

Algae ATX: Molecular and Biochemical Screening for Toxic Algae, City of Austin, Texas

Project proposal
prepared by
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1.0 Objectives

- 1.1 The purpose of this proposal is to expand the use of analytical techniques available to screen for toxins and identify the algal species that produce toxins in our drinking and recreational water supplies. After the 2019 harmful algal bloom event, an increased need is recognized to track the development of a bloom and identify and quantify algal toxins present that can negatively impact recreational uses of Austin's reservoirs. Early detection through monitoring will enable more accurate prediction of harmful bloom development and targeted management strategies toward mitigation, and hopefully, prevention. This work supports City of Austin programs to monitor waterways for nuisance algal species that may result in impairments of the beneficial uses desired by the community.
- 1.2 Objectives of the proposed work are to assist the City of Austin in the following areas:
 - 1.2.1 Expand our understanding and description of the algae species present in our streams and reservoirs by combining traditional taxonomic identifications with DNA extraction and sequencing;
 - 1.2.2 Develop a molecular library for identification of dominant or problematic species and whether those species have the potential to produce toxins of concern;
 - 1.2.3 Identify and quantify toxin(s) present in algal cells, mats, and/or water samples. Toxin(s) present will be linked to specific cyanobacterial species through molecular fingerprinting results

2.0 Background

- 2.1 Cyanobacteria blooms have been recognized as a significant emerging threat to municipal and recreational water supplies. Under certain environmental conditions, the blooms have been found to produce toxins that have been linked to skin rashes, cancer, and even death of those that come into contact with or consumes impacted waters. It is therefore essential to monitor water supplies for the presence of or potential for toxigenic cyanobacteria to impair aquatic systems. In the Lake Austin reservoir during a recent drought of record, cyanobacteria bloom magnitude and duration significantly increased, leading to concerns about the potential for the reservoir that is a drinking water source as well as popular recreational reservoir to become impacted by cyanotoxins (Bellinger et al. 2018).
- 2.2 Because of the increased cyanobacteria biomass, a pilot study identified phytoplankton

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species during blooms and screened the water for toxins (Bellinger 2018). That study did not find the presence of toxins in the water. However, it was speculated that some species may have the capability to produce toxins. This uncertainty in the potential for the species present to produce toxins led staff to seek out additional means of determining the risk of the species present to the reservoirs. In addition, the study was limited to only Lake Austin and Lady Bird Lake.

- 2.3 Based on the desire to further understand the potential for species to produce toxins as well as expand the screening of Austin's important aquatic systems, The City is looking to contract with the University of Texas which has developed the methods and techniques to isolate species of interest, analyze their DNA for the potential to produce toxins, and apply marker screening to rapidly determine if the species of concern is present in the aquatic system of interest.
- 2.4 In 2019, a distinctive cyanobacteria bloom event, consisting of cohesive mats that grow on the sediment surface and float at the surface after becoming buoyant, occurred in the upper half of the Lady Bird Lake reservoir, notably around Red Bud Isle and Auditorium shores. The harmful bloom event resulted in the death of multiple dogs after likely ingestion of algal biomass from contact with the mats. It was determined that a neurotoxin was present in the mats, with impacts consistent with the symptoms displayed by the dogs prior to their passing.
- 2.5 In light of the harmful bloom event, the scope of the previous pilot project is being expanded to build on the initial findings but to now include monitoring of cohesive cyanobacterial mats and to have toxin identification and quantitation provided in concert with the molecular screening.

3.0 Scope Of Work, 2023

The Manning Laboratory at FIU-BBC will continue generate to biochemical profiling and quantitative analysis of targeted algal toxins. These biochemical characteristics will be added to the micrographic and barcoding library for the rapid assessment of toxic algae in Austin waterways. The examination of benthic strains of microalgae will be added to these analyses, which was previously monitoring phytoplankton, exclusively.

The Manning Lab will generate micrographic and genetic barcoding libraries to develop a reference guide for the identification of microalgae strains (primarily cyanobacteria) that occur in Austin waterways. In addition to DNA barcoding, i.e., 23S, cyanolTS, ITS2, will be used for the identification of microalgal isolates from surrounding waterways.

