Chapter 8: Occurrence Workgroup Poster Abstracts

Delaware's Experience with Cyanobacteria in Freshwater Ponds

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Introduction

With the increased emphasis on HABs in estuarine environments and their potential to impact natural resources and human health, the State DNREC in 2001 initiated a program to evaluate commercially available Microcystin kits and to measure Microcystin concentrations in select freshwater ponds. Although no environmental or human health impacts associated with blue-green algal blooms have been reported, Delaware does have a large number of private and public freshwater ponds which are accessible to humans, domestic animals, and wildlife.

A Standard Operating Procedure was developed for the EnviroLogix analysis tools in order to insure accurate repeatable results. The EnviroLogix kits are based on the presence of Microcystin LR in combination with Microcystin LA, Microcystin RR, Microcystin YR and Nodularin.

Hypothesis

Since records, compiled by the State of Delaware Division of Fish and Wildlife, indicate that 14 out of 38 ponds have historically exhibited blue-green algae blooms, some of which included extensive masses of scum; it appeared possible that Microcystin concentrations could be elevated during bloom events. Since hepato-toxins produced by blue-green algae *Microcystis* and *Anabaena* had not been tested previously in Delaware freshwater ponds, it was necessary to collect data in order to demonstrate a need for routine monitoring of these surface waters.

Methods

Surface water samples were collected from 6 freshwater ponds within the State which historically exhibited blue-green algal blooms. Samples were collected adjacent to the shoreline and in surface scum when present. Presence of scum anywhere in the pond on the day of collection was recorded. Ambient water samples were analyzed for the predominance of *Microcystis* and *Anabeana* via light microscopy (100X using a 0.1 Palmer Cell Counter) and for Microcystin concentrations using commercially available Microcystin analysis kits: EnviroLogix Microcystin Tube Kit ET022 (years 2002 & 2003), Strategic Diagnostic EnviroGard Microcystin Plate Kit (2002), and EnviroLogix Microcystin Plate Kit EP022 in conjunction with the Bio Tek µQuant Spectrophotometer Plate Reader Flx800 using KC4 software (2003). Intracellular Microcystin was released by freezing the water samples for a minimum of 24 hours but less than 1 month.

Results

- High concentrations of the organism *Microcystis* is NOT necessarily a good indicator of the level of Total Microcystin in water sample.
- Two out of 88 samples from 9 ponds showed Dissolved Microcystin > 3 ppb and 3 out of 88 showed concentrations > .5 ppb but < 3 ppb using the EnviroLogix Tube Kit. This occurrence was noted in 5 separate ponds all of which were sampled on September 24, 2002.
- Five out of 18 samples analyzed with the EnviroGard Microcystin Plate Kit showed Total Microcystin > 1.00 ppb, this is the provisional upper limit of Microcystin LR established by the World Health Organization for finished drinking water. Samples showing this exceedence (1.28 ppb to 3.28 ppb)

were collected in late September (Sept. 26 and 30, 2002) at 4 separate ponds.

- One out of 33 samples from 10 ponds showed Total Microcystin concentrations > 1 ppb with the EnviroLogix Plate Kit., and 1 out of 33 samples showed Dissolved Microcystin >0.5 but < 3.0 ppb. These results are from late August and mid-September respectively and from 2 separate ponds in 2003.

Conclusions

Surface scum of freshwater ponds showed high concentrations of *Microcystis* 88% of the time, but had measurable levels of Microcystin (>0.5 ppb) only 17% of the time.

Total Microcystin measures both the Microcystin free in the water-column and that sequestered in the blue-green algae cells and as such its determination is recommended if Dissolved Microcystin concentrations approach 1 ppb.

Investigation of microcystin concentrations and possible microcystin–producing organisms in some Florida lakes and fish ponds

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Introduction

Occurrence of potentially toxic cyanobacteria blooms is very common in Florida lakes. These include blooms of *Microcystis, Anabaena, Oscillatoria* and *Cylindrospermopsis*. Among these the first three are potential microcystin producers. However, the toxicity data for many lakes are not available. We investigated 10 lakes, ranging from 4 to 424 acres in Hillsborough County, Tampa, FL over a two month period. In addition we tested samples from Lake George, St. Johns River system and two fish ponds. Here we present the data obtained from microcystin ELISA and PCR with specific primers targeted to the condensation domain of *mcyA* gene, which were designed to detect microcystin–producing *Microcystis, Anabaena* and *Oscillatoria* strains.

