

CVRWQCB – South Delta Temp. Barriers Project – Section 401 Water Quality Requirements
DWR (NCRO WQES) - Delta Regional Monitoring Program (RMP) Participation Plan

NCRO WQES Proposed HAB Monitoring Workplan 2022

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Introduction:

The collection of additional harmful algal bloom (HAB) data is part of additional requirements by the South Delta Temporary Barriers Project (TBP) Section 401 Water Quality Certification. DWR was required as part of the certification process to provide a revised Delta Regional Monitoring Program (RMP) participation plan that describes a list of proposed monitoring tasks to help the Central Valley Regional Water Quality Control Board (CVRWQCB) better understand the complex water quality questions related to the TBP monitoring program.

The list of additional HAB related Delta RMP monitoring tasks include:

1. Year-Round Visual Harmful Algal Bloom (HAB) Index Reporting – DWR DES Environmental Monitoring Program (EMP) 1-5 *Microcystis* Visual Index
2. Collection of Water Samples for Algal Bloom and/or Toxin Analyses
3. Phytoplankton Identification

Background and Purpose:

HABs are large overgrowths of algae in marine or freshwater ecosystems that have the potential to produce toxins that can harm other living organisms (Zegura et al. 2002). The Sacramento-San Joaquin Delta has observed an increasing number of HABs in the past few years, especially in the central and south Delta. These HABs have been composed primarily of the freshwater algae called cyanobacteria, or blue-green algae (BGA). Specifically, one of the most prevalent cyanobacteria in recent years is *Microcystis aeruginosa* (Lehman et al. 2017); a known producer of toxins called microcystins that negatively affect the health of aquatic organisms and can impact human health. In late 2017, as required by South Delta Temporary Barriers Projects CVWRCB Section 401 Water Quality Certification, DWR NCRO began implementing and recording a surface *Microcystis* bloom visual index value during standard water quality station visits.

Monitoring and Evaluation:

Time-period: Visual DWR EMP Visual Index Monitoring of HABs will occur year-round (Jan.-Dec.) in the South and Central Delta at all North Central Region Office (NCRO) Water Quality Evaluation Section continuous monitoring stations. Tow net samples for *Microcystis* and Van Dorn samples for phytoplankton analyses will occur during the months of known peak *Microcystis* presence (July-October) and coincide with Temporary Agricultural Barrier installation which is typically May-October, but sampling can occur outside of that window if the barrier installation timeline is altered and or visual index scores indicate earlier detection of HABs. Water samples for toxin analysis will be collected only during peak Visual Index periods (Visual Index >4) and will require consultation with CVWRCB before collection.

Location: The NCRO operates monitoring stations and monitors for *Microcystis* Visual Index from Miner Slough near Sacramento River in the northern Delta south to stations in Old River near Mountain House Creek (Figure 1). The primary study area for the TBP monitoring efforts is in the south Delta channels of Old River and Grant Line Canal near Tracy, CA. The four primary temporary barriers installed are Middle River TBP near MRX station, Grant Line Canal TBP near GLE station, lower Old River TBP near ODM, and Old River near Head TBP near OH1 station (Figure 1).



Figure 1. DWR NCRO WQES map of south Delta phytoplankton sampling locations. Four primary sampling locations MHO, GLE, ORM, and OH1 noted by orange circles.