The 2023 phase of Algae ATX will involve the extraction of environmental DNA (eDNA) to examine the complexity of algal metaphyton mats and community structure. Total eDNA will be extracted from metaphyton mat materials and the DNA will be sequenced using Illumina NovaSeq PE150 whole-genome shotgun sequencing and compared with existing databases for the determination of species diversity, presence and abundance.

- 3.1 Toxin identification and quantification. Based on the harmful algal bloom of 2019, the need has arisen for rapid, accurate identification and quantitation of toxins being produced by algae. Presence or absence of specific toxins will be linked to the genetic potential of the strain, described in the above work, to produce toxins of concern.
 - 3.1.1 Deliverables: Reports on the suite of toxins present, expressed as concentration (i.e., mass per unit volume of water) or content (i.e., mass per unit dry mass of algae).

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- 3.2 Development of robust molecular tools for the detection and identification of toxin-forming species: In addition to DNA barcoding investigations (e.g., 16S, 23S, ITS2, and toxin-related genes), examination of mat materials have revealed the complexity of the metaphyton mats containing toxigenic microalgae. Initial phases of this project have isolated individual algae for DNA barcoding analysis, although not all isolates could be maintained in culture and/or were overgrown by competing strains. Thus, we propose to develop tools for the holistic analysis of metaphyton biomass, including the microbiome (phycosphere), using environmental DNA (eDNA) and genome sequencing to examine the algal community structure, including species presence/absence and relative abundance.

3.2.1 Deliverables: Reports on DNA barcoding sequences for successful isolates.

3.2.2 Deliverables: Reports on the algal community composition at the different sampling sites, at various timepoints of the monitoring season.

4.0 Tasks

Task 1. Toxin identification and quantitation (2020-2025)

Objectives: Gas chromatography (GC) and/or liquid chromatography (LC) coupled with mass spectrometry (MS) will be utilized to identify and determine concentrations of toxins extracted from water or cyanobacterial samples collected from Austin's reservoirs. Ongoing Task.

- From 2020 – 2022, toxin analysis reports were provided weekly to City scientists for algal and water samples collected during the summer-fall months. This dataset is maintained as a real-time Excel spreadsheet for each sample site. Some of the toxin results have been published by peer-reviewed journals, e.g., Manning et al. 2020, and forthcoming manuscripts are planned for the other years, including pre- and post-Phoslock treatments to determine the effectiveness of these treatments on algal abundance and toxin production.
- This task will continue at FIU for the project years 2023 – 2025.

Task 2. Phytoplankton cyanobacterial species identification, DNA barcoding analysis (2020-2025)

Objectives: Continue study of applying molecular techniques toward rapid, certain determination of the presence of toxigenic cyanobacteria species in the water column of Austin's reservoirs. Ongoing Task.

- From 2020 – 2022, individual taxa from metaphyton materials were isolated for genetic barcoding and DNA sequencing. These data are available to City scientists through a shared cloud folder. Initial results were published in Manning, Perri, and Bellinger 2020 (Data in Brief), Manning et al. 2020 (Mendeley Data), and more recent data obtained will be published (manuscript in preparation, Perri, Bellinger and Manning et al. 2022, Toxins).
- This task will continue at FIU for the project years 2023 – 2025.

Task 3. Analysis of metaphyton eDNA to determine algal community composition (2020-2025)

Objectives: Apply molecular, sequencing, and computational techniques to analyze metaphyton eDNA from complex metaphyton material to determine the relative abundance of microbial taxa. Ongoing task.

- Following the 2019 sampling season, sequencing environmental DNA (eDNA) has been the preferred modality over single-cell isolations and sequencing, and metaphyton samples obtained from 2020 and 2021 have been processed using eDNA techniques to capture the community structure using metagenetics. These data have been reported to City scientists and the results of these analyses have been presented at national scientific meetings, i.e., PSA 2021 and JASM 2022. Moreover, we anticipate a portion of these data will be published in 2023 (manuscript in preparation, Manning, Rouzbahani, and Bellinger et al.

