TUCP and Emergency Drought Barrier Cyanotoxin Monitoring 2022 Work Plan

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Study Objectives/Questions

- What are the spatial and temporal trends in the relative abundance and cyanotoxin concentrations of cyanobacterial harmful algal blooms (cyanoHABs) in the central Delta, with specific interest in the areas around Franks Tract and Mildred Island before, during, and after the West False River Emergency Drought Barrier (EDB) is installed?
- Does the installation of the EDB promote an increase in the relative abundance and/or cyanotoxin concentrations from cyanoHABs in the Central Delta?
- Does the 2022 Temporary Urgency Change Petition (TUCP) promote an increase in the relative abundance and/or cyanotoxin concentrations from cyanoHABs in the Central Delta?
- How does the relative abundance of cyanotoxin concentrations compare annually and interannually with and without the EDB and TUCP?

Rationale/Need

California faces a multitude of environmental impacts due to climate change, one of which is the increased frequency and intensity of droughts. Current drought conditions (2018-2021) brought about the California Department of Water Resources' (DWR)requested emergency authorization for the installation of the 2021 – 2022 West False River Emergency Drought Salinity Barrier (EDB) in accordance with Governor Newsom's emergency proclamations issued on April 21 and May 10, 2021. The EDB would serve California water users by reducing the negative impacts of saltwater intrusion from the San Francisco Bay into the central and south Sacramento-San Joaquin Delta. Under drought conditions, reduced freshwater flows in the winter and spring result in the absence of flows to repel high salinity waters from the San Francisco Bay.

Installation of the EDB would allow California to conserve water by reducing the need for water releases from reservoirs used to push high salinity water downstream. Lastly, the barrier would also mitigate impacts on wildlife by maintaining important aquatic habitats for sensitive species. Low outflows in 2021 and 2022 also necessitated Temporary Urgency Change Petitions to Water Rights Decision D-1641 in June and July of 2021 and April-June of 2022. The 2022 TUCP seeks changes to permit and license conditions imposed pursuant to D-1641 that require the Projects to meet flow-dependent water quality objectives designed to protect fish and wildlife and agricultural beneficial uses in the Delta. These changes were requested because the Projects' storage and inflow may be insufficient to meet D-1641 requirements and additional operational flexibility is needed to support other Project priorities, including: minimum health and safety supplies (defined as minimum demands of water contractors for domestic supply, fire protection, or sanitation during the year); preservation of upstream storage for release later in the summer to control saltwater intrusion into the Delta; preservation of cold water to manage river temperatures for various runs of Chinook salmon and steelhead; maintenance of protections for State and federally endangered and threatened species and other fish and wildlife resources; and other critical water supply needs.

However, the installation of the drought barrier and the changes to outflow and exports associated with the TUCP will alter flows and increase residence times, promoting the growth of harmful algal blooms caused by cyanobacteria (cyanoHABs). CyanoHABs may impose threats to water quality and wildlife in several ways. This includes and is not limited to approximately 25 million Californians being affected by possible cyanotoxin releases by cyanoHABs into the water supply, potentially requiring costly water treatment options. CyanoHABs may also lead to the mortality of wildlife and domestic animals and the die-off of cyanoHABs can create anoxic conditions that may lead to substantial fish kills. Thus, the monitoring of cyanoHABs and cyanotoxins by DWR and USGS is critical to detecting and managing the potential impacts of the EDB and the TUCP.

In 2021, the Delta experienced a harmful algal bloom after the installation of the EDB, which triggered a request for additional cyanotoxin sampling for 2022 by the State Water Resources Control Board and the California Department of Fish and Wildlife. As one of the conditions of approval of the 2022 TUCO, DWR and Reclamation are required to continue a special study on the impact of the TUCP on harmful algal blooms in the Delta. Requirements for this report include measurements of cyanotoxin concentrations in areas where this TUCP Order may modify hydrodynamics to Delta waterways. This study describes the cyanotoxin monitoring being conducted in 2022 to fulfill this condition.

DWR's Division of Integrated Science and Engineering (DISE) and the North Central Region Office (NCRO) will share cyanotoxin sampling responsibilities during routine station maintenance and water quality monitoring from April through September 2022. Cyanotoxin monitoring at Franks Tract (FRK) will be conducted to assess the impact of the EDB specificatlly, while other sites in the central and south Delta (Middle River near Holt—Mildred Island (HLT), False River Near Oakley (FAL) and Holland Cut near Bethel Island (HOL)) will also be sampled for cyanotoxins to conduct a more thorough survey of HABs throughout the area most

hydrologically impacted by the TUCP. These samples will be combined with other studies of cyantoxins in the Delta being conducted by other researchers for a full assessment of HABs across the Delta and the potential impact of the drought actions.

