Running Head: Animal water quality and toxic cyanobacteria

Pond bank access as an approach for managing toxic cyanobacteria in beef cattle pasture drinking water ponds

Alan E. Wilson[a,*], Michael F. Chislock[a,b], Zhen Yang[a], Mário U. G. Barros[a], and John F. Roberts[c]

[a]Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn, Alabama, 36849 USA, wilson@auburn.edu (AEW), zhyang@niglas.ac.cn (ZY), mariobarros86@hotmail.com (MUGB)

[b]The College at Brockport – State University of New York, Department of Environmental Science and Ecology, Brockport, New York, 14559 USA, mchislock@brockport.edu

[c]Alabama Department of Agriculture and Industries, Thompson Bishop Sparks State Diagnostic Laboratory, Auburn, Alabama, 36832 USA, jfr0004@auburn.edu

[*] Corresponding author: wilson@auburn.edu, 334-246-1120

ABSTRACT:

Forty-one livestock drinking water ponds in Alabama beef cattle pastures during were surveyed during the late summer to generally understand water quality patterns in these important water resources. Since livestock drinking water ponds are prone to excess nutrients that typically lead to eutrophication, which can promote blooms of toxigenic phytoplankton such as cyanobacteria, we also assessed the threat of exposure to the hepatotoxin, microcystin. Eighty-percent of the ponds studied contained measurable microcystin, while three of these ponds had concentrations above human drinking water thresholds set by the US Environmental Protection Agency (i.e., 0.3 µg/L). Water quality patterns in the livestock drinking water ponds contrasted sharply with patterns typically observed for temperate freshwater lakes and reservoirs. Namely, we found several non-linear relationships between phytoplankton abundance (measured as chlorophyll) and nutrients or total suspended solids. Livestock had direct access to all the study ponds. Consequently, the proportion of inorganic suspended solids (e.g., sediment) increased with higher concentrations of total suspended solids, which underlies these patterns. Unimodal relationships were also observed between microcystin and phytoplankton abundance or nutrients. Euglenoids were abundant in the four ponds with chlorophyll concentrations > 250 µg/L (and dominated three of these ponds), which could explain why ponds with high chlorophyll concentrations would have low microcystin concentrations. Based on observations made during sampling events and available water quality data, livestock-mediated bioturbation is causing elevated total suspended solids that lead to reduced phytoplankton abundance and microcystin despite high concentrations of nutrients, such as phosphorus and nitrogen. Thus, livestock could be used to manage algal blooms, including toxic secondary metabolites, in their drinking water ponds by allowing them to walk in the ponds to increase turbidity.

Key words: animal health, cattle, eutrophication, microcystin, sediment, stoichiometry, total suspended solids, turbidity

1. INTRODUCTION

Good water quality is critical for the health and well-being of livestock and their associated production. Although drinking water sources vary across space and time (e.g., well water, municipal drinking water, ponds), commercial livestock in the southeastern U.S. are often limited to nearby drinking water ponds that tend to be small, shallow, warm, hyper-eutrophic, and prone to algal blooms (Straubinger-Gansberger et al. 2014). Despite the importance of livestock drinking water quality, very little is known about this water resource related to animal health (but see van Halderen et al. 1995, Nägeli et al. 1997, Veira 2007, Wilson et al. 2013, Chagas et al. 2014, Silva et al. 2014, Bichsel et al. 2016, Badar et al. 2017). In contrast, dozens of studies have documented spatiotemporal trends in water quality in recreational waterbodies (Downing et al. 2001, Beaver et al. 2014, Taranu et al. 2017).

Using the broader limnological literature, eutrophication driven by increased resources, such as light or nutrients like phosphorus and nitrogen, typically leads to higher concentrations of phytoplankton, including taxa such as cyanobacteria that may produce toxic secondary metabolites that can poison livestock (Chislock et al. 2013, Wood 2016). Smaller, shallower, and warmer systems tend to exhibit enhanced effects of eutrophication (Scheffer and van Nes 2007). The relationship between nutrient resources and phytoplankton abundance is often linear across a wide range of nutrient concentrations. However, suspended solids, either organic (e.g., phytoplankton) or inorganic (e.g., sediments), reduce water transparency that limits the availability of light deeper in the water column. Thus, at very high concentrations of phytoplankton, the relationship between nutrients and phytoplankton abundance weakens because another resource, access to sunlight, limits phytoplankton production (Vollenweider

1979). Similarly, non-linear patterns between nutrients and chlorophyll are common when inorganic suspended sediments are high.

Livestock drinking water ponds are especially vulnerable to high concentrations of phytoplankton, and in some instances the damaging effects of toxigenic cyanobacteria. This is often attributed to the excess nutrients that ponds receive through the direct (urination (primarily nitrogen) and defecation (primarily phosphorus)) and indirect (watershed livestock waste runoff) effects of livestock. Thus, traditional approaches to eutrophication management, such as nutrient control, are challenging in these ponds unless regular flushing events are possible.

A proposed method to control toxic cyanobacteria is through the use of livestock-mediated bioturbation within their own drinking water ponds. As livestock access to pond banks is feasible, and required in some instances, this method could provide farmers a simple way to control cyanobacterial blooms without increased costs. Many studies have shown, however, that livestock grazing erodes stream banks, which leads to the eventual loss of buffer strips (Agouridis et al. 2005). In addition, the effects of erosion in ponds may be worse considering the lack of flow in these systems relative to streams (Bichsel et al. 2016). However, despite higher concentrations of inorganic suspended sediments that may negatively affect livestock drinking water taste and aesthetics, additional benefits of reduced water transparency may exist through reduced toxigenic cyanobacteria.