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- 2023, Toxins special issue on the impacts of algal toxins in freshwater systems).
- This task will continue at FIU for the project years 2023 – 2025.

Funding:	FY 2023	\$99,910
	FY 2024	\$99,910
	FY 2025	\$49,517.77 (Jan 1 – March, 30, 2025)

5.0 Cost Section

Cost Rates:

A total of \$99,910 per year is budgeted to cover personnel, materials and supplies, core facilities, and indirect costs for the **Algae ATX** project.

The PI, Dr. Schonna R. Manning, will contribute 1 month of time per year (\$9251.27) to assist with the project goals and deliverables. A postdoctoral associate will be appointed 50% time (\$27,500) to help with the isolation and screening efforts (e.g., genetic and biochemical analyses) as well as data management. Another \$14,208.04 is budgeted for fringe benefits (calculated at 38.66%).

A total of \$6,000 is budgeted to cover materials and supplies, including, but not limited to: nitrile gloves, micropipette tips, glass Pasteur pipets, general reagents, agarose, agar, petri dishes, Thermo GeneClean (up to 5 large kits), Thermo GeneJet (up to 5 large kits), 500- and 1.5-mL polypropylene microfuge tubes, thin-wall PCR tubes, 96-well PCR plates, 6- and 12-well plates for cultures, and PCR primers (i.e., forward and reverse primers for 23S, 18S, ITS, anatoxin, microcystin, and strain-specific detection). Note that all materials and supplies will be purchased through FIU vendors using state educational rates. Another \$780 is budgeted for publication costs to disseminate the research findings.

A total of \$10,000 is budgeted to cover sequencing facility charges. These fees include Sanger sequencing for DNA barcoding and related PCR-based sequencing analyses (up to 625 total sequences, including forward and reverse reactions at \$8 per sequence; \$10,000 total). This will also support Next Generation Sequencing (NGS) of whole genomes for targeted algae (up to 10 strains, approx. \$500 per genome, depending on the size of the genome; \$10,000 total).

Per the FIU faculty-staff union by-laws, there is an instituted 3% salary increment per year, thus materials and supplies and publication costs were decreased in subsequent project to accommodate this increase and to stay within the pre-approved budget. The indirect costs are calculated at the current rate of 47.5%.

6.0 Term of Contract

The Contract shall commence upon execution, unless otherwise specified, and shall remain in effect for an initial term of twelve (12) months. The Contract may be extended beyond the initial term for up to four (4) additional twelve (12) month periods at the City's sole option. If the City exercises any extension option, all terms, conditions, and provisions of the Contract shall remain in effect for that extension period, subject only to any economic price adjustment otherwise allowed under the Contract.

THE UNDERSIGNED CONTRACTING PARTIES do hereby certify that: (1) the services specified above are necessary and essential for activities that are properly within the statutory functions and programs of the affected agencies of State Government; (2) the proposed arrangements serve the interest of efficient and economical administration of the State Government, and (3) the services, supplies or materials contracted for are not required by Section 21 of Article 16 of the Constitution

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of Texas to be supplied under contract given to the lowest responsible bidder.

PERFORMING AGENCY further certifies that it has the authority to contract for the above services by authority granted in Article 4413 (32e) V.C.S. and Chapter 105 of the Texas Education Code.

RECEIVING AGENCY further certifies that it has the authority to perform the services contracted for by authority granted in Article 4413 (32e) V.C.S.

7.0 Points of Contact

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FLORIDA INTERNATIONAL UNIVERSITY PROPOSAL BUDGET SHEET

Principal Investigator/Project Director:

Schonna R. Manning

Agency:

City of Austin

Budget Period Start Date:

01/01/23

Budget Period End Date:

12/31/23

Period/Year #:

