Identification of toxigenic *Microcystis* strains after incidents of wild animal mortalities in the Kruger National Park, South Africa

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ABSTRACT

The eutrophic process potentially caused by a high urine and faecal load resulting from an unusually high hippopotamus (*Hippopotamus amphibious*) density in the Nhlanganzwane Dam, Kruger National Park, South Africa, triggered a chain of events characterised by an increase in the growth of primary producers (*Microcystis aeruginosa*). This increase in *M. aeruginosa* biomass was followed by bio-intoxication incidents in wild animals. In this study, we determine if a *M. aeruginosa* bloom with a total microcystin level of 23,718 µg l^{-1} have been responsible for mortalities of megaherbivores in the Nhlanganzwane Dam. We further use microcystin molecular markers derived from the *mcy* gene cluster to identify potentially toxigenic environmental *Microcystis* strains in the dam during the occurrence of animal intoxications. The estimated total microcystin-LR daily intake by an adult male white rhinoceros (*Ceratotherium simum*) from cyanobacterial-contaminated water of the dam during the toxic event was an order of magnitude higher (754.29 µg kg^{-1} bw) in comparison with the lowest observed adverse effecting level (LOAEL) value measured for pigs in a previous study by other authors. In this study the presence of toxic cyanobacterial strains was confirmed with the use of molecular markers that detected the presence of the *mcy* gene cluster responsible for the production of toxin by *M. aeruginosa*.

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1. Introduction

Eutrophication is the natural ageing process of lakes. It is characterized by a geologically slow shift from in-lake biological production driven by allochthonous loading of nutrients, to production driven by autochthonous processes. This typically slow process over centuries can be greatly accelerated to within decades by human intervention in the natural biogeochemical cycling of nutrients within a watershed (Rast and Thornton, 1996). This eutrophication is mainly caused by the addition of nutrients from human activities, called in this context, anthropogenic eutrophication. The phenomenon is generally a consequence of intensive agricultural and industrial activities and the development of large sewage systems associated with large metropolitan areas (Pitois et al., 2001). Although there are a few previous studies that indicate eutrophication (over-enrichment) as a direct consequence of resident animals, fish and birds in freshwater bodies (Bomchul et al., 2001; Oberholster and Botha, 2007), little is known about eutrophication as a result of megaherbivores on

lake ecosystems. In many cases the direct consequences of eutrophication are the excessive growth of autotrophic organisms. In standing waters this manifests in blooms of generally undesirable cyanobacteria. Some species of cyanobacteria may produce substances that are highly toxic. The identification of a cyanobacterial genus by microscopic morphology does not indicate its potential for toxin production. Different strains of one species can be morphologically identical but differ in toxigenicity. For example, *Microcystis aeruginosa* which is the most abundant toxic cyanobacterial genus in South Africa may contain both toxic and non-toxic strains (Meissner et al., 1996; Bolch et al., 1997).

The Kruger National Park is one of the largest conservation areas in Africa and covers 19,485 km^2 on South Africa's eastern borders with Mozambique (Fig. 1). Its perimeters were entirely fenced from 1974 to 1993, when sections of the western fence bordering private wildlife reserves were removed. Megaherbivores are an important component of the fauna and include the elephant (*Loxodonta africana*), white rhinoceros (*Ceratotherium simum*) and hippopotamus (*Hippopotamus amphibius*). Annual rainfall varies from 750 mm in the south-west to 450 mm in the
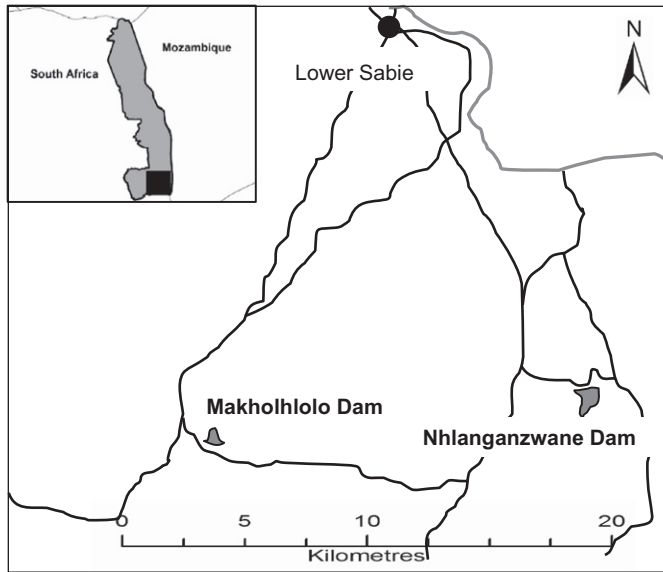
Fig. 1. Map of the Kruger National Park with cluster of two point source incidents of wildlife mortalities.