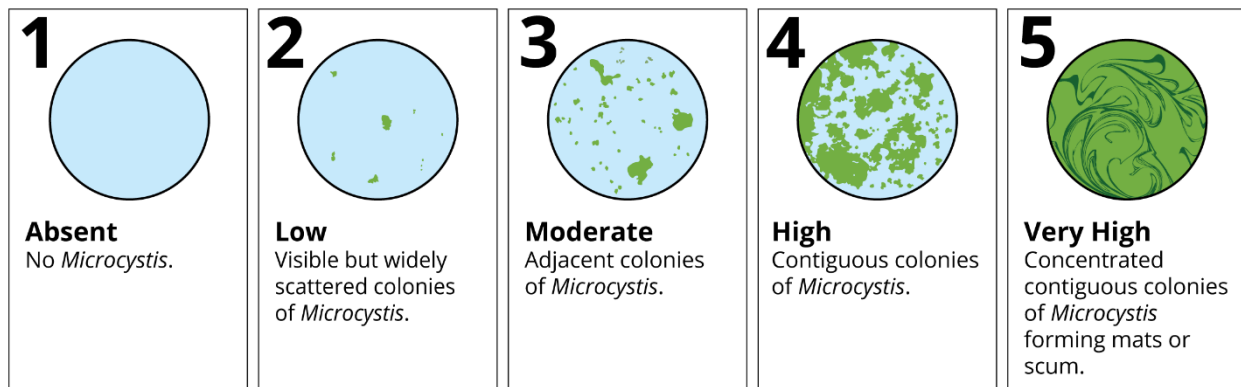
Monitoring Tasks: The following 3 tasks are the procedures which DWR staff accomplish to complete the above listed objectives to monitor HABs in the South Delta. Task 3 was drafted in the original workplan upon the creation of this project but has yet to be completed due to a lack

of funding from the CVWRCB. As funding allows, completion of task 3 objectives would be desired during events of high HAB concentrations.

Task 1. Year-Round Visual Harmful Algal Bloom (HAB) Index Reporting

DWR WQES will collect year-round visual analysis of HAB species (*Microcystis* and *Aphanizomenon*) using DWR EMP Visual Qualitative *Microcystis* Scoring Guide (1-5) while performing regular maintenance at continuous water quality stations (typically 1x per month). The primary regions of interest are the south and central Delta regions. These will be reported to the California Water Quality Monitoring Harmful Algal Bloom Portal (<https://mywaterquality.ca.gov/habs/index.html>). CVRWQCB will provide WQES staff with a modified spreadsheet for importing field data. Data will be reported to the portal monthly after typical continuous water quality station maintenance field runs. Additional photos will be taken and archived to provide visual evidence of index conditions.

Figure 3. DWR EMP Visual *Microcystis* Scoring Guide



Source: Ted Flynn, Environmental Monitoring Program

Task 2. Phytoplankton Sample Collection and Identification

DWR WQES will collect a similar sampling matrix to DWR EMP:

1. *Microcystis* sample will be collected by towing an 80- μ m plankton net towed horizontally just below the surface for 1-3 min or using bucket if thick biomass. Sample will then be placed in 50mL Amber glass bottle and preserved w/ Lugol's solution for ID and enumeration of *Microcystis* only using DWR FlowCAM and/or contracted taxonomist (See Appendix for attached protocol).
2. Ambient water sample will be collected at 1m with Van Dorn water sampler and placed in 50 mL amber glass bottle and preserved w/ Lugol's solution for ID and enumeration of all phytoplankton taxa by contracted taxonomist (i.e., BSA Environmental). This sample will correspond with regular chlorophyll-a grab sample completed at each station for corroboration with continuous sonde data.

DWR WQES plans to collect net tow samples at 4 stations (MHO, ORM, GLE, and OH1 – See Figure 1 and 2) during planned water quality station maintenance field runs. These stations are known sites of high (3-4) Visual Qualitative Scoring and cover primary South Delta flow channel corridors potentially effected by temporary barrier installation and/or water project operations. Samples will be collected at these 4 stations 4x each year, 1x per month starting July-Oct (total

net tow samples = 16 for FlowCAM or contracted taxonomist *Microcystis* ID and enumeration and 16 ambient water sample for BSA taxonomist identification).

This task will utilize DWR EMP's standard methods for phytoplankton identification, quantification, biomass estimation, and quality assurance. Samples will be analyzed with the DWR-owned FlowCAM. Samples will typically all be run through FlowCAM within 1-2 months of collection (not exceeding 3-months). Sample biovolume estimates will be completed at the end of the collection period typically by February of the following year.

