics and integrity. At the faculty level, Associate Deans (Research) provide 'local' leadership in research planning, target setting, research development and performance review. Heads of academic departments provide important leadership in research and research training. Most faculties have faculty research managers to manage the administration of research activities within the faculty. Two committees report directly to the Deputy Vice Chancellor (Research): an advisory group of senior academics that provides advice on strategic issues such as research investments and priorities and the committee of associate deans that provides advice on research policy and operational matters. There are a number of other research sub-committees which report through the committee of associate deans, and which are concerned with policy development and review as well as engagement with external regulators.[20] An important feature of these management-centric structures is the conspicuous presence of research managers and a hierarchy of managers and the absence of scientific committees with significant research steering autonomy. Management-centric structures do not have scientific committees with fully fledged research competences in the management of research activities as well as competences for the determination of research investment, policy development, research ethics and peer review. Rather, every other point in these hierarchies ultimately reports to the Deputy Vice Chancellor (Research) while the scientific and technical advisory committees play only professional advisory roles.

In contrast to the situation at University of Melbourne, the management-centric governance structures in the particular context of public research institutions in The Gambia appear to be highly centralised. At the same time, support from scientific committees is very limited. Research policy development, research investment and funding, and other critical research issues including research collaboration and partnerships, research initiatives and intellectual property management, if any, are determined and treated as administrative matters without significant input from scientists. The University of The Gambia had a Research and Strategic Committee, consisting of professionals/scientists, which steered research until 2009. From 1999 to 2009, the University of The Gambia produced significant research outputs. However, this committee, as well as other sub-departmental or faculty scientific committees, seem to have become either redundant or moribund. In the National Agricultural Research Institute, the Director General, Deputy Director General and the Director of Research manage research. Scientific committee structures with significant research competences appear to have disappeared within the same period in the Institute. In this context, a majority of respondents in the qualitative survey of data commented that:

> *We do not have these committees* [or] *evaluation committee*[s] *within the research system. Scientists are left to coordinate among themselves and decide what things to work on in a particular season, you know, and then they later come to management to make a final decision.*

Respondents also agreed that 'it is only management that dictates what one does and when' and 'if…you are not in the good books of management…they would not want to fund your activity'. It is thus understandable why, in this context, management-centric governance structures of research governance, without supportive research steering roles from scientific committees, actually inhibited research output. This finding therefore suggests that management-centred governance structures have not created 'that atmosphere for competition' because 'in having good research there must be an atmosphere of competition'. Because scientific committees do not exist, management 'does not mind much, if problems arise…they do not even scrutinise the outcome of some of the research that the scientists have produced'. Overall, these findings suggest that scientific committee models and structures of research governance tend to be in a better structural position than management-centric models and structures to steer research and handle critical research issues of peer review and governance of behaviours of researchers. A plausible explanation is that scientific committees with significant autonomy could create a more enabling and challenging research environment; better foster research collaborations and exchange of information among colleagues; understand and resolve critical research issues; and make and implement more informed research decisions than institutional management. The key strength of scientific committee structures appears to lie in consultation and consideration of a wider range of options in decision-making and implementation, as inputs could come from core scientists across

hierarchical levels of the structure. Another plausible explanation for the better performance in a steering role by scientific committee structures derives from traditional peer-based evaluation of the work of scientists and scholars. Research steering scientific committees may be more inclined to accept reforms such as those suggested by Osterloh and Frey[21] in favour of a combination of qualitative peer reviews and bibliometrics, which can balance the advantages and disadvantages of the two methods in measuring the scientists' performance[22-25]. While bibliometric indicators provide extrinsic incentives for performance, and qualitative reviews, anchored on quality, provide intrinsic motivation, the combination of the two methods could possibly improve rankings as instruments for monitoring and sanctioning, or precisely, governing the behaviour of scientists. The preference for autonomy in choosing one's own goals is important for innovative research, because it guarantees useful self-selection effects and is the most important prerequisite for intrinsic motivation.[26-28] Hence an approach which combines both qualitative peer review and bibliometrics would generally improve the governance system and allow more room for creative research to blossom. Consequently, within the context of this study, the argument is that scientific committees consisting of mainstream scholars and scientists who understand the process and content of research would more readily introduce qualitative peer evaluations (or better still, a combination of quality- and quantity-based evaluations), foster creative research and generally improve the governance system. However, there might be limitations to realising these advantages because of pressures that could arise from expectations of peers, which include biases based on views contradicting those of the mainstream scholars. Another obstacle could be discouragement among scholars from conducting and submitting creative and unorthodox research.[29,30]

Conclusion 2: *Scientific committees could mould and sustain appropriate research behaviour and practices and competences of individual researchers and research teams by fostering linkages, effective communication, and institutional socialisation and by means of peer review.*

Turning to the finding that a connection exists between research practices and behaviour and scientific committee models and structures, results from analysis of data suggest that autonomous scientific committee structures exert a moderating effect on research practices, and, for this reason, can improve the research competence of individual scientists. This moderating influence of scientific committee structures on research behaviour appears to account to some extent for the nature of interconnectedness of some of the multicausal factors of knowledge production.

After a detailed examination of the qualitative evidence, it can be reasonably asserted that, in the context of institutional research, scientific committee structures of research governance have greater impetus to encourage and enhance collaborative exchanges, foster important research expertise and behaviours and ultimately improve scientists' performance effectiveness. As deductively derived from data, the connection (or interaction) between research practices and scientific committee structures implies that these structures support the doing of research. For this reason, scientific committee models and structures of research governance could be a necessary condition for productive research practices. The argument is that the association between research practices and behaviour and effective research performance tends to be strong (or otherwise weak), depending on other conditions or factors, such as varying 'values/weights' of scientific committee structures. This implies that the effect of research behaviours on scientific performance would increase (or otherwise decrease) depending on varying levels of autonomy in steering research by scientific committees. The effect of this association changes in intensity or direction when these conditions occur, i.e. when there are variations in the value or weight of scientific committee structures. It further implies that different levels of, or changes in, decision-making competences of scientific committee structures could produce corresponding changes in the association between research practices and scientific performance. The following explanations seem reasonable.

Firstly, an important observation is that none of the previous empirical studies, including that of Schubert[1] which focused on impacts of different governance models and structures, have tested the contribution of the influence of scientific committee models and structures on scientific output. This governance model consists of research chairs and scientific committees holding autonomous decision competences. Rather, Schubert[1] found that the influence of deans and chancellors and presidents of research institutions had a positive impact on research efficiency (provided that they used their power and influence wisely). Schubert was concerned with linking relationships between inputs and outputs to governance structures in terms of efficiency, which is calculated using a field-specific differences estimator (FDH). The FDH procedure constructs an estimated frontier out of a sample of observed units, where FDH is an estimation of a production frontier, which is defined as the maximum output producible at a given input level. In this context, Schubert examined the association between new public management governance mechanisms and efficiency. In contrast, in this study, the mediating effects of autonomous scientific committee structures on research practices were examined and outcomes of research were determined using publication counts (number of scientific publications and citations) as a proxy for scientific performance (i.e. output effectiveness). Publication counts were used because comparisons were not made across research institutions, so as not to disadvantage research institutions that specialise in activities measured by other output indicators. Hence, it is argued that, in the context of institutional research, scientific committee models and structures of research governance, more than most other governance models and structures, create a more enabling and challenging research environment. In this model, described as professional self-steering, research chairs and scientific committees at different hierarchical levels could have a significant degree of autonomy to decide on key research issues. However, within the framework of institutional mission and science policy, internal guidelines and inter-institutional agreements, the research chair in the apex hierarchy could deal mainly with strategic research issues, providing the necessary institutional research leadership and coordination of research activities. Departmental scientific committees and research sections could provide support on research direction and priorities and sectoral research leadership. In brief, unlike the governance pattern often described as new public management[31-33], the scientific committee model anchors on limited control by the state, external stakeholders and management, as well as limited state and research-chair holder competences. However, the model still maintains the spirit of new public management as it gives greater steering autonomy to researchers and research teams. Thus, given these critical competences over research, and in playing a supportive role in achieving the institutional strategic research agenda, scientific committee governance structures could mould research behaviours at the level of the individual scientist by facilitating both internal and external research collaborations, shaping not only individual research behaviour and practices but also possibly the entire institutional research process. When not distracted by exigencies of institutional management and administration, and concerned primarily with research and research-related activities (e.g. teaching, research supervision and mentoring), research leaders speak the language of peers and understand their concerns. It would thus appear that they are in a better position to understand and resolve critical research issues of governance, professional information, research management and administration, grant conditions of awards and research-related contracts, contacts with relevant stakeholders, and opportunities, responsibilities, risks and benefits associated with collaborative initiatives. With appropriate competences and steering autonomy, scientific committees could take and implement more informed research decisions than institutional management or even research chairs who, when acting alone, could either ignore or overrule professional advice and inputs. For these reasons, it is argued that scientific committee structures of research governance, more so than other governance structures, could better facilitate the establishment of information exchange networks that enable researchers to make important choices concerning research topics and priorities, funding sources, publication orientation and strategies as well as communication options. This is because the information exchange networks which define

scientists' communication behaviour, tend to maximise resources, find complementary skills and expand the organisation's ability to generate and access new knowledge.[34,35] In addition to providing professional research services – which might include promoting the preparation of high-quality proposals and information to researchers on research integrity and ethics by means of guidelines, individual assistance, websites, training and workshops – scientific committees could enable scientists to improve their research and their competences. In support of this view, Osterloh and Frey[21] argued that a governance system based on qualitative evaluation of peers and supportive feedback would be able to inform researchers on how to improve their research and their competence. Peer reviews or 'evaluations are reasonable mechanisms to enhance publishing activities'[1], and develop reputations that come from quality publication, in particular creativity.[36] Based on these arguments, scientific committees could encourage the development of appropriate publishing behaviour by scientists. In brief, by fostering linkages, effective communication, institutional socialisation, and by means of peer reviews, scientific committees could mould and sustain appropriate research behaviour and practices and competences of individual researchers and research teams. This suggestion is consistent with the assertion that scientific collaboration results in the generation of new knowledge, new method and new approaches.[37] However, there may be a technical problem in determining the precise mediating effect of scientific committee structures on research practices because the association is qualitatively determined. There also is a possible danger of using this recipe to create a governance model that can be hijacked by professional oligarchs. A limitation to this study is the lack of crucial data to investigate the precise moderating effects of scientific committee structures on research behaviours of scientists; this could be considered as an area for future research. Nonetheless, the analysis yields the insight that research institutions, considering their missions, should wisely choose their governance system because of the far-reaching implications it might have for the research practices or behaviours of scientists and their knowledge production.

Conclusion

This study contributes to empirical discussions on research governance, showing that scientific committee structures with significant research steering autonomy contribute not only directly to scientific output but also indirectly through moderating effects on research practices and behaviour. By generally contributing positively to the development of appropriate research behaviour, this model and its governance structures could have profound impacts on scientific outputs. Autonomous scientific committees could mould and sustain appropriate research behaviour and practices and competences of individual researchers and research teams by fostering linkages, effective exchange of information among peers and institutional socialisation and by means of qualitative peer-based evaluations. However, it is acknowledged that, if supported by advisory groups of mainstream scientists and academics at key hierarchical levels of governance, management-centric models and structures can positively contribute to scientific output. Overall, as original research, particularly creative research, and innovations require autonomy, individuality and freedom on the part of researchers, research teams and governors of research institutions must set up suitable governance models which provide for significant decision-taking autonomy at key hierarchical levels of governance. A national research council which guides and focuses research activities according to the needs of society and industry could support such suitable governance structures, which are tailored towards the institutional mission.

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Characterisation of *Mycosphaerella* species associated with pink spot on guava in South Africa

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DATES:
Received: 18 Feb. 2013
Revised: 04 Feb. 2014
Accepted: 07 Feb. 2014

KEYWORDS:
Capnodiales; *Psidium guajava;* multi-gene phylogeny; internal transcribed spacer region

HOW TO CITE:
Jacobs A, Truter M, Schoeman MH. Characterisation of *Mycosphaerella* species associated with pink spot on guava in South Africa. S Afr J Sci. 2014;110(9/10), Art. #2013-0041, 6 pages. http://dx.doi.org/10.1590/sajs.2014/20130041

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Pink spot symptoms on guava fruit in the Lowveld region were in the past attributed to *Colletotrichum gloeosporioides*, but recently *Mycosphaerella* species were suggested to be part of a disease complex, including pink spot symptoms. During routine surveys of guava diseases in the Lowveld area of the Mpumalanga Province in South Africa, *Mycosphaerella* species were consistently isolated from guava fruit. *Colletotrichum gloeosporioides* was also retrieved, especially from older, bigger lesions. The *Mycosphaerella* isolates were compared based on their growth characteristics in culture and on DNA sequences of the internal transcribed spacer region, large subunit of the ribosomal DNA as well as the β-tubulin and translation elongation factor 1α gene regions. The phylogenetic analyses indicate that the isolates from the present study represent at least three species not previously reported on guavas. This report is therefore the first report of *Mycosphaerella* species associated with *Psidium guajava* in South Africa.

Introduction

Guava (*Psidium guajava* L.) is a fruit-bearing tree in the Myrtaceae plant family. Closely related genera include *Eucalyptus* L'Heritier and *Syzygium* R.Br. ex Gaertn. The tree is native to Mexico, the Caribbean and Central America, where it is an economically important crop.[1,2] Leading producers of guava fruit are Brazil, India and Mexico.[2] In South Africa, approximately 41 000 tonnes of guava are harvested per annum for fresh sales and processing.[3]

Several diseases affecting the health and productivity of guava plants and fruit have been reported internationally. Of these, anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., is the most common fruit disease in guava-growing countries globally.[2] In Puerto Rico, up to 50% of the guava crop (mainly from wild trees) is threatened by anthracnose, which mummifies and blackens immature fruits and rots mature fruits. Similarly, dry rot, caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (*Diplodia natalensis* Pole-Evans), has been reported to affect 40% of the crop on some trees in South India.[1]

Fruit diseases occurring on guava in South Africa include anthracnose, blossom end rot (caused by *Phomopsis psidii* Nag Raj & Ponnappa) and pink spot.[4,5] Pink spot symptoms were in the past attributed to *C. gloeosporioides*[6] – the same fungus causing anthracnose – but later the possibility of a *Guignardia* sp. as the causal agent was investigated (Schoeman, unpublished data). Recently, it has been suggested that *Mycosphaerella*-like species could be associated with the small pink and red/purple specks of the disease called pink spot. These specks have been seen on fruit in all guava production areas in South Africa, but are most severe in the Mpumalanga Province.[5]

Mycosphaerella Johanson is one of the largest genera of plant pathogenic fungi, many of which cause economically important diseases in temperate and tropical crops.[7-10] *Mycosphaerella* species occur on all aboveground plant parts including the leaves and stems of several hundred different host plants.[11] *Mycosphaerella* can spread to healthy hosts either as waterborne conidia or as airborne ascospores. Once in contact with compatible host tissue, the spores can germinate and penetrate the plant through the leaf stomata.[8]

Identification of *Mycosphaerella* species based on morphology alone is impossible. The production of very small fruiting structures with highly conserved morphology, host-specificity and poor growth in culture contribute to a lack of informative morphological characteristics. Although ascospore germination patterns[8,12] and morphology of the asexual state[13] greatly facilitate species identification, co-inhabitancy[14] makes it difficult to link the asexual state from the isolated culture to their correct sexual state as observed on the host tissue[15] using only morphological approaches.

Comparisons of DNA-based methods such as RAPDs, species-specific primers, microsatellites and DNA sequence data have in recent years been employed to distinguish between *Mycosphaerella* species, resulting in substantial changes to genus and species concepts in this group.[12,13,15-17] The majority of studies employing DNA sequence data for species identification has relied on sequence data from the internal transcribed spacer (ITS) region, large subunit of the ribosomal DNA (LSU) as well as translation elongation factor 1α (TEF) gene regions.[18-20]

The objective of this study was to identify possible *Mycosphaerella* species associated with pink spot on *P. guajava* fruit in the Lowveld area of the Mpumalanga Province, South Africa. Identification was performed using DNA sequence data of the ITS, LSU, TEF as well as the β-tubulin (BT) gene regions.

Materials and methods

Symptoms and isolations

Symptomatic guava fruit were collected from various orchards in the Mpumalanga Province of South Africa during the 2007–2009 production seasons and field observations were recorded. Fruit were surface disinfected by spraying twice with 75% ethanol, followed by air drying. Lesions of different sizes were randomly selected, aseptically removed and small (1–2 mm in diameter) segments plated on potato dextrose agar (PDA) (Biolab, Merck, Darmstadt, Germany) in Petri dishes. Plates were incubated at 25 °C, under 24-h cool white fluorescent

light for 3–4 weeks. The emerging fungal colonies were examined and selected colonies were purified for further identification.

Isolates were sub-cultured onto carnation leaf agar, malt extract agar (MEA) (Biolab, Merck, Darmstadt, Germany) and oatmeal agar (OA) for morphological grouping and determination of growth characteristics.[21] Petri dishes were incubated at 25 °C under continuous near-ultraviolet light, and examined weekly for 2 months. Mounts were prepared in lactic acid and examined using phase and bright-field phase contrast microscopy at x400 and x1000 magnification. Colony colour was assigned based on Rayner's colour chart.[22] Cultures are maintained in the South African National Collections of Fungi (PPRI collection) (Table 1).

DNA sequence comparisons

DNA extraction and amplification

All isolates obtained were grown on PDA at 25 °C for 21 days. DNA was isolated using the DNAeasy® Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's specifications. Extracted DNA was used as a template in polymerase chain reactions (PCR) to amplify a part of the ITS region using the primer set ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3')[23], LSU region (including domains D1–D3) using the primer set LR0R (5'-ACC CGC TGA ACT TAA GC-3')[24] and LR7 (5'-TAC TAC CAC CAA GAT CT-3')[25], BT region using the primer set TUB2Fd (5'-GTB CAC CTY CAR ACC GGY CAR TG-3') and TUB4Rd (5'-CCR GAY TGR CCR AAR ACR AAG TTG TC-3')[26] and TEF region using primer set EF1-728F (5'-CAT CGA GAA GTT CGA GAAGG-3') and EF2-986R (5'-TAC TTG AAG GAA CCC TTACC-3')[27].

The PCR reactions consisted of 1x Roche *Taq* reaction buffer with $MgCl_2$, dNTPs (250 μM each), primers (0.2 μM each), template DNA (25 ng) and Roche *Taq* polymerase (0.5 U) (Roche Pharmaceuticals, Berlin, Germany). The PCR reaction conditions for the amplification of the ITS gene region were an initial denaturation at 94 ºC for 2 min, followed by 35 cycles of denaturation at 94 ºC for 1 min, annealing at 52 ºC for 1 min and elongation at 72 ºC for 1 min, with a final elongation step of 72 ºC for 5 min. PCR cycling conditions for the LSU, BT and TEF gene regions differed from the above-mentioned protocol only in the annealing temperatures (62 °C for LSU and 52 °C for BT and TEF). The resulting amplicons were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany).

DNA sequencing and sequence comparisons

DNA sequences were determined from PCR amplicons using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, (Applied Biosystems, Paisley, UK) using both forward and reverse primers for each gene region. Sequences generated in this study have been deposited in GenBank (Table 1).

BLAST comparisons on the Mycosphaerellaceae database (MycoBank, www.mycobank.org) were done for the ITS, TEF, LSU and BT sequences generated. These comparisons were performed to enable the construction of data sets for phylogenetic analyses and comparisons with closely related species. Multiple sequence alignments for all gene regions were generated for our data using MAFFT version 5.[28] Gaps were treated as missing data in the subsequent analysis. Phylogenetic analysis was based on parsimony using PAUP 4.0* (Phylogenetic Analysis Using Parsimony *and Other Methods version 4).[29] Heuristic searches were done with random addition of sequences (100 replicates), tree bisection-reconnection branch swapping, MULPAR effective and MaxTrees set to auto-increase. The combinability of the data sets was determined by the partition homogenicity test.[30] Species previously isolated from Myrtaceae plant species were included in the final phylogenetic data set. The consistency and retention indices were determined for the data set. The phylogenetic tree was rooted with *Uwebraunia commune* (Crous & Mansilla) Crous as monophyletic sister outgroup to the rest of the taxa. Bootstrap analyses were performed to determine branching point confidence intervals (1000 replicates) for the most parsimonious trees generated for the respective data sets.

Results

Symptoms and isolations

Spots and lesions were observed on fruit from February to September, depending on the time of pruning, and on leaves from December to August during the 3-year inspection period. Symptoms on fruit

Table 1: *Mycosphaerella* isolates from infected guava fruit used in this study

Name	PPRI no.†	Geographical locality	GenBank accession numbers (ITS/TEF)	MycoBank BLAST result (ITS/TEF)
Mycosphaerella acaciigena	8911	Nelspruit, Mpumalanga, South Africa	JQ254924	*M. acaciigena*/*M. heimioides*
M. acaciigena	8916	Nelspruit, Mpumalanga, South Africa	JQ254928	*M. acaciigena*/*M. heimioides*
M. acaciigena	8919	Nelspruit, Mpumalanga, South Africa	JQ254923	*M. acaciigena*/*M. heimioides*
M. acaciigena	8920	Nelspruit, Mpumalanga, South Africa	JQ254922	*M. acaciigena*/*M. heimioides*
M. acaciigena	8921	Nelspruit, Mpumalanga, South Africa	JQ254927	*M. acaciigena*/*M. heimioides*
Mycosphaerella keniensis	8912	Nelspruit, Mpumalanga, South Africa	JQ254919	*M. keniensis*/ *M. heimioides*
M. keniensis	9045	Nelspruit, Mpumalanga, South Africa	JQ254918	*M. keniensis*/ *M. heimioides*
M. keniensis	9046	Nelspruit, Mpumalanga, South Africa	JQ254917	*M. keniensis*/ *M. heimioides*
Mycosphaerella heimii	8915	Nelspruit, Mpumalanga, South Africa	JQ254920	*M. heimii*/ *M. heimioides*
M. heimii	8917	Nelspruit, Mpumalanga, South Africa	JQ254925	*M. konae*/ *M. heimioides*
Mycosphaerella sp.	8913	Nelspruit, Mpumalanga, South Africa	JQ254926	*M. acaciigena*/ *M. acaciigena*
Mycosphaerella sp.	8914	Nelspruit, Mpumalanga, South Africa	JQ254916	*M. aurantia*

†*The South African National Collections of Fungi (PPRI collection).*

ITS, internal transcribed spacer region; TEF, translation elongation factor 1α.

developed before harvesting and did not increase significantly in size after harvest, but became more obvious as the fruit changed colour. Single spots originated from an initial slight depression (1–2 mm in diameter) with a brick-red margin (Figure 1a). On green fruit, lesions had a dark reddish-brown colour (Figure 1b) and as the fruit coloured, the centres became light brown and the brick red margins showed up more clearly. As a spot aged, it enlarged to about 3–4 mm in diameter with the tissue in the centre turning lighter brown, surrounded by a dark brown to purple-red margin which faded to a brick red colour (Figure 1c). Not all lesions enlarged with age, giving rise to various-sized lesions on the fruit at harvest. No fruiting structures were observed in the fruit lesions at the time of harvest. Fruit incubated in moist conditions developed excessive sporulation of a *Colletotrichum* species and no fruiting structures of fungi in the Mycosphaerellaceae could be detected.

Symptoms on leaves documented during field inspections were small (<2 mm in diameter) and visible on both sides of the leaf. These lesions were roundish and had a red-brown colour (Figure 1d). Lesions did not enlarge with age. Symptoms on leaves were most severe in July and August – at times, large areas of the leaves were covered with spots. In December to June, only a few spots per leaf were found. No further investigations were performed on these lesions.

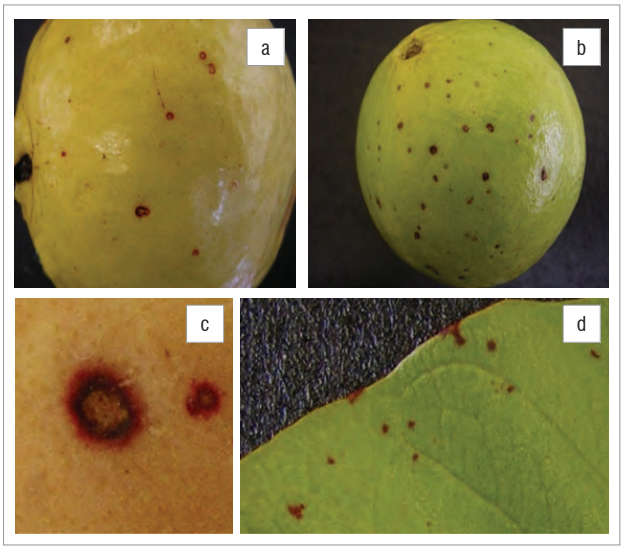

Figure 1: Symptoms of pink spot on fruit and leaves: (a) initial slight depression with brick red margin, (b) green fruit showing dark reddish brown lesion, (c) a mature spot and (d) pink spot symptoms on leaves.

Big, mature lesions on fruit yielded mostly *C. gloeosporioides*-like fungi (90%), identified based on ITS gene sequences, with only a few isolates resembling the Mycosphaerellaceae. Fewer Mycosphaerellaceae species were retrieved when lesion segments larger than 2 mm in diameter were used for isolations; however, with small (1–2 mm in diameter) lesions, 80–90% of lesion segments yielded isolates resembling fungi in the Mycosphaerellaceae.

Mycosphaerella-like cultures could be grouped into three morpho-groups based on colony morphology and colour. Cultures were, however, sterile, making it impossible to confirm their identity based on morphology. Colonies of *Mycosphaerella* group A cultures were characterised by thin, white, smooth margins (1 mm wide), sparse to moderate aerial mycelium and grey-olivaceous (21’’’’i) surfaces on OA; whereas on MEA, the colony margins were evenly edged, aerial mycelium moderate, surfaces were olivaceous-grey (23’’’’i) and the reverse was greenish black (33’’’’k).

Colonies of *Mycosphaerella* group B cultures on OA were characterised by smooth margins, moderate aerial mycelium and grey-olivaceous (21’’’i) surfaces; whereas on MEA, the colony margins were irregular but smooth with moderate aerial mycelium and olivaceous-grey (23’’’’i) surfaces with olivaceous-black (27’’’’k) reverse sides.

Colonies of *Mycosphaerella* group C isolates on OA displayed thin, white, smooth margins (1–2 mm wide), sparse aerial mycelium and pale olivaceous-grey (21’’’’d) surfaces. On MEA, the margins were smooth, aerial mycelium sparse and variable in colour, and surfaces were predominantly pale olivaceous-grey (23’’’’d) with patches of olivaceous-grey (21’’’’i) and smoke grey (19’’’d), and the reverse was dark olivaceous-grey (21’’’’i).

DNA sequence comparisons

DNA sequencing and sequence comparisons

BLAST results of sequences for the ITS, LSU, BT and TEF gene regions generated for *Mycosphaerella*-like isolates from guava fruit in this study suggested that all our isolates represented species of *Mycosphaerella* (Table 1). Parsimony analyses of the sequences for the ITS and TEF gene regions were done to determine the phylogenetic placement of the *Mycosphaerella*-like isolates from guava amongst other *Mycosphaerella* species associated with Myrtaceae plants (Table 2) – the plant family to which *Psidium guajava* belongs. Alignment by inserting gaps resulted in a total of 1153 characters used in the combined data set for the ITS and TEF gene sequences. All parsimony-uninformative and constant characters were excluded, resulting in 394 parsimony-informative characters for the combined data set. Heuristic searches on the data set generated 100 most parsimonious trees and partition homogeneity tests indicated that the two data sets could be combined (Figure 2). The LSU and BT data sets were excluded from the combined data set because the LSU data set did not resolve the relationships amongst the species (data not shown) and the BT data available to include for reference strains are limited.

Two South African isolates from guava (group A) grouped with *Mycosphaerella heimii* Bouriquet ex Crous, supported by a 90% bootstrap value (Figure 2), while three isolates (group B) grouped with *M. keniensis* Crous & T.A. Cout., although this grouping was only supported by a 74% bootstrap value. An additional five South African isolates (group C) grouped with *M. acaciigena* Crous & M.J. Wingf, supported by a 100% bootstrap value. PPRI 8914 clustered basal to the clade of *M. ellipsoidea* Crous & M.J. Wingf, *M. africana* Crous & M.J. Wingf, *M. aurantia* A. Maxwell and *M. keniensis*, while PPRI 8913 grouped basal to the *M. acaciigena* cluster, both supported by a 100% bootstrap value. Both of these isolates may represent new species. All these groupings support the MycoBank BLAST results.

Discussion

Pink spot of guava fruit in South Africa has in the past been attributed to *C. gloeosporioides.*[6] *Mycosphaerella* species were only recently suspected to be associated with the pink spot observed on guava fruit and leaves in the Lowveld of Mpumalanga, South Africa. In the current study we identified isolates from pink spot symptoms by means of phylogenetic comparisons, as opposed to morphology only. The analyses indicate that the *Mycosphaerella* isolates from fruit lesions represent at least three species not previously reported on guavas. These species are *M. heimii, M. keniensis* and *M. acaciigena. Mycosphaerella heimii* have previously been reported on *Eucalyptus* species and *M. acaciigena* on *Acacia mangium* Willd.[20,31-33] A number of *Mycosphaerella* species are associated with Myrtaceae species other than *Eucalyptus,* including *Mycosphaerella syzygii* Crous, *Mycosphaerella aequatoriensis* Petr., *Mycosphaerella eugeniae* Rehm and *Mycosphaerella metrosideri* F. Stevens & P.A. Young.[34] None of these were isolated from the infected guava trees.

In this study, we applied DNA sequences of the ITS, LSU and TEF regions. Although ITS has been shown to be insufficient for the *Mycosphaerella* species in anamorph genera such as *Cercospora* and *Septoria*, it appears to be useful for distinguishing species with *Pseudocercospora* Speg., *Ramularia* Unger and most other *Mycosphaerella* anamorph genera.[13] Our data of the ITS and TEF regions enabled us to distinguish between all the *Mycosphaerella* species included in this study, except for two isolates that grouped basal to existing clades. These isolates may represent new species. The BLAST results did, however, cluster them

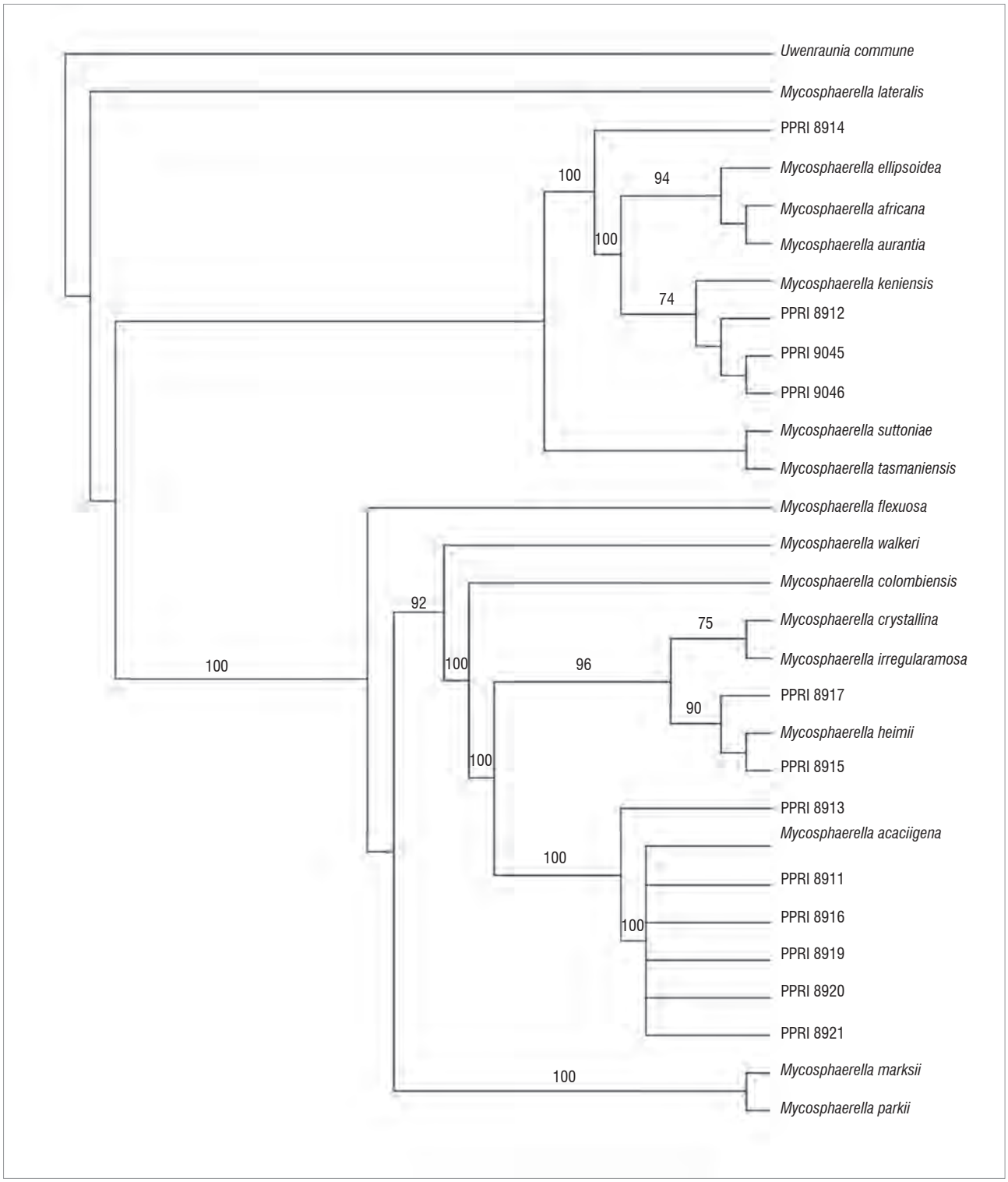

Figure 2: Phylogenetic tree produced using parsimony of the internal transcribed spacer and translation elongation factor 1α gene regions with *Uwebraunia commune* as outgroup. Bootstrap values above 70% (percentages of 1000 bootstrap replicates) are indicated above the branches of the tree (tree length=483; consistency index=0.5983; retention index=0.7798).

with individual species. The LSU data failed to resolve the phylogenetic relatedness amongst the species.

Species of *Mycosphaerella* are usually assumed to be host-specific and most species have a narrow host range.[13,14] But, in some cases, *Mycosphaerella* species have been reported from other plants, albeit in low numbers.[8] Crous and Groenewald[13] suggested that in the absence of their natural host, *Mycosphaerella* species can infect and reproduce on non-host plants or catch crops in an attempt to find the appropriate host species. They referred to the phenomenon as the 'pogo stick hypothesis'. It is possible that guava serves as only a catch crop for the associated *Mycosphaerella* species identified in the present study. Another hypothesis explaining the presence of *Mycosphaerella* species on non-host plants is adaptation to a new host plant species.[13]

Table 2: Species included in the phylogenetic analyses

Species	GenBank accession number	
	Internal transcribed spacer sequence data	Translation elongation factor 1α data
Uwebraunia commune[a]	EU514232[c]	JX500111
Mycosphaerella acaciigena[a]	AY752143[a]	GU384367
Mycosphaerella africana[b]	DQ267577[b]	DQ235099
Mycosphaerella aurantia[b]	AY509744[a]	DQ235097
Mycosphaerella colombiensis[b]	AY725533[a]	AY752183
Mycosphaerella cryptica[b]	DQ302951[b]	–
Mycosphaerella crystallina[b]	AF222839[d]	JQ733028
Mycosphaerella cyptica[b]	AY534226[a]	–
Mycosphaerella ellipsoidea[b]	AY725545[d]	JX901653
Mycosphaerella flexuosa[b]	DQ302956[b]	JX901653
Mycosphaerella heimii[a]	EF394837[a]	JX500107
Mycosphaerella heimioides[b]	DQ267586[b]	–
Mycosphaerella irregulariramosa[b]	AF309608[d]	DQ240178
Mycosphaerella keniensis[b]	AF173300[d]	DQ235100
Mycosphaerella lateralis[b]	AY725552[d]	DQ235139
Mycosphaerella madeirae[b]	AY725553[d]	–
Mycosphaerella marasasii[b]	AF309591[d]	–
Mycosphaerella marksii[b]	DQ302979[b]	DQ235135
Mycosphaerella parkii[b]	AY152599[d]	DQ235137
Mycosphaerella suttoniae[b]	DQ303053[b]	DQ240170
Mycosphaerella tasmaniensis[b]	DQ784689[b]	DQ235122
Mycosphaerella walkeri[b]	AY045501[d]	DQ235096

[a]Crous et al.[19]; [b]Hunter et al.[20]; [c]sourced from the National Center for Biotechnology Information; [d]Crous et al.[18]

Most of the South African *Mycosphaerella* isolates associated with pink spot clustered with *Mycosphaerella* species from unresolved clades, as previously indicated by Crous et al.[35] Further work is required to resolve the taxa associated with pink spot on guava in South Africa. In addition, the pathogenicity of *Mycosphaerella* isolates on guava needs to be resolved before conclusions on the host range of *M. acaciigena, M. heimii* and *M. keniensis* can be made. Characterisation of fungal isolates associated with leaf lesions observed during the 3-year period, also warrants further investigation. This report is the first of *Mycosphaerella* species associated with pink spot of guava in South Africa.

Authors' contributions

M.H.S. was the project leader. A.J. and M.T were responsible for experimental and project design and performed most of the experiments. All the authors contributed to the writing of the manuscript.

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Escherichia coli with virulence factors and multidrug resistance in the Plankenburg River

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DATES:
Received: 06 Nov. 2013
Revised: 06 Feb. 2014
Accepted: 18 Feb. 2014

KEYWORDS:
enteropathogenic; enteroaggregative; virulence genes; antibiotic resistance; pollution

HOW TO CITE:
Lamprecht C, Romanis M, Huisamen N, Carinus A, Schoeman N, Sigge GO, et al. *Escherichia coli* with virulence factors and multidrug resistance in the Plankenburg River. S Afr J Sci. 2014;110(9/10), Art. #2013-0347, 6 pages. http://dx.doi.org/10.1590/sajs.2014/20130347

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Escherichia coli is a natural inhabitant of the gut and *E. coli* levels in water are considered internationally to be an indication of faecal contamination. Although not usually pathogenic, *E. coli* has been linked to numerous foodborne disease outbreaks, especially those associated with fresh produce. One of the most common ways through which *E. coli* can be transferred onto fresh produce is if contaminated water is used for irrigation. In this study, a total of 81 confirmed *E. coli* strains were isolated from the Plankenburg River as part of three separate studies over 3 years. During sampling, *E. coli* levels in the river were above the accepted levels set by the World Health Organization and the South African Department of Water Affairs and Forestry for safe irrigation of fresh produce, which indicates that transfer of *E. coli* during irrigation is highly probable. Multiplex polymerase chain reaction screening for pathogenic gene sequences revealed one enteroaggregative positive strain and four enteropathogenic positive strains. The four enteropathogenic strains were also found to be resistant to three or more critically and highly important antibiotics and were therefore classified as multidrug resistant strains. These results show that *E. coli* with enteropathogenic potential and multiple antimicrobial resistance properties has persisted over time in the Plankenburg River.

Introduction

Escherichia coli is a natural inhabitant of the gut of humans, birds and other warm-blooded animals and is widely accepted as an indicator of faecal contamination of water. It is a robust bacterium which is genetically highly adaptable to environmental stresses, and has been shown to survive and multiply in the environment.[1,2] Based on the aforementioned, concerns have been raised in recent years regarding the status of *E. coli* as just a faecal indicator organism.[3]

Although most strains are commensal, pathogenic *E. coli* strains can contain various virulence factors and can be responsible for a variety of infections.[4] Based on the specific virulence factors present, pathogenic *E. coli* can be classified as either extra-intestinal pathogenic *E. coli* (ExPEC) or intestinal pathogenic *E. coli* (InPEC). ExPEC strains are usually able to cause infections in anatomical sites outside of the intestinal tract and are associated with urinary tract infections, neonatal meningitis and septicaemia. ExPEC, like commensal *E. coli*, can colonise the intestinal tract without causing gastroenteritis. In contrast, InPEC strains can cause different types of gastroenteritis and can be divided into six pathogenic groups: enterohaemorrhagic (EHEC); enteropathogenic (EPEC); enteroaggregative (EAEC); enterotoxigenic (ETEC); enteroinvasive (EIEC) and diffusely adherent (DAEC) *E. coli*. Each of the InPEC types has different infection mechanisms and symptoms.[4]

Foodborne disease outbreaks linked to pathogenic *E. coli,* specifically those derived from fresh produce, are increasing both in number and intensity.[5] As a result, *E. coli* is considered to be an emerging pathogen. One of the most common means by which *E. coli* can be transferred to fresh produce is via contaminated irrigation water. Recognising this potential danger, the World Health Organization (WHO) and the South African Department of Water Affairs (DWA) have set a recommended limit for irrigation water used for fresh produce of 1000 faecal coliforms/100 mL.[6,7]

Long-term monitoring studies of the Plankenburg River have revealed that although faecal coliform loads vary depending on the season, the loads are above the recommended limits.[8] The Plankenburg River flows past an informal settlement as well as through an industrial area of the town of Stellenbosch before it converges with the Eerste River and flows through an agricultural region, where the water is withdrawn for irrigation. Constant high faecal coliform levels might therefore contribute to the possible transfer of *E. coli* during irrigation, if river water is not treated prior to irrigation.

Although the presence of several potential pathogens in the Plankenburg River has been reported,[8] the occurrence and types of pathogenic *E. coli* are not known. The aim of this study was therefore to determine the number, types and antibiotic susceptibility of potential pathogenic *E. coli* present in the Plankenburg River.

Materials and methods

Sampling

Water samples from which *E. coli* was isolated were collected from the Plankenburg River (Stellenbosch, Western Cape Province) at three sites (P-0, P-1 and P-2).[8] The P-0 sampling site was about 5 km upstream of the P-1 site and was selected specifically to assess the impact of an informal settlement and industrial area on the water quality of the Plankenburg River. However, for large parts of each year, especially during the dry summer months, this site had no flowing water and therefore it was not sampled at the same frequency as the other sites. Two of the sites – P-1 and P-2 – are situated downstream of an informal settlement and industrial area and have shown high levels of faecal contamination.[8] River water sampling was done in accordance with the SANS 5667-6 method[9]; all samples were 1 L in volume.

E. coli isolation and identification

Study 1 (year 1):

Daily, for 9 days, 1 L of water was collected aseptically at site P-1. The presence of coliforms, faecal coliforms and *E. coli* were determined using the multiple tube fermentation (MTF) technique.[10] The *E. coli* broth tubes that exhibited gas production as well as fluorescence after 24 h incubation at 44.5 °C were streaked onto Eosin Methylene-blue Lactose Sucrose (L-EMB) agar (Oxoid, Hampshire, UK). Five typical *E. coli* colonies (dark purple to black colonies with a distinct metallic sheen) from each of the water samples were selected using Harrison's disc method[11] and further purified using Brilliance™ *E. coli*/coliform selective agar (Oxoid).

Study 2 (year 2):

Daily, for 14 days, 1 L of water was collected aseptically at site P-2. *E. coli* enumeration was done according to the American Public Health Association Standard Method[12] and Violet Red Bile Agar (Merck, Johannesburg, South Africa) was used for the enumeration. For further analysis, representative *Enterobacteriaceae* colonies (red and pink) were selected using Harrison's disc method.[11] The selected colonies were further purified using Brilliance™ *E. coli*/coliform selective agar (Oxoid).

Study 3 (year 3):

As part of a larger environmental study, sites P-0, P-1 and P-2 were sampled twice. Enumeration of total coliforms and *E. coli* was done according to SANS 9308[13] using Colilert 18 (IDEXX, Cape Town, South Africa). The Colilert 18 method is considered more user-friendly than the traditional MTF method used in Study 1, and it has been reported that Colilert results compare well with MTF results.[14] After incubation,[14] Quantitrays were divided into quarters and two fluorescent wells per quarter were pooled (1 mL per well to yield a maximum of 8 mL). A dilution was made from the pooled extract and spread plates were prepared on L-EMB agar (Oxoid). Five typical *E. coli* colonies from each of the water samples were selected at the highest dilution using Harrison's disc method[11] and purified using Brilliance™ *E. coli*/coliform selective agar (Oxoid).

Characterisation and confirmation of E. coli *identification*

Each isolate was streaked out on nutrient agar (Biolab, Johannesburg, South Africa), and the analytical profile index (API) 20E system (BioMérieux, Johannesburg, South Africa) was used in conjunction with Gram staining, a catalase test and growth on MacConkey medium.[15] The profile was then entered into the API Web database (BioMérieux) for species identification. Isolates were stored in 40% (v/v) glycerol at -80 °C.

Polymerase chain reaction analyses

Cell extracts of all isolates were prepared prior to the polymerase chain reaction (PCR) using the method of Altalhi and Hassen[16]. Isolates identified as *E. coli* with API were tested for the presence of the *E. coli uidA* household gene using the primer sequences of Heijnen and Medema[17] and KAPATaq™ HotStart DNA polymerase (KAPABiosystems, Cape Town, South Africa). Strains that tested positive for *uidA* were subjected to a multiplex PCR method as modified from Omar and Barnard[18] to screen for the presence of InPEC genes (Table 1). Strains identified as InPECs were also screened for the presence of ExPEC genes[19] and classified phylogenetically using the triplex PCR method of Clermont et al.[20] for *E. coli* phylogenetic group (genogroup) identification. The Kapa2G™ Fast Multiplex PCR kit (KAPABiosystems) was used for the InPEC and ExPEC multiplex methods as well as for the triplex PCR method. Positive and negative controls were included in all PCRs, and all reactions were performed in a G-storm thermal cycler (Vacutec, Johannesburg, South Africa). Primer sequences and concentrations are presented in Table 1. Reaction conditions are presented in Table 2. PCR products were all visualised with UV illumination after gel electrophoresis in agarose gels containing 1 μg/mL ethidium bromide and 0.5 x TBE buffer. Gel electrophoresis was performed at 210V/20 min for the *uid*A PCR products and the triplex PCR products, using 1.25% agarose gels and 2% agarose gels, respectively. Gel electrophoresis of the InPEC and ExPEC PCR products were conducted at 120V/90 min using 1.25% agarose gels. A 100-bp marker (Promega, Madison, WI, USA) was included for size estimation purposes.

