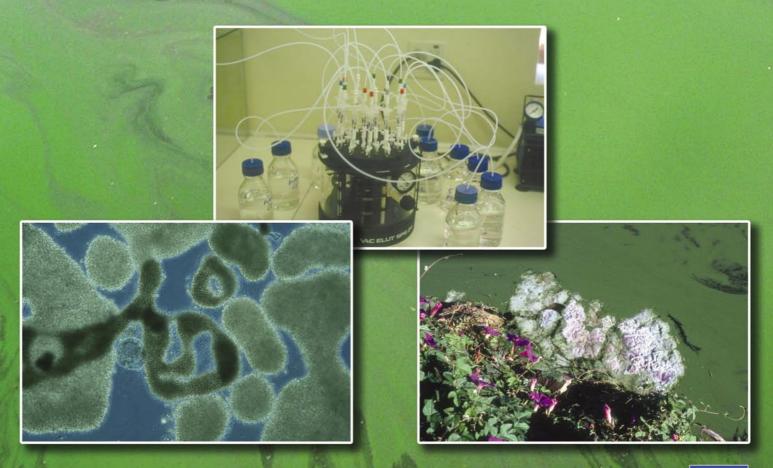
# A Research Strategy for the Detection and Management of Algal Toxins in Water Sources

William R Harding



### A RESEARCH STRATEGY FOR THE DETECTION AND MANAGEMENT OF ALGAL TOXINS IN WATER SOURCES

### **Report to the Water Research Commission**

by

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This document includes a CD containing the ancillary documents and reports forming part thereof

WRC TT 277/06

**JUNE 2006** 

Obtainable from:

Water Research Commission Private Bag X03 Gezina 0031

The publication of this report emanates from a project entitled:

"Development of a Research Strategy for the Detection and Management of Algal Toxins in Water Sources in South Africa"

(WRC Consultancy K8/576)

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ISBN 1-77005-461-8

Printed in the Republic of South Africa

### **EXECUTIVE SUMMARY**

### **BACKGROUND**

The development of problematical aggregations ('blooms') of cyanobacteria ('blue-green algae') in South Africa's surface waters is a problem that has been in existence for many decades. Cyanobacterial blooms are a symptom of the anthropogenic nutrient enrichment ('eutrophication') of water resources, a problem common to most of the world's nations. As cyanobacteria have the propensity to produce a wide variety of particularly noxious toxins, blooms of these organisms pose significant problems for, *inter alia*, human and animal health, ecosystem health, costs of water treatment and/or availability of raw potable water supply, recreational use and property values at or near affected waters. The incidence of cyanobacterial blooms and associated problems is often of an intermittent or seasonal nature, while in certain cases climatic conditions support levels of cell development that are disruptive to water resource use for much of the year.

Insofar as the bulk provision of raw potable water is concerned, and based on the available evidence, problems posed by cyanobacteria in South Africa are not of national crisis status. It may, however, be argued that the trophic status of the major dams in Gauteng is so high that a regional crisis exists. Severe and sustained algal problems are routinely experienced in a suite of 10 dams, five of which are situated in the aforementioned area of greatest population density and national economic activity. In these hypertrophic dams, such as Hartbeespoort, severe cyanobacterial problems have been commonplace for decades, and more recently similar conditions have become sustained and resilient in dams such as Rietvlei, Roodeplaat and Klipvoor. Several other dams are potentially problematical, i.e. those which are eutrophic, whereas many more are incipient eutrophic. The degree to which rural domestic and agricultural water resources are affected is currently unknown. This situation of burgeoning eutrophication is common to all countries and has resulted in eutrophication becoming the greatest threat to the future of water supply across the globe. The problem is no less severe in the southern as opposed to the northern hemisphere but, given the milder climate and warmer conditions, the duration of conditions that sustain algal development are prolonged in southern hemisphere countries.

Incidents of fatal cyanobacterial poisonings in South Africa are not uncommon, occur annually and to-date have only involved stock deaths and some domestic animal poisonings. This is a global commonality. Acute poisonings of humans have been reported from South America, Africa and Australia. The deemed health risk to humans is via chronic exposure to low levels of cyanotoxins in drinking water supplies. The threat is increasing with the spread of hitherto undetected species, such as *Cylindrospermopsis*, and the heightened risks posed by mixtures of toxins containing both tumour promotion and carcinogenic elements, such as is the case in mixed blooms of *Microcystis* and *Cylindrospermopsis* underpin cause for additional concern. New emerging problems include the production of β-N-methylamino-L-alanine (BMAA), a neurotoxic amino acid which is a possible cause of amyotrophic lateral sclerosis/parkinsonism-dementia in humans and which is produced by all known

cyanobacteria. This new discovery has obvious implications for widespread human exposure to an additional health risk.

The prevention, mitigation and control of cyanobacterial development in water supplies fundamentally resides in the effective attenuation of nutrient loading ('bottom-up' management) of surface waters. Such reductions are technologically possible and indeed have proven successful in many countries, but require the formulation of national policy to ensure their mandatory use in order to protect water storages on a strategic basis. This is, however, not a simple solution. In many cases the sheer volumes of effluent produced outweigh the levels of achievable reduction and will not bring the level of loading to the point where algal growth will be limited by nutrient availability. Accordingly, cyanobacterial research has to focus not only on prevention but also to understand the production and regulation of toxins as this occurs in nature.

Until the mid-1980s South Africa was a world-leader in research in the fields of eutrophication, cyanobacterial physiology and eco-physiology and cyanotoxins. This advantage was lost due to an astounding and ill-conceived departmental decision to afford a low-priority status to eutrophication in South Africa – and in so doing to effectively terminate funding for research. In the post-2000 era the errors of this approach have been recognized and fresh initiatives have been launched to restore lost impetus in this field of water resource management. However, South Africa now finds itself at a significant disadvantage in that it possesses very limited intellectual and infrastructural capacity in both the causative field of eutrophication and its symptomology of cyanobacterial development. However, recent global developments, coupled with the retention of sound professional relationships between a small group of local specialists and overseas research groups, afford South Africa a basis for a renewed research strategy which will rapidly offset much of the losses incurred during the past 20 years.

Research into the problem of toxic algae in South African waters is currently focused on management responses to the problem, genetics-based strain identification and eco-physiological investigations. These foci reflect commonalities in global research direction and are crucial for the regionally-specific characterization of cyanobacterial taxa, individual species and their toxins.

### **MOTIVATION**

From the foregoing it was evident that a report, providing a background, rationale and framework that will assist the Water Research Commission in determining the appropriateness of algal toxin research proposals and research needs, was required. Said report should also propose a research and management infrastructure for the specific purpose of directing and supporting toxic algae and algal toxin research, and the development of the requisite associated understanding, in South Africa. The proposed strategy has to focus on South Africa's most competent extant capabilities in this field, and integrates the existing infrastructure and research programs into a core research network. Lastly the report must be based on a comprehensive analysis of global trends in cyanobacterial research gleaned from conferences and symposia and the re-establishment of working contacts with the leading international specialists in this field.

South Africa currently lacks the basic cyanobacterial-orientated research infrastructure essential for sustaining a desirable level of applied cyanobacterial management. The water management sector is under-resourced in terms of funding, experience and analytical capacity, and the country has no dedicated analytical facility responsible for method standardization, calibration of standards and interlaboratory certification – i.e. there is no appropriately qualified or equipped laboratory to which samples may be submitted from any source for analysis. Analytical services are provided on an informal basis by certain of the larger water utilities, or by the Department of Water Affairs and Forestry (DWAF) laboratories. Reliance on the core toxin detection and quantification method, viz. HPLC (High Performance Liquid Chromatography), has largely been replaced by ELISA (Enzymelinked Immunosorbent Assay) methods that are prone to false positive results and which have not been exhaustively calibrated for South African use. There is no curated national culture collection of cyanobacterial strains. There is no mechanism for reporting of cyanobacterial incidents, nor any formal entry of those incidents that are known into a centralized database.

### PROPOSED RESEARCH STRATEGY

This analysis undertaken in support of the development of a fresh cyanobacterial and cyanobacterial toxin research strategy in South African water sources has identified two vital planning elements, viz. (i) the need to create a management and support infrastructure and (ii) the research aspects best suited to South African cyanobacterial research needs. In addition, there is a clear indication that successes will be limited should collaboration with overseas specialists and organizations not be implemented. To South Africa's advantage is its current involvement in both the Global Water Research Coalition (GWRC) and CYANONET initiatives, plus a willingness expressed by international specialists formerly associated with cyanobacterial work in South Africa to continue their association. Allied to this is the cosmopolitan nature of the cyanobacterial problems as experienced worldwide. This analysis was fortunate to have been commissioned during the year (2004) that saw the launch of the GWRC and CYANONET initiatives, as well as two key international conferences that allowed for the identification of globally-relevant research initiatives and emerging issues.

The strategy proposed here is based on comparing and contrasting the current directions and emerging issues in international cyanobacterial research with identified South African needs. From this analysis a suite of key research issues have been formulated, as follows:

### Development of a supportive and guiding research environment

Research into cyanobacteria and cyanobacterial toxins is, internationally, confined to a relatively small number of individuals and organizations, many of whom have been in working contact since the late 1980s. In South Africa the situation is much the same, although this country has experienced a significant loss of specialists to other countries and research fields, often as a consequence of the low priority and absence of research funding afforded to cyanobacterial projects. The problem of attracting and retaining specialists is exacerbated by the intermittent nature of the 'toxic algae problem', and the low level of recognition afforded to it by water utility companies and agencies. Research in South Africa is confined to programs conducted within small university research units, using shared resources,

and largely on the personal initiative and interest of the researchers concerned. South Africa has no formal eutrophication or cyanobacteria/cyanobacterial toxin teaching programs other than limited content presented within various coursework programs. Water resource managers are poorly-equipped with the awareness and understanding required to make informed decisions. Research funding and interest is limited and provided essentially by the WRC and the DWAF, with a small number of the larger metropolitan utilities having internal monitoring and applied research budgets. There is no formal engagement of the water utility industry with the research programs as they currently exist. Issues pertaining to cyanobacteria are not directly allied to eutrophication as a sub-set thereof. There are no national overarching, inter-departmental programs for research and management in either field, nor do coordinating committees exist at national level. Review and acceptance of research proposals is of an *ad hoc* nature and not subject to internationally-relevant and informed peer review.

In order to address these limitations and create an environment in which effect can be given to a cyanobacteria/cyanobacterial toxin research strategy it is proposed that the following supportive elements be created – these being based on a strong tri-partite relationship between researchers, utilities and the resource agencies (see **Figure 1**):

### 1. Personalities and leaders

This is a field that will benefit from being personality driven as the core of integrative experience in the science confined to relatively few individuals. Having the right people in the right places is crucial to success, as has been illustrated by the Australian CRC-WQT model. It is essential that devoted 'champions' be identified and tasked with ensuring that links are developed and sustained between the research field, the water utility industry and other potential sources of funding. The same champions would also, for example, be tasked with explaining the advantages of genomic-based strain identification research and development to the water industry – an application which, at first glance, might be considered by the industry as not being an operational need worthy of funding.

### 2. Rationalization and prioritization of research needs

The allocation of cyanobacterial research needs must maintain clear distinction between the "need to know" (= applied or operational research) and the "scientific interest" (= blue-sky) subfields. Scientific interest research may be allocated funding from the primary (= operational) funding pool only if there is a clear indication that the deliverable will directly inform applied research needs, and be forthcoming within a timeframe relevant to operational requirements

### 3. Funding Sources

A mix of funding sources would be most desirable to support research in this field, and creating structures appropriate to accessing this mix of funds should be a mandatory requirement. While funding raised from water supply is applied to research via the WRC, it would be more beneficial to create a wider working partnership between research and the water utilities. Here it should be noted that issues pertaining to both eutrophication and cyanobacteria have cross-cutting implications for the research thrusts of ecosystems management, water resource management,

health and agriculture – this should be taken into account both for the formulation of committees and for identifying funding sources.

#### 4. Institutional structures and skill sets

Although skill sets relevant to cyanobacteria and cyanobacterial research exist in South Africa these are highly-fragmented and lacking in any coordination of tactical or strategic planning. There are too few resources in this particular field for a situation of this nature to be effective in terms of the application of scarce research funding. There is a need to ensure the location of 'permanent' research 'units' at one or more institutions – with 'buy-in' from the parent institution - and with 'satellite' units possible at others depending on need and the interest and funding support of the parent institution. Skill sets should be continually augmented and exposed to 'real-world' problems via international collaboration and exchange of personnel (see also **5. Central Analytical Facility**). It would be to general advantage if extant and new skills were amalgamated rather than splitting up any further.

### 5. Central Analytical Facility

South Africa urgently requires the services of a reliable, multidisciplinary facility providing the full range of cyanotoxin analytical methods, standards certification, calibration services and interlaboratory calibration. There are currently no laboratories in South Africa where samples can be submitted for routine cyanotoxin analysis – other than on a non-routine and often off-line basis provided by the City of Cape Town or Rand Water.

The use of the cheaper ELISA methods, in the absence of the integrated use of HPLC confirmation, is at the least risky. ELISA is a screening technique, nothing more, and should not be utilized without accurate confirmation. As it is unlikely that there will be scope for an independently-funded service of this nature in the short to medium term this function should be 'piggy-backed' with the development of institutional structures. A core task for this facility would be the local production of toxin standards to offset the increasing difficulties experienced with the obtaining of commercially-available standards.

### 6. Overlap with 'parent' programs

Cyanobacterial problems in water resources is a consequence of eutrophication. Accordingly, the cyanobacterial research program needs to be closely allied to the national strategic eutrophication management program. Essential points of contact should focus on the extent of the problem (cyanobacterial blooms and the percentage thereof that are toxic); bottom-up and top-down management approaches, and access to funding from the eutrophication program in cases where resolution of identified problems is unlikely to be forthcoming from the eutrophication management sector itself.

### 7. Applicable models and international links

The local research advisory committee (see **8. Review Committee/SARNAT**) should contain members who are representatives of the utilities, health regulators, natural resource agencies and researchers (both university and in some cases from private industry). This mix ensures the

balance between operational and scientific interest research, and also adds an extra funding leg, namely that provided by tertiary institutions. International links should be maintained both through this model and via the Review Committee (see below).

#### 8. Review Committee/SARNAT

South Africa currently lacks the locally-based experience to confidently conduct reviews of both solicited and unsolicited proposals, especially in areas involving aspects such as genomics/cyanogenetics. This situation is further complicated by the small pool of SA specialists used as reviewers but also bidding for the same funding resources – which may create conflicts of interest. Accordingly the review "panel" (not simply two reviewers) should consist of the manager or consultant for the national strategic planning, local reviewers as available plus two or more international specialists. In all cases, but especially for unsolicited proposals, there must be a mandatory requirement on the proposer to show clearly where the expected deliverable addresses a gap in the international science, what work underpins the proposed research, and what opportunities may exist for collaboration with other local and international institutions or agencies working in the same field. The latter process will be assisted by the links maintained through the Information Management function (see below). Ideally unsolicited proposals should be submitted (confidentially) via SARNAT in the first instance, thereafter being added to the research program as solicited proposals once in a format that dovetails with the overall research strategy. All project deliverables should be subject to international peer review in instances where technical oversight is critical for the final product.

It is proposed that a national working and advisory group, the South African Research Network for Algal Toxins (**SARNAT**) be constituted to oversee and manage cyanobacterial research and management in South Africa.

