Assessing the Potential for Lyngbya Toxin Production in Lake Gaston

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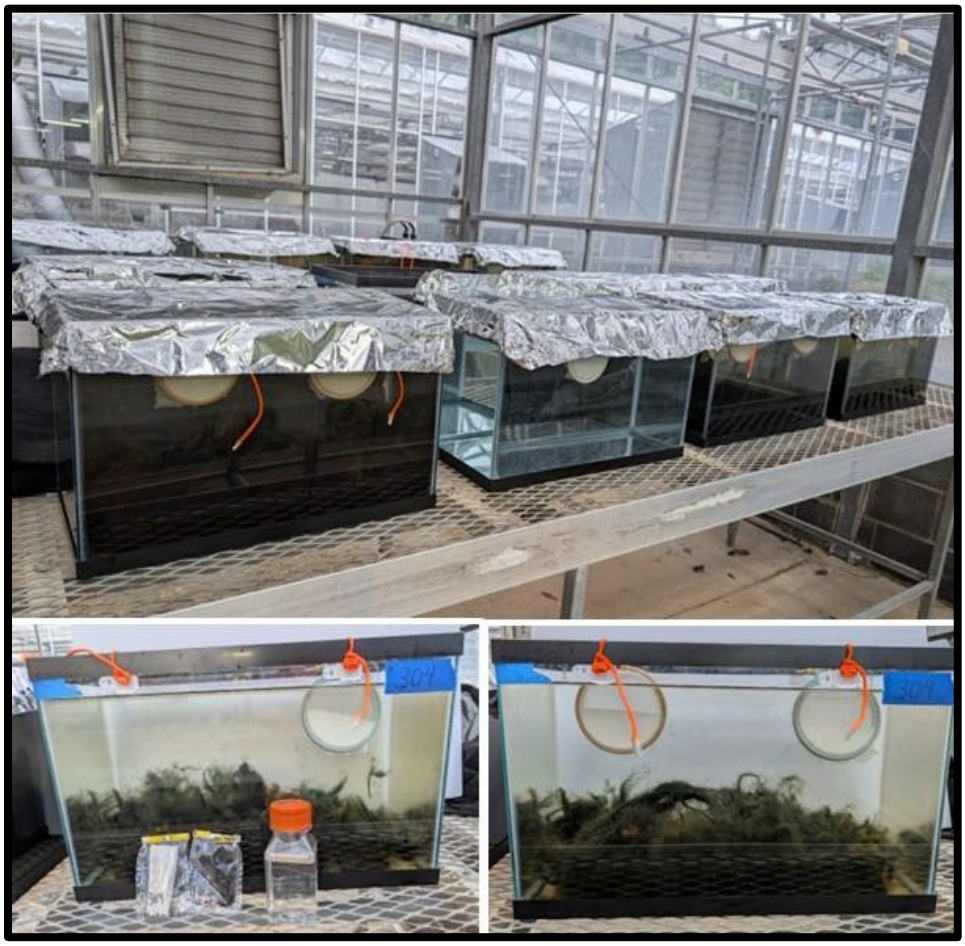
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Executive Summary

Microseira wollei (formerly known as *Lyngbya wollei*) is a filamentous cyanobacterium that is becoming more prevalent in Piedmont Reservoirs. Microseira species have previously been documented to produce numerous toxins in some environments and there is a need to develop monitoring protocols that will help better understand their associated environmental and human health risks. Understanding the dynamics of toxin production and availability, along with the utility of new sampling technologies are fundamental first steps for the development of future Lyngbya toxin monitoring programs.

The objectives of this study were to evaluate potential toxin production within Lake Gaston's Lyngbya population, determine most appropriate toxin sampling methods and understand stress-inducing scenarios that might lead to toxin release. Lyngbya was subjected to treatment types that represented common anthropogenic interactions including physical disturbance from boat propellers and chemical disturbance from algicide treatments. Toxin production and availability were determined by using standard cyanotoxin sampling methods, including water, biomass, and passive gear sampling (Solid Phase Adsorption Toxin Tracking (SPATT)).

Toxins associated with Lyngbya, *Lyngbya wollei* toxins (LTWs 1-6), decarboxylation forms of STX (dcSTX) and GTX 2 and 3 (dcGTX2-3), were all detected using at least one of the sampling methodologies. Biomass samples produced the highest rate of detection. However, these intercellular concentrations may not correlate to ambient toxin availability which is the most common route of human exposure. Of those methods that were utilized to detect ambient toxin availability (SPATT and water), water samples produced the least number of detections, most of which were duplicated in the corresponding SPATT sample. SPATT technology produced the highest rate of detections and included toxins associated with human health risks (LWT-1, -5, and dcSTX). SPATTs were also the only methodology that identified a trend of increased ambient toxins produced by Lyngbya that had been subjected to some form of injury when compared to Lyngbya that was left undisturbed.

During this study three replicates of each treatment were conducted; but, due to the budgetary constraints of sample analysis, only two replicates were analyzed for this report. Within those two replicates, relatively high temporal and spatial variation in toxin concentrations were found and therefore it is recommended that the third replicate of each sample be analyzed for cyanotoxins. We expect that these results will help address variation in the data and provide some clarity regarding temporal trends in toxin production and release following stress-inducing scenarios. These additional results will also provide the level of scientific rigor necessary for publication in a top-tier peer-reviewed scientific journal.

Overall, this study provided a better understanding of the composition, appropriate sampling methodology, and ambient release of toxins found in *Microseira wollei* in Lake Gaston. It was also successful in identifying novel sampling methods that have yet to be reported regarding detecting *Lyngbya wollei* toxins (LWTs 1-6). These findings are significant to the development of future studies addressing Lyngbya associated toxins in larger and more dynamic environments such as Lake Gaston and will assist managers in identifying appropriate protocols for future toxin monitoring programs.

Introduction

Microseira wollei (formerly known as *Lyngbya wollei* and referred to as Lyngbya in this report)) is a filamentous cyanobacteria that grows in dense mat-like formations along the benthos of freshwater environments (Foss et all 2012; Smith 2019). Blooms of this cyanobacterium have been associated with a host of environmental and human health-related risks throughout the Southeast, including the potential production and release of cyanotoxins (Meira et al. 2026). Most research associated with human health impacts related to cyanotoxins produced by Lyngbya focus on the marine species *Lyngbya majuscule,* a species in which negative impacts to human health have been reported on since the 1950's (Meira et al. 2026). Research directed at toxins produced by *Microseira wollei* has focused on detection, concentration, and environmental drivers of production (Foss et al. 2012; Hudon et al. 2016; Metz et al. 2022; Meira et al. 2026), but has left a data gap regarding potential human health risks.