Table 1: Primer sequences used for *uid*A,[17] triplex,[20] InPEC[18] and ExPEC[19] polymerase chain reactions (PCRs)

Primer†	Primer sequence (5' - 3')	Size (bp)	Primer concentration
	***E. coli* confirmation PCR[17]**		
UAL 1939b	ATGGAATTTCGCCGATTTTGC	187	0.4 μM
UAL 2105b	ATTGTTTGCCTCCCTGCTGC		
	InPEC PCR[18]		
***E. coli* control**			
Mdh01 (F)	GGTATGGATCGTTCCGACCT[21]	300	0.2 μM
Mdh02 (R)	GGCAGAATGGTAACACCAGAGT[21]		
EIEC			
L-ial (F)	GGTATGATGATGATGAGTCCA[22]	650	0.2 μM
Ial (R)	GGAGGCCAACAATTATTTCC[22]		
EPEC & EHEC‡			
L-eaeA (F)	GACCCGGCACAAGCATAAGC[22]	384	0.2 μM
L-eaeA (R)	CCACCTGCAGCAACAAGAGG[22]		
EHEC			
Stx1 (F)	ACACTGGATGATCTCAGTGG[23]	614	0.2 μM
Stx1 (R)	CTGAATCCCCCTCCATTATG[23]		
Stx2 (F)	CCATGACAACGGACAGCAGTT[23]	779	0.2 μM
Stx2 (R)	CCTGTCAACTGAGCACTTTG[23]		
ETEC			
LT (F)	GGCGACAGATTATACCGTGC[22]	450	0.2 μM
LT (R)	CGGTCTCTATATTCCCTGTT[22]		
ST (F)	TTTCCCCTCTTTTAGTCAGTCAACT[18]	160	0.2 μM
ST (R)	GGCAGGATTACAACAAAGTTCACA[18]		
EAEC			
Eagg (F)	AGACTCTGGCGAAAGACTGTATC[24]	194	0.2 μM
Eagg (R)	ATGGCTGTCTGTAATAGATGAGAAC[24]		
	Phylogenetic group PCR[20]		
chuA.1 (F)	GACGAACCAACGGTCAGGAT	279	0.2 μM
chuA.2 (R)	TGCCGCCAGTACCAAAGACA		
yjaA.1 (F)	TGAAGTGTCAGGAGACGCTG	211	0.2 μM
yjaA.2 (R)	ATGGAGAATGCGTTCCTCAAC		
TSPE4.C2.1 (F)	GAGTAATGTCGGGGCATTCA	152	0.2 μM
TSPE4.C2.2 (R)	CGCGCCAACAAAGTATTACG		
	ExPEC PCR[19]		
papA (F)	ATGGCAGTGGTGTCTTTTGGTG	717	0.2 μM
papA (R)	CGTCCCACCATACGTGCTCTTC		
papC (F)	GTGGCAGTATGAGTAATGACCGTTA	205	0.2 μM
papC (R)	ATATCCTTTCTGCAGGGATGCAATA		
sfa (F)	CTCCGGAGAACTGGGTGCATCTTAC	410	0.2 μM
sfa (R)	CGGAGGAGTAATTACAAACCTGGCA		
iutA (F)	GGCTGGACATCATGGGAACTGG	302	0.2 μM
iutA (R)	CGTCGGGAACGGGTAGAATCG		
kpsMT (F)	GCGCATTTGCTGATACTGTTG	272	0.2 μM
kpsMT (R)	CATCCAGACGATAAGCATGAGCA		

†F, forward primer; R, reverse primer

‡EHEC strains should be eaeA *positive as well as stx 1 and/or stx 2 positive*

Table 2: Thermocycling conditions used for *uid*A, triplex, InPEC and ExPEC polymerase chain reactions (PCRs)

PCR method	Thermocycling conditions
*uid*A	95 °C for 3 min, 35 cycles of 95 °C for 30 s, 59.7 °C for 30 s, 72 °C for 30 s, final extension at 72 °C for 5 min
InPEC multiplex	95 °C for 3 min, 30 cycles of 95 °C for 15 s, 55 °C for 30 s, 68 °C for 30 s, final extension at 72 °C for 5 min
Genogroup triplex	95 °C for 3 min, 30 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, final extension 72 °C for 5 min
ExPEC multiplex	95 °C for 3 min, 30 cycles of 95 °C for 15 s, 61 °C for 30 s, 68 °C for 30 s, final extension at 72 °C for 5 min

Antimicrobial resistance testing

The *E. coli* pathogens identified with PCR analysis were also screened for antimicrobial susceptibility against Ampicillin (10 μg), Cephalothin (30 ug), Chloramphenicol (30 ug), Ciprofloxacin (5 μg), Tetracycline (30 μg), Trimethoprim (2.5 μg), and Streptomycin (S) (10 μg). This screening was done using the standard antimicrobial disc susceptibility test described by the US Clinical and Laboratory Standards Institute (CLSI).[25] Inhibition zones were interpreted using the performance standards of CLSI[26] (for Ampicillin, Cephalothin, Chloramphenicol, Ciprofloxacin, Tetracycline and Streptomycin) and Andrews[27] (for Trimethoprim) and strains were classified as susceptible, intermediate or resistant. The American Type Culture Collection (ATCC) strain 25922 was included as a susceptible control in all antimicrobial resistance screening tests.

Standard cultures for PCR analysis

E. coli ATCC 25922 was used as a positive control in the *uid*A PCR and the triplex PCR. ATCC 25922 was also combined with ATCC 35218 and used as a positive control for the ExPEC multiplex PCR after the identity of bands amplified in the expected regions for *pap*A, *sfa*/*foc*, *iut*A, *kpsMT* II and *pap*C were confirmed with sequencing and BLAST identification. A combination of EPEC, EHEC, ETEC, EIEC and EAEC positive strains was used as a positive control for the InPEC multiplex PCR.

Results and discussion

E. coli *loads*

The *E. coli* loads in the Plankenburg River samples and the number of confirmed *E. coli* strains are presented in Table 3. In all instances, the *E. coli* counts exceeded the WHO recommended limit for irrigation water used for fresh produce of 1000 faecal coliforms/100 mL.[6] National guidelines set by the DWA associate risk with different *E. coli* levels in irrigation water: 'no risk' is associated with <1 *E. coli*/100 mL, 'low risk' is associated with 1–999 *E. coli*/100 mL and 'high risk' is associated with 1000–3999 *E. coli*/100 mL.[7] Considering these guidelines, the *E. coli* levels at the time of analysis qualify as unacceptably high for the most part. It would therefore be a fair assumption to conclude that, under these conditions, the transfer of microbes from the Plankenburg River via irrigation onto the surface of fresh produce is highly probable.

Table 3: *E. coli* load ranges in the Plankenburg River and number of confirmed *E. coli* isolates

Study (sampling site)	*E. coli* load range	Number of isolates
Study 1 (P-1)	29 000–790 000 MPN/100 mL	27
Study 2 (P-2)	3900–118 500 cfu/mL	30
Study 3 (P-0)	1600–20 000 MPN/100 mL	10
Study 3 (P-1)	250 000–1 000 000 MPN/100 mL	8
Study 3 (P-2)	2500–310 000 MPN/100 mL	6

E. coli *with InPEC virulence genes*

The 81 *E. coli* strains isolated from the river (Table 3) were positively identified by the API system and their identity was then confirmed using *uid*A PCR. The strains were then screened for InPEC gene sequences and the characteristics of the pathogenic *E. coli* strains identified are presented in Table 4. Of the 81 strains screened (Table 3), five pathogens were identified (one EAEC and four EPEC strains) (Table 4). This result concurs with previous reports that *E. coli* strains that carry pathogenic gene sequences represent 0.9–10% of *E. coli* present in surface waters.[28] It is interesting to note that the three EPEC strains isolated during Study 2 (Table 4) had different biochemical (API) profiles, which suggests that they are not clones.

Table 4: Intestinal pathogenic (InPEC) *E. coli* strains isolated

Strain	Source	API profile code	Genogroup	Pathotype
E. coli H45	Study 1 (P-1)	5 0 4 4 5 5 2 5 7	A_1	EAEC
E. coli A95	Study 2 (P-2)	1 0 4 4 5 7 2 5 7	$B2_3$	EPEC
E. coli A118	Study 2 (P-2)	5 1 4 4 5 5 2 5 7	B1	EPEC
E. coli A132	Study 2 (P-2)	5 0 4 4 5 5 2 5 7	$B2_3$	EPEC
E. coli S49a	Study 3 (P-2)	1 0 4 4 5 7 2 5 7	$B2_3$	EPEC

EAEC, enteroaggregative E.coli*; EPEC, enteropathogenic* E. coli

The API profiles for all the pathogenic strains showed that they differed in three respects: production of lysine decarboxylase (LDC) and L-ornithine decarboxylase (ODC) and utilisation of saccharose. Variation in these three properties has been observed before in *E. coli* isolated from untreated surface waters and soil.[29,30] It has furthermore been reported that *E. coli* can induce production of amino acid decarboxylases (such as LDC and ODC) in response to reduced pH conditions.[31] This report illustrates the highly adaptable nature of *E. coli* which helps it survive in more acidic environments.[32]

Because the PCR-based detection method of Clermont[20] was used to identify *E. coli* phylogenetic groups (genogroups), the four main groups (A, B1, B2 and D) can further be subdivided into seven subgroups to increase discrimination: A_0, A_1, B1, $B2_2$, $B2_3$, D_1 and D_2.[33] It has been reported that ExPEC strains usually belong to genogroups B2 and D, while InPEC strains that cause severe diarrhoea-related diseases (EIEC, EHEC and ETEC) are most commonly classified into genogroups A and B1.[34] *E. coli* strains that cause mild and chronic diarrhoea (EAEC, EPEC and DAEC) can belong to any of the four main phylogenetic groups.[34] It has, however, been reported that most human EPEC strains belong to genogroups B1 or B2.[35] Carlos et al.[36] examined *E. coli* from a variety of different primary hosts (human, goat, chicken, cow, sheep and pig) and found that $B2_3$ strains in particular could only be found in human samples. It was therefore concluded that the EPEC strains isolated from the river in this study were possibly of human origin, which suggests human faecal contamination of the Plankenburg River. The isolation data

indicates that the contamination might also be constant over time, as similar EPEC strains were isolated during Studies 2 and 3 (Table 4).

Health implications

The enteroaggregative (EAEC) *E. coli*, detected during the first study (Table 4), affects the small intestine and causes mild but persistent watery diarrhoea (≥14 days) in people of all ages. It is frequently found in immunocompromised individuals. As a result of mucinase activity, it can cause mild but significant mucosal damage.[4,37]

Enteropathogenic (EPEC) *E. coli*, of which four were isolated during Studies 2 and 3 (Table 4), primarily affects children and infants, causing profuse watery diarrhoea, fever and nausea. It can also cause disease in certain animals. Outbreaks in developing countries can be quite severe, with reported mortality rates of up to 30%.[37] Adult infections are rare, but possible if high infective doses are combined with substances that can neutralise gastric pH. Certain medical conditions, such as diabetes, can also make adults more susceptible to EPEC infections.[38]

EPEC attachment to the small intestine is a multi-step process which starts with initial attachment to epithelial cells and microcolony stabilisation through bundle-forming pili (BFP), intimate adherence through the production of intimin, and the formation of attaching and effacing lesions and 'pedestal'-like structures.[4,37] Intimin production is facilitated by the locus of enterocyte effacement pathogenicity island which carries the *eae* gene, and is a prerequisite for EPEC and EHEC pathogenicity. BFP is coded for by the *E. coli* adherence factor plasmid, and, although it is considered a virulence contributing factor because of its stabilising role during initial microcolony formation, it is not an absolute requirement for EPEC pathogenicity.[39] Strains that are *eae*(+) and BFP(+) are referred to as typical EPEC (tEPEC), while *eae*(+) and BFP(-) strains are known as atypical EPEC (aEPEC) strains.[40] Although symptoms of aEPEC are milder, with non-inflammatory diarrhoea and no fever or vomiting, it is, however, associated with prolonged diarrhoea (>14 days).[39] Prolonged diarrhoea for longer than 14 days is clinically associated with an increased risk for illness and death.[40] Although the EPEC strains identified in this study were all *eae* positive, no screening for BFP was done, so the strains could be either tEPEC or aEPEC. However, in both instances, the burden of disease would be significant.

Antibiotic resistance profiles and ExPEC virulence genes

The presence of pathogenic *E. coli* in this river could result in waterborne or foodborne diseases. Treatment of these diseases could be further complicated if the pathogenic isolates are also resistant to medically important antibiotics. The five InPECs presented in Table 4 were additionally screened for antibiotic susceptibility to seven clinically important antibiotics, as well as for the presence of ExPEC genes. The results are presented in Table 5.

The results showed that the InPEC strains could not be positively confirmed as ExPEC, as they did not have two or more of the ExPEC gene sequences. The EAEC strain (*E. coli* H45) did carry the ExPEC gene sequence *iut*A, which codes for the aerobactin siderophore that contributes to essential ferric iron uptake and transport in different iron-deficient environments.[41,42] It has been reported that the incidence of aerobactin genes correlates with the presence of highly virulent pathogenic *E. coli* strains.[42] It should, however, be stated that the pathogenic strains were only screened for the most abundant ExPEC gene sequences[19] and that more than 30 other ExPEC genes have been reported.[41] It is thus possible that *E. coli* H45 may be a carrier of other ExPEC gene sequences which were not tested for.

The antibiotics tested for in this study all represented different classes of antimicrobials: aminoglycosides (Streptomycin), fluoroquinolones (Ciprofloxacin), penicillins (Ampicillin), amphenicols (Chloramphenicol), cephalosporins (Cefalotin), dihydropholate reductase inhibitors (Trimethoprim) and tetracyclines (Tetracycline). Antibiotic resistance testing revealed that, although all of the InPEC strains were resistant to multiple antibiotics, the four EPEC strains (Table 5) can be referred to as multidrug resistant (MDR) strains. This conclusion is based on the accepted definition of MDR which refers to the co-resistance that a strain can have to three or more classes of antimicrobials.[43] This finding concurs with previous observations that multiple antibiotic resistances are common for EPEC.[37]

The most abundant resistances were against Ampicillin (5/5) and Trimethoprim (4/5), followed by Tetracycline (3/5), Streptomycin (2/5) and Chloramphenicol (1/5). Trimethoprim and Ampicillin resistance were furthermore observed in the InPEC strains from all three studies, which showed that Trimethoprim and Ampicillin resistance persisted among bacteria in the river over time. Tetracycline and Streptomycin resistances were limited to strains from Study 2, while Chloramphenicol resistance was only observed in the isolate from Study 3. Antibiotic resistance can either be carried on mobile genetic elements, such as plasmids, which could be easily transferred horizontally between different bacteria, or be as a result of the environmental selection of a chromosomal mutation.[44] Whether chromosomal or plasmid-based, the results showed that antibiotic-resistant *E. coli* that also carries InPEC virulence genes is present in the Plankenburg River.

E. coli strains resistant to Tetracycline, Streptomycin, Chloramphenicol and Ampicillin have also been isolated from surface and groundwaters in KwaZulu-Natal and the North-West Province.[45-47] None of these studies tested for Trimethoprim resistance. The widespread occurrence of resistance to Tetracycline, Streptomycin, Chloramphenicol and Ampicillin is not surprising, as they are 'older' antibiotics, some of which have been in use since the 1940s. The fact that antibiotics such as Tetracycline have also been widely used as growth promoters in animal production, could also have contributed to the extent to which resistance has spread in the environment.[48]

The increased antibiotic resistance of Gram-negative bacteria such as *E. coli* is considered a serious global problem, because there is no foreseeable development of new antibiotic classes in the next 10 years.[49] This concern has led to the WHO classification of Ampicillin and Streptomycin as antimicrobials of critical importance for human medicine, while Chloramphenicol, Trimethoprim and Tetracycline are considered as highly important antimicrobials.[49] The occurrence of

Table 5: Antibiotic resistance profiles and presence of ExPEC genes in the InPEC strains

Strain	Pathotype	Source	Antibiotic resistance	ExPEC genes
E. coli H45	EAEC	Study 1 (P-1)	Ampicillin; Trimethoprim	*iut*A
E. coli A95	EPEC	Study 2 (P-2)	Ampicillin; Trimethoprim; Tetracycline	–
E. coli A118	EPEC	Study 2 (P-2)	Ampicillin; Trimethoprim; Tetracycline; Streptomycin	–
E. coli A132	EPEC	Study 2 (P-2)	Ampicillin; Tetracycline; Streptomycin	–
E. coli S49a	EPEC	Study 3 (P-2)	Ampicillin; Trimethoprim; Chloramphenicol	–

EAEC, enteroaggregative E.coli*; EPEC, enteropathogenic* E. coli

E. coli strains that show resistance to these important antibiotics in the Plankenburg River is therefore a matter of concern.

The presence of antibiotic substances in the aquatic environment is increasing mostly as a result of widespread application in the fields of human medical therapy and agriculture. Antibiotic substances are never fully metabolised in humans or other animals, which means that small amounts are regularly excreted and can enter water streams directly or indirectly via faecal pollution, agricultural run-off or through discharge from wastewater treatment plants.[50,51] The concentrations are usually at sub-inhibitory levels, which contribute to the development and spread of antibiotic resistance properties among environmental bacteria. If human pathogens acquire resistance to antibiotics, a serious situation can arise which, at the very least, can result in increased disease treatment costs. Antibiotic-resistant pathogens that are resistant to multiple antibiotics might, however, also lead to increased morbidity and mortality rates.[51]

Conclusions

Escherichia coli counts in the Plankenburg River have been found to be unacceptably high and this river can thus be classified as a 'high-risk' irrigation water source. Under these conditions, transfer of *E. coli* from the water to produce during irrigation will be highly probable. MDR *E. coli* strains, harbouring intestinal pathogenic gene sequences, were isolated from the Plankenburg River on more than one occasion. Three of the *E. coli* strains carrying enteropathogenic sequences also belonged to the $B2_3$ phylogenetic group, which could indicate human faecal contamination of the Plankenburg River. Subsequently, if irrigated fresh produce contaminated with MDR pathogens is consumed raw, it might act as a direct vehicle for the transmission of disease. Treatment of these MDR pathogenic bacterial related diseases will then be negatively impacted. It is therefore in the public's interest to report the existence of multiple antibiotic-resistant pathogenic *E. coli* in the Plankenburg River, so that corrective action, specifically in terms of treatment of irrigation water prior to irrigation, can be taken as soon as possible.

Acknowledgements

We thank Dr T.G. Barnard (Water and Health Research Unit, University of Johannesburg) for kindly providing standard InPEC cultures. This study was part of a solicited research project (K5/1773) funded by the Water Research Commission and co-funded by the Department of Agriculture, Forestry and Fisheries (WRC Knowledge Review 2012_13 KSA4). Financial support provided by the National Research Foundation (IFR2008112100010) is also gratefully acknowledged.

Authors' contributions

T.J.B. and G.O.S. were the project leaders and were responsible for the project design; T.J.B. and C.L. were responsible for the experimental design and supervision; M.R., N.H., A.C., N.S. and C.L. performed the experiments; C.L. and T.J.B. wrote the manuscript; and G.O.S. made editorial contributions.

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Novel CYP2E1 haplotype identified in a South African cohort

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DATES:
Received: 11 Oct. 2013
Revised: 07 Feb. 2014
Accepted: 18 Feb. 2014

KEYWORDS:
CYP2E1; haplotype; alcohol metabolism; molecular autopsy; South Africa

HOW TO CITE:
Heathfield LJ, Dalvie S, Kalideen K, Dandara C. Novel CYP2E1 haplotype identified in a South African cohort. S Afr J Sci. 2014;110(9/10), Art. #2013-0324, 5 pages. http://dx.doi.org/10.1590/sajs.2014/20130324

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Alcohol abuse accounts for approximately 2.5 million deaths annually and is the third highest risk factor for disease and disability. Alcohol is metabolised by polymorphic enzymes and the status of an individual with respect to alcohol metabolising enzymes may have forensic relevance in post-mortems. Baseline frequencies of gene variants involved in alcohol metabolism need to be established to aid the identification of suitable population-specific polymorphisms to genotype during molecular autopsies. The principal alcohol metabolising enzymes include alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and cytochrome P450 2E1 (CYP2E1). Six single nucleotide polymorphisms (SNPs) – rs1229984G>A and rs2066702C>T in *ADH1B*, rs671G>A in *ALDH2*, and rs3813867G>C, rs2031920C>T and rs6413432T>A in *CYP2E1* – were genotyped in 150 individuals from four South African populations: Xhosa, Zulu, South African white and South African coloured. Allele frequencies for each SNP in the four population groups were 0–10% for rs1229984A, 2–12% for rs2066702T, 0–2% for rs671A, 1–4% for rs3813867C, 0–1% for rs2031920T and 3–15% for rs6413432A. Haplotype analysis revealed a novel combination of three SNPs in CYP2E1 whose effects on alcohol metabolism need further investigation. Establishment of baseline frequencies adds to our knowledge of genetic variation in alcohol metabolising enzymes and additional research is required to determine the functional significance of this novel CYP2E1 haplotype.

Introduction

Alcohol abuse accounts for nearly 2.5 million deaths annually and contributes to about 4% of all global deaths.[1] On average, 31% of South Africa's population consume alcohol; and the total amount of alcohol consumed per capita is one of the highest in the world.[2] Alcohol is the leading abused substance for the majority of South African citizens[3] and contributes to more than 60 different types of injuries and diseases[1].

Alcohol metabolism can significantly influence drinking behaviour as well as the risk of alcohol dependence.[4,5] Ethanol, the main component of alcohol, is primarily converted to acetaldehyde by alcohol dehydrogenases (ADHs). However, ADH enzymes are easily saturated, especially in chronic alcohol consumers, and, in such cases, two additional families of enzymes – cytochrome P450 family 2 subfamily E polypeptide 1 (CYP2E1) and, to a lesser extent, catalase (CAT) – become involved. Acetaldehyde is then converted to acetate via the mitochondrial isoform of aldehyde dehydrogenase (ALDH2).[6] Ethanol is eliminated from the body primarily by metabolism (95–98%) with small quantities being excreted via breath (0.7%), sweat (0.1%) and urine (0.3%).[7]

Acetaldehyde is toxic and must therefore be metabolised as soon as it is formed.[6] There is considerable evidence that accumulated acetaldehyde contributes to tissue damage by inducing mitochondrial cell apoptosis. Furthermore, acetaldehyde has been reported to form adducts with dopamine, resulting in the neurotoxin salsolinol, which is thought to contribute towards alcohol dependence.[8,9] Under normal circumstances, an estimated 1–2% of acetaldehyde enters the bloodstream,[10] but the human body encompasses an efficient defence mechanism in the form of ALDH2, an enzyme with a high affinity for acetaldehyde. However, in cases of binge drinking, an increased concentration of blood acetaldehyde has been reported to induce acute adverse effects such as facial flushing, tachycardia and severe nausea.[11] In some individuals, these symptoms lead to the dislike of alcohol,[4] while in others, the accumulation of acetaldehyde results in severe illness, ultimately leading to death. Therefore, it is of pharmacogenetics interest to investigate variation in genes coding for alcohol metabolising enzymes – ADH, CYP2E1, CAT and ALDH2. However, CAT was not included in the current study because it is responsible for less than 2% of alcohol metabolism.[5]

The functions of gene variants involved in alcohol metabolism are fairly well established and their frequencies are reasonably documented in European and Asian populations.[11,12] However, this information is lacking in South African populations. Although Warnich et al.[13] extensively reviewed allele frequencies of various CYP enzymes in Bantu-speaking South Africans, CYP2E1 was omitted. However, Li et al.[14] determined allele frequencies of various CYP2E1 polymorphisms in black male South Africans in relation to oesophageal cancer, and several other novel CYP variants have been observed in some South African populations.[14–17] It is thus of interest to ascertain the frequencies of functionally significant gene variants in all South African populations in order to predict their forensic significance in circumstances associated with alcohol metabolism. In this pilot study, we aimed to establish the frequencies of six pharmacogenetically informative single nucleotide polymorphisms (SNPs) in four South African populations.

Methods

Cohort

The cohort consisted of 150 control subjects from four South African population groups: Xhosa ($n=34$), Zulu ($n=40$), South African white ($n=44$) and South African coloured ($n=32$). Ethnicity of individuals was self-reported and informed consent was obtained from all participants. For the purposes of this study, the South African white group comprised both English-speaking and Afrikaans-speaking white individuals. It should also be noted that

some individuals in the South African coloured group exhibit mixed ancestry. The study was carried out according to the Declaration of Helsinki (2008) and was approved by the University of Cape Town's Research Ethics Committee (REC REF: 103/2009).

Genotyping

Candidate genes that are involved in alcohol metabolism and their variants were identified using published literature[14,18] and data from the 1000 Genomes Project (http://www.1000genomes.org/)[19]. *ADH1B* rs1229984G>A (p.Arg49His), *ADH1B* rs2066702C>T (p.Arg370Cys), *CYP2E1* rs3813867G>C, *CYP2E1* rs2031920C>T, *CYP2E1* rs6413432T>A and *ALDH2* rs671G>A (p.Glu504Lys) were selected. Fragments containing the SNPs of interest were amplified using the polymerase chain reaction (PCR). Each PCR mixture contained 100 ng DNA, 5 X Green Go*Taq*® reaction buffer (Promega, Madison, WI, USA), 0.5 U *Taq* polymerase (Promega, Madison, WI, USA), 0.2 mM of each dNTP (Bioline, London, UK) and 0.4 μM of each relevant primer (Table 1) in a total volume of 25 μL.[20-22] Typical cycling conditions were followed.

Table 1: Primers used for polymerase chain reaction (PCR)

Gene	SNP	Primer sequence	Reference
ADH1B	rs1229984	F: 5′-TCTAAATTGTTTAATTCAAGAAGG-3′	N/A
		R: 5′-ACTAACACAGAATTACTGGAC-3′	N/A
	rs2066702	F: 5′-GGATGGAAATAGGGTAGC-3′	20
		R: 5′-TAGAGGAGGCTGAAGACTG-3′	20
CYP2E1	rs3813867 and rs2031920	F: 5′-GTGCCAAAAACCAGAGGGAA-3′	N/A
		R: 5′-TTCATTCTGTCTTCTAACTGG-3′	21
	rs6413432	F: 5′-GAGGAGGTGTGAAAGGTC-3′	N/A
		R: 5′-TCTGTTGTCAGGCTAGAGTG-3′	22
ALDH2	rs671	F: 5′-CCCAAGAGTGATTTCTGC-3′	N/A
		R: 5′-GTCCCACACTCACAGTTT-3′	N/A

SNPs were genotyped using SNaPshot PCR, except for rs6413432 which was genotyped using restriction fragment length polymorphism (RFLP) with the restriction enzyme *Dra*1. SNaPshot PCR was carried out on cleaned, pooled PCR products, relevant SNaPshot primers (Table 2) and 1 μL Applied Biosystems SNaPshot® Multiplex Ready Reaction Mix (Applied Biosystems, Carlsbad, CA, USA) in a total volume of 10 μL. All SNaPshot reactions were carried out on the GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, CA, USA) and cycled 25 times at 96 °C for 10 s, 50 °C for 5 s and 60 °C for 30 s. Samples underwent capillary electrophoresis on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA) in the presence of GeneScan™ 120 LIZ® Size Standard (Applied Biosystems, Carlsbad, CA, USA). GeneMapper™ v3.0 (Applied Biosystems, Carlsbad, CA, USA) software was used to analyse peaks and genotypes of samples.

For the RFLP, samples were digested with 1 U FastDigest® *Dra*1 (Fermentas, Ontario, Canada) and 10X FastDigest® Green Buffer (Fermentas, Ontario, Canada) in a final volume of 30 μL and incubated at 37 °C for 1 h. Of the total cohort, 10% was sequenced to verify SNaPshot PCR and RFLP results, whereby at least one representative of each genotype was included for each SNP. Each population was represented in this selection.

Statistics

Genotypes for every SNP within each population group were tested for deviation from Hardy–Weinberg equilibrium. Fisher's exact tests were performed to examine whether there were differences in the allelic frequencies between each of the population groups under study, for each SNP independently. The online bioinformatic tool SHEsis (http://analysis.bio-x.cn/SHEsisMain.htm) was used to analyse linkage disequilibrium (LD) between the SNPs under investigation. The SHEsis platform uses a full-precise-iteration algorithm which calculates the probability of the SNPs of interest being inherited together, based on the genotype input data.[23,24]

Results

All SNPs were in Hardy–Weinberg equilibrium for each population group. Allele frequencies for the six SNPs under investigation are presented in Table 3.[19] The South African coloured population exhibited the most heterozygosity for the six SNPs while the Zulu population exhibited the least.

Genotypes for *ADH1B* rs1229984G>A and rs2066702C>T were analysed in combinations of the previously reported haplotypes *ADH1B*1*, *ADH1B*2* and *ADH1B*3* (Table 4).[25] All three haplotypes were observed in the Xhosa, South African white and South African coloured populations, but the *ADH1B*2* haplotype was absent in the Zulu population. *ADH1B*1* was the most frequent haplotype in each population.

Similarly, genotypes for *CYP2E1* rs3813867G>C, rs2031920C>T and rs6413432T>A were grouped together into the haplotypes *CYP2E1*1A, CYP2E1*5A*, *CYP2E1*5B* and *CYP2E1*6* (http://cypalleles.ki.se/cyp2e1.htm) (Table 4). In a total of six cases, *CYP2E1* rs3813867 was heterozygous G/C while *CYP2E1* rs2031920 and rs6413432 were both homozygous (i.e. rs2031920C/C and rs6413432T/T). This combination of nucleotides (i.e. rs3813867G/C, rs2031920C/C and rs6413432T/T) did not fall into a previously reported haplotype and was noted in a separate row in Table 4.

The *CYP2E1*1A* haplotype was the most common in all four populations, followed by *CYP2E1*6*. The *CYP2E1*5A* haplotype occurred in a single individual in the South African white population while *CYP2E1*5B* did not appear in any population. The newly observed combination of nucleotides occurred in each of the populations being studied and had the highest relative frequency (0.07) in the Xhosa population.

When comparing allele observations in a pairwise fashion between the four population groups, three significant results were obtained. The Zulu and the South African coloured population groups had significantly different allele frequencies for *ADH1B* rs1229984A ($p=0.009$). The *ADH1B* rs2066702T variant frequency differed significantly between the Xhosa population group and the South African white population group ($p=0.034$). Allele frequencies for *CYP2E1* rs6413432 were significantly different between the Zulu and South African white populations

Table 2: Internal primers used for SNaPshot reactions

Gene	SNP	Primer (5` → 3`)	Orientation
ADH1B	rs1229984	ATAAGTGGCTGTAGGAATCTGTC	Forward
	rs2066702	TAACCACATATATTCCTATTGCAG[T/C]ATC	Forward
CYP2E1	rs3813867	GGGCTTGGTTCAGGAGAG	Forward
	rs2031920	AAAACTATACATAAAGATTCATTGTTAATATAAAAGTA	Forward
ALDH2	rs671	AATAACAATAATATACACTCACAGTTTTCACTT	Reverse

Table 3: Distribution of allele frequencies in the four South African population groups, the 1000 Genome Project and two HapMap3 populations

	ADH1B		*CYP2E1*			*ALDH2*
	rs1229984	**rs2066702**	**rs3813867**	**rs2031920**	**rs6413432**	**rs671**
Allele	**A**	**T**	**C**	**T**	**A**	**A**
Xhosa (*n*=34;33;30;30;33;34)	0.03	0.12	0.03	0.00	0.05	0.00
Zulu (*n*=34;30;37;37;37;40)	0.00	0.10	0.01	0.00	0.03	0.00
South African white (*n*=29;30;36;36;36;42)	0.03	0.02	0.04	0.01	0.13	0.00
South African coloured (*n*=30;30;31;31;31;32)	0.10	0.05	0.02	0.00	0.15	0.02
1000 Genome Project (phase 1 populations)	0.21	0.05	0.10	0.09	0.16	0.06
HapMap3 Yoruba	0.00	0.27	0.00	0.07	0.00	0.09
HapMap3 Caucasian	0.00	0.00	0.00	0.07	0.06	0.14

Table 4: Frequencies of *ADH1B* and *CYP2E1* haplotypes in the four South African populations

Haplotype	**Single nucleotide polymorphisms (SNPs)**			**Population**			
	SNP 1	**SNP 3**	**SNP 3**	**Xhosa**	**Zulu**	**South African white**	**South African coloured**
	rs1229984 (G>A)	rs2066702 (C>T)	N/A				
*ADH1B*1*	G	C	N/A	0.73	0.8	0.9	0.73
*ADH1B*2*	A	C	N/A	0.03	0	0.07	0.2
*ADH1B*3*	G	T	N/A	0.24	0.2	0.03	0.07
	rs3813867 (G>C)	rs2031920 (C>T)	rs6413432 (T>A)				
*CYP2E1*1A*	G	C	T	0.86	0.92	0.73	0.71
*CYP2E1*5A*	C	T	A	0	0	0.03	0
*CYP2E1*5B*	C	T	T	0	0	0	0
*CYP2E1*6*	G	C	A	0.07	0.06	0.18	0.26
New haplotype	C	C	T	0.07	0.03	0.06	0.03

(p=0.030). However, the observed allele frequency distributions were no longer significantly different when the Bonferroni correction for multiple testing was applied.

The online bioinformatic tool SHEsis was used to analyse LD between the SNPs in CYP2E1 (Figure 1). The numbers in the blocks within Figure 1 indicate a LD score (D'), whereby an LD score of 100 indicates complete LD. The *p*-values in brackets indicate the level of significance of the score, calculated using Fisher's exact test.

Discussion

Pharmacogenetics has potential in the clinical setting in which drugs are dispensed for the treatment of disease; however, its use can be extended to aid forensic science, especially in the context of post-mortem in suspected drug-associated deaths. Individuals are frequently exposed to substances which have the capacity to cause death and whose metabolism is influenced by the genetic variants in their metabolising enzyme genes. The utility of pharmacogenetics in forensic science lies in its ability to reduce the miscategorisation of deaths previously classified as sudden unexpected deaths by identification of the associated gene variants that affect metabolism and lead to such deaths. In this study, we aimed to establish the baseline frequencies of variants within genes involved in alcohol metabolism within four South African populations – Xhosa, Zulu, South African white and South African coloured – for future use in predicting possible differences among the population groups in their disposition to alcohol.

Such variants are of forensic relevance as they may help in classifying the manner in which a person might have died. For example, if a post-mortem reports a lethal amount of alcohol in the deceased, it may not be readily possible to conclude whether the manner of death was suicidal or accidental[26]; but the presence of a gene variant associated with accumulation of the particular drug (e.g. ethanol) may point to unintentional accumulation (e.g. of acetaldehyde) and ultimately to accidental death. However, if the individual had a gene variant that resulted in normal metabolism of ethanol, the manner of death would

most likely be considered suicide. It has to be kept in mind that substances that inhibit or induce these enzymes are also likely to have similar effects as variant alleles. Therefore, after genotype or phenotype studies, SNPs known to affect metabolism of drugs suspected to have been taken by the deceased, could be recommended for inclusion in a potential molecular autopsy to aid determination of the manner of death.

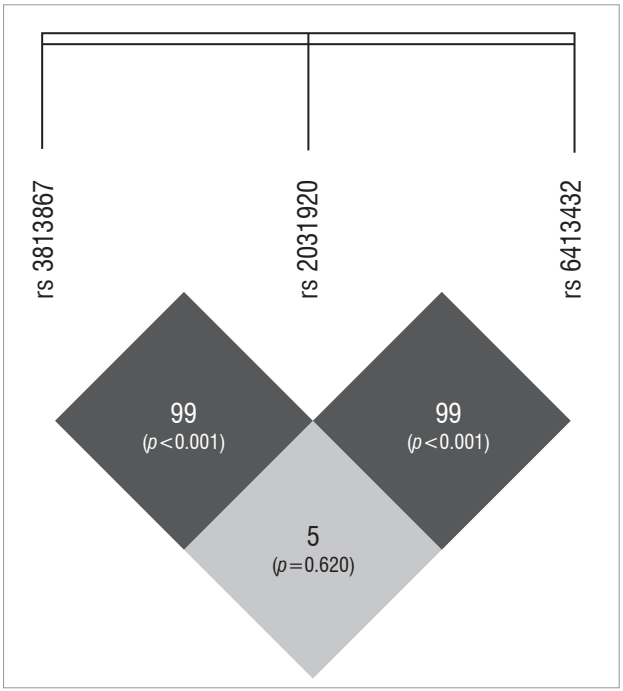

Figure 1: Linkage disequilibrium scores (D') between rs3813867, rs2031920 and rs6413432 in *CYP2E1*. Complete LD is observed where the LD score is 100. Complete linkage equilibrium is observed where the LD score is 0.

Of these SNPs with forensic relevance, the South African coloured group exhibited the most heterozygosity and was the only population group to show variation in *ALDH2* rs671. However, this finding needs to be confirmed by comparing the distribution of this variant among South African coloured individuals whose deaths were alcohol related and those who drank but whose deaths were not alcohol dependent. Other populations showed relatively little variation in these SNPs; for example, the Zulu population group exhibited variation only in *ADH1B* rs2066702 (10%), *CYP2E1* rs3813867 (1%) *and CYP2E1* rs6413432 (3%). With respect to *ADH1B*, the Zulu group presented with more of the slow ethanol-metabolising variants than the variants associated with fast metabolism.

No individual in the entire cohort contained variants for both *ADH1B* rs1229984A and *ADH1B* rs2066702T, which is consistent with the literature to date.[20] It can be speculated that either the SNPs are in complete LD or that having both nucleotide changes is incompatible with life.

Although the possibility exists that *CYP2E1* haplotypes with different combinations of the three SNPs (rs3813867 (G>C), rs2031920 (C>T) and rs6413432 (T>A)) could occur, current literature does not provide evidence for this. Watanabe et al.[27] demonstrated that rs3813867G and rs2031920C are in complete LD. However, a different combination of variants (*CYP2E1* rs3813867G/C, *CYP2E1* rs2031920C/C) was detected in six individuals in this study, suggesting that a recombination event might have occurred that resulted in the novel haplotype.

One can speculate that if the rs2031920T allele leads to increased transcriptional activation of CYP2E1, and the rs3813867C polymorphism has little effect on transcription,[28] individuals with the novel allele detected in this study could more likely display CYP2E1 activity within the normal range. However, functional studies would be needed to confirm this speculation.

The outcome of SHEsis indicated a D' value of 99 for SNPs rs2031920 and rs3813867, indicating that the SNPs are in almost complete LD ($p<0.001$). The disruption of the former haplotype could be a result of a recombination event and occurred at low frequencies (≤ 7%) in all four population groups in the study. Non-African populations are known to have larger haplotype blocks as a result of founder effects subsequent to migration out of Africa. Since the out of Africa migration, fewer recombination events have occurred as there have been fewer generations from the new founder group. As a consequence, larger haplotype blocks are inherited by successive offspring,[29] which may explain the presence of the novel allele in the Xhosa and Zulu populations. In the South African coloured group, the novel allele could be a result of admixture, resulting in gene flow between black South Africans, the San, the Khoikhoi, western Europeans, Indonesians and Indians who settled in the Cape in the 17th century.[30] Therefore, if the recombination event occurred in the African ancestor and the allele was passed to the South African coloured individual in this way, the presence of the novel allele in the South African coloured population is explained. However, this explanation is unlikely as the frequency for the novel allele is second highest for the South African white population group who are of European descent. Rather, a second recombination event could have occurred in the European population group after the out of Africa migration, resulting in the South African coloured group inheriting the allele from the South African white population.

A heritage analysis by Greeff in 2007 revealed that the Afrikaner population also has numerous contributors of genes, including Madagascans, African slaves and Indians,[31] which could possibly account for the presence of the haplotype in the South African white population.

Lee et al.[32] undertook a comprehensive study and reported global patterns of allele and haplotype frequencies in *CYP2E1.* They genotyped 11 polymorphisms across *CYP2E1* in 50 population groups and showed that allele, haplotype and LD patterns varied greatly among geographical regions. Lee et al.[32] identified extensive genetic variation in Africa and reported 16 common haplotypes in addition to various residual haplotypes. An in-depth and direct comparison of haplotype results cannot be made as classification of haplotypes by Lee et al.[32] was not done according to the CYP allele database (http://www.cypalleles.ki.se/), as was done here. However, the *CYP2E1* SNPs in this study correspond to markers 2 (rs3813867), 3 (rs2070672) and 9 (rs6413432) in Lee et al.'s[32] study and fall into 'core groups' A, A and B, respectively. The proposed recombination event in this study was between markers 2 and 3, both of which were grouped into core A by Lee et al.[32] It therefore seems unlikely that the novel haplotype proposed in this study has been previously observed, as they both form part of the same core group in this global study.

A limitation of this study was the small sample sizes used for each population group, which may have resulted in an inflation of statistical type 1 and type 2 errors. Furthermore, the self-reported ethnicity of participants also poses a limitation, as baseline frequencies may not have been truly representative of the actual populations. This method was deemed suitable for this pilot study, but a more reliable method to determine ethnicity should be included in subsequent studies.

Baseline frequencies for six informative polymorphisms in genes involved in alcohol metabolism were obtained for four South African populations: Xhosa, Zulu, South African white and South African coloured. The findings reported here add to knowledge in the field, offer a potential utility in the forensic setting and provide a platform for future studies in the area.

Acknowledgements

This work was supported by the University of Cape Town and the National Research Foundation of South Africa.

Authors' contributions

All authors contributed significantly to the study and agreed to the submission of the manuscript. L.J.H. carried out all laboratory experimental work, analysis of results and statistics and wrote the

manuscript. S.D., K.K. and C.D. provided conceptual input and were responsible for the project design. C.D. was the project leader.

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IziNambuzane: IsiZulu names for insects

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DATES:
Received: 15 Sep. 2013
Revised: 22 Feb. 2014
Accepted: 25 Feb. 2014

KEYWORDS:
traditional indigenous knowledge; cultural entomology; folk taxonomy; language policy; lexicographic standardisation; trilingual entomological dictionary

HOW TO CITE:
Cockburn JJ, Khumalo-Seegelken B, Villet MH. IziNambuzane: IsiZulu names for insects. S Afr J Sci. 2014;110(9/10), Art. #2013-0292, 13 pages. http://dx.doi.org/10.1590/sajs.2014/20130292

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We provide a tool for communicating about insects in isiZulu to facilitate research and knowledge sharing in the fields of indigenous knowledge, cultural entomology, environmental education and community extension involving isiZulu speakers. A total of 213 different names for 64 insect specimens were encountered among a sample of 67 respondents in 11 communities distributed across the province of KwaZulu-Natal, South Africa. This list includes 93 names that can be considered core isiZulu vocabulary and which are widely used to identify insects that are agriculturally, medically, domestically, culturally or ecologically common or significant. Substantial variation was found regarding the names for particular insects, especially between regions, suggesting dialectal differences between isiZulu speakers. Grammatical and social variation in names was also recorded. This study highlights interdisciplinary teamwork in the field of indigenous knowledge research and the influences affecting the standardisation of South African languages for technical and scientific work.

'We have really everything in common with America nowadays, except, of course, language.'
Oscar Wilde, The Canterville Ghost (1887)

Introduction

Research into indigenous knowledge is a fairly new field that aims to connect the knowledge of local communities on one hand and professionals in disciplines including science and community development on the other.[1-5] An example is the development of agricultural pest control technology from traditional African farming practices.[6] As professionals in science and technology start to understand indigenous knowledge systems, they can develop a more accurate and profound understanding of their counterparts in the stakeholder community. In turn, science and technology can become more accessible and more acceptable to the anticipated users.[2,4,5] The perception of science and indigenous knowledge as disparate entities, each apparently misunderstood by the other, could be ameliorated by identifying, examining, explaining and evaluating indigenous knowledge.[5,7] We have deployed these insights in this study in the context of South African entomology by compiling a list of names in the isiZulu language (Guthrie Bantoid Language number S42[8-11]) that are used in KwaZulu-Natal to identify insects that are agriculturally, medically, domestically, culturally or ecologically common or significant in that province.

The Constitution of the Republic of South Africa, *Act 108 of 1996*, recognises 11 official languages, and research into these languages has become a priority.[12-14] IsiZulu is the home language of about 23% of South Africans, and is spoken or understood by a substantial percentage of people in all nine provinces in South Africa as their first or second language.[15] It is most prevalent in KwaZulu-Natal, where about 78% of the population speaks isiZulu as a first language.[15] It is a Level 4 (Educational) language (http://www.ethnologue.com/language/zul) on the Expanded Graded Intergenerational Disruption Scale (EGIDS[16]), which means that literacy in isiZulu is being sustained through a system of public education. This indicates a need to gather and formalise indigenous knowledge of terminology in this language, and to compile isiZulu texts and standardise vocabulary for use in every field of science and technology in the country (cf. Djité[13]; Mufwene[14]). The need for inclusion of indigenous languages and standardisation of names for specific plants and animals for use in environmental research, education and communication in South Africa has been recognised repeatedly (e.g. Feely[17]).

Two isiZulu language boards – one constituted under the *African Languages Board Act of 1977* and the other under the *Pan South African Language Board (PanSALB) Act of 1995*, in the apartheid and post-apartheid eras, respectively – have made hardly any progress as yet with regard to the standardisation of the language as envisaged by the governments in either era. PanSALB works cooperatively with the Department of Arts and Culture's National Language Service Terminology group, and is actively concerned with lexicography and terminology. In 2001, PanSALB set up new language-specific bodies for each of the official languages, including the isiZulu National Language Body. Additionally, a number of grassroots initiatives have formed in response to this need. One of the most inspiring of these is the 'Project for the Study of Alternative Education in South Africa (PRAESA) – Growing biliteracy and multilingualism' (http://www.praesa.org.za). It is our foremost aim in this study to support and contribute to efforts by these bodies and non-governmental grassroots initiatives to systematise and standardise entomological terminology in isiZulu.

The names collected during this study were analysed to determine those most frequently used, and to investigate whether different regions have different names for the same insect. The second aim of publishing this study was therefore to provide a tool to enable an enlarged circle of participants, including researchers, scientists, educators and non-professional citizens, to engage more profoundly and effectively in elementary and advanced communication about insects and entomology. Internationally standardised biological nomenclature ('scientific names'[18]) has been used as a frame of reference to minimise ambiguity regarding the identities of the insects and to promote reproducibility in the identification and naming of insects.

English (one of the six EGIDS Level 0 (International) languages, along with Arabic, Chinese, French, Russian and Spanish; http://www.ethnologue.com/) was selected as the language for communicating this study. Even though the proportion of participants and people in general speaking it as a first language at the national level is relatively low (about 8%), English has high instrumental value nationally because it is the most commonly widespread and geographically evenly distributed second or third language in the country. It is the language of learning and teaching

at most South African schools and tertiary educations under the new national education curriculum that makes the teaching of two languages compulsory at school level, and it is the *lingua franca* both of national government, business and commerce, and of science and education in most parts of post-colonial Africa and abroad.[19(p.28)] Being able to translate isiZulu entomological names into English gives access to a wealth of entomological information (e.g. Scholtz and Holm's reference work[20]) to isiZulu speakers. We therefore offer a means to translate between scientific, isiZulu and English names.

Materials and methods

Sample selection

Specimens of the 64 insect taxa used by Mkize et al.[21] to gather isiXhosa names of insects were selected (Table 1). These insects covered a broad range of insect diversity, and included species that are likely to be familiar because they are common or have agricultural, medical, medicinal, cultural or other significance to people. The selection covered the insects included in the children's book by Uys and McLarty[22] (Table 1), which focuses strongly on taxa of significance to humans, and which might be considered a core set of taxa.

The study was limited to the province of KwaZulu-Natal, South Africa, as it is the area in which the isiZulu language originated and is spoken indigenously and where it is therefore likely to be at its purest and least adulterated by other languages. A total of 11 sites was selected throughout the province (Figure 1), representing a compromise between logistical accessibility and geographical coverage. The interview sites can be found at the following localities: Berg Reserves (29°02'54''S, 29°24'22''E), Commondale (27°17'41''S, 30°53'15''E), Elandskraal (28°28'02''S, 30°28'19''E), Hluhluwe (28°03'49''S, 32°09'35''E), Keate's Drift (28°51'33''S, 30°30'22''E), Khula Village (28°22'17''S, 32°22'22''E), Mbongolwane (28°56'11''S, 31°13'48''E), Muden (28°58'11''S, 30°24'03''E), Ntunjambili (28°55'46''S, 30°56'47''E), Richmond (29°56'12''S, 30°17'27''E) and Winterton (28°55'47''S, 29°30'03''E).