Task 3. Collection of Water Samples for Algal Bloom and/or Toxin Analyses

DWR WQES will collect a water sample at sites for toxin analysis **only** when Visual Qualitative Scoring is high (4 - 5) and only when CVWRCB provides directive and funding. DWR WQES will contact CVRWQCB to make final decision on whether to collect samples for toxicity (Contacts: Janis Cooke (CVWQCB) 916-464-4672). Samples will be collected in 250 mL amber glass bottles provided by CVWQCB. DWR WQES will contact CVWQCB to coordinate sample drop-off and/or pick-up from laboratory facilities. Samples will typically be held overnight in the WQES laboratory refrigerator or in cooler on ice and picked up the next morning (hold time ~24hours).

Data Management and Analysis:

Data Management and Accessibility

Collected data is stored both physical field datasheets and electronic form. Electronic data is stored on DWR shared drives, cloud-based drives, and external hard drives all of which are housed in DWR facilities on secure servers which experience daily data back-ups. Datasets are maintained by DWR staff and available upon request from Technical Lead and/or Project Manager.

Deliverables

1. Monthly Visual Harmful Algal Bloom (HAB) Index Reporting
2. Phytoplankton Identification and Enumeration Data
3. Annual summary report of years visual scores and HAB biovolume calculations as well as dataset will be delivered in the first quarter of the following year
4. Final Report at end of 401 Certification (every 5 years) summarizing and analyzing the projects visual scoring and biovolume calculations.

Data Analysis

Annual analysis of collected data would consist of 3 main analyses.

1. A monthly average of the HAB visual index over the 12-month periods collected at all the TBP stations.
2. A biovolume estimation of *Microcystis* collected during task 2 at our 4 target stations. Estimation would be completed by one of the following procedures:
 - a. FlowCAM processing and classification by DWR staff utilizing EMP's protocols (see Appendix IV for analysis computations)
 - b. By outside contracted taxonomic expert in identifying *Microcystis* and other HAB causing phytoplankton
3. A percentage summary of total phytoplankton identified from ambient water samples collected at our 4 target stations over the 4-month sampling period

Further investigation would occur during the final report which could include but is not limited to:

1. Investigation into correlation or causation of HAB abundance in relation to Water Quality and Flow Data collected by NCRO office
2. Statistical analysis of potential trends seen over the project's lifespan
3. Comparing results with other HAB related data collected from throughout the Delta

Funding

All labor and equipment associated with this HAB monitoring project will be funded by Department of Water Resources Operations and Maintenance (O&M) Temporary Barriers Project and Lower San Joaquin South Delta Branch. The primary program manager contacts on the resource agreements for this work are Karen Tolentino (Karen.Tolentino@water.ca.gov 916-902-9897), Bill McLaughlin (William.Mclaughlin@water.ca.gov 916-902-9899), and Jacob McQuirk (Jacob.McQuirk@water.ca.gov (916) 902-9905).

Stakeholder and Agency Coordination

DWR commits to working alongside CVWRCB as well as partners throughout the Interagency Ecological Program to coordinate HAB monitoring efforts and sharing of knowledge to provide the best available results in monitoring the Sacramento San-Joaquin Delta.

References:

Lehman, P. W., Kurobe, T., Lesmeister, S., Baxa, D, Tung, A., Teh, S. J. 2017. Impacts of the 2014 severe drought on the Microcystis bloom in San Francisco Estuary. Harmful Algae 63:94-108.

Zegura B, Sedmak B, and Filipi M. 2002. "Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2." Toxicon, 41, 41-48.

Appendix:

I. 2019 *Microcystis* Field Sampling SOP

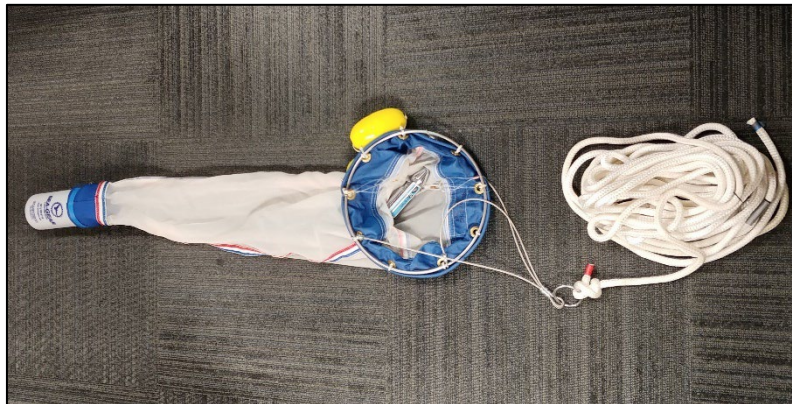
Purpose:

NCRO sampling for *Microcystis* biovolume and toxins (when appropriate) as part of the 401c permit for temporary barriers in the South Delta.

A. *Microcystis* net setup

Equipment:

- *Microcystis* net - 0.3 m wide, 80 μ m mesh plankton net
- 1 L Plastic Cod End
- 40 ft rope
- General Oceanic 2030R6 Mechanical Flow meter (w/ Low Velocity Rotor)
- Floatation Buoy
- Zip-ties



1. Prepare the flow meter for use by adding water to the body of the meter as directed by manufacture. Attach flow meter to the mouth of the *Microcystis* net using zip-ties. Be sure to attach the flow meter to the net's ring so that it sits in the center of the net and the propeller falls inside of the net.
2. Attach floatation buoys to the top of the *Microcystis* net ring using zip-ties. Attach enough flotations to allow the top of the net's ring to sit at the surface of the water when launched.
3. Attach a plastic wide mouth bottle to the end of the *Microcystis* net. Be sure to tighten any loose bolts that are holding down the hose clamp ring.
4. Tie one end of the 40 ft rope to the steel wires at the mouth of the net. Prepare rope for launch by securely attaching rope to vessel used for towing.



B. *Microcystis* Net Tow samples

Equipment:

- *Microcystis* net - 0.3 m, 80 µm mesh plankton net, flow meter, line, cod end, floats
- 1L amber glass bottle
- 1 Labeled 60ml amber glass bottle with 0.5 mL Lugol's Iodine solution
- 2000 mL graduated cylinder
- 100 mL graduated cylinder
- *Microcystis* tow and toxin data sheet, pencil
- Funnel
- Bucket
- DI water refill and wash bottle

Procedure:

1. Bottle Preparation: Label the bottle before collecting samples with station name, date (mm/dd/yy), sampling method ('net tow'). Add in 0.5 mL of Lugol's Iodine Solution to 60 mL phytoplankton bottle.
2. Read the flow meter value and write down the start value.
3. With the *Microcystis* net, flow meter, line, and cod end attached and ready, drop the net in the water just below the surface of the water without spinning the fin on the flow meter and let the current take it out 50 feet away. If current is slack, slowly spool out line into the water as boat operator drives forward. There should be tape or a visible marker on the line indicating when you are 50 feet away.
4. Once the net reaches 50 feet, gently pull the net in. Try not to break any *Microcystis* colonies
5. Repeat steps 3 and 4
6. Record the ending flow meter reading.
7. Gently rinse the net from the outside using the DI wash bottle to rinse any big colonies into the cod end.
8. Remove the cod end from the net. Pour the cod end water into a 2000 mL graduated cylinder and record the volume.
9. Pour the water from the graduated cylinder into the 1L glass amber bottle if collecting additional sample during high HAB concentration events.
10. At all times, be gentle with the sample. Gently swirl the 1L amber bottle containing net tow water. Using the 100 mL graduated cylinder, measure out 50 mL of the *Microcystis* Net Tow sample. Be careful not to spill any sample. Use a small funnel for sample transfers if needed.
11. Tightly cap the Net tow 60 mL bottle and invert several times to ensure the mixture of Lugol's Solution. Place in a Ziploc bag. Store the samples in a safe place that will avoid damage to the bottles and keep out of direct sunlight. **Do NOT ice or freeze samples.**
12. Once finished, tightly screw on the lid of the 1L glass amber bottle, and place on wet ice in the cooler.