north-east, with 80% received during the summer months of October to March (Eckhardt et al., 2000).

With the establishment of the Kruger National Park in 1898, animals concentrated during the dry season around five perennial rivers (Pienaar, 1970). Between these rivers only a few natural perennial water sources existed and most of the water sources were unreliable and depended on seasonal rainfall events (Pienaar, 1970). In the early 1930s, the Kruger National Park water stabilisation programme started, but gained particular momentum in the 1960s after fencing of the western boundary of the park, which blocked the migration routes of certain herbivores to this area that previously acted as dry season grazing. Until the 1980s more than 300 boreholes were drilled, 50 earth dams were constructed and various seasonal and perennial rivers dammed as part of the water provision programme to ensure all year round reliable surface water throughout the park (Gaylard et al., 2003).

During the autumn and early winter of 2005 rangers of the Kruger National Park found several carcasses in the region of one of these artificial water points, namely the Nhlanganzwane Dam that is situated in the extreme south eastern corner of the Kruger National Park, to the south of Lower Sabie restcamp (Fig. 1). Blood smears sent to the State Veterinary laboratory at Skukuza were negative for anthrax. Due to the decomposed state of the carcasses, it was not possible to perform necropsies, and the cause of death could not be determined. After discovery of a fresh zebra carcass in May 2005, a detailed necropsy was performed and a full range of laboratory tests were conducted by the State Veterinary Services and the Onderstepoort Veterinary Institute. The microscopic lesions in the liver as well as formation of intracytoplasmic eosinophilic globules in some hepatocytes and proliferation and accumulation of a lipofuscinous pigment in Kuffer cells especially in the centrilobular areas were compatible with a diagnosis of Microcystis intoxication (Bengis et al., 2008). Mouse toxicity tests were also strongly positive. During the time of the discovery of the carcasses, water levels at all the dams in the region were unusually low and approximately 100 hippopotami inhabited the Nhlanganzwane Dam. The large density of hippopotamus in the dam may have resulted in eutrophication which most probably has given rise to the visible cyanobacterial bloom. A total of 52 carcasses were detected from February to July 2005 consisting of 7 white rhinoceros, 2 lions, 2 cheetahs, 9

zebras, 23 wildebeest, 1 hippopotamus, 1 giraffe, 5 buffalo, 1 warthog and 1 kudu. Mortalities decreased to zero after July 2005 when the overgrazed area surrounding Nhlanganzwane Dam was subject to a low intensity accidental veldt fire. This resource depletion resulted in the dispersal of herbivores from the area, including the hippopotamus population, which dispersed to the Sabie and Crocodile Rivers. Two years later, in June 2007, mortalities of white rhinoceros, zebras and wildebeest where again reported from the area of the Nhlanganzwane Dam. Microscopic lesions in the livers of these dead animals were compatible with a diagnosis of Microcystis intoxication (Bengis et al., 2008). Once again there was a visible cyanobacterial bloom as well as a large resident hippopotamus population in the dam.