1

Level 3 Budgetary Account	Level 4 Budgetary Account	Account Code	(List each separately with title)	Base Salary	% Effort CY	% Effort AY	% Effort Summer	12 month	9 month	Summer	Total	
		(F) = Faculty (A) = Administrative	Position New Exists	A.1. - FACULTY / ADMINISTRATIVE SALARY (fringe at 38.66%)								
S77100 - Salaries & Wages (Salary & Fringe) \$50,959.31	P77100 - Salaries & Wages	771001-771139 (F) 771140-771149 (A)	1.000Schonna Manning	\$ 82,000.00			33%	\$ -	\$ -	\$ 9,251.27	\$ 9,251.27	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	1.00	\$ -	\$ -	\$ 3,576.54	\$ 3,576.54	
		771001-771139 (F) 771140-771149 (A)	2.000Rakib Rashel	\$ 55,000.00	50%			\$ 27,500.00	\$ -	\$ -	\$ 27,500.00	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			6.00	0.00	0.00	\$ 10,631.50	\$ -	\$ -	\$ 10,631.50	
		771001-771139 (F) 771140-771149 (A)	3.000					\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		771001-771139 (F) 771140-771149 (A)	4.000					\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		771001-771139 (F) 771140-771149 (A)	5.000					\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		771001-771139 (F) 771140-771149 (A)	6.000					\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		771001-771139 (F) 771140-771149 (A)	7.0000	\$ -				\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		771001-771139 (F) 771140-771149 (A)	8.0000	\$ -				\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		771001-771139 (F) 771140-771149 (A)	9.0000	\$ -				\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		A.2 - Staff Salary (fringe at 61.69%)										
		771150-771159 (S)	1.0000	\$ -				\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		771150-771159 (S)	2.0000	\$ -				\$ -	\$ -	\$ -	\$ -	
		PERSON MONTHS & FRINGE BENEFITS FOR PERSONNEL ABOVE			0.00	0.00	0.00	\$ -	\$ -	\$ -	\$ -	
		Total New & Existing Positions:			00							
		TOTAL SENIOR PERSONNEL SALARIES & WAGES (SECTION A): \$ 36,751.27										
		TOTAL SENIOR PERSONNEL FRINGE BENEFITS (SECTION A): \$ 14,208.04										
		P77150-OPS	Object Code	Head Count	B. OTHER PERSONNEL - FRINGE AT 5.31% EXCEPT FOR GRADUATE STUDENTS AT 11.90%, OPS STUDENTS AT 0.00%							
			771500-771549	1.N/A	Other Professionals							
			771500-771549	2.1	Graduate Student Assistants							
			771500-771549	3.0	Student OPS							
			771500-771549	4.N/A	Secretarial-Clerical (SECRETARIAL-CLERICAL WILL REQUIRE A DIRECT CHARGE EXEMPTION FORM AT PROPOSAL TIME)							
			771500-771549	5.N/A	Other							
			Total Head Count		1	TOTAL OTHER PERSONNEL SALARIES & WAGES(SECTION B): \$ -						
		771660-771669		TOTAL OTHER PERSONNEL FRINGE BENEFITS (SECTION B): \$ -								
		TOTAL SALARIES & WAGES (A+B): \$ 36,751.27										
		P77156 - Fringe	TOTAL FRINGE BENEFITS: \$ 14,208.04									
		TOTAL SALARIES, WAGES, AND FRINGE BENEFITS (S77100): \$ 50,959.31										
		S77100 - Operating Expenses (Unrestricted Items) \$16,776.28	Object Code	C. EXPENSES - IN ALPHABETICAL ORDER BY BUDGET CATEGORY.								
P71121 - Domestic Travel	711211-711226		1	Travel - Domestic								
P71123 - Foreign Travel	711230-711239		2	Travel - Foreign								
P71119 - Long Distance Telephone Calls	711190-711199		3	Long Distance Telephone Calls								
P77300 - Materials and Supplies	773600-773699		4	Educational & Lab Supplies								
	773500-773599		5	Motor Fuel & Lubricants								
	773900-773910		6	Other Materials & Supplies								
	773912 - 773999		7	Recharge Center Charges								
	773911		8	Printing & Reproduction								
P71190 - Other Operating Expenses	711021-711031		9	Freight								
	711360-711399		10	Insurance								
	773001-773002			Miscellaneous Expenses (Other)								
	711075-711077			*** Must Provide Amount & Associated Details Below ***								