Methods

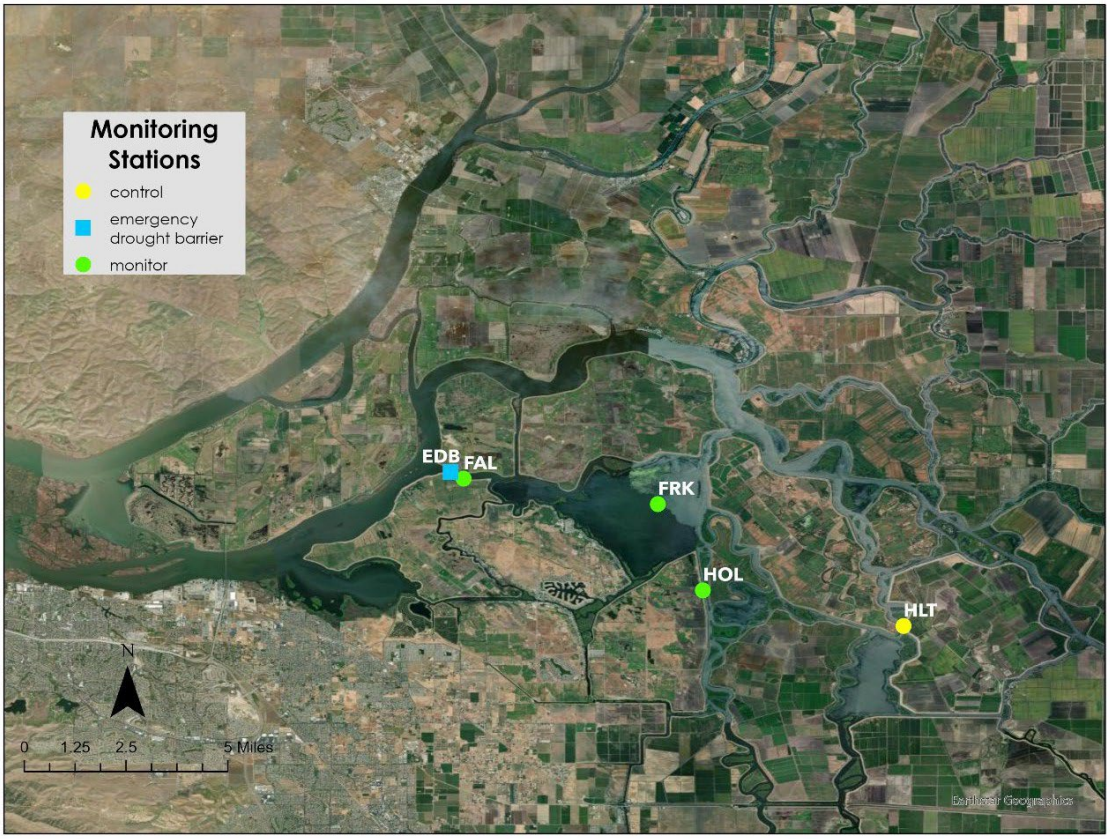

Figure 1. Station map of monitoring and control stations and the Emergency Drought Barrier.

Water Quality Monitoring

Routine continuous monitoring of water quality with YSI EXO2 sondes will be conducted at all stations with parameters as listed in Table 1. Field measurements will also be taken upon arrival at each station to document ambient conditions as cyanotoxin samples are collected. Maintenance of YSI EXO2 sondes will occur typically monthly (or every 3-5 weeks) following protocols from the NCRO Water Quality Evaluation Section Field Manual at False River near Oakley (FAL), Holland Cut near Bethel Island (HOL), and Middle River near Holt—Mildred Island (HLT) (DWR 2020). Additionally, discrete water samples will be collected at these same sites during monthly site visits for analysis by Bryte Lab for chlorophyll-a, total suspended solids, and standard nutrients (Table 1). Nutrients will be collected at FRK every 2 weeks. Measurements of turbidity with Secchi depth and visual *Microcystis* index values will also be taken alongside discrete samples. Sondes at FRK will be managed and maintained following DISE SOPs by the

Continuous Environmental Monitoring Program.

Table 1. Stations with continuous water quality sondes

StationCode	Station Name	Latitude	Longitude	Sensors
FAL	False River near Oakley	38.05547	-121.667	Chlorophyll, DO, Specific Conductance, Water Temperature, Turbidity
HOL	Holland Cut Near Bethel Island	38.01582	-121.582	DO, Specific Conductance, Water Temperature, Turbidity
HLT	Middle River near Holt	38.00308	-121.511	Chlorophyll, Specific Conductance, Water Temperature, Turbidity
FRK	Franks Tract Mid Tract	38.04642	-121.598	Chlorophyll, DO, Specific Conductance, Water Temperature, Turbidity, pH

Table 1. Discrete sampling constituents

Constituents
chlorophyll a (µg/L)
pheophytin a (µg/L)
dissolved chloride (mg/L)
dissolved bromide (mg/L)
dissolved ammonia (mg/L as Nitrogen)
dissolved nitrite + nitrate (mg/L as Nitrogen)
dissolved organic nitrogen (mg/L as Nitrogen)
total Kjeldahl nitrogen (mg/L as Nitrogen)
dissolved organic carbon (mg/L as Carbon)
total organic carbon (mg/L as Carbon)
dissolved orthophosphate (mg/L as Phosphorus)
total phosphorus (mg/L as Phosphorus)