Emphasis was placed on selecting respondents in rural communities (who tend to speak 'deep Zulu', *isiZulu esijulile*; 'high Zulu', *isiZulu esiphakeme*) over urbanised respondents (who tend to speak urban

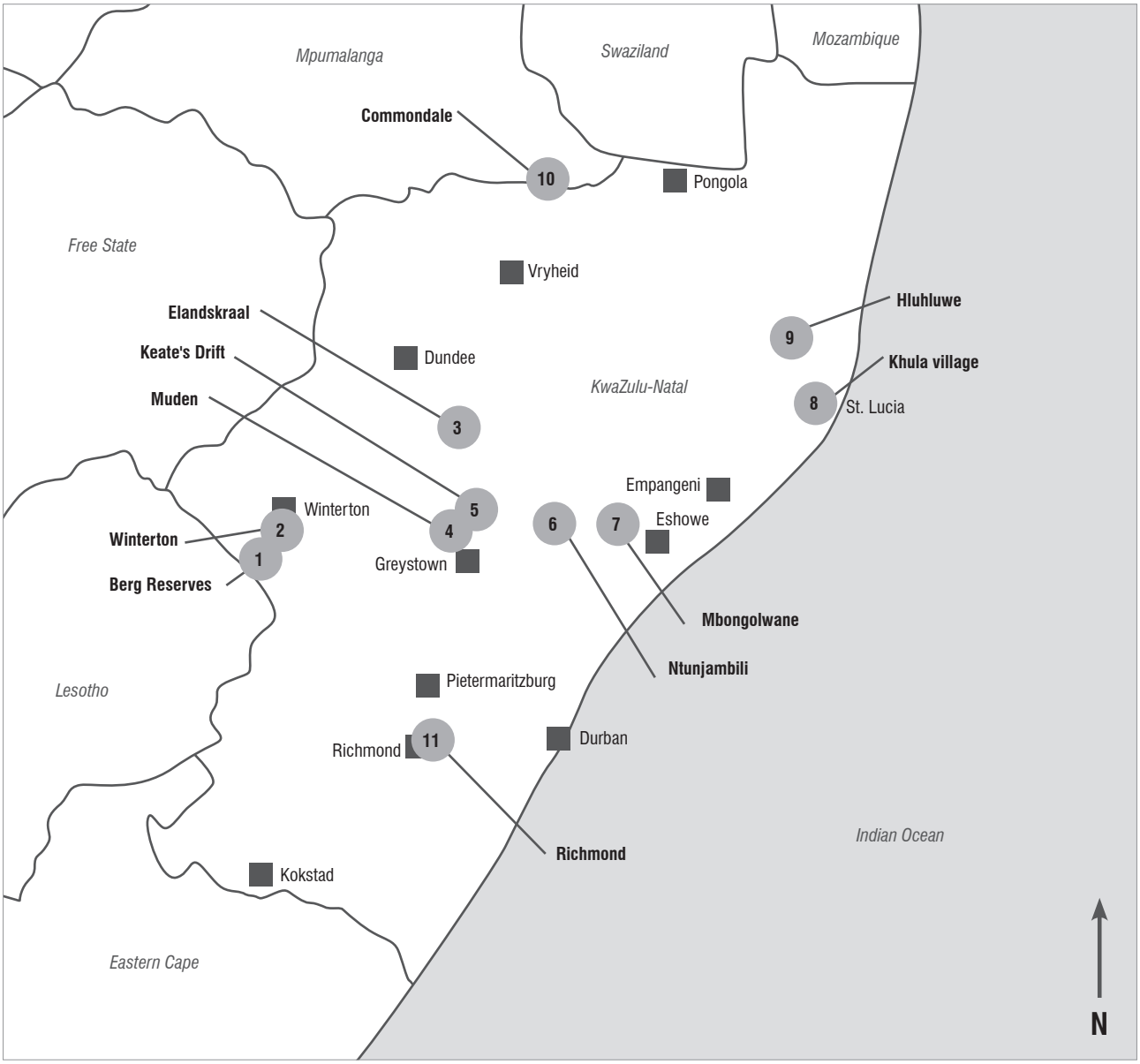

Figure 1: Localities of survey sites. Provinces and neighbouring countries are shown in grey italics; ■ = towns; ○ = survey sites, numbered and shown in bold. Exact latitudes and longitudes of the interview sites are provided in the text.

Table 1: List of insect specimens used in interviews, and of names listed by Doke et al.[33] and Uys and McLarty[22]. The names are sorted phylogenetically so that entomologists can assess the taxonomic coverage.

Scientific name		English name	isiZulu name	
Order	Family		Doke et al.[33]	Uys and McLarty[22]
Thysanura	Lepismatidae	fishmoth	umvunya wasezincwadini	inyundu yasezincwadini
Ephemeroptera	Baetidae	mayfly	(none)	(none)
Odonata	Synlestidae	damselfly	ujekamanzi	(none)
	Aeshnidae	dragonfly	ujekamanzi	amazekamanzi
Dermaptera	Forficulidae	earwig	umkhothane	umkhothane onezimpondo ezinde
Orthoptera	Grylidae	cricket	inyekevu	isihlonono, udambi
	Stenopalmatidae	sand cricket	inyendle	(none)
	Tettigoniidae	katydid	igawozi	isilokazane
	Acrididae	grasshopper	idiye	amaboni
	Pyrogomorphidae	foam grasshopper	intothoviyane	intithoviyane
	Pneumoridae	bladder grasshopper	(none)	(none)
	Pamphagidae	rain grasshopper (female)	uquqululu	(none)
		rain grasshopper (male)	uquqululu	(none)
Phasmatodea	Phasmatidae	stick insect	umtwanawezulu	izintethe ezisanduku
Mantodea	Mantidae	praying mantis	isithwalambiza	amaqaqa
Blattaria	Blaberidae	wingless cockroach	igugu	(none)
	Blatidae	American cockroach	iphela	amakokoloshe, amaphela
	Termitidae	termite (winged)	inhlwa	amaye
		termite (workers)	umuhlwa	amaye
Psocodea	Menoponidae	louse	umkhuphe	izintwala
Hemiptera	Aphididae	aphid	i-afidi	izintwala zezimbali
	Diaspididae	scale insect	ukhwekhwe lwezilokazanyana olubulala izithombo	(none)
	Cicadidae	cicada	isihlonono	isihlonono esinephiko elisawolintshi
	Cimicidae	bedbug	imbungulu	imbungulu yasembhedeni
Hymenoptera	Ichneumonidae	ichneumonid wasp	umuvi	(none)
	Pompilidae	spider-hunting wasp	umuvi	umuvi ozingela izicabucabu
	Sphecidae	mud wasp	umuvi	umuvi wodaka
	Apidae Apinae	honeybee	inyosi	inyosi yoju
	Apidae Xylocopinae	carpenter bee	uhlobo olukhulu lwenyosi ezakhela yodwa	inyosi engumbazi
	Vespidae Eumeninae	potter wasp	umuvi	umuvi wodaka
	Vespidae Vespinae	paper wasp	umuvi	umuvi wephepha
	Formicidae	ant (worker)	itsheketshe	(none)
		thief ant (queen)	(none)	(none)
Coleoptera	Gyrinidae	whirligig beetle	(none)	(none)
	Geotrupidae	dung beetle	inkuba	ibhungane elibubende
	Scarabaeidae Melolonthinae	Christmas beetle	ibhungane	(none)
	Scarabaeidae Dynastinae	rhinoceros beetle	ibhungane	ibhungane elingubhejane
	Scarabaeidae Cetoniinae	fruit chafer	ibhungane	ibhungane lezithelo zasengadini
	Scarabaeidae Scarabaeinae	dung beetle	inkumablongwe	ibhungane elibubende
	Tenebrionidae	toktokkie beetle	umzifisi	ibhungezi limthende
	Meloidae	blister beetle	ibhungane	(none)
	Lampyridae	glowworm	imfinyezi	imfinyezi kanye nomsundu ocwebezelayo
	Elateridae	click beetle	(none)	(none)
	Curculionidae	weevil	(none)	(none)
	Coccinellidae	ladybird	isilokazana esincane esibomvu esinamachashazi amnyama	ibhungane eliwugugumbe
	Cerambycidae	longhorn beetle	umpondompondo	(none)
	Chrysomelidae Bruchinae	bean weevil	(none)	(none)
	Chrysomelidae Cassidinae	tortoise beetle	ibhungane	(none)
Neuroptera	Myrmeliontidae	antlion (adult)	inkunzi yomhlaba	(none)
		antlion (larva)	inkunzi yomhlaba	(none)
Lepidoptera	Psychidae	bagworm (larva)	umahambanendlwana	umahambanendlwana
	Sphingidae	hawk moth	uvemvane	inyundu enombala osasiliva okufana noklebe
	Noctuidae	owlet moth	uvemvane	izinyundu
	Papillionidae	citrus swallowtail	uvemvane	izivemvane
	Pieridae	white butterfly	uvemvane	izivemvane
Diptera	Tipulidae	crane fly	(none)	umlenzemide
	Culicidae	mosquito	umiyane	umiyane
	Asilidae	robber fly	(none)	impukane eyisigebengu
	Syrphidae	hover fly	inyosi	(none)
	Muscidae	house fly	impukane	impukane yasendlini
	Sarcophagidae	flesh fly	impukane	(none)
	Calliphoridae	bluebottle fly	imvimvi	(none)
		greenbottle fly	imvimvi	(none)
Siphonaptera	Pulicidae	flea	izeze	amazeze

Zulu, *isiZulu sasedolobheni*) because *traditional* indigenous knowledge is generally believed to be retained better in rural areas than in urban settings.[21,23] In total, 67 people were interviewed, five to eight from each site (Table 2). Older respondents were chosen preferentially, as one might expect them to have accumulated a greater knowledge of insects' names.[21]

Data collection

Various isiZulu speakers familiar to particular local communities accompanied the interviewer (JJC) to introduce them to the prospective respondents, to facilitate the observation of appropriate local etiquette, and to interpret, explain and clarify where necessary. The interviewer also spoke isiZulu, which contributed to normalising the situation and promoting communication. To expose potential methodological problems, two pilot interviews were conducted before commencing data collection.[24,25] The study was approved by the Department of Zoology and Entomology, Rhodes University.

In adherence to the principle of observing and respecting the dignity and privacy of each interviewee, interviews started with explaining the goals of the interview and asking permission to continue.[25-27] Relevant biographical details including age, education and employment were recorded on questionnaire sheets by the interviewer if the respondent was explicitly willing to share that information. Ten of the respondents were nature conservation personnel, who could be expected to have a more detailed and accurate knowledge of insects' names because of their apparently greater interest in, or exposure to, nature. This subset of respondents was also analysed separately to provide qualitative cross-validation of the list of isiZulu names.

Each respondent was then asked to identify the preserved specimens of insects (Table 1), and to share any additional information about each insect, e.g. its agricultural, medical, domestic, cultural or other significance. The insects were selected for their ubiquity or significance to humans. The specimens were each numbered for ease of reference and data capture and placed in wooden field boxes suitable for travelling. Several strategies were used to increase the quality of the interview data. One interviewer (JJC) carried out all of the interviews to promote uniformity. Respondents were interviewed individually whenever possible to ensure independence of opinion, although this was not always possible as a result of local etiquette and custom. Leading questions and questions with yes/no answers were avoided, and interviewees were given opportunity to expand freely on their basic identification by giving additional information.[24,26-29] The use of names of insects in other languages (isiXhosa, seSotho, English, etc.) were avoided during interviews to forestall potential sources of confusion.

Spelling was transcribed phonetically by the interviewer in consultation with the accompanying isiZulu translator using the standard Roman notation for isiZulu click consonants (c = |, dental click; q = !, alveolar click; x = ‖, [bi]lateral click) and alveolar lateral fricatives (hl = ɬ, voiceless; dl = ɮ, voiced) (http://isizulu.net/p11n/).

Analysis and cross-validation

The profiles of respondents were summarised with simple descriptive statistics[25] and bivariate linear regression.

The completeness of the sample, at least for KwaZulu-Natal, was assessed using sample accumulation curves[30,31] and a response-frequency histogram[32] was constructed to explore how the names could

Table 2: Biographical profiles of amaZulu respondents in 11 communities located in KwaZulu-Natal (Figure 1)

	Berg Reserves	Commondale	Elandskraal	Hluhluwe	Keate's Drift	Khula Village	Mbongolwane	Muden	Ntunjambili	Richmond	Winterton	Total	
												N	%
Number of respondents													
Total	5	7	6	8	6	5	7	6	5	6	6	67	100
Women	0	2	3	5	3	3	3	5	3	0	4	31	46
Men	5	5	3	3	3	2	4	1	2	6	2	36	54
Age group (years)													
20–39	2	1	3	2			4	1				13	19
40–59	1	4	2	2		2		1	1	1	5	19	28
60–69	2	1	1	2	6	3	1	1	4	4	1	26	39
70+		1		2			2	3		1		9	14
Formal education													
Undisclosed		1										1	1.5
None		1	6	4	4	5	3	4	2	5	4	38	57
Primary	3	3		2	1			1	3	1	2	16	24
Secondary	2	2		1	1		4	1				11	16
Tertiary				1								1	1.5

be simplistically categorised into personal, local and standard names according to how many people reported them[21]. When a name was reported by only two respondents who were interviewed at the same time, they were deemed to be non-independent samples and were classified as personal names for this study. When a name was reported by only two respondents but they were from distant sites, these names were regarded as rare knowledge rather than personal names; four such names were encountered.

Two additional published sources of insect names in isiZulu were consulted to further gauge the completeness of the sample and for comparative purposes: an *English-Zulu Zulu-English Dictionary*[33] and a children's book on invertebrates, *My First Book of Southern African Creepy-Crawlies*[22], which included isiZulu names (Table 1).

Results and discussion

Respondents' profiles

Many people were willing and even enthusiastic to be respondents. The occupational backgrounds of the selected respondents included induna (1 person), councillor (2), farmer (2), homekeeper (14), farmworker (7), security guard (1), shopkeeper (1), teacher (1), nature conservation personnel (10), traditional healer (1), variously employed (17) and unemployed (10). Over half (57%) of the respondents had no formal education and only 11% had formal education above primary level (Table 2), implying that few of the respondents might have learned 'standard' names from such formal sources. Respondents provided 18–38 names each, and on average an individual knew 28 names. The same name was often indicated for more than one specimen by an individual. No one could name every specimen; individual respondents identified an average of 8 (0–18) specimens as familiar but did not know a name for them, and 7 (0–17) specimens as unknown to them, indicating that it was unlikely that respondents made up names during the interviews.

About 53% of the respondents were over 60 years of age (Table 2, Figure 2). Contrary to expectations, there was no overall correlation between a respondent's age and the size of their reported entomological vocabulary, irrespective of gender (Table 3). Younger men tended to report significantly more names, primarily in the non-personal category. While older women tended to report proportionately more personal names (Figure 2), this was not statistically significant (Table 3). Many of the respondents mentioned that they learned insects' names while playing in the fields as children and could only barely remember some of them because they have not had much contact with them since their childhoods.

Table 3: Pearson's correlation coefficients for the relationship between respondents' ages and the numbers of names they reported. Correlations set in bold are statistically significant at $\alpha = 0.05$.

	All names	Non-personal names	Personal names
Women	0.122848	0.025456	0.194084
Men	**-0.387923**	**-0.337790**	-0.239872
All	-0.166607	-0.188450	-0.028460

Only three respondents were living more than 60 km from their place of birth, so migration was unlikely to have introduced allochthonous dialectal names to a site.

Completeness of sample

In total, 213 names were collected. A sample accumulation curve constructed without permutation (Figure 3a) shows that the number of interviews (5–8) at each site was sufficient to represent each site because the curves flattened off within each site. When names classified as personal (i.e. reported by only one person, or two people in the same interview) were excluded, the unpermuted sample accumulation curve indicated that there were 93 non-personal names, and that the sample was adequate. A response-frequency histogram (Figure 4) suggested that about five more non-personal names might be found. In the 6 years subsequent to our field work, we discovered only one more name – *isihlava* – which is used widely for stem borers (Lepidoptera: Noctuidae) that attack maize and sugar cane.[34] In areas north and northeast of the Thukela River and northeast of the Phongolo River, isiZulu speakers call dragonflies (Odonata) *ibhebhamanzi* or *amabhebhamanzi*, but these terms were not reported to the interviewers, apparently because they are considered impolite.

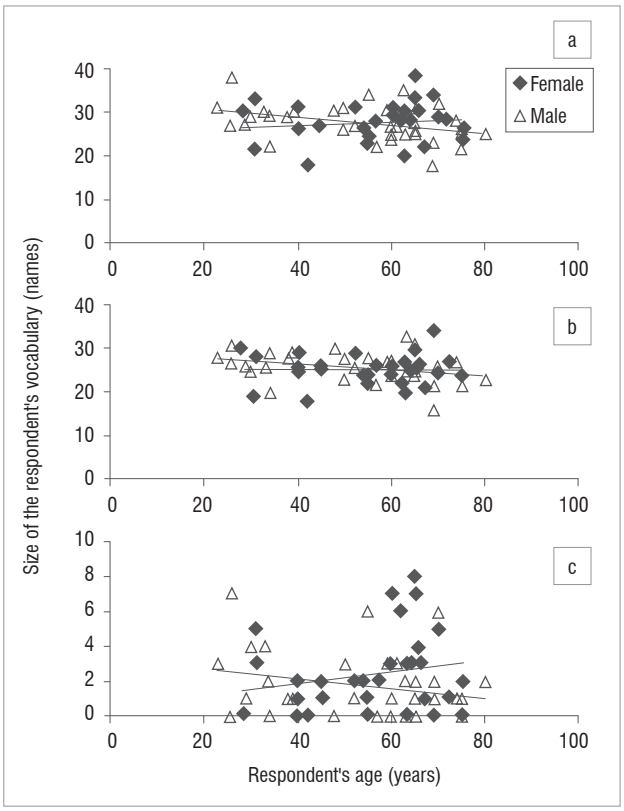

Figure 2: Relationships between age and vocabulary size of respondents in terms of (a) all names, (b) non-personal names and (c) personal names. The trend lines reflect the correlations reported in Table 3.

Comparing the sample accumulation curve of this study with that of a sample of isiXhosa names for the same insects,[21] there are fewer non-personal names (93) in isiZulu, whereas for the isiXhosa study the curve appeared to be reaching a plateau at about 116 non-personal names. The study of isiXhosa involved only eight sites and 51 respondents.[21]

Interviewing nature conservation personnel did not elicit a greater number of names (an average of 49 names versus an average of 50 names from other respondents; two-tailed *t*-test with unequal variances, $t = 0.65$; $p = 0.524$), nor did the respondents contribute a set of words that was much different from that provided by other respondents. This finding indicated that such specialists would not be a useful source against which to check for errors, perhaps because most of them were in the younger age groups. The respondents who seemed to impart the greatest knowledge of insects with the most confidence were elderly women who had been farmers all their lives.

Of the names collected in the present study, 56% matched the names of Doke et al.[33] either exactly or with some qualification (i.e. variations in pronunciation [= spelling] or grammar or by the addition of descriptive phrases) (Table 4). The total percentage of matches with the list of names of Uys and McLarty[22] was lower (36%), and 59% of the names in that list had no match with those in the present study. The lists of names from Doke et al.[33] and Uys and McLarty[22] both include descriptive names of insects, i.e. lengthy phrases which describe the insects but are unlikely to be a standardised name for that insect in isiZulu (Table 1).

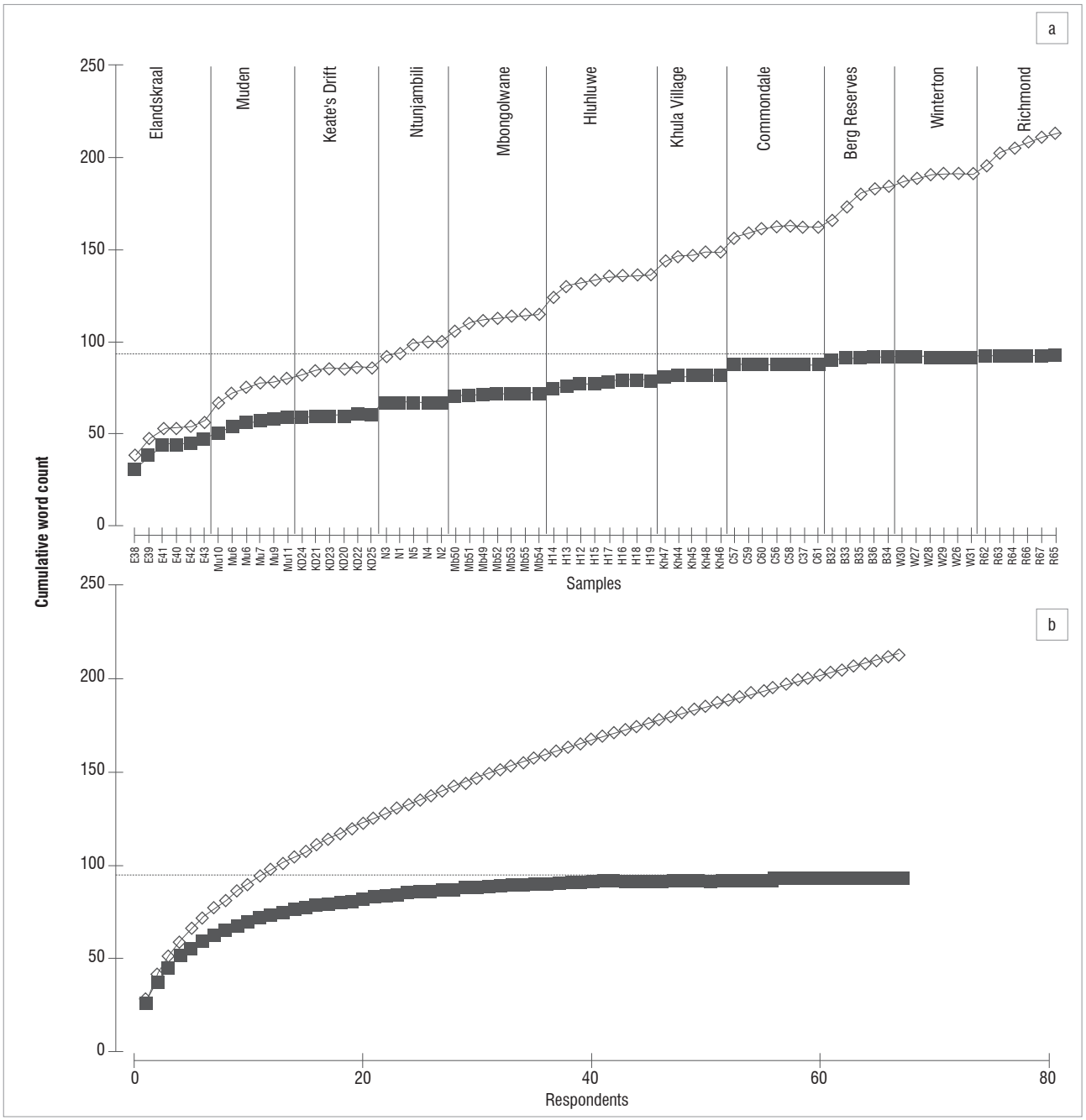

Figure 3: (a) Sample-accumulation curves constructed without permutation of samples. The flattening of the curves within each locality indicates that they are adequately surveyed. (b) Sample-accumulation curves constructed using 999 sampling permutations, used to estimate how many isiZulu entomological names there may be. The sample of non-personal names (i.e. those reported by at least two respondents from different sites; solid symbols) reaches an apparent asymptote at 93 names (dotted line), while the sample is still growing when personal names (i.e. names reported by only one respondent) are included.

We interpret these statistics collectively to mean that a sufficiently large sample had been collected here for a substantial list of reliable names to be compiled confidently and for the further inference of interesting patterns.

Patterns of variation

A quarter of the names (56 names, 26%) are 'general' names known by more than seven respondents and are widespread across all regions and all communities (Figure 4), and may be interpreted as core vocabulary in isiZulu. Another 37 'local' names (Figure 4), defined as names known by two to eight respondents from neighbouring sites,[21] were responsible for much of the small, sharp 'leaps' in numbers in the sample accumulation curve (Figure 3a), and for 17% of the names. These names are particularly characteristic of the Richmond site, which has one name (*unogwensi*) used by all respondents from this site, but which was not used at any other site, and three more names used almost exclusively at this site and by most of its respondents.

A total of 120 names were classified as 'personal', known by only one or two (non-independent) respondents (Figure 4), and the sample accumulation curves (Figure 3) did not provide an estimate of the potential total. Bryant[35] also noted that people using Bantoid languages have, like many other language families elsewhere, shown a high degree

of inventiveness and ingenuity in sculpting their languages. Personal names were not evenly distributed amongst the respondents, but were over-represented among older women and younger men (Figure 2). A potential explanation for this pattern lies in the amaZulu tradition of *ukuhlonipha*, the respectful avoidance of speaking the names, or even syllables of the names, of significant people such as elders, leaders or in-laws.[36-38] When a speaker needs to use a name affected by this tradition, they must substitute a different syllable or pick a replacement name. Such individualised creativity can be expected to particularly affect the names used by older women (with more relatives by marriage) and younger men (with relatively more seniors), relative to their counterparts, and therefore fits the pattern in Figure 2. Unfortunately, we could not follow up this speculation.

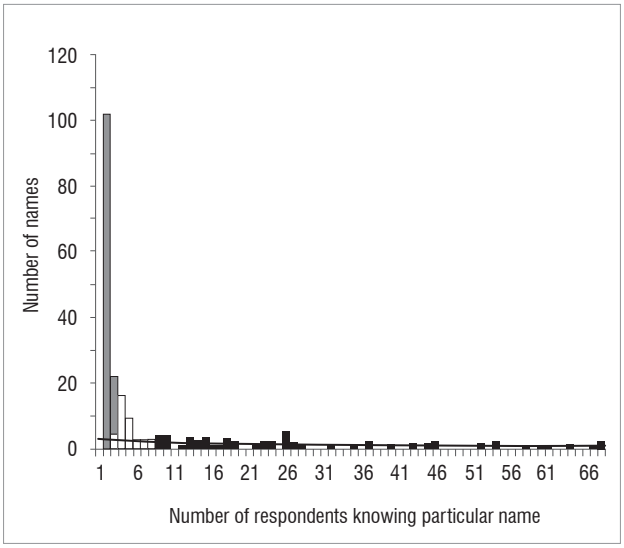

Figure 4: Response-frequency histogram used to estimate that about five cosmopolitan isiZulu names for insects are yet to be 'discovered'. The black histogram bars represent cosmopolitan names, and the point at which the black line fitted to them intersects with the *y*-axis provides an estimate of how many names are known by 0 people in the sample, i.e. await 'discovery'.

Table 4: Comparison of isiZulu insect names collected in the present study with two reference sources of names

Number of names in lists	Doke et al.[33]	Uys and McLarty[22]
Exact matches	34 (53%)	8 (13%)
Near matches (spelling or other variation)	2 (3%)	15 (23%)
Inexact matches (different taxa)	6 (9%)	3 (5%)
No match	22 (34%)	38 (59%)

There is also a great deal of variation in the grammatical structure of the isiZulu words for insects, which has also been noted for isiZulu plant names.[39] For example, dragonflies (Odonata: Anisoptera) are most commonly indicated by the stem *-jekamanzi*, but the prefix (and therefore the noun class) of this varies depending on the geographical region in focus. The prefixes *isi-* (plural *izi-*) and *u-* (plural *o-*) were found to be used with the stem *-jekamanzi* (Tables 5, 6). Grammatically, isiZulu birds' names can be classified into three categories according to the structure of the word stem, namely single-, complex- and compound-stemmed names.[40] Amongst the insects' names, examples of each of these categories of names can also be found: simple-stemmed name: *idiye* (Orthoptera: Acrididae: grasshopper); complex-stemmed name: *umayifisa* (Coleoptera: Tenebrionidae: toktokkie beetle); and compound-stemmed name: *imfundamakhwela* (Coleoptera: Gyrinidae: whirligig beetle).

Finally, there may be social differentiation in the use of names for insects. Although not reported by the respondents, alternative names may be given to insects by children, boys and girls alike, for example *ufudu* (which means 'tortoise' in English) for tortoise beetles (Coleoptera: Chrysomelidae: Cassidinae), which adults would call *umanqolwane* (BKS, personal observations). Parallel examples occur in other languages, e.g. in Hausa (e.g. little girls' *kabar mazaa daddahee* versus adults' *adinidma* for locust[7]) and in English (children's 'bow-wow' versus adults' 'dog').

Taxonomic resolution

Attempts to compare non-scientific naming systems (often termed 'folk taxonomies'[4,5,41,42]) such as those found in isiZulu or English with any terminological systems of biological nomenclature have ultimately been rejected as ineffective and undesirable. This situation is especially true when it comes to trying to promote and support interrelated networks of knowledge systems within a given context in post-colonial Africa.[43] Speakers of an indigenous African language like isiZulu perceive and experience such attempts as biased, presumptuous and exclusively Eurocentric.[44,45] Penel[7] noted that the biggest difference between the two knowledge systems, in his case between the nomenclature of the non-Bantoid Hausa language of Niger and Nigeria and Linnean biological nomenclature, is that the latter is divided into genus and species, and Hausa names are not. This is also true for isiZulu and English names. The Linnean system is also hierarchical, nested and governed by an internationally mandatory, bilingual, published code,[18] and is generally practised in an explicitly phylogenetic framework, all of which distinguish it from folk taxonomies.

An example of the incommensurability of isiZulu folk taxonomy and biological nomenclature is the term *izinambuzane*, which refers to insects in general, but is also applied to moles and cane rats in some areas (JJC, personal observations). However, there are some parallels between names in isiZulu and the ranks of Linnean classification. It appears that isiZulu names do not go beyond the taxonomic resolution of family-level identification, and are more easily comparable to names accorded the Linnean taxonomic rank of 'order'. For example, *ujekamanzi* corresponds to the order Odonata (dragonflies and damselflies), *ibhungane* / *ibhungezi* / *ibhungayezi* to the Coleoptera (beetles *sensu stricto*), depending on which region the speaker is in, and *umnyovu* / *umuvi* to the Hymenoptera (wasps, but excluding ants and bees). This taxonomic resolution does vary though; for example, the order Diptera (flies) is not given one general name, but is rather sub-divided into *umiyane* / *unongxi* for mosquitoes (of the family-ranked taxon Culicidae) and *impukane* for blow flies and house flies (families Calliphoridae and Muscidae, respectively). This pattern seems to indicate that more specific names are given to insects which are of particular intimate significance, for example honeybees, mosquitoes and house flies. A similar pattern is seen in other languages, including English and isiXhosa.[21]

It is not clear to what extent misidentification is involved here, but there is some evidence of it. For example, the hoverfly (Diptera: Syrphidae) was often named *inyosi*, the name given to honeybees (Hymenoptera: Apidae: *Apis mellifera*) (Tables 1, 5, 6), presumably because hoverflies mimic honeybees sufficiently well to confuse observers even though they have only one pair of wings like other flies. Crane flies, robber flies and antlions were all referred to as *ujekamanzi* (Tables 5, 6), although the reference to water (*-manzi*) in that name clearly aligns it with the biology of the dragonflies and damselflies that it also denotes (Tables 5, 6). However, all of these specimens were large, with elongated abdomens and clear wings, so *ujekamanzi* may be understood to designate a physical form rather than a specific taxon, in analogy to the terms 'pest' and 'bug' and 'germ' in English folk taxonomy, *inunu* in isiZulu or *gogga* in Afrikaans.

Names that indicate insects at the species level refer to particular insect species that have intimate contact with the lives of isiZulu speakers. For example, honeybees (Hymenoptera: Apidae: *Apis mellifera*), greenbottle

Table 5: Dominant isiZulu entomological names collected from interviews. 'isiZulu name 1' refers to the most common name per specimen across all regions and 'isiZulu name 2' refers to the second most common name across all regions (where this differs from isiZulu name 1 and is known by five or more people). '–' indicates that no isiZulu name showed consistency (fewer than five people knew the name), often because that particular insect was not known to the respondents.

English name	isiZulu name 1		isiZulu name 2	
	Singular	Plural	Singular	Plural
ant	itsheketshe	amatsheketshe	intsheketshe	amantsheketshe
ant, thief ~ (queen)	ihlwabisi	amahlwabisi	umnyovu	iminyovu
antlion (adult)	ugogo	ogogo	–	–
antlion (larva)	ujekamanzi	ojekamanzi	–	–
aphid	–	–	–	–
bagworm	umahambanendlwane	omahambanendlwane	–	–
bee, carpenter ~	ibhungezi	amabhungezi	ibhungane	amabhungane
bee, honey ~	inyosi	izinyosi	–	–
beetle, blister ~	ibhungezi	amabhungezi	ibhungane	amabhungane
beetle, Christmas ~	ibhungezi	amabhungezi	ibhungane	amabhungane
beetle, click ~	ibhungezi	amabhungezi	–	–
beetle, dung ~	ibhungezi	amabhungezi	ibhungane	amabhungane
beetle, dung ~	ibhungezi	amabhungezi	ibhungane	amabhungane
beetle, longhorn ~	umzondo	imizondo	–	–
beetle, rhinoceros ~	ibhungezi	amabhungezi	ibhungane	amabhungane
beetle, toktokkie ~	ibhungezi	amabhungezi	ibhungane	amabhungane
beetle, tortoise ~	–	–	–	–
beetle, weevil ~	ibhungezi	amabhungezi	umayifisa	omayifisa
beetle, whirligig ~	imfundamakhwela	izifundamakhwela	–	–
bug, bed ~	imbungulu	izimbungulu	–	–
butterfly, citrus swallowtail	uvemvane	izimvemvane	ivemvane	amavemvane
butterfly, white	uvemvane	izimvemvane	ivemvane	amavemvane
cicada	isihlonono	izihlonono	–	–
cockroach, American ~	iphela	amaphela	igugu	amagugu
cockroach, wingless ~	igugu	amagugu	–	–
cricket	inyekevu	inyekevu	inyendle	izinyendle
cricket, sand ~	inyendle	izinyendle	–	–
damselfly	ujekamanzi	ojekamanzi	–	–
dragonfly	ujekamanzi	ojekamanzi	isijekamanzi	izijekamanzi
earwig	umkhothane	imikhothane	umbhelekendlane	imibhelekendlane
fishmoth	ubuthethe	ubuthethe	–	–
flea	izenze	amazenze	izeze	amazeze
fly, bluebottle ~	impukane	izimpukane	–	–
fly, crane ~	ujekamanzi	ojekamanzi	–	–
fly, flesh ~	impukane	izimpukane	isibawu	izibawu
fly, greenbottle ~	impukane	izimpukane	–	–
fly, house ~	impukane	izimpukane	–	–
fly, hover ~	inyosi	izinyosi	–	–
fly, mosquito ~	umiyane	omiyane	unonxi	ononxi
fly, robber ~	ujekamanzi	ojekamanzi	–	–
fruit chafer	ibhungezi	amabhungezi	ibhungane	amabhungane
glowworm	imfinyezi	izimfinyezi	ukhanyikhanyi	okhanyikhanyi
grasshopper	iboni	amaboni	idiye	amadiye
grasshopper, bladder ~	intothoviyane	izintothoviyane	–	–
grasshopper, foam ~	intothoviyane	izintothoviyane	intethe	izintethe
grasshopper, rain ~ (female)	ugqugqululu	ogqugqululu	isihlonono	izihlonono
grasshopper, rain ~ (male)	ugqugqululu	ogqugqululu	isihlonono	izihlonono
katydid	intethe	izintethe	igawozi	amagawozi
ladybird	ibhungezi	amabhungezi	–	–
louse	ubukhuphe	ubukhuphe	intwala	izintwala
mayfly	umniyane	omniyane	–	–
moth, hawk ~	uvemvane	izimivemvane	ivemvane	amavemvane
moth, owlet ~	uvemvane	izimvemvane	isiphaphalazi	iziphaphalazi
praying mantis	isithwalambiza	izithwalambiza	umashisindlu	omashisindlu
scale insect	–	–	–	–
stick insect	isithwalambiza	izithwalambiza	–	–
termite (winged)	inhlwabusi	izinhlwabusi	inkulungwane	obhobholwane
termite (worker)	umuhlwa	imihlwa	–	–
wasp, ichneumonid ~	–	–	–	–
wasp, mud ~	umnyovu	iminyovu	umuvi	imivi
wasp, paper ~	umnyovu	iminyovu	–	–
wasp, potter ~	umnyovu	iminyovu	–	–
wasp, spider-hunting ~	umnyovu	iminyovu	–	–
weevil, bean ~	imbovane	izimbovane	impehlwa	imiphehlwa

flies (Diptera: Calliphoridae) and bedbugs (Hemiptera: Cimicidae: *Cimex lectularius*) are well known by the majority of respondents and each have one universal name (Figure 5). The honeybee is known for its honey and its sting; the greenbottle fly for frequently infecting food and cattle's wounds; and the bedbug for uncomfortable bites. These names are amongst the most geographically universal, which is an interesting correlation that is very convenient for practical reasons, especially in applied entomology.

Linguistic standardisation

As in isiXhosa, in which over half of the insects had more than one name,[21] isiZulu may have several names for the same insect, which poses a potential challenge for proponents of the linguistic standardisation of this dynamic language. To decide which name was dominant, and whether there was enough consistency within the sample to assign one dominant name to an insect, i.e. if a name is culturally stable, we used the criterion that a name must be known by at least five people throughout the sample (i.e. 10%). Table 5 provides the apparent core list of isiZulu names for insects compiled under this criterion. For some insects, no consistent name could be recorded and it was therefore conservatively assumed that the name was not agreed by the respondents we interviewed.

As highlighted by the response-frequency histogram (Figure 4), there is some regionalism that cannot be ascribed to differences in the local faunas because the interviews were standardised by using the same set of specimens. For the regional list (Table 6), the most common name must be known independently by at least two people in that community. Independent use of a name within a community by more than two people, which was not mentioned in other areas, clearly illustrates how different names are used in different areas. For example, in most of the regions surveyed, the word *izenze* was used for flea (Siphonaptera) but in the area southeast of the Drakensberg and northwards to northeast of the Thukela River, the word *izeze* was more common (Figure 6). Fortunately, the regional names are not mutually exclusive, and an isiZulu-speaker anywhere will understand both *izeze* and *izenze*. The case of *ujekamanzi* and *ibhebhamanzi* is mentioned above. These examples are most likely a form of dialect, which is described by Kaschula and Anthonissen[46] as mutually intelligible forms of a language that differ systematically across geographical or social gradients. According to the literature, two to four dialects are usually attributed to isiZulu, including *isiZulu phaqa* (proper Zulu), *isiZulu sasezansi* (south Zulu) and *isiZulu sasemadolobheni* (urban Zulu) spoken north and south of the Thukela River (Guthrie number S42A), *isiZulu saseGoli,* Transvaal Zulu (S42B), and perhaps the extinct Lala (S406).[8-11]

However, not all geographical variants are as similar as these examples, and some regions show an overlap in regional names that are less likely to represent mutually intelligible dialectal variation. For example, at Mbongolwane, almost as many respondents called butterflies (Lepidoptera: Papilionidae and Pieridae) *isiphaphalazi* as called them *uvemvane*. Mbongolwane lies between sites northeast of the Thukela River (Hluhluwe, Khula Village) that use *isiphaphalazi* and sites southeast of the Thukela River (Muden, Ntunjambili, etc.) that favour *uvemvane*. Such differences mark the onset of development of dialects into separate languages.

Linguistic standardisation is part of the mandate of the Pan South African Language Board, which must reconcile its aims with all of these aspects of diversity and evolution in isiZulu entomological names, which also occur in isiZulu ornithological names[40], isiZulu botanical names[39], isiXhosa entomological names[21], folk taxonomies worldwide and language in general. Traditionally, dictionaries have been seen by their users (and often also their writers) as arbiters of standardised usage and spelling, thus accorded a prescriptive role. Modern lexicography recognises that languages are dynamic and evolutionary, an insight that has led to dictionaries becoming linguistically descriptive, rather than prescriptive,[47] but they can still be compiled for linguistic standardisation agendas.

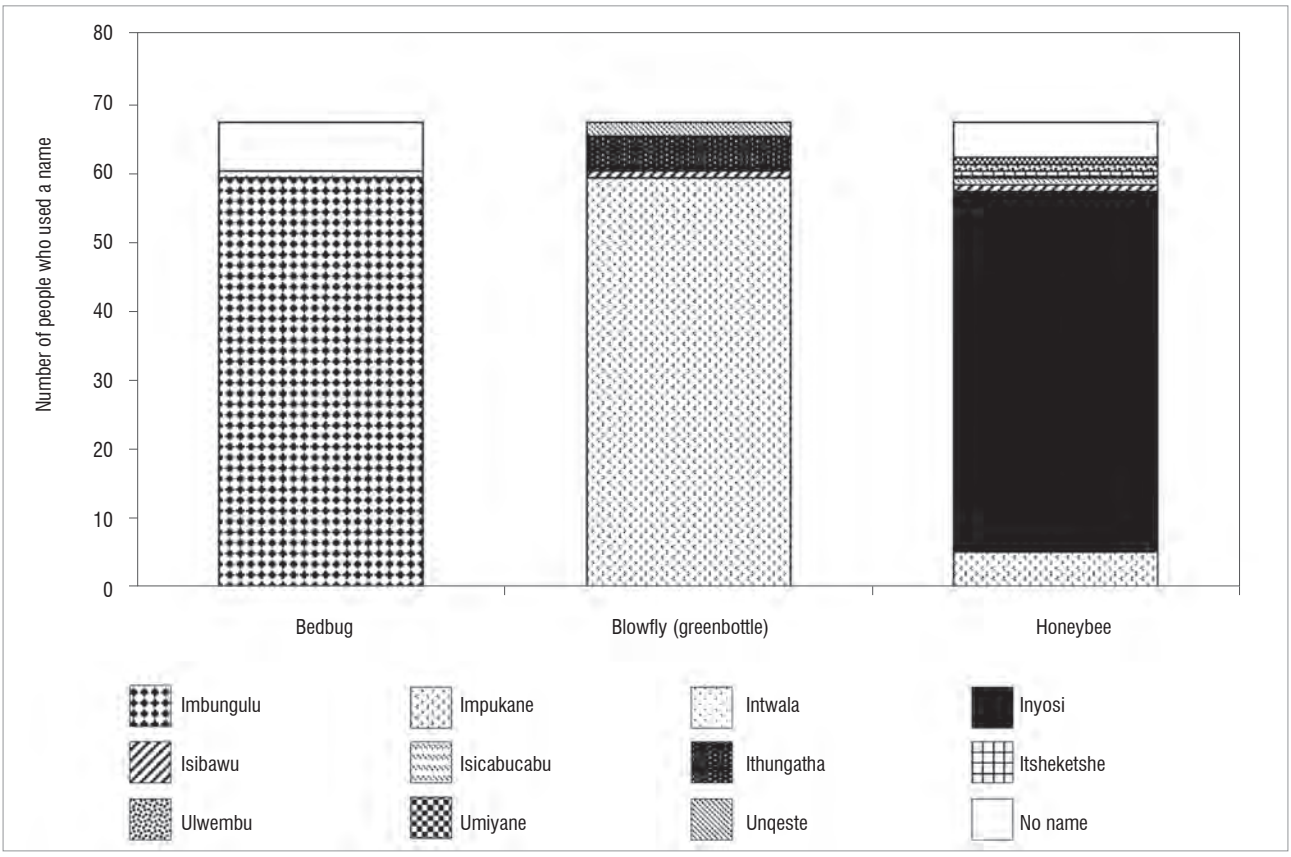

Figure 5: Examples of insects that have one cosmopolitan name that is used consistently across all regions.

