

## RECOGNITION OF AN IMPORTANT WATER QUALITY ISSUE AT ZOOS: PREVALENCE AND POTENTIAL THREAT OF TOXIC CYANOBACTERIA

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**Abstract:** Zoo animals may be particularly vulnerable to water sources contaminated with cyanobacterial toxins, given their nonvoluntary close association with this resource. However, the prevalence and potential threat of toxic cyanobacteria in this setting are unknown. Several otherwise-unexplained yellow-bellied slider (*Trachemys scripta scripta*) deaths were documented in a zoo moat with recurring blooms of toxic *Microcystis aeruginosa*. Furthermore, an extremely high and potentially lethal concentration of the hepatotoxin microcystin (166 ng/g) was found in the liver of a necropsied turtle that died in this moat. A subsequent monthly survey of water quality revealed detectable concentrations of microcystin in all moats (0.0001 to 7.5 µg/L), with moats higher than 1 µg/L being significantly higher than the threshold for safe drinking water recommended by the World Health Organization. These results demonstrate that cyanobacterial blooms are an important water quality issue in zoos, and future research is necessary to identify potential associations between water quality and zoo animal health and effective moat management strategies.

**Key words:** Animal health, eutrophication, hepatotoxin, microcystin, phosphorus.

### BRIEF COMMUNICATION

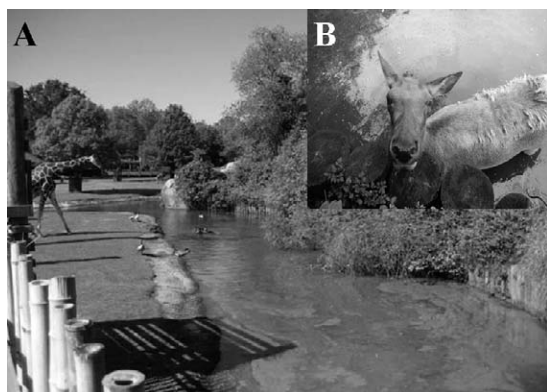
Poisonings of domestic animals and wildlife by blooms of toxic blue-green algae (i.e., cyanobacteria) are well documented throughout the world and date back to Francis' observation in 1878 of dead livestock associated with a bloom of the cyanobacterium *Nodularia*.<sup>7,11</sup> Zoo animals (and domestic animals, in general) may be particularly vulnerable to poisoning by water sources contaminated with cyanobacterial toxins given their involuntary close association with this resource (Fig. 1). However, the potential threat of toxic cyanobacteria to animal health and reproduction in this setting is unknown. The objectives of this study were to determine concentrations of the hepatotoxin microcystin in the livers of deceased zoo animals and to evaluate the prevalence of cyanobacterial blooms and associated toxins by surveying water quality in moats with zoo animal access.

During the summer of 2011, several dead yellow-bellied sliders (*Trachemys scripta scripta*)

were found in a perimeter moat. This moat was found to contain elevated nutrient concentrations (total phosphorus = 987 µg/L), a dense bloom of the toxic cyanobacterium *Microcystis aeruginosa*, and levels of the cyanotoxin microcystin (15 µg/L) that were well above preliminary guidelines for safe drinking water set by the World Health Organization (1 µg/L).<sup>5</sup> Microcystin concentrations in algal cells from moat water were quantified using enzyme-linked immunosorbent assay (ELISA [ADDA]; ABRAXIS, LLC, Warminster, Pennsylvania 18974, USA; and plate reader model ELx808, BioTek Instruments, Inc., Winooski, Vermont 05404, USA) after extraction from Pall A/E filters (Pall Corporation, Ann Arbor, Michigan 48103, USA) with 75% aqueous methanol.<sup>1</sup> ELISA was also used to measure the microcystin content (i.e., ng microcystin/g tissue dry weight) in liver tissues using standardized methods.<sup>12</sup>

Microcystin levels found in the liver of a necropsied yellow-bellied slider (166 ng/g) were comparable to lethal levels found in other animals.<sup>3,5,11</sup> Microscopic examination of this turtle revealed moderate hepatic atrophy, severe intestinal nematodiasis, and systemic Spirorchiid infection. The other turtle carcasses noted during this time were too severely autolysed to provide reliable data as a result of high air and water temperatures (>35°C and 30°C, respectively). Thus, the findings from the necropsy of one turtle do not provide a definitive link between high microcystin concentrations and the observed

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**Figure 1.** (A) Dense blooms of toxic cyanobacteria (*Microcystis aeruginosa*) in zoo animal perimeter moats present both an aesthetic and potentially dangerous situation for zoo animals. (B) Zoo animals may be particularly vulnerable to poisoning by water sources contaminated with cyanobacterial toxins (e.g., the hepatotoxin microcystin), given their close association with this resource.