SPATT Monitoring at Franks Tract (FRK)

Solid Phase Adsorption Toxin Tracking (SPATT) samplers will be deployed at Franks Tract station and swapped every 2 weeks. SPATT samplers are devices used to collect time-integrated data on toxin presence using resin beads that adsorb dissolved toxins in a body of water (Kudela 2020). SPATT samplers will be used in conjunction with discrete whole water sampling for cyanotoxins. USGS will construct SPATT samplers for deployment by DWR following the Standard Operating Procedures for SPATT assembly (Kudela 2020). SPATT samplers will be provided to DWR by USGS fully assembled with the resin mesh enclosed within its embroidery

hoop with each sampler individually stored in ultrapure water to prevent desiccation in zip lock bags (Fig 2a). USGS will also provide sample labels for retrieved SPATT samplers (Fig 3d). See field SOP for detailed procedures in Appendix A (DWR 2022).

SPATT Sampling

Samplers will be transported on wet ice to the field and deployed at FRK in a 6-inch PVC pipe and attached to a plastic-coated steel cable with a zip tie (Fig 2b). SPATT samplers will be submerged at approximately 1-meter below the surface (approximately the same depth as the stations continuous YSI EXO2 sonde) and oriented perpendicular to the flow of water. After the 2-week deployment period, samplers will be retrieved and swapped with a new SPATT sampler. The outgoing SPATT sampler will be rinsed in native water to remove any debris. To store the SPATT sampler, the resin bag will be removed from the embroidery hoop (Fig 3b) and stored completely flat in two plastic zip lock bags (Fig 3c), then placed on ice for transport back to the lab (Appendix A, DWR 2022).

SPATT Storage

SPATT samplers will be stored in the DISE EMP -20°C freezer until retrieved by USGS. Note the SPATT retrieval date and time on the SPATT log adjacent to the EMP freezer.

Figure 2. a) outgoing SPATT, b) attach outgoing SPATT to steel cable, c) outgoing SPATT ready for deployment.

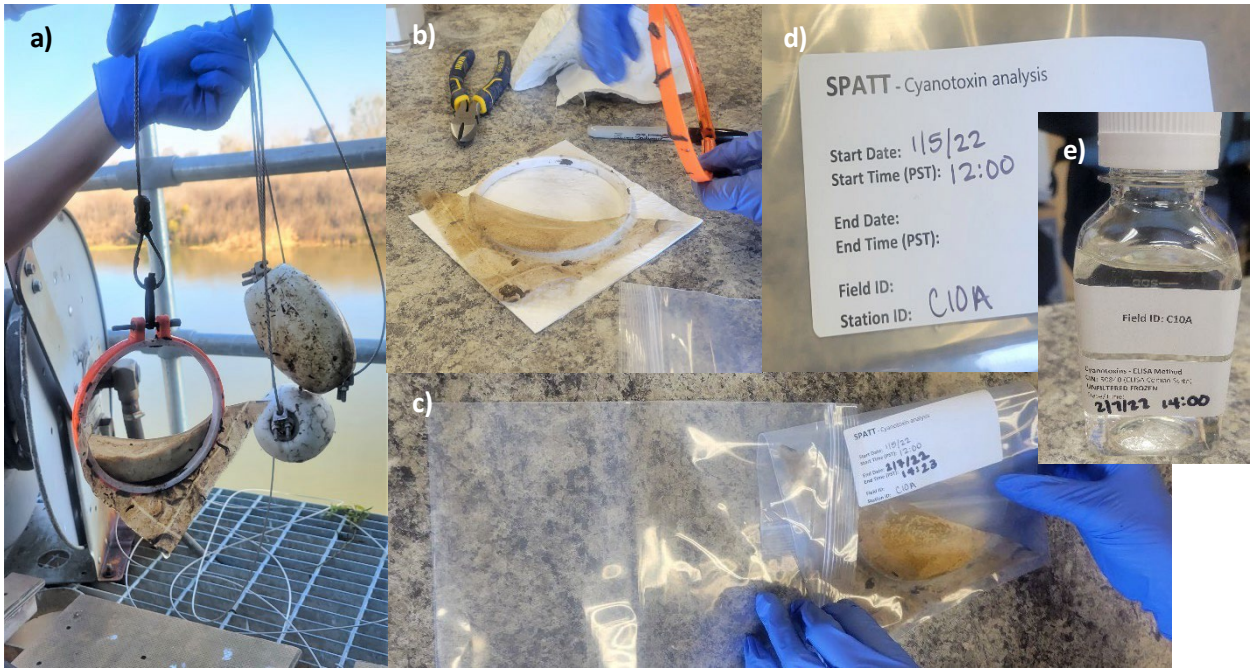

Figure 2. a) SPATT retrieval, b) SPATT bag removed from embroidery hoop, c) SPATT sampler double bagging, d) SPATT label, e) cyanotoxin water sample label.