Hypothesis

We propose that many of the lakes in Florida that have high cyanobacteria populations will have microcystins.

Methods

Water samples were collected from each lake with a vertical integrating sampling tube. Sub–samples were taken for phytoplankton counts, chlorophyll measurements, ELISA and PCR. ELISA was performed with the Envirologix Microcystin Plate Kit. Primer pairs, mcyA–CD 1F and mcyA–Cd 1R were used in the PCR reaction.

Results

In the case of Hillsborough County lake samples, microcystins were detected in 3 out of 10 lakes. These were Cedar Lake, Lake Brant and a scum sample on the shore of Lake Magdelene. Microcystin concentrations were 0.11, 1.84 and 18.58 $\mu g.l^{-1}$, respectively. ELISA measurements are compared with the results obtained from the PCR analyses. The possible organisms that might be producing microcystins in these samples are discussed.

Conclusion

In addition to taxonomic identification and assessment of population dynamics of cyanobacteria present in water bodies, it is also vital to determine toxin concentrations and toxin producing organisms. ELISA and PCR are two fast and inexpensive methods that can perform these functions.

Potentially toxic cyanobacteria in Chesapeake Bay estuaries and a Virginia lake

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Introduction

Since 1985, phytoplankton populations have been monitored monthly in Chesapeake Bay (U.S.A) and its tidal estuaries. This surveillance has identified 29 potential toxin producing phytoplankters in these waters which includes representative taxa of dinoflagellates, diatoms, cyanobacteria, and raphidophytes (Marshall 1995, Marshall et al. 2005). Although toxic events attributed to these taxa have been rare, their wide spread presence is noted, with evidence for increased seasonal bloom events indicated for several species in our records. Local studies of HAB's in Virginia's regional lakes have been minimal. However, annual blooms of *Microcystis aeruginosa* have occurred annually in the Potomac River, a major tributary of Chesapeake Bay. This species produced a bloom ($>10^6$ cells ml^{-1}) in 2004 that lasted from June through August and included an extensive area of the river, with microcystin levels consistently recorded at >3 ppb. Decomposition products and wind blown algal masses accumulated along the river shore; these conditions and the high microcystin levels temporarily closed recreational usage in some regions of the river.

Results

M. aeruginosa is a common algal component of the tidal rivers in this region, with significant concentrations annually present in the tidal fresh and oligohaline river sections. Its development farther downstream and into Chesapeake Bay increases during the summer months and is enhanced by periods of extended rain and increased river flow. Other potentially toxic cyanobacteria identified in these rivers have included: *Anabaena affinis*, *A. recta, A. solitaria, Aphanizomenon flos-aquae, A. issatschenkoi, Microcystis firma, Planktothrix agardhii, P. limnetica,* and *P. limnetica* f. *acicularis*.. Long term trend analysis from 1985-2004 indicates there are significant increases in biomass and abundance of cyanobacteria within the James, Rappahannock, and York Rivers in Virginia, and as well in Chesapeake Bay. In addition, Lake Burnt Mills, a shallow reservoir of 288 ha located in southeastern Virginia experienced an extensive cyanobacteria bloom in July 2005. This bloom persisted for several days and was produced by *Microcystis aeruginosa* and *M. wesenbergii*, with concentrations of 3.5×10^5 and 22.9×10^5 cell ml^{-1} respectively. During this period microcystin levels near shore exceeded 3 ppb.

Summary

Long term trend analysis of phytoplankton populations in three Virginia rivers and Chesapeake Bay indicate an increase in the abundance of biomass of cyanobacteria has occurred in these waters since 1985. Among these cyanobacteria are populations of potentially toxic species. The most common bloom producing taxon within this group is *Microcystis aeruginosa*. Microcystin concentrations have been associated with these blooms in the Potomac River. Continual monitoring of these rivers and Chesapeake Bay will continue for the presence harmful algal species. In addition, greater surveillance of these bloom producers in regional lakes has taken place and will continue in the future.