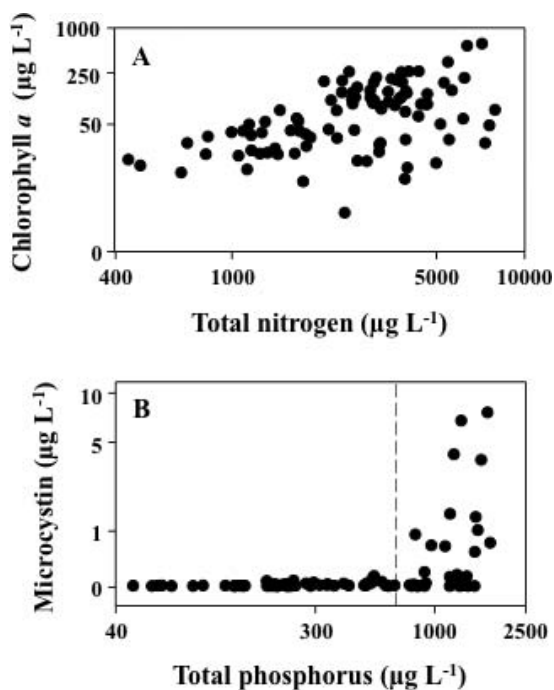
mortalities. In subsequent months, no turtles were observed in this moat, possibly as a result of high levels of turtle mortality. In contrast, an adjacent moat with low microcystin concentrations had abundant yellow-bellied sliders as well as soft-shelled and snapping turtles (*Apalone mutica* and *Chelydra serpentina*, respectively). Thus, another possibility is that turtles from the moat with high microcystin migrated to other moats with lower levels of toxic cyanobacteria. In any case, the concurrence of impaired water quality and the turtle deaths raise concerns about the prevalence of microcystin and its potential effects on animal health.

Microcystin content in liver tissues was also measured from a random sample of animals that died in the summer of 2011 as a result of causes unrelated to water quality. The first example case was a 14-yr-old bighorn sheep (*Ovis canadensis*) with severe heart disease, and the second was a stillborn elephant (*Loxodonta africana*) calf that likely died as a result of fetal hypoxia. The bighorn sheep and the maternal elephant both had exposure to moats containing microcystin (0.04  $\mu\text{g/L}$  and 2.9  $\mu\text{g/L}$ , respectively, at the times of death). Microcystin content in the liver of the stillborn elephant calf was below the detection limit ( $<0.3$  ng/g), while there was detectable but very low microcystin content in the liver of the bighorn sheep (0.5 ng/g). A direct comparison of the microcystin liver content of aquatic reptiles that spend significant time in water (e.g., turtles) with that of terrestrial animals is not prudent;

these data do, however, provide a reference point for future studies.

Given the potential threat of toxic cyanobacteria to zoo animal health, a monthly survey of water quality in nine animal perimeter moats and the water source reservoir was conducted from April to October of 2012 to evaluate water quality and the prevalence of the toxin microcystin and in order to understand patterns in the occurrence of toxic cyanobacterial blooms. Dissolved oxygen and temperature were measured in situ with a handheld meter (Hydrolab MiniSonde 4a, Hach, Loveland, Colorado 80539, USA). Depth-integrated water samples were collected with a tube sampler (inside diameter = 51 mm) stored on ice in 2-L plastic bottles and processed in the lab to measure nutrients (total nitrogen and total phosphorus), algal biomass (as chlorophyll *a*), algal species composition (via light microscopy), and microcystin using standard limnologic protocols.<sup>4</sup> Multiple linear regression with a backward elimination variable selection procedure was then used to determine the best predictors of chlorophyll *a* using data from all moats across all sampling dates. Dissolved oxygen, temperature, total nitrogen, total phosphorus, nitrogen-to-phosphorus ratio, and interactions between temperature and total nutrients (nitrogen and phosphorus) were included as possible predictors. All data were examined for normality and homogeneity using quartile–quartile and residual plots and were subsequently log-transformed to meet the assumptions of the model. As relationships between predictors and microcystin were nonlinear (Fig. 2B), classification and regression tree (CART) modeling was conducted.