Table 6: IsiZulu names across all regions sampled (= most frequent name per specimen in each region; '–' indicates that there was no consistent name for that specimen, i.e. a name known by at least two people)

English name	Commondale	Hluhluwe	Khula Village	Mbongolwane	Elandskraal	Muden	Keate's Drift	Ntunjambili	Richmond	Berg Reserves	Winterton
ant	intsekentse	itsheketshe	itsheketshe	itsheletshe	itsheketshe	itsheketshe	itsheketshe	itsheketshe	intsekentse/ itsheketshe	itsekentse	itsekentse
ant, thief (queen)	intsheketshe	ihlabusi	ihlabusi	isiqandi	itsheketshe	ihlwabisi	ihlwabisi	ihlwabisi	umnyovu	umnyovu	umnyovu
antlion (adult)	ugogo amanzi	ugogo	–	–	ugogo	–	ugogo	–	–	ugogo	–
antlion (larva)	–	ujekamanzi	uvemvane	ujekamanzi	ujekamanzi	ijekamanzi	isijekamanzi	ujekamanzi	–	ujekamanzi	ujekamanzi
aphid	–	–	isinambuzane	–	–	–	–	–	–	–	–
bagworm	umahambanendl-wane	umahambanendl-wane	inkukudleni	umahambanendl-wane	umahambanendl-wane	–	umahambanendl-wane	umahambanendl-wane	umahambanendl-wane	umahambanendl-wane	umahambanendl-wane
bee, carpenter	ibhungane	ibhungane	ibhungane	ibhungane	inyosi	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungayezi
bee, honey	inyosi	inyosi	inyosi	inyosi	inyosi	–	inyosi	inyosi	inyosi	inyosi	inyosi
beetle, blister	umashisindlu	ibhungezi	ibhungane	ibhungane	ibhungezi	ibhungezi	ibungezi	ibhungezi	ibhungezi	–	ibhungayezi
beetle, Christmas	ibhungane	ibhungezi	ugadleni	ibhungane	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungayezi	ibhungayezi
beetle, click	ibhungane	–	umtingweni	–	ibhungezi	ibhungezi	–	ibhungezi	ibhungezi	isangoma	isangoma
beetle, dung	umnyovu	ugadleni	ugadleni	ibhungane	–	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungayezi	ibhungayezi
beetle, dung	ibhungane	ugadleni	ungadleni	ibhungane	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungayezi/ inkubablongwe	ibhungayezi
beetle, longhorn	ibhungane	umzondo	umzondo	ibhungane	umzondo	umzondo	umzondo	umzondo	ibhungezi	–	uchothovane
beetle, rhinoceros	ibhungane	ugadleni	ugadleni	ibhungane	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungayezi	ibhungayezi
beetle, toktokkie	ibhungane	ugadleni	ungadleni	ibhungane	ibhungezi	isiginqa	umayifisa	ibhungezi	ibhungezi	ibhungayezi	ibhungayezi
beetle, tortoise	–	–	–	–	–	ibhungezi	–	–	–	uchothovane	–
beetle, whirligig	inyekevu	imfundamakhwela	imfundamakhwela	imfundamakhwela	imfundamakhwela	imfundamakhwela	inhlabamakhwela	imfundamakhwela	intlagamakhwela	imfundamakhwela	imfundamakhwela
bug, bed	imbungulu	imbungulu	imbungulu	imbungulu	imbungulu	imbungulu	imbungulu	imbungulu	imbungulu	imbungulu	imbungulu
butterfly, citrus swallowtail	uvemvane	uvemvane	isiphaphalazi	uvemvane	uvemvane	uvemvane	uvemvane	uvemvane	uvevane/uveveshane	ivemvane	ivemvane
butterfly, white	uvemvane	uvemvane	isiphaphalazi	uvemvane	uvemvane	uvemvane	uvemvane	uvemvane	uveveshane	ivemvane	ivemvane
cicada	inyekevu	isihlonono	isihlonono	isihlonono	–	isidlonono	isidlanono	–	–	isibawu	isibawu
cockroach, American	iphela	ikokoloshe	iphela	iphela	iphela	ikokoloshe	igugu	igugu	igugu/ ibhungezi	iphela/ iphelagugu	iphelagugu
cockroach, wingless	ibhungane	igugu	igugu	igugu	igugu	igugu	igugu	igugu	ibhungezi	–	iphelagugu
cricket	ibhungane	inyekevu	umaqhatshel-esendeni	inyendle	umaqhantshel-esendeni	umaqhantshela	inyendle	inyendle	unogwintsi	inyekevu	inyekevu
cricket, sand	inyekevu	–	uklembe	inyendle	–	inyendle	inyendle	uvete	unogwentsi/ unogwintsi	–	–
damselfly	–	ujekamanzi	uvemvane	ujekamanzi	ujekamanzi	ujekamanzi	isijekamanzi	ujekamanzi	isidungamanzi	–	–
dragonfly	–	ujekamanzi	uvemvane	ujekamanzi	ujekamanzi	isijekamanzi	isijekamanzi	ujekamanzi	isidungamanzi/ uveveshane	ujekamanzi	ujekamanzi
earwig	umkhothane	–	–	intlava	umkhothane	–	–	umbhelekendlane	umbhelekendlane	umkhothane	umkhothane

Continued on next page

English name	Commondale	Hluhluwe	Khula Village	Mbongolwane	Elandskraal	Muden	Keate's Drift	Ntunjambili	Richmond	Berg Reserves	Winterton
fishmoth	umsundu	–	iphela	iphela	–	ubuthethe	ubuthethe	ubuthethe	–	–	–
flea	izeze	izenze	izeze	izeze	izenze	izenze	izenze	izenze	izenze	izeze	izeze
fly, bluebottle	impukane	impukane	impukane	impukane	impukane	impukane	impukane	impukane	impukane	impukane	impukane
fly, crane	–	–	uvemvane	–	ubhephamanzi	ujekamanzi	isijekamanzi	–	isidungamanzi	–	umdozolwane
fly, flesh	impukane	impukane	impukane	impukane	impukane	isibawu	impukane	impukane	impukane	impukane	impukane
fly, greenbottle	impukane	impukane	impukane	impukane	impukane	impukane	impukane	impukane	ithungatha/ impukane	impukane	impukane
fly, house	ubukhupe	impukane	impukane	–	impukane	impukane	impukane	impukane	imbuzane	umpukane	impukane
fly, hover	inyosi	inyosi	inyosi	inyosi	inyosi	inyosi	inyosi	inyosi	inyosi	inyosi	inyosi
fly, robber	–	ujekamanzi	ujekamanzi	ujekamanzi	ujekamanzi	isijekamanzi	isijekamanzi	ujekamanzi	–	ujekamanzi	–
fruit chafer	ibhungane	ibhungezi	ugadleni	ibhungane	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungezi	ibhungayezi	ibhungayezi
glowworm	umnyovu	imfimyezi	inkanyezi	isikhanyikhanyi	umagqamemnya-meni	ukhanyikhanyi	umagqamemnya-meni	ukhanyikhanyi	–	umsundu	umsundu
grasshopper	intethe	iboni	iqhwagi	iqhwagi	idiye	iboni	iboni	idiye	iqhwagi	idiye	idiye
grasshopper, bladder	–	–	–	–	intothoviyane	intothoviyane	igawozi	umkhonya	–	–	–
grasshopper, foam	intothoviyane	intothoviyane	intothoviyane	intothoviyane	intothoviyane	intethe	intothoviyane	intothoviyana	intothoviyane	intothoviyane	intothoviyane
grasshopper, rain (female)	intethe	isihlonono	isihlonono	iqhwagi	ugqugqululu	ugqugqululu	ugqugqululu	isihlonono	–	idiye	idiye
grasshopper, rain (male)	intethe	isihlonono	isihlonono	iqhwagi	idiye	ugqugqululu	ugqugqululu	isihlonono	–	idiye/intethe	idiye
katydid	–	–	ugogo	idiye	–	igawozi	igawozi	intethe	intethe	idiye	idiye
ladybird	ibhungane	–	imfinyezi	–	–	–	imfinyezi	ibhungezi	–	–	–
louse	ibhungane	ubukhuphe	ubukhuphe	ubukhuphe	ubukhuphe	intwala	ukhuphe	–	ukhuphe	ubukhuphe	ubukhuphe
mayfly	umiyane	–	–	–	umniyane	–	–	–	imbuzane	indozolwane	–
mosquito	umdozolo	umiyane	umiyane	umiyane	unongxi	unongxi	unongxi	umiyane	–	umiyane	umiyane
moth, hawk	uvemvane	uvemvane	uvemvane	umvemvane	uvemvane	uvemvane	uvemvane	uvemvane	uvevane	ivemvane	ivemvane
moth, owlet	–	isiphaphalazi	isiphaphalazi	uvemvane	uvemvane	uvemvane	uvemvane	uvemvane	uvevane	ivemvane	ivemvane
praying mantis	umvunya	isithwalambiza	isithwalambiza	iisthwalambiza	isithwalambiza	isithwalambiza	isithwalambiza	isithwalambiza	–	umashisumuzi	umashisindlu
scale insect	–	–	–	–	–	–	–	–	ukhwekhwe	–	–
stick insect	umashisumuzi	isithwalambiza	isithwalambiza	isithwalambiza	isithwalambiza	isithwalambiza	isithwalambiza	isithwalambiza	isithwalamnqwaza	–	umashisindlu
termite (winged)	inhlwabusi	inhlwabusi	–	ihlwabusi	inhlwabusi	inkulungwane	inkulungwane	inhlwabusuku	inkulungwane	ubhobholwane	ubhobholwane
termite (worker)	intuthwane	–	unina	umuhlwa	umuhlwa	umuhlwa	umuhlwa	–	inhlwa	–	umuhlwa
wasp, ichneumonid	imfundamakhwela	–	–	umiyane	–	–	umnyovu	–	–	–	iqendevane
wasp, mud	ibhungane	umnyovu	umuvi	umuvi	umnyovu	umnyovu	umnyovu	umnyovu	umnyovu	umnyovu	umnyovu
wasp, paper	ibhungane	umnyovu	umuvi	isiqandi	umnyovu	umnyovu	umnyovu	isiqandu	inxenyane	umnyovu	umnyovu
wasp, potter	umnyovu	umnyovu	umivi	umuvi	umnyovu	umnyovu	umnyovu	umnyovu	umnyovu	umnnyovu	umnyovu
wasp, spider-hunting	umnyovu	umnyovu	umuvi	umuvi	umnyovu	umnyovu	umnyovu	umnyovu	umnyovu	umnyovu	umnyovu
weevil	ibhungane	umayifisa	umayifise	–	ibhungezi	–	umayifisa	ibhungezi	umazifise/ ibhungezi	–	–
weevil, bean	–	impehlwa	impehlwa	–	impehlwa	imbovane	–	impehlwa	impehlwa	–	imbovane

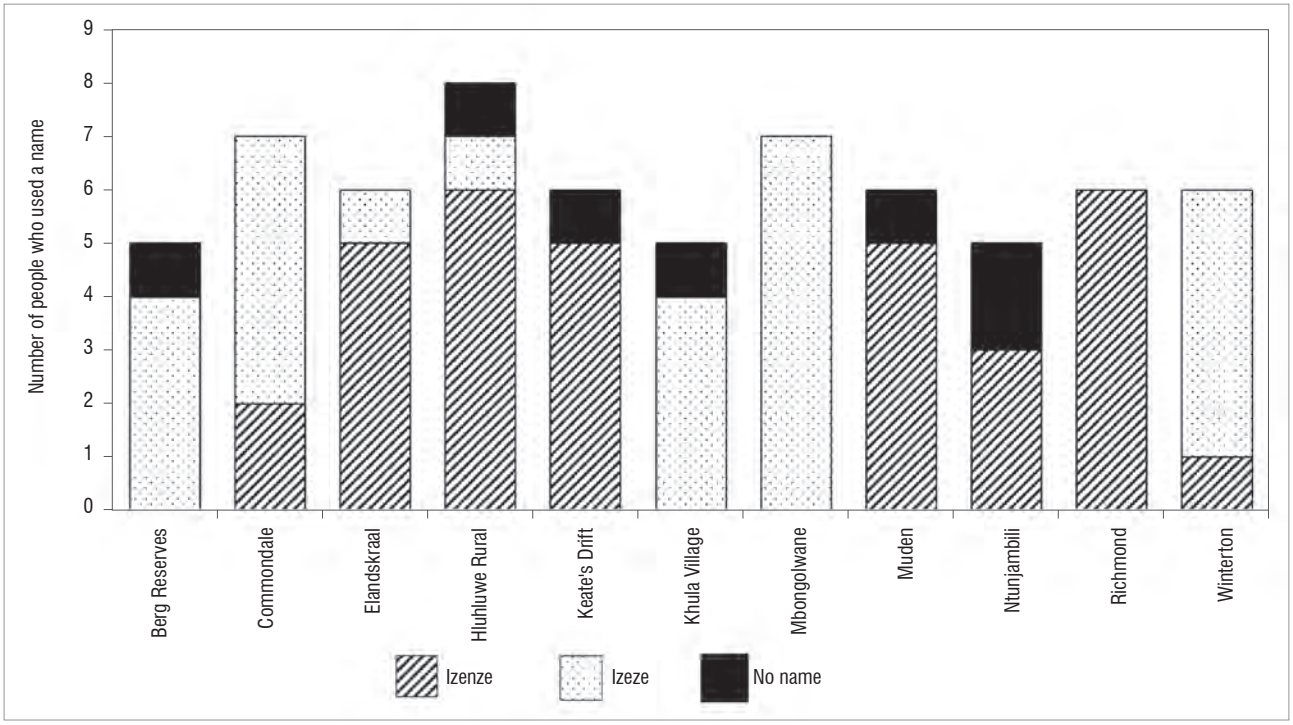

Figure 6: The isiZulu name for fleas (Siphonaptera: Pulicidae) varies across regions. The term *izenze* is more common in the centre of the province (Elandskraal, Muden, Keate's Drift, Ntunjambili, Hluhluwe), and *izeze* is more common in the peripheral coastal (Mbongolwane, Khula Village) and mountain (Berg reserves, Winterton, Commondale) regions (cf. Figure 1).

Ideally, lexical variations that achieve a certain level of universal intelligibility cannot be overlooked when compiling a descriptive dictionary. *Isihlonipho*, the alternative term resorted to in a particular instance, poses an interesting challenge to defining an appropriate level of universality for this selection criterion because although names generated through it are frequently personal and apparently rife (e.g. Figure 3), they are also widely understood. Because the exhaustive description of isiZulu might appear impracticable, Koopman[39] suggested that one mentions the general phenomenon of high variation and catalogues only the key variants of each name. Presumably lexicons aimed at standardisation would ignore much of the dialectal, cultural, social and grammatical richness of languages like isiZulu because of their distinctive agenda.

Conclusion

This list could be a valuable reference for professionals working on reciprocal technology transfer in the fields of applied entomology, environmental education, and agricultural, veterinary and medical extension work in communities in which isiZulu is spoken predominantly. It also facilitates further studies of cultural entomology and research into isiZulu folklore relating to insects. This is of interest as insects play a major role in human society: as pests and transmitters of diseases, and as a useful and beneficent presence that is intricately linked to human livelihood.[48]

This study will hopefully prove fruitful as a research model even though it was not focused on technical linguistic issues. It highlights the need for interdisciplinary teamwork in the field of indigenous knowledge research and the challenges facing the standardisation of South African languages.

Acknowledgements

Foremost, we thank the 67 respondents who sat patiently with the field researchers and generously shared their knowledge of insects. We also thank Milton Ngeju (Department of Zoology and Entomology, Rhodes University) for letting us use the departmental collection of preserved insect specimens; Matthias Bodenstein, Rob Crausaz, Sigrid Dedekind, Ncane Ndulini, Vic Schütte, Anittah Sithole, Reneé Stockil and Roland Vorwerk for assisting us in making contact with local Zulu communities; Kevin and Stella Cockburn for transport and accommodation in the field, and encouragement; Stella Cockburn for assistance in compiling the list of isiZulu names and for proofreading; Adrian Koopman (University of KwaZulu-Natal) for access to his unpublished work on isiZulu animal and plant names; Helen Henninger (INRIA, Antibes, France) for pointing out the custom of resorting to isiHlonipo; Bernadette Hubbart for reference material; and Rhodes University for funding.

Authors' contributions

J.J.C. (student) led and conducted the majority of the research, including the design, data collection through interviews, analysis and write-up. B.K.-S. provided input into the writing process, in particular with the history of the isiZulu language development. He also ensured language accuracy of the lists of names collected. M.V. supervised the student and provided conceptual input into the research design and process, and assisted with the analysis and write-up.

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Prescription patterns of enzyme-containing products in South Africa over a 2-year period

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DATES:
Received: 14 Dec. 2013
Revised: 22 Feb. 2014
Accepted: 26 Feb. 2014

KEYWORDS:
enzymes; drug utilisation review; prescriptions; enzyme-based therapeutics; hyaluronidase

HOW TO CITE:
Truter I. Prescription patterns of enzyme-containing products in South Africa over a 2-year period. S Afr J Sci. 2014;110(9/10), Art. #2013-0385, 5 pages. http://dx.doi.org/10.1590/sajs.2014/20130385

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Enzymes are traded in five categories, namely medical (intervention), diagnostic (detection and quantification), molecular biology, biofuel and industrial. Therapeutic enzymes have been investigated for different uses, for example, for the treatment of genetic disorders, blood clotting disorders, cancer and infectious diseases and for burn debridement. No studies on the prescription of enzyme-containing products in South Africa could be found. Enzymes are classified in the *Monthly Index of Medical Specialities* under digestants, enzymes and fibrinolytics. The primary aim of this study was to investigate the prescription patterns and cost of enzyme-containing products in South Africa. A private health-care medicines claims database for 2010 and 2011 of approximately 4.5 million records was analysed retrospectively. Enzyme-containing products constituted a small percentage of medical insurance claims (only 0.02% of approximately 4.5 million claims for products and procedures), yet they were relatively expensive. A total of 906 products was prescribed at a cost of almost ZAR2 million over the 2 years. Hyaluronidase was the most frequently prescribed (60.04%), followed by pancreatin-containing products (34.66%). Pancreatin (lipase/protease/amylase) is primarily used in the management of pancreatic exocrine insufficiency. The average cost per hyaluronidase prescription paid by the medical insurance schemes was ZAR280. Other enzyme-containing products prescribed were imiglucerase, alteplase and tenecteplase. Imiglucerase was overall the most expensive. Alteplase, tenecteplase and streptokinase are antithrombotic enzymes that are used in the treatment of acute myocardial infarction or ischaemic stroke. Streptokinase, regarded as the most affordable antithrombotic enzyme, was not prescribed during the period under study. With the growing opportunities for enzymes for therapeutics, the use of enzyme-containing products which are comparatively expensive require cost-effectiveness studies.

Introduction

Enzymes are natural proteins that catalyse chemical reactions, converting a specific set of reactants (substrates) into specific products. Enzymes are highly specific and have several applications in different industries, such as the paper, starch, leather, pharmaceutical, baking, beer brewing, detergent, and wine-making industries.[1] Enzymes are traded in five distinct categories, namely medical (intervention), diagnostic (detection and quantification), molecular biology, biofuel and industrial. Based on their application, enzymes can be categorised into two major categories[1]: industrial enzymes and medical enzymes. The differences between industrial and medical enzymes are given in Table 1.

Table 1: Differences between industrial and medical enzymes[1]

Industrial enzymes	Medical enzymes
Produced in large quantities	Produced in small quantities
Partially purified but at an optimum	Extensively purified
Economic concerns are very important	Excellent functionality
Used as catalysts; hence, functionally, industrial enzymes are catalytic	Used to treat various diseases; hence, functionally, medical enzymes are therapeutic
Source of industrial enzymes is microbial and recombinant	Source of medical enzymes is mainly human or animal and recombinant

The market for enzymes in medicine is growing. The global market for industrial enzymes was valued at USD2.9 billion in 2008 and reached about USD3.1 billion in 2009.[1] In contrast to this, the global market for medical enzymes was estimated at USD6 billion in 2010, and it is growing to an estimated USD7.2 billion in 2015.[2] Therapeutic enzymes are the biggest segment in terms of revenue generated.[2] This sector was valued at USD5.3 billion in 2010 and is expected to increase to USD6.3 billion in 2015.[2]

The variety of enzymes and their potential therapeutic applications are considerable.[3] Some examples of enzymes which have realised the potential to become important therapeutic agents are asparaginase, hyaluronidase, ribonuclease, streptokinase and urokinase.[3] Enzymes as medicines (therapeutic enzymes) have two important features that distinguish them from all other types of medicines.[4] Firstly, enzymes often bind and act on their targets with great affinity and specificity, and, secondly, enzymes are catalytic and convert multiple target molecules to the desired products.[4] These two features make enzymes specific and potent medicines that can accomplish therapeutic biochemistry in the body that small molecules cannot. Enzymes can often be used for treatments complementary to those by small molecules, without one necessarily being better than the other. Each has its own

best application. These characteristics have resulted in the development of many enzyme-containing medicines for a wide range of disorders.

For the past 50 years, therapeutic enzymes have been investigated for the treatment of genetic disorders, blood clotting disorders, cancer and infectious diseases, as well as for burn debridement, amongst others, and have been registered as 'orphan drugs' or 'therapeutic interventions'.[4] The field has developed rapidly, and, in 1987, the US Federal Drug Administration approved the first recombinant enzyme drug alteplase, which is a human tissue plasminogen activator.[4] Even though products classified under 'Enzymes' in the *Monthly Index of Medical Specialities* (MIMS) [5] are reimbursed by medical aid insurance schemes in South Africa, no drug utilisation studies could be found in the literature on the prescription, usage patterns and cost of these products despite the fact that they are relatively expensive and deemed of national importance. MIMS[5] lists most of the pharmaceutical products in the South African market, especially those regularly prescribed, and is regarded as a standard reference source of available medicines in South Africa.

Enzyme-containing products are included in MIMS Category 27.0.0 (Enzymes), but are also referred to in MIMS Categories 8.3 (Fibrinolytics) and 12.1 (Digestants).[5] Only six enzyme active ingredients are listed in these categories – hyaluronidase, imiglucerase and pancreatin and three antithrombotic enzymes (alteplase, tenecteplase and streptokinase).[5,6] This list is limited, even though there are more types of enzymes used in therapeutics in other parts of the world, either as registered pharmaceuticals or as orphan drugs.[4]

Four of the six enzyme-containing products are prescription only (Schedule 4) medicines as classified in the *Medicines and Related Substances Act, No 101 of 1965* (as amended) in South Africa; the exceptions are hyaluronidase and pancreatin, which are Schedule 1 medicines (available over the counter in pharmacies).[5-7] All these products are for parenteral administration, except the pancreatin-containing products.

Hyaluronidase (hyaluronoglucosaminidase EC 3.2.1.35) is a glycosidase hydrolysing 1,4-linkages between *N*-acetyl-β-*D*-glucosamine.[8] The enzyme is extracted from ovine or bovine testes as the protein is present on the posterior head and the acrosomal membrane of mammalian sperm.[9] Recently, recombinant forms of hyaluronidase (produced by the combining of material from more than one origin), such as rHuPH20, have been introduced onto the market.[9,10] Hyaluronidase modifies the permeability of connective tissue through the hydrolysis of hyaluronic acid, which temporarily decreases the viscosity of the cellular cement and promotes diffusion of injected fluids or of localised transudates, thus facilitating their absorption. It is used as an adjunct to increase the absorption and dispersion of other injected drugs[10], for hypodermoclysis, for improved resorption of subcutaneously administered radiocontrast media in urography, for the effective decrease of injected depots of hyaluronic acid in aesthetic surgery[11], and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents. It is used off-label for the treatment of vitreous haemorrhage and diabetic retinopathy. Sodium hyaluronate 10 mg/mL is included in the Standard Treatment Guidelines and Essential Medicines List for South Africa (Hospital Level Adults).[12] It is used as an ocular peri-operative pharmaceutical product and is classified under 'Surgical and diagnostic products' (Section 18.8).[12] It is therefore used in the public health sector in hospitals, but no data on the total number of prescriptions could be found.

Imiglucerase is a recombinant DNA-produced analogue of human β-glucocerebrosidase[4] (EC 3.2.1.45). The enzyme hydrolyses the beta glycosidic links in glucocerebroside that is an intermediate in lipid metabolism. A mutation in the glucocerebrosidase gene leads to the disorder known as Gaucher's disease (a lysosomal storage disease) that occurs in the absence of glucocerebrosidase activity. The use of glucocerebrosidase in enzyme replacement therapy is the first of its kind using an exogenous enzyme targeting its natural site of activity in the body.[4]

Pancreatin is an extract from ovine pancreas and contains lipases (pancreatic triacylglycerol lipase EC 3.1.1.3), α-amylase (EC 3.2.1.1), proteases (trypsin EC 3.4.21.4) and chymotrypsin (EC 3.2.21.1) in varying proportions. Pancreatin is used to treat pancreatic insufficiencies (both prescription and over-the-counter) as well as in the treatment of fat malabsorption in HIV patients and pancreatic insufficiency in cystic fibrosis patients (where the lipases are from recombinant maize[4]).

The antithrombotic enzymes are tissue plasminogen activators that are used to remove blockages in blood vessels in acute ischaemic strokes, myocardial infarctions and pulmonary oedemas. Alteplase and tenecteplase (EC 3.4.21.68) are serine proteases of human origin that cleave plasminogen to plasmin, which is the enzyme responsible for clot breakdown. Streptokinase (EC 3.4.24.29) produced by various strains of streptococci is able to bind and activate plasminogen in a non-proteolytic manner to break down fibrin clots.

In the light of the identification of medical enzymes as an important research focus for South African academia and industry, this study identified trends in the prescription of medical enzymes in South Africa. The primary aim of the study was to investigate the prescription patterns and cost of products classified as enzymes in a South African private health-care insurance claims database over a 2-year period.

Methodology

A retrospective, cross-sectional drug utilisation study was conducted on the database of a private medical insurance scheme administrator in South Africa. According to the South African Board of Healthcare Funders that represents 72 health insurers in South Africa, only 16% of the South African population is covered by medical insurance.[13] This figure equates to 3.5 million insured members and their 4.6 million dependants. The remainder of the South African population, that is 39.9 million people, is dependent on the government's medical services.

Data covered 2010 and 2011 and included medication, procedures and devices (a total of 2 126 264 records for 2010 at an amount claimed of ZAR173 812 440.86, and 2 298 312 records for 2011 at an amount claimed of ZAR169 127 258.13). Each medication record contained information on the age and gender of the patient, with a unique number to identify each patient, the date of the prescription, detailed information on the dispensed drug (name, package size, formulation, strength and quantity), price and various reimbursement variables.

MIMS[5] was used to identify and classify the medicines. All records for 'Enzymes' (MIMS Category 27.0.0 Enzymes (8.3; 12.1)) were extracted, as well as records in Categories 8.3 (Fibrinolytics) and 12.1 (Digestants).[5] Microsoft Access® and Excel® were used to analyse the data. Basic descriptive statistics were calculated. The cost indicated is the amount that was paid by the respective medical aid insurance schemes and may differ from the single exit price (SEP)[14] that is used in South Africa, as not all medical aid insurance schemes cover the full costs of these products and co-payments may have to be made by patients. At the time of the study (at the juncture between 2010 and 2011), EUR1.00 was equal to ZAR9.38, USD1.00 was equal to ZAR7.64 and GBP1.00 was equal to ZAR11.48.

Limitations of the study were that no clinical information or diagnoses were available in the database, and that only data of patients served by the private health-care sector in South Africa were included. Also, only products containing enzymes that were prescribed during 2010 and 2011 are discussed in the results section, although more trade name products have since become available on the South African market. Permission to conduct this study was obtained from the Research Ethics Committee (Human) of the Nelson Mandela Metropolitan University (ethics clearance number: H08-HEA-PHA-005).

Results and discussion

Enzyme-containing products constituted only 0.02% of the approximately 4.5 million claims for products and procedures paid for by the medical insurance schemes interrogated in this study, and 0.57% of cost. Of the enzyme-containing products available for prescription in South Africa,

only five were prescribed and submitted for reimbursement by the medical insurance company whose database was interrogated.

In the 2 years under study, a total of 906 products (525 in 2010 and 381 in 2011) were prescribed at a cost of ZAR1 956 948.76 with an average cost of ZAR2 159.99 (SD=R9 929.54) to 579 patients. The large standard deviation is because of the wide variation in cost between the different trade name products, with some products relatively inexpensive (the lowest average cost per product was ZAR82.34) and other products extremely expensive (the highest average cost per product was ZAR62 470.48). Patients were prescribed on average 1.56 (SD=1.65) products over the 2 years. The 579 patients identified included 292 female and 287 male patients. The average age of patients was 55.17 (SD=16.00) years (female: 55.53 (SD=17.71) years; male: 54.81 (SD=15.27) years). Nearly two-thirds of patients (64.59%) were between 40 and 69 years of age.

The different enzyme-containing product classes that were dispensed and paid for are given in Figure 1. Most products were prescribed in MIMS Category 27.0.0, accounting for 62.69% of the number of prescriptions for enzymes and 80.99% of the total amount claimed for enzymes over the 2 years. About half of the products (52.21%) were dispensed by private hospitals.

Only two enzyme-containing products were prescribed in MIMS Category 27.0.0[5], namely Cerezyme® (imiglucerase) powder for injection (200 units/mL and 400 units/mL) and Hyalase® injection (hyaluronidase 1500 iu/ampoule, 10 ampoules per package). Only 24 prescriptions were dispensed for imiglucerase (12 prescriptions for 200 units/mL in 2010 and 12 prescriptions for 400 units/mL in 2011) (see Table 2).

Hyaluronidase was the most frequently prescribed (60.04% of all enzyme products), followed by pancreatin-containing products (34.66%). A total of 544 prescriptions for hyaluronidase injections were dispensed (273 products in 2010 and 271 products in 2011) to 376 patients at a total cost of ZAR152 611.61 (average cost of ZAR286.85; SD=ZAR54.29). The average age of patients was 59.47 (SD=14.40) years. Nearly all the injections were dispensed in private hospitals or by unattached operating theatres (day clinics). Only two prescriptions for hyaluronidase were dispensed by ophthalmologists. Patients received an average of 1.45 (SD=0.61) prescriptions for hyaluronidase over the 2 years, with patients in the 50–59-year age group receiving the highest average of 1.52 prescriptions. The average cost per product paid by the medical aids was ZAR280.54 (ZAR279.22 in 2010 and ZAR281.86 in 2011). The SEP (unit price) on 12 January 2012 for hyaluronidase was ZAR283.40 (effective from 22 May 2010).[14]

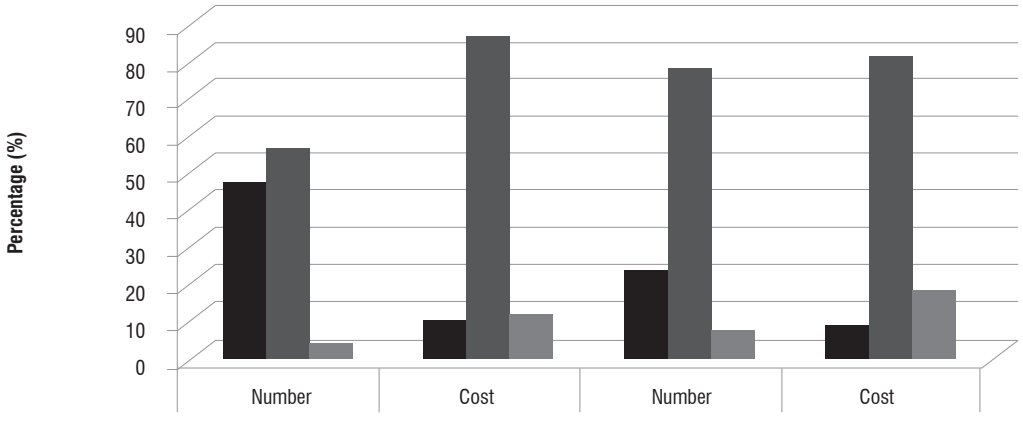
Hyaluronidase is classified in the Anatomical Therapeutic Chemical / Defined Daily Dose (ATC/DDD) Index as an enzyme under 'Blood and Blood Forming Organs (Other Haematological Agents)'.[6,15] It is used off-label for the treatment of vitreous haemorrhage and diabetic retinopathy. It is not possible to speculate on the reason for its use. Recently, it was reported that administering recombinant human hyaluronidase (rHuPH20) with meal-time insulin injections could help improve blood sugar control in people with type-1 diabetes (the combination led to smaller rises in glucose levels than treatment with insulin lispro alone).[16]

Imiglucerase was overall the most expensive (an average cost of ZAR58 103.26 for the 200 units/5 mL vials and ZAR62 470.48 for the 400 units/5 mL vials prescribed, and a total cost of ZAR697 239.17 for the 200 units/5 mL vial and ZAR749 645.73 for the 400 units/5 mL vial). Imiglucerase, used in the treatment of Gaucher's disease, is also listed in MIMS Category 26.0.0 (Biologicals)[5] and in ATC Group A16AB02.[6, 15]

Ten prescriptions were dispensed for alteplase at an average cost of ZAR6572.60 per prescription. Alteplase is used as fibrinolytic therapy in acute myocardial infarction within 6 h of symptom onset, as thrombolytic treatment in patients with acute massive pulmonary embolism and haemodynamic instability, as thrombolytic treatment of acute ischaemic stroke initially within 3 h after the onset of stroke symptoms and after the exclusion of intracranial haemorrhage.[5]

Tenecteplase, of which 14 injections were dispensed, is also used as thrombolytic therapy in acute myocardial infarction as soon as possible after symptom onset but within 6–9 h of symptom onset. The average amount claimed per injection was ZAR11905.72 for 8000 units and ZAR13091.24 for 10 000 units.

The pancreatin-containing products are available from pharmacies and are indicated as supplementation for pancreatic exocrine insufficiency caused by chronic pancreatitis, cystic fibrosis or partial pancreatectomy.[4] The formulation with dimethicone is used for abdominal distention due to cumulative gas and foam, in hepatic and biliary dysfunction and in post-operative flatulence and pre-gastrointestinal radiologic



	2010 Number	2010 Cost	2011 Number	2011 Cost
12.1.0 Digestant	44.76	7.25	20.73	6.20
27.0.0 Enzymes	53.71	83.85	75.07	78.50
8.3.0 Fibrinolytics	1.52	8.90	4.20	15.30

Figure 1: Number of products and amount claimed (in ZAR) of the different classes of enzyme-containing products as a percentage of the total number and the total cost of all enzyme-containing products (n=906).

Table 2: Number of enzyme active ingredients and total amount claimed for each enzyme active ingredient

Active ingredients	Number		Both years		Amount claimed (in ZAR)		Both years		SEP (in ZAR) (unit price)†
	2010	2011	Number	%	2010	2011	Amount	%	
12.1.10 Digestants									
Pancreatin 170 mg and dimethicone 80 mg tablets (100 tablets)	34	2	36	3.97	3491.27	77.84	3569.11	0.18	3.01
Pancreatin 170 mg and dimethicone 80 mg tablets (25 tablets)	163	10	173	19.09	12990.68	1253.39	14244.07	0.73	3.01
Pancreatin capsules - enzyme activity per capsule amylase 8000, lipase 10 000, protease 600 Ph Eur units (100 capsules)	30	47	77	8.50	8 939.75	16 886.69	25 826.44	1.32	5.69
Pancreatin capsules - enzyme activity per capsule amylase 18 000, lipase 25 000, protease 1000 PIP units (100 capsules)	8	20	28	3.09	29 689.96	38 675.92	68 365.88	3.49	13.68
27.0.0 Enzymes									
Hyaluronidase 1500 iu/ampoule injection (10 injections)	270	274	544	60.04	74 156.62	78 454.99	152 611.61	7.80	283.40
Imiglucerase powder for injection vial (200 units/5 mL)	12	0	12	1.32	697 239.17	0	697 239.17	35.63	1 534.90
Imiglucerase powder for injection vial (400 units/5 mL)	0	12	12	1.32	0	749 645.73	749 645.73	38.31	1 534.90
8.3.0 Fibrinolytics									
Alteplase in 2333 mg dry solvent (50 mg vial + solvent kit)	3	7	10	1.10	17 318.85	48 407.10	65 725.95	3.36	6 050.89
Tenecteplase vial (8000 units) injection	1	2	3	0.33	11 346.37	24 370.78	35 717.15	1.83	12 185.39
Tenecteplase vial (10 000 units) injection	4	7	11	1.21	51 926.23	92 077.42	144 003.65	7.36	13 579.34

†Single exit price (SEP)[14] as on 12 January 2012.

examination preparation.[5] These products were mostly dispensed by pharmacies and in private hospitals and were relatively inexpensive.

In the database being interrogated, no prescriptions were encountered for streptokinase, which is indicated for severe myocardial infarction and is regarded as the most affordable antithrombotic enzyme.[6] In the SEP file of January 2012,[14] streptokinase was indicated as 'not approved' and it was not prescribed during the period under study.

Conclusion and recommendations

No studies could be found in the literature on the prescription patterns of enzyme-containing products in South Africa. Therefore, the aim of this study was to investigate these patterns as well as the cost of enzyme-containing products in South Africa using a private medical insurance scheme database. A limitation of this study was the absence of diagnoses in the database, which did not allow for the determination of the reason for the use of the various enzyme-containing products.

Considering the increased emphasis on therapeutic enzymes and the growing global market for enzymes, it is noteworthy that medical enzymes only constituted 0.02% of reimbursements from the medical claims database. Whilst it may be difficult to speculate on the underlying reasons, both cost and familiarity with enzymes may play a role in the prescription patterns found. Medicinal products containing enzymes are relatively expensive and warrant further studies into their cost-effectiveness. Only one trade name product was prescribed for each enzyme-containing product in this study (although some trade names had more than one dosage strength or pack size). It will be interesting to monitor how prescription patterns and cost will be affected when more trade name products are introduced. In the absence of other drug utilisation studies with which to compare the results, this study can be regarded as a baseline study and further studies are recommended.

Acknowledgements

We thank the medical insurance scheme administrator for providing the data for the study and Dr Michael Graz of Biophys Ltd (United Kingdom) for conceptualising the study and for his assistance with the article.

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Advances towards the development of a cloud-resolving model in South Africa

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DATES:
Received: 03 May 2013
Revised: 19 Sep. 2013
Accepted: 27 Feb. 2014

KEYWORDS:
atmospheric modelling; thunderstorms; TOGA COARE; microphysics schemes

HOW TO CITE:
Bopape MM, Engelbrecht FA, Randall DA, Landman WA. Advances towards the development of a cloud-resolving model in South Africa. S Afr J Sci. 2014;110(9/10), Art. #2013-0133, 12 pages. http://dx.doi.org/10.1590/sajs.2014/20130133

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Recent advances in supercomputing have made feasible the numerical integration of high-resolution cloud-resolving models (CRMs). CRMs are being used increasingly for high-resolution operational numerical weather prediction and for research purposes. We report on the development of a new CRM in South Africa. Two bulk microphysics parameterisation schemes were introduced to a dynamical core of a two-dimensional Non-hydrostatic σ-coordinate Model (NSM) developed in South Africa. The resulting CRM was used to simulate two 12-day periods and an 8-day period observed during the Tropical Oceans Global Atmosphere Coupled Ocean-Atmosphere Response Experiment. The response of the NSM to the large-scale forcing which occurred over the three periods, and which included both suppressed and active convection, was examined. The NSM is shown to be able to capture the differences in the three experiments and responds correctly to the large-scale forcing (i.e. it is able to distinguish between suppressed and active regimes). However, the model simulations are cooler and drier than the observations. We demonstrate progress made in the development of a CRM in South Africa, which can be used to study the attributes of convective rainfall over the region.

Introduction

Non-hydrostatic atmospheric models have been used primarily for research purposes since the 1960s, as their application to operational weather forecasting and climate simulation was hindered by computational restrictions. Powerful computers with faster processing capabilities and enhanced memory are now making it possible for atmospheric models to be used operationally at resolutions at which the hydrostatic assumption is not applicable. These technological developments have led to an international trend to develop non-hydrostatic models. In South Africa, non-hydrostatic model development is taking place at the Council for Scientific and Industrial Research (CSIR) in collaboration with the University of Pretoria (UP)[1-3] and at the University of Cape Town (UCT)[4]. Further model development, made possible by the availability of the Centre for High Performance Computing resources in South Africa, is on coupling atmospheric and oceanic components developed elsewhere on hydrostatic scales.[5,6] Randall[7] noted in his essay on a university's perspective on global climate modelling, that it is important that model development is undertaken by many research centres to accelerate the generation of new ideas.

Model development activities in South Africa were abandoned in the mid-1990s when policy changes at the institutions at which the development took place favoured the use of advanced models developed elsewhere. The South African Weather Service (SAWS) currently uses the United Kingdom's Meteorological Office Unified Model for short-range forecasting. The CSIR currently uses the Conformal Cubic Atmospheric Model developed in Australia. The US National Center for Atmospheric Research (NCAR) Weather Research and Forecasting (WRF) model is used at UCT, SAWS, UP and in the private sector. Engelbrecht et al.[1,2] developed a dynamical core of the Non-hydrostatic σ-coordinate[8] Model (NSM). The development of the NSM, which started in 2002 through funding from the Water Research Commission, revived numerical weather model development activities in South Africa.[9] In this paper, we report on the further development of the NSM, through the introduction of moisture and microphysics schemes to the original adiabatic kernel to make the explicit simulation of clouds possible.

Non-hydrostatic models that include the necessary physics to simulate ensembles of clouds explicitly over a large enough domain are called cloud-resolving models (CRMs) or cumulus ensemble models.[10] To be able to simulate clouds explicitly, CRMs employ microphysics parameterisations which are grouped into bin and bulk approaches. The majority of CRMs use bulk microphysics parameterisations (BMPs) to simulate clouds explicitly,[11-13] because BMPs are computationally economic compared to bin approaches. BMP schemes specify a functional form for the particle distribution and usually predict the mixing ratios of as few as possible water substance classes. In a BMP scheme, the various cloud microphysical processes responsible for transferring the water substance from one species to another are parameterised. CRMs can also employ multi-moment BMP schemes by predicting more than one moment of the particle size distribution.[14,15] A double moment scheme predicts both the particle mixing ratio and the concentration, while a single-moment scheme predicts only the particle mixing ratio. The benefit of multi-moment schemes is that they should be applicable across a wider range of environments. Multi-moment schemes are starting to be applied in numerical models[13,14]; however, their increased computational cost as a result of the prediction of a multiple-moment discourages their use in real-time numerical weather prediction.[16]

CRMs can be used to study the response of thunderstorms to large-scale circulations. To do so, CRMs are driven with large-scale observations similar to the procedure that is followed when testing cumulus parameterisation schemes with a single column model.[10,17,18] This method constrains the domain-averaged horizontal velocities to follow the observed values and thereby provides a means for controlling the cloud system dynamics by the large-scale momentum and shear. Synoptic and mesoscale motions play a major role in the formation, maintenance and structure of thunderstorms. The tropical–temperate trough, which is associated with the northwest–southeast aligned cloud bands, is a major synoptic rainfall-producing weather system over southern Africa.[19-21] In this paper, we introduce two microphysics schemes with the purpose of investigating the adequacy of these schemes for simulating the convective response to large-scale forcing, as well as the sensitive dependence

of the simulated convective dynamics and precipitation to different microphysics schemes.

The use of observations from the experimental campaigns has contributed to the improvement of models and has also shown that CRMs are useful tools to study cloud systems.[18,21,22] In this study, forcing data from the Tropical Oceans Global Atmosphere Coupled Ocean-Atmosphere Response Experiment (TOGA COARE) were used.[23-25] The case study presented here was investigated by the Precipitating Cloud Systems Working Group of the Global Energy and Water Cycle Experiment Cloud System Study.[24,26,27] The objective of this case study was to examine the role of the convective process in moistening the atmosphere during the suppressed phase of the Madden–Julian oscillation and to assess the impact of moistening on the subsequent evolution of convection in numerical simulations.[23,24,27] In this study, the NSM with the newly added microphysics schemes will be used to simulate the suppressed and active periods of TOGA COARE in order to test the NSM's ability to respond to the large-scale forcing. We first describe the basic equations of the model and the two microphysics schemes.

Model, data and methods

The basic equations of the Non-hydrostatic σ-coordinate Model

Engelbrecht et al.[1,2] derived the NSM equation set as the σ-coordinate equivalent to the pressure coordinate equations of White[28]. These equations have been modified to reflect the introduction of moisture and microphysics processes. σ is defined based on the full pressure field (p) as

$$\sigma = \frac{p - p_T}{p_{surf} - p_T} = \frac{p - p_T}{p_S} \qquad \text{Equation 1}$$

where p_T is the prescribed pressure at the model top and p_{surf} is the full surface pressure and $p_s = p_{surf} - p_T$.

$$\frac{Du}{Dt} + \frac{\partial \varphi}{\partial X} - \sigma \frac{\partial \varphi}{\partial \sigma} \frac{\partial \ln p_s}{\partial X} = 0 \qquad \text{Equation 2}$$

$$\frac{R}{g} \frac{D}{Dt}\left[\frac{\omega T}{p}\right] + g + \frac{p}{p_s} \frac{g}{RT} \frac{\partial \varphi}{\partial \sigma} = 0 \qquad \text{Equation 3}$$

$$\frac{\partial u}{\partial x} + \frac{\partial \dot{\sigma}}{\partial \sigma} + \frac{D \ln p_s}{Dt} = 0 \qquad \text{Equation 4}$$

$$\frac{DT}{Dt} - \frac{RT\omega}{c_p p} = S_h \qquad \text{Equation 5}$$

$$\frac{Dq_x}{Dt} = S_x,\ x = 1,\ldots,n \qquad \text{Equation 6}$$

$$\frac{Dq_x}{Dt} = S_x + \frac{\partial}{\partial \sigma}(\rho V_x q_x) \frac{\partial \sigma}{\partial z},\ x = n+1,\ldots,n+k \qquad \text{Equation 7}$$

In Equations 2 to 7, the total derivative is given by

$$\frac{D}{Dt} = \frac{\partial}{\partial t} + u \frac{\partial}{\partial x} + \dot{\sigma} \frac{\partial}{\partial \sigma} \qquad \text{Equation 8}$$

where x represents the horizontal coordinate and t is time. All differentiations with respect to time and the horizontal coordinate are carried out at constant σ. The horizontal component of the wind is u; ø is the geopotential height, gz, z being geometric height; T is temperature; $\sigma = D\sigma/Dt$ and $\omega = Dp/Dt$. R is the gas constant for dry air and $\kappa = R/c_p$, with c_p the specific heat of dry air at constant pressure. The development in this study was performed on a two-dimensional model.

The horizontal (Equation 2) and vertical (Equation 3) momentum equations take on the same form as for dry air. The continuity and thermodynamic equations are given by Equations 4 and 5, respectively. Microphysics processes change a particular water class, and also change the temperature through the release and absorption of latent heating. The change in temperature as a result of the microphysics processes is represented by the term on the right hand side of Equation 5. When moisture is introduced, water continuity equations (Equations 6 and 7) have to be solved for all the non-falling and falling water classes in the model, respectively. q_x represents the different mixing ratios with x being the place holder for different species of the water substance. S_x represents the microphysics processes that act as sources and sinks for q_x. The BMP schemes introduced to the NSM use five or six water classes, as discussed in the next subsection.

A variable Ω is defined based on the relation between the fields σ and ω as:

$$\Omega = \frac{\omega}{P} = \frac{p_s}{\sigma p_s + p_T}\left(\sigma \frac{D \ln p_s}{Dt} + \dot{\sigma}\right) \qquad \text{Equation 9}$$

Equations 2 to 7 may be combined to obtain an elliptic equation (Equation 10) in the geopotential. An extra term (i.e. last term) appears in the elliptic equation as a result of the microphysics processes (compared with the dry adiabatic form of the equation).

$$\frac{\partial^2 \varphi}{\partial x^2} + \frac{\partial}{\partial \sigma}\left[s^2 \frac{\partial \varphi}{\partial \sigma}\right] - 2\sigma\left[\frac{\partial \ln p_s}{\partial x}\left(\frac{\partial^2 \varphi}{\partial x \partial \sigma}\right)\right] +$$

$$\frac{\partial}{\partial \sigma}\left(\sigma^2 \frac{\partial \varphi}{\partial \sigma}\right)\left(\frac{\partial \ln p_s}{\partial x}\right)^2 - \frac{\sigma}{p_s}\frac{\partial \varphi}{\partial \sigma}\left(\frac{\partial^2 p_s}{\partial x^2}\right) =$$

$$2 \frac{\partial u}{\partial x}\frac{\partial}{\partial \sigma}\left(\frac{\Omega p}{p_s}\right) - \frac{2}{p_s}\frac{\partial u}{\partial \sigma}\frac{\partial}{\partial x}(p\,\Omega) -$$

$$\frac{\partial}{\partial \sigma}\left(sg - \frac{p}{p_s}\Omega^2 \frac{1}{\gamma}\right) - \frac{\partial}{\partial \sigma}\left(\frac{S_h p \Omega}{p_s T}\right) \qquad \text{Equation 10}$$

Here, $s = (\sigma + p_T/p_s)(g/RT) = (p/ps)(g/RT)$, $\gamma = c_p/c_v$ and S_h is heating or cooling from latent heat release or absorption, respectively. Equation 10 is needed during the numerical solution of governing Equations 2 to 7. The split semi-Langragian method is used to solve the quasi-elastic σ-coordinate equations.

The microphysics schemes

Two microphysics schemes that are used to forecast the changes in mixing ratios as a result of microphysics processes and sedimentation were obtained from the NCAR WRF model.[29] The two schemes – the classic and the new – are described below.

The classic scheme

Chen and Sun[30] developed a BMP that is based on Lin et al.[31] and Rutledge and Hobbs[12]. The scheme was developed at Purdue University (Indiana, USA) and is known as the PURDUE-LIN scheme. It includes six classes of the water substance, namely water vapour, cloud water, cloud ice, rain, snow and graupel. Chen and Sun[30] also applied the saturation adjustment of Tao et al[32]. All precipitating fields are assumed to fall at their mass-weighted fall speed. Cloud water and cloud ice are assumed to be monodispersed and non-falling. The scheme can be used either with five water classes (i.e. excluding graupel) or with all six classes. The PURDUE-LIN scheme was chosen because it is considered to be a classic scheme, on which most recent schemes are based. There are studies that suggest that PURDUE-LIN is an outlier with the largest biases compared with other schemes[33]; however, other studies, based on the most complete data sets of tropical convection, have shown the scheme to be comparable in performance with more recently developed schemes.[34,35]

The new scheme

A new BMP scheme was recently developed at Stony Brook University (New York, USA) using the PURDUE-LIN scheme as a starting point.[36] The developers of the scheme called it SBU-YLIN. The SBU-YLIN scheme includes five prognostic equations, for water vapour, cloud water, cloud

ice, rain and precipitating ice. Snow and graupel share the same category and hence the same processes, which makes the scheme cheaper to run. The SBU-YLIN scheme uses a generalised gamma distribution to describe size distribution of cloud water droplets $N_c = N_{0c} D^{\mu} e^{-\lambda D}$ where N_{0c} is the intercept, μ is the shape parameter and λ is the slope. Rain and its related parameterisations are similar to those of the PURDUE-LIN scheme. Cloud ice is assumed to be monodispersed, similar to the PURDUE-LIN scheme; however, ice is allowed to fall. Dry snow, rimed snow and graupel are included in the precipitation ice category through the introduction of varying riming intensity parameters. The SBU-YLIN scheme was chosen because, although it is newer and improved, it is also cheaper to run compared to the classic scheme, which makes it ideal for numerical weather prediction purposes.

Data and methods

The simulations were carried out for three periods: 28 November to 10 December 1992 (A0 experiment), 09 to 21 January 1993 (B0 experiment) and 21 to 29 January 1993 (C0 experiment); all of which were observed using TOGA COARE. TOGA COARE is an observational and modelling program aimed at understanding (1) the basic processes that maintain the warmest waters of the oceans and (2) the role that warm water plays in determining the mean state and variability of climate. The TOGA COARE intensive observing period took place from 01 November 1992 to 28 February 1993 in the near equatorial western Pacific Ocean, part of a region commonly referred to as the warm pool.[25]

The large-scale advective tendencies of potential temperature and water vapour, which were provided six hourly, were made by Ciesielski[37]. The NSM's simulated horizontal average winds (u) were relaxed towards the observed horizontal wind (u_{obs}) with a timescale (τ) of 2 h, applied at every time step (Equations 11 and 12). The temperature and water vapour tendencies were interpolated linearly to the NSM's vertical grid and every advection time step and applied directly to the NSM (Equations 13 and 14).

$$\frac{Du}{Dt} + \frac{\partial \varphi}{\partial x} - \sigma \frac{\partial \varphi}{\partial \sigma} \frac{\partial \ln p_s}{\partial x} - \left(\frac{\partial u}{\partial t}\right)_l = 0 \qquad \text{Equation 11}$$

$$\left(\frac{\partial u}{\partial t}\right)_l = -\frac{< u > - u_{obs}}{\tau} \qquad \text{Equation 12}$$

$$\frac{DT}{Dt} - \frac{R_d T \omega}{c_p p} = S_h + radhr + \left(\frac{\partial T}{\partial t}\right)_l \qquad \text{Equation 13}$$

$$\frac{Dq_v}{Dt} = S_v + \left(\frac{\partial q_v}{\partial t}\right)_l, x = 1, \ldots, n \qquad \text{Equation 14}$$

Similarly to Woolnough[23], the large-scale forcing was applied only up to the 150 hPa level. Active and suppressed periods were defined by the nature of the large-scale forcing applied. The suppressed periods were defined by periods when the large-scale forcing was acting to dry and warm the column. The active periods were defined by periods during which there was substantial cooling and moistening of nearly the entire column by the large-scale forcing. Each of the three experiments is characterised by deep convection in the first 2 days to spin-up the model, followed by suppressed conditions, and then a transition period, ending with deep convection of different lengths as shown in Table 1. The experiment was run initially for Day 1 of the simulation, with updated large-scale tendencies. The simulated average temperature and water vapour mixing ratio in x were then replaced with the initial condition temperature and water vapour mixing ratio at every advection time step of 10 s. In this way, perturbations were generated and used with the initial conditions and the runs were resubmitted and allowed to simulate for a number of days for each experiment. The sea–surface temperatures and surface pressure were prescribed at every time step. Surface fluxes were calculated using aerodynamic equations as described in Holtslag and Boville[38], which allow moisture from the ocean back into the atmosphere. A 2K/day cooling was applied throughout the troposphere. Simulations were made with the PURDUE-LIN and SBU-YLIN schemes. The PURDUE-LIN was run for two cases: with graupel (PURDUE-LIN1) and without graupel (PURDUE-LIN2).