Expanding existing harmful algal blooms surveillance systems: canine sentinel

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Introduction

There have been several episodes in the United States in the past several years in which dogs have died after having been exposed to cyanobacterial (blue green algae) blooms. Reports of their deaths preceded any reports of human illness related to exposure to these blooms. Investigations of these as well as other animal deaths have shown them to be related to exposure to cyanobacterial toxins in these blooms. Documented deaths have occurred in both domestic animals (pets and livestock) and in wildlife. Some of the factors that may account for the susceptibility of dogs to illness and death from cyanobacterial toxins may include increased exposure to bloom waters during the summer months and the size of the animals relative to the dose of toxin that they received. While the poisoning and death of these dogs is disheartening, the reporting of canine deaths associated with exposure to bloom waters serves as an important tool for preventing human exposure and for reducing further animal exposure to cyanobacterial toxins.

Hypothesis

The reporting of dead dogs will not detect potentially harmful algal blooms.

Methods

During the past year, the North Carolina Harmful Algal Blooms (HAB) and the Veterinary Public Health Programs coordinated a sentinel surveillance system to detect acute lethal poisonings of dogs. This program encourages practicing veterinarians to voluntarily report any deaths of dogs that they think might be related to exposure to blue green algae blooms. Veterinarians were notified of this program by use of an internet–based " Listserv" communication tool.

Results

Over 800 veterinarians were informed about the rationale for reporting dog deaths and they were provided with an educational flyer to further document and reinforce this message. No dogs deaths have been reported to date. One dead waterfowl incident was reported by a concerned citizen, but the presence of a harmful algal bloom or toxins was not determined.

Conclusions

At this time the HAB and Veterinary Public Health programs are planning to expand the Canine Sentinel Surveillance Program to include additional animals (livestock and wildlife), other state and local public health agencies, and to encourage other states to take part in this program. The North Carolina Cooperative Extension Program is one example of another governmental agency that is being encouraged to participate in this surveillance program. The Canine Sentinel Surveillance Program will be used to help detect occurrences of deaths in animals. This program should also reduce the number of animal poisonings due to algal toxins by increasing public awareness of this potential problem and through preventive measures to reduce the exposure of animals to algal toxins in public recreational waters of North Carolina. The increased public awareness may also reduce the potential for human exposure to algal toxins.

Use of embedded networked sensors for the study of cyanobacterial bloom dynamics

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Introduction

Traditional monitoring techniques are limited in the development of predictive models for aquatic microbial populations that requires very fine spatial and temporal resolution of data. The need for continuous (or real-time remote) monitoring of the environment combined with the desire for directed (intelligent or autonomous) sampling has prompted the development of a sensor network. The network incorporates low-energy demand, and highly adaptable sensors which exploit recent advances in computer networking and robotics to process sensor data and ensure high data fidelity. The coordination of stationary sensor nodes and mobile sensing using a sampling robot allow for efficient collection of samples from features of interest, as exemplified in a recent study of a cyanobacterial bloom in Lake Fulmor, California.

Hypothesis

The application of Embedded Networked Sensing (ENS) technology to monitor cyanobacterial bloom in a lake environment will provide new observational capabilities with unique information on the distribution and/or behaviors of planktonic assemblages.

Methods

The sensor network (NAMOS: Networked Aquatic Microbial Observing System) consists of 10 stationary buoys and one mobile robotic boat. Each buoy is equipped with a computer, sensor suite, and wireless communication. They are networked and communicate with each other and a shore-based station via wireless ethernet. Onboard sensors include a thermistor array for measuring water temperature to 3 m depth and a fluorometer capable of detecting chlorophyll (chl) *a* concentrations from 0.5-500 µg/L. The robotic boat is equipped with similar sensors and processing capabilities in addition to a water sampler capable of taking six 4-ml samples. The robotic boat is autonomously controlled using information obtained from the network.