The water quality survey revealed a large range of algal biomass (as chlorophyll *a*) across all the moats (8–465  $\mu\text{g/L}$ ), with the highest chlorophyll *a* concentrations observed in nutrient-rich moats (Fig. 2A), particularly during the summer months (multiple linear regression, total nitrogen  $\times$  temperature interaction:  $F_{1,82} = 36.36$ ,  $P < 0.0001$ ). Chlorophyll *a* is a widely used standard for water quality (higher concentrations usually indicate lower water quality) and is a proxy for algal biomass. In general, chlorophyll *a* concentrations for most moats were well above those typically observed in recreational water bodies and were indicative of poor water quality.<sup>10</sup> Maximum chlorophyll *a* concentrations (465  $\mu\text{g/L}$ ) were comparable to the highest levels observed for a wide range of temperate lakes in North America (596  $\mu\text{g/L}$ ).<sup>10</sup> Microcystin was also prevalent, with detectable concentrations observed in all nine



**Figure 2.** The relationship between (A) total nitrogen and chlorophyll *a* concentration and (B) total phosphorus and microcystin concentration for zoo moats. Data are plotted on log–log scales. The best predictor of chlorophyll *a* was the interaction of temperature and total nitrogen ( $F_{1,82} = 36.359$ ,  $P < 0.0001$ ), with all other variables being eliminated (Final model:  $\log \text{chlorophyll } a = 0.609 \times \log \text{temperature} - 1.239 \times \log \text{total nitrogen}$  ( $R^2 = 0.299$ )). The apparent threshold total phosphorus concentration at which elevated concentrations of microcystin ( $>1 \mu\text{g/L}$ ) became prevalent was  $\sim 670 \mu\text{g/L}$  based on classification and regression analysis.

moats and the source reservoir, and maximum concentrations of 3.8 and 7.5  $\mu\text{g/L}$  were observed in two of the moats with the highest nutrient concentrations (total phosphorus = 1,600 and 1,700  $\mu\text{g/L}$ ). Furthermore, the microcystin concentration (15  $\mu\text{g/L}$ ) in the moat where turtle deaths occurred was considerably higher than the maximum microcystin concentrations reported for water bodies in the southeastern (5  $\mu\text{g/L}$ ) and midwestern United States (4.5  $\mu\text{g/L}$ ).<sup>8</sup> These levels were also significantly higher than levels suggested for safe drinking water (1  $\mu\text{g/L}$ ).<sup>5</sup> Interestingly, CART modeling revealed an apparent boundary in the relationship between total phosphorus and microcystin concentrations in the moats (Fig. 2B). When total phosphorus concentrations in moats were below  $\sim 670 \mu\text{g/L}$ , microcystin concentrations were low (i.e., below 0.1  $\mu\text{g/L}$

L). However, when total phosphorus concentrations were above this threshold, high levels of microcystin (i.e., microcystin  $> 1 \mu\text{g/L}$ ) were more common. This suggests that to effectively reduce the risk posed by microcystin, nutrient concentrations should be reduced below this level. Many of the moats were actively managed by zoo staff throughout the sampling period, primarily because of aesthetic concerns related to the algal blooms (Fig. 1B). Management strategies varied from moat to moat and included treatment with algaecides (e.g., copper sulfate), addition of dyes to stain the water, regular flushing, and draining-refilling the moats.

These results demonstrate an important but unexplored water quality issue at zoos. Despite active management by zoo staff, cyanobacterial blooms, microcystin, and impaired water quality were prevalent in many of the nutrient-rich perimeter moats. Zoo animals may thus be particularly vulnerable to issues related to water quality, including acute and chronic exposure to the hepatotoxin microcystin.<sup>3,11</sup> The direct and indirect effects of chronic exposure of animals to cyanotoxins are unknown. Cyanobacterial species produce a variety of known toxic compounds, and there may be numerous unidentified secondary metabolites that potentially affect animal health. Subacute or chronic exposures to known and unknown cyanobacterial secondary metabolites may have complex effects.<sup>5,11</sup> In the case of this study, stress from exposure to high levels of microcystin and poor water quality (as indicated by high nutrients and chlorophyll *a*) may have increased the susceptibility of turtles to infection (e.g., severe intestinal nematodiasis and systemic *Spirorchid* infection). Managers and veterinarians should be aware of the potential effects of toxic cyanobacteria and water quality. Further evaluation of cyanobacterial toxins, particularly for aquatic organisms such as turtles and fish, is justified, as aquatic animals may serve as indicator organisms for potential toxin-related risks to other zoo animals. While the use of algaecides is effective in reducing the abundance of cyanobacteria, they can also be highly toxic to many zoo animals, in addition to harming a variety of nontarget aquatic organisms.<sup>2</sup> Cell-bound toxins can also be released into the water as a result of cyanobacteria death post-algaecide treatment.<sup>2</sup> Reduction and diversion of excess nutrients, increasing natural shade around the moats, and removing planktivorous fish when possible to promote grazing by large-bodied zooplankton herbivores (e.g., *Daphnia*) may help to reduce the

abundance of toxic cyanobacteria and improve water quality.<sup>4,6,9</sup> Because of the presumption that animals will enter, drink, or otherwise ingest or be exposed to water if it is present within a moat in their display, alternative clean drinking water sources must be made available to zoo animals when none of these approaches are feasible. Future studies are necessary to determine the risks and potential associations between water quality and animal health, and greater attention to water quality in zoos is necessary.

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