### 9. Project Management

The present WRC Reference Group project management function is deemed unsuitable for this specialized area of water research - in that it does not afford the required intensity and frequency of interaction and progress evaluation that is necessary to activate and sustain a meaningful program of research. Long periods between meetings of individuals who, at the present time, are likely to have inadequate levels of experience in the particular field, is contraindicated to the need to rapidly and comprehensively develop capacity. Accordingly it is recommended that the Steering Committee function be largely replaced by the appointment of a Task Manager. This role should be fulfilled by someone with appropriate experience of the international cyanobacterial research environment *per se* who is tasked with regular 'on mission' visits to the institution or unit where the work is being conducted in order to gain first hand knowledge and experience of the progress made, constraints encountered and the like. The Task Manager would then report back both to the WRC Manager and the Reference Group. Should problems requiring specialization it would the Task Managers role to direct and facilitate contact with other specialists or organizations able to help, or to comment on the results or progress to-date.

### 10. Information Management Function

Insofar as cyanobacterial research and management is concerned South Africa suffers from a critical information gap. In the absence of one or more dedicated research units holistically-operating in this field, such as the CSIR or the former NIWR might have been expected to do, an enormous volume of published material has gone unnoticed by the SA scientific community, and continues to do so. This paucity extends to the non-published 'grey-literature' environment. In order to address this it is recommended that an Information Management Function (IMF) (= literature watch) be commissioned on a consultancy basis. This consultancy should be tasked with (i) undertaking – using an appropriate publications search package - an historical analysis of publications for the period 1985-2005, cataloging these by category and listing on a local website; (ii) maintaining this on a monthly basis thereafter using the same approach; and (iii) maintaining a watch and links with other research organizations, units and institutes regarding the publications of reports, reviews and etc. The IMF function should be closely allied to the information resources of the CYANONET initiative.

### 11. Ensuring continuity of program management skills

While there are several small research groups in South Africa involved with some or other aspect of research pertaining to cyanobacteria, the level of current and all-encompassing experience in the fields of limnology, eutrophication, cyanobacteria and their ecological associations, cyanobacterial toxins and cyanogenetics is limited to perhaps one or two individuals. As this particularly research field is not attracting new scientists this is not a sustainable situation. Accordingly, at program management level efforts should to identify appropriate personnel for mentoring. It is anticipated that with the development of the strategic cyanobacterial and eutrophication programs that 'new blood' will be attracted to this field of water resource management.

### 12. Education and Training

The current level of understanding of both eutrophication and cyanobacteria in South Africa is currently inadequate for decision making and informed stakeholder involvement. A concerted effort should be made to address this information gap, and to ensure that the relevant understanding is extrapolated across all policies and practices having influence on water resources management.

### Strategic Research Framework

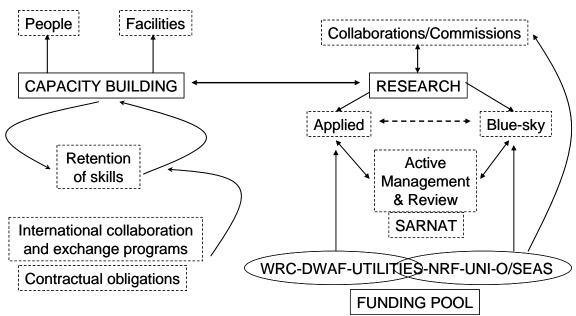


Figure 1: Proposed elements of South African toxic algal research framework

### SUGGESTED RESEARCH INFRASTRUCTURE BASED ON EXISTING CAPABILITIES:

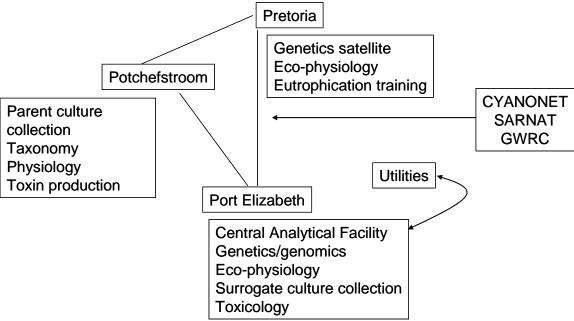


Figure 2: Proposed integration of tertiary research facilities

### Specific components of the proposed research strategy

There are three core aspects within the proposed research strategy, namely:

- (i) those pertaining to the eco-physiology of cyanobacteria, and cyanotoxin production most notably the questions surrounding why cyanobacteria produce toxins, and what triggers this response;
- (ii) physiology and genetics (population and related phylogenetic studies);
- (iii) those issues relating directly to applied (operational management as opposed to research *per se*) issues.

### Physiological and eco-physiological studies

The physiology and related research aspects have been slow to provide concrete answers, not least in South Africa. The bulk of the research work previously undertaken in South Africa has been concentrated in this area, and some is currently being undertaken at the North West University (Potchefstroom Campus). Much of the research is still widely cast ("shotgun") and based on the premise that indicators may be identified on exhaustive correlative analysis. It is, however, unlikely that new information on cyanobacterial growth and development in dams, which will translate directly into practical management options, will be forthcoming from this research arena in the short – to medium term. However, the complexity of the problem is such that it is doubtful that practical and pragmatic outcomes relevant to operational management of both water storages and water treatment plants will be forthcoming in the near future. Notable exceptions here are (a) the growing pool of strong evidence, from several countries including South Africa, that foodweb management may be of considerable use in the alleviation of ecosystem degradation brought about by sustained eutrophication; and (b) the discerned impacts of the presence of cyanobacteria, and/or cyanobacterial toxins, on aquatic biota, and the need to quantify same. This aspect may be extended to include consideration of chronic impacts on livestock and impacts on agriculture, including aquaculture.

It is recommended that only those scientific interest proposals that can show, with a high level of confidence, a deliverable or deliverables that will significantly advance the scientific understanding and hasten developments in the operational arena, be considered eligible for funding from the WRC.

By contrast the examination of natural populations (ecophysiology), linked to their genetic identities (speciation) and inter-regional and continental comparisons, has considerable potential for the rapid development of a management-linked understanding of cyanobacteria. Increasingly within this field is work aimed at genomics-based identifications of cyanobacterial species based on the presence of the gene sequences involved in toxin biosynthesis. Allied to this is the use of DNA-based tests to differentiate between geographically-remote local species, as well as to compare the genetic identities of South Afrian isolates of e.g. *Microcystis aeruginosa*, *Anabaena circinalis*, *Planktothrix agardhii*, *Cylindrospermopsis raciborskii* and *Nodularia spumigena* with those already identified in Australia.

### Operational research

Operational research is currently aimed at the provision of response guidelines (the Alert Levels and Methods Manuals, treatment protocols and the like).

#### **Recommended research elements**

In addition to the development of a research environment for this field and cognizant for the need to establish collaborative joint funding agreements where similar work has or is being done, it is recommended that the WRC consider proposals within the following current or emerging issues for funding during the initial years of this strategic program. In formulating this list it has been assumed that information on incidence and demographics of cyanobacterial occurrence is not a 'research' issue but rather a component of monitoring and management (see Development of a Supporting Research Environment, above):

- growing development of cyanogenetics, the need for qualified taxonomic identifications and the value that these hold for the development of management directives and tools, has an absolute requirement for a professionally-maintained and unadulterated culture collection. The creation and curatorship of this collection has obvious overlaps with the aforementioned need to create a Central Analytical Facility given infrastructural and staffing overlaps, as well as the ability to attract additional funding. Such a collection should be created at a primary location, with a second mirror collection at another institution or unit. The primary location should be responsible for genetic identification and genetic characterization, while the second should be tasked with classic morphological/phonetic identifications. The same facility should also provide for storage of samples from poisoning incidents for later analysis.
- 2. Genetic/molecular techniques for identifying toxic species. These are deemed critical to the development of predictive capacity for bloom management as derived from population dynamic studies linked to toxin production studies. Work in this area requires the availability of the above-mentioned culture collection. With respect to the application of PCR assays it is essential that these be applied to naturally occurring populations and not just laboratory cultures that have been in culture for a long time.
- 3. Similarities in trans-regional and trans-continental species identity (phylogenetic relatedness). Similarities between South Africa and Australia, should be investigated given the level of understanding of the genetics of the common problematical species, and toxin production, by the Australian CRC. Species eligible for immediate attention would be *Microcystis aeruginosa*, *Anabaena circinalis*, *Planktothrix agardhii*, *Cylindrospermopsis raciborskii* and *Nodularia spumigena*. An understanding of local speciation characteristics should be established prior to comparison with strains from other continents.

- **4. Regulation of cyanotoxin production**. The development of an understanding of cyanotoxin production triggers and mechanisms has the potential to provide information on how to deal, at least in part, with the problem (this given that prevention of cyanobacterial overgrowth through effective nutrient attenuation and management at or near source should be the fundamental management approach but recognizing that for many waters the level of attenuation required to bring algal development under control may not be attainable in the short to medium term).
- **5. Development of the wider potential of PCR techniques for use in cyanogenetics**. Research in this arena should identify the relevance and accuracy of 'rapid' toxin or toxicity-indicator assessment techniques based on PCR methods.
- **6. Health risks and risks posed by mixtures of species**. It is recommended that South Africa contribute to the global understanding of risks to animal and human health by becoming directly involved the work being undertaken by the Australian CRC on this topic. This contribution should be financial directed through capacity building and the supply of South African material for testing, or for the performance of parallel testing in South Africa. The already in effect GWRC proposal for this has reference.
- 7. Epidemiology and chronic exposure risks. Humans and animals with chronic exposure to cyanotoxins, particularly the hepatotoxins, do not die of liver complications. Death from pneumonia is more likely. What is clear is that chronic exposure to microcystins influences both morbidity and death. This chronic exposure risk may be of greater importance in a population with a high prevalence of the TB:HIV-AIDS co-epidemic (see also Risks to rural supplies). The emergence of BMAA as a new 'slow toxin' health risk underscores the need for epidemiological investigations in communities where the raw potable water is known to contain cyanobacterial blooms or near-permanent aggregations of cyanobacteria.
- **8. Risk to rural domestic and agricultural water supplies.** The possible impacts on human health in rural areas remote from controlled water supplies should be established. Coupled to this should be the provision of an awareness program amongst farmers and rural communities.
- **9. Foodweb manipulation**. There are strong and positive indications from several countries, including South Africa, that foodweb manipulation plays a strong and beneficial attenuation role in eutrophic waters. The efficacy of this approach, the mechanisms and dynamics thereof strongly merit investigation with the cyanobacterial management sphere of interest.
- 10. Bioaugmentation products. There are a number of unsubstantiated bioaugmentation "silver bullet' cures for eutrophication commercially available. Most of these appear to be based on freeze-dried bacterial mixtures ostensibly aimed at the small-scale fish breeding market. These products have unproven efficacy in impacted natural or semi-natural systems, and are unsupported by any identified proven scientific validation. There has also been a marked unwillingness by the suppliers of these products to participate at their own cost in any local field trials. While the 'let the buyer beware' concept should prevail here the costs of these

products are not insubstantial and may be purchased in desperation by uniformed clients approached by these companies. Accordingly it would be in the national interest to investigate the use of these products, ideally in collaboration with the companies marketing same.

- 11. Algicides. The use of algicides, most commonly copper sulphate, to control algal growth in South Africa is limited to the management of irrigation canals and some farm dams. The practice is generally unacceptable in that the accumulation of copper from repeated use has a sustained and negative impact on aquatic biota. Notwithstanding this the use of algicides may be indicated in certain once-off applications to address an acute situation, and with the provision that effective attenuation and mitigation measures will be installed to prevent a recurrence of an algal bloom. While application guidelines for copper sulphate are well established there are a range of algicides of various formats registered in the USA which may be of relevance in South Africa, but which would require evaluation prior to local registration
- **12. Bacterial predation.** The potential for the algicidic actions of bacteria on cyanobacterial blooms as an invocable biomanipulation procedure is largely unknown, and results to-date have been less than promising. Pursuance and funding of research in this field should be subject to international experience and direction.
- 13. Verification of analytical screening methods for local application. With respect to the need for a Central Analytical Facility, the validity of screening methods such as ELISA for local use should be exhaustively tested. The application of ELISA as a stand-alone method is not suitable in cases where unknowns exist this precludes quantification of toxin concentration.
- 14. Analytical techniques for the detection of cyanotoxins in fish tissue and marine filter-feeding organisms. Aquaculture in South African impounded waters is a developing industry and requires analytical support if the products are to be successfully marketed. Here it should be noted that techniques adequate for quantifying tastes and odours in fish flesh are available in South Africa but not on a readily-available basis.

### **Non-research elements**

- 1. Recreational use guidelines. The greatest risk of acute toxicity lies with human and animal exposure to algal scums. This requires a more complex suite of evaluation criteria than is applied to treated water. While South African guidelines need to be developed for this it is recommended that the comprehensive Australian, as well as the WHO guidelines, be used to provide direction for same and to expedite the local delivery of an adapted product.
- 2. Alert Level and Operational Methods manuals. Although local contracts for this type of product have already been commissioned, the quality and content of same is unfortunately not of an international standard, and South Africa is not by any means a world leader in cyanotoxin analytical techniques. As put forward by the 2004 GWRC meeting it would be useful to combine efforts of this nature into single, internationally-relevant products.

### CONCLUSIONS AND RECOMMENDATIONS

The situation analysis component of this strategic plan clearly reveals that South Africa must place itself in a position in which it clearly understands the issues and threats posed by the presence of cyanobacteria in the water supplies of this country. This conclusion is founded not only on local experience but on the global, cosmopolitan nature of the problem. While South Africa has lost some ground in this particular field this is not unique as the levels of resources developed to understand and combat this problem are sparse worldwide. South Africa is, however, extremely fortunate to be in a position where collaborative and cooperative links to existing programs in other countries are readily accessible.

With respect to the research thrusts (KSAs) of the Water Research Commission toxic algae and algal toxin research needs cut across all domains.

The nature and extent of the 'cyanobacterial problem' is generally poorly understood and prone to misinterpretation and misinformation – both of which can significantly retard positive progress. A considerable amount of effort remains to be devoted to developing a cross-cutting level of awareness of the causes and effects of problematical cyanobacterial growth in surface waters. It is recommended that the outcomes of this initial strategic plan be communicated to the key public and private role-players and affected parties by means of briefing workshops – as opposed to the simple dissemination of this document. The nature of, and issues surrounding, cyanobacteria and their toxins (toxic algae in general) are best communicated in person by someone closely familiar with the topic. This personal approach is likely to provide a far greater stimulus for engaging with and seeking 'buy-in' from the water supply industry, be this government, utilities or city and town administrations. This recommendation is aligned with the need for this initiative to be 'championed' – as discussed in this report.

### **ACKNOWLEDGMENTS**

This analysis was developed through an unsolicited Water Research Commission (WRC) consultancy (WRC K8/576) awarded to Dr W R Harding of DH Environmental Consulting.

The kind support and interaction from and with the following persons is gratefully acknowledged:

### International:

Mike Burch (CRC for WQT), Don Bursill (CRC for WQT), Paul Alan Cox (Institute for Ethno-Medicine, USA), Wayne Carmichael (Wright State U), Ingrid Chorus (UBA), Peter Donlon (WSSA), Ian Falconer (CRC for WQT), Geoff Codd (U. Dundee), Andrew Humpage (CRC for WQT), Gayle Newcombe (CRC for WQT), Stephanie Rinck-Pfeiffer (United Water), Wido Schmidt (TZW), Frans Schulting (GWRC), Glen Shaw (Griffith U), Dennis Steffensen (CRC for WQT), Hans Uitkilen (FHI Norway), Roelof Veeningen (STOWA).