In this study, we surveyed the water quality of 41 drinking water ponds in beef cattle pastures throughout Alabama during one summer to (1) document variation in water quality of the ponds, (2) assess the threat that the hepatotoxin, microcystin, poses to livestock, (3) determine if water quality patterns observed for well-studied systems, including freshwater lakes and reservoirs, occur in livestock drinking water ponds, and (4) use these data to develop best

management practices for livestock producers to minimize the threat of toxic cyanobacteria in surface drinking water sources.

2. MATERIALS AND METHODS

During August and September 2011, near surface (~0.5 m depth) water quality samples were collected from 41 shallow livestock (primarily beef cow-calf populations) drinking water ponds located throughout Alabama. Livestock had direct access to all ponds. In most cases, multiple integrated samples were collected with a long, rigid tube sampler at a depth of 0.5 m from a single location on the shore to avoid disturbing the sediments and surface algal bloom scums that can collect near shore. In four cases, multiple integrated samples were collected from a single location using a boat or dock at a depth of 0.5 m using the same tube sampler. All sampling sites were selected because they were considered to be representative of the entire waterbody, thus obvious visual differences (e.g., algal scums collected on the windward side of the pond) were avoided. Samples were stored in acid-washed plastic bottles on ice in a cooler until they were processed in the laboratory for algal abundance (measured as chlorophyll), total suspended solids, nutrients (total phosphorus and total nitrogen), the hepatoxin, microcystin, and phytoplankton identification. Chlorophyll (µg/L) was determined fluorometrically (Turner Designs Trilogy with a non-acidification module) after extracting filters in 90% ethanol for 24 hours in the dark at 4ºC (Sartory and Grobbelaar 1984). Lugol's preserved (1% by volume) phytoplankton were assessed by scanning an entire Palmer chamber (~175 µl sample volume) to determine the top five genera based on abundance (Chislock et al. 2014). One sample had too much inorganic sediment to accurately evaluate the phytoplankton community. Total suspended solids (mg/L) and inorganic suspended solids (mg/L) were determined after weighing tared filters with suspended solids that were dried at 50ºC for at least 48 hours or combusted at 550ºC for at least 2 hours until filter weights stabilized (USEPA 160.2), respectively. Organic suspended solids (mg/L) were calculated by subtracting inorganic suspended solids from total

suspended solids. Nutrient concentrations were determined by spectrophotometry using colorimetric (total phosphorus; µg/L) and ultraviolet (total nitrogen; µg/L) standard methods (Gross and Boyd, 1998). Microcystin concentration (µg/L) in the seston was quantified using enzyme-linked immunosorbent assay (ELISA) (An and Carmichael, 1994) after extraction from filters with acidified 75% aqueous methanol. Eight of the 41 ponds had undetectable microcystin concentrations. Although these undetectable microcystin concentrations were not included in later statistical analyses, associated data with these samples are provided in each figure for visualization purposes using grey symbols. Light attenuation was not measured in all of the ponds due to sampling constraints, such as muddy and wet banks (due to livestock trampling) and very shallow depths (prevented boat sampling). However, Secchi depth (a well-studied metric of transparency; Davies-Colley and Smith 2001) was measured in 26 ponds.

Prior to regression analyses, data were log-transformed to reduce heteroscedasticity given the large variation in water quality parameters across the 41 ponds (Figures 1, 2). Results from polynomial regression analyses (specifically, quadratic) were compared to linear regression analyses since many of the correlations were unimodal. When comparing results between linear and polynomial regression analyses, Akaike Information Criterion (AICc) values, corrected for small sample sizes, were primarily used to compare the fit of regression lines. However, we also considered *P* values and coefficients of determination (R^2) when comparing the fit of linear or quadratic regressions. In situations where *P* values and R^2 were similar, the simplest model (i.e., linear regression) was selected. Principal component analyses (PCA; all values log-transformed prior to analysis) was used to simplify the multivariable dataset into two principal components for 37 of the ponds that had complete datasets for the following parameters, chlorophyll (µg/L), microcystin (µg/L), total phosphorus (µg/L), total nitrogen (µg/L), total suspended solids (mg/L),

organic suspended solids (mg/L), inorganic suspended solids (mg/L), and N:P (by weight). For PCA, we wanted to include all available ponds and used the microcystin concentration of 0.001 µg/L for ponds where this toxin was undetectable to contrast ponds with and without measurable microcystin. All analyses were conducted using SYSTAT 13 or R studio (version 3.4.1).

3. RESULTS

Water quality varied widely across 41 livestock drinking water ponds in Alabama (Figures 1, 2), although all ponds could be classified as eutrophic to hyper-eutrophic. Livestock had direct access to all of the ponds and were observed in the ponds during most sampling events (Figure 2). For example, large ranges were observed for all water quality parameters, including chlorophyll (15 - 697 µg/L; median = 113 µg/L), total suspended solids (10 - 2,745 mg/L; median = 35 mg/L), inorganic suspended solids (0.4 - 2,461 mg/L; median = 11 mg/L), organic suspended solids (3.5 - 284 mg/L; median = 26 mg/L), % inorganic material in the total suspended solids (2 - 90%; median = 45%), % organic material in the total suspended solids (10 - 98%; median = 56%), total phosphorus (62 - 1,644 µg/L; median = 175 µg/L), total nitrogen (666 - 13,888 µg/L; median = 1,960 µg/L), and N:P (0.9 - 37.3, by weight; median = 10.5). One or more toxigenic cyanobacterial genera, including *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, and *Planktothrix*, were abundant in 76% (31/41) of the surveyed ponds. Moreover, microcystin concentration was detectable in 80% (33/41) of the ponds but varied by over three orders of magnitude when detectable (range: 0.0046 – 2.50 µg/L; median = 0.013 µg/L).