The objectives of this study were (1) to use PCR assays to assess the presence of possible toxigenic strains of M. aeruginosa Kützing ex Lemmermann in Nhlanganzwane Dam in the Kruger National Park, around which two mortality clusters in wild animals, associated with cyanobacterial intoxication, occurred in the dry seasons of 2005 and 2007. (2) To unravel the animal mortalities in the region of this dam, we investigate some limnological conditions of the dam at the time of the intoxication event, as well as the role played by megaherbivores associated with the dam.

2. Materials and methods

2.1. Sampling protocol and enumeration of phytoplankton species

One sampling trip was undertaken in July 2007 during the occurrence of the cyanobacterial intoxication event. Sampling was performed in triplicate with 200 ml polyethylene bottles at three different sampling sites by scooping up surface water ($+10$ cm depth) within the littoral zone of the Nhlanganzwane Dam. Sampling were conducted in areas where cyanobacteria cells accumulated (surface film $+4$ cm thick) as well as in the littoral zone of the Makhohlolo Dam that was uninhabited by hippopotami and contained very low concentrations of cyanobacterial cells (2.3×10^2 cells ml^{-1}) and a low water level that was not suitable for hippopotami. The latter served as a control for this study and was within the region of the Nhlanganzwane Dam. Water samples were transferred from the field to the laboratory in a dark coolbox. Phytoplankton species composition and community structure was assessed from 10-ml aliquots sampled from 200-ml water samples after it was fixed with lugol's solution. Colonies of Microcystis were disintegrated by ultrasonication prior to counting (40 impulses/s over 4 min for a 10-ml sample) (Kurmayer et al., 2003). Phytoplankton cells were identified and counted from transects in a Sedgewick–Rafter sedimentation chamber with an inverted microscope. Identification and counting of phytoplankton were carried out according to procedures outlined by Lund et al. (1958) and Padisak (1993). While general algal species identification was carried out using Wehr and Sheath (2003).

2.2. Pigment and physical/chemical determination

Physical/chemical analyses were conducted from subsamples of the triplicate 200 ml polyethylene bottles. These 200 ml surface water samples were taken at a depth of 10 cm at the same three sampling sites in the littoral zone of both dams. Chlorophyll-a was assessed from a 50-ml aliquots sampled from 200-ml surface water samples. Chlorophyll-a was .extracted from lyophilized GF filters ($20\,\mu$m) and kept in N,N-dimethylformamide for 2 h at room temperature. Chlorophyll-a was then measured photospectrometrically at 647 and 664 nm according to the calculations of Porra et al. (1989). Nutrient concentrations were analyzed from 100-ml aliquots sampled from 200-ml surface water samples using the American Public Health Association, American Water Work Association, and Water Pollution Control Federation (1998) classical spectophotometric methods. Temperature, dissolved oxygen (DO) and conductivity of the water column were measured at a depth of 10 cm at all three sampling sites in both dams with a Hach^{TM} sension 156 portable multiparameter (Loveland, CO, USA). To determine the trophic state of the Nhlanganzwane and Makhohlolo Dams during the poisoning incidence in 2007, inorganic nitrogen (IN), total nitrogen (TN), total phosphorus (TP), chlorophyll-a as well as the TN:TP ratio were used following the trophic state index of Vollenweider (1968) and Forsberg and Ryding (1980).

We obtained mean monthly air temperatures and rainfall patterns of 2005 and 2007, from stations within or near the Southern region of the Kruger National Park from the South African Weather Bureau, and amalgamated these to derive the mean annual temperature regime for the region.

2.3. Cyanobacterial reference strain culturing

The toxic, axenic *M. aeruginosa* strain PCC 7806, obtained from the Institute Pasteur (PCC; Paris, France) was used in this study as reference strain. The *Microcystis* field strain was cultured in liquid BG-11 medium at 24 °C under continuous illumination of approximately 60 μmol photons (PAR) $m^{-2}s^{-1}$. Light intensity was measured with a Cole Parmer Model DLM2 light meter (Vernon Hills, Illinois, USA) at a pH of 8.0 on a daily basis. All cultures were routinely screened for contamination by streaking samples on TYG agar and in TYG broth. Cells were harvested at late-exponential growth phase.