Saxitoxins (STX) are highly toxic members of the Paralytic Shellfish Toxin (PSTs) family, a group of naturally occurring neurotoxins that can be produced by select cyanobacteria species (Genenah 1981; Cusick and Sayler 2013). There are over 50 known natural saxitoxin analogs, including neurosaxitoxin (neoSTX), gonyautoxins (GTX1-6), C-toxins, and decarbamylated forms of STX, neoSTX, and GTX (dcSTX; dcneoSTX; and dcGTX1-6) (Wiese 2010). Several of these STX derivatives are associated with Lyngbya in freshwater environments, including decarbamoylgonyautoxins 2 & 3 (dcGTX2 and dcGTX3), decarbamoylsaxitoxin (dcSTX), and six novel saxitoxin analogs (*Lyngbya wollei* toxins (LWTs 1-6)) (Onodera et al. 1997, Foss et al. 2012; Smith 2019). Each of these toxins may pose distinct hazards to human health, including neurotoxicity (STX) and dermatotoxicity (LWTs). However, the degree of toxicity varies among these analogs and derivatives (Genenah 1981; Cusick and Sayler 2013).

While specific health-related hazards have not been associated with all six *Lyngbya wollei* toxins, they appear to have a lower toxic potential compared to other toxin groups produced by Lyngbya thereby presenting a lower risk to human health (Foss et al. 2012; Poirier-Larablie et al. 2020). Against a standard mouse assay, individual LWTs ranged from nontoxic (LWTs 1, 4, and 6) to the most toxic of the LWTs (LWT-5) (Onodera et al. 1997; Foss et al. 2012). Onodera et al. (1997) found that LWT-5 produced similar mortality results as STX in a standard mouse assay but had 14% the relative potency when compared to its STX carbamoyl counterpart. However, environmental conditions (increased pH and temperature) have been found to disrupt the stability of LWTs resulting in degradation to other STX derivatives (Metz et al. 2022). Degradation of LWT-5 to dcSTX is of particular concern due to the resulting increased toxicity potential of this biotransformation. In fact, when compared to STX, dcSTX has 50% the relative potency which can increase net toxicity of the surrounding environments by a factor of 4 (Onodera et al. 1997; Metz et al. 2022). Although LWT-1 was found to not induce mortality in standard mouse assay, it is the most abundant toxin produced by Lyngbya and has a dermatotoxicity that has been linked to dermal reactions (Hudon et al. 2016; Poirier-Larablie et al. 2020).

Like other cyanobacteria, the presence of Lyngbya biomass does not inherently indicate toxin production and/or release (Foss et al. 2012). Previous studies focused on *Lyngbya wollei* toxins have determined the presence and concentration of toxins through the breakdown of Lyngbya biomass (Foss et al. 2012; Hudon et al. 2016; Poirier-Larabie et al. 2020; Metz et al. 2022). However, cyanobacterial toxins compartmentalize between intercellular (particulate) and extracellular (soluble) forms, and the ambient release of toxins from inter- to extracellular has been associated with physical breakdown or lysis of the cellular structures (Codd et al. 2010). Therefore, neither the presence of toxins within

Lyngbya filaments nor their associated concentrations may accurately reflect conditions in the surrounding aquatic environment.

Standard cyanotoxin monitoring programs routinely utilize discrete water samples to determine current ambient toxin concentrations within a water body. These concentrations are then directly used to determine appropriate advisory types and levels regarding any water use restrictions (Virginia Department of Health, 2025). Due to the challenges of temporal and spatial heterogeneity related to toxin release, some monitoring programs have begun incorporating Solid Phase Adsorption Toxin Tracking (SPATT) technology to better understand toxin dynamics (Roué et al. 2018). This technology uses a porous resin to passively absorb ambient toxins present in the water column (MacKenzie 2010). When combined with discrete samples, SPATT technology can help facilitate an integrated approach to monitoring that enables managers to detect the presence of toxins, even when concentrations are below detection limits at the time of water sample collection (Roué et al. 2018). Successful toxin absorption depends on the type of resin used, the length of deployment, and environmental conditions. Although SPATT technology can not universally detect all cyanotoxins, it has been proven to successfully detect Paralytic Shellfish Poisoning (PSP) toxins (Lane et al. 2010; Rodriguez et al. 2011). It is important to note that the successfully detected ambient toxins originated from planktonic cyanobacteria, and their application to *Lyngbya wollei* toxins has yet to be reported.

There is a need to develop monitoring protocols that will help better understand environmental and human health risks associated with *Microseira wollei* (Lyngbya) freshwater environments (Meira et al. 2026). Understanding the dynamics between intra- and extracellular toxin availability and the utility of new sampling technologies to monitor *Microseira wollei* (Lyngbya) toxin releases is a fundamental first step for the development of future monitoring programs.

The objectives of this project were to 1) characterize potential toxin production within Lake Gaston's Lyngbya population, 2) understand the relationship between standard toxin sampling methods and the detection of Lyngbya toxins, and 3) understand the temporal timeline of toxin release following stress-inducing scenarios.

Methods

In September 2025, fresh Lyngbya biomass was collected from a single site location (Sledge Creek) at Lake Gaston, NC/VA, and transported to North Carolina State University (NCSU) for establishment in a mesocosm environment under controlled greenhouse conditions (Figure 1). Lyngbya (600 g) was randomly placed into 12 10-gallon glass aquaria, filled with reconstituted soft water (ASTM International 2022; 30 L), and covered with aluminum foil to prevent evaporation and cross-contamination. After an establishment period (48 hrs), representative samples were collected from each lyngbya mat (n=9), and cellular viability measurements were determined by visual observation under 40x magnification, to ensure that the condition of the mat material exceeded 90%. Lyngbya was subjected to three treatment types that represented common anthropogenic interactions in a randomized block design: a) undisturbed, b) injury by emulsification of filament walls (i.e. boat propeller), or c) injury by lysis of viable cells (i.e. algaecide treatment) (Figure 2). Emulsification of Lyngbya was standardized across treatment aquaria (n=3) using three 3-second pulses with an emersion blender to physically break filament walls. To replicate Lyngbya mortality and lysis of viable cells during chemical treatment, copper-based algaecide and surfactant (Captain XTR® and AMP®) were administered at the maximum label rate

of 1 mg/L Cu to each aquarium in this treatment (n=3). To ensure that any potential toxin release was associated with the presence of Lyngbya, reference aqauria that only contained soft water without the addition of Lyngbya were included as a negative control (n=3). The final treatment included aquaria (n=3) in which Lyngbya was added to ASTM soft water and left undisturbed. The test began on September 29, 2025, following the establishment period. Baseline samples (T:0) were collected before any administered treatments; additional samples were collected at 48 hours after treatment (48 HAT), 10 days after treatment (10 DAT), and 3 weeks after treatment (3 WAT). Three replicates of each treatment were conducted; however, due to the budgetary constraints of sample analysis, only two replicates were analyzed for this report.