Results

Over the course of a 4-day NAMOS deployment, the chl *a* concentration showed high temporal variability. Cyclic daily variations in subsurface chl *a* fluorescence were observed with a peak between the hours of midnight and 5 am. During this time period, chl *a* concentrations increased from a day-time average of ≈2.5 µg/L to >6 µg/L. *Spirulina* sp. strongly dominated the phytoplankton community. The sensor network also detailed the spatial distribution of photosynthetic organisms along the length of Lake Fulmor, indicating increased concentrations of chl *a* towards the southwest end of the lake.

Conclusion

The presence of daily variations in chl *a* concentration at all static node stations implies a strong vertical migratory behavior of phytoplankton in the lake, most likely *Spirulina*. Accumulations of chl *a* in the southwest corner of the lake suggest reduced mixing or increased nutrients in this deeper, more protected area. And finally, the ability to resolve this trend at several points along Lake Fulmor and over the course of several days, and the combination of these data with autonomously collected water samples, demonstrates a marked improvement over traditional point sampling techniques.

Bloom and toxin occurrence

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Fresh water is a renewable but a finite and vulnerable resource and essential to sustain life, development and the environment. In fact 97.3 percent of earth's water is saline and 2.7 per cent is fresh water. 70 per cent of this fresh water is frozen in ice caps of Antarctica and green land and balance is present either as soil moisture or underground moisture. As a result less than 1 per cent of all the water on earth is accessible for direct human use. This water is found in lakes, rivers, reservoirs etc. Only this tiny portion of the planet's water is recycled by nature's cycle.

Water is getting scarce due to rising population, rapid urbanization and growing industrial demands. People dump wastes, untreated sewage and chemical discharges, which pollute the sources of water like rivers, lakes, ponds and even underground resources. The most significant issues of water ecosystem is the eutrophication and deteriorating water quality including the development of numerous harmful algal blooms. Significant impacts of these blooms are high biomass, visible surface scums, loss of submerged aquatic vegetation and benthic habitat. Harmful cyanobacterial blooms produce toxins and affect commercial species like fish etc. Non–toxic blooms affect the benthic flora and fauna due to decreased light penetration. These blooms also affect the recreational activities of humans.

Microcystis aeruginosa is the most common cyanobacterial HAB not only in US but also all over the world in fresh, eusturine and marine waters. Other toxic blooms formed by *Lyngbya majuscula, Schozothrix calcicola, Oscillatoria nigroviridis* cause swimmer's itch and these are commonly found in tropical and sub–tropical sea waters. *Anabaena flosaquae, Aphanozomenon flosaquae* are the common cyanobacterial toxic blooms. *Gleotrichea intermedia, Aphanothece gelatinosa, Anabaena iyengarii, Cylindrospermum stagnale, Scytonema javanicum, Scytonema simplex, Oscillatoria princes, Nodularia, Lyngbya martinsiana, Phormidium anomala, Nostoc commune* are the other common toxin producing cyanobacterial blooms.

Cyanobacterial blooms which are toxic in fresh water may not be necessarily toxic marine environment or vice versa. In general toxic cyanobacterial blooms are same or similar all over the world. According to the literature 25 per cent of cyanobacterial blooms produce toxins. Cellular target phytoplankton toxins are ichyotoxins, neurotoxins, hepatotoxins, hemolysins and cytotoxins. Most commonly observed toxins all over the world are:

- Paralytic shellfish poisoning toxins (PSP) – water soluble neurotoxins.
- Amnestic shellfish poisoniong toxins (DSP) –water soluble neurotoxins
- Neurotoxic shellfish poisoning toxins (NSP) _lipid soluble brevetoxins
- Ciguatera fish poisoning toxins (CFP) –lipid soluble heat stable.

These are the most commonly observed toxins in US and all over world in marine and eustarine waters. If the trend of eutrophication continues in the same manner cyanobacterial HABs will increase proportionately and pose a greater threat not only to natural ecosystems but also to the human health. Therefore, there should be legislative actions to ensure that efforts to achieve nutrient reduction and establish a water quality standard.

Public education is one of the major tools other than scientific research in efforts to minimize the impacts of Cyanobacterial HABs and their toxins in marine, eustarine and fresh waters.