### South and southern African:

Colin Archibald (KZN Consulting), Peter Ashton (CSIR), Chris Dickens (Umgeni Water), Tim Downing (NMMU), Meiring du Plessis (WRC), Sandra du Plessis (NWU Potch), Hein du Preez (Rand Water), Johan Grobbelaar (UOFS), Jan Joubert (OVRI), Lindah Mhlanga (U Zimbabwe), Steve Mitchell (WRC), Annatjie Moolman (WRU), Anna Maria Oberholster (U Pretoria), Gordon Shepherd (MRC), Carin van Ginkel (DWAF-RQS).

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### DEVELOPMENT OF A RESEARCH STRATEGY FOR THE DETECTION AND MANAGEMENT OF ALGAL TOXINS IN WATER SOURCES

### 1. INTRODUCTION

South Africa, through activities underwritten by the Water Research Commission (WRC) and the Department of Water Affairs and Forestry (DWAF) has recently restored the need for research into eutrophication prevention and management to a position of high priority (e.g. Walmsley, 2000; Van Ginkel et al., 2001; Rossouw and Harding, 2005). Notwithstanding this resurgence of effort, there exists more than a decade of inactivity within this vital area of water resource management in South Africa, during a time when eutrophication became recognized as the highest ranking threat to surface water resources (both marine and freshwater) by many countries. Allied to this have been significant collaborative global advances into cyanobacterial research and management, and movement towards consensus on how best to deal with the potentially-devastating problem of sustained occurrence of toxin-producing cyanobacteria in raw potable, livestock watering and irrigation supplies. It is important to recognize here that eutrophication in South Africa does not currently constitute a national threat, rather eutrophication crisis situations are limited to 10 dams identified by the Trophic Status Project (DWAF, 2001). However, eutrophication should be regarded as an emerging issue, one which has the potential to impact severely and negatively on the water resources of this country (see Walmsley, 2001).

The core objective of this project was to develop a national and internationally-relevant strategic cyanobacterial and cyanobacterial toxin research and development program for South Africa. This program will serve to direct the most appropriate selection of research initiatives and allocation of research funding in this field, and crucially take cognizance of similar programs being conducted elsewhere, and maximize the use of mutually-beneficial, internationally-collaborative efforts, whereever possible. Only in this manner can the optimization of scarce expertise and funding be achieved. The program must strive to build on the substantial national database of information, and to expand the availability of human resources and education-based understanding of this subject. The national level of understanding of eutrophication in general, and cyanobacteria in particular, is exceedingly poor. In particular the proposed program should seek to redress the local knowledge gap that arisen in this field during the past decade. The program should, on an on-going basis, remain abreast of developments in the management of eutrophic waters in this country, as well of similar initiatives elsewhere. The strategy to be developed crucially needs to encompass an optimum education and resource development component, integrated with eutrophication management initiatives. Additionally the proposed strategy needs to incorporate cross-cutting research and management considerations that span water, agriculture, human and animal health and ecosystem health.

### 2. PROJECT APPROACH

### 2.1 SCOPE

The creation and implementation of this research strategy is intended to derive maximum benefit from existing and potential working links and international cooperation, rather than having to conduct a bottom-up research endeavour in the absence of any supportive norms – there is no need to commit scarce funding resources to the 'reinvention of wheels'. This project seeks to develop an internationally-relevant yet locally-focused research strategy for the detection and management of algal blooms and algal toxins in water supplies. This strategy will:

- take note of international direction emanating from the UK, USA, Scandinavia, Germany, Australia, Poland and Japan;
- assess local human resource capacity, skills and infrasture;
- determine current and envisaged local research needs;
- integrate findings with those of parallel and 'parent' (= eutrophication) research and management programs;
- maintain local relevance although drawing on international experience to preclude repetition of work for which the results would otherwise be directly relevant to the South African situation;
- will be developed in collaboration with prominent international specialists, and incorporating international direction presented at
  - (i) the participative Global Water Research Coalition Workshop (GWRC, Adelaide, Australia, May 2004);
  - (ii) the 6<sup>th</sup> International Conference on Toxic Cyanobacteria (ICTC, Bergen, Norway, June 2004);
  - (iii) the 11<sup>th</sup> International Conference on Harmful Algal Blooms (HABs) (Cape Town, November 2004); and
  - (iv) the inaugural meeting of the international CYANONET<sup>1</sup> representative management group (Scotland, November 2004).

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<sup>&</sup>lt;sup>1</sup> CYANONET is a UNESCO-supported international initiative that aims to create a web-based 'clearing house' of information pertaining to cyanobacteria, their toxins, incidences of toxicosis, literature databases, management practices and the like. CYANONET will not only provide a linked forum for cyanobacterial researchers, but also provide an information and skills transfer service to the global community. Phase I of establishing CYANONET entails setting up regional (by continent) communication networks and the preparation of regional reports.

### 2.2 APPROACH

For the envisaged South African algal toxin research strategy to be successful the plan needs to:

- have a clear understanding of international research focus areas;
- be aware of past and present research efforts, their outcomes and their relevance in terms of South African strategic planning;
- understand the level of available skills and resources; where capacity building is a shortcoming to
  further progress; and to what extent South African researchers can make innovative and relevant
  contributions to this research field.

In order to identify, define and rank a relevant suite of algal toxin research aims for South Africa, the following approach was followed (relevant project Deliverables in parentheses)<sup>2</sup>:

- identification of the major algal toxin research role players in South Africa (3,4);
- evaluation of the human and infrastructure capacity currently available in the field of algae and algal toxin research and management in South Africa (3,4);
- identify research needs as envisaged by South African researchers (3,4);
- list and document key information gaps and research needs identified through institutional assessments (3,4);
- establish dialogue with international researchers and research institutions regarding the processes and policies underpinning algal toxin and management research in their respective countries (3,4);
- develop preliminary working associations with international specialists for the purposes of seeking review and comment on South African direction in this field (1-5);
- attendance and participation in the GWRC Cyanobacterial workshop. The core focus of the GWRC is the achievement of research goals through the promotion of inter-partner collaboration. The fact that many of the GWRC partners are prominently-active in the field of cyanobacterial research and management rendered this meeting extremely important for this project (2);
- identification of current research foci and strategies apparent from the material presented at the 6<sup>th</sup> ICTC meeting. The triennial gatherings of the ICTC constitute the premier meeting of specialists and institutions involved in cyanobacterial research and management (1);
- convening of a workshop on the formulation of South Africa's algal toxin research program (first advertised presentation of the summary findings and intentions to the scientific community). This workshop provided the last open opportunity to identify additional issues pertinent to this project, and also to provide overlap with algal toxin specialists from the marine disciplines (this was last possible during 1998 when this author delivered a plenary presentation on developments in the freshwater algal toxin field at the Swakopmund Benguela Upwelling meeting (5);

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<sup>&</sup>lt;sup>2</sup> Copies of the reports for the individual deliverables are appended to this report in electronic (Adobe .pdf format). This document encompasses Deliverables 3 and 4 as the final synthesis.

- attendance and presentation at the inaugural meeting of the UNESCO CYANONET initiative. This
  meeting allowed for a comparison and contrasting of the level of cyanobacterial understanding in
  South Africa to be compared with that in the rest of Africa and the world, and for the incorporation
  of the global situation and needs assessment in this report. In so doing this analysis contains the
  most up-to-date inter-regional assessment and details of research and management programs
  worldwide (6);
- compare and contrast South African and international algal toxin research and management initiatives. Identify those projects that would be best undertaken at the local level and those which would benefit from international collaboration (3,4);
- identify those projects that require national implementation (for example: risk assessment and exposure evaluations) (3,4);
- identify and list human resource, infrastructure and over-arching management and associated policy needs required to support the implementation of a strategic initiative for algal toxin research and management in South Africa (3,4).

Finally the research strategy must assign research needs as being "academic", i.e. valid scientific-interest research which is of a longer-term nature and with no immediate prospects of providing management guidelines or solutions, or "applied" – being those research needs directly allied to the management of water resources and from which identified management "tools" will or are likely to become available in the short term.

### 3. BACKGROUND

South Africa was not been involved in "mainstream" cyanobacterial science and research for an extended period (~1985-2000). The reason for this is related to a conscious decision (*circa* 1985) by the Department of Water Affairs and Forestry (DWAF) to downgrade the importance associated with eutrophication, and the intermittent nature of cyanobacterial "incidents" – this despite the buildup of international efforts to address what has been deemed to be the most serious threat to global water resources (e.g. Conservancy Association, 2003). The cessation of the CSIR-driven Hartbeespoort Dam eutrophication research program (NIWR, 1985) heralded the end of an era of world-class efforts in this field. A summary of South Africa's policy and approach to eutrophication, and the gaps between policy and implementation, is provided in Walmsley (2000). Encouragingly renewed efforts by both the DWAF and the Water Research Commission (WRC) have seen the initiation of a number of new research- and management-directed initiatives.

Excessive development of cyanobacterial aggregations, or "blooms" are in the main symptomatic of uncontrolled eutrophication. Accordingly the formulation of cyanobacterial research and management programs require that the cause and effect pathway be considered in an holistic fashion – the one cannot be considered without the other – with cyanobacterial issues essentially being a 'subset' of the greater problem that is eutrophication. The problem is of a global and entirely generic nature, as is evidenced by the recent CYANONET appraisal and other surveys (discussed further hereunder). Both eutrophication and its symptomology, specifically the development of cyanobacterial blooms and the

production of toxins and/or tastes and odours, have cross-cutting research and management implications across the fields of water, agriculture (including aquaculture), human and animal health and ecosystem conservation. Both encompass a very high potential for placing a severe drain on economic resources across the spectrum of water supply, economic, recreational and tourism and natural resources.

South Africa experiences problematical blooms of algae as do many of the world's nations (see **4. The Global Perspective**). Although toxic blooms and animal deaths occur annually, the problem is of an intermittent nature and not currently of national import – although several dams are seriously affected these are in the minority when compared to the total storage of raw potable water in this country. What is of significance in the South African situation is that the (currently) worst affected waters are situated around and supply water to the most populous hub of economic development. The problem is, however, an emerging issue, likely to get worse, and there is no information that indicates the present extent of the problem within the rural domestic and agricultural sectors.

Prior to 1985 South Africa occupied an acclaimed and internationally-central position in cyanobacterial research (see Harding & Paxton, 2001). The period during which South Africa was absent from the cyanobacterial research arena was especially negative in that not only was this momentum lost, it was during this time that a marked surge in published information and the development of international collaborations occurred worldwide [see **Figure 1**]. During this same period the cyanobacterial research and management role moved from the nationally-funded arena to a few local authorities or water utilities, notably the City of Cape Town, Rand Water and Umgeni Water. This move was driven by an identified need to ensure safe water supplies by these bulk providers of potable water. The first entirely dedicated cyanobacterial toxin laboratory (in Africa) was established in Cape Town in 1994 and later similar facilities were created at Rand Water and the Water Utilities Corporation (WUC) in Botswana. In addition, cyanotoxin analytical capability existed at the DWAF Roodeplaat laboratories as well as at Umgeni Water.

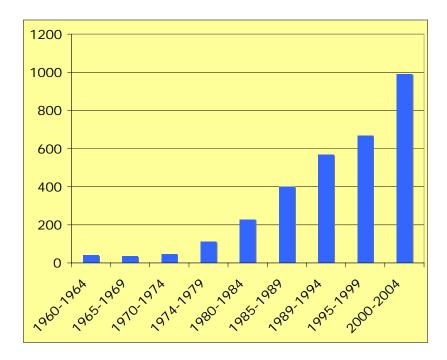


Figure 1: Increase in US-based rate of cyanobacterial-related peer-reviewed publications per 5-year intervals, 1960-2004, with particular emphasis on the period 1980-2004 (Source: Wayne Carmichael).

As identified by Walmsley (2000) "the country's [SA] water resource managers need to develop national strategies and guidelines for eutrophication control, but do not have relevant quantitative knowledge of:

- the national eutrophication problem in terms of its extent and trends;
- the sources of nutrients and the levels entering aquatic systems; and
- the actual social and economic costs of the problem on a national basis".

As has been identified by Walmsley and others, the problems of eutophication and its consequences, real and potential, are not new in South Africa and were identified for management action many years ago (e.g. Toerien, 1978; DWAF, 1979). While the potential risks have long been identified they have also been ignored or, at least, afforded a low priority status – this in contrast to the global recognition of the potential magnitude of the problem.

Subsequent to the release of the Walmsley (2000) report significant steps have been taken to start to address these issues through the means of consultancy investigations. However, South Africa is not served at the tertiary or technicon education level by any courses devoted to eutrophication, impoundment management and allied considerations; nor is there any central 'analytical agency' responsible for quality control, method development, provision of standards or inter-laboratory calibration. While the aforementioned local authority personnel kept some track of new developments, this was within the exigencies of their appointments – and without dissemination to the wider scientific or research communities. Despite the best of intentions many individuals working with cyanobacteria in South Africa during the past 15 years have lacked any formal training in limnology, eutrophication or cyanobacterial science – this placing severe constraints to progress. This lack of human resources

capacity development and the absence of any form of 'champion' proved to be a significant drawback leading to South Africa 'losing touch' with the rest of the world insofar as cyanobacterial research is concerned. Despite the examples set by various countries in terms of limiting phosphorus disposal to rivers and dams, South Africa lacks any form of policy in this regard.

During the 1990s significant developments were achieved in the analytical detection of cyanobacterial toxins, particularly the hepatotoxins – extending the detection capability to the nanogram level and including the recovery of toxins from complex biological tissue. The use of ELISA [Enzyme-linked Immunosorbent Assay] started to gain precedence over the more accurate yet more time-consuming use of HPLC [High Performance Liquid Chromatography] or HPLC-MS [Mass Spectrometry]. During the early 2000s the detection of toxicity in single colony isolates became possible, and the onset of the new millennium saw a surge of interest and effort applied to pcr-based genomics evaluations.

Cyanobacterial research and development is practiced at a very small number of centres of excellence scattered around the world. The number of these, and the personalities associated with them, has not changed significantly during the past 25 years. Despite the potential threats and risks associated with the development of cyanobacteria in water supplies, surprisingly little attention or funding is allocated to research in this field. The reasons for this are not hard to find: toxic cyanobacterial blooms are not reported at a frequency that results in their becoming recognized as a common problem requiring of urgent attention - at least not in South Africa - this notwithstanding the fact that an analysis of incidents and reports for the period 1990-2000 declared that "cyanobacterial problem events are widespread, frequent, prevalent and typically seasonal. Water resources subject to eutrophication commonly experience problems" (Downing and Van Ginkel, 2004). The fervour that typically surrounds publicized poisonings usually lasts only as long as the press decides the incident to be newsworthy - for example the loss of an entire dairy herd of 400 animals at Kareedouw (Harding and Van Halderen, 1998, unpublished data) attracted national television coverage, yet nil attention from any national department. Problematical blooms are infrequent and as yet in SA, have not resulted in reported and confirmed effects on humans. Animal deaths that can be reliably linked to cyanotoxins occur annually, but in the absence of education and any form of incident reporting the actual incidence remains unknown. With respect to bulk water supplies several of South Africa's dams are impacted by cyanobacteria, with some - such as the notorious Hartbeespoort, having been so for several decades (Trophic Status Report, DWAF 2001). Certain major rivers are impacted by cyanobacterial development, notably in Northern and Kwa-Zulu Natal provinces.