2.4. Microcystin analysis

For cyanotoxin analysis, a volume representing 50 mg dry weight of the environmental strains biomass was filtered through glass fiber filters, air dried and stored in the dark at room temperature. Cyanotoxicity was determined with an enzyme-linked immunosorbent assay (ELISA), using a Quanti™ Kit for microcystins (EnviroLogix, USA). Results were obtained by reading the absorbance using a microtitre plate reader set (Thermo Labsystems, USA) at 450 nm within 30 min after the addition of the stop solution. It has been estimated that the average cellular microcystin-LR content for *Microcystis aeruginosa* in laboratory cultures is approximately 0.2 pg/cell (range, 0.07–0.3 pg; Mole et al., 1997). On this basis, the total intracellular microcystin (T_{cm}) of the bloom was determined for the period of intoxication by animals using the following equations:

$$T_{cm} = I_c \times C_a \tag{1}$$

where I_c is the average intracellular microcystin-LR (pg/cell) and C_a the cyanobacterial cell abundance (cells ml^{-1}) of the bloom.

The daily total microcystin-LR intake (D_m) by different wild animals during the occurrence of intoxication event was determined by the following equations:

$$D_m = \frac{T_m \times W_r + E_m}{M_{bw}} \tag{2}$$

where T_m is the total intracellular microcystin-LR (μg l^{-1}), E_m the total extracellular microcystin-LR (μg l^{-1}), W_r the daily water requirements of a animal (litres) and M_{bw} the average mean body weight (kilogram).

2.5. Assessing toxigenicity by PCR

DNA was extracted from the environmental strains as well as from the reference strain PCC 7806 using DNAzol®-Genomic DNA Isolation reagent following the manufacturers' procedures (Molecular Research Center, Inc., USA). PCR amplification was performed in a GeneAmp2400 thermocycler (Perkin-Elmer Cetus, Emeryville, California, USA). The thermal cycling protocol included an initial denaturation phase at 94 °C for 2 min, followed by 35 cycles. Each cycle began with 30 s at 96 °C followed by 30 s at the annealing temperature of T_M °C for the specific primer pairs (Ouahid et al., 2004, 2005; Oberholster et al., 2006), and 30 s at 72 °C for the elongation step. The PCR reaction was terminated by maintaining samples at 72 °C for 7 min. The amplification reaction contained a 10× amplification buffer with 1.5 mM MgCl$_2$, 0.2 mM dNTPs, 20 pmol of each primer and 1 U Taq DNA polymerase, and 3–5 ng purified DNA in a final volume of 20 μl. Electrophoresis of the PCR products was carried out in 2% agarose gels in TAE buffer (0.04 M Tris–acetate–0.001 M EDTA [pH 8.0]), and the DNA was stained with ethidium bromide and visualized and photographed under UV transillumination.

2.6. Data analysis

In this study, results were recorded on standard Excel spreadsheets for data processing, and statistical analysis was performed using SYSTAT® 7.0.1 (1997). Statistical differences were analyzed calculating Pearson correlation and a *t* test using the Sigma Plot (Jandel Scientific) program. Values of $p \leqslant 0.05$ were regarded as significant.

3. Results

3.1. Species composition

The surface water samples at the three sampling sites collected in July 2007 revealed a cyanobacterial abundance of 1.1×10^8 cells ml^{-1} with the occurrence of one cyanobacterial morphospecies *M. aeruginosa* (Wehr and Sheath, 2003) in the Nhlanganzwane Dam. In terms of abundance, the phytoplankton community in the Nhlanganzwane Dam was dominated by the cyanobacteria *M. aeruginosa*, followed by Chlorophyceae (most abundant species:

Scenedesmus armatus). Euglenophyceae (e.g. *Phacus longicauda*) occurred in very low numbers. The total number of phytoplankton species encounter in all samples combined were 16 species of which 9 species occurred in the Makhohlolo Dam.