Cyanotoxin Monitoring

Cyanotoxin will be sampled at FRK every 2 weeks concurrently with SPATT exchanges. FAL, HOL, and HLT will be sampled every 4 weeks. In the event of an algal bloom[1], cyanotoxin sampling will occur every 2 weeks at FAL, HOL and HLT. USGS will provide sample bottles for NCRO and DISE for cyanotoxin samples collected from FRK. Sample bottles for FRK will be pre-labeled with the field station, date, and time (Fig 3e).

A DWR subcontractor, GreenWater Laboratories, will analyze cyanotoxin samples from FAL, HOL and HLT. Sample bottles will be labeled directly on the bottles with a waterproof pen (e.g., Sharpie) with the date and time of collection, name of the water body, and station ID. Samples from FRK will be analyzed at Lumigen Instrument Center, a subcontractor of USGS and DSP.

Cyanotoxin Sample Collection

Cyanotoxin samples will be collected from the surface of the water using a sampling pole, bucket, or van dorn. Sample bottles will be triple rinsed with sample water then dispensed into 250 mL plastic sample bottles. Sample bottles will be filled to the 250 mL line to allow for enough headspace for expansion during freezing. Cyanotoxin samples will then be placed on ice for transport.

Cyanotoxin Sample Storage

Samples collected at FRK will be frozen in the EMP -20°C freezer until retrieved by USGS. Upon collection by USGS samples will be frozen at -80°C.

All other stations (FAL, HOL, HLT) will be refrigerated (not frozen) for up to 2-3 days prior to shipping to GreenWater. Note: samples will not be frozen as they cause cells to lyse and will not be viable for GreenWater's Potentially Toxicogenic Cyanobacteria (PTOX) screening.

Cyanotoxin Sample Shipping

Samples from FAL, HOL, HLT will be shipped to GreenWater Laboratories. A sampling schedule will be sent to GreenWater approximately two weeks prior to the start of cyanotoxin sampling (around mid-March) to allow GreenWater enough time to ship sampling kits prior to field sampling. Sampling kits will include a Styrofoam cooler with freeze packs and sample bottles. Bryte and Weck labs will be notified of sampling events and COCs will be provided to them via email.

Sample bottles will be placed in a plastic bag in the cooler. Bubble wrap and extra freeze packs will be used as needed to cushion the sample bottles and prevent samples from shifting during transport.

Coolers will be dropped off and shipped via FedEx standard overnight shipping (not priority or

[1] An algal bloom will be identified when the water temperature is greater than 19 C and a visual Microcystis index is 4 or 5. Or, when satellite data show a cyanobacterial index of 3.2 or greater, or when fluoroprobes read a cyanobacterial concentration of > 20 ug/L.

first overnight shipping, since they may arrive too early for GreenWater to receive). Shipping overnight will not occur on Fridays, as GreenWater will not receive samples on the weekends. When dropping off samples, GreenWater's FedEx account number and shipping address as well as the mailing address for the West Sacramento DWR office will be provided.

Samples from FRK will be shipped approximately monthly to Lumigen Instrument Center. Sample bottles will be packed to avoid breakage and shipped with dry ice to keep samples frozen. Samples will be shipped priority overnight.

Two different laboratories are being used for this study to provide continuity with existing data sets. All resulting toxins will be compared to thresholds for recreational use advisories, and any differences between the laboratories should be small in comparison with the advisory thresholds. Both Lumigen Instrument Center and GreenWater Laboratories are will respected and have provided high quality data for many years. Additional information on quality control procedures can be found in our QAPP.

Sample Analyses

GreenWater will conduct a Potentially Toxigenic Cyanobacterial (PTOX) screening of cyanotoxin samples to determine which cyanotoxins to test. Taxonomists at Greenwater will use an inverted microscope to inspect the sample for presence of cyanobacteria in the genera *Microcystis, Aphanizomenon, Cylindrospermum, Dolichospermum, Planktothrix*, and other potentially toxigenic taxa. Based on the taxa identified, Greenwater will use appropriate analytical chemistry techniques to determine whether any toxins are present (Table 2). Results from GreenWater's analyses will be emailed to DWR.

Table 2. Methods for analyzing samples for cyanotoxins used by GreenWater Laboratories.

Constituent	**Lab Method**
Microcystins/nodularins	Ada ELISA (Abraxis) EPA Method 546 & Ohio EPA Division of Environmental Services 701.0
Saxitoxin	Saxitoxin specific ELISA (Abraxis Procedure Number 52255B)
Anatoxin-a	Liquid Chromatography Mass Spectrometry
Cylindrospermopsin	Liquid Chromatography Mass Spectrometry

Samples from FRK will be analyzed by liquid chromatography and tandem mass spectrometry for different variants in the toxin classes: microcystins, anabaenopeptins, nodularin, anatoxins, saxitoxins (Table XX). A subset of approximately 20% of samples from FRK will also be analyzed by ELISA for microcystin/nodularin, saxitoxin, anatoxin, and cylindrospermopsin by BSA Environmental Labs.

