HARMFUL ALGAL BLOOMS AND TOXIN PRODUCTION IN GEORGIA PONDS

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Abstract. Cyanobacterial toxins have been implicated in fish, wildlife, livestock and human mortality events. Cyanobacteria blooms, which often produce toxins, are exacerbated by hot, dry weather. The majority of Georgia, with the exception of the coastal Plain and the Northwestern corner, is currently in severe to exceptional drought conditions. In May 2012 we began receiving reports of livestock deaths associated with algal blooms. Based on clinical signs and algal screening, we documented four cattle deaths at one central Georgia pond with a dense *Microcystis aeruginosa* bloom (4.4 x10^6 cells/mL) and microcystin (i.e. algal produced toxin) concentrations in excess of 142 ppb. Since this incident, we received and screened numerous water samples from livestock drinking water ponds throughout the state. We documented cyanoblooms, predominantly *Microcystis aeruginosa*, in the majority of ponds screened (11/14) and microcystins were present in the majority of samples screened for toxin (7/9). The livestock deaths have highlighted an important issue for Georgia farmers and pond owners that will likely be increasingly prevalent under projected climatic models. We have continued our outreach effort by establishing an official algal screening and cyanotoxin testing service through the existing Agricultural and Environmental Services Laboratories at UGA. This testing service will enable us to better serve the citizens of our state and provide a platform to disseminate information aimed at improving water resource management.

INTRODUCTION

Excessive nutrient enrichment in watersheds can create harmful algal blooms (HABs) in aquatic systems, including ponds, which are frequently used for livestock drinking water and irrigation reserves. HABs are often characterized by exponential growth of planktonic cyanobacteria (commonly referred to as "blue green algae") which produce many odorous or bioactive secondary metabolites (Carmichael 1992). Cyanobacteria have many specialized adaptations that enable them to exist and flourish in environs that are inhospitable to most organisms. For instance, cyanobacteria are capable of regulating their position in the water column to access nutrients and prevent photooxidative damage, as well as fix nitrogen (Walsby and Booker, 1980; Klemer et al., 1996). Members of this phylum can store abundant limiting nutrients, such as phosphorus, thus providing them an additional competitive advantage over green algae (Stewart 1967). Nutrient loading, increased hydraulic retention time, and moderate to high temperatures, which frequently co-occur in Georgia farm ponds, create an ideal environment for these cyanobacteria to proliferate (Wicks and Thiel, 1990). Formation and persistence of a HAB can negatively affect livestock health and the health of other organisms, including humans, utilizing the water resource.

Cyanotoxins, which include cytotoxins, dermatoxins, neurotoxins, and hepatotoxins, vary in chemical structure, threshold dose and ultimate physiologic effect(s) (Sivonen and Jones 1999). Acute exposure to cyanotoxins can cause death (often very rapidly, typically via respiratory failure or hepatic injury). Microcystins (MCs), which include over 70 structural variants, are the most commonly implicated cyanotoxins in human and animal poisonings (Jochimsen et al. 1998, and references therein).

Pet and livestock exposures to HABs may be enhanced by both cyanobacterial characteristics and animal behavior (van de Mere et al. 2012). Gas vacuoles within cyanobacterial cells increase buoyancy leading to development of thick surface scums and accumulations on the leeward edge of waterbodies. These accumulations form mats or dried crusts which are consumed, in some instances intentionally and/or preferentially, by animals (Lopez and Costas 1999). Other animal behaviors, such as coat grooming and licking or spending extended time in the water (i.e. to "cool off") may increase exposure to cyanotoxins.

Clinical signs of HAB exposure vary depending on which toxin the animal ingests. Animals exposed to toxic doses of MCs, which are potent hepatotoxins, may exhibit pale mucous membranes, shock, and bloody diarrhea, and can die within a few hours following exposure. Postmortem examination in animals with suspected MCs toxicosis is characterized by hepatic enlargement, intrahepatic haemorrhage and dissocation and later necrosis of hepatocytes (Puschner et al. 1998; Beasley et al. 1989).

Although a definitive diagnosis is possible, proper tissue and environmental samples must be taken within an

appropriate timeframe to accurately assess species composition and toxin and/or to process tissues for histology or other analyses. Early identification of an HAB is also critical for farmers so animals can be removed from the contaminated water source. Here we detail a HAB-related cattle mortality investigation and identify and address important shortcomings in this process.