NOTE: Bucket – If the *Microcystis* is thick, then the flow meter will not function well, and bucket sampling might be necessary. Try to get a representative sample of the surface water and scum – don't scrape the scum to get more *Microcystis*. Place the *Microcystis* sample in a 1L amber glass bottle and place on ice.

C. *Microcystis* Net Tow samples for phytoplankton bottles

Equipment:

- *Microcystis* tow and toxin data sheet, pencil
- Van Dorn water sampler
- 2000 mL graduated cylinder
- 100 mL graduated cylinder
- 1 Labeled 60ml amber glass bottle with 0.5 mL Lugol's Iodine solution
- Small funnel for sample transfers

Procedure:

1. Bottle Preparation: Label the bottle before collecting samples with station name, date (mm/dd/yy), sampling method ('van dorn'). Add in 0.5 mL of Lugol's Iodine Solution to 60 mL phytoplankton bottle.
2. Rinse the van Dorn 3x by dropping it into the water and pulling it back up just above the surface of the water to empty.
3. Using a Van Dorn, collect a water sample 1 meter below the surface of the water.
4. Measure out 50 mL of sample using a 100 mL graduated cylinder and place in 60 mL bottle.
5. Tightly cap the phytoplankton bottle and invert several times to ensure the mixture of Lugol's Solution. Place in a Ziploc bag. Store samples in a safe place that will avoid damage to the bottles and keep out of direct sunlight. **Do NOT ice or freeze phytoplankton samples.**
6. Record data by adding a checkmark to the assigned box on the datasheet. Rinse the graduated cylinder 3x with DI water for the next site.

D. Ambient Water –Toxin samples

Equipment

- 0 - 0.3 m Van Dorn water sampler
- 1 L amber glass bottle

Important: inspect the van Dorn rope for frays or other weak points at the beginning of each sampling day. You do not want to lose the sampler due to rope break.

Procedure:

1. Bottle Preparation: Label the bottle before collecting samples with station name, date (mm/dd/yy), sampling method ('van dorn').
2. Rinse the van Dorn 3x by dropping it into the water and pulling it back up just above the surface of the water to empty.

3. Lower the van Dorn right below the surface of the water column and collect water sample by dropping the messenger to release the rubber end covers. Do not try to collect *Microcystis*, just the water below the surface.
4. Pull up the van Dorn and triple-rinse a 1-L amber glass bottle before filling it all the way up. Place the bottle on wet ice in the cooler.
5. Record data by adding a checkmark to the assigned box on the datasheet and prep the van Dorn for the next site.

E. Sonde readings and additional notes

Note: This step is not required by the Central Valley Regional Water Quality Control Board, but is additional information being collected by WQES staff.

1. Using a calibrated EXO field sonde, take a field reading 1 meter below the surface while collecting the *Ambient water – Toxin Samples*. Record the following parameters: Temperature, Dissolved Oxygen percent and concentration, Specific Conductivity, pH, Turbidity (FNU), Blue Green Algae (ug/L), and Chlorophyll (RFU and ug/L).
2. Record HAB visibility based on WQES' HAB visibility scale (1=not visible, 2= low, 3= medium, 4= high, 5= extreme)

F. Sample Processing

1. The 60 mL **Van Dorn** samples are to be stored in a dimly lit place until it can be processed for phytoplankton analysis. WQES is working with DISE to conduct phytoplankton analysis. Lugol's Iodine solution allows for storage of the sample up to four months after collection.
2. The 1 L *Microcystis* tow net samples can be discarded after the 60 mL subsample is taken. Please be aware that staff at UC Davis may want to conduct additional analysis on these samples. It is important that you communicate with all stakeholders, assuring the sample is no longer needed before discarding collection. The 60 mL **Net Tow** bottles must be stored in a dimly lit place until it can be processed through the FlowCAM for analysis through LT300 and GT300 lens or analyzed via microscopy by outside taxonomist. Lugol's Iodine solution allows for storage of the sample up to four months after collection. See FlowCAM protocol for procedure or contract language for taxonomist.
3. Enter all data collected from field run into an excel sheet including: Date, Time of collection, Site, Net Tow volume, Flow Meter reading of 100 ft tow, Sonde readings, and any additional notes. This file should be shared with all stakeholders.