Table 3. FRK cyanotoxin analyses

Toxin class	Variants / congeners
Microcystins	D-Asp3-Dhb7-RR, MC- RR, MC-YR, M C-HtyR, MC-LR, Dha-LR, D-Asp3-LR, Leu1 LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF
Anabaenopeptins	Anabaenopeptin A, Anabaenopeptin B, Anabaenopeptin F, Oscillamide Y
Nodularin	Nodularin R
Anatoxins	Anatoxin-a, Dihydroanatoxin, Homoanatoxin-a
Saxitoxins	Saxitoxin, Neosaxitoxin, Desamidoylneosaxitoxin
Cylindrospermopsin	Cylindrospermopsin, 7-epi-Cylindrospermopsin

Epiphytic CyanoHAB Monitoring

A subset of the 4 stations will be sampled to detect potential cyanoHABS on submerged aquatic vegetation (SAV). SAV samples will be collected within a 2-meter radius of the water quality station. Leaves of the SAV will be scraped and those scrapings will be collected in deionized water, see Appendix B (DWR 2022b). Samples will be transported back to the West Sacramento DWR office on ice.

Epiphytic cyanoHAB storage and shipping

Epiphytic HAB samples will be stored and shipped to GreenWater in an identical manner to cyanotoxin water samples collected at FAL, HOL and HLT (see Cyanotoxin Sample Shipping section above).

Data analyses

- Compare cyanotoxin levels between the control site (HLT) and monitoring sites (FRK, FAL and HOL) before, during, and after the EDB installation.
- Compare cyanotoxin levels over time during years with and without TUCPs.
- Time series visualizations of continuous water quality data (temperature, chlorophyll a, turbidity, specific conductance, flow, stage height) before, during, and after the EDB installation
- Investigate potential relationships between continuous water quality data and discrete cyanotoxin samples and time-integrated SPATT samples

Budget

SPATT samplers and laboratory analyses of whole water and SPATT samples will be covered by USGS for FRK. Discrete water samples (chlorophyll-a and total suspended solids) are covered under routine monitoring and nutrient samples are covered under EDB monitoring.

- Journal publication costs
- Additional supplies
    - Zip ties (to attach SPATT samplers)
    - Extra bubble wrap for shipping
    - Extra freeze packs for shipping

Table 4. GreenWater Whole Water Sample Processing Costs

Analytes and Analysis	**Cost per sample**	**Discounted cost (more than 1 sample)**
PTOX screening (waived if follow up analyses are performed)	$125	$125
Anatoxin-a-LC-MS/MS	$200	$150
Cylindrospermopsin ELISA	$200	$150
Microcystins ELISA	$125	$100
Saxitoxins ELISA, LC-MS/MS	$175	$150
BMAA LC-MS/MS (beta methylamino-L-alanine)	$325	$275

May 1 -Nov 30 = 31 weeks → 1 water sample/4 weeks ≈ 7 samples/station

HLT & HOL— 7 samples/station x 2 stations x $825/sample = **$11,550***

FAL—7 samples/station x 1 station x $1025/sample = **$7,175****

* This estimate assumes more than 1 sample will be submitted $825/sample (if all analytes are processed). Samples may range from $125-825 depending on the PTOX screening recommendations.
**Note for FAL, this will be the only station sampled on the Central Delta North run, so cost per sample won't be discounted and will range from $125-1,025.

Table 5. GreenWater Phytoplankton Identification & Enumeration Costs

Analysis	**Cost per sample**
Potentially Toxigenic (PTOX) Cyanobacteria Screen	$125
Qualitative Algal Identification	$150
Cyanobacteria ID & Enumeration	$250
Total Algal ID & Enumeration	$300
Algal ID, Enumeration & Biovolume	$375

7 samples/station x 3 stations x $300/sample = **$6,300**

Cyanotoxin and algal ID and enumeration grand total = 11,550 + 7,175 + 6,300 = **$25,025**

Resources

Estimated internal staff hours Oct 2021 - Nov 2022

Staff	Division/Section	Roles	Hours pre-barrier Oct 2021-Mar 2022	Hours during barrier/month Apr-Nov 2022	Total Hours
Rosemary Hartman	DISE/ Synthesis, Resiliency & Adaptive Management	Analysis, writing, planning			
Ted Flynn	DISE/Discrete Environmental Monitoring	Advise			
Morgan Martinez		Task support		8 -16	
Scott Waller	DISE/Continuous Environmental Monitoring	Advise			
Michelle Nelson		Task Support		8 - 16	
Andrew Tran	DISE/Continuous Environmental Monitoring	Task Support		8-16	
Daphne Gille	Estuarine Science & Monitoring				

Peggy Lehman	Estuarine Science & Synthesis	Microcystis/HABs expertise			
Shaun Philippart	Environmental Monitoring & Assessment	Advise			
Jared Frantzich	Regional Assistance/Water Quality Evaluation	Advise			
Tyler Salman		Task support		16	
Elena Huynh		Sample coordination & logistics, task support		32 * 8 = 256	2048