Given that such few studies have examined algal toxins in livestock drinking water sources (but see van Halderen et al. 1994, Nägeli et al. 1997, Viera 2007, Wilson et al. 2013, Chagas et al. 2014, Silva et al. 2014, Bichsel et al. 2016, Badar et al. 2017) and that no microcystin concentration threshold currently exists for livestock drinking water, we used a recently established 10-day microcystin drinking water health advisory threshold (0.3 µg/L) for humans (<6 years of age) created by the U.S. Environmental Protection Agency (USEPA 2015). This USEPA-based threshold is conservative considering that it is based on a lowest observed

168 adverse effect level (50 µg/kg/day) in tested rats and includes a total uncertainty safety factor of
169 1000 (USEPA 2015). The USEPA did not identify a no observed adverse effect level of
170 microcystin in drinking water (USEPA 2015). Nine percent (3/33) of the ponds with detectable
171 microcystin had concentrations that exceeded this human health advisory threshold when
172 sampled. However, it is important to note that that there are no established thresholds for
173 microcystin concentrations in livestock drinking water. We contend that more research is needed
174 in this area.

175 Certain water quality patterns for the livestock ponds were consistent with past
176 observations made in recreational waterbodies (Sarnelle et al. 2010). For example, positive
177 correlations between total nutrients and algal abundance (Figure 1A; $R^2 > 0.63$, $P < 0.00001$) are
178 common considering that resources, such as phosphorus and nitrogen, are important for
179 phytoplankton growth and that phosphorus and nitrogen comprise ~1% and ~6% of algal dry
180 biomass, respectively (Duarte 1992). Thus, more nutrients tend to promote more phytoplankton.
181 Interestingly, the log-transformed correlations between nutrients and chlorophyll were
182 significantly non-linear (Figure 1A) suggesting that other resources, such as light, were limiting
183 phytoplankton at the high end of the chlorophyll range. Although the relationship between total
184 nitrogen and chlorophyll is presented as linear (linear AICc = 3.87, quadratic AICc = 6.33), the
185 R^2 and *P-value* for the linear and polynomial regressions were nearly identical ($R^2 = 0.630$, $P <$
186 0.0000001).

187 As light penetrates water, it is reduced (i.e., light extinction) as a function of heat loss and
188 scatter associated with turbidity from phytoplankton (organic) or sediment (inorganic). Indeed,
189 across 26 ponds sampled, transparency of the ponds was poor (Secchi depth mean = 38 cm;
190 range = 5-101 cm) and negatively correlated with chlorophyll concentration (log transformed

191 data; R^2 = 0.527, P = 0.000027). Indeed, we found a significant unimodal relationship between
192 total suspended solids (index of total turbidity) and algal abundance (measured as chlorophyll)
193 (Figure 1B; R^2= 0.245, P = 0.0052) where total suspended solids peaked around 100 mg/L,
194 while the pond with the highest TSS (2,745 mg/L) had no detectable microcystin despite high
195 chlorophyll (195 µg/L). Remarkably, euglenoids were abundant in the four ponds with the
196 highest chlorophyll concentrations (>250 µg/L) and dominated the phytoplankton communities
197 in three of these ponds.
198 Although the relationship between inorganic suspended solids and algal abundance was
199 weak (Figure 1C; F = 1.79, R^2 = 0.045, P = 0.188), there was strong positive relationship
200 between organic suspended solids and chlorophyll (Figure 1D; F = 33.43, R^2 = 0.468, P <
201 0.00001), as expected. Moreover, the percent contribution of inorganic materials that composed
202 the total suspended solids increased with higher concentrations of total suspended solids (Figure
203 1E; F = 13.81, R^2 = 0.267, P = 0.00065). The decline in organic material that composed total
204 suspended solids was stronger and less variable (Figure 1F; F = 65.09, R^2 = 0.631, P <
205 0.0000001) than the pattern observed for inorganic suspended solids. Clearly, suspended
206 sediments negatively impacted phytoplankton production in the ponds studied. And, our
207 observations of livestock in the drinking water ponds during sampling events suggest that high
208 turbidity is a result of livestock bioturbation inside or outside (through erosion and runoff) of the
209 ponds (Figure 2).
210 Relationships between microcystin and other water quality parameters did not always
211 align with earlier observations in recreational waterbodies (Beaulieu et al. 2014). For example,
212 non-linear relationships were observed between microcystin and chlorophyll (Figure 3A; R^2 =
213 0.129, P = 0.125), total phosphorus (Figure 3C; R^2 = 0.158, P = 0.083), and total nitrogen

(Figure 3C; $R^2 = 0.211$, $P = 0.0362$). In other words, higher concentrations of phytoplankton or nutrients did not result in higher concentrations of microcystin. A significant linear relationship was observed for microcystin and N:P (Figure 3D; $R^2 = 0.227$, $P = 0.0068$), while no relationship existed for total suspended solids (Figure 3B; $R^2 = 0.0071$, $P = 0.647$). Sites without detectable microcystin were included in both figures (grey symbols) for visualization purposes. Although there was a tendency for sites with non-detectable microcystin to be at the lower end of the range, in many cases microcystin was not detected despite high chlorophyll, TSS, and/or nutrients (Figure 3).

Principle component analysis highlighted some interesting patterns when synthesizing the dataset. The first two PCA components explained more than two-thirds of the variance in water quality data (70.22%; Figure 4). Component 1 (x-axis) was positively influenced by most variables, including chlorophyll, total phosphorus, total nitrogen, total suspended solids, inorganic and organic suspended solids, and microcystin. N:P was not correlated with component 1. Component 2 (y-axis) showed a strong influence of N:P and microcystin but was also positively affected by total nitrogen and chlorophyll and negatively affected by total phosphorus, and total and inorganic suspended solids. Organic suspended solids were poorly correlated with component 2. The similar vectors for organic suspended solids and chlorophyll makes sense considering we expect most organic suspended solids to be phytoplankton. Finally, the disconnect between chlorophyll and microcystin further supports our observation regarding the non-linear relationship between phytoplankton abundance and algal toxicity.