3.2. Physicochemical measurements

The physicochemical conditions measured in the Nhanganzwane Dam in July 2007 were total nitrogen, inorganic nitrogen, total phosphorus, total microcystin, chlorophyll-*a*, dissolved oxygen and water temperature. Data measured in the Nhanganzwane Dam in July 2007 were the following: total nitrogen, 13,600 μg l^{-1}; inorganic nitrogen, 2171 μg l^{-1}; total phosphorus, 1210 μg l^{-1}; soluble reactive phosphorus, 129 μg l^{-1}; total microcystin level, 23,718 μg l^{-1}, chlorophyll-*a*, 580 μg l^{-1}, dissolved organic carbon, 6.1 mg l^{-1} dissolved oxygen, 2.13 mg l^{-1} and surface water temperature 22 °C. According to the near surface water average nutrient concentration index of Vollenweider (1968) and Forsberg and Ryding (1980) for classification of the trophic states of the dam during the time of the animal mortalities, the dam was hypertrophic in July 2007. A significant positive relationship ($r = 0.98229$; $p \leqslant 0.02$) between the high cyanobacterial cell abundance and high soluble reactive phosphorus concentration (129 μg l^{-1}) was observed. The soluble reactive phosphorus concentration in Nhlanganzwane Dam exceeded the level of 50 μg l^{-1} considered conducive to cyanobacterial blooms (Wasson et al., 1996). The Makhohlolo Dam, on the other hand, had low total nitrogen (660 μg l^{-1}) and total phosphorus (21 μg l^{-1}) concentrations, indicating a mesotrophic system (Table 1). The average temperature range of the surface water in July 2007 was 20–22 °C. Rainfall data revealed a dryer winter season in 2005 than in 2007 with high air temperatures in both cases.

Table 1
Physicochemical measurements of raw surface water of the Nhanganzwane and Makhohlolo Dams in July 2007 ($n = 1$).

Physicochemical variables:	Nhanganzwane Dam	Makhohlolo Dam
Total Nitrogen	13,600 μg l^{-1}	660 μg l^{-1}
Inorganic nitrogen	2171 μg l^{-1}	183 μg l^{-1}
Total phosphorus	1210 μg l^{-1}	22 μg l^{-1}
Soluble reactive phosphorus	129 μg l^{-1}	6.2 μg l^{-1}
Total microcystin	23,718 μg l^{-1}	0.317 μg l^{-1}
Chlorophyll-*a*	580 μg l^{-1}	3.8 μg l^{-1}
Dissolved oxygen	2.79	4.38
Dissolved organic carbon	6.1 mg l^{-1}	1.3 mg l^{-1}

The Nhanganzwane Dam was occupied by a large resident of hippopotamus population during this time.

Table 2
Estimated average daily intake of cellular microcystins by different herbivores at Nhlanganzwane Dam, July 2007.

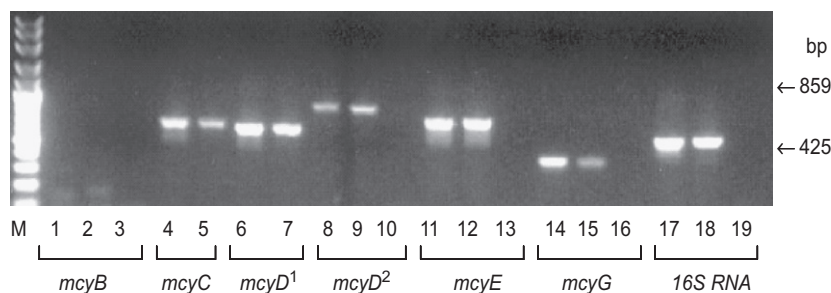| Herbivores	Mean weight at maturity in kg	Water requirement in litres per day	Average daily microcystin-LR intake (μg kg^{-1} d)
White rhinoceros male	2100	72	754.29
White rhinoceros female	1600	72	990
Zebra: Burchells	240	12	1100
Warthog	30	3.5	2566.6
Wildebeest: black	160	7	962.5