Timeline

Nov 2021—Feb 2022—Planning and drafting of study plan

April 2022 first week—Emergency Drought Barrier will be closed

April 2022—Nov 2022—Data collection

Nov 2022—Removal/opening of EDB

Dec 2022—Begin data visualization and analysis

2022 Timeline

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Coordination and planning meetings	General coordination meeting												
	HAB control meetings												
Field Monitoring	Continuous SpCond, Temp, Turbidity, DO, Chlorophyll/phycocyanin,pH												
	SPATT samples												
	Discrete cyanotoxin grab samples												
	Epiphytic HAB samples												
	Discrete grab samples (Chlorophyll-a, TSS, nutrients, Secchi Depth)												

Deliverables	Final report special study of barrier effect and TUCP on HABs and aquatic weeds												
	Preliminary draft results of EDB monitoring and analysis												
	Status report covering monitoring period June – Dec 2021												
	Comprehensive report covering monitoring June 2021 – Dec 2022									Fall / Winter 2023			
Presentations	IEP Annual Meeting												
	Bay-Delta Science Conference 2023												
	IEP Directors Meeting												
	IEP Stakeholders Meeting												
	CAMT and CSAMP meetings												

Locations

Table 6. Station Information

Station Name	Station Code	Latitude	Longitude
Franks Tract	FRK	38.04642	-121.59810
Middle River near Holt--Mildred Island	HLT	38.00310	-121.51080
False River near Oakley	FAL	38.05580	-121.66690
Holland Cut near Bethel Island	HOL	38.01640	121.58190

References

DWR. 2022. Cyanotoxin and SPATT Sampling Field Standard Operating Procedures. California Department of Water Resources. North Central Region Office. State of California.

DWR. 2022b. Epiphytic HAB Sampling Field Standard Operating Procedures. California Department of Water Resources. North Central Region Office. State of California.

DWR. 2020. Resources Assessment Branch Water Quality Evaluation Section Field Manual. California Department of Water Resources. North Central Region Office. State of California.

Kudela, Raphael. 2020. Standard Operating Procedure for Solid Phase Adsorption Toxin Testing

(SPATT) Assemblage and Extraction for Freshwater and Brackish Harmful Algal Toxins.

Appendices

Appendix A. NCRO Cyanotoxin Sampling Field Standard Operating Procedures 2022

Sampling Plan (April – November 2022)

Franks Tract (FRK) SPATT and cyanotoxin sampling:

1st sampling event

- C-EMP swap out SPATT during sonde exchange visit
- C-EMP collect cyanotoxin water sample
- C-EMP process/filter nutrients at West Sac office

2nd sampling event

- NCRO swap out SPATT 2 weeks from the last swap
- NCRO collect cyanotoxin water sample
- NCRO process/filter nutrients at West Sac office

FAL, HOL, HLT cyanotoxin sampling:

- NCRO collect cyanotoxin water samples once a month
- NCRO process/filter nutrients at West Sac office

Sampling Equipment/Supplies

- Van Dorn
- 250 mL cyanotoxin PETG clear bottles (2 per station at FRK)—supplied by USGS
- 250 mL cyanotoxin plastic bottles (all other stations)
- Outgoing SPATT sampler—supplied by USGS
- Zip ties
- Clippers/cutters (to remove zip ties)
- Cooler with wet ice
- Zip lock bag for retrieved SPATT bag (2 per station)

Deployment of SPATTs

1. Always wear fresh (clean) gloves when handling SPATTs.
2. Transport outgoing SPATTs to the field on wet ice.
3. Fresh SPATT samplers supplied by USGS are stored in double zip lock bags with approximately 100 mL of ultrapure water to prevent resin from drying out (Fig 1a).
4. Visually inspect the SPATT to make sure there are no obvious holes in mesh bag that may allow resin to escape and to make sure it is securely fastened in the embroidery hoop.

5. SPATTs should be secured at the same depth as the continuous sonde where sensor measurements are taking place. The SPATT should be secured at a fixed depth and should not rise and fall with the tide. Secure the SPATT with a zip tie located at the top of the embroidery hoop (Fig 1b) and cut off any excess zip tie.
6. Lower the secured SPATT sampler (Fig 1c) into the PVC housing. The embroidery hoop should be kept upright in the water column – perpendicular to flow - so that water can move through the resin in the mesh bag. The resin will adsorb cyanotoxins present in the water.
7. Plumb bobs (small weights) can be secured to the embroidery hoop to prevent the SPATT from floating back up to the surface.
8. Note deployment date/time along with any relevant information on the SPATT label.