Samples taken for toxin analysis, included water, biomass, and passive gear sampling (SPATT), and were used to assess potential toxin production and availability (Figure 3). For ambient water grab samples, sterile PETG media bottles were utilized to collect 250 ml of surface water. Freshly prepared ASTM soft water (250 ml) was added back to the aquaria to maintain 30 L of water throughout the experiment. For biomass analysis, 10 grams of Lyngbya (excess water removed by light pressure with gloved hands) was subsampled and placed in sterile sampling bags. These samples were drawn from multiple locations throughout the established "mat" to account for variability within the biomass. For passive gear collections, a series of SPATTS were suspended at the top of each aquarium (Figure 1). For the physical and chemical treatments, two SPATTS were suspended simultaneously in all aquaria and collected at 48 HAT and 10 DAT (independently). At 10 DAT, a third SPATT was freshly suspended in the aquaria from 10 DAT to 3 WAT. SPATTS were constructed using embroidery hoops to encase 3 g (dry weight) of Supelco HP20 Diaion Resin in 100 um Nitex bolting cloth.

All samples were frozen post-collection and stored at -20 °C prior to being shipped to GreenWater Laboratories in Palatka, FL for toxin analysis. Their analysis was performed on all samples using LC-MS/MS for the Saxitoxin Suite (including C1/C2, GTX 1 – 6, dcGTX2/3, dcSTX, NEO, and STX) and *Lyngbya wollei* toxins (LTW 1-6).

Results

A total of 76 samples (water, SPATT, and biomass) were analyzed for the standard saxitoxin suite and *Lyngbya wollei* toxins. There was no detection of STX or its natural analogues neurosaxitoxin (neoSTX), gonyautoxins (GTX1-6), C-toxins, or decarboxylaized neurotoxin (dcNEO) reported for any sample. Toxins associated with Lyngbya, *Lyngbya wollei* toxins (LTWs 1-6), decarboxylation forms of STX (dcSTX) and GTX 2 and 3 (dcGTX2-3), were all detected in a minimum of one sample. Biomass samples produced the greatest frequency of detections, with all expected Lyngbya-related toxins reported (LWTs 1-6, dcSTX, dcGTX2-3), and 100% of samples had at least one positive toxin detection (Table 1). SPATT technology had the second-highest rate of detection (72%), but dcGTX2-3 and LWTs-2,3 were not reported utilizing this sampling method (Table 1). Water samples collected post baseline (T:0) produced the least number of detections, with a total of 6 (33%) samples testing positive for the following toxins (LWT-1, 5, and 6, and dcSTX) (Table 1). Prior to treatments, there were no toxins detected within any of the water samples. Due to relative variability in detection rates and concentrations, the two replicates were reported independently of one another (Table 1; Figures 4-6).

Overall, ambient detections (SPATT and water) of cyanotoxins increased after Lyngbya was subjected to an injury-inducing event (emulsification or algaecide treatment) (Table 1; Figure 4-6). Release of toxins

was not observed in undisturbed Lyngbya at any time point, except for LWT-6 at 48 HAT and 10 DAT (Table 1; Figures 4-6). For Lyngbya subjected to injury, toxins were observed at all time periods (48 HAT, 10 DAT, and 3 WAT), but temporal release was dependent on the injury type (Table 1). Utilizing SPATT technology, at 48 HAT, a single toxin detection (LWT-6) was reported in emulsified Lyngbya whereas multiple LWTs (1, 4, 5, and 6) were reported for algaecide-treated Lyngbya. At 10 DAT, ambient detections of dcSTX and LWT-1, 4, 5, 6 were observed for both emulsified and treated Lyngbya (Table 1). At 3 WAT, LWT-1, and -6 were detected for each injury type, but LWT-4 was only reported for algaecide-treated Lyngbya. Water samples corroborated these findings, with 5 out of 6 total samples indicating simultaneous detections with the corresponding SPATT, and it is worth noting that all of these occurred with Lyngbya that was emulsified (Table 1). At 10 DAT, LWT-1, 5, 6, and dcSTX toxins were reported for both SPATT and water samples, and at 3 WAT the same simultaneous findings were reported for LWT-1. dcSTX at 3 WAT was the only toxin in which detection was reported within the water sample and absent within the SPATT.

Among the LWTs, LWT-2 and -3 had the least number of detections, were the only two LWTs not found in all biomass samples, and neither were detected using ambient sampling methods (Table 1). Replicates 1 and 2 displayed different temporal trends in toxin concentrations, and while the first replicate indicated a possible temporal trend for undisturbed and emulsified Lyngbya, this occurrence was not repeated in the second replicate (Figure 4). No other temporal or treatment-related trends were found for LWT-2 and 3. LWT-6 had the greatest number of SPATT detections of all reported toxins (72%) and was one of four toxins to be detected within water samples (Table 1). Ambient detections were reported for both undisturbed and injured Lyngbya, but was not correlated with concentrations reported within biomass samples (Figure 5). Biomass concentrations ranged from 347 ppb to 6,750 ppb, but there were no obvious trends related to treatment type. For both replicates, LWT-6 did increase with time within emulsified Lyngbya, but no other temporal trends were observed. At 3 WAT, the highest biomass concentration of LWT-6 was reported; similarly, this was also reported for LWT-4, which is a biotransformation product of LWT-6. Overall, LWT-4 was reported at lower concentrations than LWT-6 and did not indicate any temporal or treatment-related trends. Like LWTs-2 and -3, decarboxylation forms of GTX 2 and 3 (dcGTX2-3) were only observed in Lyngbya biomass, but not all samples, and were not detected in ambient forms. Both replicates exhibited higher concentrations of both GTXs in undisturbed Lyngbya when compared to damaged Lyngbya and these concentrations increased greatly between 48 HAT and 3 WAT (Figure 6).