4. DISCUSSION

Water quality of livestock drinking water ponds is poorly understood despite its obvious importance for animal health and production (but see van Halderen et al. 1994, Nägeli et al. 1997, Viera 2007, Wilson et al. 2013, Chagas et al. 2014, Silva et al. 2014, Bichsel et al. 2016, Badar et al. 2017). We found large variation in the water quality of the 41 livestock drinking water ponds surveyed during the summer, although there was a tendency of the ponds to be highly eutrophic and productive (Figure 1A). Such patterns are not surprising given the expected nutrient inputs through livestock defecation and urination. Concentrated animal feeding operations would only exacerbate nutrient additions and concomitant eutrophication issues (Chagas et al. 2014).

Phytoplankton communities varied across the 41 ponds but tended to be dominated by cyanobacteria or euglenoids (between both taxa: 93% (38/41)). These findings make sense considering that both taxa perform well in hyper-eutrophic environments with abundant organic matter. Interestingly, the patterns between chlorophyll and nutrients, including phosphorus and nitrogen, had a tendency to be non-linear especially at higher concentrations (Figure 1A), suggesting another resource was limiting algal production. Turbidity from phytoplankton (organic) or sediment (inorganic) can negatively impact phytoplankton and cyanobacterial toxin production, even in eutrophic systems, through greater light extinction (Davies-Colley and Smith 2001). Across the 26 ponds sampled, there was a strong negative correlation between Secchi depth and chlorophyll concentration in the ponds (log transformed data; $R^2 = 0.527$, $P = 0.000027$). These data support our contention that reduced light limited phytoplankton growth in many of the studied ponds despite abundant nutrient resources. Moreover, the non-linear relationship between total suspended solids data and chlorophyll (Figure 1B) suggest that

inorganic suspended sediments represented a higher proportion than phytoplankton with increasing total suspended solids. Indeed, the percent contribution of inorganic material increased with higher concentrations of total suspended solids (Figure 1E). Thus, our data suggest that livestock access to drinking water ponds limits phytoplankton abundance and concentrations of the hepatotoxin, microcystin, under elevated nutrient concentrations. Given that livestock were regularly observed in the ponds during sampling events (Figure 2) and that their movement would directly disturb pond sediments as well as promote the erosion of pond banks, we contend that livestock could be used to manage the presence of toxigenic phytoplankton taxa.

Bioaccumulation of the hepatotoxin, microcystin, in livestock has been documented in the past (van Halderen et al. 2014, Badar et al. 2017). Although microcystin concentration data in the drinking water ponds were not provided in Badar et al. (2017), >80% of the studied cows and buffaloes had measurable microcystin in their blood and >90% of the animals suffered from liver irregularities. These findings are consistent with microcystin poisoning (Dawson 1998, Carmichael et al. 2001, Wood 2016). van Halderen et al. (2014) described three livestock poisoning events associated with toxic cyanobacteria. Thus, livestock are clearly vulnerable to microcystin through direct interactions with their drinking water (Mez et al. 1997, Nägeli et al. 1997, Falconer 2001). In our study, toxigenic cyanobacteria dominated in 76% of the ponds and the hepatotoxin, microcystin, was measured in 80% of the surveyed ponds. Of these ponds, three cases exceeded the human drinking water thresholds for microcystin (0.3 µg/L) recently established by the U.S. Environmental Protection Agency which served as our reference given that no microcystin threshold currently exists for livestock drinking water. It is important to

stress that the implication of this microcystin threshold to livestock is unclear based on available data in the literature.

Considering that we were not targeting surface scums, livestock could be prone to significantly higher concentrations of algal toxins when consuming water near pond edges. The 20% of the ponds that lacked detectable concentrations of microcystin spanned a large gradient in most of the other water quality parameters (Figure 3). Thus, a high concentration of phytoplankton (measured as chlorophyll) or nutrients did not necessarily relate to high concentrations of microcystin (Figure 3). In fact, many of the water quality patterns with microcystin were not linear, in contrast to a large number of limnological studies that have documented linear relationships in a variety of regions and aquatic ecosystems (Beaulieu et al. 2013). Considering that cyanobacterial toxins, including microcystin, are generally found within toxigenic phytoplankton cells (unless when cells lyse and toxins are released into surrounding water) and produced in response to a variety of external stimuli (e.g., nutrient limitation, reduced light, and/or consumers), large variation between microcystin and several water quality parameters (Figure 3) is not surprising (Taranu et al. 2017). Interestingly, the patterns for microcystin and chlorophyll (Figure 3A), total phosphorus (Figure 3C), and total nitrogen (Figure 3C) were non-linear (and nearly unimodal in some cases) across the range of concentrations measured. We are not aware of any studies documenting such dramatic water quality patterns in livestock drinking water ponds. These findings suggest that some factor (or factors) was limiting phytoplankton and/or toxin production. Contrasting relationships between chlorophyll and inorganic (Figure 1C) or organic (Figure 1D) suspended solids show that suspended sediments reduce light transparency, which negatively affects phytoplankton production.

303 In our study, results from the principal components analysis further supported our
304 findings that chlorophyll and microcystin or total suspended solids are not strongly correlated
305 (Figure 4). For example, principal components vectors for microcystin and chlorophyll were not
306 parallel while the vectors for microcystin and organic suspended solids were almost
307 perpendicular (Figure 4). Had these parameters been strongly correlated, this analysis would
308 have shown similar directions for relevant analytes.
309

5. CONCLUSION

Based on patterns between suspended solids (total, inorganic, and organic) and algal abundance and our observations during sampling events, we contend that livestock access to ponds promotes bioturbation that reduces light availability to phytoplankton, including toxigenic taxa. Consequently, livestock farmers should be aware that livestock-mediated bioturbation can be a tool for controlling toxic cyanobacteria in their own drinking water ponds. Although such an approach counters current advice from research and extension publications (Agouridis et al. 2005, Derlet et al. 2010, Evans et al. 2006), suspended sediments may serve multiple functions related to cyanobacterial toxins. First, increased turbidity will limit light penetration, which should reduce phytoplankton production even under hyper-eutrophic conditions. Second, some variants of microcystin bind preferentially to sediment, thus the presence of some sediments may serve as a sink for dissolved cyanobacterial toxins (Wu et al. 2012, Song et al. 2015). As with all management approaches, we encourage farmers to directly work with extension agents to regularly test livestock drinking water sources to determine the presence and abundance of toxigenic organisms, including cyanobacteria.