Table 1: The duration of events expected during the different days simulated for experiments A0, B0 and C0

Period	A0 (12 days total)	B0 (12 days total)	C0 (8 days total)
Spin-up/ deep convection	Days 1–2 (2 days)	Days 1–2 (2 days)	Days 1–2 (2 days)
Suppressed	Days 3–5 (3 days)	Days 4–6 (3 days)	Days 3–4 (2 days)
Transition/ recovery	Days 6–10 (5 days)	Day 7 (1 day)	Day 5 (1 day)
Deep convection	Days 11–12 (2 days)	Days 8–11 (4 days)	Days 6–8 (3 days)

The first number/range represents the day of the simulation on which the event occurred, while the number in brackets represents the total number of days for which each event was observed.

Results

The simulations are initially compared with TOGA COARE observations and then the simulations made with the different microphysics schemes are compared with one another. Such comparisons enable us to determine if the NSM simulations are closer to reality, and whether the response to the large scale is dependent on the microphysics schemes. A combination of simulations made with PURDUE-LIN1 and PURDUE-LIN2 is called PURDUE-LIN simulations. The A0 experiment is discussed in detail, whereas the B0 and C0 experiments are discussed only briefly for comparison with the A0 experiment.

Comparison with TOGA COARE observations

Temperature

The simulations were compared with the reanalysed full fields generated by Ciesielski[37] and therefore correspond fully with the initial conditions and forcing fields used to make the simulations. Figure 1a shows observed temperatures over the 12-day period of the A0 experiment, while Figure 1b shows how the temperature changed over the 12-day period with respect to the initial conditions. The troposphere is generally warmer compared to the initial conditions. Almost throughout the 12-day period, there is a cooler region at a level of about 900–700 hPa. The simulated temperature with all the schemes (Figure 1c, 1e and 1g) decreases significantly in the first few hours of the simulation. The heat seems to be transported from the lower parts of the troposphere to the upper parts. This transport is represented by a much warmer upper troposphere and lower stratosphere compared to the initial conditions in Figure 1d, 1f and 1h. All the microphysics schemes simulate a much cooler region between the 700 hPa and 900 hPa levels which is simulated as a layer much thicker than that observed. This result suggests that the mechanism that leads to a cooler region in the observations is simulated by the NSM even though the simulated layer is much thicker.

The cooling in the first few hours of the simulation during the spin-up period is also simulated in the B0 (Figure 2c, 2e and 2g) and C0 experiments (Figure 2d, 2f and 2h). The cooling in the simulations is much stronger than in the observations (Figure 2a and 2b). This result suggests that the model reaches a steady state with lower temperatures in the lower troposphere and higher temperatures in the upper troposphere and lower stratosphere. Increases in temperature do occur at certain times beyond the first day of simulation; however, the temperature values are not able to recover to levels comparable with the observations and initial conditions. The NSM is able to capture the differences in the experiments. The observations show that the atmosphere is generally cooler in the C0 experiment compared to the initial conditions during the 8-day period (Figure 2b). The NSM is able to capture that the C0 experiment (Figure 2 column 1) is much cooler compared to its initial state, while the A0 and B0 experiments are less cooler compared to their initial states (Figures 1 and 2 column 2). The NSM simulates much cooler conditions during Days 6 to 8 under all the microphysics schemes in the B0 experiment, although this situation is not observed.

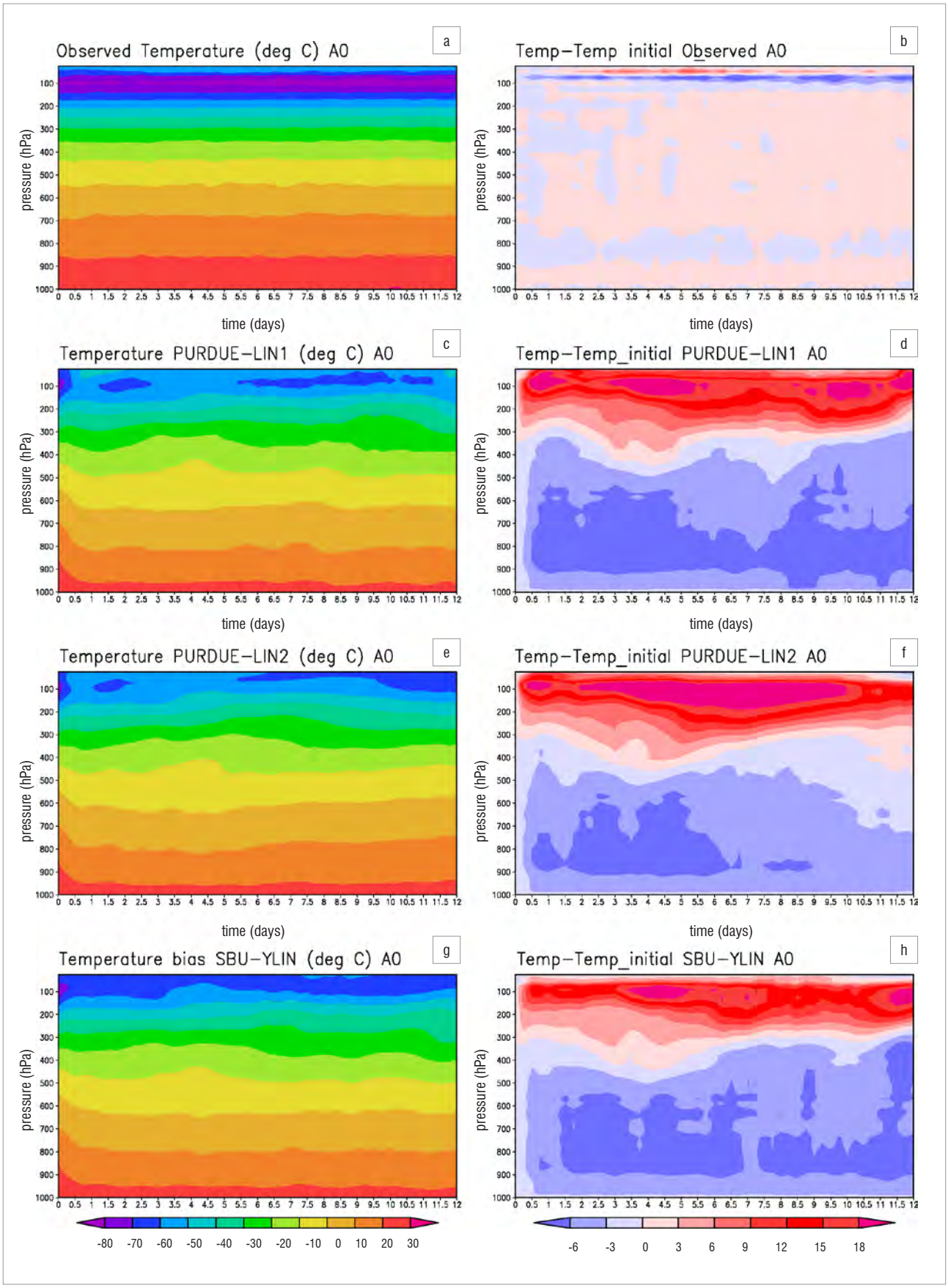

Figure 1: The temporal evolution of (a) observed temperature and (b) observed temperature relative to the initial conditions over a 12-day period with height expressed in pressure coordinates. (c,e,g) The temporal evolution of the simulated temperature by the different microphysics schemes and (d,f,h) temperature relative to the initial conditions for the A0 experiment.

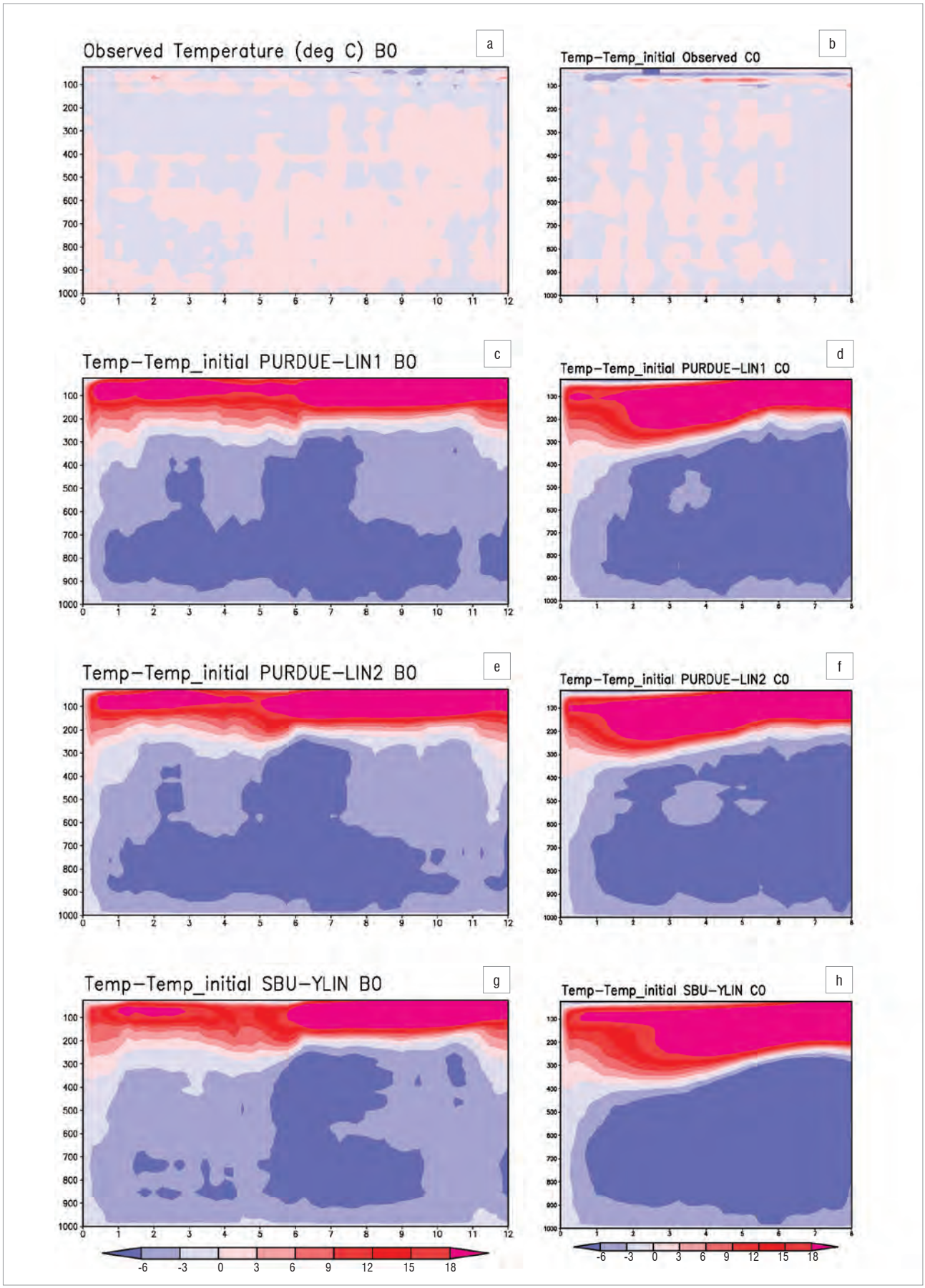

Figure 2: The temporal evolution of temperature relative to the initial conditions for (a) B0 and (b) C0 experiments. The simulated temporal temperature evolution with different microphysics schemes (c,e,g) for the B0 experiment and (d,f,h) for the C0 experiment.

Specific humidity

The deep convection periods in the A0 experiment are observed to be characterised by moister conditions in the upper troposphere compared to other days of the simulation period (Figure 3b). This finding is in agreement with those of Lucas and Zipser[39] who studied the TOGA COARE observations and found that during rainy periods, the mid-troposphere was rather moist with relative humidity in the order of 70%, while during periods without widespread precipitation, the opposite was seen – with a relative humidity of ~40% in the mid-troposphere. The specific humidity is higher everywhere in the lower troposphere up to the 800 hPa level throughout the 12-day period compared to the initial conditions. The moister region corresponds to the cooler region seen in the temperature figures. This result suggests that hydrometeors generally evaporate in this layer, subsequently increasing the amount of water vapour in the atmosphere while cooling the atmosphere as a result of latent heat absorption.

The A0 experiment simulated atmosphere becomes much drier than the initial conditions and observations in the first few hours of the simulation, and then it recovers at some point, but not to the magnitudes found in the initial conditions or observations (Figure 3d, 3f and 3h). This feature is also found in the B0 and C0 experiments (not shown). The cooler and drier troposphere correspond because cooler air carries less water vapour than warmer air. Although much drier than the observations, there is a layer close to the 900 hPa level that is less dry in comparison to layers below and above in the A0 experiment. The last 2 days of the simulation are less dry compared to the rest of the 12 days, which suggests that the NSM responds well to the large-scale forcing (Figure 3d, 3f, 3h and 3i). The simulated specific humidity values in the C0 experiment are smaller compared to the other two experiments, and are similar to differences seen in observations, consistent with temperature differences.

Horizontal winds

The horizontal winds were observed to be westerly in the most part of the troposphere and lower stratosphere during the first deep convection period in the A0 experiment (Figure 4a). The simulated winds compare well with the observed winds in the troposphere (Figure 4c, 4e and 4g) suggesting that the relaxation of the simulated winds to the observations was applied successfully. The differences are bigger at the top of the domain where the relaxation was not applied.

Updrafts and hydrometeor simulations

Deep convection or spin-up period

The large-scale forcing applied to the model in the first 2 days of simulation in all three experiments was done in order to allow deep convection to spin-up the model. In the A0 experiment, convection forms before the end of the first 2 h of simulation, using all three microphysics schemes (Figure 4d, 4f and 4h). As soon as the hydrometeors start forming, the simulated maximum updrafts start to appear different. In all three simulations with different microphysics schemes, the updrafts are stronger on the first day of simulation than on the second day of simulation; on the second day, the updrafts are stronger in the PURDIE-LIN1 simulation and weaker in the SBU-YLIN simulations.

The simulations suggest that the PURDUE-LIN scheme simulates stronger cold pools that are able to trigger stronger storms compared to the SBU-YLIN scheme. Simulated temperature differences (not shown) show that in the first 2 days of the simulation, the PURDUE-LIN2 and SBU-YLIN schemes are generally warmer closer to the surface than the PURDUE-LIN1 simulation. These temperature differences confirm the presence of a stronger cold pool. The SBU-YLIN scheme is the warmest along the surface. The higher temperatures in the SBU-YLIN scheme compared to the PURDUE-LIN schemes extend into the middle and higher troposphere from about 12 h until 24 h in the PURDUE-LIN2 simulation and 26 h in the PURDUE-LIN1 simulation. The higher temperatures suggest the presence of more ice in the SBU-YLIN scheme simulation, which is associated with more latent heat release in its formation. The values of the total mixing ratio of ice (Figure 5) confirm that more ice is simulated by the SBU-YLIN scheme.

The PURDUE-LIN1 scheme acts quicker to remove cloud water and ice from the atmosphere because of the presence of graupel. This is confirmed by the amount of simulated ice by this scheme which is the least of all the schemes (Figure 5b). The SBU-YLIN hydrometeors fall slower and hence produce weaker downdrafts and cold pools. PURDUE-LIN1 simulates the least amount of liquid water, while PURDUE-LIN2 simulates the highest amount of liquid water. This difference is because graupel and rain water in the PURDUE-LIN1 simulation remove cloud water from the atmosphere faster than would be possible in the PURDUE-LIN2 and SBU-YLIN schemes. The simulated liquid water is generally deeper in the SBU-YLIN scheme simulations. The NSM is also found to adequately respond to the large-scale forcing during the spin-up period in both the B0 and C0 experiments (Figure 6).

The suppressed period

The simulated maximum updrafts in the suppressed period are much smaller compared to those in the spin-up period in both the A0 and C0 experiments when using all three microphysics schemes (Figure 4d, 4f, 4h and Figure 6d, 6f, 6h). Some ice particles are simulated, but they are much smaller compared to the active and transition periods (e.g. Figure 5). Woolnough et al.[23] found the suppressed period to be dominated by shallow convection with some updrafts penetrating above the melting level. The updraft during the suppressed period in the B0 experiment (Figure 6c, 6e and 6g) is stronger than those in the A0 and C0 experiments, with more ice and liquid water simulated in the B0 experiment. The suppressed period in the B0 experiment forms part of the period for which the large-scale forcing was suspected to have errors.[23] Temperatures in the SBU-YLIN scheme are generally higher than the PURDUE-LIN temperatures in the troposphere, suggesting that the cooler and drier biases that are found in the simulations are stronger in the PURDUE-LIN simulations.

Transition period

The simulated maximum updrafts are stronger in the transition period compared to the suppressed period in both the PURDUE-LIN1 and SBU-YLIN simulations and in the A0 and C0 experiments. No recovery is simulated in the PURDUE-LIN2 simulations in the A0 experiment, which illustrates the need for graupel in the simulations. The temperature differences show that the PURDUE-LIN2 is generally much warmer compared to the PURDUE-LIN1 and SBU-YLIN during the transition period. Maximum updrafts in the B0 experiment during the transition period are smaller than during the suppressed period. Woolnough et al.[23] found a steady increase in precipitation in the simulations and observations during the transition between the suppressed period and the active period. There is a general recovery in the level of simulated specific humidity in all the simulations during the transition period.

Active period

The maximum updrafts are generally stronger in this period compared to the suppressed and transition periods in all three experiments. In the A0 experiment, SBU-YLIN simulates the strongest updrafts during the first day, and much weaker updrafts in the second day of simulation. PURDUE-LIN1 simulates the highest values during the second day of the active period. PURDUE-LIN2 simulates the smallest values of maximum updrafts and it is also found to be warmer than simulations with two other microphysics schemes. All three schemes simulate the atmosphere reasonably, and can all potentially be used for operational forecasting.

Summary, conclusions and recommendations

A CRM is under development in South Africa. Two bulk microphysics parameterisation schemes were added to a dynamical core of the NSM. The two schemes were obtained from the NCAR WRF model and are called the PURDUE-LIN and SBU-YLIN schemes. The CRM presented here was used to simulate cloud evolution over three periods, which all started with deep convection to spin-up the model, followed by a period during which convection is suppressed, a transition period and a second period of deep convection. Two of the periods were 12 days (A0 and B0), while the third was 8 days (C0). The deep convection,

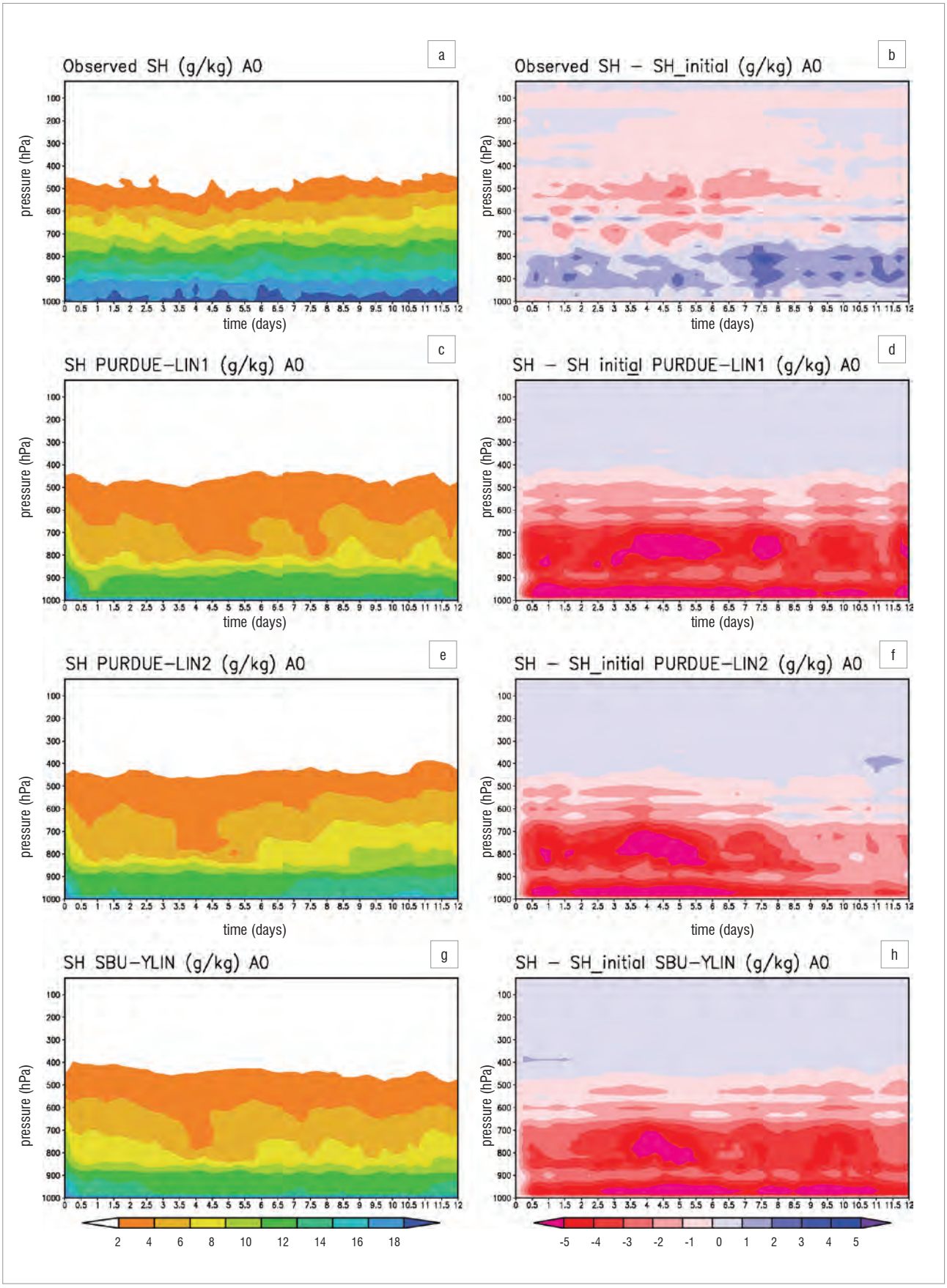

Figure 3: The temporal evolution of the (a) observed specific humidity and (b) observed specific humidity relative to the initial conditions. (c,e,g) The temporal evolution of the simulated specific humidity by the different microphysics schemes and (d,f,h) specific humidity relative to the initial conditions for the A0 experiment.

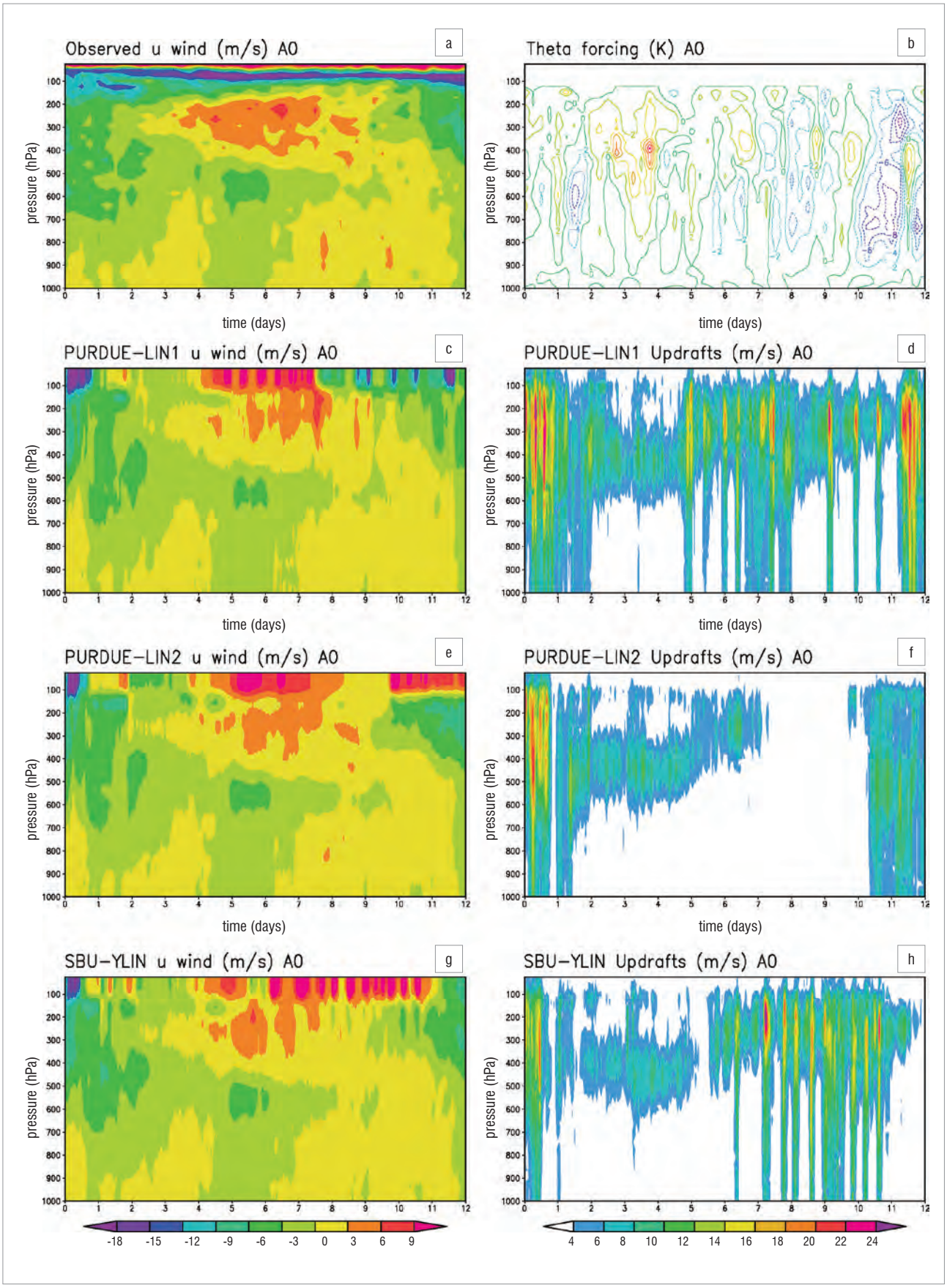

Figure 4: The temporal evolution of the (a) observed and (c,e,g) simulated average horizontal wind by the different schemes. (b) The large-scale warming or cooling applied to the Non-hydrostatic σ-coordinate Model over a 12-day period. (d,f,h) The temporal evolution of the simulated maximum updrafts across the domain by the different schemes over a 12-day period for the A0 experiment.

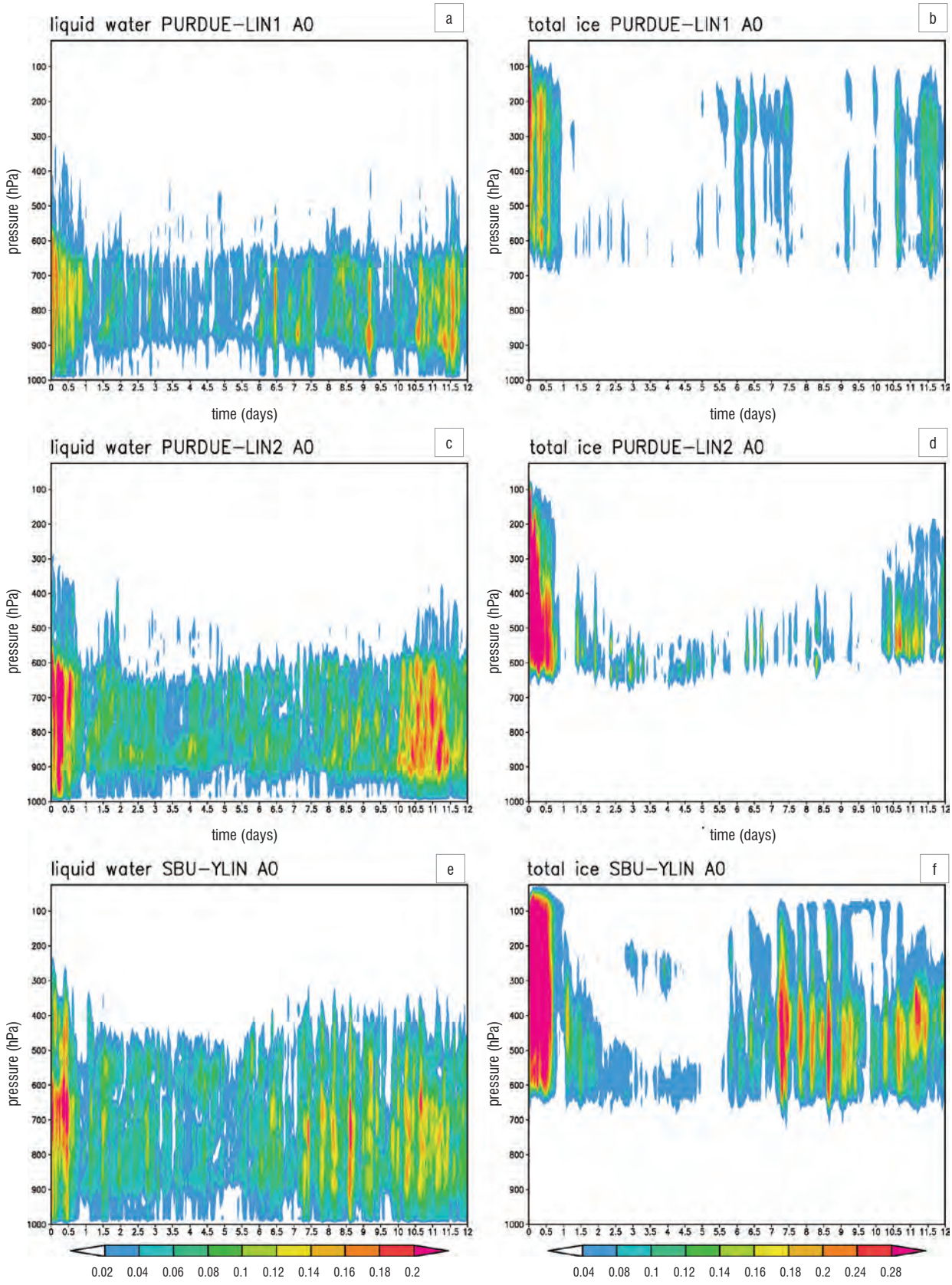

Figure 5: The temporal evolution of the simulated (a,c,e) liquid and (b,d,f) ice mixing ratios by the different microphysics schemes for the A0 experiment.

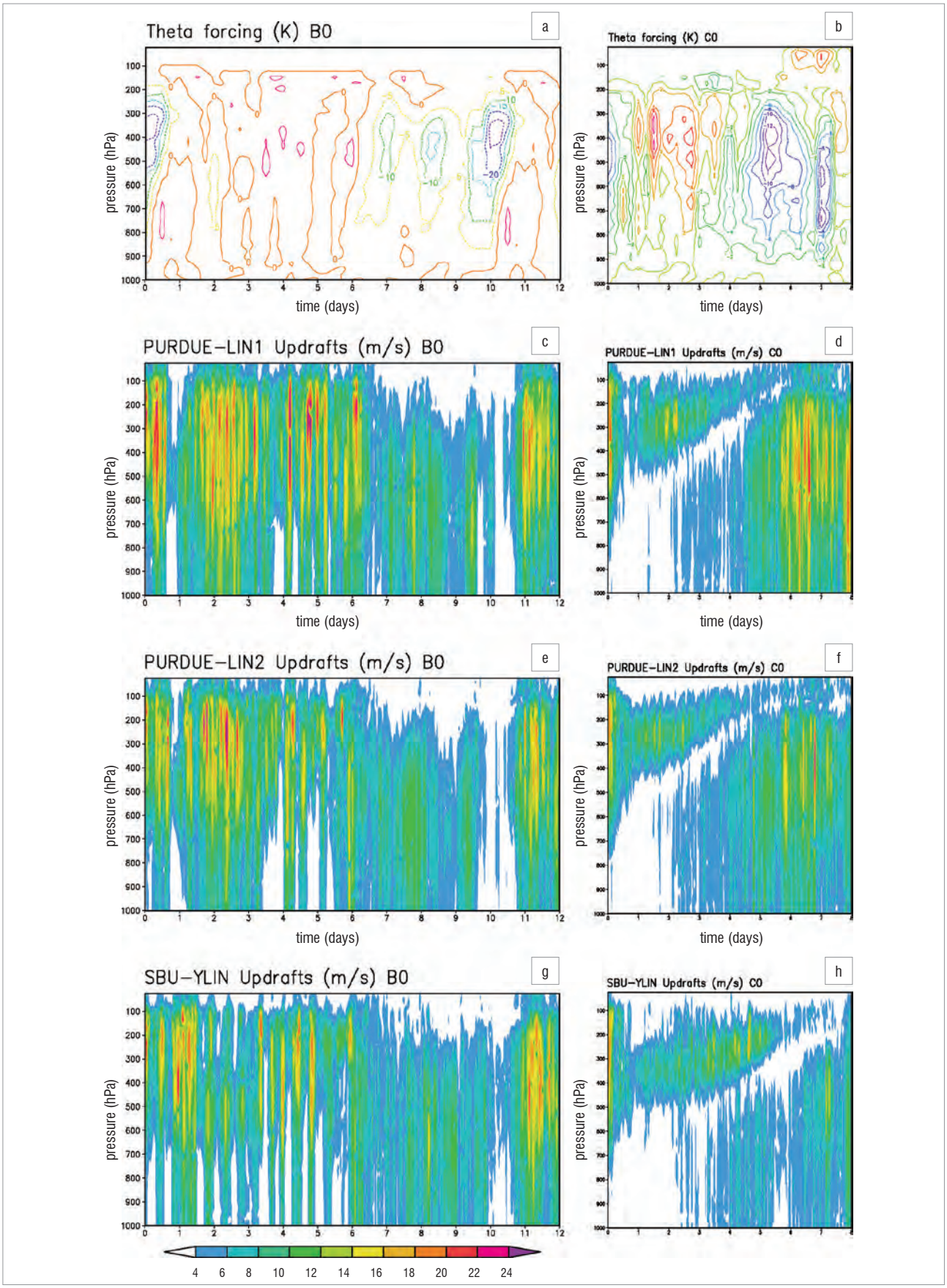

Figure 6: The temporal evolution of the large-scale warming and cooling in the (a) B0 and (b) C0 experiments. The temporal evolution of the simulated maximum updrafts across the domain by the different schemes (a,c,e) over a 12-day period for the B0 experiment and (b,d,f) 8-day period for the C0 experiment.

transition and suppressed periods within the longer periods were all of different durations. The PURDUE-LIN scheme was run both with graupel (PURDUE-LIN1) and without graupel (PURDUE-LIN2).

The NSM with newly added microphysics is shown to be able to capture general differences in the experiments. The simulations were found to be colder and drier compared to observations, for all the experiments performed and for all options of microphysics schemes used. The PURDUE-LIN simulations display a larger cold bias than the SBU-YLIN simulations. Previous studies have shown that PURDUE-LIN1 tends to overpredict precipitation because it unrealistically converts snow to graupel which falls out quicker.[40,41] Our results also show that PURDUE-LIN simulates more rain than does SBU-YLIN. Moreover, in the lower levels, the melting of graupel and stronger downdrafts induced by graupel are likely contributing to the larger cold bias in PURDUE-LIN1 simulations.

Of the three observed periods, A0 was generally the warmest throughout the troposphere but cooler in a layer between 900 hPa and 600 hPa. The model captured the existence of this relatively cool layer, but overestimated its depth. The C0 experiment represented the observed atmosphere that evolved into the coolest state (relative to the observations). The NSM realistically simulated this evolution of the large-scale state. The different amounts of hydrometeors influence the temperature as a result of latent heating or absorption, which then determines how the atmosphere responds when the large-scale forcing is applied. In general, the NSM captured the differences in the suppressed, transition and active regimes, with some simulations being more realistic than others. In general, all three microphysics schemes were comparable, with the scheme without graupel being least realistic.

Further development of the NSM is continuing, with the implementation of a sophisticated radiation scheme almost completed. Preliminary results show a slightly warmer lower troposphere, which is an improvement over the simulations without a radiation scheme. The results presented here illustrate progress towards the development of a CRM that can be used to study the attributes of cumulus cloud and convective rainfall over the southern African region.

Acknowledgements

The CSIR is acknowledged for funding this work through a parliamentary grant and Young Researcher Establishment Funds. The Centre for High Performance Computing (CHPC) as well as the Applied Centre for Climate and Earth System Science (ACCESS) are also acknowledged for their support. Two anonymous reviewers are acknowledged for their valuable comments.

Authors' contributions

M.M.B. undertook the development of the model and experiments; F.A.E. supervised and helped throughout the study; D.A.R. advised and helped throughout the study; and W.A.L. supported the study and helped with the writing of the manuscript.

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Trace element composition of two wild vegetables in response to soil-applied micronutrients

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DATES:
Received: 31 Oct. 2013
Revised: 17 Jan. 2014
Accepted: 02 Mar. 2014

KEYWORDS:
wild vegetables; soil; fertiliser; micronutrient concentration; fortification

HOW TO CITE:
Mavengahama S, De Clercq WP, McLachlan M. Trace element composition of two wild vegetables in response to soil-applied micronutrients. S Afr J Sci. 2014;110(9/10), Art. #2013-0339, 5 pages. http://dx.doi.org/10.1590/sajs.2014/20130339

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Wild vegetables are an important commodity in the subsistence farming sector. They are considered to be rich in micronutrients and can therefore be used to overcome inadequate nutrition. However, research on micronutrients in wild vegetables remains limited and sporadic. In this study, we evaluated the responses of two wild vegetables – *Corchorus olitorius* and *Amaranthus cruentus* var. Arusha – to micronutrients added to the soil in comparison with a reference crop, Swiss chard (*Beta vulgaris* var. *cicla*). Swiss chard concentrated significantly ($p<0.01$) higher amounts of Cu, Zn and Mn in the leaves than did the wild vegetables. Variations in micronutrients among the vegetables were greater for Zn (72–363 mg/kg) and Mn (97.9–285.9 mg/kg) than for Cu (8.8–14 mg/kg). *C. olitorius* had the least capacity to concentrate Mn and Zn in the leaves. However, *C. olitorius* concentrated significantly more Fe (327 mg/kg) in the leaves than did *A. cruentus* (223 mg/kg) or *B. vulgaris (*295 mg/kg). The mean per cent S concentration in the leaves ranged from 0.26% in *C. olitorius* to 0.34% in *A. cruentus* and *B. vulgaris*. We conclude that the different vegetables had different abilities to concentrate Cu and Zn in the order *B. vulgaris* > *A. cruentus* > *C. olitorius*. These results seem to contradict the belief that wild vegetables have an inherent ability to concentrate mineral micronutrients in their tissues.

Introduction

A review of micronutrients in diets in South Africa and other developing countries by Steyn and Herselman[1] revealed widespread shortages. They concluded that the poor micronutrient status among the poor is caused by insufficient and undiversified diets and compounded by soils of low micronutrient status. There are three main ways in which micronutrients can be added to foods: (1) food fortification or supplementation, (2) genetic bio-fortification and (3) agronomic biofortification.[2] Food fortification is widespread and efficient for those who can afford fortified foods, but is not applicable to most rural populations in Africa, which produce most of their staple crops, vegetables and meat at their farms and therefore do not generally purchase micronutrient fortified foods. Biofortification through conventional breeding shows promise, but research is currently limited to a small range of staple crops.[3] Genetic biofortification is still mostly at the experimental stage, and even when it becomes viable, it might be some time before the genotypes that result are widely available and affordable to subsistence farmers. Agronomic biofortification, which is the increase in plant tissue micronutrient content through soil or foliar application of fertilisers, also involves the purchase of these mineral fertilisers and is only viable in commercial agriculture. Another strategy, which is the long-term goal of our present study, is to use locally available wild, semi-domesticated and domesticated vegetable species that have the ability to concentrate the mineral micronutrients Zn, Cu and Fe, even from depleted soils. The possibility of exploiting the variability in shoot mineral content among different plant species in combating micronutrient under-nutrition has been discussed in detail by Broadley et al.[4,5]

Our targeting of wild vegetables is based on the knowledge that they are widely available and relatively easily accessible to poor subsistence farmers. These wild vegetables (also known in South Africa as African leafy vegetables, indigenous vegetables, *morogo* and *imfino*) are variously reported to be superior to conventionally cultivated vegetables such as Swiss chard (*Beta vulgaris* var. *cicla*) and cabbage (*Brassica oleracea* var. *capitata*)[6,7] in micronutrients, including beta-carotene and minerals such as Zn, Fe and Cu. These vegetables are important to subsistence farmers, especially the poor, some of whom rely on wild vegetables as a major form of relish used to complement and accompany staple meals such as *phutu, pap, ugali* and *sadza*.[8]

A recent (2012) report on the importance of African leafy vegetables reaffirmed the potential of these vegetables in the eradication of micronutrient under-nutrition.[7] The report particularly highlighted the prevalence of vitamin A and Fe deficiencies in rural parts of South Africa. Although Zn deficiency was reported as not being well documented in developing countries[9], including South Africa[7], the World Health Organization has identified Zn as one of the most serious deficiencies in the past decade.[10] The consumption of fruits and micronutrient-rich vegetables has been reported to be low, leading to monotonous and micronutrient-poor diets in several provinces of South Africa.[7,11] It is against such a background that promotional and research efforts for wild vegetables need to be taken seriously.

Wild vegetables are widely reported to have superior nutritional properties with respect to micronutrients compared to conventional vegetables such as cabbage (*Brassica oleracea* var. *capitata*) and *Beta vulgaris* var. *cicla*.[12-16] There is wide variability in the nutritional composition reported for these vegetables in different studies. The claim that uncultivated indigenous vegetables could have superior micronutrient levels to cultivated conventional vegetables suggests that there is the possibility of increasing their micronutrient content if micronutrients are added to the soil.[17] For most conventional crops, research has established critical points for deficiency or excess (toxicity).[18] Such information is important in crop production if correct amounts of fertilisers are to be applied. The toxicity risks associated with applying excessive amounts of micronutrients to the soil were extensively reviewed by White et al.[18] The levels of nutrients applied in crop production also determine the ability of a food crop to supply nutrients to humans. Deficiencies of these micronutrients in the soil also lead to reduced crop yields, thereby presenting a double problem: reduced amounts of food of poor nutritional quality.[17] Some studies that have reported the superior nutrient composition of wild vegetables in South Africa[12-16] were not controlled experiments and involved

collection from the wild or purchasing from the market and conducting tests. The aim of the present study was to compare, under well-defined conditions, the accumulation of Zn, Cu and Fe in the leaves of two leafy wild vegetable species commonly consumed in the rural areas of South Africa – wild okra (*Corchorus olitorius)* and pigweed (*Amaranthus cruentus* var. Arusha) – and a reference vegetable crop that is also widely eaten – Swiss chard (*Beta vulgaris* var. *cicla*).

Materials and methods

The experiment was conducted on potted plants in a greenhouse at the University of Zululand (28°51'S; 31°51'E). The growing medium used was soil collected from the university farm. The soil has been classified as Glenrosa soil form.[19] Prior to filling the pots, the soil was sieved through a 13-mm diamond mesh wire mounted on a wooden frame, to homogenise it and to remove large stones, clods, sticks and grass.

Treatments

There were two factors in the study – vegetable species and micronutrient fertiliser. Vegetable species were of three types: *Corchorus olitorius*, *Amaranthus cruentus* var. Arusha and *Beta vulgaris* var. *cicla*. Micronutrient fertiliser had four levels: 0 kg/ha (control), 5 kg/ha, 10 kg/ha and 15 kg/ha each of Cu, Fe and Zn mixed and applied in the form of $CuSO_4.5H_2O$, $FeSO_4.7H_2O$ and $ZnSO_4.7H_2O$ (Table 1). The combination of the two factors resulted in a 3x4 factorial experiment with 12 treatments arranged in a randomised complete block design with three replications. The micronutrient, basal fertiliser and nitrogen top dressing rates were calculated per plant based on a standardised plant density of 100 000 plants per hectare for the three vegetable species. Because basal fertiliser was not a treatment in this study, a general basal fertiliser of 5 g per plant of NPK 2:3:2 (14) was added pre-plant to all the pots resulting in 0.2 g N, 0.3 g P and 0.2 g K applied per plant. Lime ammonium nitrate (LAN, 28% N) top dressing fertiliser was also applied 10 days after transplanting to all pots at a rate of 3 g per plant resulting in 0.84 g of N applied per plant. Each pot contained 2.4 kg of air-dried soil. The micronutrient treatments were added 15 days after transplanting.

Plant management

Seedlings of each of the three test species were germinated and grown for 38 days in the commercial growth medium Hygromix® (Hygrotech Sustainable Solutions, Pretoria, South Africa) in polystyrene trays. Seedlings of uniform size were selected and transplanted into moist soil in which basal fertiliser had been applied pre-plant as described above. One seedling was planted per pot and there were four pots for each treatment. Thereafter, plants were watered in a way that avoided leaching of the nutrients from the soil. There were no plant mortalities such that at harvesting time each treatment had four plants. Samples of the youngest fully expanded leaves (blade and petiole) were taken 26 days after the application of micronutrients for chemical analysis. Samples were harvested from all the four plants per treatment so as to collect enough samples for analysis. Leaf samples were washed in distilled water soon after harvesting. Excess water was allowed to drain off for 4 h before the samples were placed in new brown paper bags and dried in the oven at 60 °C until they had attained uniform mass.

Chemical analyses of youngest fully expanded leaves

Dried plant samples were milled in a Retsch ZM200® mill (Retsch GmbH, Haan, Germany) to pass through a 0.5-mm sieve. They were then submitted for analysis to the Fertiliser Advisory Services of the South African Sugar Research Institute, Mount Edgecombe, KwaZulu-Natal.

Measurement of leaf nutrients

All the analysed elements were determined according to methods described by Wood et al.[20] For Zn, Cu, Fe, Mn and S, a 1-g dried leaf sample was digested in 15 mL nitric acid followed by 5 mL perchloric acid. After digestion, the resultant mixture was filtered and then made up to 50 mL using water. The elements were then determined using atomic absorption spectrophotometry. S was determined colorimetrically. For N, P, K, Ca and Mg, a 0.25-g dried leaf sample was digested in 2 mL selenised sulphuric acid for 1.5 h in a Kjeldatherm block digester at a temperature of 370 °C. After digestion, the samples were diluted by adding water to a volume of 1 L and K, Ca and Mg were determined by atomic absorption spectrophotometry; N and P were determined colorimetrically.

Soil analyses

Information on physico-chemical attributes of the soil (Table 2) was obtained prior to fertiliser application. Soil pH was determined in 0.01 M $CaCl_2$. Exchangeable acidity was determined by titration after extraction with 1 M KCl. The macronutrients P, K, Ca, Mg and Ca and micronutrients Fe, Cu, Zn and Mn were determined using the method described by Van der Merwe et al.[21] after extraction by the Ambic–2 extraction method using EDTA-di-ammonium solution. Truog-extractable P was determined colorimetrically after extraction with 0.02 N sulphuric acid. Si was determined as described by Miles et al.[22] after overnight extraction with 0.01 M $CaCl_2$.