All three sampling methods detected Lyngbya-associated toxins reported to have adverse human health effects (dcSTX and LWT-1, and -5) (Table 1). SPATTs detected ambient release of LWT-1 for algaecide-treated Lyngbya at 48 HAT (replicate 1 only), 10 DAT, and 3 WAT. For Lyngbya that was emulsified, LWT-1 was detected simultaneously in SPATT and water samples at 10 DAT and 3 WAT (replicate 2). Emulsified Lyngbya also reported higher LWT-1 concentrations when compared to algaecide-treated Lyngbya at 10 DAT and 3 WAT for both replicates, but no other temporal or treatment trends were observed (Figure 4). LWT-5 was also reported at its highest concentration in biomass at 3 WAT for both undisturbed and injured Lyngbya (both replicates) (Figure 5). In the second replicate, SPATTs detected ambient release of LWT-5 from algaecide-treated Lyngbya at 48 HAT and 10 DAT. There were also simultaneous detections by SPATT and water samples for emulsified Lyngbya at 10 DAT (Figure 5). LWT-5 concentrations steadily increased in undisturbed Lyngbya throughout the study and, apart from emulsified Lyngbya at 3 WAT, were equal or greater to concentration reported for injured Lyngbya (both

replicates). Relatively high variation in biomass concentrations of dcSTX, a product of LWT-5 biotransformation, was found between replicates, and neither indicated trends related to time, treatment type, or ambient release (Figure 6). However, both replicates documented that toxin concentrations in biomass at 3 WAT were greater in injured Lyngbya when compared to Lyngbya that was left undisturbed. Both replicates also reported SPATT detection for algaecide-treated Lyngbya at 10 DAT, but not 48 HAT or 3 WAT. For emulsified Lyngbya, the two replicates detected concentrations at different time points. For replicate 1, dcSTX was detected in the water sample at 3 WAT, which was the only time a toxin was independently reported for water samples and not for SPATTs. Replicate 2 reported ambient detection at 10 DAT by both SPATT and the water sample.

Discussion

Analysis of experimental replicates 1 and 2 reported herein provide intriguing results about Lake Gaston Lyngbya toxin production and availability. We now have a better understanding of the composition, appropriate sampling methodology, and ambient release of toxins found in *Microseira wollei* in Lake Gaston. Most importantly, this study was able to identify novel sampling methods that have yet to be reported regarding detecting *Lyngbya wollei* toxins (LWTs 1-6). However, further data support and resolution from the analysis of experimental replicate 3 samples would further strengthen these findings.

Previous studies that reported on toxin dynamics related to freshwater Lyngbya utilized biomass sampled from *in situ* mat formations to characterize composition and concentration of *Lyngbya wollei* toxins (LWTs 1-6), decarboxylation forms of STX (dcSTX), and GTX 2 and 3 (dcGTX2-3) (Foss et al. 2012, Metz et al 2022). As in these studies, we found all the aforementioned toxins expected for Lyngbya in samples from Lake Gaston. While biomass may provide information on the intercellular toxin potential of Lyngbya, these levels have not been found to correlate with extracellular levels and ambient toxin potential (Poirier-Larabie et al. 2020). Knowledge of intercellular concentrations would inform potential concerns of direct ingestion, but it is unclear if any organisms actively graze on Lyngbya (Foss et al. 2012).

While discrete surface water samples are the gold standard for determining cyanotoxin concentrations for planktonic cyanobacteria forms, using this methodology for Lyngbya and Lyngbya-associated toxins presents challenges. Hudon et al. (2016) and Poirier-Larabie et al. (2020) found that LWT-1 concentrations were positively correlated with *in situ* Lyngbya biomass levels and were greatest in samples that were collected from water directly overlying benthic mat formations. Unlike these studies, we found that water samples taken within a foot of biomass and from an enclosed aquaria environment produced the least number of detections when compared to other ambient sampling methods. However, the biomass utilized for this study (600 grams) may not have been large enough to produce sufficient levels of toxin for release to water or were simply below analytical detection limits. Another potential issue with utilizing water samples to detect Lyngbya toxins is that benthic mats have been reported in depths of up to 9 feet in Lake Gaston (Baumann, personal observation), presenting an issue of determining appropriate collection depth. While these greater water depths would decrease the likelihood of direct human contact with ambient toxins, they would not prevent exposure to water utility intakes, resident organisms, or other biological receptors.

SPATT technology has been used to detect various aquatic toxins, but to our knowledge, this is the first study to report the detection of *Lyngbya wollei* toxins (LWT-1, 4, 5, and 6) in ambient form using this methodology. In addition to determining toxin presence, SPATTs detected ambient forms at a higher rate than standard discrete water samples. This increased detection probability resulted in the identification of an increase in ambient toxins produced by Lyngbya that had been subjected to some form of injury when compared to Lyngbya that was left undisturbed, a finding that was not seen using standard water or biomass or water samples.

A topic of concern is the potential adverse effects of Lyngbya presence and toxin release on human health, but appropriate sampling methods and an understanding of their limitations are still needed. Foss et al. (2012) note that direct skin contact is the most common route of human exposure to Microseira wollei toxins, but chronic exposure through drinking water is also a concern (Hudon et al. 2016). Because intercellular concentrations found within Lyngbya filaments show a weak correlation with cyanotoxin concentrations in the overlying water (Poirier-Larabie et al. 2020), cyanotoxin concentrations within Lyngbya filaments may not be appropriate for determining human risk factors. Given the close association between ambient toxin concentrations and benthic Lyngbya mat formations, surface water samples may also misrepresent the hazard potential posed by freshwater Lyngbya. Based on our findings, we suggest that SPATT technology continue to be explored for its utility in developing standard monitoring programs directed at Lyngbya. This experiment showed that under ideal environmental conditions, SPATTs produced a high level of analytical detections, although it could not explain possible trends in concentration.

Relatively large temporal and spatial variability have been reported for toxin concentrations measured from *in situ* biomass (Hudon et al. 2016). This variability has been linked to variables associated with both biotic and abiotic factors, such as water quality parameters, bathymetry of the system, and density of benthic mat formation and viability (Hudon et al. 2016; Metz et al. 2022). While this experiment did not address the influence of environmental factors on toxin production and detection, it provides a crucial first step in determining the efficacy of different sampling methods for Lyngbya-associated toxins. We expect that some of our observed temporal and spatial variation in toxin concentrations will be clarified upon the analysis of the third set of samples from the remaining replicate. Moreover, our findings from this experiment will be useful in developing future studies to address larger and more dynamic environments such as in Lake Gaston or other similar reservoirs.