Fig. 2. Separation of PCR amplicons obtained with *Microcystis aeruginosa* strain PCC7806 (lanes 1, 4, 6, 8, 11, 14 and 17) and an environmental strain from Kruger National Park (lanes 2, 5, 7, 9, 12, 15 and 18) using different primer sets after analysis on a 2% agarose gel. Lanes 3, 10, 16 and 19 represents the negative control (i.e. PCR reaction without genomic DNA template). $M = 100$ bp ladder (O'RangeRular™ 100 bp DNA Ladder).

3.3. Microcystin analysis

The total microcystin level determined in July 2007 by ELISA was 23,718 μg l^{-1} while the extracellular microcystin-LR level was 2.1 μg l^{-1}. To the author's knowledge, the microcystin level measured in the Nhlanganzwane Dam in July 2007 is one of the highest levels ever measured to date in South Africa. In a previous study on Hartbeespoort Dam (during the period August 2003 to May 2004) by the South African Department of Water Affairs and Forestry, the maximum total microcystin value recorded to date was 14,400 μg l^{-1}, while low levels of cyanobacteria toxins persist thought the year at levels above 10 μg l^{-1} (Van Ginkel, 2004).

The microcystin levels measured in other dams within the region of the Nhlanganzwane Dam during the time of the animal mortalities in 2007 were much lower (Mpanama Dam, 0.581 μg l^{-1} and Makhohlolo Dam, 0.317 μg l^{-1}). The possible daily total microcystin-LR intake per body weight calculated for different animals consuming the shallow surface mixed layer (10 cm depth) of contaminated water from the Nhlanganzwane Dam during the period July 2007 were very high (Table 2.).

3.4. Assessing toxigenicity by PCR

The PCR products on the basis of the six *mcy* gene primers used, reveal the presence of the polyketide synthase genes (Fig. 2), which are ultimately responsible for the synthesis of the characteristic microcystin amino acid Adda (3-amino-9-methoxy-2,6,8-trimethyl-10 phenyldeca-4,6-dienoic acid), a 20-carbon amino acid that is important for the toxicity of microcystins (Harada et al., 1996). Measurement of the total microcystin level by ELISA confirms the presence of microcytin toxins in all the samples tested and was concurrent with the results obtained from the PCR analysis.

4. Discussion

The occurrence of the toxic bloom of *M. aeruginosa* and incidence of animal mortalities in 2007 permitted comparison of the dam conditions and identification of ecological drivers that promoted the occurrence of a toxic cyanobacterial bloom. Microscopic identification of the bloom composition revealed the dominance of cyanobacteria and the occurrence of very low numbers of other phytoplankton species. There are two plausible explanations for this phenomenon; firstly, *M. aeruginosa* blooms can reduce the growth of other phytoplankton because their surface habitat limits light transmission into the water column and allows them to out compete other phytoplankton that cannot tolerate the high light and the water temperatures at the surface (Robarts and Zohary, 1992). Secondly, the high suspended solids (112 mg l^{-1}) in the water column and stratification generate an underwater light climate that favors cyanophytes like *Microcystis* (Dokulil and Teubner, 2000; Bonnet and Poulin, 2002).