Data analysis

Analysis of variance was performed on the data using the Genstat 12 statistical package to test for significant treatment effects. Statistical significance was evaluated at $p<0.05$. Where the *F*-tests were significant, the treatment means were separated using the least significant difference test.[23,24]

Results and discussion

The effects of the micronutrient mixture on leaf nutrient concentration varied according to plant species and application rate. *B. vulgaris* concentrated Cu, Zn and Mn in the leaves significantly ($p<0.01$) more than did A. *cruentus* and *C*. *olitorius* (Table 3). The variations among the vegetables in these three micronutrients were greater for Zn (72–363 mg/kg) and Mn (98–286 mg/kg) than they were for Cu (9–14 mg/kg). In a previous study, in which Swiss chard was grown in several different types of soil in South Africa,[1] wide variations in micronutrient concentrations (2.72–152.12 mg/kg for Cu and 11.9–623.8 mg/kg for Zn) were reported. In that same study, Swiss chard samples from fruit and vegetable markets and shops had Cu levels of 9.4–111.7 mg/kg and Zn levels of 34.0–816.0 mg/kg.[1] The large discrepancies between our study and that of Steyn and Herselman's[1]

Table 1: Combinations of the sulphates of Cu, Fe and Zn to achieve the desired micronutrient treatments used in the experiment

Amount of each of elemental Cu, Fe and Zn applied (kg/ha)	Amount of the sulphate of each of Cu, Fe and Zn in grams applied per plant to achieve desired rate of element in kg/ha based on a standardised plant population of 100 000 plants/ha		
	$ZnSO_4.7H_2O$	$CuSO_4.5H_2O$	$FeSO_4.7H_2O$
0	0	0	0
5	0.22	0.25	0.20
10	0.44	0.50	0.40
15	0.66	0.75	0.60

in leaf Cu and Zn concentration ranges of Swiss chard are difficult to explain. Similar variations in Zn concentrations in plant tissues used for food were also reported in other studies.[25] In the present study, *C. olitorius* had the least capacity to concentrate Mn and Zn in the leaf, which suggested that this vegetable is a less satisfactory candidate for agronomic biofortification of these micronutrients. However, *C. olitorius* leaves concentrated significantly more Fe (327 mg/kg) than did *A. cruentus* (223 mg/kg) or *B. vulgaris (*295 mg/kg).

Table 2: Soil physico-chemical analysis results of the soil used in the study

Soil attribute	Units	Value
pH (0.01 M $CaCl_2$)	–	4.58
Phosphorus	mg/L	10.1
Potassium	mg/L	169.9
Calcium	mg/L	1432.3
Magnesium	mg/L	525.4
Sodium	mg/L	104.3
Exchangeable acidity	cmol/L	0.19
Total cations	cmol/L	12.55
Acid saturation	%	1.51
Exchangeable sodium	%	3.6
Calcium/magnesium ratio	–	1.65
Zinc	mg/mL	2.4
Copper	mg/mL	2.7
Manganese	mg/mL	6.3
Iron	mg/mL	395
Silicon	mg/mL	28.54
Clay estimate	%	23
Organic matter estimate	%	4.3
Volume weight	g/mL	1.23

Table 3: The main effect of vegetable plant species on leaf concentrations of Cu, Fe, Zn and Mn

	Concentration (mg/kg)			
Vegetable	**Cu**	**Fe**	**Zn**	**Mn**
Corchorus olitorius	9.2^{b}	327^{a}	72^{c}	97.9^{c}
Amaranthus cruentus var. Arusha	8.8^{b}	223^{b}	235^{b}	199.8^{b}
Beta vulgaris var. *cicla*	14.3^{a}	295^{a}	363^{a}	285.8^{a}
Significance	**	*	**	**
Least significant difference	2.4	70	63	28.9

**significant at p<0.05; **significant at p<0.01*

Means followed by different letters in the same column are significantly different at p<0.05 according to the least significant difference test.

Leaf Cu, Zn and S concentrations increased with increasing application rate, whereas Fe concentrations did not show a defined pattern (Table 4). The application of micronutrient fertilisers did not affect the concentration of macronutrients Ca, K, Mg, P and N (not shown), but there were significant ($p<0.05$) differences in the concentrations of Ca, Mg, P and S among the plant species tested (Table 5). The general trend of *B. vulgaris* > *A. cruentus* > *C. olitorius* in terms of leaf micronutrient content was also observed for the macroelements Mg and P (Table 5). However, the trend was reversed for Ca concentration, with *C. olitorius* leaves having three times the Ca content of *B. vulgaris*. However, a regression analysis of this negative relationship showed that it was not significant. The concentration of shoot Ca and Mg and their variations across different plant families have been investigated in comprehensive studies.[4,5] These studies revealed that the order Caryophyllales, to which *Amaranthus* and Swiss chard belong, has a tendency towards high shoot Mg levels, resulting in lower Ca:Mg ratios, as observed in *B. vulgaris* in the present study.

Table 4: The main effect of soil-applied micronutrients on leaf Cu, Fe, Zn and Mn concentrations of *Beta vulgaris* var. *cicla, Corchorus olitorius* and *Amaranthus cruentus* var. Arusha

	Concentration (mg/kg)			
Micronutrient mixture amount† (kg/ha)	**Cu**	**Fe**	**Zn**	**Mn**
0	4.69^{c}	280	60^{c}	0.23^{c}
5	9.27^{b}	274	218^{b}	0.32^{b}
10	14.75^{a}	312	277^{ab}	0.34^{a}
15	14.49^{a}	258	338^{a}	0.34^{a}
Significance	**	ns	**	**
Least significant difference	2.80	–	72	0.02

*ns, not significant; *significant at p<0.05; **significant at p<0.01*

Means followed by different letters in the same column are significantly different at p<0.05 according to the least significant difference test.

†Micronutrient mixture consisted of the following treatments: 0 kg/ha (control), 5 kg/ha, 10 kg/ha and 15 kg/ha each of elemental Cu, Fe and Zn mixed together and applied to the soil as $CuSO_4.5H_2O$; $FeSO_4.7H_2O$ and $ZnSO_4.7H_2O$.

Table 5: Macronutrient composition of three vegetable species in response to micronutrient fertiliser added to soil

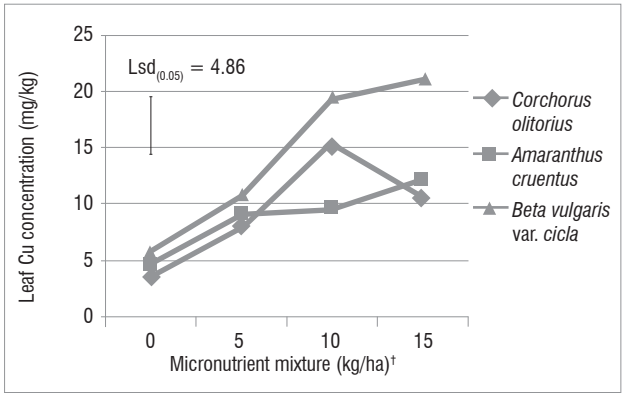
	Concentration (mg/kg)					
Vegetable	**Ca**	**K**	**Mg**	**P**	**N**	**S**
Corchorus olitorius	1.77^{a}	3.15	0.30^{b}	0.35^{c}	3.86	0.27^{b}
Amaranthus cruentus var. Arusha	1.37^{ab}	3.33	1.01^{a}	0.51^{b}	3.80	0.34^{a}
Beta vulgaris var. *cicla*	0.55^{b}	3.50	1.04^{a}	0.61^{a}	3.84	0.34^{a}
Significance	*	ns	**	**	ns	**
Least significant difference	0.83	–	0.08	0.09	–	0.05

*ns, not significant; *significant at p<0.05; **significant at p<0.01*

Means followed by different letters in the same column are significantly different at p<0.05 according to the least significant difference test.

There were significant ($p<0.05$) interactions (Figure 1 and 2) among plant species and fertiliser rate in terms of leaf Zn and Cu. In all three vegetables, the leaf Cu concentration increased with Cu addition up to 10 kg/ha but declined at 15 kg/ha (Figure 1). The decline in leaf Cu concentration at 15 kg/ha Cu was more marked in *C. olitorius* than in Swiss chard and *A. cruentus*. The reason for the decline in Cu concentration in *C. olitorius* at high application rates in the current study is not known but could be a result of toxicity or simply the inherent inability of *C. olitorius* as a species to accumulate the microelement, as alluded to by several researchers.[4,5,18,25,26] Swiss chard concentrated more Zn and Cu at all fertiliser application rates (Figures 1 and 2), which contradicted

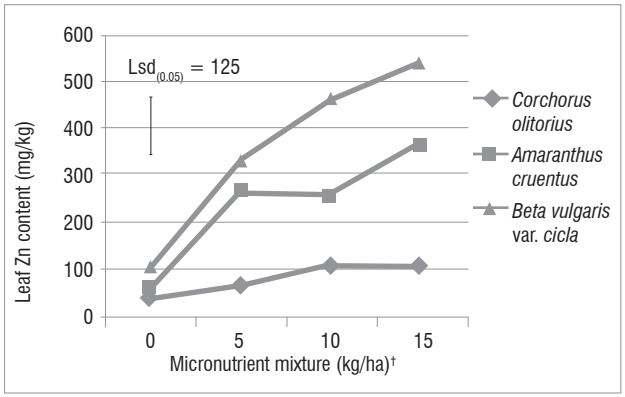
our hypothesis that wild vegetables have a greater inherent ability to accumulate micronutrients from the soil than the widely cultivated exotic vegetables. Nonetheless, the wild vegetables also responded positively to the incremental application of the two micronutrients, which supported our postulate that the addition of micronutrients would result in increased micronutrient concentrations in wild vegetables leaves. Thus, when the vegetables were grown in soil with augmented micronutrient content, they concentrated increased quantities from the soil.



†*Micronutrient mixture consisted of increasing quantities of each of elemental Cu, Fe and Zn mixed together and applied to the soil as* $CuSO_4.5H_2O$; $FeSO_4.7H_2O$ *and* $ZnSO_4.7H_2O$.

LSD, least significant difference

Figure 1: Concentration (mg/kg) of Cu in the leaves of two wild vegetables – *Corchorus olitorius* and *Amaranthus cruentus* var. Arusha – and a conventional vegetable, Swiss chard (*Beta vulgaris* var. *cicla*) after application of 0 kg/ha, 5 kg/ha, 10 kg/ha or 15 kg/ha micronutrient mixture.



†*Micronutrient mixture consisted of increasing quantities of each of elemental Cu, Fe and Zn mixed together and applied to the soil as* $CuSO_4.5H_2O$; $FeSO_4.7H_2O$ *and* $ZnSO_4.7H_2O$.

LSD, least significant difference

Figure 2: Concentration (mg/kg) of Zn in the leaves of two wild vegetables – *Corchorus olitorius* and *Amaranthus cruentus* var. Arusha – and a conventional vegetable, Swiss chard (*Beta vulgaris* var. *cicla*) after application of 0 kg/ha, 5 kg/ha, 10 kg/ha or 15 kg/ha micronutrient mixture.

The concentrations of micronutrients tested in this study could be considered high, or even toxic to crops. We did not evaluate toxicity in this study, but observed no visible toxicity symptoms. The concentrations of micronutrients obtained from the leaf analysis results after applying micronutrient fertiliser were much higher than those reported for wild vegetables collected from the wild.[12-16] However, not all micronutrients present in crops are available for uptake by humans. Some are complexed in unavailable forms by various biomolecules.[2,25] It is therefore necessary to investigate if the increase in leaf micronutrient level has a positive effect on bioavailability of the micronutrients in wild vegetables. Micronutrients are generally applied in smaller quantities than macronutrients,[27] but mostly in commercial agriculture. There is a danger of accumulation of micronutrients to hazardous levels in soils associated with high application rates of micronutrient fertilisers such that some countries have statutory maximum limits of micronutrients to be applied to soils to prevent accumulation to toxic levels.[18] The nutrient content of most soils in the subsistence farming sector is generally not well known, yet there is a belief among agriculturalists that micronutrients occurring naturally in the soil are adequate for crop production. In contrast, in commercial agriculture, in which the nutrient status of soils is well known, farmers periodically apply micronutrients, either as foliar sprays or chelates to soils.[28]

Conclusions

The different vegetable species investigated demonstrated different abilities to take up Cu and Zn, in the order Swiss chard > *A. cruentus* > *C. olitorius*, and they responded to soil-applied micronutrients by taking up more from the soil, as more was supplied, but up to a certain point. The trend *B. vulgaris* > *A. cruentus* > *C. olitorius* was observed for the macroelements Mg and P but it was reversed for Ca concentration, with *C. olitorius* leaves containing three times the Ca content of *B. vulgaris.* Our results contradict the current claim that wild vegetables have superior micronutrient content to exotic vegetable species.

Acknowledgements

We thank the Research Committee of the University of Zululand for financial support and the Agricultural Research Council – Vegetable and Ornamental Plant Institute for supplying the *Corchorus olitorius* and *Amaranthus cruentus* seeds.

Authors' contributions

All authors contributed to the research and write-up. S.M. was responsible for the design and conduct of the experiments, and the data collection and analysis. W.P.d.C. and M.M. were responsible for overall supervision of the project.

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Long bone cross-sectional geometric properties of Later Stone Age foragers and herder–foragers

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DATES:
Received: 05 Dec. 2013
Revised: 02 Mar. 2014
Accepted: 04 Mar. 2014

KEYWORDS:
South Africa; Holocene; bioarchaeology; regional variation; habitual physical activity

HOW TO CITE:
Cameron ME, Pfeiffer S. Long bone cross-sectional geometric properties of Later Stone Age foragers and herder–foragers. S Afr J Sci. 2014;110(9/10), Art. #2013-0369, 11 pages. http://dx.doi.org/10.1590/sajs.2014/20130369

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Diaphyseal cross-sectional geometry can be used to infer activity patterns in archaeological populations. We examined the cross-sectional geometric (CSG) properties of adult Later Stone Age (LSA) herder–forager long bones from the inland lower Orange River Valley of South Africa (n=5 m, 13 f). We then compared their CSG properties to LSA forager adults from the coastal fynbos (n=23 m, 14 f) and forest (n=17 m, 19 f) regions, building on a previous report (Stock and Pfeiffer, 2004). The periosteal mould method was used to quantify total subperiosteal area, torsional strength, bilateral asymmetry and diaphyseal circularity (I_{max}/I_{min}) at the mid-distal (35%) location of upper arms (humeri) and the mid-shaft (50%) location of upper legs (femora). Maximum humerus and femur lengths were similar among the three samples, suggesting that adult stature was similar in all three regions. When compared to the previous study, CSG property values obtained using the periosteal mould method correlated well, and there were no significant differences between data collected using the different methods. No statistically significant differences were found among the humerus or femur CSG properties from the different regions. This finding suggests that all individuals undertook similar volitional habitual activities in regard to their upper limbs, and also had similar degrees of terrestrial mobility. These results indicate relative behavioural homogeneity among LSA foragers and herder–foragers from South Africa. The small degree of regional variation apparent among the three samples may reflect local ecology and the subsistence demands affecting populations in these different regions.

Introduction

Reconstruction of physical activity among Later Stone Age foragers

Holocene Later Stone Age (LSA) forager skeletons have been recovered archaeologically most often from the southern and southwestern coasts and coastal forelands of the South African Cape (Figure 1). They date from ca. 10 000 bp to the historical era.[1,2] While these LSA populations share artefact traditions[2,3], there are some pertinent ecological differences between coastal regions that appear to have been consistent throughout the Holocene[2].

Along the southwestern Cape coast, fynbos ecology predominates (Figure 1), featuring succulents, a diverse plant base of geophytes and fruits, and a Mediterranean climate with rainfall of 200–600 mm per annum. Small browsers, with some larger grazing ungulates, dominate the faunal assemblages. Along the southern coast, afro-montane forest conditions predominate, although fynbos vegetation also persists (Figure 1). While the climate is similar to the fynbos, the evergreen canopy in the forest is continuous and rainfall is higher (500–1200 mm per annum). The forest has small browsers as well, although some larger species inhabited this area during the Holocene. Diverse marine resources are available in both regions.[1,2] The southwestern region will be referred to as the 'fynbos' and the southern region will be referred to as the 'forest,' following the geographical definitions provided by Morris[1].

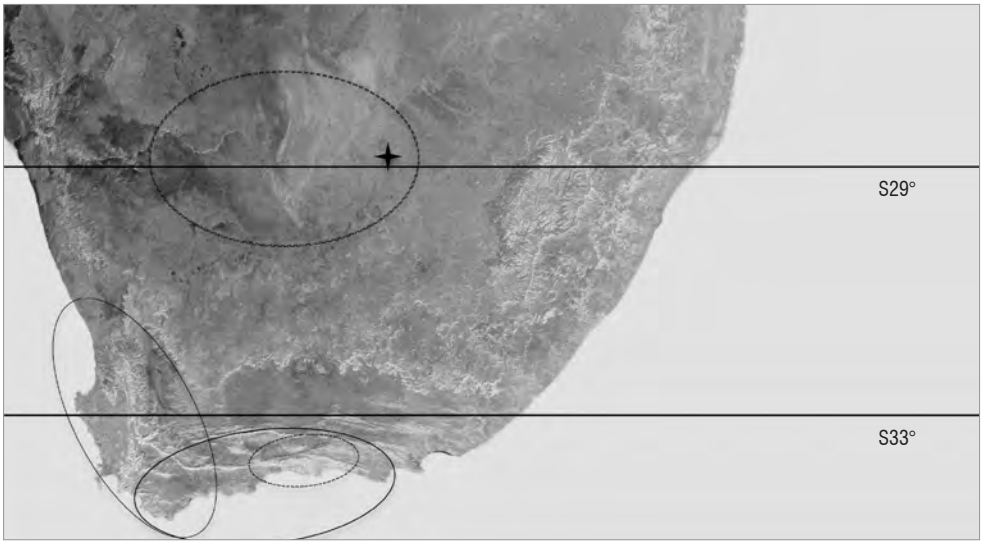

Figure 1: Map of southern Africa with forest, fynbos and lower Orange River Valley regions approximated. The fynbos region is indicated by the solid outline, the forest region by the small dotted outline and the lower Orange River Valley by the dashed outline. Black lines indicate approximate latitude markers and the star indicates the approximate location of Koffiefontein.

The size and shape of adult LSA forager skeletons have interested many researchers. Studying a sample available 20 years ago, Smith et al.[4] examined changes in LSA forager shaft diameter, cortical bone thickness, maximum humerus length and maximum femur length to detect if changes in diet and lifestyle associated with contact between forager and pastoralist groups may have impacted forager health and activity patterns between the hypothesised pre- and post-contact periods. They noted that cortical bone thickness and maximum bone lengths increased in the post-contact period, particularly in forager men, although these increases only reached significance for a few of the measures considered. About 15 years ago, Churchill and Morris[5] examined differences in the habitual activities of LSA foragers from the fynbos, forest and savannah regions of South Africa by examining musculoskeletal stress markers. They predicted that physical activity and musculoskeletal stress markers would be higher in the forest, reflecting higher resource abundance. They reported a stronger pattern among men than among women. Ledger et al.[6] used computed tomographic (CT) scans to compare long bone diaphyseal cross-sectional geometric (CSG) properties of LSA foragers to a sample of historical skeletons from Cape Town and a modern cadaveric collection. They found that male and female foragers had strong lower limbs and appeared to have been highly mobile and active compared to the more recent populations. Stock and Pfeiffer[7] also examined variation in the habitual physical activity patterns of male and female LSA foragers from the fynbos and forest by analysing long bone diaphyseal CSG properties.

Cross-sectional geometric properties are presumed to reflect the strength and shape of the long bone. These properties develop during life, and are influenced by the intensity and frequency of physical activities undertaken. Consequently, they reflect the habitual activities and biomechanical patterns of an individual.[8] Stock and Pfeiffer[7] found differences in upper and lower limb strength between the men and women of the forest and fynbos samples. They concluded that there was a greater disparity in the intensity of manual labour between forest men and women than between fynbos men and women. Bilateral asymmetry differences between the men of these two regions suggested that forest men may have more commonly used spears for hunting and fynbos men may have more frequently used light-draw bows.[7] These conclusions built on previous research into upper limb strength asymmetries in past populations and contemporary athletes.[9,10] A conclusion of spear use, which is reflected in greater limb strength asymmetry, is consistent with the artefacts documented from the forest region.[9,10] Small points, typical of fynbos assemblages, are consistent with the type of bow-and-arrow hunting ethnographically associated with the San foragers of southern Africa.[3,7] Men from both the forest and fynbos displayed femur CSG properties consistent with levels of terrestrial mobility higher than those of women. Femur data further showed that women from the forest had CSG properties consistent with higher levels of terrestrial mobility than women from the fynbos.[7]

That study of the fynbos and forest regions has subsequently been used to compare habitual physical activities among diverse Holocene foragers. Stock[11] compared the upper and lower limb CSG properties of LSA foragers to an early historical Andaman Island sample, as Andaman Islanders represent another small-bodied foraging population. He found that LSA foragers displayed greater lower limb CSG property values, likely as a result of greater terrestrial mobility, while Andaman Islanders displayed higher upper limb CSG property values, which was attributed to a high proportion of water-based activities, such as canoeing and swimming. He also compared LSA CSG properties to those of Yahgan foragers from Tierra del Fuego in South America. The Yahgan foragers displayed higher upper limb CSG property values, which may also reflect substantial watercraft use and swimming activities. Carlson et al.[12] compared the CSG properties of Australian Aborigine foragers to those of the LSA. Australian Aborigines displayed generally weaker upper and lower limb CSG property values, suggesting that LSA foragers pursued terrestrial mobility-based foraging activities at higher intensities.[12]

In sum, LSA foragers appear to have been more terrestrially mobile than other groups, yet do not appear to have regularly engaged in activities requiring intense upper limb strength. With the exception of male arm strength, physical activity patterns were relatively consistent between two regions. To elaborate on this line of research, a LSA skeletal sample from a third region – the lower Orange River Valley – will be assessed. The inclusion of this sample will help to establish the range of variation in habitual physical activities that may have existed among geographically diverse Holocene populations of South Africa.

Later Stone Age herder–foragers of the lower Orange River Valley

The lower Orange River Valley is both geographically and ecologically distinct from the two more southerly coastal areas (Figure 1). The lower Orange River Valley will be referred to as the 'inland' region, as distinct from the fynbos and forest regions. The inland area is semi-arid, with less predictable rainfall and more extreme temperatures than the Cape. Ecologically, it is marginal between the Sweet Grassveld and Karoo types.[13] Vegetation is relatively bushy and uneven, although it is capable of sustaining livestock if there is sufficient rainfall.[13] Geophytic plants dominate the edible plant species. The lower Orange River Valley has a high proportion of browsers relative to areas with heavier rainfall where grazers may be more common.[14] Therefore, inland foragers may have had distinctive diets, reflecting the ecological differences among the three areas being compared. Inland population density appears to have been much lower, likely because of sporadic rainfall and low availability of reliable resources.[2]

LSA skeletons have been recovered from Koffiefontein and Augrabies Falls, both located in the inland lower Orange River Valley.[14] These sites date to the southern African proto-historical period, with one burial near Koffiefontein dating to 390 ± 50 bp (uncalibrated) radiocarbon years.[14] Many skeletons from this region were found near Type-R settlements.[14,15] These settlements are associated with what has been characterised as a relatively sedentary lifestyle in which food resources were acquired through pastoralism as well as foraging.[14] During this period, Khoe pastoralist and Bantu-speaking (black African) agriculturalists also occupied the lower Orange River Valley. Type-R settlement sites show some evidence of close contact and trade with Bantu-speaking agropastoralists.[14]

Morris[14] studied the craniometric and dental characteristics of LSA herder–foragers from the lower Orange River Valley. Herder–foragers from this region display craniometric characteristics consistent with other LSA and San populations, implying that, genetically, inland men and women remained a unique population despite the presence of non-foraging groups in this area.[14] Morphological evidence of some Bantu admixture was also noted, suggesting intermarriage with Bantu-speaking agriculturalists.[14]

An analysis of lower Orange River Valley (inland) LSA herder–forager postcranial skeletons may help to clarify the types of habitual activities undertaken by this group, and determine if their activities were distinct from other LSA foragers as a result of cultural differences, such as the incorporation of pastoralism. As herder–foragers may supplement their foraging efforts with provisions supplied by animal herds, lower mobility demands may have been placed on the legs, reflecting less searching for food resources. Upper limb strength could also be less crucial compared to other LSA groups, if less vigorous food processing was required. Examining whether inland herder–foragers had habitual physical activities similar to those of related foragers from the other regions may also help clarify whether contact with non-foraging populations affected herder–foragers' habitual activities.[2,16-18]

Biomechanical analyses of long bone shafts

Studies investigating the relationship between physical activity and bone remodelling are based on assumptions of bone functional adaptation. In this framework, bone remodelling is stimulated by the physical deformation of bone tissue through positive and negative feedback. Heighted physical activity and resulting tissue deformation leads to bone tissue deposition, while lessened physical activity leads to the resorption of bone tissue.[19] Analyses of CSG properties, which characterise the distribution of bone tissue around long bone diaphyses,

have been applied to skeletal materials originating from archaeological contexts, to reconstruct habitual activities undertaken by past human populations.[6-8,12,20-22]

CSG analyses focus on the application of beam theory to the quantification of long bone CSG properties.[8,23–25] Information typically comes from long bone diaphyseal cross-sections in which periosteal and endosteal contours are modelled using CT scanners or a combination of silicone moulds and biplanar radiographs.[8,23-26] However, while these analyses are accurate, high-resolution scanners are often unavailable in remote research locales and images may be expensive to produce.[8,23,26]

In response to these issues, the accuracy of external methods for quantifying CSG properties has been explored by examining the efficacy of relying solely on periosteal contours, represented by periosteal moulds.[23-25] Because the strength of a long bone diaphysis is dependent on the amount and distribution of cortical bone present, a greater distance between the periosteal boundary and the diaphyseal centroid indicates a long bone's enhanced capacity to resist bending and torsional loading.[8,23,25] Periosteal contours have been shown to have a stronger influence on a bone shaft's biomechanical characteristics than do endosteal contours.[8,23,25] Moulding of periosteal contours with silicone impression material is non-invasive, does not damage skeletal tissue and relies on no biomedical technology. Validation of this approach for South African LSA foragers would corroborate other reports indicating that external methods can be used to obtain valid CSG properties, without reliance on images of endosteal contours.[21-24]

Aims

The accuracy of the periosteal mould method was examined by comparing newly generated values with previous values from the same LSA long bones used in a previous study by Stock and Pfeiffer[7]. The sample sizes for the fynbos and forest populations were also increased, as more skeletons had become available for analysis since Stock and Pfeiffer's study.[7] Then, using this method, a new geographic comparison of CSG properties among LSA forager populations from the fynbos, forest and inland regions of South Africa was undertaken, to look for behavioural patterns among the samples. We sought to determine whether inland herder–foragers display habitual activity patterns comparable to those of foragers that did not practise any pastoralism. The results obtained in this study for the fynbos and forest samples should remain consistent with those of Stock and Pfeiffer[7] despite the increase in sample sizes. Variation in habitual activity patterns may be low among and between the three samples, regardless of the incorporation of pastoralism inland, if LSA foraging is characterised by behavioural homogeneity.

Materials and methods

Later Stone Age forager and herder–forager samples

The samples include the humeri and femora of 91 adults (45 males, 46 females; Table 1) originating from archaeological sites in the fynbos (n=23 m, 14 f), forest (n=17 m, 19 f) and inland (n=5 m, 13 f) regions. The skeletons have been radiocarbon dated to between 10 000 bp and 300 bp (uncalibrated) (Table 1). CSG properties of many of the LSA foragers from the fynbos and forest have previously been examined but the sample sizes have increased since then. There are newly added fynbos skeletons (n=14 m, 9 f) and newly added forest skeletons (n=2 m, 1 f). Age and sex estimations are based on skeletal morphology, as observed by collaborating bioarchaeologists L.E. Doyle and C. Merritt who also documented postcranial dimensions.

The craniometric stability and homogeneity observed among LSA skeletons[27] indicate that the three samples can be considered as a single population. To confirm postcranial size homogeneity across regions, one-way analyses of variance (ANOVAs) for maximum humerus and femur lengths were conducted for all samples.

Assessment of cross-sectional geometric properties

Periosteal moulds were taken using Exaflex Heavy Body silicone impression material (GC America, Alsip, IL, USA) at the mid-distal (35%) location of all humeri (bilaterally) and at the mid-shaft of one femur per individual, with the right femur preferred. Researchers commonly use the 35% location on the humerus shaft, thus avoiding large muscle attachment sites and allowing effective assessment of bilateral asymmetry. The left femur was used when the right was missing or incomplete. Assuming symmetry of the lower legs, values from left femora were included with values from the right femora in the analysis. Once mid-shaft and mid-distal locations had been identified for the femora and humeri, respectively, these locations were marked with string, and then the quick-drying silicone impression material was applied to the external surface of the bone. The moulds were then removed, resulting in a representation of the diaphyseal (shaft) cross section.

Anatomical planes of orientation were marked on each mould during the moulding process to help maintain correct orientation during digitisation. The moulds were digitised using an Epson flatbed scanner, including a ruler for scale. Periosteal contours were estimated by manually tracing along the edges of digitised moulds using a drawing tablet. The traced images were analysed using the ImageJ platform[28] with the Moment Macro v1.3 plug-in[29]. CSG properties were calculated for each bone (Table 2).

The CSG variables examined in this study are listed in Table 2. Total subperiosteal area (TA) is a direct indicator of diaphyseal cross-sectional size. It influences the polar second moment of area, which reflects the bending strength of a long bone.[8] TA also represents the most accurate measure that can be derived from the periosteal mould method. Torsional strength (J) represents the robustness of a diaphysis and is the best indicator of a bone's capacity to withstand diverse mechanical loads.[8] There is some error associated with J when the periosteal mould method is applied, because of the exclusion of medullary cavity dimensions. This inaccuracy limits the comparability of J calculated using this method to values of J reported in other studies that have generated this value from periosteal and endosteal information.[22,24] Values of J calculated from periosteal contours alone, however, correlate highly with values of J calculated from CT scans or direct sectioning.[23,25] Periosteally derived J values can therefore be used to compare long bone strength characteristics within a sample. Humerus bilateral asymmetry (BA) in TA and J were quantified as percentage values, as was sexual dimorphism (Table 2).

Diaphyseal shape was assessed by quantifying the ratio of maximum to minimum bending strengths (I_{max}/I_{min}) as a measure of diaphyseal circularity. I_{max}/I_{min} is a robust indicator of diaphyseal shape, as it compares two non-fixed axes.[8] A value of I_{max}/I_{min} that is close to unity represents a circular diaphysis, in which uniform loading has occurred around the long axis of the diaphysis, while values greater than one indicate greater loading in the direction of the I_{max} value.

Different body size standardisation methods were applied to humerus and femur CSG properties (Table 2). While the upper limbs are not weight bearing, humerus CSG values must be scaled to account for differences in bone lengths. Humerus CSG properties were standardised to body size using theoretical equivalents for the product of bone length and body mass based on bone length alone. The powers of 3 for standardising cross-sectional areas and 5.33 for torsional strength were used as they provide a strong correlation between humerus length and body size in *Homo sapiens*.[30]

Femur CSG properties must be standardised in a way that incorporates estimated body mass, as mass affects axial, bending and torsional loading in weight-bearing long bones.[30] TA and J were standardised to body mass using formulae incorporating body mass and bone length.[11,22] Estimated body mass was calculated using the McHenry[31] formula in which femur head diameter is used (Table 2). This formula is preferred for assessing body mass in small-bodied samples, like LSA foragers.[32,33]

To determine the accuracy of the periosteal mould method, data from the Stock and Pfeiffer[7] study were compared to data from the present study.

Table 1: Skeletons included in this analysis, organised by region

Site	Catalogue number	Sex	Uncalibrated radiocarbon date	Lab number
Fynbos sample				
Groot Brak River	NMB1204	F	2210±35	Pta-8744
Gordon's Bay	SAM-AP1443	M	2050±50	Pta-2309
Noordhoek	SAM-AP4308	M	2170±60	Pta-4404
Kommetjie	SAM-AP4720	M	2195±80	GX-13179
Peer's Cave	SAM-AP4692	M	6891±37	OxA-17376
Hermanus	SAM-AP4790	M	1610±150	Pta-2163
Pearly Beach	SAM-AP4901	M	1900±40	Beta-241154
Kleinsee	SAM-AP4931	M	3750±60	Pta-4827
Melkbosch	SAM-AP5035A	M	630±50	Pta-4813
Melkbosch	SAM-AP5041	F	2010±50	Pta-4376
Cape Point	SAM-AP5075	M	2530±60	Pta-4669
Ysterfontein	SAM-AP5083	F	1490±55	Pta-926
Saldanha	SAM-AP5095	F	2660±70	Pta-4674
Byneskranskop	SAM-AP6049	M		
Byneskranskop	SAM-AP6050	F	1480±50	Pta-2855
Byneskranskop	SAM-AP6051	F	3190±50	Pta-2969
Saldanha	SAM-AP6147	M	2920±60	Pta-8774
Melkbosstrand	SAM-AP6260A	F	2120±60	Pta-9069
Melkbosstrand	SAM-AP6264	M	1950±60	Pta-9073
Melkbosstrand	SAM-AP6317	M	2970±60	Pta-8807
Melkbosstrand	SAM-AP6318	M	3310±60	Pta-8741
Melkbosstrand	SAM-AP6319	F	3200±35	Pta-8752
St Helena Bay	SAM-AP6331	F	790±90	TO-8953
Melkbosstrand	SAM-AP6332	F	980±50	Pta-8767
Melkbosstrand	SAM-AP6334	M	1400±50	Pta-8790
Saldanha	SAM-AP6372C	F	2470±40	UGAMS2803
Saldanha	SAM-AP6372D	M	2340±40	UGAMS2804
Kommetjie	UCT097	M	1560±40	Pta-4828
Bloubergstrand	UCT220	M	2100±21	Pta-5678
Faraoskop	UCT385	F	2130±60	Pta-5281
Faraoskop	UCT390	M		
Faraoskop	UCT394	M	2150±70	Pta-4964
Voelvlei	UCT582	F	740±40	Pta-7178
Cape Point	UCT591	M	2460±40	Beta-263612
Elands Bay	B263609	M	3230±40	Beta-263609
Bloubergstrand	BLOUB.	M		
Stompneus Bay	STOMPNEUS	F		
Forest sample				
Matjies River Cave	NMB1241A	F	2790±60	Pta-6958
Matjies River Cave	NMB1241B	F	3290±90	Pta-6950
Matjies River Cave	NMB1273	M	3050±60	Pta-6942
Matjies River Cave	NMB1274	M	5120±50	Pta-6981
Matjies River Cave	NMB1342	F	9688±36	OxA-V-2064-56
Matjies River Cave	NMB1437	M	4940±70	Pta-6947

Continued on next page

Table 1 continued

Site	Catalogue number	Sex	Uncalibrated radiocarbon date	Lab number
Robberg Cave	NMB1639	F	2590±60	Pta-6965
Robberg Cave	NMB1640	F	4120±60	Pta-6983
Plettenberg Bay	NMB1704	F	760±50	Pta-6963
Plettenberg Bay	NMB1705A	F	2780±60	Pta-6964
Matjies River Cave	NMBSkel1	F	2280±60	Pta-6952
Matjies River Cave	NMBSS1	F		
Matjies River Cave	NMBSS2	F	5370±70	Pta-6976
George	SAM-AP34	M	2310±25	Pta-6599
Robberg	SAM-AP1131	F	4030±110	TO-8401
Robberg	SAM-AP1145	M	3210±70	Pta-2284
Robberg Cave	SAM-AP1871	F	3310±60	Pta-2273
Robberg Cave	SAM-AP1878A	M	2170±20	Pta-6592
Robberg Cave	SAM-AP1878B	F	2620±35	Pta-2145
Robberg Cave	SAM-AP1879	M	3440±60	Pta-2283
Robberg Cave	SAM-AP1889	M	2310±50	Pta-6594
Robberg	SAM-AP1893	M	2360±20	Pta-6613
Robberg	SAM-AP3021	F	4030±60	Pta-6595
Robberg	SAM-AP3026	F	3980±60	Pta-7925
Drury's Cave	SAM-AP4734B	M	7050±80	Pta-6632
Bokbaai	SAM-AP4813	F	2140±45	Pta-4204
Knysna	UCT107	M	2290±50	Pta-6815
Oakhurst Rock Shelter	UCT199	M	6180±70	Pta-3718
Oakhurst Rock Shelter	UCT201	F	5990±70	Pta-4226
Oakhurst Rock Shelter	UCT204	M	4530±20	Pta-4449
Oakhurst Rock Shelter	UCT206A	M	5450±70	Pta-4367
Oakhurst Rock Shelter	UCT206B	F		
Oakhurst Rock Shelter	UCT212	F		
Oakhurst Rock Shelter	UCT214	M	4900±60	Pta-4467
Plettenberg Bay	UCT254	M	1270±50	Pta-6820
Nelson Bay Cave	UCT347	M	3236±33	OxA-V-2055-35
Lower Orange River Valley sample				
Koffiefontein	MMK203	M		
Koffiefontein	MMK204	F		
Koffiefontein	MMK206	F		
Koffiefontein	MMK213	F		
Koffiefontein	MMK228	F		
Koffiefontein	MMK229	M		
Koffiefontein	MMK231	M		
Koffiefontein	MMK235	F	390±50	Pta-2894
Koffiefontein	MMK236	F		
Koffiefontein	MMK237	F		
Koffiefontein	MMK250	F		
Koffiefontein	NMB1208	F		
Koffiefontein	NMB1215	F		
Koffiefontein	NMB1216	M		
Augrabies Falls	NMB1420	F		
Vaalbank, Riet River	NMB1655	F		
Vlakfontein	NMB1657	F		
Vlakfontein	NMB1659	M		

Table 2: Definitions and formulae for cross-sectional geometric properties and standardisation protocols used in this study

Cross-sectional property	Abbreviation	Biomechanical significance	Unit of measurement
Total area	TA	Influences second moment of area	mm^2
Maximum second moment of area	I_{max}	Maximum bending strength	mm^4
Minimum second moment of area	I_{min}	Minimum bending strength	mm^4
Diaphyseal circularity index	I_{max}/I_{min}	Directionality of bending strength	
Polar second moment of area	J	Torsional strength	mm^4
Formulae used to calculate and standardise cross-sectional geometric properties			
Bilateral asymmetry (%)	% BA	100% X [(maximum-minimum)/minimum]	Stock and Pfeiffer[7]
Sexual dimorphism (%)	% SD	100% X [(male-female)/female]	Stock and Pfeiffer[7]
Humerus:			
Total cross-sectional area	TA ^	(Total area/length^3) X 10^8	Ruff et al.[30]
Torsional strength	J ^	(Second polar moment of area/length$^{5.33}$) X 10^{12}	Ruff et al.[30]
Femur:			
Body mass	BM	2.239 X FH - 39.9	McHenry[31]
Total cross-sectional area	TA*	(Total area/ estimated body mass in kg) X 10^2	Stock[11]
Torsional strength	J*	[Second polar moment of area/(bone length in mm x estimated body mass in kg)] X 10^2	Stock[11]

Definitions adapted from Ruff[8]

One of the authors (MEC) scanned all periosteal moulds, both those retained from Stock and Pfeiffer's[7] research and those that were newly obtained. CSG properties from the retained moulds, calculated by MEC, were compared to the original measurements on file. This calibration exercise assures consistency between the studies. I_{max}/I_{min} and J were compared between individuals assessed in both studies using two-tailed paired *t*-tests. Results from the Stock and Pfeiffer[7] study were also regressed using a reduced major axis linear regression to determine the correlation between data obtained from the same long bones using the two methods. After establishing homoscedasticity between samples using Levene's test for the equality of variances, independent samples *t*-tests (Student's *t*-tests) were used to compare TA, J and I_{max}/I_{min} between men and women within each region for both humeri and femora. To investigate ecological variation, samples from the fynbos, forest and inland regions were compared using one-way ANOVAs. For all statistical comparisons, α was set at 0.05 for detecting significant differences between samples.

Results

Comparison between methods

A strong correlation exists between data from Stock and Pfeiffer[7] and data obtained from the same long bones using solely periosteal contours. Both I_{max}/I_{min} ($r^2 = 0.82$) and J ^ ($r^2 = 0.91$) correlate strongly. When compared using a two-tailed paired samples *t*-test, there are no significant differences between the I_{max}/I_{min} ($p = 0.174$) or J ($p = 0.972$) values obtained using the different methods. This result validates further comparison of CSG properties calculated using periosteal contours, allowing expansion of the sample sizes from the fynbos and forest regions.

Linear dimensions of Later Stone Age adult skeletons

When compared using a one-way ANOVA, no significant differences are evident among the inland, fynbos and forest samples for maximum humerus lengths ($p = 0.189$) and maximum femur lengths ($p = 0.281$) (Figure 2). The samples are homogenous in terms of linear dimensions. The inclusion of the values from the inland sample expands the prior observation that LSA forager adults are small bodied.[34]

Humerus cross-sectional geometric properties

In absolute terms, inland men and women display the highest humerus total area (TA ^) values (Table 3). In all three regions, men display higher absolute TA ^ humerus values than women but the differences do not reach significance in any region. Comparing the magnitude of sexual dimorphism by region, it is greatest between the right humeri of the forest men and women. The magnitude of dimorphism is lower and is comparable for inland and fynbos men and women. Sexual dimorphism is the lowest between the left humeri of forest men and women.

With regard to humerus torsional strength, men show higher J ^ than women, and in terms of regions, inland men and women display the highest J ^ values (Table 3; Figure 3). There is a significant difference between the right humeri of forest men and women ($p = 0.00138$). Sexual dimorphism is again greatest between the right humeri of forest men and women. Values are lower and comparable for the fynbos and inland men and women, except for left humeri J ^ , for which forest men and women display the lowest sexual dimorphism. These results suggest that fynbos and inland men and women experienced similar patterns in diaphyseal loading intensity, different from those in the forest.

TA ^ and J ^ bilateral asymmetry values are similar for inland men and women. This result differs from the fynbos and forest regions, where men and women display different bilateral asymmetry values (Table 3; Figure 4). The difference between the sexes in bilateral asymmetry is trending towards significance for the forest men and women ($p = 0.089$ for TA ^ and $p = 0.068$ for J ^). Inland women display greater bilateral asymmetry than women from the other two regions, and inland men display lower bilateral asymmetry than other men.

There is a significant difference in the circularity (I_{max}/I_{min}) of the right humeri between men and women in the fynbos ($p = 0.003$; Table 3). The difference between the right humeri of men and women when the forest

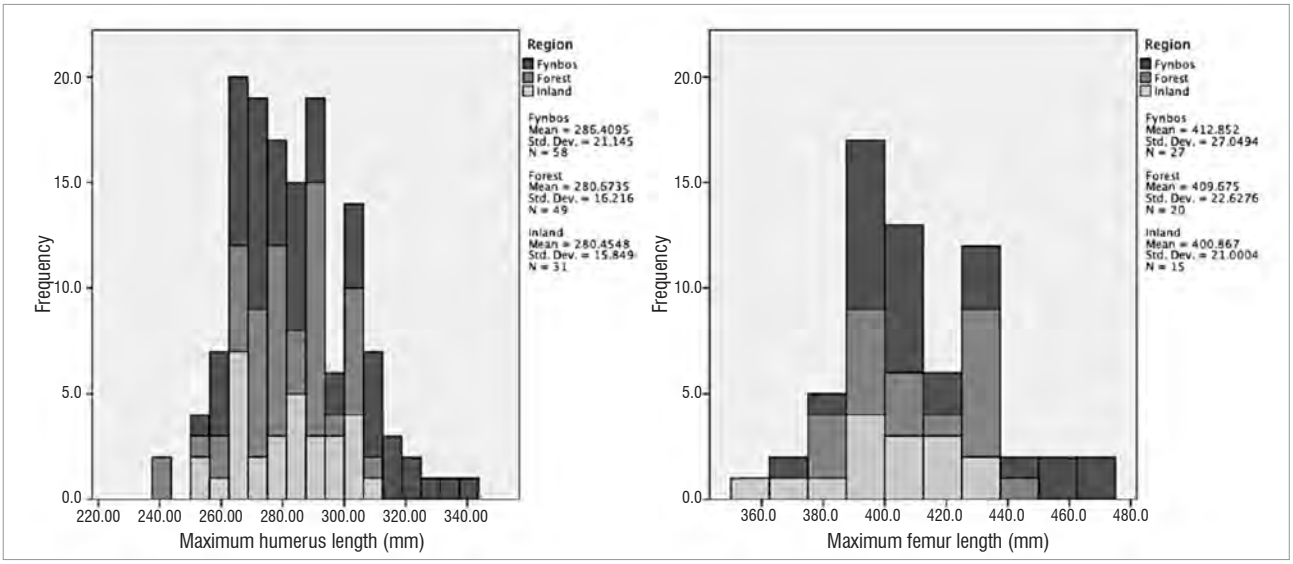

Figure 2: Histograms plotting maximum humerus lengths (mm) and maximum femur lengths (mm), organised by region.

and fynbos samples are pooled is also significant ($p=0.002$). There are no significant differences between inland men and women for either side. This suggests less differentiation between inland men and women in the distribution of diaphyseal loading, compared to the other regions.

Femur cross-sectional geometric properties

Table 4 documents femur total area (TA*) for all regions. The TA* values of men are higher than women in all regions; however, there are no significant differences between the sexes. The highest male TA* values are found among inland men, while the highest female values are evident among forest women. The degree of sexual dimorphism between men and women is comparable for the inland and fynbos samples, and is lower between forest men and women.

Table 4 and Figure 5 display femur torsional strength (J*) values for all regions. Men have higher J* than women in all regions. Male J* values are similar regardless of regional origin. Forest men and women display the highest J* values when compared to the other regions. Sexual dimorphism is comparably high between the J* values of inland and fynbos men and women: the difference between fynbos male and female J* values reaches statistical significance ($p=0.044$). Sexual dimorphism is relatively low between forest men and women, because forest women have relatively high J* values when compared to those of inland and fynbos women. These results suggest that forest male and female femora were loaded with relatively similar intensities, while inland and fynbos male femora were loaded at higher intensities than female femora.

The femur circularity (I_{max}/I_{min}) results are displayed in Table 4. Men have higher I_{max}/I_{min} (less circular femora) than women in all regions. The I_{max}/I_{min} values are comparable between the fynbos and forest samples: men of these two regions have very similar I_{max}/I_{min}, as do women. Fynbos men have the highest I_{max}/I_{min} (the greatest deviation from circularity) when compared to men from the other regions, while forest women have the highest female I_{max}/I_{min} values. Inland men and women, however, display slightly lower values than the other two regions. The inland sample also displays less sexual dimorphism.

Comparisons to foragers from other parts of the world

Because of the somewhat different methods used for body size standardisation and biomechanical strength assessment, only values for humerus and femur I_{max}/I_{min} can be readily compared with published values from foragers who lived in other parts of the world. This variable is relatively resistant to observer error and methodological differences, and it reflects bone adaptation to habitual loading.[23] I_{max}/I_{min} does not need to be standardised based on body size, thereby simplifying comparisons. Australian Aborigine humerus CSG properties could not be compared to the LSA data, as Carlson et al.[12] took all measurements at the humerus mid-shaft (50%) rather than mid-distally (35%).

The patterns reported in previous comparisons of LSA CSG properties have been maintained (Table 5). LSA foragers display average to low humerus I_{max}/I_{min} when compared to Andaman Islanders and Yahgan foragers, and high femur I_{max}/I_{min} when compared to these two samples as well as Australian Aborigines. High femur I_{max}/I_{min} values in LSA foragers relative to other mobile forager samples confirm the prior observation that terrestrial mobility was high amongst this group relative to other foraging populations.

Discussion

LSA foragers represent a good sample for this type of analysis, as there is good preservation of discrete individual skeletons. By including skeletons from three regions of South Africa, this work includes a diverse sample that holistically represents active foragers and herder–foragers in South Africa across the past 10 000 years. Achieving humerus CSG property values that correlate well to those of Stock and Pfeiffer[7] using the periosteal mould method validates the use of this new, simpler technique. Although there are some differences among and between the three South African regions compared, there is also evidence of behavioural homogeneity within the LSA.