Toxins associated with Lyngbya, *Lyngbya wollei* toxins (LTWs 1-6), decarboxylation forms of STX (dcSTX) and GTX 2 and 3 (dcGTX2-3), were all detected using at least one of the sampling methodologies. Biomass samples produced the highest rate of detection. However, these intercellular concentrations may not correlate to ambient toxin availability which is the most common route of human exposure. Of those methods that were utilized to detect ambient toxin availability (SPATT and water), water samples produced the least number of detections, most of which were duplicated in the corresponding SPATT sample. SPATT technology produced the highest rate of detections and included toxins associated with human health risks (LWT-1, -5, and dcSTX). SPATTs were also the only methodology that identified a trend of increased ambient toxins produced by Lyngbya that had been subjected to some form of injury when compared to Lyngbya that was left undisturbed.

Conclusion

As occurrences of Lyngbya increase across the Southeastern United States, so do questions related to ecological and human health impacts. Scientists from the toxicology and human health fields are particularly interested in how these results may influence future standard monitoring operations and risk analysis. This experiment was successful in identifying the toxin profile for Lake Gaston Lyngbya, as well as, the appropriate methods for monitoring these toxins. The increased ambient presence of toxins after Lyngbya was damaged, either physically or chemically, during this experiment highlights the need for such monitoring. The analysis of study results from two of the three replicates provides encouraging insight into toxin dynamics and methodological questions. However, we recommend that the third replicate of each sample collected during this experiment be analyzed for cyanotoxins, as soon as practical. A third replicate will also provide the level of scientific rigor necessary for publication of this experiment and its results in a top-tier peer-reviewed scientific journal; the importance of these results has already been made clear and is being sought after within the local and regional scientific community.

Future research should focus on expanding these methodologies from mesocosms to *in situ* situations in a reservoir environment. This study provided proof of concept for validating appropriate sampling methods; it does not address the potential influence of environmental factors on detection rates of these toxins in Lake Gaston or elsewhere.

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Tables and Figures

Table 1. Standard toxin sampling methods including water, biomass, and passive gear sampling (SPATT) that were used to detect *Lyngbya wollei* toxins (LTWs 1-6) decarboxylation forms of STX (dcSTX) and GTX 2 and 3 (dcGTX2-3). Two replicates of each treatment were analyzed and "T" indicates detection in first replicate and "t" indicates detection in second replicate and "Tt" represent detection in both replicates.

	T:0		48 hr			10 DAT			3 WAT		
	Biomass	**Water**	**Biomass**	**SPATT**	**Water**	**Biomass**	**SPATT**	**Water**	**Biomass**	**SPATT**	**Water**
Undisturbed											
LWT -1	Tt		Tt			Tt			Tt		
LWT -2			T			T			Tt		
LWT -3	T		Tt			Tt			Tt		
LWT -4	Tt		Tt			Tt			Tt		
LWT -5	Tt		Tt			Tt			Tt		
LWT -6	Tt		Tt	T		Tt	T		Tt		
dcSTX	Tt		Tt			Tt			Tt		
dcGTX2	Tt		Tt			Tt			Tt		
dcGTX3	Tt		Tt			Tt			Tt		
Emulsified											
LWT -1	Tt		Tt			Tt	t	t	Tt	t	t
LWT -2			T								
LWT -3	Tt		Tt			t					
LWT -4	Tt		Tt			Tt	t		Tt		
LWT -5	Tt		Tt			Tt	t	t	Tt		
LWT -6	Tt		Tt	Tt		Tt	Tt	t	Tt	t	
dcSTX	Tt		Tt			Tt	t	t	Tt		T
dcGTX2	Tt		Tt						Tt		
dcGTX3	Tt		Tt			Tt			Tt		
Algaecide -Treated											
LWT -1	Tt		Tt	t		Tt	Tt		Tt	Tt	
LWT -2											
LWT -3	t		t						T		
LWT -4	Tt		Tt	t		Tt	Tt		Tt	Tt	
LWT -5	Tt		Tt	t		Tt	t		Tt		
LWT -6	Tt		Tt	Tt		Tt	Tt		Tt	Tt	
dcSTX	Tt		Tt			Tt	Tt		Tt		
dcGTX2	Tt		t						T		
dcGTX3	Tt		Tt			t			Tt		