During South Africa's cyanobacterial 'doldrums' no new specialists, possessing of a robust and potentially-sustainable interest in eutrophication and/or cyanobacteria have emerged – this lack being entirely understandable given the above-outlined lack of a research environment within which to work. Continuity of effort insofar as the national interest is concerned has remained confined to one or two dedicated specialists providing largely unpaid services. During the same period there was steady progress away from mainstay analytical techniques (HPLC) towards cheaper, more rapid methods such as ELISA and protein phosphatase inhibition (PPI). While this method is justifiable in terms of available skills, ease of use, costs per sample and rates of sample throughput, it does contain appreciable room for error – both false positives and negatives. Accordingly its use should always be

backed-up using HPLC or other definitive technique. The provision of such second-tier methods should exist in a central facility that is continually 'on-line'. However, no national laboratory exists in SA, and few commercial local authorities can afford to dedicate equipment and personnel to what is often a non-routine application. The use of ELISA in South Africa is rendered more problematic by the fact that it's use has yet to be comprehensively evaluated in terms of range, linearity and specificity of response.

From the work undertaken during this project it is apparent that South Africa's old international allies in the cyanobacterial research and management arenas remain positively and warmly disposed towards renewed collaborative efforts. As will become apparent from the analysis provided hereunder collaboration must comprise an essential component of any successful return by South Africa to prominence and capability in this field.

During the early 2000s cyanobacterial research in South Africa followed the international trend towards genomics-based investigations. With minor exceptions – the work at the University of Port Elizabeth being a case in point – this research was more a case of run before learning to walk – i.e. the analytical skills and resources were inadequately developed for the task at hand. Some projects incorporated a mixture of environmental assays – seemingly in the hope that something would show up on correlative analysis. To-date much of the work in this area has been of relatively poor standard and lacking in local and international relevance and would have benefited from scrutiny by an appointed international panel in the absence of relevant proposal appraisal skills and experience from within the local scientific community.

### 4. THE GLOBAL PERSPECTIVE

The recent global assessment undertaken by the International Steering Committee for CYANONET provides a clear indication of the generic nature of the cyanobacterial problem. The following text is extracted *verbatim* from a global synthesis prepared by this author (Harding, 2005) for the CYANONET First Phase Report to UNESCO.

"The output from this first phase of CYANONET provides the first truly global synthesis of the 'state of the planet' insofar as cyanobacteria and cyanotoxins are concerned. Cyanobacterial aggregations (blooms, scums and mats), are among the most common and best known symptoms and consequences of anthropogenic eutrophication. They form a common characteristic of surface waters, worldwide. An awareness of cyanobacterial mass populations blooms, and their associated toxicity among limited scientific and other professional groups has spanned a period of more than 100 years, and indeed may be discerned in the wider community even earlier according to non-scientific/non-technical literature and popular culture. However, such awareness has increased and declined with time, as associated poisoning incidents have occurred and their immediate impacts have passed. Recognition of cyanobacterial blooms and their health significance has increased over the last 15 years, although as indicated in this exercise, this awareness is very patchy, and apparently lacking in many parts of the world. It is only quite recently that the threat to inland and coastal surface waters posed by nutrient enrichment has been catapulted to the forefront of water resource management on all continents – this especially during the past 50 years. The eutrophication of lakes, impoundments and other surface

waters stems from a global commonality - an anthopogenic deterioration in surface water quality that became particularly apparent during the 1950s. This led to, typically, an order of magnitude increase in the availability of nutrients, particularly in lakes, reservoirs, rivers and estuaries, and a resultant increase in observed cyanobacterial and algal development incidence from the 1960s onwards.

While eutrophication remains a cosmopolitan threat, its geographic extent is, with the exception of some countries, poorly known. There have been successes in reducing eutrophication at individual waterbodies in certain countries, notably in areas of Europe where stringent and mandatory regulations on effluent composition and discharge have been in effect for many years. Elsewhwere, especially in the arid regions of the southern hemisphere, the problem is burgeoning in developing countries where access to potable water and wastewater disposal servicing are unavailable to many millions of people. The findings of this CYANONET situation assessment support the inference that eutrophication is both widely spread and under-recognised across the World's freshwater environments.

Phase 1 of the CYANONET Project obtained reports from at least 65 countries, throughout Africa, the Americas, Europe, Asia and Australasia, of occurrences of cyanobacterial mass populations. From this it is clear that all reporting countries have experienced or routinely experience cyanobacterial presence in a range of surface waters – and in many cases to a problematical extent. The availability of information is, however, highly variable. With the exception of South America, Africa and parts of Asia, access to scientific and management information and reports are relatively readily available for North America, some parts of Europe, Australia, New Zealand and highly developed parts of Asia. They are far less available in much of Africa and for parts of Latin America and Asia. A minority of countries per continent are relatively well-resourced in terms of cyanobacterial monitoring and awareness. Awareness of the threats posed by eutrophication, and its consequences for health and water supply, is limited or non-existent in many developing countries. For example, language, cultural and conflict barriers render communication and information exchange difficult on the African continent. Nonetheless, it may be reliably predicted that most of the, as yet non-reported countries, will confirm the widespread occurrence of cyanobacterial mass populations development and associated problems.

The presence of cyanotoxins is common to all reporting countries included in this survey. The microcystins are the most commonly reported family of cyanotoxins in all countries. In cases where comprehensive analyses for multiple classes of cyanotoxins have been performed (e.g. in Scandinavia), this may be due to the dominance of these hepatotoxins among the cyanotoxins in the waterbodies of a particular country. The less frequent reporting of cyanobacterial neurotoxins may be due to their lower occurrence in intensively investigated countries. However, it is also likely to be due to limitations in the ability to detect, identify and quantify cyanobacterial neurotoxins.

Occurrences of the more recently recognized cyanotoxin cylindrospermopsin are reported from countries on all continents. Reported producers of cylindrospermopsin, including some, but not all populations of *Cylindrospermopsis* and several other cyanobacterial genera, are also available from all continents. This is a developing knowledge field to which CYANONET may be able to contribute in the future via its expanding network.

For most countries reporting the detection of cyanotoxins, HPLC forms the mainstay of analytical competence, increasingly augmented by immunoassays (ELISA). More sophisticated methods are also practiced, although only at a limited number of centres of excellence. Globally the availability of monitoring and analytical facilities, appropriately trained personnel and indeed even facilities capable of undertaking reliable cyanobacterial identification and enumeration, is severely under-resourced. The non-reporting of cyanotoxins, unless specifically not found, may in most cases be due to the non-availability of the necessary facilities and skills. Inter-laboratory comparability and validation practices are almost non-existent - this being rendered extremely problematical by the limited availability of laboratory facilities, necessary expertise in cyanobacteriology and cyanotoxin analysis and especially of cyanotoxin standards and reference materials.

Reported health impacts caused by cyanotoxins involve humans, animals, birds and fish. Most reports involve domestic and wild animals, with mortalities being extremely common. A considerable number of both lethal and non-lethal effects on animals may go unreported due to a lack of awareness or a commonality in symptomology with better known causes, e.g. tick-induced biliary. The role of cyanotoxins in the mass mortalities of Lesser Flamingos in Africa provides an example of the hazards of the toxins to an endangered wildlife species. Allelopathic effects and impacts occurring at the primary and secondary production levels within aquatic ecosystems are known, but as yet poorly understood. With respect to humans, reports of external (skin, ear and eye) problems, e.g. blistering and dermal irritation, are commonly associated with cyanobacterial blooms in most reporting countries, as are gastroenteric symptoms. Elevated incidences of primary liver cancer (PLC) associated with untreated drinking water containing microcystins were reported from China some years ago. The present survey has been informed of a further suspected association between increased elevated PLC and microcystin-exposure in a European country (Serbia). The strength of theis association requires further investigation.

The only known confirmed case of human mortalities clearly attributable to cyanotoxins has been that reported by Brazil (the 1996 Caruaru incident). However, an unconfirmed report from Kenya documents 100 human deaths at Lake Embu attributed to cyanotoxins. The latter report provides a timely example of the need for the establishment of a communication and support network such as that being envisaged for CYANONET. Reports from Asia, Africa, South America and Australia have documented problems posed by cyanotoxin accumulation in marine and estuarine filter-feeders. Other countries where aquaculture is a major industry have concerns regarding cyanotoxin accumulation in invertebrates and fish tissue, as well as for the associated accumulation of taste and odour compounds (e.g. geosmin) – which renders the marketable product unpalatable.

The determination of the precise extent of adverse impacts on human health is limited by the lack of epidemiological investigations across all reporting countries, with a small number of exceptions (principally China, Australia). It is commonly recognized that conducting such investigations on human study groups is constrained by a large number of factors, not least public panic in cases where sustained cyanobacterial blooms are common in drinking water supplies. In other instances, notably from South Africa, confusion is generated by the presence of very high and potentially lethal

concentrations of cyanotoxins in unprotected water sources, yet with no recognized evidence of chronic or acute effects in humans and animals consuming the affected water.

Negative impacts of cyanobacteria, and in many cases cyanotoxins, on water treatment and supply are common to all reporting countries. Increased costs of raw potable water treatment are incurred by increased levels of process blockages, tastes and odours, and the presence of cyanotoxins. In a minority of countries, this is addressed by additional tertiary treatments (e.g. activated carbon or ozone). Financial costs preclude such retro-management in poorer countries or communities. The majority of affected water resources are partly safeguarded by rudimentary or primary treatment processes with little to no protection against cyanotoxins entering the reticulated supply system. Countries in Africa and South America, where many millions of inhabitants do not have access to safe drinking water are particularly at risk. This problem is exacerbated in the drier regions. On a single country assessment, China experiences a very high degree of water resource impairment. The large-scale use of algicides, including the ecosystem-damaging copper sulphate, continues to be implemented in both some developed and developing countries, whereas such chemicals are legally banned in others.

The occurrence of cyanobacterial blooms significantly and increasingly constrains the recreational use and potential of many waterbodies in countries on all continents. In countries such as South Africa, which has an absolute requirement on dams for water supply, eutrophication from polluted return flows has severely limited the overall usability of several major storages. Holland and Norway, for example, experiences increasing loss of use of recreational waters during the summer months. Temporary closures of waterbodies to recreational activities, with consequent losses to amenity and local economies occur in some countries e.g. in Europe and Australia, where cyanobacterial populations and cyanotoxins are monitored and recreational safety guidelines are applied. A minority of countries have well researched and developed recreational guidelines for cyanobacteria.

The availability of management tools and guidelines to reduce cyanotoxin health risks is limited, in the global context, to the information and advice provided by the World Health Organization (WHO) and a minority of national policies. Several countries have produced various guidelines and management protocols, but these are variable in validity, content and scope. Some of these are straightforward adoptions of the guidelines derived by the WHO. A minority of countries, e.g. Brazil, have gone further and recently invoked national legislation to limit microcystin concentrations in drinking water. These national regulations are variants of the WHO drinking water (lifetime exposure) guideline value of 1 µg microcystin-LR per litre. In some cases, the legal instruments apply only to the single microcystin variant 'microcystin-LR', whereas others apply potentially to the wider range of microcystins. Several express the guideline as encompassing all variants expressed as 'microcystin-LR equivalents'. Yet other countries, with a relatively high level of expertise in the risk management of cyantoxins, have not introduced legislation to control their concentration in potable water, but prefer to abide by a guideline-based system. Which of these approaches are the most appropriate in terms of health protection and practicability may become apparent with in the coming years as research on cyanotoxins continues and further practical experience is developed and shared.

This assessment has shown that wide differences exist throughout the world in the necessary levels of recognition, knowledge and preparedness to address the problems which cyanobacteria and cyanotoxins present to water availability, amenity, safety and health. There are also major geographical and institutional differences with respect to the availability of required skills, information, experience and technology for transfer. However, there is a high potential for sharing such resources through awareness promotion, education, training, with adaptation to accommodate regional and local characteristics and needs. This approach is distinctly lacking at present and is the central justifying aim for CYANONET.

The problems posed by eutrophication and its symptoms are cosmopolitan, and their reduction would clearly be well-served by the development and dissemination of appropriate and internationally-relevant control policies and information resources. At local (country) level there is an identified need for tertiary level specialist training and human resource capacity building. Much of the global knowledge resource on cyanobaceria and cyanotoxin control and risk management is localized within a very small group of individuals, mostly located at universities and research institutes associated with the water industry. This experienced group does not appear to be well-placed for the sustained transfer of knowledge and technology through the attraction of young specialists and scientists - possibly as a consequence of the low level of attention devoted to this aspect of water quality management by many governments. This in itself surprising given the complete dependence of humanity on water supplies for survival.

### 5. BASIS FOR A SOUTH AFRICAN ALGAL TOXIN RESEARCH STRATEGY

### 5.1 PRE-IDENTIFIED SOUTH AFRICAN INFORMATION AND RESOURCE NEEDS

### 5.1.1 Macro-biological Research Thrust (WRC)

A WRC-convened workshop<sup>3</sup> was held during October 2000 to identify research needs relevant to all WRC research thrusts ("cross-cutting" needs). The following broad research objectives were identified:

- need to identify and address knowledge and information gaps, especially with regard to algal physiology and metabolism, but also including training, treatment and management issues;
- establish standard methods for algal sampling and counting;
- problems posed by toxins, tastes and odours and disinfection by-products;
- monitor the incidence and dynamics of algal development;
- develop a Best Practice guide for algal management.

The objectives were subsequently condensed into two categories of needs, these being Top, Major and Long-term Priorities, as follows:

<sup>&</sup>lt;sup>3</sup> Notes on a workshop held at the [Johannesburg] Airport Conference Center, 16 October 2000

### Top Priority

- Need for a central 'help-line' or information on call;
- Standardized methodologies based on universally-accepted protocols (sampling, monitoring, counting, etc.);
- Quantification of the extent of the problem;
- Development of a coordinated research program.

### Major Priority

- Causes and dynamics of algal toxin production;
- Species-based contributions to toxins and tastes and odours;
- Best Practice Guides:
- Alternative, cost-effective treatments.

### Long-term

• Establishment and maintenance of a national culture collection;

This workshop also identified that any national program on algae should maintain links to the following:

- Eutrophication program;
- Water treatment utilities;
- International research centres;
- Other cross-cutting research programs;
- River Health Programme;
- Ecological research initiatives;
- Resource Management;
- Medical Research Council;
- Veterinary Research;
- Tertiary education institutions.

### 5.1.2 The "Toxic Algal Forum"

During the late 1990s an industry-led initiative to sustain impetus and information sharing in the field of cyanobacteria and cyanobacterial toxins was convened under the founding auspices of Rand Water, and championed by the WRC. Despite the best-intentions of this group the attempt failed shortly after its inception – mainly as a result of the lack of national direction and support.

### 5.1.3 Eutrophication understanding and management

The development of cyanobacteria in water sources is a direct symptom of unmanaged eutrophication. While the need for research into the ecological associations of toxic algae, the production of toxins and

the management thereof is unquestioned, the ultimate control of the problem needs to focus on the cause, and primarily nutrient generation at the level of the catchment and the transfer thereof into streams, rivers, wetlands, ponds and dams. Walmsley (2000)<sup>4</sup>, in his report to the Water Research Commission, provides a detailed list of research topics, summarized as follows:

- Assessment and prediction of eutrophication problems in water resources;
- Nutrient loading;
- Role and efficacy of wetlands in retaining nutrients especially wetlands impacted by elevated anthropogenic nutrient loads;
- Nutrient dynamics in reservoirs (dams);
- Nuisance plant growths (algae and macrophytes);
- Other factors influencing the expression of eutrophication (hydrology, suspensoids, climate);
- Assessment and development of experimental eutrophication management techniques;
- Development of information management systems.