METHODS

Field investigation. In May 2012 our laboratory received a report of cattle deaths potentially associated with a HAB in a Dacula, Georgia farm pond (33°56'20.48"N, 83°49'53.65"W). Two cows were found dead 20 May 2012 and a third cow was recumbent with bloody diarrhea; this animal died the following day. The remaining five cows were then confined in a different pasture and watered with well-water via trough. A fourth cow, which had been in the pasture with the pond, began displaying similar clinical signs (recumbency, bloody diarrhea, anorexia) and was moved to a paddock. Oral antibiotics were administered and the animal continued to lose weight [~22.7 kg (50 lbs)] and died 3 weeks post-exposure (pers comm. Troy Pickerel, DVM).

During the initial visit, the attending veterinarian noted the pond appeared "grass green" and suggested the farmer contact a UGA extension agent to facilitate getting the water evaluated for algal toxin(s) (pers comm. Troy Pickerel, DVM). We were contacted by Dr. Lawton Stewart, UGA (Animal and Dairy Science Dept) and visited the property 30 May 2012 to collect water samples for screening.

The 2.4 ha (5.9 acre) pond is approximately 40 years old and is a typical watershed farm pond with an earthen dam. The pond has an average depth of 1.2 m (3.9 ft) with eroded banks. Little herbaceous riparian vegetation was present besides mixed forage grasses (*Cynodon sp.* and *Paspalum sp.*) and some emergent aquatic plants, Alligator weed (*Alternanthera philoxeroides*) and water primrose (*Ludwigia hexapetala*) along the shoreline. The entire pond was opaque and bright green in color.

Species determination. We conducted identification and abundance estimates ≤2h post collection or receipt of the sample(s). Algae were identified morphologically to genus and species according to Prescott (1964). Using brightfield microscopy, we performed identifications and abundance estimates with a hemocytometer.

Toxin analysis. During our initial visit to the Dacula pond (30 May) we evaluated pond water on site for MCs with a commercially available field test kit (1 ppb Source Drinking Water with QuikLyse™, www.abraxiskits.com). The bloom persisted so an additional sample was collected 13 June and shipped on ice overnight to Clemson University for toxin analysis using an ELISA test kit (Quantiplate™ Kit for Microcystins, EnviroLogix™, www.envirologix.com, Envirologix QuantiPlate EP022; Molecular Devices, Spectromax M2).

Additional sites. Due to coverage of the above event by both television and print media, our laboratory began receiving unsolicited water samples from ponds across the state. Sample collection protocol and field conditions varied widely among samples. All samples were from pond owners that either used their pond to water cattle (10/14), for recreational fishing (3/14), or both (1/14). The pond owners were concerned about cyanotoxins and therefore we simply evaluated the samples using microscopy for the presence of toxigenic species. If toxigenic species were noted, we tested for MC presence with a commercially available field test kit.

RESULTS

Species identification. *Microcystis* colonies (*Microcystis aeruginosa*) dominated the Dacula pond sample (4.40E+06 cells/mL) and a few cells of *Scenedesmus* sp. were present (Table 1).

Table 1. Pond water screening results from multiple sites throughout Georgia**; samples**, received from May 2012-September 2012.

County	Primary use	Toxigenic Species*	Toxin	Suspected cattle poisoning **
Dade	Cattle watering	*Microcystis aeruginosa*	No	2 dead*
Emanuel	Recreational fishing	*M.aeruginosa*	Yes	
Grady	Cattle watering	none	–	8 dead*
Gwinnett	Cattle watering	*M.aeruginosa*	Yes	4 dead
Gwinnett	Cattle watering	*M.aeruginosa*	Yes	
Gwinnett	Cattle watering	*M.aeruginosa*	Yes	
Gwinnett	Cattle watering	*M.aeruginosa*	Yes	
Gwinnett	Cattle watering	none	–	
Gwinnett	Both	*M.aeruginosa*	Yes	
Gwinnett	Recreational fishing	*M.aeruginosa*	Not screened	
Habersham	Cattle watering	None	–	sick
Johnson	Cattle watering	none	–	4 dead*
Morgan	Recreational fishing	none	–	
Spaulding	Cattle watering	*M.aeruginosa, Aphanizomenon flos-aquae*	No	2 sick

*Toxigenic species noted were all at bloom level (>1x106cells/mL)
**Suspected cattle poisonings: Dead and sick cattle were reported by owners. Tissue samples were either not processed for histology (3/4) or were inconclusive. Clinical signs, lack of gross lesions, presence of toxigenic cyanobacterial species and/or toxin suggest algal toxicosis reasonable diagnosis or contributing factor.

Toxin analysis. The field test kit indicated the Dacula pond water sample collected 30 May 2012 contained microcystins >5ppb, the upper limit of the test strip. Analysis of the 13 June 2012 sample sent to Clemson University contained 142 ppb of MCs.