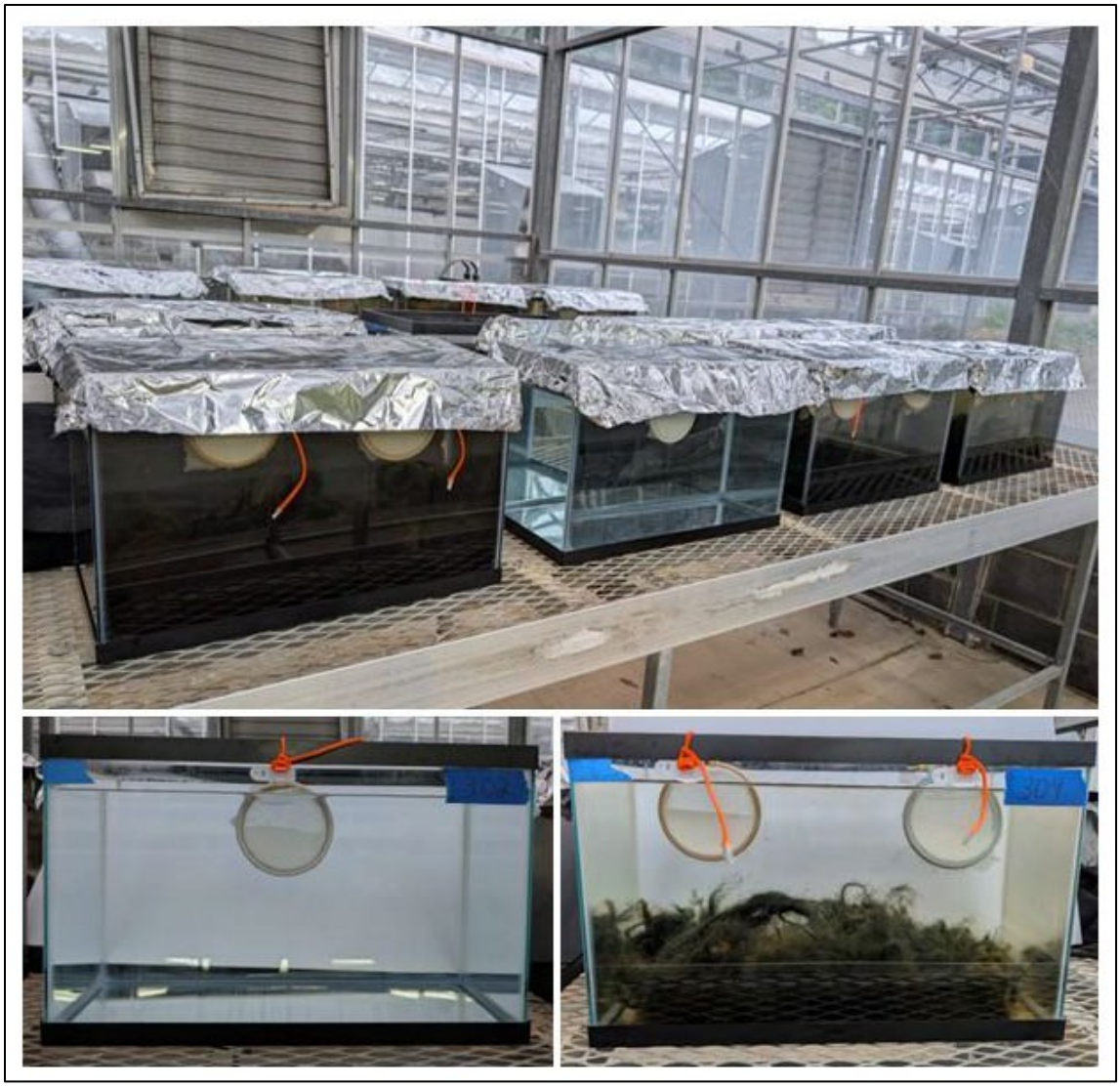


Figure 1. Images of the mesocosm greenhouse trial used to assess potential production and release of Lyngbya and saxitoxin suite toxins. Standard toxin sampling methods included water, biomass, and passive gear sampling.

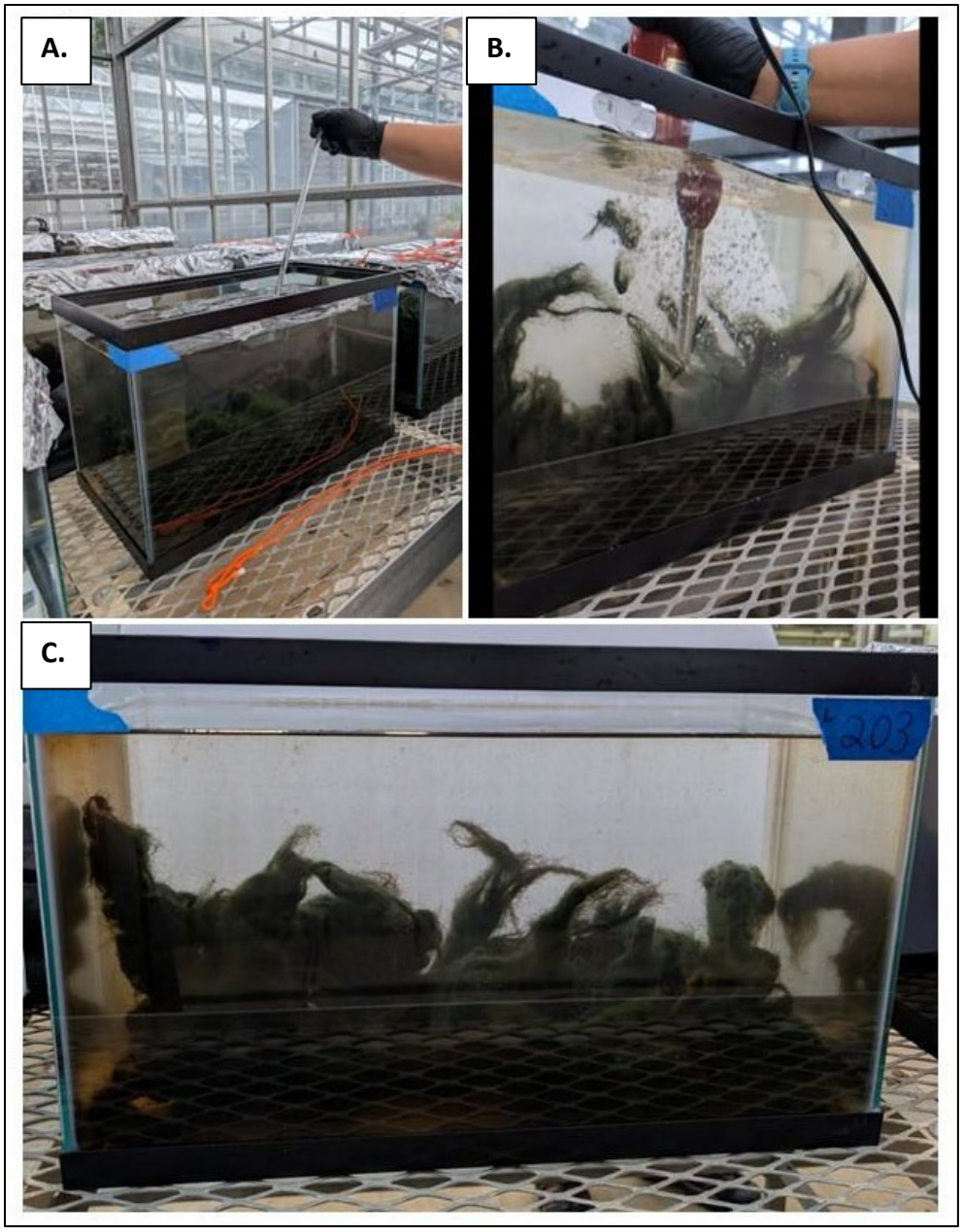


Figure 2. Three experimental treatments were used to assess Lyngbya toxin production response to stress events. Lyngbya was (A) exposed to a maximum label rate of copper-based algaecide (Captain XTR®and AMP®), (B) emulsified using an immersion blender, or (C) left undisturbed (ie., non-treated).



Figure 3. Three sampling methods were used to assess Lyngbya toxin production response to stress events. Standard toxin sampling methods included SPATT (left), biomass (middle), and water (right).