	711900-711989		11	Specify								
P711902	12		Software License (requires a direct charge exemption form)									
Participant Support Costs Total "MUST BE ENTERED IN THE PARTICIPANT SUPPORT WORKSHEET"										\$ -		
P71100 - Participant Payments	711991		13	Participant Payments								
P71150 - Patient Care Cost	711510		14	Patient Care Costs								
P77200 - Professional Fees	772101-772102											
	772104-772126											
	772131-772132											
	772141											
	772151-772157											
P77201 - Consultants	772301-772303	15	Professional Fees									
P77210 - Consultants	772103	16	Consulting Services/Agreements									
P71140 - Rent Expense Other Than Buildings	711401-711406	17	Rent Expense Other Than Buildings									
P76100 - Repairs and Maintenance	761001-761006	18	Repairs & Maintenance									
P76800 - Scholarships	768201-768211	19	Scholarships									
P76830 - Stipends	768301	20	Stipends									
TOTAL OPERATING EXPENSES (S77100): \$ 16,776.28												
S77295 - Subcontractors > \$25K \$0.00	P77295 - Subcontractor Amounts over \$25K	772952	21	*** Subcontractor Budgets Must Be Entered in The 'Project Subcontractor Budgets' Worksheet ***								
S7729U - Subcontractors < \$25K \$0.00	P7729U - Subcontractor Amounts under \$25K	772951	22	*** Subcontractor Budgets Must Be Entered in The 'Project Subcontractor Budgets' Worksheet ***								
S76840 - Tuition \$0.00	P76840 - Tuition	768400-768409	23	Tuition Payments								
Restricted Expenses - In alphabetical order by budget category.												
RED Items are part of F&A and are normally not allowed on Federal Grants. RED items will require a DIRECT CHARGE EXEMPTION FORM.												
S7710R - Restricted Operating Expenses (Restricted Items) \$0.00	P77220 - Advertising Services	772201-772204	24	Advertising Services								
	P72110 - Books & Film	721101-721103	25	Books & Films								
	P77320 - Building and Construction	773201-773212	26	Building & Construction								
	P71117 - Cellular Phones	711171	27	Cellular Phones								
	P77218 - Construction Services	772181-772185	28	Construction Services								
	P77382 - Data Processing Supplies	773821	29	Data Processing Supplies								
	P77381 - Hazardous Chemicals/Waste	773810	30	Hazardous Chemicals/Waste								
	P77380 - Food Products	773801	31	Food Products								
	P71116 - Local Telephone Calls	711196	32	Local Telephone Calls								
	P71145 - Memberships & Subscriptions	711451-711454	33	Membership & Subscriptions								
	P71130 - Moving Expenses	711301-711304	34	Moving Expenses								
	P77330 - Office Supplies	773301-773305	35	Office Supplies/Computer Supplies								
	P71101 - Postage	711011-711013	36	Postage								
	P71144 - Rent Expense Buildings	711440	37	Rent Expense Buildings								
	P71118-Telephone Equipment	711180-711189	38	Telephone Equipment								
	TOTAL RESTRICTED OPERATING EXPENSES (S7710R): \$ -											
	S72120 - Equipment & Other Capital Exp (Equipment/OCO) \$0.00	D. PERMANENT EQUIPMENT & BOOKS OR OTHER LIBRARY RESOURCES (list item and dollar)										
P72100 - Other Capital Outlay		721200-721999	amount for each item exceeding \$5,000									
Total Permanent Equipment (S72120):							\$ -					
TOTAL DIRECT COSTS (A through D): \$ 67,735.59												
S75700 - Facilities & Administrative Expense \$32,174.41	E. INDIRECT COSTS											
	P75700 - Indirect Costs	757003	Rate:	47.50%	INDIRECT COST BASE						\$ 67,735.59	
	Type:				MTDC - Modified Total Direct Cost	Indirect Cost:					32,174.41	
TOTAL INDIRECT COSTS (S75700): \$ 32,174.41												
G. TOTAL DIRECT AND INDIRECT COSTS \$ 99,910.00												

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