Cyanotoxins in the tidewaters of Maryland's Chesapeake Bay: The Maryland Experience

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Introduction

Cyanobacteria blooms were noted in the Potomac River and the upper Chesapeake Bay during the 1950s and 1960s coincident with the invasion of water milfoil. Since 1985, cyanobacteria blooms have been documented in the tidal tributaries of Chesapeake Bay almost annually during summer months by the Maryland Department of Natural Resources (MDNR) long-term comprehensive water quality monitoring program. During September 2000, an extensive late summer bloom of *Microcystis* on the Sassafras River, however, was among the first blooms tested for cyanotoxins in Maryland and results were positive for elevated concentrations of microcystin. The microcystin levels (591.4-1041 $ug*g^{-1}$ dry wt) led to the Kent County Health Department closing a public beach in the bloom area for the remainder of the year, the first beach closure in the history of the state due to detected levels of cyanotoxins.

Hypotheses

We hypothesized that elevated cyanobacterial toxin levels were common features of the annual blooms in the tidal tributaries and multiple toxins would be present. Such findings of an increased diversity of toxic HABs and CyanoHABs would represent expanded management concerns regarding human water-related activities and living resources effects for the Bay.

Methods

From 2002 to 2004, MDNR conducted cyanotoxin surveys working with Dr. Wayne Carmichael (Wright State University, Dayton, OH) and Dr. Greg Boyer (State University of New York College of Environmental Science and Forestry, Syracuse, NY). We examined water samples from tidal regions of the Chesapeake Bay with elevated concentrations (>10,000 cells/ml) of cyanobacteria.

Results

Microcystin, anatoxin-a and saxitoxin were detected from tributaries throughout Maryland tidewaters at wet weight concentrations of 0.34-657.9 ug*L^{-1} (n=40), 0.009-3 ug*L^{-1} (n=6) and 0.003 ug*L^{-1} (n=1), respectively. Mean and median concentration were 35.24 ug*L^{-1} and 5.04 ug*L^{-1} for microcystin and 0.54 ug*L^{-1} and 0.05 ug*L^{-1} for Anatoxin-a. In 100% of *Microcystis* bloom samples tested (concentrations > 10,000 cells*ml^{-1}) there were detections of microcystin. Anatoxin-a and saxitoxin testing has been uncommon.

Conclusions

1. Microcystin concentrations exceeded the WHO drinking water standard of 1 ug*L^{-1} with 85% of test samples. Anatoxin-a and saxitoxin have also been detected in the open waters of the tidal tributaries of the Chesapeake Bay system.
2. The findings increase the range of habitats where potential human health and living resource threats due to aquatic born toxins must be considered by management agencies in Maryland.
3. County health departments again closed beaches in 2003 and 2004 in response to recommendations from Maryland's Interagency Harmful Algae Task Force regarding the detected levels of cyanotoxins. State resource agency efforts to alert the public regarding timing and location of bloom waters as well as potential risks to human health, pets and livestock included 1) HAB webnews articles on the State resource agency websites 2) State Press Releases linked with MDNRs "Eyes on the Bay" water quality monitoring website, and 3) print, radio and TV news coverage of the issues.

Harmful Algal Blooms and Cyanotoxins in Metropolitan Water District's Reservoirs

Izaguirre G

The Metropolitan Water District of Southern California (MWDSC) supplies drinking water to about 18 million people in six counties in the coastal plain of southern California. MWDSC is composed of 26 member agencies, which are cities or regional water agencies. Its two sources of water are the Colorado River and water from northern California, called State Project Water (SPW), delivered through the California Aqueduct. MWDSC operates three reservoirs in Riverside County: Lake Mathews, Lake Skinner and Diamond Valley Lake. The former is the terminal reservoir of the Colorado River Aqueduct; the other two reservoirs are supplied with a blend of the two waters. In addition, the state Department of Water Resources owns and operates Silverwood Lake, Lake Perris and Castaic Lake, three combined drinking water and recreational lakes that receive State Project water. Metropolitan regularly receives water from Castaic Lake (northwest of Los Angeles) and Silverwood (in the San Bernardino Mountains), and occasionally also uses water from Lake Perris.