II. *Microcystis* Sampling Gear Checklist

Microcystis NCRO Sampling 2019

Boat Check List	Date	Date	Date	Date
Field sheets and pencils				
Clip board				
Van Dorn sampler				
Microcystis net, float, flow meter, line, cod end				
Large funnel (optional)				
2000mL graduated cylinder				
Small transferring funnel				
One Ziploc bag for phyto bottles				
Ice chest with sample bottles (below)				
Two 1L amber glass bottle per station				
Two 60mL phytoplankton bottle per station				

III. 2019 *Microcystis* Field Data Sheet

Microcystis Tow & Toxin Sheet

Site	Date	Time	Net Tow Volume (1-2 L glass amber bottle)	Phytoplankton		Ambient Water (0.3m van dom; 1-2L glass amber bottle)	HAB visual score (1 - 5)	Microcystis Flow Meter 100 ft tow		Notes
				Net Tow	Van Dom			Start	Stop	

HAB visual Scoring index: 1=not visible, 2= low, 3= medium, 4= high, 5= extreme

Additional Notes:

IV. FlowCAM Analysis Computations

Microcystis Net Tow

Microcystis was sampled using an 80 µm mesh plankton net with a General Oceanics’ mechanical flow meter attached to the opening of the net (0.3 m in diameter). The following formulae were used to calculate the water volume that passed through the *Microcystis* net (from “General Oceanics Digital Flowmeter Mechanical and Electronic Operators Manual”).

Values needed	Description
Start and end of flow meter values	Before casting the net and flow meter into the water, the starting flow meter value is recorded. Once the net is pulled out of the water, the ending flow meter value is recorded.
Net diameter = 0.3 meter	Measurement from opening of the net
Standard Speed Rotor Constant = 26,873	Given value from manufacturer
Formulae:	
$\text{DISTANCE in meters} = \frac{\text{Difference in COUNTS} * \text{Rotor Constant}}{999999}$	
$\text{VOLUME cubic meters} = \frac{3.14 * (\text{Net Diameter})^2 * \text{Distance}}{4}$	
MA net water VOLUME in Liters = Volume cubic meters * 1000	Convert VOLUME cubic meters to VOLUME Liter for easier computation when calculating Final Chlorophyll Value (below)

Calculating Default Values for Sites with No Difference Values

Updated 08 August 2019

To calculate the water volume that passes through the *Microcystis* net, both START and ENDING flow reading are necessary; however, error in reading can occur due to flowmeter not spinning or getting stuck. When this occur for a site, a default value is calculated for the net volume difference (ENDING – START). Below is the calculation on how to obtain the default value if a site is missing a difference value.

Refer to **Field data Microcystis net comps 02182016.xlsx** for example.

File location: F:_Microcystis 2015\Analysis\Bryte data

Site BI on 10/14/15 did not have a difference value because the flowmeter got stuck. A default value was generated by averaging the difference values for BI from all sampling events.

$$\text{mean difference} = \frac{(\text{sum of difference value for BI from all sampling events})}{\# \text{ of sampling event with actual difference value}}$$

$$1449 = \frac{(1059 + 1392 + 1363 + 1707 + 2030 + 1145)}{6}$$

Updated 08 August 2019

Final Chlorophyll a Volume

The following calculations were performed to determine the final chlorophyll [$\mu\text{g}/\text{L}$] volume in the *Microcystis* net tow sample. Chlorophyll a from here on is abbreviated as chla.