ACKNOWLEDGEMENTS

We would like to thank the Alabama Cooperative Extension System agents, including George Tabb, Jimmy Jones, Jimmy Smitherman, Richard Conway, and Tinsley Gregg, and Alabama beef producers for helping us get access to livestock drinking water ponds. We also appreciate the help of Vernon Anderson during sample collection and Riley Buley, Edna Fernandez, and Zhen Yang for comments that improved earlier versions of the manuscript. This project was supported by the Alabama Agricultural Experiment Station and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

REFERENCES

Agouridis, C.T., Workman, S.R., Warner, R.C., Jennings, G.D., 2005. Livestock grazing management impacts on stream water quality: A review. Journal of the American Water Resources Association 41, 591-606.

An, J.S., Carmichael, W.W., 1994. Use of colorimetric protein phosphatase inhibition assay and enzyme-linked immunosorbent-assay for the study of microcystins and nodularins. Toxicon 32, 1495-1507.

Badar, M., Batool, F., Khan, S.S., Khokhar, I., Qamar, M.K., Yasir, C., 2017. Effects of microcystins toxins contaminated drinking water on hepatic problems in animals (cows and buffaloes) and toxins removal chemical method. Buffalo Bulletin 36, 43-55.

Beaulieu, M., Pick, F., Palmer, M., Watson, S., Winter, J., Zurawell, R., Gregory-Eaves, I., 2014. Comparing predictive cyanobacterial models from temperate regions. Canadian Journal of Fisheries and Aquatic Sciences 71, 1830-1839.

Beaver, J.R., Manis, E.E., Loftin, K.A., Graham, J.L., Pollard, A.I., Mitchell, R.M., 2014. Land use patterns, ecoregion, and microcystin relationships in US lakes and reservoirs: A preliminary evaluation. Harmful Algae 36, 57-62.

Bichsel, D., De Marco, P., Bispo, A.A., Ilg, C., Dias-Silva, K., Vieira, T.B., Correa, C.C., Oertli, B., 2016. Water quality of rural ponds in the extensive agricultural landscape of the Cerrado (Brazil). Limnology 17, 239-246.

Carmichael, W.W., Azevedo, S., An, J.S., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K., 2001. Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. Environmental Health Perspectives 109, 663-668.

360 Chagas, C.I., Kraemer, F.B., Santanatoglia, O.J., Paz, M., Moretton, J., 2014. Biological water
361 contamination in some cattle production fields of Argentina subjected to runoff and
362 erosion. Spanish Journal of Agricultural Research 12, 1008-1017.
363 Chislock, M.F., Doster, E., Zitomer, R.A., Wilson, A.E., 2013. Eutrophication: Causes,
364 consequences, and controls in aquatic ecosystems. Nature Education Knowledge 4, 10.
365 Chislock, M.F., Sharp, K.L., Wilson, A.E., 2014. *Cylindrospermopsis raciborskii* dominates
366 under very low and high nitrogen-to-phosphorus ratios. Water Research 49, 207-214.
367 Davies-Colley, R. J., Smith, D. G., 2001. Turbidity, suspended sediment, and water clarity: a
368 review. Journal of the American Water Resources Association 37, 1085-1101.
369 Derlet, R.W., Goldman, C.R., Connor, M.J., 2010. Reducing the impact of summer cattle grazing
370 on water quality in the Sierra Nevada Mountains of California: a proposal. Journal of
371 Water and Health 8, 326-333.
372 Downing, J.A., Watson, S.B., McCauley, E., 2001. Predicting Cyanobacteria dominance in lakes.
373 Canadian Journal of Fisheries and Aquatic Sciences 58, 1905-1908.
374 Duarte, C.M., 1992. Nutrient concentration of aquatic plants - patterns across species. Limnology
375 and Oceanography 37, 882-889.
376 Evans, D.M., Redpath, S.M., Elston, D.A., Evans, S.A., Mitchell, R.J., Dennis, P., 2006. To
377 graze or not to graze? Sheep, voles, forestry and nature conservation in the British
378 uplands. Journal of Applied Ecology 43, 499-505.
379 Falconer, I.R., 2001. Toxic cyanobacterial bloom problems in Australian waters: risks and
380 impacts on human health. Phycologia 40, 228-233.