In response to the recommendations of the Walmsley Report, and a subsequent workshop to identify eutrophication research and capacity building needs (Walmsley 2001) a number of eutrophication-directed projects have been initiated by the WRC or DWAF. These are:

- A Guide to Conduct Eutrophication Assessments for Rivers, Lakes and Lacustrine Wetlands (WRC K5/1343);
- Nutrient Management: Development of Best Practice Guidelines and Implementation Strategies to Support the Management of Eutrophication in South Africa. (DWAF, commencing April 2005);
- Development of a Model to Assess Costs Associated with Eutrophication (WRC, commencing April 2005);
- National Eutrophication Monitoring Program (DWAF, 2002);
- Trophic Status Project and the Assessment of the Trophic Status Report (DWAF, 2001).

### 5.1.4 Geographic extent of the problem in South Africa

Three studies have examined the geographic extent of eutrophication and cyanobacterial spread in South Africa, namely:

- Trophic Status Project (DWAF, 2001);
- National survey of the incidence of cyanobacterial blooms and toxin production in South African impoundments (DWAF, 2004) and;
- Cyanobacterial Monitoring: 1990-2000 Evaluation of South African Data (WRC, 2004).

<sup>&</sup>lt;sup>4</sup> See also results of followup workshop (Walmsley, 2001).

With respect to toxic algae and algal toxin research needs these reports concluded that there is a need for:

### (a) DWAF(2004):

- South Africa to develop standardized sampling and analyses methods;
- analytical laboratories, both for algal identification and cyanobacterial toxin analyses, to be
  established on a regional, provincial or CMA level for more efficient data acquisition and
  dissemination of information;
- a National Cyanobacterial Monitoring Programme, possibly as part of the National Eutrophication Monitoring Programme, and inclusive of all water resources;
- the integration of biological and meteorological research to understand and estimate the consequences of climate variations on [aquatic] ecosystems in South Africa;
- sampling surveys on small farm dams and irrigation systems should be initiated on a regional level to determine the potential hazard to rural domestic water users and agricultural users;
- eutrophication management strategies need to be developed on a National and Regional level and implemented to reduce toxic cyanobacterial bloom development in South Africa's freshwater resources and to ascertain safe domestic, agricultural and recreational water supplies.

### (b) 1990-2000 Evaluation (WRC, 2004):

### Information needs

- increased national coverage on eutrophication and associated algal and cyanobacterial status;
- increased monitoring frequency and coverage for toxin and geosmin/MIB;
- an integrated national monitoring and data analysis system so that stakeholders have access to up-to-date information on all areas and water resources;
- information on applicable alert level structures;
- information on analytical methods;
- coordinated training on analytical methods;
- nationally-standardised monitoring procedures.

### Recommendations for research

- Development of improved predictive models of cyanobacterial growth and toxin/taste-andodour-compound production;
- Standardisation and inter-lab comparisons of analytical and sampling methods;
- Development of an all-encompassing (including all aspects of water quality) national alert level structure for application with minimal alteration by each management body;
- The correlation of trends in frequency and severity of cyanobacterial problem events with climatic variation;

• Evaluation and modification of existing predictive models based on available data from South African impoundments.

### 5.1.5 Cyanobacterial Research in South Africa

A comprehensive history of cyanobacterial research and related activities in South Africa pre-2000 is contained in:

• Cyanobacteria in South Africa. A Review. (WRC, 2001).

### 5.2 INTERNATIONAL RESEARCH DIRECTIONS

Prominent examples in international cyanobacterial research have originated in the United Kingdom (e.g. UKEA, 2000), Australia (CRC WQT 1998) and Europe (WHO, 1999). More recently approaches concentrating mainly on algal detection, identification and appropriate water treatment approaches have been augmented by attention to recreational management issues. The most comprehensive example of progress here has been that produced by the Australian NHMRC.

### 5.2.1 Collaborative approaches – the GWRC example

As will be apparent from the global perspective provided by the CYANONET synthesis, the level of scientific effort applied to issues of algal toxin research and management is confined to a few centers of excellence. A dominant analytical and interpretive presence exists on all continents except Africa. Collaborative links exist between many of these centers.

The mode of operation of the GWRC is based on international cooperation and collaboration between its partner members, a model that is ideally suited to the derivation of maximum benefit from scarce personnel and infrastructure resources. As is also apparent from the findings of the CYANONET synthesis the nature and characteristics of the 'algal toxin problem' are globally-generic – this in itself emphasizing the value of allied rather than individual approaches to the solving of common problems. With perhaps minor exceptions it is clearly-apparent that inadequate research progress will be made in the absence of international collaboration.

The issue of toxic algae is a priority focus of the GWRC. The May 2005 workshop formed part of a project aimed at reviewing and exchanging information on the present knowledge on the management of toxic algae, and to develop an allied collaborative research strategy. The aims of the workshop (full details contained in GWRC, 2004) were to:

- (i) establish the critical issues of concern for the managers of water supplies;
- (ii) foster a collaborative approach between groups concerned about the management of toxic algae;
- (iii) ensure current research undertaken by various groups is complimentary, rather than duplication;

- (iv) develop a more co-ordinated approach to global toxic algae research, with consequent benefits to all organisations involved;
- (v) identify current research needs and strategic research directions.

The identified research needs emanating from this (GWRC) workshop are summarized as follows:

### Impact of toxic algae

- Exchange of information relevant to impacts on agriculture;
- Impacts of toxic algae on the aquatic environment (ecosystem health);
- Guideline information based on toxicological studies.

### Monitoring

- Technology transfer on sampling methodology;
- Exchange of information on inter-laboratory testing of methods;
- Coordination of development on certification of standards;
- Support of development of an 'Occurrence Database', e.g. CYANONET;
- State-of-the-Science and development of Online Monitoring.

### Source water management

- Consolidation of knowledge into a guidance manual;
- Non chemical means for control of formation or algal toxins.

### Water treatment

- Consolidation of knowledge into a guidance manual;
- Coordination of on-going projects.

### 5.2.2 Proposed GWRC Research Strategy

The outcome of the GWRC meeting was the formulation of a set of three proposals (included *verbatim* below) for approval and funding allocation by the GWRC Managing Committee. The GWRC partners who will undertake the work, when approved, are indicated in parentheses:

**GWRC Proposal 1:** Toxicological studies for guideline formulation for short-term exposures to cylindrospermopsin and microcystins. Evaluation of reproductive toxicity of cylindrospermopsin (CRC WQT).

Well-defined guidelines assure appropriate treatment and monitoring expenditure. Cyanobacterial blooms have an impact on economic activity associated with affected water-bodies. Recreational guidelines define when there is, or is not, a real threat to public health. There are no WHO-approved guidelines for cylindrospermopsin although de facto water treatment targets are being incorporated into performance contracts, and several countries have already included them in their legislated or approved

guidelines. This will lead to inadequate protection of public health and potentially increased costs for water treatment and analyses. The same concerns apply to recreational conditions. There is an urgent requirement for studies into the adverse health effects of short-term oral exposures. Such data have application for both drinking water and recreational guideline formulation. Current guidelines for drinking water do not address short-term or intermittent oral exposures. The cyanotoxins of primary concern are cylindrospermopsin and microcystins. Microcystin analogues other than mLR need to be assessed to determine the applicability of current drinking water guidelines to actual microcystin occurrence. Animal studies addressing potential carcinogenicity are either underway (microcystins) or planned (cylindrospermopsin) by the US National Toxicology Program and so are not contemplated in this proposal. Potential reproductive toxicity of cylindrospermopsin also needs to be assessed before comprehensive guidelines can be formulated.

**GWRC Proposal 2:** Towards applying the most appropriate technologies for on-line and direct monitoring of recreational, source and treated waters impacted by toxic cyanobacteria (TZW).

Cyanobacterial cells, cell by-products, taste and odour compounds and toxins are important indicators for monitoring, early warning and modelling for harmful impacts of cyanobacteria. New technology could provide remote detection and cheap, rapid detection for early warning of toxins. The current knowledge on developing technologies such as real-time PCR, selective fluorescence measurements, mass spectroscopy, remote sensing and lake diagnostic systems indicate that this approach is feasible. The water quality framework requires that the critical control points are continuously monitored to assess effectiveness of barriers and risk from hazards. The rapid detection of toxin outbreaks is essential for risk management. In the first phase of the project the existing knowledge will be assembled and based on new promising technologies methods for toxin monitoring will be developed. In the second phase of the project the technologies will be validated and deployed to the pilot or field environment.

### **GWRC Proposal 3:** *Guidance manual for the management of toxic algae* (CRC, WRC)

Over the past 20 years significant research has been conducted into the management of cyanobacteria and the toxins they produce. The work has been published in hundreds of papers, reviews, reports and books. Presently no single document exists that has consolidated this vast knowledge into a guidance manual that can be easily used by water suppliers in the management of toxic cyanobacteria; however, a number of research groups are planning to produce such a guide within the next two years. While most of these groups will still produce their own "regional" guide, the objective of this proposal for the GWRC is to combine and consolidate the information in these guides to produce a truly international document, written in a way that all levels of the water industry can appreciate. The GWRC will then be able to take advantage of the combined knowledge such a document would represent, and the information contained in the regional manuals would be enriched with additional views and approaches.

# 5.2.3 6<sup>th</sup> International Conference on Toxic Cyanobacteria

The following research directions and emerging issues could be identified from the proceedings of, and discussions held at, the 6<sup>th</sup> ICTC meeting (June 2004). The list is not presented in any order of priority:

- (i) the problem of increasing incidence of *Cylindrospermopsis*<sup>5</sup> and the associated production of the alkaloid toxin cylindrospermopsin;
- (ii) the need for guideline levels for cylindrospermopsin (mammalian toxicity);
- (iii) a clear rationalization and prioritization between "need to know" and "scientific interest" research;
- (iv) an increase in the number of applied contributions pertaining to water resource management and treatment <sup>7,8</sup>:
- (v) concern about the effects of global warming/climate change on the frequency and severity of cyanobacterial-related water quality incidents;
- (vi) increased attention to bioaccumulation (foodchain) issues 10;
- (vii) risks posed by exposure to multiple toxins, in particular combinative mixtures of microcystin and cylindrospermopsin<sup>11</sup>;
- (viii) relationships between cyanotoxins and microbial pathogens;
- (ix) new interest in degradative pathways examining the breakdown of cyanotoxins both by bacteria <sup>12</sup> and molluscs such as mussels;
- (x) the risks posed by neurotoxicity <sup>13</sup>;

<sup>&</sup>lt;sup>5</sup> Cylindrospermopsis is a filamentous cyanobacterium producing cylindrospermopsin is a small molecular size alkaloid carcinogenic toxin. During recent years there has been a global increase in the prevalence of this alga in surface waters. It presents particular management difficulties in that it can fix atmospheric nitrogen, does not form scums or odours, demonstrates tolerance to salinity and is an effective storer of phosphorus – i.e. it can find a selective advantage in low P environments that no longer support *Microcystis*. Isolated yet potentially-problematical appearances of *Cylindrospermopsis* have occurred in South Africa in recent years, the most notable being the seeding of the Orange River system downstream of the confluence with the Harts River.

<sup>&</sup>lt;sup>6</sup> An outcome of the GWRC Workshop on Toxic Cyanobacteria was a proposal to undertake the necessary studies to quantify the toxicity of cylindrospermopsin to mammals (to be reported in K8/576/Deliverable 2).

<sup>&</sup>lt;sup>7</sup> There is now additional clarity on the efficacy of chlorine-based removal of microcystins from drinking water supplies. The use of chlorine remains unsuitable for treating waters containing anatoxin-a.

<sup>&</sup>lt;sup>8</sup> Interestingly no references were made to geosmin or 2 MIB – this suggesting that taste and odour problems are no longer a major focus in the cyanobacterial research and management arena, and that taste and odour management has been relegated to simply being a function of water treatment.

<sup>&</sup>lt;sup>9</sup> In plants, fish, shellfish, molluscs, birds and mammals. There is clear evidence that the foodweb acts both as a sink and as a vector for cyanotoxins.

<sup>&</sup>lt;sup>10</sup> Novel studies show cyanotoxins being vectored to humans via bats, and via the surfaces of aquatic plants (e.g. *Hydrilla*) into waterfowl.

<sup>&</sup>lt;sup>11</sup> Given that mixed blooms of *Microcystis* and *Cylindrospermopsis* commonly occur, and as microcystin is a tumour promoter and cylindrospermopsin a carcinogen, the level of risk to human health is likely to be exacerbated. Cylindrospermopsin is also produced by other genera of cyanobacteria such as *Aphanizomenon ovalisporum* and *Umezakia natans*.

<sup>&</sup>lt;sup>12</sup> 16 strains of bacteria that can degrade microcystins and nodularin have been characterized.

Routine screening for the presence of neurotoxins such as anatoxin-a remains rare, largely due to the unavailability of standards, short half-life of the toxin, and problems with analytical applications.

- (xi) increased awareness of ecological impacts both by toxins and other compounds such as protease inhibitors on biota, particularly on growth rates and reproductive ability of zooplankton, and osmoregulation of a variety of aquatic animals;
- (xii) genomics-related studies in a variety of directions <sup>14,15</sup>;
- (xiii) purpose and role of microcystins in cyanobacterial ecology physiological (e.g. iron-binding), nucleotide synthesis inhibitor, signalling and/or zooplankton [grazing] inhibitor<sup>16</sup>;
- (xiv) continuing attention to the development of detection methods for cyano- and other toxin compounds 17,18,19,20;
- (xv) attention to inter-laboratory standardization of cyanotoxin detection and quantification methods<sup>21</sup>;
- (xvi) threats posed by cyanobacteria to recreational use of surface waters<sup>22</sup>;
- (xvii) the need for multidisciplinary and/or collaborative<sup>23</sup> approaches to cyanobacterial research and management;
- (xviii)a clear indication that significant problems are being encountered at ambient phosphorus levels that are considerably lower than is commonly believed<sup>24</sup>. Allied to this are management initiatives setting very low levels of in-lake phosphorus as management targets<sup>25</sup>;
- (xix) an apparent lack of any in-depth limnological training and awareness. Only in a few cases was the observed behaviour of cyanobacterial populations correctly linked to biophysical lake and reservoir forcing functions;
- (xx) a heightened awareness and acceptance of the role of biomanipulation<sup>26</sup> (food web manipulation) in offsetting the effects of eutrophication;

<sup>22</sup> This aspect of cyanobacterial management is receiving especial attention in countries such as Australia, New Zealand, Holland and Norway. Despite its Scandavian, north-temperate location, cyanobacterial problems currently affect drinking water and recreational use water resources for, respectively, 2% and 13% of the Norwegian population.

<sup>&</sup>lt;sup>14</sup> Cyanobacterial genetics is a rapidly-developing field that is generating interesting but as yet somewhat inconclusive answers. Research needs in this specific area will receive special attention during the development of this strategic plan. Attention continues to be focused on the Mcy gene array.

<sup>&</sup>lt;sup>15</sup> The dominant global centres for cyanobacterial genetics appear to be Finland (Kaarina Sivonen) and Germany (various researchers).

<sup>&</sup>lt;sup>16</sup> The debate surrounding why cyanobacteria produce toxins, supported by a large number of studies over many years, continues but the answer remains elusive. Microcystins require considerable amounts of cellular energy to produce, have complex synthesis pathways and are extremely old in evolutionary terms – all of which tends to suggest a very specific function.

<sup>&</sup>lt;sup>17</sup> Despite claims made during the past few years the development of a "field-kit" test approach has not been fulfilled.

<sup>&</sup>lt;sup>18</sup> Advances have been made that allow nanogram-level detection limits. Other refinements include the ability to accurately determine low levels of cyanotoxin in feathers and human hair. These advances offer not only additional scope for forensic work but may support non-invasive epidemiological studies.

<sup>&</sup>lt;sup>19</sup> Significant advances have been made in reducing analysis times for resolving individual toxins.