The upper limb

The humerus torsional strength values of forest men and women suggest that forest men pursued activities that relied heavily on their right arms, while the forest women did not. Fynbos men and women also undertook different types of activities from one another. In both scenarios, these observations may be related to the foraging activities of women, including their bilateral use of digging sticks, and the hunting activities of men, which may be more lateralised. The observation that only two humerus measures in men and women differed significantly among the three samples supports the assertion that there is low variation in the activities these three populations undertook using their upper limbs.

Upper limb strength is slightly higher among inland men and women, which suggests that higher loads may have been placed on the diaphyses of this sample. While pastoralism was hypothesised to reduce labour intensity, the opposite effect was observed. Higher intensity workloads may have been associated with the challenge of acquiring resources in the semi-arid environment of the lower Orange River Valley. Herder–foragers may also have increased resource acquisition to cope with competition from non-foraging groups who were also occupying

Table 3: Comparison of humerus total subperiosteal area (TA^), humerus torsional strength (J^), humerus bilateral asymmetry (%BA) in total subperiosteal area (TA^) and torsional strength (J^), humerus maximum versus minimum bending strengths (I_{max}/I_{min}), and per cent sexual dimorphism (%SD) for each property between the lower Orange River Valley (inland), fynbos and forest samples

Region		Men			Women			%SD
		n	Mean	s.d.	*n*	Mean	s.d.	
TA^ (mm²)								
Fynbos								
Right humerus 35%	TA^ (mm²)	17	887.3	165.4	12	804.8	182.0	10.3
Left humerus 35%	TA^ (mm²)	18	842.7	141.9	11	754.9	159.8	11.6
Forest								
Right humerus 35%	TA^ (mm²)	13	809.0	70.2	14	705.2	90.0	**15.9**
Left humerus 35%	TA^ (mm²)	13	727.3	104.8	15	724.7	111.9	0.36
Inland regions								
Right humerus 35%	TA^ (mm²)	5	**917.6**	137.0	10	**822.3**	162.9	11.6
Left humerus 35%	TA^ (mm²)	4	**871.1**	155.8	13	**779.9**	145.7	11.7
J^ (mm⁴)								
Fynbos								
Right humerus 35%	J^ (mm⁴)	17	588.4	209.7	12	481.7	206.7	22.2
Left humerus 35%	J^ (mm⁴)	18	521.4	157.8	11	422.4	158.0	**23.4**
Forest								
Right humerus 35%	J^ (mm⁴)	10	479.8*	137.4	14	353.8*	82.6	**40.0**
Left humerus 35%	J^ (mm⁴)	10	408.4	104.6	15	373.8	105.3	0.83
Inland								
Right humerus 35%	J^ (mm⁴)	5	**600.1**	164.8	10	**494.8**	172.2	21.3
Left humerus 35%	J^ (mm⁴)	4	**544.3**	182.2	13	**443.4**	152.8	22.8
%BA								
Fynbos								
	% BA of TA^	15	9.98	7.84	9	5.82	5.09	
	% BA of J^	15	**21.5**	17.9	9	12.1	10.4	
Forest								
	% BA of TA^	10	**13.9**	14.7	12	4.72	6.70	
	% BA of J^	10	20.7	14.0	12	9.70	14.6	
Inland								
	% BA of TA^	4	7.34	5.54	10	**7.49**	5.24	
	% BA of J^	4	16.2	11.3	10	**15.0**	10.6	
I_{max}/I_{min}								
Fynbos								
Right humerus 35%	I_{max}/I_{min}	17	1.20*	0.13	12	**1.38***	0.16	
Left humerus 35%	I_{max}/I_{min}	18	**1.26**	0.15	11	**1.35**	0.18	
Forest								
Right humerus 35%	I_{max}/I_{min}	13	1.20	0.14	14	1.29	0.16	
Left humerus 35%	I_{max}/I_{min}	12	1.18	0.15	16	1.26	0.16	
Pooled regions								
Right humerus 35%	I_{max}/I_{min}	30	1.21*	0.13	26	1.33*	0.16	
Left humerus 35%	I_{max}/I_{min}	30	1.23	0.15	27	1.30	0.17	
Inland								
Right humerus 35%	I_{max}/I_{min}	5	**1.26**	0.13	10	1.34	0.22	
Left humerus 35%	I_{max}/I_{min}	4	1.20	0.084	13	1.27	0.11	

***Bold**=largest when comparing between region.*

** = Significant difference between men and women (independent samples t-test).*

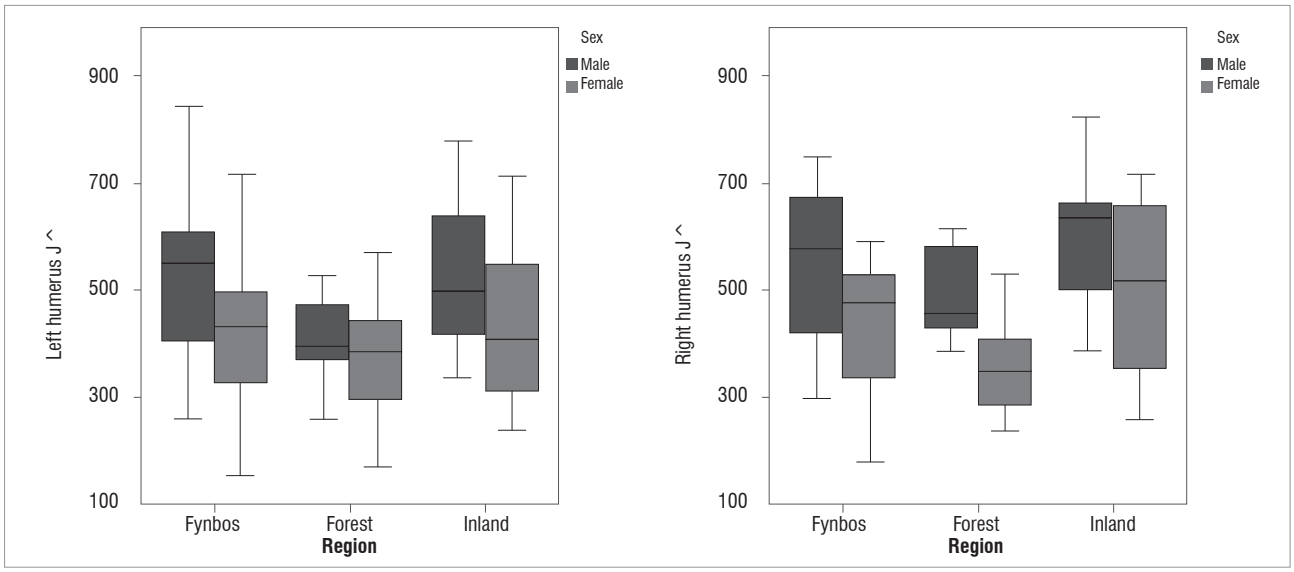

Figure 3: Comparison of humerus torsional strength (J^, mm^4) for the lower Orange River Valley (inland), fynbos and forest samples.

Table 4: Comparison of femur total subperiosteal area (TA*), torsional strength (J*), maximum versus minimum bending strengths (I_{max}/I_{min}) and per cent sexual dimorphism (%SD) for lower Orange River Valley (inland), forest and fynbos samples

Region		Men			Women			%SD
		n	Mean	s.d.	*n*	Mean	s.d.	
Fynbos								
Femur 50%	TA* (mm^2)	18	928.7	119.4	9	872.8	95.4	6.41
	J* (mm^4)	18	176.0*	35.8	9	147.9*	24.3	19.0
	I_{max}/I_{min}	18	**1.77**	0.25	9	1.55	0.38	**14.2**
Forest								
Femur 50%	TA* (mm^2)	12	922.9	46.8	8	**920.7**	117.1	0.11
	J* (mm^4)	12	**181.0**	23.5	8	**163.8**	29.1	10.5
	I_{max}/I_{min}	12	1.76	0.42	8	**1.58**	0.22	11.4
Inland	TA* (mm^2)	3	**947.7**	57.4	12	887.1	81.0	**6.83**
Femur 50%	J* (mm^4)	3	167.5	21.0	12	139.5	25.8	**20.1**
	I_{max}/I_{min}	3	1.51	0.18	12	1.42	0.28	6.34

Bold=*largest when comparing between regions.*

** = Significant difference between men and women (independent samples t-test, p=0.043983).*

this region. If herder–foragers in the lower Orange River Valley were supplementing the resources of non-foraging groups with foraged foods – a dynamic suggested by Wadley[35] based on observations at the Jubilee Shelter in Magaliesburg – herder–foragers may have intensified resource collection to meet these demands.

Inland male and female bilateral asymmetry and diaphyseal shape values are similar, suggesting that men and women participated in similar types of activities. These results differ from those of the forest and fynbos regions, where differences in strength asymmetry and shape were previously attributed to the different activities associated with hunting for men and foraging for women.[7] The convergence in bilateral asymmetry and diaphyseal shape between the sexes in the inland sample suggests that men and women from the lower Orange River Valley participated in activities that were more similar than those of foragers from the fynbos and forest. However, the inland sample is small.

The lower limb

The greater lower limb strength values observed in men relative to women are consistent with values reported in previous studies. Lower limb strength is comparable between the men of all three regions, which is unexpected given the differences in terrain: in terms of topographic relief, the forest is the most mountainous, followed by the fynbos, then inland.[7] This result suggests that fynbos and inland men must have been very mobile and traversed considerable distances to have acquired similar lower limb strength values as men from the more rugged forest region.

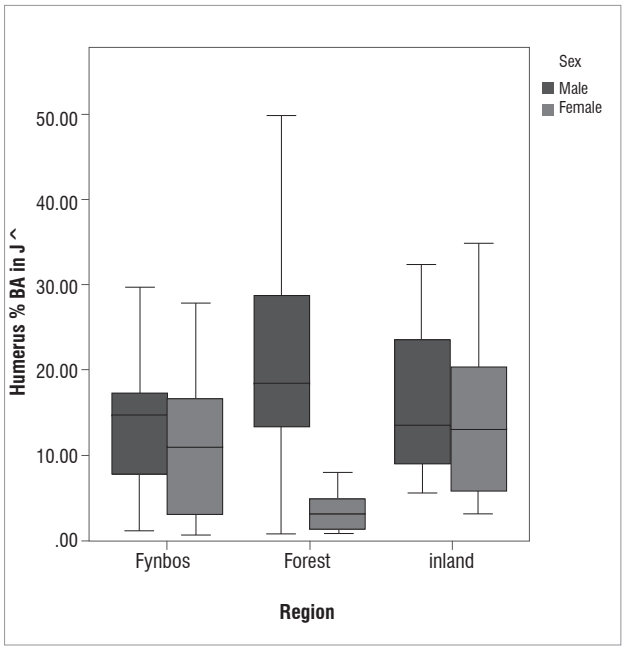

Figure 4: Comparison of humerus bilateral asymmetry (%BA, see Table 3) in torsional strength (J^) for the lower Orange River Valley (inland), fynbos and forest samples.

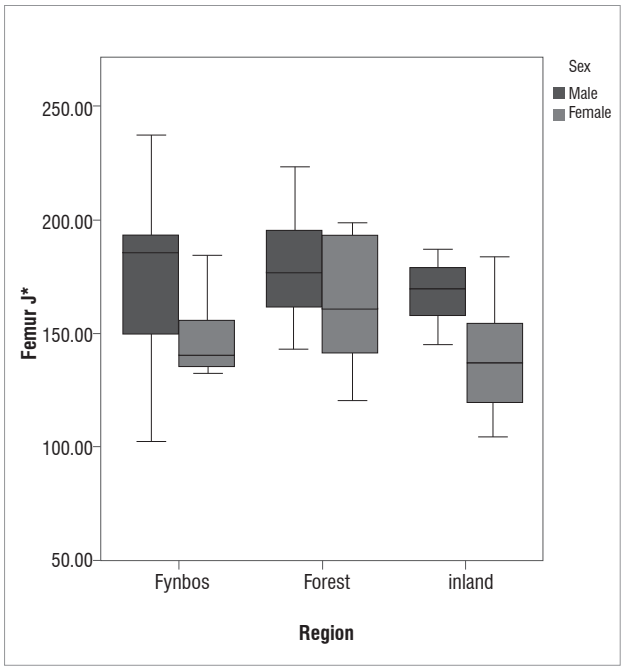

Figure 5: Comparison of femur torsional strength (J^, mm^4) for the lower Orange River Valley (inland), fynbos and forest samples.

Forest women display femora that are more robust than those of inland and fynbos women. This suggests that they may have been more terrestrially mobile than their fynbos and Karoo counterparts. These results indicate a lower degree of sex-based differentiation in terrestrial mobility in the forest during the LSA as compared to other regions of the southern African Cape. The shared pattern of higher sexual dimorphism in femoral CSG properties between men and women of inland and fynbos regions suggests that a similar sex-based differentiation of terrestrial mobility may have occurred in these two regions. This may reflect a similar division of labour between men and women in terms of mobility. However, limited conclusions can be drawn because of the small sample sizes.

Table 5: Comparison of Holocene forager humerus and femur I_{max}/I_{min}. Later Stone Age humeri have similar or lower I_{max}/I_{min} than other Holocene foragers, while Later Stone Age femora have higher I_{max}/I_{min} than other Holocene foragers.

	Men				Women			
	Later Stone Age	Australian Aborigine	Andamanese Islanders	Fuegian	Later Stone Age	Australian Aborigine	Andamanese Islanders	Fuegian
Left humeri mid-distal I_{max}/I_{min}	1.22	–	1.26	1.33	1.29	–	1.36	1.24
Right humeri mid-distal I_{max}/I_{min}	1.21	–	1.21	1.30	1.34	–	1.30	1.35
Femoral mid-shaft I_{max}/I_{min}	1.68	1.47	1.32	1.40	1.52	1.38	1.27	1.27

Summary and conclusions

In the inland, forest and fynbos regions, men participated in activities that increased humerus and femur biomechanical loading as compared to women, regardless of local ecology. Women's upper arm bilateral symmetry and shaft non-circularity in the forest and fynbos regions suggests that women participated in low-intensity activities with non-equivalent loading around the shaft. In contrast, men and women of the lower Orange River Valley engaged in activities with heavier upper arm biomechanical loading.

Relatively little behavioural variability is evident among foragers and herder–foragers from the fynbos, forest and inland regions of South Africa, despite the ecological differences of these regions and the probable incorporation of some pastoralism among the inland foragers. The lack of statistically significant differences between the upper and lower limb strength and shape characteristics of these three samples suggests that many similar physical behaviours were commonly undertaken. The inland forager–herder sample from a semi-arid region displays CSG property values that are similar to LSA foragers who did not possess livestock and lived in more resource-rich environments.

The interpretation of results from this study is limited by the small size of the inland herder–forager sample. As the lower Orange River Valley individuals have long bone lengths that are similar to those of the small-bodied foragers from the fynbos and forest, pastoralism does not appear to have affected body size. However, further investigation is needed to explore possible relationships among foraging, pastoralism and body size. As there appear to be meaningful patterns in the CSG properties of these individuals, further investigation into the inland population is warranted. As interaction with non-foraging groups may have also affected the habitual activities of inland populations, the CSG properties of individuals from neighbouring, non-LSA groups could also be examined. This examination would help clarify if inland herder–foragers displayed slightly different manual activities from non-foraging populations. Further investigation of local resource availability, geography, hydrology, and topography in each region could clarify the relationships between observed patterns in terrestrial mobility and these factors.

The similarity between the CSG properties of the three samples supports an assumption of behavioural and physical activity homogeneity among foraging populations, regardless of ecological variation, or the incorporation of pastoralism. These results are generally consistent with those from previous investigations into the physical activities of LSA foragers. They reinforce a growing impression that 'trekking' was a core

adaptation that was particularly important among hunter–gatherers of southernmost Africa.

Acknowledgements

We thank L. Elizabeth Doyle and Catherine Merritt for collecting the diaphyseal moulds used in this study and Professor Michael Schillaci for his comments. We also thank the curating institutions for permission to study their collections (National Museum Bloemfontein, Iziko Museums of South Africa and McGregor Museum). In all instances, curatorial procedures of the respective institutions were followed. No permits were required for the described study, which complied with all relevant institutional regulations. The Social Sciences and Humanities Research Council provided funding for this research.

Authors' contributions

S.P. arranged for the observations to be made at various curation facilities in South Africa and was responsible for the broad concept on which the research is based; M.E.C. organised and analysed the data; and both authors wrote the manuscript.

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Tufa stromatolite ecosystems on the South African south coast

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DATES:
Received: 09 Jan. 2014
Revised: 03 Mar. 2014
Accepted: 05 Mar. 2014

KEYWORDS:
tufa stromatolites; microbialites; South Africa; barrage pools; waterfall deposits

HOW TO CITE:
Perissinotto R, Bornman TG, Steyn P-P, Miranda NAF, Dorrington RA, Matcher GF, et al. Tufa stromatolite ecosystems on the South African south coast. S Afr J Sci. 2014;110(9/10), Art. #2014-0011, 8 pages. http://dx.doi.org/10.1590/sajs.2014/20140011

Following the first description of living marine stromatolites along the South African east coast, new investigations along the south coast have revealed the occurrence of extensive fields of actively calcifying stromatolites. These stromatolites have been recorded at regular distances along a 200-km stretch of coastline, from Cape Recife in the east to the Storms River mouth in the west, with the highest density found between Schoenmakerskop and the Maitland River mouth. All active stromatolites are associated with freshwater seepage streams flowing from the dune cordon, which form rimstone dams and other accretions capable of retaining water in the supratidal platform. Resulting pools can reach a maximum depth of about 1 m and constitute a unique ecosystem in which freshwater and marine organisms alternate their dominance in response to vertical mixing and the balance between freshwater versus marine inflow. Although the factors controlling stromatolite growth are yet to be determined, nitrogen appears to be supplied mainly via the dune seeps. The epibenthic algal community within stromatolite pools is generally co-dominated by cyanobacteria and chlorophytes, with minimal diatom contribution.

Introduction

The first extant marine tufa stromatolites along the southern African coast were described in the early 2000s from Cape Morgan[1,2] and later investigated in some detail from a geochemical and geomorphological point of view[3]. Located on a dolerite headland shaped into a wave-cut platform, these stromatolites consist of continuous, extensive laminar growths or discrete accretions bridging gaps between separate boulders. In either case, the formation results in enclosed rock pools capable of trapping carbonate-rich groundwater seeps. Although other rare, isolated examples of similar formations have been reported to occur from Port Elizabeth to Tofu in Mozambique,[3] the recent discovery of numerous and closely spaced living stromatolites on the coastline south of Port Elizabeth appears to be extraordinary. While the Cape Morgan headland includes about 50 stromatolite colonies, each of about 3 m^2 on average,[3] the formations mapped thus far to the west of Cape Recife include 540 colonies, ranging in cover from <1 m^2 to >10 m^2.

Stromatolites are important because they are regarded as the oldest type of calcified formations in which cyanobacteria play a major role in the deposition of calcite crystals, either directly on the cell surface or, more commonly, through inclusion within the mucilaginous sheath that surrounds the cell.[4,5] The process requires $CaCO_3$ supersaturation of the water, which only occurs in today's marine environment in a few special circumstances, e.g. under states of hypersalinity, excess evaporation or mixing of extremely different water types.[3,6,7] As stromatolites date back in the fossil record at least 2.7–3.5 billion years, the study of the few extant colonies still remaining in the marine environment may be instrumental in understanding the hydrospheric conditions that prevailed at the onset of life on earth.

Southern African tufa stromatolites are regarded as unique in their nature, because they typically occur at the interface between freshwater seepage points and the marine penetration.[3] The closest type to those discovered on the southeast coast of South Africa are the tufa deposits recently reported from the southwest coast of Western Australia[8] and from the Giant's Causeway of Northern Ireland[9]. In Australia, most tufa formations are associated with inland spring discharges, whereas the South African types are all upper intertidal to supratidal in position, with strong and regular marine influence. By comparison, the Northern Ireland formations have a very limited thickness and are seldom inundated by marine waters.[9]

Here we report a preliminary account of the nature and extent of the newly discovered tufa stromatolites on the Eastern Cape south coast, including basic observations on their location, extent, structure and ecological functioning. More comprehensive investigations will be undertaken during the next few years within a project recently initiated at the Nelson Mandela Metropolitan University, in collaboration with the South African Environmental Observation Network and Rhodes University.

Materials and methods

Three different tufa pools of the 'barrage' type were selected for the preliminary study. These pools were located at representative sites along the coastline, from south of Cape Recife (Pool A, 34°02'42.13''S, 25°34'07.50''E) to Schoenmakerskop (Pool B, 34°02'28.23''S, 25°32'18.60''E) and on the eastern outskirts of Seaview (Pool C, 34°01'03.16''S, 25°21'56.48''E) (Figure 1). Pool A was located far from any residential disturbance, while Pool B was just below the village of Schoenmakerskop and likely affected by anthropogenic effluents. Pool C was situated just below the eastern residential extension of the village of Seaview and was, therefore, potentially most affected by leaching of anthropogenic effluents.

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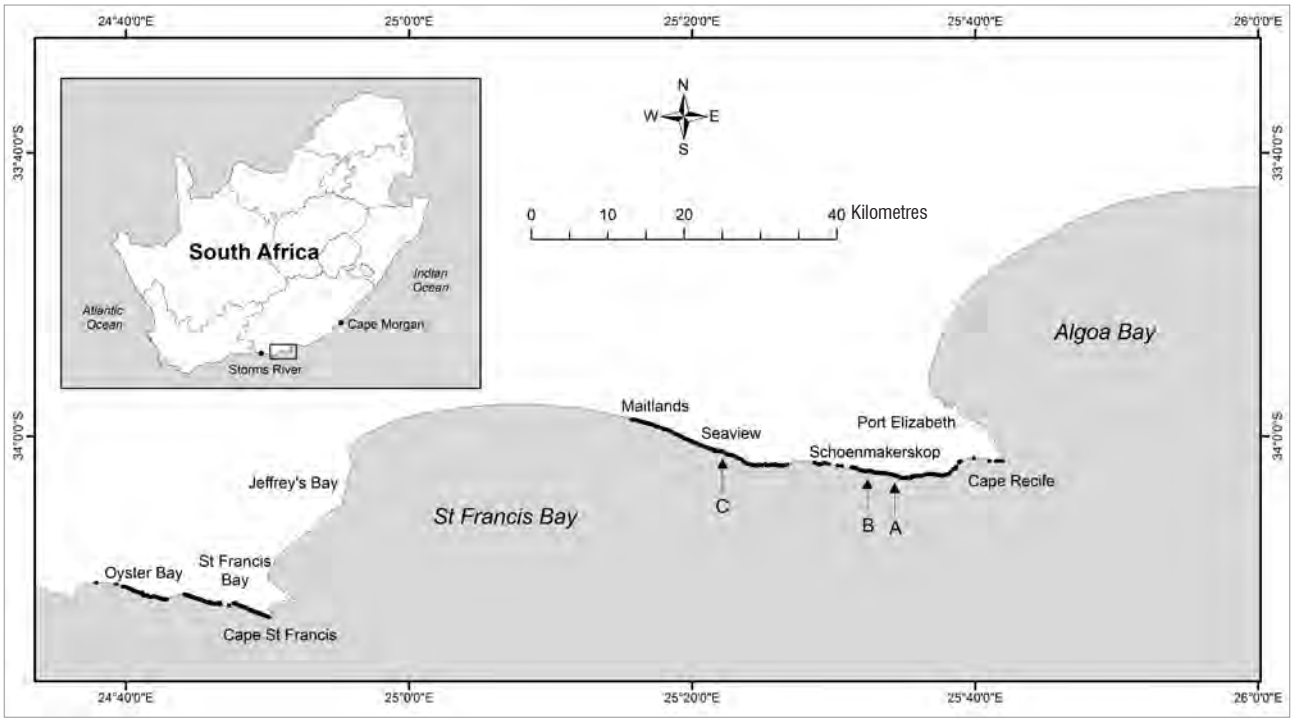

Figure 1: Study site map showing the location of freshwater seeps between Cape Recife and Oyster Bay (black dots). The location of the pools that were studied in more detail are indicated by A, B and C.

Continuous recording of temperature and salinity at the three selected pools was initiated in April 2013, using Star Oddi DST CT (http://www.star-oddi.com/) probes deployed in crevices at the bottom of each pool. Data were downloaded after 6 months. A full survey of the three pools was undertaken during 13–15 October 2013, when a range of physico-chemical parameters was measured using a YSI 6600-V2 multiprobe system (YSI Incorporated, Yellow Springs, OH, USA). These parameters include depth, temperature, salinity, conductivity, pH, dissolved oxygen and turbidity. Samples for nutrients and chlorophyll-*a* analyses were also collected from the water column, along with epibenthic macrofauna, macrophytes, algae and cores for microphytobenthic chlorophyll-*a* analysis.

Percentage composition of algal classes was estimated both in the water column and at the sediment surface using a bbe Moldaenke FluoroProbe (bbe Moldaenke, Schwentinental, Germany) fitted with a dual bentho-pelagic switch system. FluoroProbe is an in-situ fluorometer that uses the spectral fluorescence approach to discriminate among major algal classes on the basis of selective excitation of accessory pigments (which differ between the major taxonomic groups of algae).[10,11] Sediment cores to a standard depth of 50 mm were collected using a stainless-steel corer (17 mm internal diameter) pushed through the tufa deposits with a hammer. Cores were fractionated into 5 x 10 mm units and again extracted in 30 mL of 90% acetone solution for 48 h. Fluorescence readings were taken using a Turner 10-AU (Turner Designs, Sunnyvale, CA, USA) narrow-band system. Dissolved inorganic nitrogen and phosphorus were measured by determining the concentration of ammonium, nitrate and nitrite, and orthophosphate, respectively, using standard colorimetric methods.[12] Qualitative samples of benthic macrofauna, fish, micro- and macroalgae as well as macrophytes were collected at each pool, to identify key constituents of barrage pool communities.

For microbial diversity studies, approximately 0.8 g of stromatolite material from an upper and lower elevation of Pool B (Figure 1) was crushed using a sterile pestle and mortar in liquid nitrogen. Genomic DNA was extracted from crushed material using the PowerSoil DNA Extraction kit (MoBio Laboratories Inc., Carlsbad, CA, USA) and variable regions 4 and 5 of the bacterial 16S rRNA gene were amplified through a polymerase chain reaction (PCR) using fusion primers including a multiplex identifier tag as well as a nucleotide sequence required for the sequencing reaction. PCR products were analysed by 454-pyrosequencing (454 sequencing platform, Roche Products, Johannesburg, South Africa). Primer tags, sequence reads shorter than 200 bp, reads containing ambiguous nucleotides as well as reads with homopolymeric runs longer than seven nucleotides, were culled from the data set using Mothur[13]. The remaining 5733 sequences were classified using the Naïve Bayesian rRNA classifier algorithm[14,15] against the Ribosomal Database Project (http://rdp.cme.msu.edu/) and visualised in Xcel or MEGAN v4[16]. Sequence data were submitted to the Sequence Read Archives hosted at the National Center for Biotechnology Information, with accession number SRX478035.

Results and discussion

Range and extent of tufa stromatolites

Reconnaissance surveys have established that the newly discovered stromatolite fields of the southern Cape coast are spread across approximately 200 km of coastline from east of the Storms River mouth (34°1'12.14''S, 23°54'10.66''E) to west of Cape Recife (25°42'6.40''S, 34°1'45.15''E) (Figure 1). Thus they are among the most extensive tufa deposits currently occurring along any coastline (for comparison, Western Australia's deposits extend to 90 km[8] and those in Northern Ireland to 25 km[9]). A preliminary survey of a 10-km section of the coastline between Cape Recife and Maitlands revealed 341 freshwater seeps (a density of one in every 25 m), 90% of which exhibited stromatolite deposits. Of the 346 freshwater seeps (a density of one in every 41 m) in a 14-km section between Cape St Francis and Oyster Bay, about 58% formed tufa deposits of some kind. All active deposits are supratidal to upper intertidal in position and receive regular inflow of seawater, either as wave overtopping at high spring tide or wave splash during storm surges.

Structure of stromatolite formations

Both 'barrage pools' with downstream rimstone dams (Figure 2a) and 'waterfall deposits' (*sensu* Forbes et al.[8]) (Figure 2b) occur at regular intervals along the coastline, with the former type generally dominating both in terms of frequency and areal extent. Additionally, 'shell

conglomerates' form under higher flow conditions, when fast-flowing streams with a high $CaCO_3$ content end up directly onto a beach made up of shell grit, pebbles and sand, with calcification eventually cementing everything together. Barrage pools seem restricted to flat coastline areas, with very few developing along steep areas or where a longer distance between seep and the shore is involved. Conversely, waterfall deposits are most abundant on steep slopes, where freshwater seeps among rocks, from waterfalls or the occasional pump house (Figure 2b).

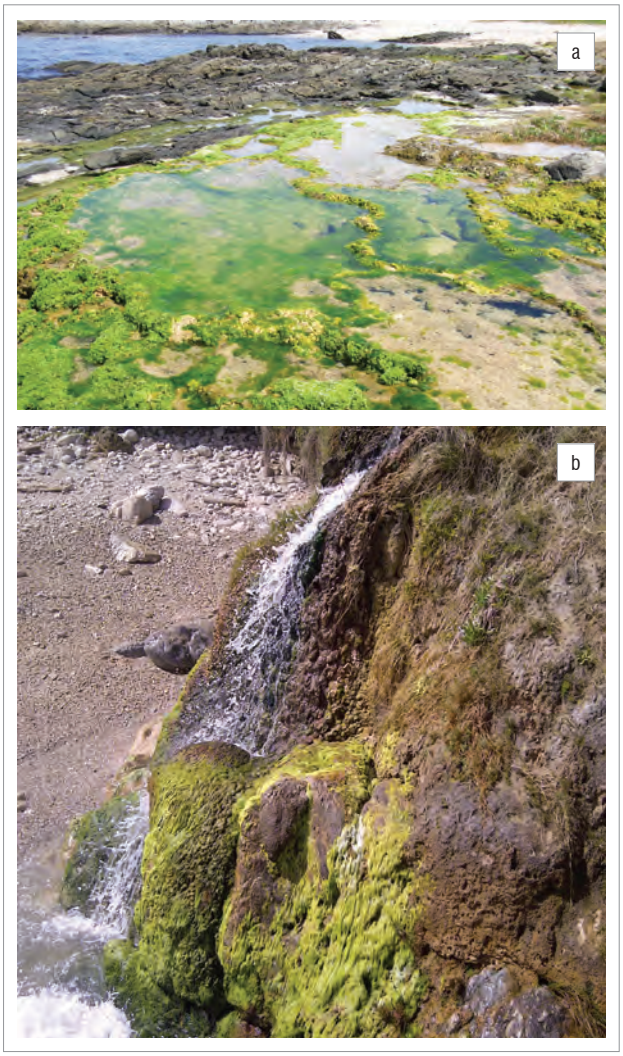

Figure 2: (a) Barrage pools with downstream rimstone dams exhibiting extensive chlorophitic growth at the marine interface at Schoenmakerskop on 15 October 2013 (photo: Lynette Clennell) and (b) waterfall deposits formed from a pump house outflow at Rebelsrus on 17 October 2013 (photo: Thomas G. Bornman).

All three mesofabric structures recognised earlier in the Cape Morgan fields[2,3] can be observed in the south coast formations. These structures include 'colloform growths', most often found underwater in the deeper parts of the barrage pools. They exhibit a typical multiconvex lamination with irregular margins probably imparted by bioerosion.[17] 'Laminar/columnar deposits' occur mainly at pool margins, as boundstone connecting bedrock outcrops to form rimstone dams with a thickness not unusually in excess of 1 m (Figure 2a). 'Pustular formations' are partially emergent and occur in shallow water around pool rims or as waterfall deposits (Figure 2b). Their morphology is irregular with disrupted lamination probably imparted by wave or current action. In all formations, the typical laminar structure observed previously in the Morgan Bay stromatolites is also found, with a micritic network composed mainly of radial laminae separated by thinner, longitudinal climax laminae.[2,3]

Stromatolite ecosystem dynamics

The barrage pools are regularly inundated with seawater (Figure 3), with frequency depending on the elevation of the formation. An increase in marine overtopping events was evident in winter, coinciding with frontal storm events. The variability in salinity is further increased by the volume of freshwater input. Pool A, although subjected to almost daily marine overtopping events in winter, also receives a high flow of fresh water that quickly replaces the seawater. The less frequent marine overtopping events in Pool B have a longer residence time as a result of lower freshwater inflow, thereby creating more stable conditions for biota (Figure 3).

Haloclines and stratification develop in calm conditions between the upper freshwater lid and the heavy saline water at the bottom (Table 1). From upstream seepage pools through to the barrage pools and the downstream pools a progressive trend of pH increase, from about 7.2 to about 9.1, can be observed, indicating CO_2 degassing and $CaCO_3$ precipitation.[3] Inside barrage pools, diurnal oxygen levels can reach extremes in excess of 345% or 27.87 mg/L supersaturation at the interface between sediment and water, as a result of photosynthesis by the benthic algal community (Table 1).

In the three pools sampled during the study, dissolved inorganic nitrogen was dominated overwhelmingly by the nitrate + nitrite fraction, accounting for 99% of the total nitrogen. A comparison of nutrient contents in barrage pools versus upstream seepage pools and downstream marine adjacent pools, shows that there was a high dissolved inorganic nitrogen content in the fresher waters, while no differences were observed in the distribution of dissolved inorganic phosphorus. There was, however, a gradient of increasing concentration from Pool A (Cape Recife) to Pool C (Seaview) (Figure 4), which appears to be correlated with the intensity of residential settlements in proximity of the pools. The nitrogen is in the most oxidised form and highest concentration in the upper pools, hence the nutrient source fuelling stromatolite productivity appears to be in the seepage water.

Total core (50 mm thick) benthic microalgal biomass in stromatolite tufa formations was in the range of 197–853 mg (chl*a*-eq)/m^2. Of this biomass, 80.5% was found in the upper 10 mm, decreasing sharply with depth to 7.6% at 20 mm, 5.5% at 30 mm, 4.4% at 40 mm and 2.2% at 50 mm (Figure 5). In terms of algal classes, in barrage pools benthic algae were dominated by chlorophytes with 51–55% of total epilithon, followed by cyanobacteria with 43–48% and diatoms with only 0–2.7% (Table 2). Chlorophyte activity appeared to peak immediately after marine overtopping events, subsiding during periods of no marine penetration. In the water column, phytoplankton classes were dominated by chlorophytes with 2–49% of the total, followed by cyanobacteria with 6–20%. 'Yellow substances', presumably from leaching dune vegetation, accounted for the bulk of the fluorescent signal (Table 2).

The bacterial assemblage associated with the upper and lower stromatolite formations sampled from Pool B was dominated by Cyanobacteria, Bacteroidetes and Proteobacteria (Figure 6a). Verrucomicrobia, Planctomycetes and Chloroflexi also occurred in significant numbers but were not as numerically dominant. The bacterial diversity profiles of stromatolites from Western Australia and the Bahamas, whilst containing large numbers of Bacteroidetes and Cyanobacteria, are dominated by Proteobacteria.[18-21] By contrast, stromatolites from Spain contained higher abundances of Cyanobacteria followed by Bacteroidetes and Proteobacteria.[22] Closer examination of the dominant cyanobacterial operational taxonomic units (OTUs) in the stromatolites from Pool B shows a single OTU, classified as *Lyngbya*, representing almost 60% of the cyanobacterial reads within the upper pool stromatolite (Figure 6b). By contrast, the cyanobacterial OTUs within the stromatolite from the lower pool are more evenly distributed with dominant genera of *Lyngbya, Plectonema,* and *Leptolyngbya*. With respect to the Proteobacteria found within the stromatolites in this study, the bulk of the reads was assigned to the Alphaproteobacteria (Figure 6b). Of interest is the occurrence of photosynthetic genera in the lower pool stromatolites (e.g. *Rhodobacter* and *Erythrobacter*), as well as the distinctive difference in distribution patterns of the proteobacterial

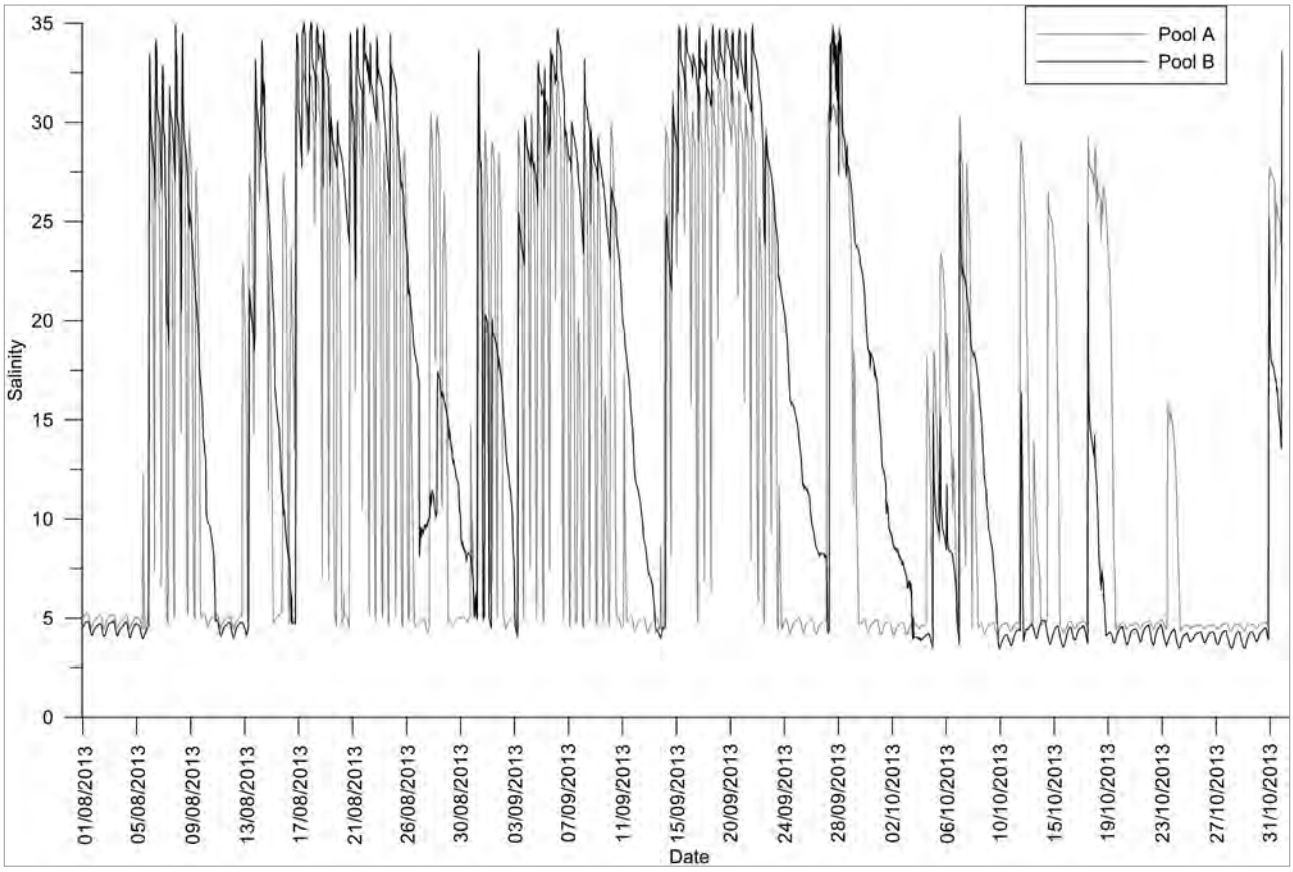

Figure 3: Hourly salinity readings from the bottom of Pools A and B from 01 August to 31 October 2013. The lower limit of the instrument is ~5.

Table 1: Range of physico-chemical parameters in stromatolite pools measured during October 2013

	Temperature (°C)	Salinity	Specific conductivity (mS/cm)	pH	Dissolved oxygen (mg/L)	Turbidity (NTU)
Pool A	18.84–19.76	0.93–6.17	1.826–10.85	7.26–8.1	8.46–16.29	0.6–3.1
Pool B	23.05–24.36	0.77–5.19	1.525–9.271	8.1–8.61	13.11–20.56	0.5–1.2
Pool C	20.78–24.99	1.05–21.61	2.051–34.33	8.01–9.09	7.03–27.87	0.2–1.5

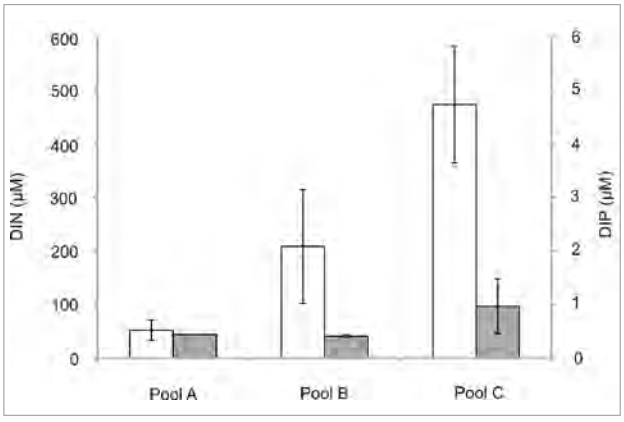

Figure 4: Dissolved inorganic nitrogen (DIN, white bars) and phosphorus (DIP, grey bars) concentrations (±SD) measured in stromatolite pools during October 2013.

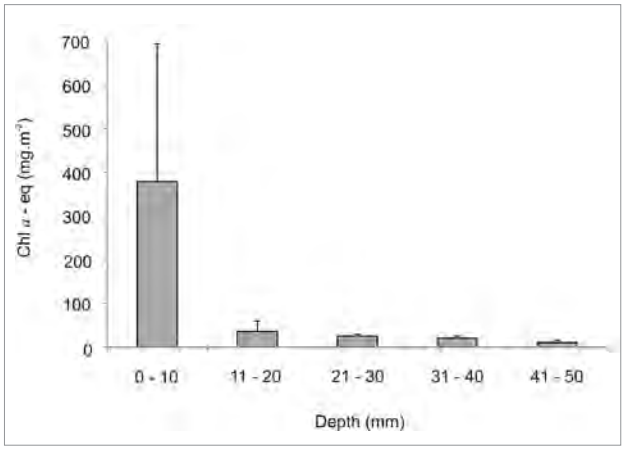

Figure 5: Average chlorophyll-*a* profile of cores (+SD) taken at stromatolite Pools A–C during October 2013.

Table 2: Relative contribution (%) of total biovolume for algal classes discriminated by the bbe Moldaenke FluoroProbe in the water column and at the sediment surface of stromatolite Pools A–C during October 2013

		Sediment surface			Water column				
		Chlorophyta	Cyanophyta	Diatoms†	Chlorophyta	Cyanophyta	Diatoms†	Cryptophyta	Yellow substances
Pool A	Lower	57	43	0	54	3	14	4	25
	Middle	52	48	0	2	20	0	0	78
	Upper	61	39	0	1	26	0	0	73
Pool B	Lower	56	44	0	58	3	0	2	37
	Middle	56	44	0	49	9	0	0	42
	Upper	52	48	0	26	25	0	0	49
Pool C	Lower	63	35	2	6	29	0	0	65
	Middle	54	43	3	48	6	0	0	46
	Upper	60	36	4	5	7	0	0	88

†*Diatoms: Bacillariophyta*

genera between the two stromatolite samples. Stromatolite formation is hypothesised to be cyclical in nature, consisting of the formation of microbial mats dominated by Cyanobacteria, which leads to the rapid production of exopolymeric substances which bind sediment granules. This formation is then followed by a hiatus characterised by a decrease in sedimentation and an increase in mineral precipitation, during which heterotrophic bacteria increase in abundance.[23-25] Both samples examined in this study had high abundances of Cyanobacteria and heterotrophic Bacteroidetes and Proteobacteria (Figure 6a). The lower pool sample exhibited higher relative abundances of Cyanobacteria and phototrophic purple bacteria, suggesting that it is still within the active sedimentation phase, whilst the increased heterotrophic component in the upper pool indicates a possible transition from sedimentation to hiatus phase.

Plants and macroalgae from the tufa stromatolite environments were sampled in the period between overtopping events, during which the systems were highly stratified. The resulting salinity gradient allowed for the occurrence of eight different macroalgal species, ranging from marine to typically freshwater species within the span of a single barrage pool complex (ca. 10–30 m). *Spirogyra* sp. was common near the inflow point to the barrage pools, while *Ulva intestinalis* and *U. prolifera* were particularly abundant near the outflow where the influence of the sea was greater. Overall, the macroalgal species recorded in the barrage pool systems show strong similarities to the macroalgae of Eastern Cape estuaries.[26] Similarly, the macrophytes associated with the barrage pool systems included typical freshwater wetland species[27,28], as well as plants normally associated with Cape Estuarine Saltmarsh vegetation[29]. Based on the macrophyte species data, the tufa stromatolite environment appears to have more in common with estuarine systems than with freshwater wetlands.

With few exceptions, metazoan communities have generally been regarded as incompatible with the very existence of stromatolites.[6,30,31] However, a diverse macrofauna community characterises the stromatolite pools of the Eastern Cape, with euryhaline species occurring throughout the system and typical freshwater and marine species alternating with each other for pool dominance, or even coexisting in different layers of each pool. A case in point is provided by the true crabs (brachyurans), which exhibit abundances of a typical freshwater species, *Potamonautes perlatus* (Figure 7a), next to a typical marine intertidal dweller, *Cyclograpsus punctatus* (Figure 7b). The two can be found occasionally in the same barrage pool, but at different depths, with the marine species generally under rocks in the deeper parts where the denser saline water sinks. Mass mortality of *C. punctatus* has been observed when pools become fresh water dominated as a result of an imbalance of flows from the two sources or when turbulent conditions force mixing and the breakdown of haloclines in the water column. Stromatolite pools appear to be colonised by a unique suite of common fish species, most of which are usually associated with estuaries. It is unknown to what extent these unique habitats serve as a fish nursery. In the fresher pools, there are often frogs and dense aggregations of tadpoles.