381 Gross, A., Boyd, C.E., 1998. A digestive procedure for the simultaneous determination of total
382 nitrogen and total phosphorus in pond water. Journal of the World Aquaculture Society
383 29, 300-303.
384 Mez, K., Beattie, K.A., Codd, G.A., Hanselmann, K., Hauser, B., Naegeli, H., Preisig, H.R.,
385 1997. Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on
386 alpine pastures in Switzerland. European Journal of Phycology 32, 111-117.
387 Naegeli, H., Sahin, A., Braun, U., Hauser, B., Mez, K., Hanselmann, K., Preisig, H.R., Bivetti,
388 A., Eitel, J., 1997. Sudden deaths of cattle on Alpine pastures in South-Eastern
389 Switzerland. Schweizer Archiv Fur Tierheilkunde 139, 201-209.
390 Sarnelle, O., Morrison, J., Kaul, R., Horst, G., Wandell, H., Bednarz, R., 2010. Citizen
391 monitoring: Testing hypotheses about the interactive influences of eutrophication and
392 mussel invasion on a cyanobacterial toxin in lakes. Water Research 44, 141-150.
393 Sartory, D.P., Grobbelaar, J.U., 1984. Extraction of chlorophyll a from freshwater phytoplankton
394 for spectrophotometric analysis. Hydrobiologia 114, 177-187.
395 Scheffer, M., van Nes, E.H., 2007. Shallow lakes theory revisited: various alternative regimes
396 driven by climate, nutrients, depth and lake size. Hydrobiologia 584, 455-466.
397 Silva, A.C., Souza, A.M., Dutra, I.S., 2014. Occurrence of blue-green algae in the drinking water
398 of extensively raised cattle. Pesquisa Veterinária Brasileira 34, 415-420.
399 Song, H.H., Coggins, L.X., Reichwaldt, E.S., Ghadouani, A., 2015. The importance of lake
400 sediments as a pathway for microcystin dynamics in shallow eutrophic lakes. Toxins 7,
401 900-918.

Straubinger-Gansberger, N., Kaggwa, M.N., Schagerl, M., 2014. Phytoplankton patterns along a series of small man-made reservoirs in Kenya. Environmental Monitoring and Assessment 186, 5153-5166.

Taranu, Z.E., Gregory-Eaves, I., Steele, R.J., Beaulieu, M., Legendre, P., 2017. Predicting microcystin concentrations in lakes and reservoirs at a continental scale: A new framework for modelling an important health risk factor. Global Ecology and Biogeography 26, 625-637.

USEPA, 2015. 2015 Drinking water health advisories for two cyanobacterial toxins. In: Agency, U.S.E.P. (Ed.), Washington, D. C.

van Halderen, A., Harding, W.R., Wessels, J.C., Schneider, D.J., Heine, E.W.P., van der Merwe, J., Fourie, J.M., 2014. Cyanobacterial (blue-green algae) poisoning of livestock in the western cape province of South Africa. Journal of the South African Veterinary Association 66, 260-264.

Veira, D.M., 2007. Meeting water requirements of cattle on the Canadian prairies. Rangeland Journal 29, 79-86.

Vollenweider, R.A., 1979. Concept of nutrient load as a basis for the external control of eutrophication process in lakes and reservoirs. Zeitschrift Fur Wasser Und Abwasser Forschung-Journal for Water and Wastewater Research 12, 46-56.

Wilson, A.E., Chislock, M.F., Doster, E., Wright, R.A., Kottwitz, J.J., Walz, H., Rose, H., 2013. Toxic algae threaten livestock health. The Alabama Cattleman 6, 16-17.

Wood, R., 2016. Acute animal and human poisonings from cyanotoxin exposure - A review of the literature. Environment International 91, 276-282.

424 Wu, X.Q., Wang, C.B., Xiao, B.D., Wang, Y., Zheng, N., Liu, J.S., 2012. Optimal strategies for
425 determination of free/extractable and total microcystins in lake sediment. Analytica
426 Chimica Acta 709, 66-72.
427

FIGURE LEGENDS

Figure 1. The relationship between chlorophyll *a* concentration (µg/L) and (A) total phosphorus (hexagons) and total nitrogen (triangles), (B) total suspended solids (mg/L), (C) inorganic suspended solids (mg/L), and (D) organic suspended solids (mg/L) for livestock drinking water ponds as well as the relationship between total suspended solids (mg/L) and (E) % inorganic or (F) % organic material in the total suspended solids. Data are plotted on log-log scales. Grey boxes indicate sites without detectable microcystin, a cyanobacterial hepatotoxin, that were included in the statistical analyses. Quadratic regression fit the data better for (A) total phosphorus ($F = 35.55$, $R^2 = 0.658$, $P < 0.000001$) and (B) total suspended solids ($F = 6.004$, $R^2 = 0.245$, $P = 0.0052$) while linear regression fit the data better for (C) inorganic suspended solids ($F = 1.79$, $R^2 = 0.045$, $P = 0.188$), (D) organic suspended solids ($F = 33.43$, $R^2 = 0.468$, $P < 0.00001$), (E) % inorganic material in the total suspended solids ($F = 13.81$, $R^2 = 0.267$, $P = 0.00065$), and (F) % organic material in the total suspended solids ($F = 65.09$, $R^2 = 0.631$, $P < 0.0000001$). Linear regression also fit the data as well as quadratic regression for (A) total nitrogen ($F = 61.33$, $R^2 = 0.630$, $P < 0.000001$) and was chosen since it is a simpler model.