<sup>&</sup>lt;sup>20</sup> New data are providing information on the [mammalian] toxicity of lipopolysaccharides (LPS).

<sup>&</sup>lt;sup>21</sup> Access to accurately-quantified standards remains a problem.

During the development of this project it was clearly apparent that, given the limited number of specialists and cyanobacterial research units worldwide, and the global commonalities that are characteristic of cyanobacterial developments, the most appropriate and cost-effective manner to deal with the problem will be through maximizing collaboration opportunities, both in terms of personnel and funding.

<sup>&</sup>lt;sup>24</sup> Problems are experienced with low ( $\sim 1 \, \mu g \, \ell^{-1}$ ) yet persistent concentrations of toxins in bulk water supplies – this akin to the problems that have been experienced in South Africa for many years in dams such as Theewaterskloof and Voelvlei (W R Harding, personnel observations and research).

<sup>&</sup>lt;sup>25</sup> The Lake Erie program has set an extremely low target of 10 μg  $\ell^{-1}$  total phosphorus, and P-load reduction efforts have thusfar reduced in-lake concentrations to between 25 and 30 μg  $\ell^{-1}$ .

- (xxi) the need for a clear by country or region demographic understanding of the pervasiveness of cyanobacterial dominance in water supplies<sup>27</sup>;
- (xxii) the need for awareness-raising and education programs both for eutrophication and its symptomology such as cyanobacteria and their toxins;
- (xxiii)problems with world terrorism issues and restrictions being placed not only on the transport of samples and standards, but also the threat of cyanobacterial toxins as Biological Warfare Agents<sup>28</sup>.

# 5.2.4 11<sup>th</sup> International Conference on Harmful Algal Blooms (HABs)

Subsequent to the 6<sup>th</sup> ICTC, and following interactions with various specialists, the following condensed list of South African research areas was derived for workshop presentation at the 11<sup>th</sup> HAB meeting (November 2004)<sup>29</sup>:

- (i) Health risks and impacts;
- (ii) Emergence of new problem species;
- (iii) Regulatory and management approaches;
- (iv) Standardization of methods/certification of standards;
- (v) Non-chemical means of control / foodweb management;
- (vi) Marine freshwater experience / is there an overlap?
- (vii) Cyanotoxin accumulation in fish and shellfish;
- (viii) Taxonomy/cyanogenetics.

From the 11<sup>th</sup> HAB workshop the following additional research topics were identified:

- (i) Protection of rural potable supplies;
- (ii) Education and awareness training aimed at water treatment operators, the recreational water use environment, agriculture and the general public<sup>30</sup>;
- (iii) Updating of available taxonomic keys (see also pt v);
- (iv) Toxin supply and validation;
- (v) Training in general taxonomy of phytoplankton in general and cyanobacteria in particular;
- (vi) Risk assessments;
- (vii) Need for a national analytical facility;

<sup>26</sup> Biomanipulative approaches invoke alterations to "top-down" pressures on the lake foodweb, typically increasing predation on cyanobacterial phytoplankton. This approach has been very successful in Scandinavia, and is currently being applied in South Africa at Hartbeespoort Dam.

<sup>27</sup> The Peoples Republic of China is an example: Reportedly between 50 & 80% of 2759 lakes in China are trending towards becoming eutrophic.

<sup>28</sup> For example, conflicting legal situations exist in the Czech Republic where one law governs the allowable concentration of microcystin in water supplies, while another outlaws the compound in terms of anti-terrorism regulations.

<sup>29</sup> See Consolidated Project Direction as of end August 2004. Deliverable Report 2, August 2004, pg. 10.

<sup>&</sup>lt;sup>30</sup> The overriding importance of the need to attend to the lack of education in the fields of eutrophication in general and cyanobacteria in particular has already been identified within the framework of this project, but was not included for discussion at this workshop.

- (viii) Inclusion of protective WHO guideline in SANS guidelines (example of Brazil);
- (ix) Algal-based water treatment processes;
- (x) Need for recreational guidelines.

#### 5.2.5 The 'BMAA' issue

The non-protein amino acid β-N-methylamino-L-alanine (BMAA) is deemed to be a possible cause of the amysotrophic lateral sclerosis/parkinsonism-dementia complex in humans. Production of BMAA has been identified in *Nostoc* but recently (2004) has been deemed to be possibly associated with all known groups of cyanobacteria (Cox *et al.*, 2005). This has significant implications for widespread human exposure and several countries have already implemented the establishment of analytical facilities and screening trials of their cyanobacterial populations. This discovery places research on BMAA high on the priority research list.

#### 5.2.6 International research links

Information on international research interests (current or future) was polled by means of a letter to various leading specialists, universities, institutions and organizations (see Table 1). 16 positive responses were obtained from 22 submissions (73%).

Table 1: Process of engagement with international specialists as at end-January 2005

NAME	AFFILIATION	email address	Response	Dialogue started
Briggs, Dr Lyn	Agresearch, NZ	lyn.briggs@agresearch.co.nz	No	-
Burch, Mr Mike	AWQC	Mike.burch@sawater.com.au	See B	ursill
Bursill, Prof Don	AWQC	don.bursill@sawater.com.au	Yes	Yes
Carmichael, Dr Wayne	Wright U, USA	Wayne.carmichael@wright.edu	Yes	Yes
Chorus, Dr Ingrid	UBA Germany	Ingrid.chorus@uba.de	Yes	Yes
Codd, Prof Geoff	U Dundee	g.a.codd@dundee.ac.uk	Yes	Yes
Donlon, Dr Peter	WSSA	Peter.donlon@wsaa.asn.au	Yes	Yes
Falconer, Prof Ian	CRC WQT	<u>ifalconer@medicine.adelaide.edu.au</u>	Yes	Yes
Fastner, Dr Jutta	FEA Germany	<u>Jutta.fastner@uba.de</u>	See C	horus
Hasan, Dr Misha	AwwaRF	mhasan@awwark.com	No	-
Humpage <sup>31</sup> , Dr Andrew	AWQC	andrew.humpage@sawater.com.au	Yes	Yes
MacPhail, Dr Robert	USEPA	Macphail.robert@epamail.epa.gov	No	-
Marsalek, Dr Blahoslav	U. Brno	marsalek@brno.cas.cz	No	-
Newcombe <sup>32</sup> , Dr Gayle	AWQC	Gayle.newcombe@sawater.com.au	Yes	Yes
Rinck-Pfeiffer, Dr	Anjou Recherche	Stephanie.rinck-pfeiffer@uwi.com.au	Yes	Yes
Stephanie				
Saint, Dr Chris	AWQC	chris.saint@sawater.com.au	No	-
Schmidt <sup>33</sup> , Dr Wido	TZW Germany	Schmidt@tzw-dresden.de	Yes	Yes
Shaw, Dr Glen	AU NRCET	g.shaw@mailbox.uq.edu.au	Yes	Yes
Sivonen, Dr Kaarina	U Helsinki	Kaarina.sivonen@helsinki.fi	No	-
Steffensen, Dr Dennis	CRC WQT	dennis.steffensen@sawater.sa.gov.au	See B	ursill
Utkilen, Dr Hans	Norwegian IPC	Hans.utkilen@fhi.no	Yes	Yes
Veeningen, Mr Roelof	STOWA Nlands	rveeningen@wetterskipfryslan.nl	Yes	Yes

<sup>&</sup>lt;sup>31</sup> GWRC Health Impacts link person.

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<sup>&</sup>lt;sup>32</sup> GWRC Operational Guidelines link person.

<sup>&</sup>lt;sup>33</sup> GWRC Monitoring Procedures link person.

Key to acronyms: AWQC (Australian Water Quality Centre); UBA (Umwelt Bundes Amt); WSSA (Water Services Association, Australia); CRC WQT (CRC Water Quality and Treatment); FEA (Federal Environment Agency); AwwaRF (American Water Works Association Research Foundation); USEPA (United States Environmental Protection Agency); TZW (Water Technology Centre, Dresden); AU NRCET (Australian National Research Centre for Environmental Toxicology).

## 5.2.7 International precedents of collaborative working arrangements

2004 saw the launch of two new collaborative initiatives in cyanobacterial research and management, these being the GWRC and CYANONET ventures (see elsewhere in this report as well as the reports for Deliverables 1 and 5, respectively. The 2004/5 period saw the TOXIC program finalizing its findings and outcomes (the first phase of TOXIC will be completed during mid-2005).

# 6. SOUTH AFRICAN CYANOBACTERIA AND CYANOBACTERIAL TOXIN RESEARCH INTEREST AND CAPACITY

#### 6.1 SOUTH AFRICAN RESEARCH INTEREST AND ABILITY

Local research interests (current or future) were polled by means of a letter forwarded to various individuals, universities, institutions and organizations (see Table 2). Based on the findings reported by Downing and Van Ginkel (2004) only those water utilities and/or local authorities known to be active, or have been active, with cyanobacterial projects were approached. 14 responses were forthcoming from 20 submissions (70%).

Table 2: Process of engagement with South African specialists as at end-January 2005

NAME	AFFILIATION*	email address	Response	Dialogue started	
Allanson, Prof Brian	Rhodes University	ba11@pixie.co.za	No	=	
Archibald, Mr CGM	KZN Consulting	colbar18@telkomsa.net	Yes	Yes	
Ashton, Dr Peter	CSIR	pashton@csir.co.za	Yes	No current interest	
Day, Dr Jenny	UCT	jday@botzoo.uct.ac.za	Yes	No current interest	
Dickens, Dr Chris	Umgeni Water	chris.dickens@umgeni.co.za	Yes	Yes	
Downing, Mr Tim	UPE	bcatgd@upe.ac.za	Yes	Yes	
Du Plessis, Dr Sandra	NWU (Potch)	plbsdp@puknet.puk.ac.za	Yes	Yes	
Du Preez, Dr Hein	Rand Water	hdupreez@randwater.co.za	Yes	Yes	
Grobbelaar, Prof JU	UOFS	grobbeju@sci.uovs.ac.za	Yes	Yes	
Joubert, Dr Jan	Toxicolgy, OVRI	johanj@moon.ovi.ac.za	Yes	No current interest	
Kruger, Mrs MJF	Midvaal Water	mjfkruger@mweb.co.za	No	-	
Mhlanga, Ms Lindah	U Zim (Harare)	lmhlanga@africaonline.co.zw	Yes	Yes	
Oberholster, Dr A-M	UP	ambothao@mix1.up.ac.za	Yes	Yes	
Pieterse, Prof AJH	NWU (Potch)	plbajhp@puknet.puk.ac.za	See du P	Plessis, Dr Sandra	
Roos, Dr Jan	UOFS	roosjc@sciuovs.ac.za	No	-	
Scott, Dr Willem	Private	wescott@iafrica.com	No	-	
Shephard, Dr Gordon	MRC	gordon.shephard@mrc.ac.za	Yes	No current interest	
Van Baalen, Mrs L	Rand Water	lgrobler@randwater.co.za	See d	lu Preez, Hein	
Van Ginkel, Carin	DWAF RQS	vanginkelc@dwaf.gov.za	Yes	Yes	
Van Halderen, Dr Andre	OVRI (former)	vanhalderna@maft.gov.za	See Jo	oubert, Dr Jan	

Key to acronyms: CSIR (Council for Scientific and Industrial Research); UCT (University of Cape Town); UPE (University of Port Elizabeth); NWU (North West University); UOFS (University of the Orange Free State); OVRI (Onderstepoort Veterinary Research Institute); UP (University of Pretoria); MRC (Medical Research Council); DWAF RQS (Department of Water Affairs and Forestry Resource Quality Services); U Zim (University of Zimbabwe).

#### 6.2 CURRENT SOUTH AFRICAN RESEARCH FOCI

Cyanobacterial research in South Africa is currently focused in four areas, these being:

- 1. The development of Alert Levels Framework<sup>34</sup> and Methods Manuals for use primarily in the Water Utilities Sector. These products are being developed by Rand Water. Stemming from the 2004 GWRC initiative a proposal has been made to amalgamate the South African product with the contributions from other countries, and with the Australian CRC drafting the product. This proposal is based on the well-founded premise that the content of such manuals is globally-generic, and that there is no longer a need for individual nations to produce stand-alone documents that most often simply re-write those of other countries;
- 2. PCR- (polymerase chain reaction) –based identification of cyanobacterial strains. The objective is to be able to genetically-identify geographically-unrelated strains of South African cyanobacteria thereby allowing for the early (predictive) identification of the presence of toxic strains. This work is being undertaken at the UP & NMMU;
- 3. Physiological and cyanotoxin production aspects ecophysiology of algal nutrition. This work (essentially 'blue-sky' investigations) is being undertaken at the NWU as part of this institutions long-standing 'Vaal River' program and is of a long-term nature. The work also embodies a PCR-based strain identification component. The project is allied to parallel work being undertaken at the NMMU, and in which the role of nitrogen uptake in regulating microcystin content (regulation of toxin production) of *Microcystis aeruginosa* is being examined. This component of the project has been hailed as both innovative and practically valuable by overseas researchers. The project also includes a third component linked to the capacity-building PCR-work being carried out at the UP;
- 4. Bacteriological predation on microcystins (UP).

With the exception of the initial work being undertaken on bacterial predation the research directions are common to the international research framework. The recently-completed genomics projects were unnecessarily repetitious and unfortunately yielded little of augmentative value. This may be discounted against capacity building provided that the skills are retained and appropriately integrated into future research of this nature. There are some worrying doubts as to the true axenicity of algal cultures that are being used in South African research – a fundamental concern that casts all subsequent results into doubt. The preparation and maintenance of truly axenic cultures is a specialized function that requires the support of a national culture collection.

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<sup>&</sup>lt;sup>34</sup> An Alert Levels Framework Manual, based on the UK and Australian response frameworks, was developed for South Africa in 1995 by the DWAF in consulation with the University of Dundee, Scotland (Quibell and Codd, 1995). For reasons unknown this document was never finalized.

#### 6.3 RESEARCH FACILITATION AND 'CHAMPIONING'

Cyanobacterial and cyanobacterial toxin research in South Africa is currently not championed – either by a committee or by a representative specialist. The bulk of research funding deployed in this field is provided by the Water Research Commission. In the absence of a strategic plan, the commissioning of research projects has been of a haphazard nature and not fully cognizant of international developments and progress. Other research funding, both in-kind and otherwise, is provided by certain water utilities, either for applied research or the maintenance and operation of analytical equipment. There is no formal partnership with the Water Utilities, or with overseas research institutions other than the *ad hoc* research agreement between the NWU and Finland (recently completed). Other collaborative links between individual research units and overseas groups are being established – but not as part of a national program. The lack of an over-arching, eutrophication management research program which embodies cyanobacterial research, is considered a major constraint to progress.

# 6.4 INFORMATION, TRAINING SKILLS AND RESOURCES

Information, training skills and resources, both in terms of personnel, funding and infrastructure, are sorely lacking in South Africa. Cyanobacterial research does not possess a 'research career' profile, and students are not attracted to the science other than for relatively short-term commitments to post-graduate projects at M.Sc level. The limited interest by water utilities and the low level of funding directed towards applied research is seen as a major constraint to attracting, training and retaining personnel. Research is 'unit'-based at three universities, these being the UP, NWU and NMMU. Other resources exist at the UOFS. Despite previously prominent roles in this field the CSIR no longer has any interest; the UP veterinary faculty and the OVRI have indicated to this project that they have no active research interests other than some work on developing in-house cytotoxicity testing; and the Medical Research Council no longer has active capacity.