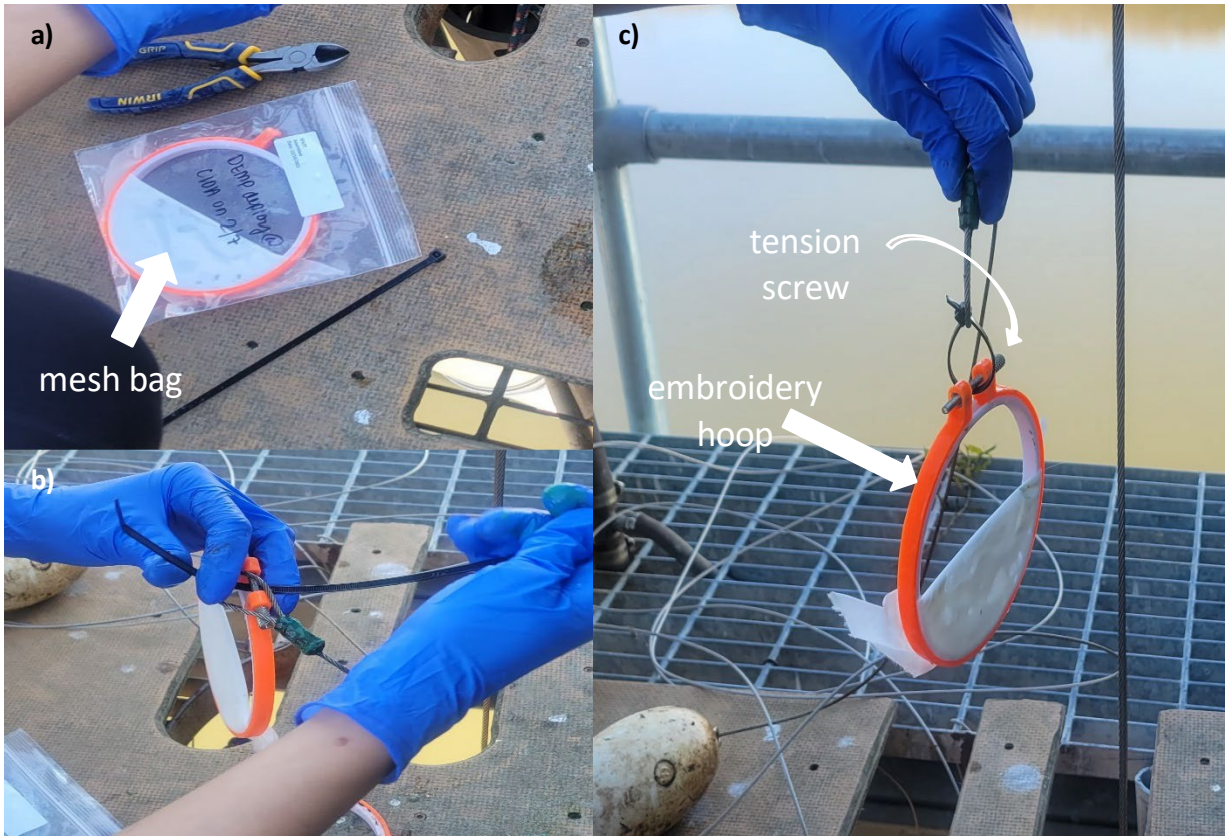

Figure 1. a) outgoing SPATT, b) attach outgoing SPATT to steel cable, c) outgoing SPATT ready for deployment.

Retrieval of SPATTs

1. Always wear fresh (clean) gloves when handling SPATTs.
2. Collect the SPATT sampler (Fig 2a) every two weeks.
3. Upon retrieval remove SPATT mesh bags from the embroidery hoop by loosening the metal tension screw (Fig 2b). Rinse the bags in native water to remove debris.
4. Shake off excess water and place the mesh bag into double zip lock bags. **Important:** Bags must be labeled with station name, date deployed, time deployed, date retrieved, and time retrieved (Fig 2d).
    a. SPATT resin bags must be stored lying completely flat (avoid folding corners of the SPATT bag) in the zip lock bags (Fig 2b).

5. Embroidery hoops can be discarded.
6. Transport bagged SPATTs on wet ice back to the office and store in the EMP lab freezer until they can be picked up by USGS.
7. Deploy a fresh SPATT according to instructions above.
8. At the West Sacramento EMP lab, note the date and time that the outgoing SPATT sampler was deployed as that information will be needed upon its retrieval label in 2 weeks.

Collection of Whole Water Samples for Cyanotoxins Analyses

1. Collect water with a Van Dorn water sampler.
    a. Triple rinse the Van Dorn by lowering the open Van Dorn to 1 meter, then pull the Van Dorn up to empty. Repeat 2 more times.
    b. Send the messenger to the Van Dorn at a 1-meter depth
2. Triple rinse the 250 mL sample bottles by dispensing a small quantity of water from the Van Dorn. Close the sample bottle top and shake the bottle. Pour out the rinse water and repeat two more times.
3. Dispense 250 mL of water from the Van Dorn into the triple rinsed sample bottle.
4. **FRK** samples (for USGS Analysis):
    a. Write the date and time of collection on the label of the 250 mL bottles (Fig 2e).
    b. Repeat steps 2-3 with the second sample bottle.
5. **FAL, HOL, HLT** samples (for GreenWater contractor):
    a. Record the time of collection on a datasheet.
6. Transport the 250 mL samples in a cooler on wet ice back to the office.
7. Store FRK samples in the EMP freezer until they can be picked up by USGS.
8. Store samples from all other sites in the refrigerator.