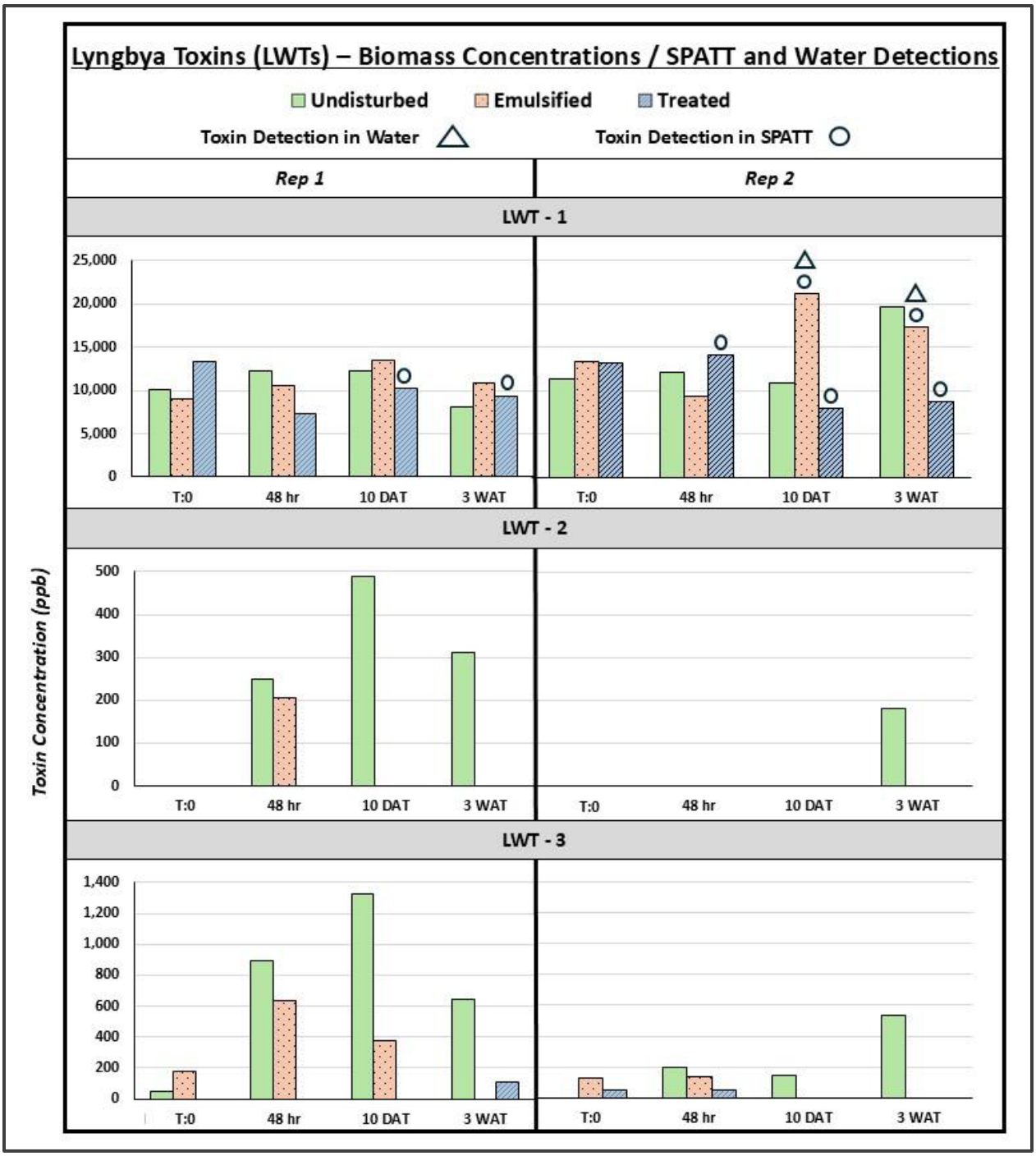


Figure 4. Toxin detection and associated concentrations of *Lyngbya wollei* toxins (LWTs 1-3). Toxin concentrations were reported for Lyngbya that was treated with algaecide, emulsified, or left undisturbed at three collection times: 48 HAT, 10 DAT, and 3 WAT. Bars represent toxin concentration within biomass, circles (**o**) indicate positive toxin detection by SPATTS, and triangles (**Δ**) indicate positive toxin detection in water samples for that treatment type and collection time.