# 6.5 STRENGTHS AND WEAKNESSES ANALYSIS

This analysis was undertaken on a comparative basis using the Australian, European and UK models. Of necessity this analysis, as it pertains to the symptomology of eutrophication, must consider strengths and weaknesses related to the cause and effect pathway. The most appropriate means of mitigating against cyanobacterial development remains effective attenuation of nutrient (phosphorus) loading to surface waters.

# Weaknesses

# Eutrophication<sup>35</sup>

- Sustained lack of an over-arching national eutrophication management program ('national eutrophication management strategy');
- Loss of intellectual capacity;

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<sup>&</sup>lt;sup>35</sup> Please also refer to the conclusions drawn by Walmsley (2000).

- Lack of coordinated<sup>36</sup>, inter-departmental understanding (Depts Water Affairs, Health, Agriculture, Economics);
- Absence of any coordinated and regionally-relevant teaching and instruction program on eutrophication and eutrophication management in South Africa;
- Absence of formal guidelines and supportive materials to direct reservoir management, within an arid environment where polluted return flows constitute a significant portion of the annual water budget;
- Absence of any policy on mandatory phosphorus removal from effluents, or the use of phosphatefree detergents and other cleaning products in priority catchments;
- Absence of eutrophication content from departmental (DWAF) training and awareness courses and materials;
- Inadequate level of understanding, at national and regional decision making levels, of the causes and consequences of eutrophication, and how to best address these;
- Lack of currency with research and management developments elsewhere;
- Outdated Best Management Practices;
- Non-implementation of research directions as pilot studies to determine effectiveness;
- Non-integration of eutrophication concepts and needs into relevant policies and guidelines<sup>37</sup>;
- Absence of a national eutrophication curriculum<sup>38</sup>;
- Lack of public awareness;
- Lack of coordination of related research needs (eutrophication, cyanobacteria and cyanobacterial toxins and human/animal health);
- Lack of a national ethos directed towards mitigating what is potentially the greatest single threat to water supply in South Africa.

#### Cyanobacteria and cyanobacterial toxins

- Absence of a national management and/or monitoring and reporting strategy for cyanobacteria and cyanobacterial toxins;
- Loss of intellectual capacity;
- Lack of coordinated, inter-departmental understanding (Depts Water Affairs, Health, Agriculture, Economics);
- Absence of any formal and coordinated teaching program pertaining to cyanobacteria and cyanobacterial toxins;
- Critical lack of a national research advisory committee comprised of key international and local research specialists;
- Project review and approval by a very small and relatively inexperienced pool of specialists, many of whom also bidding for research funds in this field;
- Fragmented national research initiatives;

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<sup>&</sup>lt;sup>36</sup> See Walmsley (2001).

<sup>&</sup>lt;sup>37</sup> See Walmsley (2001).

<sup>&</sup>lt;sup>38</sup> Various institutions (for example North West and Free State Universities) include some detail on eutrophication in their MSc level training courses. Development of intellectual capacity from within undergraduate programs is critically lacking.

- Absence of cyanobacterial content from departmental (DWAF) training and awareness courses and materials;
- Absence of a culture of information sharing (polarized research initiatives within an underpopulated research framework);
- Sustained low level of international research collaboration;
- Outdated research and management approaches;
- Lack of user-friendly (non-specialist) cyanobacterial identification tools;
- Ignorance and suspicion of proven biomanipulation (= "trophic cascade" or "top-down" management approaches);
- Lack of appropriate differentiation between "blue-sky" and "applied" research needs, this resulting in inappropriate allocation of funding;
- Absence of a national champion in a field lacking in experienced and informed specialists this both nationally and internationally;
- Absence of a national centre of analytical excellence responsible for due diligence-based direction on methods, standards, inter-laboratory calibrations;
- Absence of [national] capacity for routine and non-routine sample analyses;
- Absence of a curated reference collection of South African cyanobacterial strains;
- No integration of water utility companies and water providers in general with cyanobacterial research and management needs;
- Use of analytical techniques such as ELISA, non-calibrated for the local application, in the absence of foundation methods (HPLC);
- Non-dedicated research infrastructure (shared resources at Universities);
- No recruitment of young scientists to this research field;
- Lack of public awareness.

# Strengths

## Eutrophication

- Renewed institutional commitment to the problem;
- Recent formulation and implementation of eutrophication status (Trophic Status Project) and eutrophication monitoring program (NEMP);
- National Eutrophication Assessment Protocol (NEAP) and the Eutrophication Assessment Manual (WRC, 2005, in preparation);
- Planning for nutrient abatement and cost of eutrophication studies.

#### Cyanobacteria and cyanobacterial toxins

- Renewed institutional commitment to the problem, with small yet dedicated group of researchers;
- Recent assessments of the geographic extent of cyanobacterial-related problems in water resources;
- Development of an Alert Levels Response Manual for water treatment operators;
- Formulation of a strategic research plan (this report);
- Participation in GWRC-led initiatives;

- Limited collaboration with overseas research units;
- Capacity development in cyanogenetics.

#### 6.6 THE SOUTH AFRICAN vs INTERNATIONAL CYANOBACTERIAL RESEARCH **MATRIX**

Based on the information identified above, a matrix of previously-identified South African research needs, compared with the current international perspective, was compiled (see Table 3 on page 43). This analysis allows for identification of overlaps between coincident research and management needs, as well as for those issues not covered by one or the other.

This analysis reveals the following parity of research needs:

- A research plan that clearly differentiates between "need to know" and "scientific interest (blue sky)" objectives, excepting in cases where the latter will specifically inform applied research and/or management objectives;
- Maximization of collaborative (local and international) approaches;
- Cyanobacterial strain identification based on genomic methods;
- A curated national culture collection of cyanobacterial strains;
- Detailed information on the demographics of cyanobacteria and cyanobacterial toxins in South Africa, inclusive of the rural domestic and agricultural sectors;
- A national<sup>39</sup> analytical center, equipped with both staff and with capability in all major toxin detection methods (HPLC, HPLC-MS, ELISA and PCR). This center would also be responsible for standards certification, inter-laboratory calibration and analyst training/accreditation;
- The need for user-friendly taxonomic keys<sup>40</sup>;
- Overlap with eutrophication management initiatives;
- National risk assessments, inclusive of the level of exposure in the rural domestic and agricultural sectors;
- Developing of capacity in genomics skills;

Studies on cyanobacterial ecology and toxin production;

- Influence of climate change on algal physiology and formation of algal aggregations;
- Skills development and information transfer ('capacity building').

<sup>39</sup> A national, as opposed to more than one regional, center is proposed given the need for investment in staff

equipment measured against the anticipated workload.

40 Attempts to produce taxonomic keys have been undertaken on two previous occasions, yet in neither case did the funder take the product through to completion.

### Parity of non-research needs

- The need for a national database and incident reporting center or mechanism (for example webbased);
- A detailed, internationally-relevant methods manual (standardized methods, and continued development thereof, for the range of actions from sampling to toxin analyses);
- An 'Alert Levels Framework' for use by water treatment utilities;
- Algal management guidance manual ("what to do when....").

International research needs and emerging issues **not** identified in the consolidated South African analysis were:

- Impacts of toxic algae on agriculture, including aquaculture;
- Impacts of toxic algae on the aquatic environment and/or individual biota;
- Refinement of guidelines based on toxicological studies;
- Additional guidelines for new toxins (e.g. cylindrospermopsin);
- Geographic spread of problematical species (e.g. Cylindrospermopsis);
- Exposure risks posed by mixtures of toxins, for example microcystin (a tumour promoter) and cylindrospermopsin (an alkaloid carcinogen) in the same environment;
- Relationships between cyanotoxins and microbial pathogens;
- Degradative pathways for cyanotoxins;
- Risks posed by neurotoxicity (incidence, difficulties in analytical procedures);
- Non-chemical means of algal control (see also foodweb management);
- Development of regulatory approaches;
- Involvement of water utilities and coordination of generically-relevant projects;
- Bioaccumulation of cyanotoxins (particular with respect to aquaculture);
- Foodweb management (biomanipulation).

Unidentified non-research needs

• Recreational use (safety) guidelines.

#### 7. PROPOSED COMPONENTS FOR A SOUTH AFRICAN RESEARCH STRATEGY

The analysis undertaken in support of a fresh cyanobacterial and cyanobacterial toxin research strategy in South African water sources has identified two vital planning elements, viz. (i) the need to create a management and support infrastructure and (ii) the research aspects best suited to South African cyanobacterial research needs and capabilities. In addition, there is a clear indication that successes will be limited should collaboration with overseas specialists and organizations not be implemented. To South Africa's advantage is its current involvement in both the Global Water Research Coalition (GWRC) and CYANONET initiatives, plus a willingness expressed by international specialists

formerly associated with cyanobacterial work in South Africa to continue their association. Allied to this is the commonality of the cyanobacterial problems as experienced worldwide.

#### 7.1 THE PROPOSED RESEARCH STRATEGY:

Research into cyanobacteria and cyanobacterial toxins is, internationally, confined to a relatively small number of individuals and organizations, many of whom have been in working contact since the late 1980s. In South Africa per se the situation is much the same, although it has experienced a significant loss of specialists to other countries and research fields as a consequence of the perceived low priority and absence of research funding. The problem of attracting and retaining specialists is exacerbated by the intermittent nature of the 'toxic algae problem', and the low level of recognition afforded to it by water utility companies and agencies. Research in South Africa is confined to programs conducted within small university research units, using shared resources, and largely on the personal initiative and interest of the researchers concerned. South Africa has no formal eutrophication or cyanobacteria/cyanobacterial toxin teaching programs other than limited content presented within coursework programs. Water resource managers are poorly-equipped with the awareness and understanding required to make informed decisions. Research funding and interest is limited and provided essentially by the WRC and the DWAF, with a small number of the larger metropolitan utilities having internal monitoring and applied research budgets. There is no formal engagement of the water utility industry with the research programs as they currently exist. Issues pertaining to cyanobacteria are not directly allied to eutrophication as a sub-set thereof. There are no national overarching, inter-departmental programs for research and management in either field, nor do coordinating committees exist at national level. Review and acceptance of research proposals is of an ad hoc nature and not subject to internationally-relevant and informed peer review.

In order to address these limitations and create an environment in which effect can be given to a cyanobacteria/cyanobacterial toxin research strategy it is proposed that the following supportive elements be created – these being based on a strong tri-partite relationship between researchers, utilities and the resource agencies (see Figure 2):

# 7.1.1 Development of a research environment

## 1. Personalities and leaders

This is a field that will benefit from being personality driven as the core of integrative experience in the science confined to relatively few individuals. Having the right people in the right places is crucial to success, as has been illustrated by the Australian CRC-WQT model. It is essential that devoted 'champions' be identified and tasked with ensuring that links are developed and sustained between the research field, the water utility industry and other potential sources of funding. The same champions would also, for example, be tasked with explaining the advantages of genomic-based strain identification research and development to the water industry – an application which, at first glance, might be considered by the industry as not being an operational need worthy of funding.

# 2. Rationalization and prioritization of research needs

The allocation of cyanobacterial research needs must maintain clear distinction between the "need to know" (= applied or operational research) and the "scientific interest" (= blue-sky) subfields. Scientific interest research may be allocated funding from the primary (= operational) funding pool only if there is a clear indication that the deliverable will directly inform applied research needs, and be forthcoming within a timeframe relevant to operational requirements.

#### 3. Funding Sources

A mix of funding sources would be most desirable to support research in this field, and creating structures appropriate to accessing this mix of funds should be a mandatory requirement. While funding raised from water supply is applied to research via the WRC, it would be more beneficial to create a wider working partnership between research and the water utilities. Here it should be noted that issues pertaining to both eutrophication and cyanobacteria have cross-cutting implications for the research thrusts of ecosystems management, water resource management, health and agriculture – this should be taken into account both for the formulation of committees and for identifying funding sources.

#### 4. Institutional structures and skill sets

Although skill sets relevant to cyanobacteria and cyanobacterial research exist in South Africa these are highly-fragmented and lacking in any coordination of tactical or strategic planning. There are too few resources in this particular field for a situation of this nature to be effective in terms of the application of scarce research funding. There is a need to ensure the location of 'permanent' research 'units' at one or more institutions – with 'buy-in' from the parent institution - and with 'satellite' units possible at others depending on need and the interest and funding support of the parent institution. Skill sets should be continually augmented and exposed to 'real-world' problems via international collaboration and exchange of personnel (see also **5. Central Analytical Facility**). It would be to general advantage if extant and new skills were amalgamated rather than splitting up any further.

# 5. Central Analytical Facility

South Africa urgently requires the services of a reliable, multidisciplinary facility providing the full range of cyanotoxin analytical methods, standards certification, calibration services and interlaboratory calibration. There is currently no-where in South Africa where samples can be submitted for routine cyanotoxin analysis – other than on a non-routine and often off-line basis provided by the City of Cape Town or Rand Water (the latter services are not dedicated, have questionable accountability value and are shared with other analytical needs). The use of the cheaper ELISA methods, in the absence of the integrated use of HPLC confirmation, is at the least risky. ELISA is a screening technique, nothing more, and should not be utilized without accurate confirmation. As it is unlikely that there will be scope for an independently-funded service of this nature in the short to medium term this function should be 'piggy-backed' with the development of institutional structures. A core task for this facility would be the local production of toxin standards to offset the increasing difficulties experienced with the obtaining of commercially-available standards.

# 6. Overlap with 'parent' programs

Cyanobacterial problems in water resources is a consequence of eutrophication. Accordingly, the cyanobacterial research program needs to be closely allied to the national strategic eutrophication management program – strategic alliances need to be formed between associated programs. Essential points of contact should focus on the extent of the problem (cyanobacterial blooms and the percentage thereof that are toxic); bottom-up and top-down management approaches, and access to funding from the eutrophication program in cases where resolution of identified problems is unlikely to be forthcoming from the eutrophication management sector itself.

# 7. Applicable models and international links

The local research advisory committee should contain members who are representatives of the utilities, health regulators, natural resource agencies and researchers (both university and in some cases from private industry). This mix ensures the balance between operational and scientific interest research, and also adds an extra funding leg, namely that provided by tertiary institutions. International links should be maintained both through this model and via the Review Committee (see below).

#### 8. Review Committee/SARNAT

South Africa currently lacks the locally-based experience to confidently conduct reviews of both solicited and unsolicited proposals, especially in areas involving aspects such as genomics/cyanogenetics. This situation is further complicated by the small pool of SA specialists used as reviewers but also bidding for the same funding resources – which may create conflicts of interest. Accordingly the review "panel" (not simply two reviewers) should consist of the manager or consultant for the national strategic planning, local reviewers as available plus two or more international specialists. In all cases, but especially for unsolicited proposals, there must be a mandatory requirement on the proposer to show clearly where the expected deliverable addresses a gap in the international science, what work underpins the proposed research, and what opportunities may exist for collaboration with other local and international institutions or agencies working in the same field. The latter process will be assisted by the links maintained through the Information Management function (see below). All project deliverables should be subject to international peer review.

It is proposed that a national working and advisory group, the South African Research Network for Algal Toxins (SARNAT) be constituted to oversee and manage cyanobacterial research and management in South Africa.

# 9. Project Management

The present 'Steering Committee' or 'Reference Group' project management function is unsuitable for this specialized area of water research in that it does not afford the required intensity and frequency of interaction and progress evaluation that is necessary. Long periods between meetings of individuals who, at the present time are likely to have inadequate levels of experience in the particular field, is contraindicated to the need to rapidly and comprehensively

develop capacity. Accordingly it is recommended that the Steering Committee function be largely replaced by the appointment of a Task Manager. This role should be fulfilled by someone with appropriate experience of the international cyanobacterial research environment per se who is tasked with regular 'on mission' visits to the institution or unit where the work is being conducted in order to gain first hand knowledge and experience of the progress made, constraints encountered and the like. The Task Manager would then report back both to the Program Manager and the Reference Group. Should problems requiring specialization it would the Task Managers role to direct and facilitate contact with other specialists or organizations able to help, or to comment on the results or progress to-date.