Additional sites. The majority of samples were received from regions experiencing extreme drought (Figure 1). Seven (7/14) samples were shipped to our laboratory and one (Grady County) had been previously frozen, making species identification difficult. We identified toxigenic cyanobacteria in five (5/7) samples but only detected MCs in one of the shipped samples (Table 1). All Gwinnett County (7/14) samples were either directly delivered to the laboratory or collected by laboratory personnel. We identified *M. aeruginosa* in six (6/7) samples and detected MCs in all samples (5/5) that were screened for this toxin (Table 11). One sample with a *M. aeruginosa* bloom was not screened for toxin because our test kit supply had been depleted.

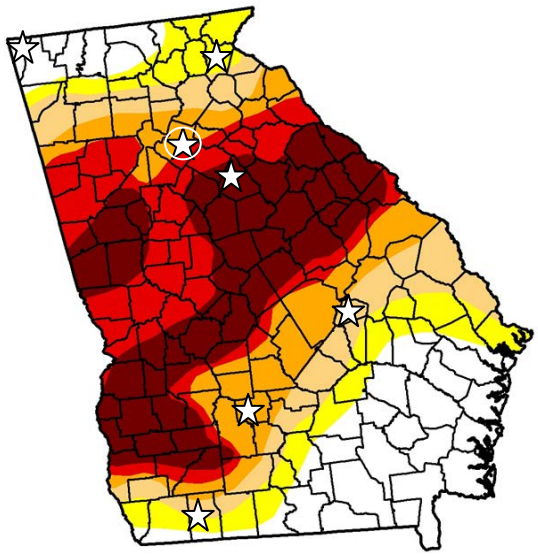

Figure 1. The majority (13/14) of pond water samples we received and screened for toxigenic cyanobacteria species were from areas experiencing some form of drought as delineated by the USDA drought monitor (USDA 2012). The circled star denotes Gwinnett county: we received and screened multiple (n=6) samples from this area.

Prior to these events the University or state government did not offer an affordable publicly available service for algal identification and toxin screening. Through collaboration with UGA's existing testing services performed at the Soil, Plant and Water Laboratory and the Feed and Environmental Water Laboratory, we developed such a service that became available to the public in February 2013. This effort included a detailed outreach letter to extension agents, sampling protocol, and materials for water sample collection and shipping. This screening service is now available for a fee of $30.00 for algal identification and $45.00 for toxin analysis (and species identification). The submitter will receive an electronic report with results, interpretation, and recommendations.

DISCUSSION

Excess phosphorus availability is a major factor driving cyanobacterial growth rates and can increase toxin production by these species (Sivonen and Jones 1999). Over 60% of Georgia cattle farms are small (1-99 acres) family owned operations and their fertilization practices are not regulated. Therefore, best management practices, which include soil testing to determine the appropriate type and fertilization rate, may not be followed (USDA: Economic Research Service, 2011). Watering livestock in ponds is a common practice and is an alternative to in-creek or trough watering. It is likely to become more common in light of recent well permit issue restrictions in drought-stricken Southwest Georgia (Turner-Georgia EPD, 2012). These factors, coupled with the availability of poultry litter (a common source of inexpensive fertilizer), can result in high levels of soil and sediment phosphorus. Farm ponds serve as a catch basin for allochthonous nutrient inputs from pasture fertilization and animal waste. During rain events or via direct input nutrients are deposited into the ponds and can promote HAB formation.

Rapid diagnosis and/or toxin identification is necessary for farms to prevent additional animal deaths. Many samples we received this summer had little to no site characteristics data and had been collected several days prior to shipment. Microbial activity and photolysis are the primary means of MC degradation (Lawton et al. 2011). An expedited system to process these samples is now in place and will likely increase our detection probability and allow us to give farmers this critical warning.

It is important to recognize that exposure to cyanotoxins may not be limited to warm spring and summer conditions. Four heifers died after drinking from a pasture pond with a dense *Microcystis* "bloom" in Michigan in mid-October when temperatures ranged from 37°C (mid-50s°F) to 0°C (30°F) (Fitzgerald and Poppenga 1993). The fundamental Dacula pond *Microcystis* "bloom" (Gwinnett County) persisted from May until late November when average air temperatures ranged from 18.3°C (65°F) to 4.4°C (40°F) (Bartelme unpublished data). Even slight increases in global temperatures due to climate

change may increase the likelihood that Georgia, and other Southern states, will have favorable conditions to promote HABs year round. Therefore, cyanotoxin poisoning should be considered during pet and livestock diagnostic investigations without consideration of seasonality.

This report serves to document a livestock mortality event following an exposure to MCs. The media attention this case received generated concern and provided a platform to educate farmers and pond owners about the risks of livestock and pet HAB exposure. With a direct avenue and outreach effort now in place, we hope to effectively assist and educate Georgia pond owners and livestock producers with cyanotoxin concerns in a timely manner.

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