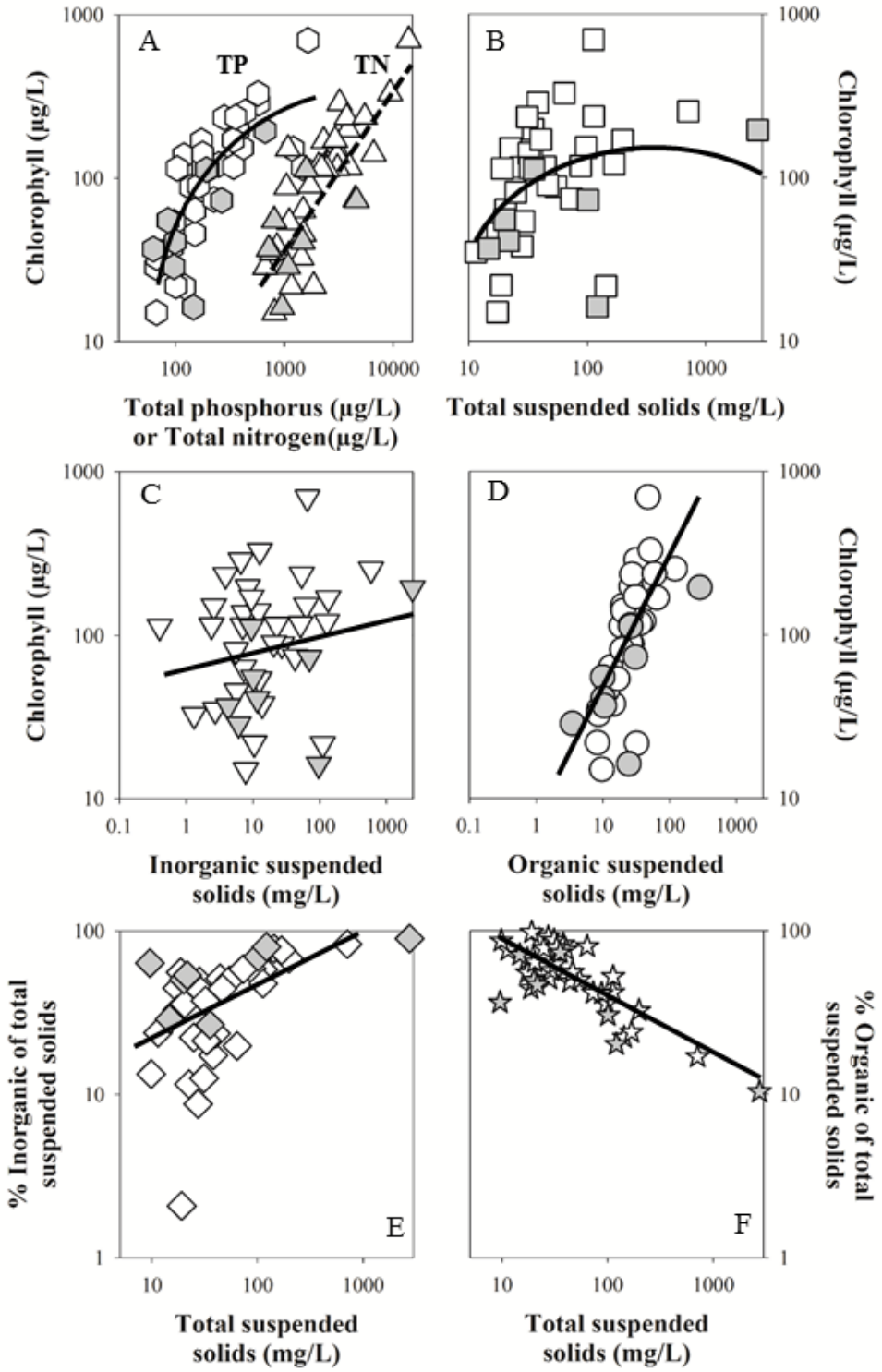
Figure 2. Photos of livestock in two different drinking water ponds. Note the high sediment turbidity in the upper panel and algal scum near the pond edge in the lower panel.

Figure 3. The relationship between microcystin concentration and (A) chlorophyll *a* concentration (µg/L), (B) total suspended solids (mg/L), (C) total phosphorus (hexagons) and total nitrogen (triangles), or (D) total nitrogen-to-total phosphorus (N:P; by weight) for livestock drinking water ponds. Data are plotted on log-log scales. Grey symbols indicate sites without

detectable microcystin that were not included in the statistical analyses but are shown for visualization purposes. Quadratic regression fit the data better for (A) chlorophyll (F = 2.23, R^2 = 0.129, P = 0.129), (C) total phosphorus (F = 2.718, R^2 = 0.158, P = 0.083), and (C) total nitrogen (F = 3.745, R^2 = 0.211, P = 0.036) while linear regression fit the data better for (B) total suspended solids (F = 0.215, R^2 = 0.0071, P = 0.646) and (D) N:P (F = 8.499, R^2 = 0.227, P = 0.0068),

Figure 4. Principal components analysis (PCA) factor map based on the following livestock drinking water pond parameters, chlorophyll (µg/L), microcystin (µg/L), total suspended solids (mg/L), inorganic suspended solids (mg/L), organic suspended solids (mg/L), total phosphorus (µg/L), total nitrogen (µg/L), and total nitrogen-to-total phosphorus (N:P; by weight). Arrow lengths and directions are indicative of parameter weight for each principal component. All data were log-transformed prior to analysis. Percentage of error explained by each principal component is provided in parentheses along each axis.