Metropolitan has a long history of algal problems, in the form of planktonic blooms and benthic proliferations. The main concern is taste and odor, specifically the compounds geosmin and 2-methylisoborneol (MIB), which impart a disagreeable flavor to the water and cannot be easily removed by conventional treatment methods. All of the reservoirs listed above have experienced algal blooms of one kind or another, including in some cases known toxigenic species. In addition, Lakes Mathews, Skinner, Perris, and Diamond Valley have developed benthic mats that have resulted in severe off-flavor problems.

In 1996, an AWWARF project on the occurrence of algal toxins in raw and treated waters in the United States and Canada was initiated, perhaps the first serious effort by the U.S. drinking water industry to assess the extent of the problem. This study showed that at least one type of cyanotoxin, the microcystins, can be found in many water sources in the U.S., sometimes even in treated waters, albeit at low concentrations (Carmichael, 2001).

Metropolitan was a participating utility in this study, and the results indicated that microcystins could be found in cyanobacterial bloom material from various source-water reservoirs, and in the corresponding plant influents and in some cases even the effluents. However, the levels were generally low, and no further monitoring was done on our system until the summer of 2001, when there was a severe bloom of *Aphanizomenon* in Silverwood Lake and of *Microcystis* in Lake Skinner. Samples of bloom material were sent to the laboratory of Dr. Gregory Boyer at SUNY in Syracuse, NY. Two of the *Microcystis* samples had relatively high microcystin levels, while the *Aphanizomenon* samples had no significant levels of any of the tested toxins. These results, though not surprising (the bloom samples giving the highest results were fairly concentrated samples), prompted concern regarding the need for more regular monitoring of these compounds in the water. In view of the likelihood that cyanotoxin monitoring will be required under the UCMR, this concern would appear justified.

The results of the 2001 samples prompted the development of a cyanotoxin monitoring program at Metropolitan's Water Quality Laboratory. This monitoring utilized two ELISA test kits for microcystin (Envirologix Inc., Portland, ME), a plate kit and a tube kit. The former was used primarily for testing water samples, while the latter was used for screening bloom samples, benthic algal samples and cultures. In addition, many samples were sent to Dr. Boyer's lab under a contract with Metropolitan. This was for confirmation of microcystin and identification of the variant. Also, since we are unable to test for toxins other than microcystin, the contract lab was needed to test for these other toxins, e.g., cylindrospermopsin and anatoxin-a.

We now have two years' worth of data on cyanotoxins in our system. Microcystin has been found in varying concentrations in surface water from all six reservoirs that were sampled. The concentrations ranged from 0.116 μg/L to 55.27 μg/L, although most of the samples were closer to the lower end of this range. The highest values were all from samples of concentrated bloom material, usually dominated by *Microcystis*. However, the majority of the water samples tested had no detectable microcystin or were just barely over the detection limit of 0.147 μg/L. The WHO guideline for drinking water is 1.0 μg/L.

In the summer of 2003, we also began testing benthic algal material from the shallows of various reservoirs, and in the process of this testing found that a cyanobacterium that is very common in three of those reservoirs produces microcystin. Eighteen isolates of this organism (a *Phormidium* sp.) were sent to Dr. Boyer's lab for verification, quantitation and identification of the toxin, and twelve of them were confirmed as strong microcystin producers. The variant was microcystin-LR in most cases. In addition, seven sediment samples were analyzed, and some of them had relatively high concentrations of microcystin, the highest being 287.95 μg/g dry wt, and the lowest 1.23 μg/g dry wt. A paper on this work is in the process of being reviewed by two collaborators in preparation for submission to a journal.

The benthic microcystin producer is the only source of the toxin found to date in our system other than *Microcystis*, and may be significant in being a more permanent “inhabitant” than the more transient and seasonal planktonic sources. Moreover, this organism grows intermingled with cyanobacteria that produce odorous compounds like geosmin and 2-methylisoborneol. These benthic proliferations are periodically treated with copper sulfate to control taste-and-odor problems. Application of the algicide to these organisms can in theory release the microcystin into the water, potentially affecting water supplied to several treatment plants.

No other toxins have been found in significant levels in Metropolitan’s waters. Blooms of *Anabaena flos-aquae* and *A. lemmermanii* have been tested for anatoxin (at Dr. Boyer’s lab), but all were negative. However, an *Anabaena* isolated from Castaic Lake in 1999 was found to produce anatoxin-a.