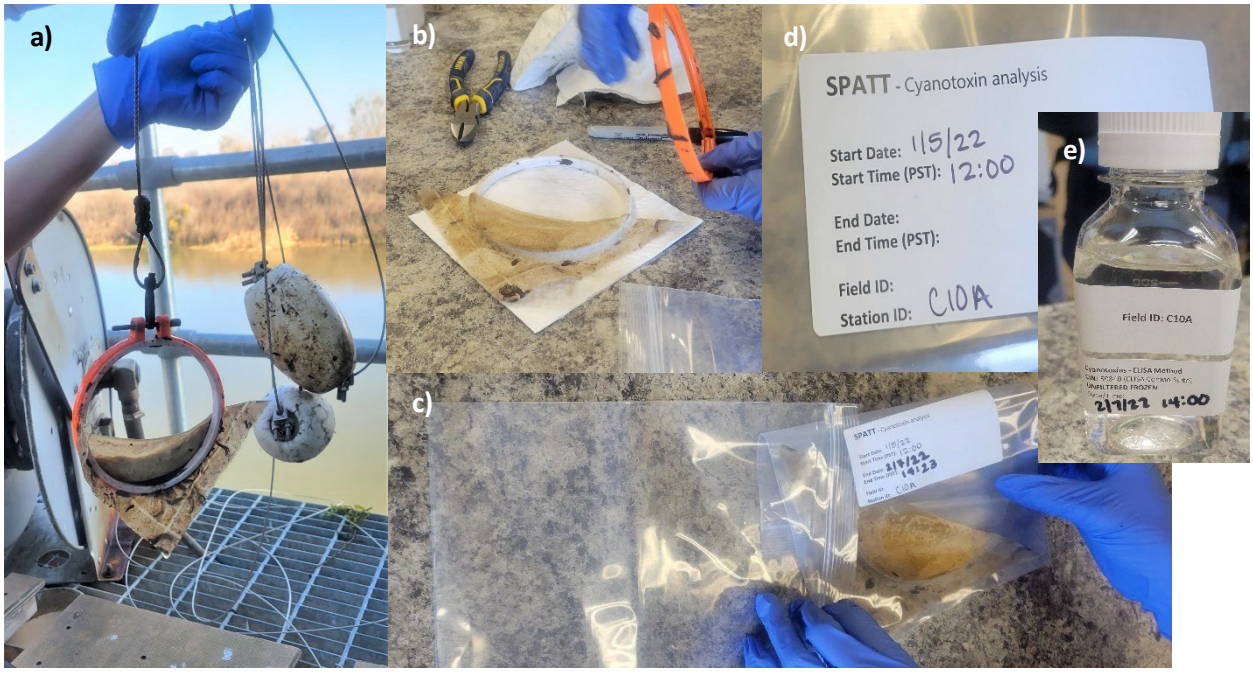

Figure. 2. a) SPATT retrieval, b) SPATT bag removed from embroidery hoop, c) SPATT sampler double bagging, d) SPATT label, e) cyanotoxin water sample label.

FRK Sample Pick-up

Once a month, USGS will pick up the retrieved SPATT samplers and 250 mL cyanotoxin samples from the West Sacramento office and will store samples in a -80 °C freezer.

FAL, HOL, HLT Samples

Samples will be stored for up to 2-3 days in the NCRO Water Quality Lab refrigerator prior to shipping to GreenWater via FedEx standard overnight shipping.

Appendix B. Epiphytic HAB Sampling Field Standard Operating Procedures

Equipment

- Sampling pole or rake
- Clippers
- Razor blades
- 250 mL sample bottles
- Squirt bottle with deionized water (DI)
- Ruler or measuring tape
- Plastic work surface (a plastic container lid or tray)
- ½ pint bottle or ~ 100 mL beaker
- Zip lock bags
- Cooler with wet ice

Methods

Pre-collection Preparation:

Label each (DI) triple rinsed bottle with a station identification code, sampling date and sample type ("epiphytic phyto") with a waterproof marker (e.g., Sharpie).

Field Collection:

1. Use a sampling pole or rake to grab submerged aquatic vegetation (SAV) found within a 10-meter radius of a water quality station. Clip vegetation from the sampling pole if needed.
2. Select the dominant plant species to sample for HABs and record sampling date, time, and species of vegetation.
3. Follow species-specific steps to standardize sampling of varying plant morphologies.

Egeria

1. Isolate a 4-cm segment of the plant to sample. Cut off the top 4 cm of the plant and discard in the appropriate receptacle (to prevent fragments from propagating). Cut a 4 cm segment of the stem with its associated leaves.
2. Triple rinse your plastic work surface and half pint bottle or beaker with deionized (DI) water.
3. Scrape the leaves from the 4-cm stem fragment on both sides with a razor blade, transferring any material from the blade into a half pint bottle or beaker. If needed, trim the leaves off the stem to make scraping easier.

4. Pour the scraped material into the sample bottle. Ensure that all the scraped material is transferred into the sample bottle by rinsing the razor blade, work surface, and sides of the half pint bottle or beaker with DI water into the sample bottle. Fill up the rest of the sample bottle with DI up to the 250 mL mark.
5. Store the sample bottle a zip lock bag and place on ice for transport back to the lab.

Storage and Shipping

Samples will be stored for up to 2-3 days in the NCRO Water Quality Lab refrigerator prior to shipping to GreenWater via FedEx standard overnight shipping.