467 *Figure 1*

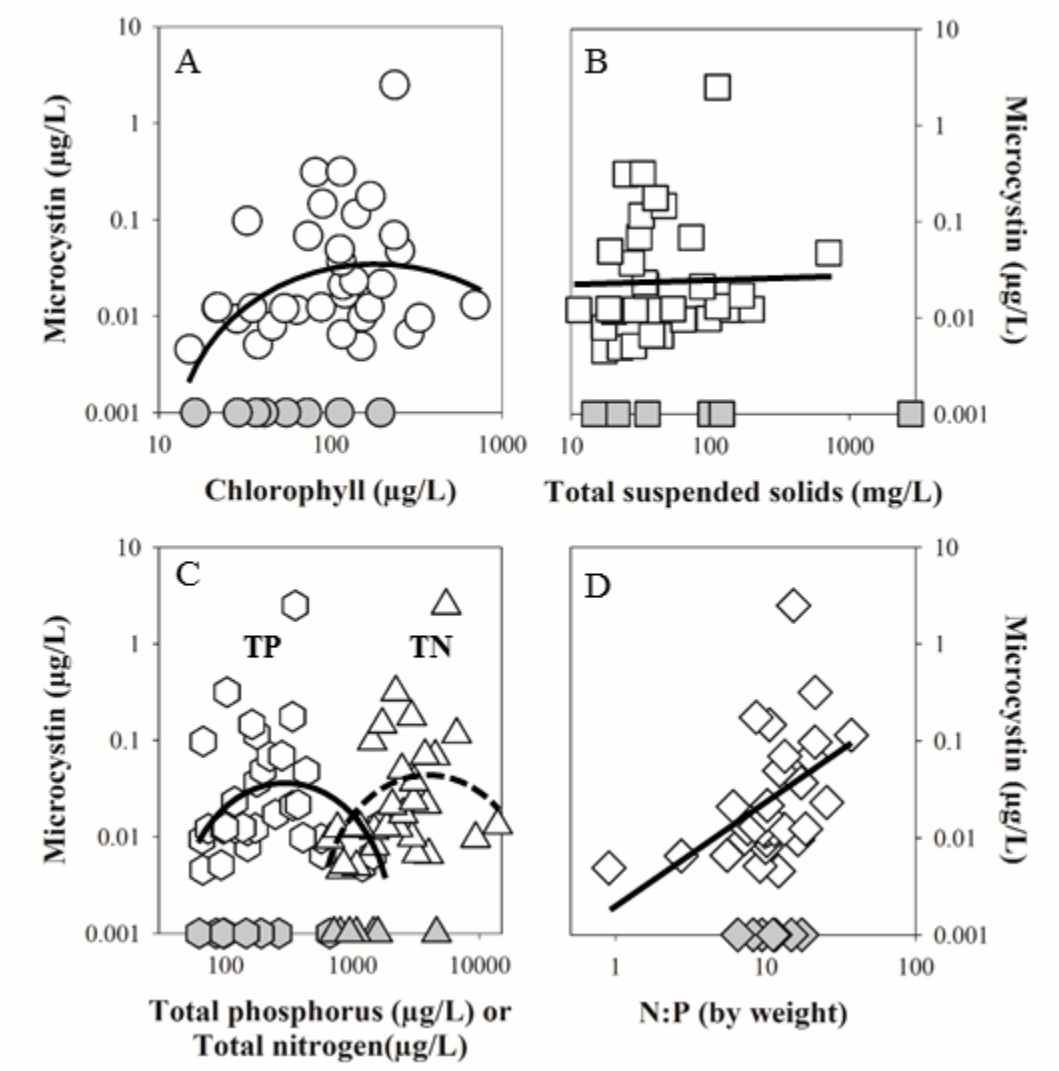


468

469

Figure 2

474 *Figure 3*

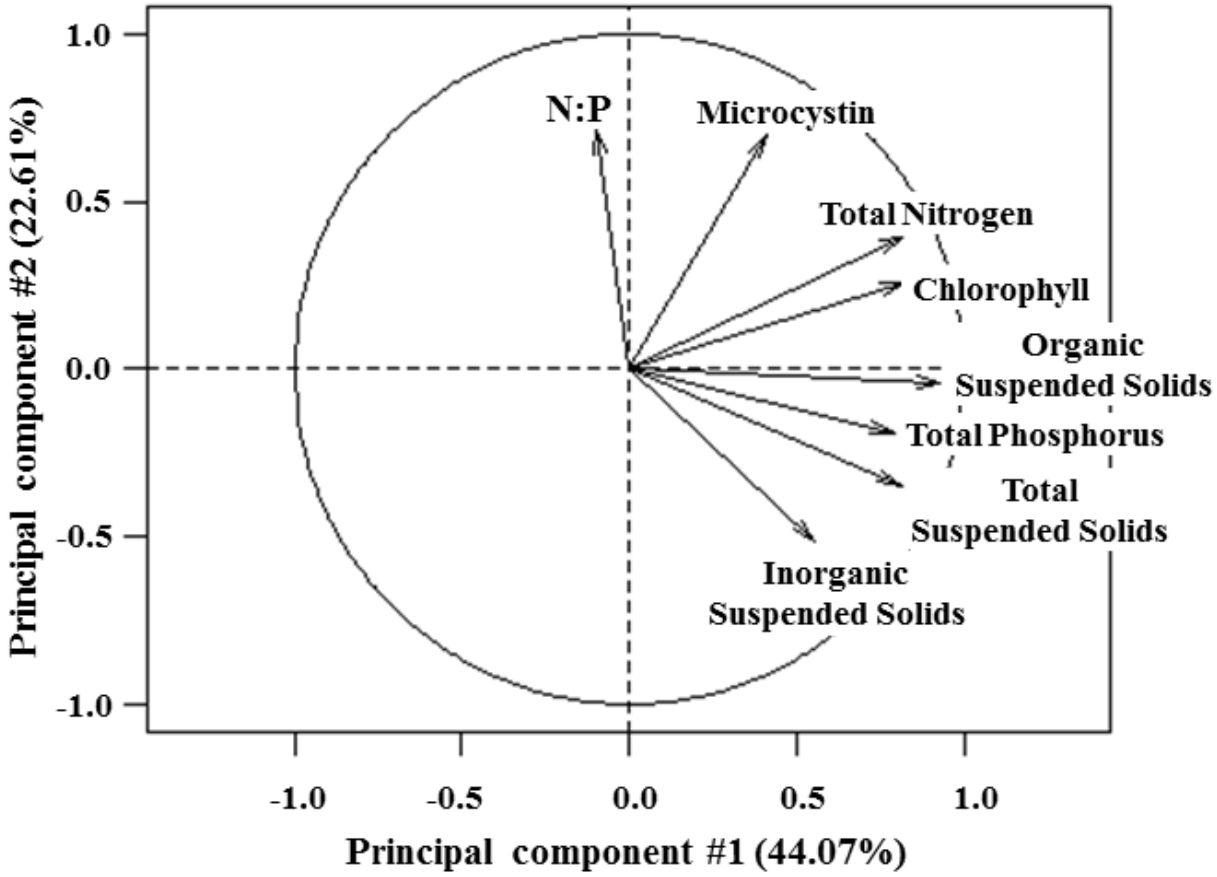


475

476

477 *Figure 4*

478



479