Values needed	Description
Bryte chla [$\mu\text{g}/\text{L}$]	Value from water quality chlorophyll sampling
Cod volume [mL]	Total <i>Microcystis</i> net tow sample volume that was collected in the brown square sampling bottle
$\text{Cod volume [L]} = \frac{\text{Cod volume [mL]}}{1000}$	total sample volume converted from mL to L for easier calculation
MA net water volume [L]	obtain this MA net water volume from the <i>Microcystis</i> net tow computations (see formulae above for method)

Formulae	Description
$\text{Net chla } [\mu\text{g}] = \text{Bryte chla } \left[\frac{\mu\text{g}}{\text{L}} \right] * \text{sample vol from cod [L]}$	obtain the net chla by multiplying the Bryte chla and cod volume values together
$\text{Final chla } \left[\frac{\mu\text{g}}{\text{L}} \right] \text{ in net tow} = \frac{\text{Net chla } [\mu\text{g}]}{\text{MA net water volume [L]}}$	Calculate final chla in the net tow sample by dividing the net chla into the MA net tow water volume

FlowCAM Total Microcystis Biovolume

Microcystis water samples were collected in phytoplankton bottles and preserved with 0.5 mL of Lugol’s solution. The samples were processed with the FlowCAM using two size fractions: greater than 300-micron particles and less than 300-micron particles (See FlowCAM Standard Operating Procedure for more details). Each sample was processed 2 to 3 times. Microcystis spp. were then identified and enumerated for total biovolume. The following calculations were performed to determine the total Microcystis biovolume for each site per sampling event.

Values needed	Description
Cod volume [L]	Total Microcystis net tow sample volume that was collected in the brown square sampling bottle
MA net water volume [L]	MA net water volume from the Microcystis net tow computations (see formulae above for method)
Sample vol from cod (L)	Total Microcystis net tow sample volume that was collected in the brown square sampling bottle
Dilution factor 1 to X	The dilution value from when processing the Microcystis sample with the FlowCAM
Count (Microcystis aeruginosa, Microcystis flos-aquaes, Microcystis wesenbergii)	Obtain the count of Microcystis species after classification and QA/QC processing. Each species is counted separately and added together later for the final count
Particles per ml, summary	Obtain this value after classification and QA/QC processing. The value can be found when exporting the classification summary into an excel file
Average biovolume, ABD (Area Based Diameter) (Biovolume → BV)	Obtain this value by exporting the Microcystis classification summary from FlowCAM

Calculations	Unit
Total mass = count * avg BV	$\mu\text{m}^3 = \text{count} * \mu\text{m}^3$

Updated 08 August 2019

$Correction\ factor = \frac{particles\ per\ mL}{count}$	$\frac{Particles}{mL} = \frac{Particles}{mL * count}$
BV/mL = total mass * correction factor	$\frac{\mu m^3}{mL} = \mu m^3 * \frac{Particles}{mL}$
Dilution correction = BV/ml * dilution factor	$\frac{\mu m^3}{mL} = \frac{\mu m^3}{mL} * dilution\ factor$
Total BV in brown bottle = dilution correction * sample vol from cod * 1000	$\mu m^3 = \frac{\mu m^3}{mL} * L * 1000$
Final BV/L = total BV/L / MA net water volume	$\frac{\mu m^3}{L} = \frac{\mu m^3}{L}$
Repeat all steps to calculate the biovolume/L for all runs and <i>Microcystis</i> spp.	

If a replication has more than one *Microcystis* spp. (ex: *Microcystis aeruginosa* and *Microcystis flos aquaes*), add the biovolume together. Obtain the mean value for each sampling event by averaging the biovolume from all the replications. Obtain the total *Microcystis* BV/L by adding the GT300 and LT300 BV/L together. Example below.

	Date	Site	Station	Rep	size fraction	Microcystis aeruginosa final BV/L	Microcystis flos aquae final BV/L	Microcystis wesenbergii final BV/L	Microcystis LT / GT300 BV/L
1	8/14/15	MI	7	a	gt300	4.38E+08	5.52E+06	0.00E+00	4.43E+08
2	8/14/15	MI	7	b	gt300	7.72E+07	2.40E+06	0.00E+00	7.96E+07
3	8/14/15	MI	7	c	gt300	4.68E+07	1.35E+07	0.00E+00	6.03E+07
4	8/14/2015	MI	7	b	lt300	6.34E+05	0.00E+00	0.00E+00	6.34E+05
5	8/14/2015	MI	7	a	lt300	5.95E+07	7.66E+06	0.00E+00	6.72E+07