The high level of inorganic nitrogen (2171 μg l^{-1}) in the upper water column is possibly the direct impact of urination and faecal eutrophication by the high density of Hippopotami that inhabited the dam during the time of the intoxication event in 2007. The release of nitrogen from the decaying dung and urine of the hippopotami, possibly supplied the cyanobacteria with a slow but steady supply of NH$_4^+$-N, sufficient for maximum growth and bloom formation. An increased of NH$_4^+$-N availability can also stimulate buoyancy in cyanobacteria and might be a secondary effect related to the growth phase (Spencer and King, 1987). However, the levels of inorganic nitrogen (183 μg l^{-1}) in the Mpanama Dam during the time of the animal mortalities in 2007 were much lower than in the Nhlanganzwane Dam, possible due to the absence of Hippopotami because this dam was to shallow to support hippos. This in turn may have cause the nonappearance of a *Microcystis* bloom in the Mpanama Dam. The surface water temperature of 20–22 °C and the high levels of total microcystin (23,718 μg l^{-1}) is comparable with observations by Van der Westhuizen and Eloff (1985) in laboratory culture strains, which indicate the toxicity of *M. aeruginosa* grown at 20 °C is much higher than that at 15, 28 and 38 °C. However, Orr and Jones (1998) reported in their study a direct linear correlation between cell division and microcystin production rates in all microcystin-producing cyanobacteria regardless of the environmental factors that is limiting cell division.

In order to explain the mortalities of the white rhinoceroses, but not the hippopotami during July 2007, their association patterns with water were studied. Water intake by white rhinoceroses occurs mainly at night-time (Owen-Smith, 1973). During daylight hours, a higher photon irradiance allows *Microcystis* cells to accumulate carbohydrates. This increases their net cell density, and they lose buoyancy and sink, only to float up later to the surface due to the reduction of light and turgor pressure. In addition, under low light conditions (late afternoon), cyanobacteria increase their buoyancy by forming more gas vesicles and accumulate in high numbers in the surface water, since a linear relation between the loss of buoyancy and carbohydrate content and buoyancy state and the light dose received were reported for *Microcystis* cells by previous authors for both culture (Kromkamp and Mur, 1984) and in natural populations (Visser et al., 1996; Oberholster et al., 2005, 2006). As night falls *Microcystis* cells cannot change their buoyancy due to low light intensity and therefore stay in the surface water near shore where the white rhinoceroses drink. This phenomenon of vertical migration patterns by cyanobacteria colonies at night and early morning were previously observed in studies by Oberholster et al. (2006) and Pearson (1990).

Unlike white rhinoceroses, hippopotami have strong temporal and spatial constraints on their foraging behavior. Hippopotami

are temporally contained in that they forage primarily at night (Laws, 1968). This is the time period when cyanobacterial cell concentrations are the highest in the surface water and exposure to cyanobacteria by oral ingestion is most likely. Although hippopotamus populations normally spend a large proportion of day time immersed in water (Schwarm et al., 2003) they congregate in deeper water where cyanobacteria colonies are suspended in the water column. Generally microcystin concentrations are much higher at sites where toxic cyanobacteria accumulate, rather than in the open water. The accumulation of cyanobacterial colonies is the result of their positive buoyancy and wind-driven hydrodynamics, with leeward shorelines and embankments being the likely sites for surface scum accumulation.

Furthermore, the common hippopotamus represents the largest extant terrestrial foregut fermenter (Owen-Smith, 1988). Their daily intake and gut capacity per body weight is lower than other megaherbivores of comparable size, which most likely stems from their very long ingesta retention times recorded by Foose (1982). Due to the tubular nature of the forestomach and the long ingesta retention time, cyanobacterial cells ingested while drinking or swimming have sufficient time to mix with digestible nutrient-fiber and general organic matter which could reduce the toxicity levels of the toxic *Microcystis* cells. The possible total average microcystin-LR daily intake for a male white rhinoceros, consuming contaminated cyanobacterial water from the Nhlanganzwane Dam in July 2007 was $754.29\,\mu g\,kg^{-1}$ bw. In a previous study conducted by Falconer et al. (1994) they determine that the lowest observed adverse effecting level (LOAEL) of microcystin in pigs were $280\,\mu g\,kg^{-1}$ bw, with general liver injury (evident from histopathology and changes in serum enzymes) occurring at two higher dose levels. Laboratory studies by Fawell et al. (1994, 1999) indicate that the lowest observed effecting level (LOEL) of microcystin-LR by oral route in mice was $(LD_{50})\ 5000\,\mu g\,kg^{-1}$ bw. We assume from the above data in this study, that the high level of $23{,}718\,\mu g\,l^{-1}$ measured with ELISA as well as the microcystin LOAEL data established for pigs by Falconer et al. (1994), indicate that the possible cause of the mortalities of the white rhinoceroses, zebra, wildebeest and other species, in the region of the Nhlanganzwane Dam, was most likely due to acute rather than chronic exposure to microcystin toxins.