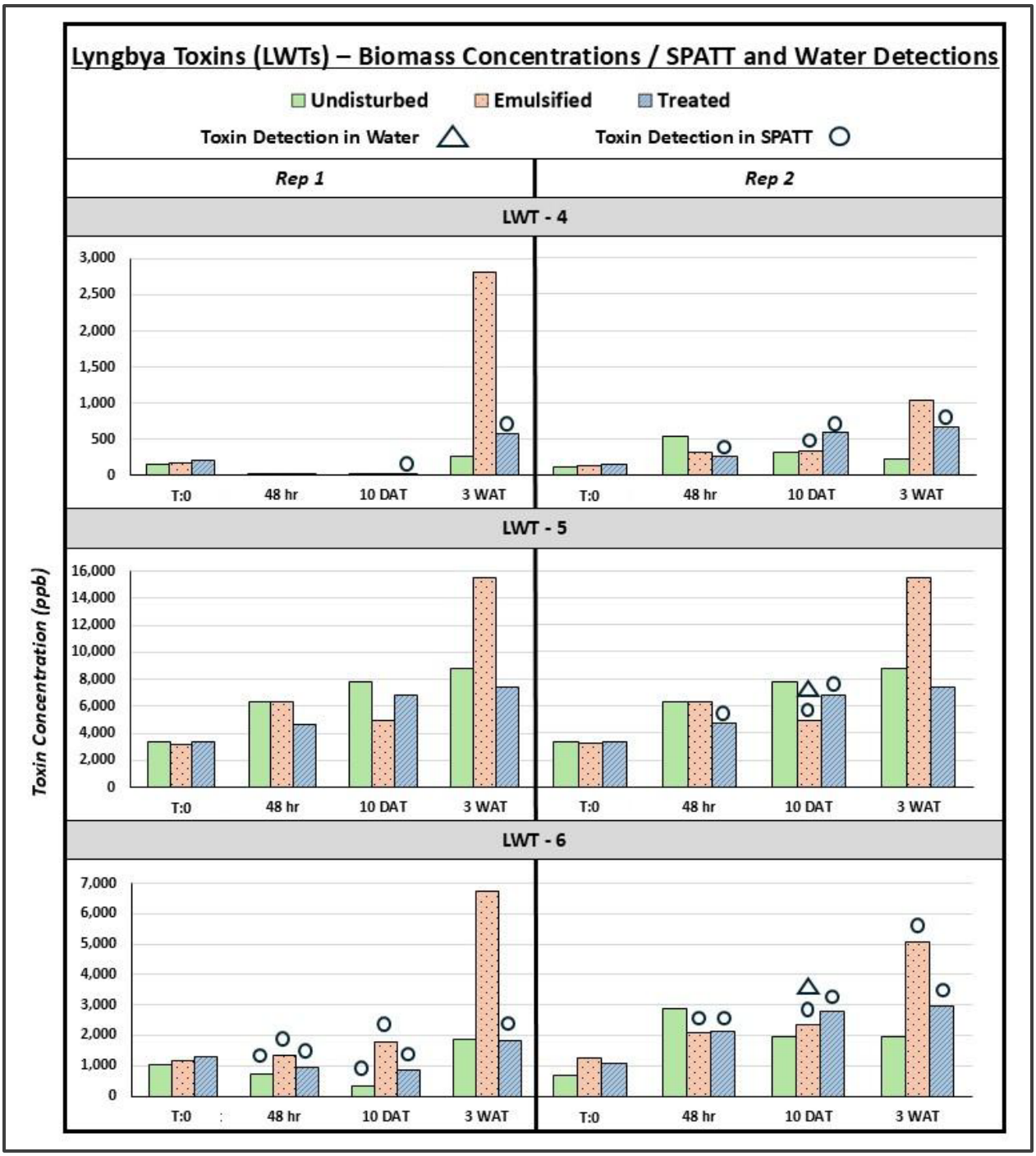


Figure 5. Toxin detection and associated concentrations of *Lyngbya wollei* toxins (LWTs 4-6). Toxin concentrations were reported for Lyngbya that was treated with algaecide, emulsified, or left undisturbed at three collection times: 48 HAT, 10 DAT, and 3 WAT. Bars represent toxin concentration within biomass, circles (**o**) indicate positive toxin detection by SPATTS, and triangles (**Δ**) indicate positive toxin detection in water samples for that treatment type and collection time.

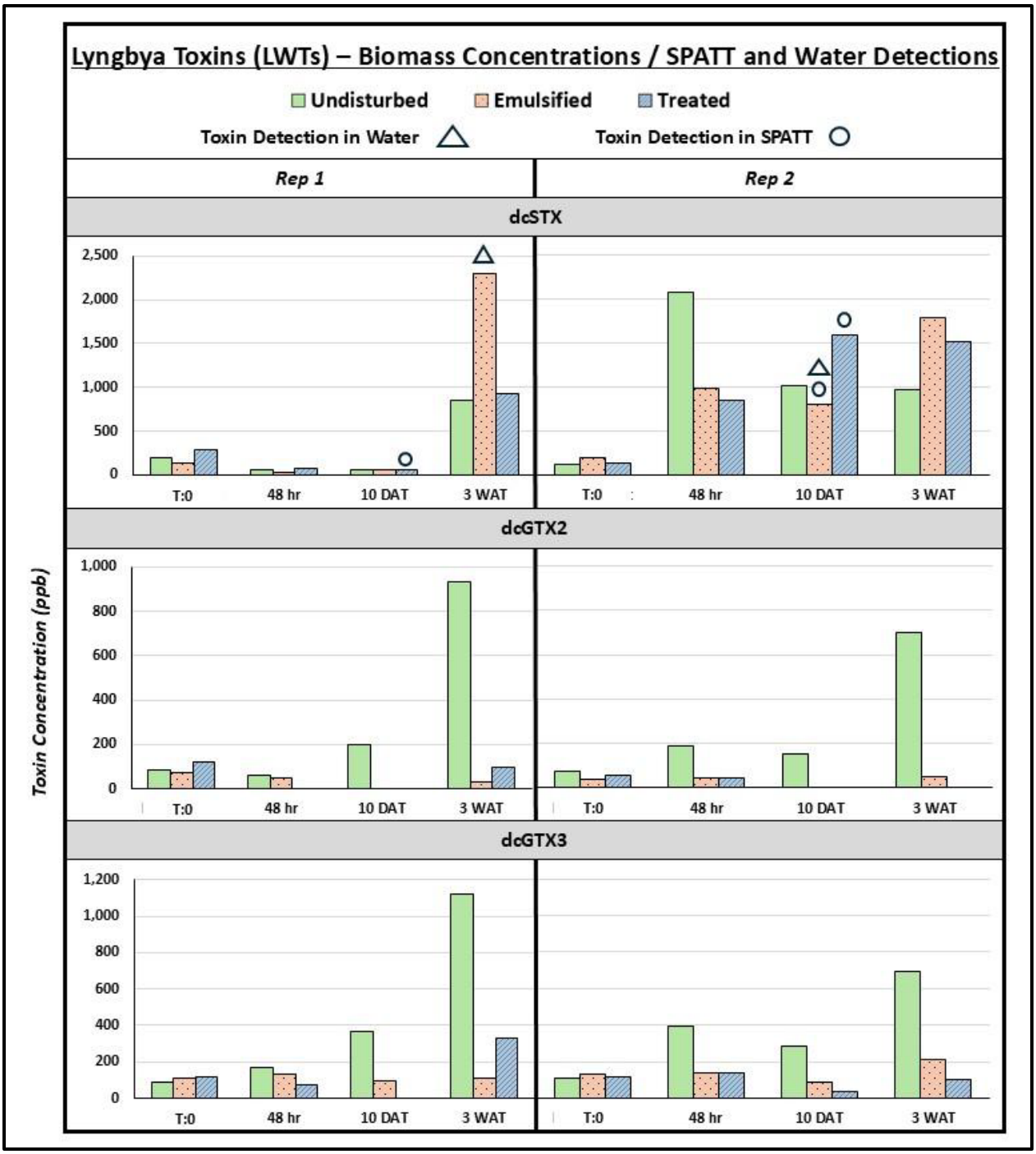


Figure 6. Toxin detection and associated concentrations of decarboxylation forms of STX (dcSTX) and GTX 2and 3 (dcGTX2-3). Toxin concentrations were reported for Lyngbya that was treated with algaecide, emulsified, or left undisturbed at three collection times: 48 HAT, 10 DAT, and 3 WAT. Graphs represent toxin concentration within biomass, circles (**o**) indicate positive toxin detection by SPATTS, and triangles (**Δ**) indicate positive toxin detection in water samples for that treatment type and collection time.