Most extant tufa stromatolite deposits around the world are already protected. Those occurring on the southwest coast of Western Australia, resembling most closely the South African type, are registered as 'Threatened Ecological Communities' with the status of endangered

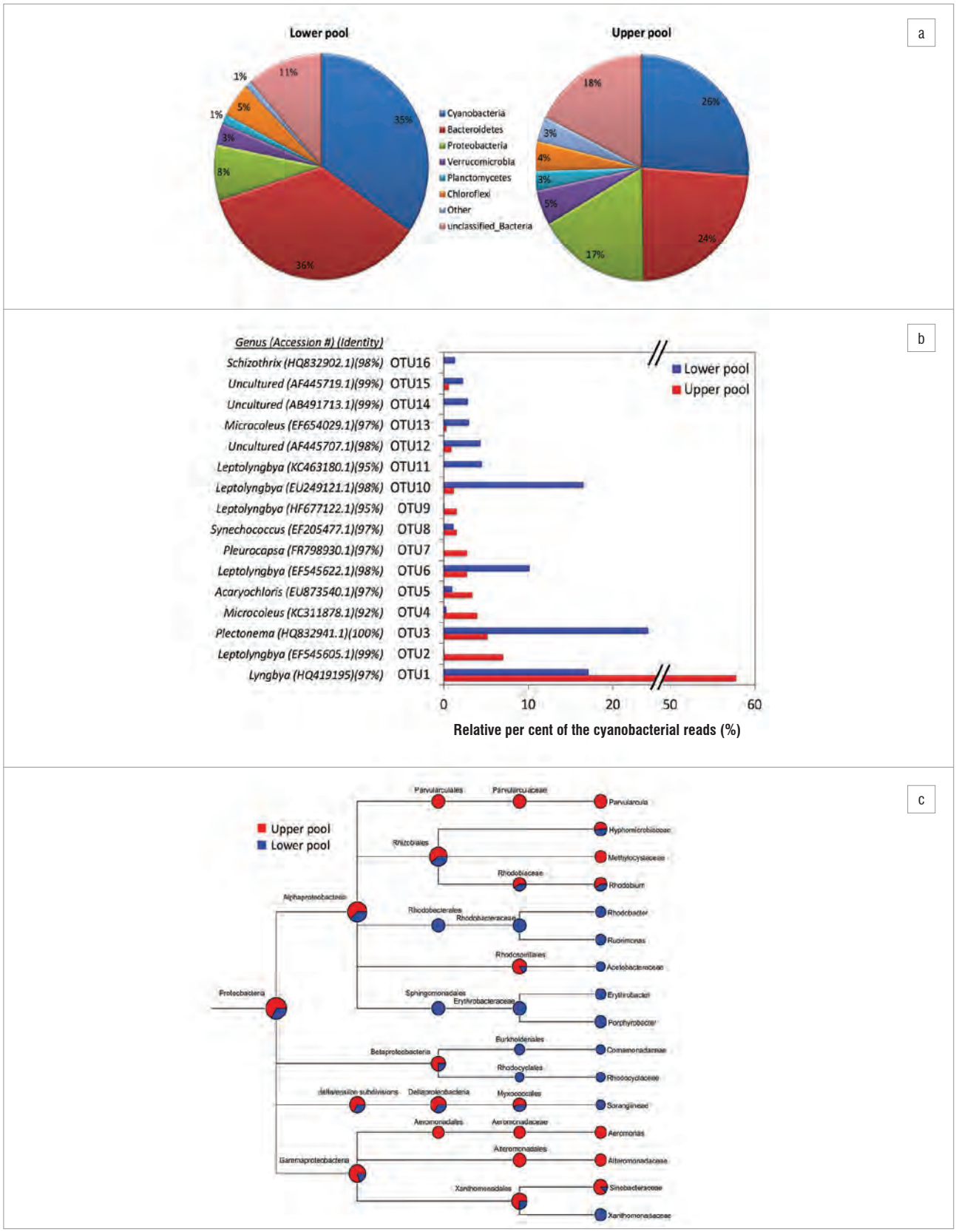

Figure 6: Microbial diversity in stromatolites from the upper and lower reaches of Pool B based on the assignment of 16S rRNA sequences to taxonomic rankings against the Ribosomal Database Project. (a) Relative abundance of the reads assigned to the taxonomic ranking of phylum. (b) Relative abundances of the ten most dominant cyanobacterial operational taxonomic units (OTUs) determined at a distance level of 0.03 using Mothur. BLAST analysis of the OTUs against the NCBI database was done to ascertain the closest genus to which the OTU could be classified (the accession number of the NCBI-deposited sequence and the percentage identity of the sequence match against a deposited sequence is indicated in brackets). (c) Taxonomic breakdown of the reads assigned to the phylum Proteobacteria using MEGAN software (data sets were normalised for comparative purposes).

ecosystems.[8] Special 'Ecological Water Requirements' have been allocated to them, in order to secure their long-term sustainability. Because of threats of residential encroachment, groundwater extraction, coastal development and invasion by alien species, it would be advisable for South Africa to follow the Australian example and place all tufa stromatolite formations under statutory protection.

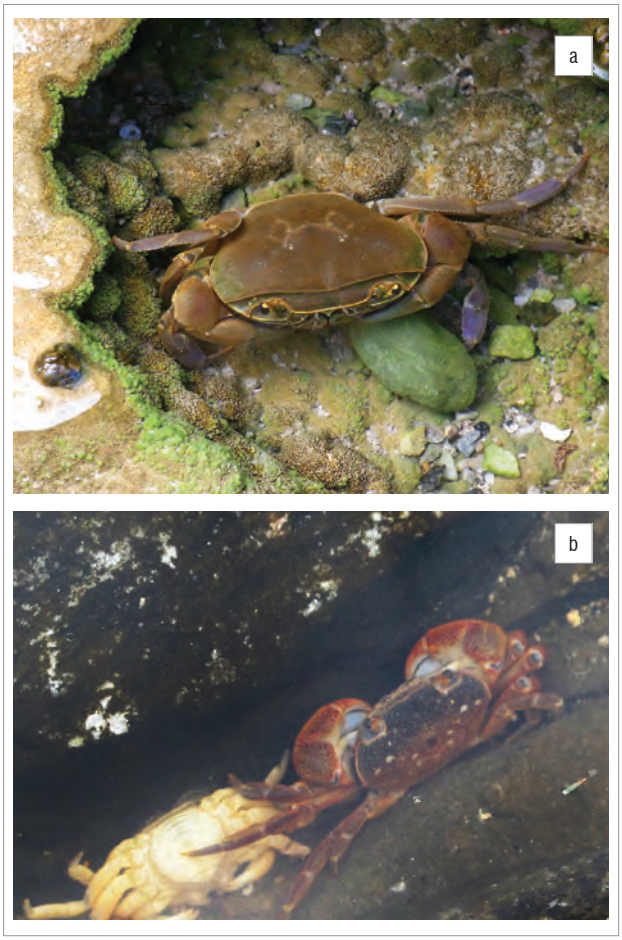

Figure 7: (a) *Potamonautes perlatus* among stromatolite formations in the upper freshwater layer of the barrage pool and (b) *Cyclograpsus punctatus* in the deeper layer of the barrage pool, with individuals dying of osmotic stress as freshwater seepage inflow increases and salinity decreases (photos: Lynette Clennell, Schoenmakerskop, 6–13 October 2013).

Acknowledgements

This work is based on the research supported by the South African Research Chairs Initiative (SARChI) and the South African Environmental Observation Network (SAEON) of the Department of Science and Technology (DST) and the National Research Foundation (NRF) of South Africa. Any opinion, finding, conclusion or recommendation expressed in this material is that of the authors and the NRF does not accept any liability in this regard. Microbial diversity studies were funded by a grant from the Rhodes University Sandisa Imbewu Fund.

Authors' contributions

R.P., T.B. and P-P.S. were the project initiators and co-ordinators, and were responsible for the sampling design and the compilation of physico-chemical and algal/macrophyte components; N.A.F.M. was responsible for the sampling design and sample collection and analysis and compiled the physico-chemical graphs; R.D. and G.M. were responsible for the sampling design and microbial analyses; N.S. was responsible for sample collection and fish analysis; and N.P. was responsible for sample collection and invertebrate analysis.

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Open access in South Africa: A case study and reflections

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DATES:
Received: 01 Apr. 2014
Revised: 11 June 2014
Accepted: 08 July 2014

KEYWORDS:
academic publishing; scholarly communication; journals; online presence

HOW TO CITE:
Czerniewicz L, Goodier S. Open access in South Africa: A case study and reflections. S Afr J Sci. 2014;110(9/10), Art. #2014-0111, 9 pages. http://dx.doi.org/10.1590/sajs.2014/20140111

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In this paper, we locate open access in the South African higher education research context where it is, distinctively, not shaped by the policy frameworks that are profoundly changing research dissemination behaviour in other parts of the world. We define open access and account for its rise by two quite different routes. We then present a case study of journal publishing at one South African university to identify existing journal publishing practices in terms of open access. This case provides the springboard for considering the implications – both positive and negative – of global open access trends for South African – and other – research and researchers. We argue that academics' engagement with open access and scholarly communication debates is in their interests as global networked researchers whose virtual identities and online scholarship are now a critical aspect of their professional engagement.

Introduction

Many South African researchers are unfortunately encountering open access for the first time in negative terms: through expensive article-processing charges (APCs), through the inaccurate definition of gold open access as 'author-pays' and through the discourse of regulatory compliance which is such anathema to the ethos of academic freedom and academic rigour which all scholars hold dear. Not surprisingly, researchers' responses range from downright negative to extremely sceptical, and there is a general lack of clarity regarding either the value proposition or the practical effects.

We provide a case study of journal research publishing from one South African university, showing where and how publishing has taken place over a 6-year period, particularly in terms of whether and how open-access publishing occurs. The case provides an opportunity to reflect on the realities, opportunities and challenges for South African research and researchers.

What is open access?

Open access as a concept has been in existence for over a decade, officially defined for the first time in the Budapest Declaration of 2001.[1] While differences in definition do exist, usually because of specific foci or interests, Peter Suber's is generally regarded as definitive: 'Open access (OA) literature is digital, online, free of charge, and free of most copyright and licensing restrictions'[2]. There are numerous misconceptions about what open access means, the most common being that copyright is lost or given away. The opposite is in fact true in most cases, as true open-access publishing is based on legally open licences, a form of copyright permission in which the author(s) retains copyright and specifies permitted uses; in this way a publisher can still publish the work without owning the copyright. The most widely used licences are Creative Commons licences, all of which require author attribution.[3] Open licences are, in fact, more aligned with academic freedom and agency than traditional copyright agreements because authors keep their copyright and determine licences for use on their own terms; under conventional agreements researchers invariably sign away their copyright to publishers.

There are two types of open access: gold and green. Gold open access means publishing in an open-access journal so that the article is freely and openly available from the time of publication (as indeed is the entire journal), and green open access means making a version of the published article (often a pre-print or post-print) available through a repository, sometimes after an embargo period.

Reasons for open access

Open access as an issue came into existence for two main reasons: the expense of subscriptions to bundled journal databases (known as 'the serials crisis'),[4] and, simultaneously, a movement arguing for publically funded research to be made available freely to the public who had paid for it (premised on the existence of the Internet making this possible in ways previously impossible). These two reasons are aligned with different philosophical approaches: one economic and the other democratic.

As access to journals has moved from print to online, there has been a move from accessing individual titles to accessing bundles, whether or not all the individual titles included in that bundle are wanted. Over the past decades, prices have skyrocketed, with average spending on journals rising by 302% from 1986 to 2004.[5] Individual titles are extremely expensive, as seen in Table 1.[5]

Researchers have been largely protected from this crisis because they have no direct involvement in journal purchasing, which is undertaken through institutional libraries. They therefore have no immediate reason to exert pressure on publishers to lower prices or to adopt business practices that are more favourable to researchers and the public.

The economic model accepts the concept of knowledge as a commodity; here the primary interest of commercial publishers is to their shareholders rather than to the research community, and in this they are successful. Despite the purported disruption to the industry, the main academic publishers' operating margins rose to a 39% profit margin in 2013.[6] However, all academic journal publishers are not the same in terms of ownership or intent, and

this is reflected in their prices: for-profit journals are priced 10–15 times higher than not-for-profit publisher titles.[7]

Table 1: The average 2013 price† (per title) for online journals in the Web of Science index

Discipline	Average price per title (USD)
Chemistry	3906
Physics	3500
Astronomy	2308
Biology	2163
Engineering	1942
Botany	1885
Zoology	1884
Health science	1661
Geology	1513
Maths and computer science	1366
Technology	1318
Food science	1284
General science	1202
Agriculture	1120
Geography	965
Social sciences	818
Education	778
Psychology	774
Military and naval sciences	751
Business and economics	746
Sociology	721
Political science	620
Anthropology	589
Recreation	581
Law	555
Library science	493
General works	472
Arts and architecture	455
History	433
Philosophy and religion	426
Language and literature	361
Music	278

Table adapted from Bosch and Henderson[5] (source: LJ Periodicals Price Survey 2013).
†Prices represent print + free online, online only and the first tier of tiered pricing.

A parallel argument for open access is founded on the democratic principles of knowledge creation and dissemination, supported by new technologies, and generally premised on the concept of knowledge as a commons. This is exemplified in the Budapest Declaration[1]:

> *An old tradition and a new technology have converged to make possible an unprecedented public good. The old tradition is the willingness of scientists and scholars to publish the fruits of their research in scholarly journals without payment, for the sake of inquiry and knowledge. The new technology is the internet. The public good they make possible is the world-wide electronic distribution of the peer-reviewed journal literature and completely free and unrestricted access to it by all scientists, scholars, teachers, students, and other curious minds.*

Whichever the approach, questions are being asked about the role of publishers in a digital age, especially in academic publishing where scholars provide unpaid-for services through the undertaking of the research itself, the peer-review process and often the editing of the research outputs too; the Cost of Knowledge movement[8] is one example of researchers engaging forcefully with commercial publishers on these issues. Many publishers have responded by changing their focus from content to services, and many universities already provide publishing services for journals and even books. In short, there is a growing consensus, shared by a diverse group of stakeholders, that the traditional scholarly communication system is 'broken' and not in sync with the changing practices of researchers in a digitally mediated age. That open access will form part of a scholarly communication system in transition is not in dispute – the questions are 'how?' and 'in whose interests?'

The value proposition

The value of open access sometimes gets lost in bureaucratic squabbling and regulatory nitpicking, but generally there is little dispute about its merit. Open access is beneficial for research universities (their rankings and impact measures improve); for funders whose missions of creating and sharing knowledge are realised; for the research process which sees efficiencies, immediacy and transparency; and for the development imperatives of universities and societies at large for which the scholarly resources of universities are made available to all.[9]

Open access is essential for visibility and has proven valuable for citations: in a meta-study of 35 studies surveyed, 27 showed a citations advantage (the percentage increase ranged from 45% to as high as 600%).[10] For those from less developed countries, the effect is more profound: the influence of free access on citations has been shown to be twice as large for the poorer countries in the developing world compared to richer countries, as measured by per capita gross national income.[11]

Open access is now a mainstream issue in the global north, and plays a central role in Latin America. What of South Africa?

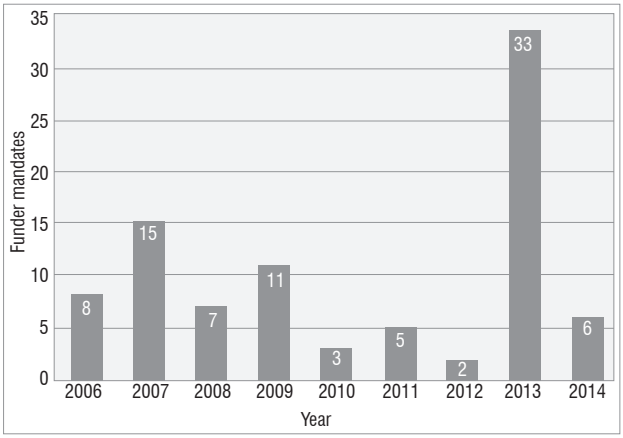
The current global policy environment

These issues might have remained of general interest in ways that did not affect local researchers but for the dramatic change in the funder policy environment during 2013 which saw a major shift to open-access publishing as a condition of grant funding. The Registry of Open Access Repositories Mandatory Archiving Policies (ROARMAP)[12] lists 90 funders who had such a mandate at the time of writing: these include research councils, government agencies (many US departments, for example) as well as the entire European Commission (Figure 1).

These mandates have given rise to consternation, debate, some jubilation, some anxiety and a great deal of confusion. The academic fear is largely that researchers are being told where to publish, which in reality they are not: an analysis of funder open access policies around the world showed that only in one case was publishing in an open-access journal a requirement; in all other cases, depositing in a repository was an option (the Finch report in the UK has since also shifted to a green/gold choice).[13] Wallander and colleagues[13] analysed 48 mandatory

funder policies and found that 33 required green (repository based) open access; 14 required either green or gold, and only one preferred gold (i.e. open-access journals) – but only 'where available'.

These trends in our local context are usefully explored through the case of one South African university, shaped by researchers' nexus in both national and international networks.



Adapted from ROAR Funder Mandate Graph (May 2014)

Figure 1: The number of funder mandates for open-access publishing per year recorded in the Registry of Open Access Repositories Mandatory Archiving Policies (ROARMAP).[12]

The South African context

South African researchers face challenges in terms of funding: the average research and development (R&D) intensity (R&D as a percentage of GDP) for Organisation for Economic Co-operation and Development (OECD) countries was 2.4% in 2009, while few developing countries had reached 1%.[14] All researchers have problems accessing research[15]; in the African and South African context, the limited availability of research is a serious problem,[16] one even worse for researchers not affiliated to universities and research institutions. The cost of access is already so high, and the situation is exacerbated by worsening exchange rates: it would be dramatically worsened by the proposed VAT on digital media which would see university libraries' purchasing power reduced by a further 40%.[17]

Researchers also face challenges in terms of the dissemination and visibility of their research: a study reported in 2013[18] showed the almost entire invisibility via a Google search of South African research in an area where it is known that much research has been undertaken. The visibility issue is ironically about to become much worse when the funder policies requiring open access in the global north are implemented, and what is found online (by researchers who expect everything to be found online[19]) is research from the global north and not local, southern research. Geo-political knowledge inequality, already an acknowledged problem[20,21], is about to be exacerbated through poor online access and limited discoverability as well as through new bottlenecks to participation[9,22]. Online visibility is not a form of vanity, it is now an essential requirement for participation in knowledge creation networks.

At the time of writing, South African research funders either in or outside of government structures – including the National Research Foundation (NRF), the Department of Science and Technology (DST) and the Department of Higher Education and Training (DHET) – had not taken a definitive stand on open access and no such similar open-access publishing requirement is exerted on South African funded research. Universities therefore make strategic decisions at the institutional level, shaped partly by global research funding contracts and individual institutional missions. A handful of South African universities have adopted open access policies, including the University of Cape Town whose policy sets the scholarly dissemination default to open, encourages all scholarship to be made available, and requires journal articles, theses and dissertations to be deposited,[23] while the University of Pretoria and Stellenbosch University have led the way in developing open repository infrastructure and content. A notable exception to this general lack of engagement to date is the Academy of Science of South Africa's SciELO SA open-access publishing platform[24] funded by DST and endorsed by DHET. Through this platform, 40 South African accredited journals were openly accessible at the time of writing, with many more under evaluation for inclusion on the platform. Journals on the SciELO platform are also indexed by the Web of Science. The increase in visibility for these journals has been dramatic in several cases. One such journal reports increased readership since going open access: 'To compare… an average issue in say 2007 with an issue now: we would print 200 copies and send perhaps 100 to subscribers and journal affiliates and sell perhaps 50 out of hand: a total circulation of 150. Now our issues collectively get about 10 000 article hits a year.' (Bank A 2014, written communication from journal editor, April 24).

Open access is making a difference to journal editors in South Africa, but what about academics? Are South African researchers publishing in open-access journals?

A case study: Journal publishing at the University of Cape Town

An analysis was undertaken of the top 20 journals in which the University of Cape Town's (UCT) research output was published. This analysis is of particular interest given that half of UCT's journal output was in these 20 journals.

The focus of the analysis was on the form of publishing and the open-access status of the top journals in which UCT publishes. Information about the open-access status of journals was obtained from the Directory of Open Access Journals[25] on 17 May 2013. All costs were converted to South African rands (ZAR) using the Google exchange rate calculator[26] on 11 June 2013 (rates: GBP1 = ZAR15.71; USD1 = ZAR10.04; EUR1 = ZAR13.36). Costs per issue were calculated by dividing the annual subscription cost by the number of issues per year. The analysis is based on Mouton's[27] 'UCT Research Performance Assessment' which covers the years 2006 to 2011. While this is an institution-wide report it is skewed towards the sciences; the output of the humanities is not entirely reflected as a majority is published as books and monographs, and disciplines (such as Computer Science) which favour conference proceedings are also not well represented. It is of note that the sciences and health sciences make up 60–70% of the total output of the sample.

The top 20 journals

We examined the top 20 journals in which UCT research was published between 2006 and 2011[27] and analysed the types of journals in which the work was published (open access, subscription or hybrid, at the time of analysis in 2013) and the publishers of these journals. The costs – in terms of subscription fees or APCs – are provided where it was possible to ascertain them (the costs provided are those at the time of analysis in 2013). Table 2 summarises this information.

Figure 2 shows the types of journals in the top 20 by category.

Figure 3 shows the number of articles published in the top 20 journals (equivalent to half of the total output) according to type (note that the number of full papers for each journal was obtained from Mouton's[27] report 'UCT Research Performance Assessment' presented at the UCT Research Indaba of 08 May 2013).

Accredited lists

All of the top 20 journals appear on accredited lists: 19 are indexed in Web of Science and 1 is indexed in the International Bibliography of the Social Sciences (IBSS) (as of 2013). A total of 8 journals are on the DHET-accredited list (some of which are also on the Web of Science), and 4 journals are available through the open-access platform SciELO SA – *South African Medical Journal, South African Journal of Science*, *South African Journal of Surgery* and *Water SA*.

Table 2: Top 20 journals: case study summary table

Journal	Publisher	Number of full papers (relative proportion)	Listed on the DOAJ as open access? (Creative Commons licence)	Not open access/no open-access options	Hybrid?	Article processing charges for open-access articles	Subscription costs and number of issues (per year) (†supplied by UCT libraries)
1. *South African Medical Journal*	Health and Medical Publishing Group and the Medical Association of South Africa	293 (10.28%)	Yes (CC-BY-NC)			None	ZAR1044 (print) 12 issues
2. *African Journal of Marine Science*	NISC and Taylor & Francis	120 (4.21%)			Yes	ZAR10 000	ZAR1495† (print + online) 4 issues
3. *PLoS One*	PLOS	104 (3.65%)	Yes (CC-BY)			USD1350	n/a
4. *Monthly Notices of the Royal Astronomical Society*	Oxford Journals	91 (3.19%)			Yes	GBP1450 USD2550 EUR2175	GBP5213 (print + online) GBP4656 (online) GBP4745 (print) 12 issues
5. *Minerals Engineering*	Elsevier	82 (2.88%)			Yes	USD2500	USD2145 (print) USD1429.87 (online – 5 users) 15 issues
6. *South African Journal of Science*	Academy of Science of South Africa	76 (2.67%)	Yes (CC-BY)			? (not indicated on site)	ZAR1000 (print) 6 issues
7. *AIDS*	Lippincott Williams & Wilkins	64 (2.25%)			Yes	USD3000	USD3155 (not stated what this includes) 18 issues
8. *Ostrich*	NISC and Taylor & Francis	57 (2.00%)			Yes	ZAR10 000	ZAR1135 (print + online) ZAR995 (online) 3 issues
9. *British Journal of Sports Medicine*	BMJ Publishing Group	55 (1.93%)			Yes	GBP1950	(institutional price not on site) 18 issues
10. *Physical Review D*	American Physical Society	54 (1.90%)			Yes	USD1700	USD7695 (online) USD10505 (print + online) for large research institutions 24 issues
11. *South African Law Journal*	JUTA	50 (1.76%)		Yes		n/a	ZAR939 4 issues
12. *International Journal of Tuberculosis and Lung Disease*	International Union Against Tuberculosis and Lung Disease	50 (1.76%)		Yes		n/a	EUR5000 (print + online) 300 – 700 end users 12 issues
13. *Lancet*	Elsevier	49 (1.72%)			Yes	USD5000	EUR1604† (print) USD1629† (online) 52 issues
14. *South African Journal of Surgery*	Association of Surgeons of South Africa, Health and Medical Publishing Group and the Medical Association of South Africa	47 (1.65%)	Yes (CC-BY-NC)			None	n/a 4 issues
15. *JAIDS – Journal of Acquired Immune Deficiency Syndromes*	Lippincott Williams & Wilkins	46 (1.61%)			Yes	USD3000	USD2588 (print + online + iPad) 15 issues
16. *South African Journal of Psychology*	Psychological Society of South Africa	46 (1.61%)		Yes		n/a	Not stated 4 issues
17. *Marine Ecology – Progress Series*	Inter-Research	44 (1.54%)			Yes	(1) Free access: EUR500 (1–8 pages,) EUR800 (9–14), EUR1000 (>14) (2) Open access under CC-BY licence: EUR900 (1–8), EUR1200 (9–14), EUR1400 (>14)	EUR5714† 25 volumes
18. *Journal of Infectious Diseases*	Oxford Journals	44 (1.54%)		Yes		n/a	GBP2153 (print + online) GBP1721 (online) – for 1000-9999 end users 24 issues
19. *CNS Spectrums*	Cambridge University Press	42 (1.47%)		Yes		n/a	Not stated 12 issues
20. *Water SA*	Water Research Commission	41 (1.44%)	(free online)	Yes (free online)		n/a	Not stated 4 issues

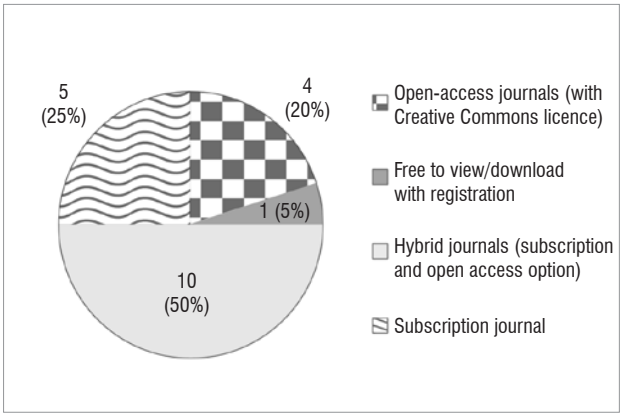


Figure 2: Number (%) in each category of the top 20 journals in which authors from the University of Cape Town publish.

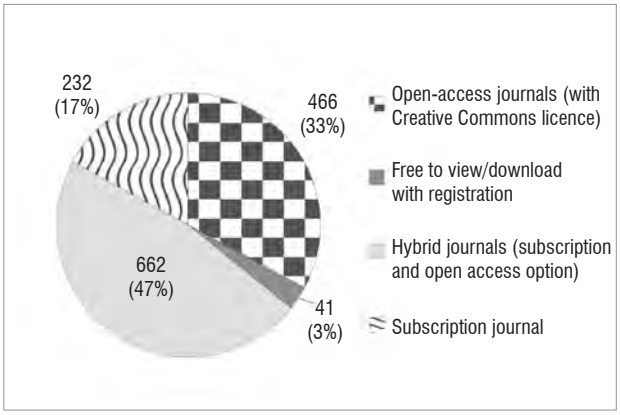


Figure 3: Number of articles published in the top 20 journals in which authors from the University of Cape Town publish (by category).

Free online and open access

Of the 20 journals, 5 are freely available online: *South African Medical Journal*, *PLoS One*, *South African Journal of Science, South African Journal of Surgery* and *Water SA*. The publications by UCT authors in these five journals make up 19.69% of the total relative proportion of all UCT articles published. Four of these five journals are available through SciELO SA, four are formally open-access journals (i.e. freely available online and openly licensed under Creative Commons licences) – 18.25% of the total relative proportion of UCT articles are published in these journals – and one is freely available online with registration (1.44% of the total relative proportion of UCT articles are published in this journal).

Of the open-access journals, two are available under Creative Commons Attribution (CC-BY) licences – which means anyone can 'distribute, remix, tweak, and build upon' the work provided that the original author is credited.[3] Others can profit from the work, as there is no restriction on commercial use. The other two are available under the Creative Commons Attribution Non-Commercial (CC-BY-NC) licence – which means anyone can 'distribute, remix, tweak, and build upon' the work provided the original author is credited and the work is not used commercially.[3]

There are no costs for publishing in three of these four open-access journals, and, interestingly, all three journals are local. UCT authors published 416 full papers (i.e. 14.6% of the total) in these three no-costs-for-publishing journals.

The remaining open-access journal has an APC. *PLoS One* charges an APC of ZAR13 800.65 per article. With 104 full papers published in *PLoS One*, the total possible cost (disregarding fee reductions, fee waivers, etc.) is ZAR1 435 267.60. It would require an interrogation of each of these articles to find out what the actual costs were for these 104 papers, given that UCT authors do apply for waivers and reductions, and also that APCs may be paid by co-authors from countries for which there are block grants for APCs.

The publishers of these open-access journals are: Health and Medical Publishing Group and the Medical Association of South Africa (co-publishers) (*South African Medical Journal);* PLOS (*PLoS One*); Academy of Science of South Africa (*South African Journal of Science*); Association of Surgeons of South Africa, Health and Medical Publishing Group and the Medical Association of South Africa (co-publishers) (*South African Journal of Surgery*) and the Water Research Commission (*Water SA*). It is of note that these open-access journals are largely published by associations rather than for-profit publishing companies.

Hybrid journals

There are 10 journals (i.e. half) which are available via subscription with an option to make a specific article openly accessible – that is, hybrid journals. These journals are *African Journal of Marine Science*, *Monthly Notices of the Royal Astronomical Society*, *Minerals Engineering*, *AIDS*, *Ostrich*, *British Journal of Sports Medicine*, *Physical Review D*, *Lancet*, *JAIDS* and *Marine Ecology – Progress Series*, and are published by NISC and Taylor and Francis (co-publishers), Oxford Journals, Elsevier, Lippincott Williams & Wilkins, BMJ Publishing Group, American Physical Society and Inter-Research (Table 2).

The subscription costs *per issue* (where it was possible to ascertain) for these journals range from approximately ZAR373 to ZAR6919 (for print + online); ZAR418 to ZAR6298 (for print only); ZAR320 to ZAR6180 (for online only); ZAR1763 (for print + online + iPad) and ZAR1791 to ZAR3100 (not stated what this cost includes). Therefore, an overall cost range per issue is ZAR320 to ZAR6919. In addition to subscription costs, all of these hybrid journals also charge APCs, ranging from ZAR10 000 to ZAR51 113 per article, for open-access articles.

The publications by UCT authors in these 10 journals make up 23.23% of the total relative proportion of UCT articles published.

It is these journals which can be described as 'double dipping', as they benefit from both subscriptions and APCs.

Subscription access

Five of the journals are available via subscription only with no open-access option. These journals are *South African Law Journal*, *International Journal of Tuberculosis and Lung Disease*, *South African Journal of Psychology*, *Journal of Infectious Diseases* and *CNS Spectrums*. These journals are published by a mixture of publisher types: JUTA, International Union against Tuberculosis and Lung Disease, Psychological Society of South Africa, Oxford Journals and Cambridge University Press.

The subscription costs (where it was possible to ascertain) for these journals range from ZAR234 (for print only) to ZAR5652 (print and online for 300–700 end users) per issue. Three of the journals do not provide a subscription cost on their website and the costs were also not available through the UCT library. The publications by UCT authors in these five journals make up 8.14% of the total relative proportion of UCT articles published.

Observations

The case study of publishing at UCT provokes several interesting points including the extent of existing publishing in open-access journals, the limited requirement for APCs, the nature of hybrid journals and the possibilities for green route open access in the South African context.

Open-access journals

The finding that nearly a quarter of the top 20 journals are open-access journals is of note given that the South African policy environment does not require or encourage open access. Articles in these open-access journals account for a third of those published in the top 20 journals. It is

perhaps unsurprising that these are all science-aligned journals as open-access publishing has been more predominant in the sciences to date.[28]

It may come as a surprise that of these open, freely available journals, only one – *PLoS One* – charges an APC. This finding means that of the open-access articles published ($n=466$), only 23% (104) required APCs. The misconception that open access equals 'author pays' is possibly caused by the muddying of the waters of the hybrid journals which offer APCs in addition to subscriptions, as seen in 10 of the 20 journals in this case study.

However, 'pure' open-access journals have a number of funding models, including membership fees, sponsorship and subscriptions (for print copies). The different options, as well as how they are utilised by different-sized publishers, are shown in Figure 4, adapted from Dallmeier-Tiessen et al.[29]

The fully funded APC model is considered advantageous because it makes publishing costs more transparent to researchers and engages them with the realities of research dissemination. In a recent (2014) study,[30] it was noted that 'there is evidence from a variety of sources that APC price is a consideration for many researchers and is helping moderate APC prices'. This is a good thing for the system as a whole, especially in the South African situation, in which, for open access APCs to be effective, it is essential that institutions see cost savings through funds not spent on subscriptions, especially given that there are no APC funds forthcoming from the government, as elsewhere.

Some South African universities (e.g. Stellenbosch[31]) have provided their own in-house APC funds to support open-access publishing. Whether these funds are specifically for open-access journals or can be used for hybrid journals is relevant, as it determines whether or not the institution is paying the publisher twice – once to write and publish and once to access and read. In addition, publishers like PLOS have a fee waiver policy for those who cannot afford the full, very expensive costs of APCs.[32] PLOS, as well as other publishers, also have a system of waivers based on a country's economic status, with reduced APCs for those below a specific per capita GDP: South Africa does not benefit from these blanket waivers although individual researchers can request waivers.

While legitimate and reputable open-access journals such as *PLoS One* have been in existence for several years, the policy shift to open access has seen an explosion in vanity publishers and 'predatory' journals which assure scholars of publication at a cost, regardless of peer review or quality. These publishers and journals have given all open-access journals a bad reputation. Of course, the criteria of quality – in the form of reputable editorial boards and rigorous peer-review processes – pertain to both open-access and propriety journals. Indeed, there are many poor quality proprietary journals, and scams occur in both – the recent case of computer-generated 'gibberish' research papers being removed from the archives of reputable and well-known commercial publishers after the papers were shown to be fakes is just one example.[33] The increasingly open research domain enabled by the online environment is one possible piece in the real rise in the number of retractions in high-impact journals in recent years.[34] South African researchers could reap the benefits of the additional checks that choosing open and digital publishing options enables.

Hybrid journals

Hybrid journals require particular attention, because they are a lose-lose situation for universities in that they pay twice (and perhaps a win-win for those publishers who are comfortable with being paid twice). The finding that 50% ($n=10$) of the top 20 journals in which UCT research is published, containing 47% ($n=662$) of the articles considered in this case study, are hybrid journals should be of particular concern to UCT, as well as to other South African universities. In some cases, research funders provide funds for APCs, an example being the Wellcome Trust whose recent data shows dramatically the extent to which double charging is taking place. A report of a 1-year period (October 2012 to September 2013) showed that academics spent GBP3.88 million to publish articles in journals with immediate online access – of which GBP3.17 million (82% of costs, 74% of papers) was for publications that universities would then be charged for again. Only GBP0.70 million of the charity's GBP3.88 million was used for publishing in a 'pure open-access' journal. Specifically, the Wellcome Trust paid nearly GBP1 million to Elsevier, and over GBP500 000 to Wiley-Blackwell to make articles freely available on point of publication, in journals that a university library would also be trying to find money to pay subscription fees.[35]

The dysfunctional nature of the hybrid journal market has been recently described in some detail by Björk and Solomon[30] who express concern about the transparency of the process of subscription reductions when aligned with APCs, especially in light of the fact that

> *reductions in the list prices of individual titles are almost meaningless since the bulk of the publishers' subscription revenue comes from multi-year bundled contracts or 'big deals', the details of which often are hidden behind non-disclosure agreements.*[30(p.39)]

They also raise as concerns the mechanisms for reducing subscription costs for individual universities or consortia in direct proportion to the hybrid APCs paid by them. They point out that such agreements may be difficult both to negotiate and implement. In short, efforts to keep

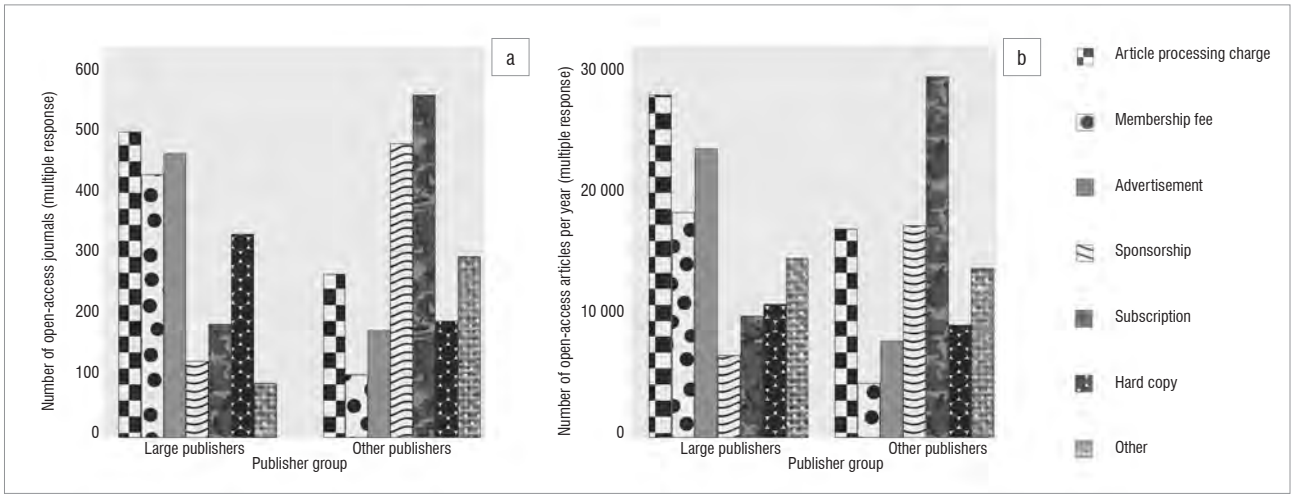


Figure 4: The number of (a) journals and (b) articles as a function of the income source of publishers, for large publishers and other publishers. 'Large' publishers are defined as those that publish more than 50 journals or more than 1000 articles per year.[29]

commercial publishers in the scholarly cycle and good intentions to enable open access are being derailed by complicated and bureaucratic processes with unanticipated consequences which may well undermine the good they set out to achieve.

One might be tempted to suggest that, in light of the complexities of the implementation in other countries, South African researchers are well enough served by closed, paywall proprietary journals. However, these journals are becoming unaffordable even for elite institutions, and the research that is published in them is effectively lost to all without financial and technical access. Through this traditional route, research uptake is thwarted and research investment wasted – a situation that South Africa can ill-afford.

Paywall and hybrid journals – the repository route

An examination of the 20 journals in the UCT case study shows that most (n=18) allow for versions to be deposited in institutional or disciplinary repositories (as ascertained by searching on Sherpa Romeo), although embargo periods may apply. Of these 18 journals, 14 allow for pre-print archiving (3 of the open-access/free online journals, 9 of the hybrid journals and 2 of the subscription journals), 11 allow for post-print archiving with no restrictions (4 of the open-access/free online journals, 5 of the hybrid journals and 2 of the subscription journals) and 6 allow for publisher's version to be archived (4 of the open-access/free online journals, 2 of the hybrid journals and none of the subscription journals). Another study[36] which looked at an extremely large sample (1.1 million articles) across a variety of publishers found that the majority of articles was legally eligible for repository deposit. Approximately half of the articles could be shared at the time of publication either as the accepted manuscript or as the publisher's version and this number increased to 80.4% after 1 year from publication.

Under pressure from academics globally, publishers have agreed that a version of a journal article can be deposited legally in authors' institutional repositories or on their own websites. These kinds of institutional mandates came to a peak a few years ago in the global north, and many publishers automate linkages to institutional scholarly communication structures, sometimes after a specified embargo period. This route in South Africa would make a real difference to the availability of local research online.

In recent years, there has been increased attention paid to such mandates in South Africa and elsewhere in Africa (for example, several Kenyan universities now have open access mandates[9]), as universities have become more attuned to the necessity of guarding and taking responsibility for the presentation and dissemination of their own resources. Universities with robust scholarly communication infrastructures and expertise can and should play a significant role in preserving and disseminating the journal scholarship of their universities through their own efforts and expertise.

The role of repositories in online visibility is proven in numerous studies with its value particularly emphasised in developing country contexts. In the South African situation, that same study (mentioned earlier) which found such limited visibility of South African research online found that one article appeared on Google and Google Scholar top 10 results. This article was accessible only through a subscription of USD593 for 12 issues or by online access to the single article for 24 h at a cost of USD31.50, but it had been legally deposited into a university repository from where it had been downloaded 2356 times at the time of writing. Ironically, the journal in which the article was published subsequently offered an open-access publishing option, at a cost to the author of USD3000.[18]

Together with such mandates has been increased attention to the building of scholarly communication infrastructures to support not only the deposit of journal articles but the full gamut of scholarly communication and research dissemination activities enabled in a digitally mediated age of scholarly social media and online participation. It is beyond the scope of this piece to discuss the intricacies of infrastructure and repositories; suffice to say that they provide a valuable mechanism for South African researchers wishing to improve their online visibility, share their scholarly output online, extend their research networks and make their work available to all with Internet access.

Concluding discussion

There is no question that open access is now firmly part of the global knowledge creation and dissemination landscape. It is the present

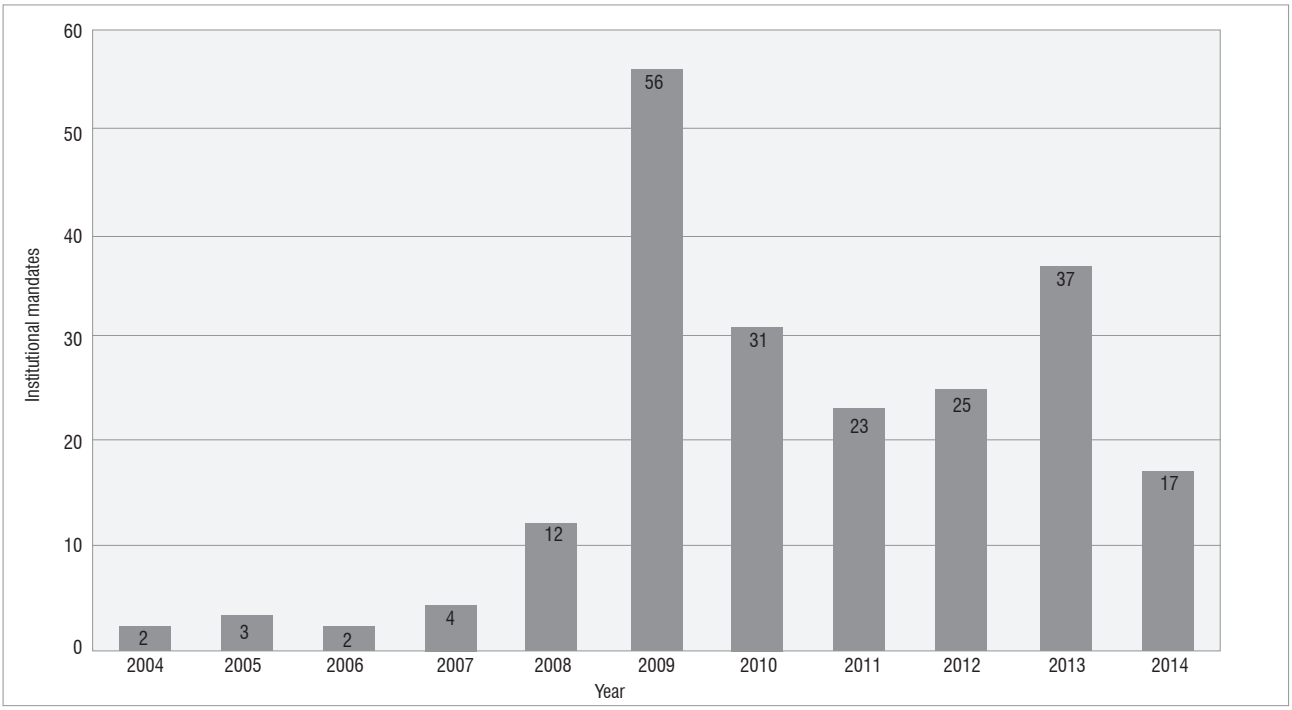


Figure 5: The number of institutional mandates on open-access publishing per year recorded in the Registry of Open Access Repositories Mandatory Archiving Policies (ROARMAP)[12].

muddied and muddled transition which makes the terrain hard to read, especially as stakes are claimed and interests fought over. South African scholars need to understand the shape of the shifting landscape and engage with the debates to ensure that their own interests are being addressed. A worst case scenario for South African researchers would be a lose-lose situation in terms of both access and participation. It would be debilitating for local researchers for access to northern research to become straightforward, but opportunities for participation by southern researchers to be reduced. Access to southern research is likely to be even further reduced as local researchers' publishing options might be restricted by financial gatekeeping at the outset. While sweeping changes in the global north will see more northern research freely available to all online, the danger for locals is twofold: firstly, that they may be limited in their opportunities to publish (especially by expensive APCs) and, secondly, that their own research drowns in the worsening invisibility of the online discoverability sphere.

The transition does not have clear sign posts; indeed, many believe that the present moment is a turning point for open access with a tug of war between a publisher-driven future and a researcher-driven future. South African researchers especially have a vested interest in understanding and engaging with the issues.

What does this mean for South African scholars? Academics need to cast a sharp eye on the choices they are offered and differentiate between types of journals (whether fully open access or hybrid) and between open access options. Doing so is premised on a realisation that taking control of research dissemination is increasingly in academic interests. Historically, research ended when an output was published; now the publication is dynamic and at the centre of a virtuous cycle of participation, online representation and the co-production of knowledge in a borderless world.

Central to full participation and engagement is copyright. There is less reason than ever before for academics to give away their copyright, when legal alternatives exist which give publishers the right to publish while academics keep the copyright on their own work and specify their own conditions. Even publishers are quietly coming to realise this; it is no coincidence that so many are changing their business models to provide research-related services and tools in new areas such as text mining, referencing and research collaboration.

A strategy that exists immediately for academics is to deposit their work in institutional and disciplinary repositories as well as properly curated websites. The expertise and costs associated with professionally preserved content are borne by informational professionals with the necessary proficiencies. Assuring that scholarly content is online and visible is not only essential for personal scholarly presence, it is also a part of ensuring equity of representation and realigning lopsided geopolitical knowledge resources online. Representation matters – because what is found online increasingly shapes what is known and what can be known. Knowledge which can be found online becomes that which is considered legitimate, normalising it and giving it power.

How the current tensions and debates in scholarly communication play out is of import to academics who should get involved in open access negotiations at the policy level: the outcomes really matter to researchers. Engagement between academics and government departments (such as DHET and DST) around open access policies and funding in the South African research environment is a key requirement going forward. The lack of a national open access policy in South Africa hinders the development, growth and availability of local research, which is in stark contrast to the strong national legislative leadership shown by countries in Latin America such as Mexico, Peru and Argentina, which have all passed national open access policies in the last 18 months, with Brazil's in the offing. National government also needs to step in at the resource and system levels. While the SciELO SA initiative is laudatory, it must be only the first step in developing and supporting new business models for scholarly publishing in the public interest. In the same vein, regulations and allocations for research dissemination (including APCs) also need urgent state attention.

Collaboration between researchers and universities in negotiations with publishers to change terms of agreement will also strengthen the interests of academia. Negotiations with stakeholder groups would of necessity include publishers and most especially should acknowledge the importance of publishing skills which are critical no matter where they are located. But fine-tuning is needed to differentiate between publishers: they are not the same and their *raison d'être* differs, profoundly determining their behaviour. Difficult questions can and must be asked, and the terms of agreement renegotiated. All this must happen while simultaneously ensuring that the highest quality academic standards are maintained and that fraudulent opportunists are firmly nipped in the bud.

As the entire global ecosystem changes, academics need to participate in global conversations about the changing nature of research dissemination in order to ensure that voices from developing countries are inserted and heard. Northern-focused gazes rarely serve the needs of research and social development in the global south; decisions may not be feasible or may privilege those with more access and with the tools to facilitate visibility and participation. While hybrid economies and ecologies can and do exist (the open source community is a fine example), academics need to intercede to ensure that commercial interests serve academic interests first and foremost, and not the other way around.

Within universities, academics need to lobby for changes in performance assessment and promotion mechanisms in order to widen the types of knowledge production acknowledged by the rewards system. Academics now can benefit from new formats and exploit the read-write affordances of new technologies, and participatory, open or collaborative academic roles and outputs are now possible; but academic evaluation systems have been slow to acknowledge and reward them.

As Poydner put it so well, what is so exhilarating about a research-driven model is that it is future focused, and so has the potential to produce forms of scholarly communication more suited to the networked environment[37]; it holds out the promise of a new 21st century scholarly communication system, not a retrofitted 20th century system. We would add that, for the opportunities to be realised, it is essential that they are determined through a genuinely global conversation to create scholarship shaped by academic rigour and quality, disciplinary frameworks and research imperatives, not determined by geographical borders, technical and other inequalities or commercial gains.

In short, we argue that despite the muddled representations of open access discourses in the South African research terrain, researchers should firmly engage with the specificities of open-access publishing through both journals and repository deposit. Engaging with open access is an important way of taking control of the fruits of academic labour – essential for individual scholars and the universities whose mandates of knowledge creation and dissemination they serve as well as the broader community which needs access to the latest research to grow, benefit from and participate in a global body of knowledge.

Acknowledgements

We thank Johan Mouton and the UCT Research Office for the UCT publication data analysed here. Two anonymous reviewers are also thanked for their insightful comments and suggestion.

Authors' contributions

Both authors contributed equally to the manuscript.

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Open Access Week is celebrated globally in October. Czerniewicz and Goodier discuss open access in the South African higher education research context in their article on page 97 (image design by Nadine Wubbeling).

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