Although the mentioned microcystin intake value of different animals in this study is a (realistic) rough estimate, there are other factors that could have played a role e.g. the water intake of animal's increases during their gestation period. Hence, few studies of this nature exist and it is known that the water intake of cattle increases by about 50% in the last 4 months of their gestation period (Meyer et al., 1996). Furthermore, we speculated that the wild animals that died in July 2007 of cyanobacterial intoxication, has drunk dam water down wind to avoid the attention of predators. It is usually in these areas where cyanobacterial scum accumulates. In addition, Lopez Rodas and Costas (1999) found in their study that mice preferred to consume rather dense cultures of a toxic strain of *M. aeruginosa* than low-density cultures or clear water. They have confirmed their findings in the field with observations from several reservoirs in Spain.

A shortfall of this study however, was that only a single sampling trip was undertaken during the occurrence of the wild animal intoxications in July 2007, and we are aware that cyanobacterial blooms can vary substantially between seasons, weeks or even days. These variations might be caused by changes in species composition, production of different toxins with varying toxicity by one clone or other reasons influenced by environmental factors (Benndorf and Henning, 1989). However, the only time there is assessment of surface water in conservation areas like the Kruger National Park, is when there are deaths of wild animals. This is also a major problem when it comes to diagnosis of cyanobacterial poisoning in wildlife, since carcasses of these animals are usually found decomposed or partially consumed by scavengers. However, the detection of fresh animal carcasses in June 2007 made it possible to associate the *Microcystis* bloom with the wildlife mortalities.

In the present study, six *mcy* gene primers where used to help improve toxigenicity typing of *M. aeruginosa* strains, which is the dominant genus causing most cyanobacterial-related animal mortalities in South Africa (Oberholster et al., 2006). These six primers were selected, since in a previous study conducted on 12 dams by Botha and Oberholster (2007) they concluded that not all existing published primers available in literature were competent under South African environmental conditions. All the primers used in this study showed that they were competent under South African environmental conditions and were existing primers that were designed to amplify gene fragments that included a consensus sequence for a characteristic domain (nonribosomal peptide sythetase operon and polyketide synthase operon) plus regions flanking that sequence (Dittmann et al., 1997; Nishizawa et al., 1999, 2000; Tillett et al., 2000, 2001). The outcome of the PCR products confirms the occurrence of various *mcy* genes in the *M. aeruginosa* strains isolated from the Nhanganzwane Dam in 2007 that could be correlated with their ability to synthesize microcystins (Hui et al., 2002).

5. Conclusion

In the Kruger National Park setting we found that conditions which favor the excessive cyanobacterial growth such as the abnormal high winter temperatures, high levels of nutrients in the water caused by defecation and urination of a relatively large resident hippopotamus population as well as stagnant water all played a role in the development of the toxic cyanobacterial bloom that occurred in the Nhanganzwane Dam. Under such conditions, artificial dams in the Kruger National Park need to be monitored in order to predict these biotoxicity events. The present study also shows that existing reliable and simple PCR methodology can be used for the rapid screening of the presence of genetoxic strains in artificial water points in conservation areas. Monitoring by PCR methodology could be used as a proactive hazard identification technique to alert animal health professionals and wildlife managers to improve their preparedness and assist in the management of toxic *Microcystis* bloom events, as was experienced in the case of the Nhlanganzwane Dam.

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