# 10. Information Management Function

Insofar as cyanobacterial research and management is concerned South Africa suffers from a critical information gap. In the absence of one or more dedicated research units holistically-operating in this field, such as the CSIR or the former NIWR might have been expected to do, an enormous volume of published material has gone unnoticed by the SA scientific community, and continues to do so. This paucity extends to the non-published 'grey-literature' environment. In order to address this it is recommended that an Information Management Function (IMF) (= literature watch) be commissioned on a consultancy basis. This consultancy should be tasked with (i) undertaking – using an appropriate publications search package - an historical analysis of publications for the period 1985-2005, cataloging these by category and listing on a local website; (ii) maintaining this on a monthly basis thereafter using the same approach; and (iii) maintaining a watch and links with other research organizations, units and institutes regarding the publications of reports, reviews and etc. The IMF function should be closely allied to the information resources of the CYANONET initiative.

# 11. Ensuring continuity of program management skills

While there are several small research groups in South Africa involved with some or other aspect of research pertaining to cyanobacteria, the level of current and all-encompassing experience in the fields of limnology, eutrophication, cyanobacteria and their ecological associations, cyanobacterial toxins and cyanogenetics is limited to perhaps one or two individuals. As this particularly research field is not attracting new scientists this is not a sustainable situation. Accordingly, at program management level efforts should to identify appropriate personnel for mentoring. It is anticipated that with the development of the strategic cyanobacterial and eutrophication programs that 'new blood' will be attracted to this field of water resource management.

# 12. Education and Training

The current level of understanding of both eutrophication and cyanobacteria in South Africa is currently inadequate for decision making and informed stakeholder involvement. A concerted effort should be made to address this information gap, and to ensure that the relevant understanding is extrapolated across all policies and practices having influence on water resources management.

# Strategic Research Framework

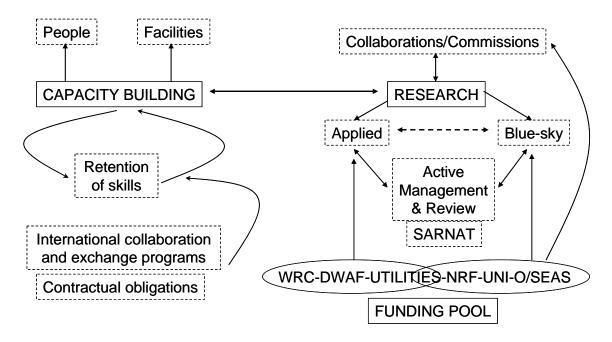


Figure 2: Proposed elements of South African toxic algal research framework

# SUGGESTED RESEARCH INFRASTRUCTURE BASED ON EXISTING CAPABILITIES:

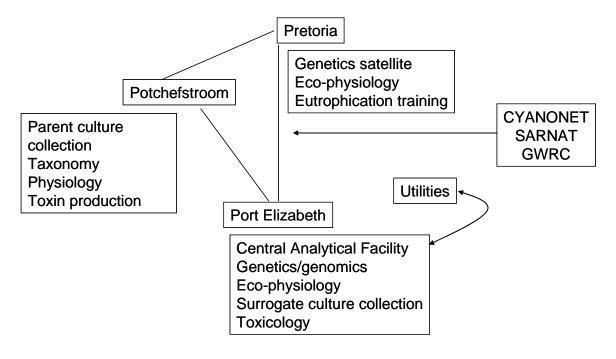


Figure 3: Proposed integration of tertiary research facilities

# 7.1.2 Specific Components of the Research Strategy

There are three core aspects within the research strategy, namely:

- (i) those pertaining to the eco-physiology of cyanobacteria, and cyanotoxin production most notably the questions surrounding why cyanobacteria produce toxins, and what triggers this response;
- (ii) Physiology and genetics (population and related phylogenetic studies);
- (iii) those issues relating directly to applied (operational management as opposed to research *per se*) issues.

## Physiological and eco-physiological studies

The physiology and related research aspects have been slow to provide concrete answers, not least in South Africa. The bulk of the research work previously undertaken in South Africa has been concentrated in this area, and some is currently being undertaken at the NWU. Much of the research is still widely cast ("shotgun") and based on the premise that indicators may be identified on exhaustive correlative analysis. It is, however, unlikely that new information on cyanobacterial growth and development in dams, which will translate directly into practical management options, will be forthcoming from this research arena in the short – to medium term. However, the complexity of the problem is such that it is doubtful that practical and pragmatic outcomes relevant to operational management of both water storages and water treatment plants will be forthcoming in the near future. Notable exceptions here are (a) the growing pool of strong evidence, from several countries including South Africa, that foodweb management may be of considerable use in the alleviation of ecosystem degradation brought about by sustained eutrophication; and (b) the discerned impacts of the presence of cyanobacteria, and/or cyanobacterial toxins, on aquatic biota, and the need to quantify same. This aspect may be extended to include consideration of chronic impacts on livestock and impacts on agriculture, including aquaculture.

It is recommended that only those scientific interest proposals that can show, with a high level of confidence, a deliverable or deliverables that will significantly advance the scientific understanding and hasten developments in the operational arena, be considered eligible for funding from the WRC.

By contrast the examination of natural populations (ecophysiology), linked to their genetic identities (speciation) and inter-regional and continental comparisons, has considerable potential for the rapid development of a management-linked understanding of cyanobacteria. Increasingly within this field is work aimed at genomics-based identifications of cyanobacterial species based on the presence of the gene sequences involved in toxin biosynthesis. Allied to this is the use of DNA-based tests to differentiate between geographically-remote local species, as well as to compare the genetic identities of South Afrian isolates of e.g. *Microcystis aeruginosa*, *Anabaena circinalis*, *Planktothrix agardhii*, *Cylindrospermopsis raciborskii* and *Nodularia spumigena* with those already identified in Australia.

#### Operational research

Operational research is currently aimed at the provision of response guidelines (the Alert Levels and Methods Manuals, treatment protocols and the like).

#### 7.1.3 Recommended Research Elements

In addition to the development of a research environment for this field and cognizant for the need to establish collaborative joint funding agreements where similar work has or is being done, it is recommended that the WRC consider proposals within the following current or emerging issues for funding during the initial years of this strategic program. In formulating this list it has been assumed that information on incidence and demographics of cyanobacterial occurrence is not a 'research' issue but rather a component of monitoring and management (see Development of a Supporting Research Environment, above):

#### 1. Creation of a curated and duplicated national cyanobacterial culture collection.

The present growing development of cyanogenetics, the need for qualified taxonomic identifications and the value that these hold for the development of management directives and tools, has an absolute requirement for a professionally-maintained and unadulterated culture collection. The creation and curatorship of this collection has obvious overlaps with the aforementioned need to create a Central Analytical Facility given infrastructural and staffing overlaps, as well as the ability to attract additional funding. Such a collection should be created at a primary location, with a second mirror collection at another institution or unit. The primary location should be responsible for genetic identification and genetic characterization, while the second should be tasked with classic morphological/phonetic identifications. The same facility should also provide for storage of samples from poisoning incidents for later analysis.

# 2. Genetic/molecular techniques for identifying toxic species.

These are deemed critical to the development of predictive capacity for bloom management – as derived from population dynamic studies linked to toxin production studies. Work in this area requires the availability of the above-mentioned culture collection. With respect to the application of PCR assays it is essential that these be applied to naturally occurring populations and not just laboratory cultures that have been in culture for a long time.

# 3. Similarities in trans-regional and trans-continental species identity (phylogenetic relatedness).

Similarities between South Africa and Australia, should be investigated given the level of understanding of the genetics of the common problematical species, and toxin production, by the Australian CRC. Species eligible for immediate attention would be *Microcystis aeruginosa*, *Anabaena circinalis*, *Planktothrix agardhii*, *Cylindrospermopsis raciborskii* and *Nodularia spumigena*. An understanding of local speciation characteristics should be established prior to comparison with strains from other continents.

# 4. Regulation of cyanotoxin production.

The development of an understanding of cyanotoxin production triggers and mechanisms has the potential to provide information on how to deal, at least in part, with the problem (this given that prevention of cyanobacterial overgrowth through effective nutrient attenuation and management at or near source should be the fundamental management approach – but recognizing that for many waters the level of attenuation required to bring algal development under control may not be attainable in the short to medium term).

## 5. Development of the wider potential of PCR techniques for use in cyanogenetics.

Research in this arena should identify the relevance and accuracy of 'rapid' toxin or toxicity-indicator assessment techniques based on PCR methods.

#### 6. Health risks and risks posed by mixtures of species.

It is recommended that South Africa contribute to the global understanding of risks to animal and human health by becoming directly involved the work being undertaken by the Australian CRC on this topic. This contribution should be financial – directed through capacity building – and the supply of South African material for testing, or for the performance of parallel testing in South Africa. The already in effect GWRC proposal for this has reference.

#### 7. Epidemiology and chronic exposure risks/BMAA.

Humans and animals with chronic exposure to cyanotoxins, particularly the hepatotoxins, do not die of liver complications. Death from pneumonia is more likely. What is clear is that chronic exposure to microcystins influences both morbidity and death. This chronic exposure risk may be of greater import in a population with a high prevalence of the TB:HIV-AIDS co-epidemic (see also **Risks to rural supplies**). The emergence of BMAA as a new health risk underscores the need for epidemiological investigations in communities where the raw potable water is known to contain cyanobacterial blooms or near-permanent aggregations of cyanobacteria.

# 8. Risk to rural domestic and agricultural water supplies.

The possible impacts on human health in rural areas remote from controlled water supplies should be established. Coupled to this should be the provision of an awareness program amongst farmers and rural communities.

# 9. Foodweb manipulation.

There are strong and positive indications from several countries, including South Africa, that foodweb manipulation plays a strong and beneficial attenuation role in eutrophic waters. The efficacy of this approach, the mechanisms and dynamics thereof strongly merit investigation with the cyanobacterial management sphere of interest.

### 10. Bioaugmentation products.

There are a number of bioaugmentation "silver bullet' cures for eutrophication commercially available. Most of these appear to be based on freeze-dried bacterial mixtures ostensibly aimed at the small-scale fish breeding market. These products have unproven efficacy in impacted natural or semi-natural systems, and are unsupported by any identified proven scientific validation. There has also been a marked unwillingness by the suppliers of these products to participate – at their own cost - in any local field trials. While the 'let the buyer beware' concept should prevail here the costs of these products are not insubstantial and may be purchased in desperation by uniformed clients approached by these companies. Accordingly it would be in the national interest to investigate the use of these products, ideally in collaboration with the companies marketing same.

#### 11. Algicides.

The use of algicides, most commonly copper sulphate, to control algal growth in South Africa is limited to the management of irrigation canals and some farm dams. The practice is generally unacceptable in that the accumulation of copper from repeated use has a sustained and negative impact on aquatic biota. Notwithstanding this the use of algicides may be indicated in certain once-off applications to address an acute situation, and with the provision that effective attenuation and mitigation measures will be installed to prevent a recurrence [of an algal bloom]. While application guidelines for copper sulphate are well established there are a range of algicides of various formats registered in the USA which may be of relevance in South Africa, but which would require evaluation prior to local registration.

# 12. Bacterial predation.

The potential for the algicidic actions of bacteria on cyanobacterial blooms as an invocable biomanipulation procedure is largely unknown, and results to-date have been less than promising. Pursuance and funding of research in this field should be subject to international experience and direction.

# 13. Verification of analytical screening methods for local application.

With respect to the need for a Central Analytical Facility, the validity of screening methods such as ELISA for local use should be exhaustively tested. The application of ELISA as a stand-alone method is not suitable in cases where unknowns exist – this precludes quantification of toxin concentration.

# 14. Analytical techniques for the detection of cyanotoxins in fish tissue and marine filter-feeding organisms.

Aquaculture in South African impounded waters is a developing industry and requires analytical support if the products are to be successfully marketed. Here it should be noted that techniques adequate for quantifying tastes and odours in fish flesh are available in South Africa but not on a readily-available basis.

### 1. Recreational use guidelines.

The greatest risk of acute toxicity lies with human an animal exposure to algal scums. This requires a more complex suite of evaluation criteria than is applied to treated water. While South African guidelines need to be developed for this it is recommended that the comprehensive Australian, as well as the WHO guidelines, be used to provide direction for same and to expedite the local delivery of an adapted product.

### 2. Alert Level and Operational Methods manuals.

Although local contracts for this type of product have already been commissioned, the quality and content of same is unfortunately not of an international standard, and South Africa is not by any means a world leader in cyanotoxin analytical techniques. As put forward by the 2004 GWRC meeting it would be useful to combine efforts of this nature into single, internationally-relevant products.

#### 8. CONCLUSIONS AND RECOMMENDATIONS

The situation analysis component of this strategic plan clearly reveals that South Africa must place itself in a position in which it clearly understands the issues and threats posed by the presence of cyanobacterial in this countries water supplies. This conclusion is founded not only on local experience but on the global, cosmopolitan nature of the problem. While South Africa has lost some ground in this particular field this is not unique as the levels of resources developed to understand and combat this problem are sparse worldwide. South Africa is, however, extremely fortunate to be in a position where collaborative and cooperative links to existing programs in other countries are readily accessible.

With respect to the research thrusts (KSAs) of the Water Research Commission toxic algae and algal toxin research needs cut across all domains.

The nature and extent of the 'cyanobacterial problem' is generally poorly understood and prone to misinterpretation and misinformation – both of which can significantly retard positive progress. A considerable amount of effort remains to be devoted to developing a cross-cutting level of awareness of the causes and effects of problematical cyanobacterial growth in surface waters. It is recommended that the outcomes of this initial strategic plan be communicated to the key public an private roleplayers and affected parties by means of briefing workshops – as opposed to the simple dissemination of this document. The nature of, and issues surrounding, cyanobacteria and their toxins (toxic algae in general) are best communicated in person by someone closely familiar with the topic. This personal approach is likely to provide a far greater stimulus for engaging with and seeking 'buy-in' from the water supply industry, be this government, utilities or city and town administrations. This recommendation is aligned with the need for this initiative to be 'championed' – as discussed in this report.

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# DOCUMENTS AND REPORTS ON CD

The CD provided (see pocket inside back cover) with this report contains the following files:

- 1. Copies of the individual reports prepared for Deliverables 1,2, 5 & 6
  - Deliverable 1: Includes report on the 2004 GWRC Workshop
  - Deliverable 2: Includes report on the 6<sup>th</sup> ICTC Conference (2004)
  - Deliverable 5: Includes report on the 11<sup>th</sup> HAB Conference (2004)
  - Deliverable 6: Includes report on the inaugural CYANONET workshop (2004).
  - Deliverables 3&4: This report (WRCK8/576 Consolidated Final Report).
- 2. MS Word file containing the CyanoHab literature reference list.
- 3. Report on the GWRC workshop.
- 4. CYANONET inaugural report to UNESCO.
- 5. The 'Bergen Booklet' on international cyanobacterial regulatory approaches.
- 6. PNAS April 2005 article on BMAA.
- 7. Project Presentation to the GWRC workshop.
- 8. Project Presentation to the 11<sup>th</sup> HAB meeting workshop).
- 9. Project Presentation to the WRC KAS Directors (May 2005).
- 10. Copy of the draft Australian Recreational Health Guidelines.