2019 NC DWR Chlorophyll *a* Round-Robin

Currently, 60.3 miles and 131,105 acres of surface waters in North Carolina are impaired due to elevated levels of chlorophyll *a*, a chemical parameter used to assess algal productivity (2018 Final 303(d) List). These are impairments that require Total Maximum Daily Load (TMDL) development and increased regulation, often at significant costs to both the State and the stakeholders in the affected watershed. It is important that the North Carolina Division of Water Resources (NC DWR) understands the quality of the data used to make these decisions.

The NC DWR initiated the first chlorophyll *a* round-robin in August 2007 because commercially available Proficiency Testing Samples did not include the extraction component required in chlorophyll *a* analysis methods. The round-robin involved the State's certified laboratories, as well as other academic and government laboratories. Seventeen participating laboratories in 2007 analyzed eight freshwater samples for chlorophyll *a* concentrations. The first round-robin results indicated significant inconsistencies with the quality of the data. The Division used the results of that round-robin to work with laboratories and improve analyses.

The data presented within this report represents the thirteenth chlorophyll *a* round-robin. Nineteen laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh-area waterbodies.

Methodology

Sample Collection

On July 31, 2019, NC DWR staff collected a batch of eight surface water grab samples from six local waterbodies. Samples were placed in light-protected carboys and transported on ice to NC DWR's Water Sciences Section (WSS).

At WSS, each of the eight samples was split into nineteen 500 mL subsamples using a churn splitter. Every sample was churned for two minutes prior to splitting and was continually churned during the split. The splitter faucet was purged prior to sample collection. The order in which the subsamples were split from the main sample was randomized in an effort to control bias. Subsamples were put into amber HDPE bottles, then placed on ice and were either delivered to laboratories by NC DWR staff (in-state laboratories) or shipped overnight (out-of-state laboratories) to meet holding times.

Analysis

Participating laboratories were asked to analyze the eight samples according to their Standard Operating Procedures for chlorophyll *a* analysis and to complete a questionnaire concerning the analysis. The answers to most of the questionnaire questions are presented on pages 3 through 8. Statistical analyses and results of the data are presented graphically on pages 9 through 11.

Participating Laboratories

Participating laboratories were referred to by random letter identification throughout the round-robin. The order of letters is alphabetical and does not represent the order of the following list.

ALS Environmental – Jacksonville
Charlotte Water – Environmental Laboratory Services
Environment 1, Inc.
Environmental Conservation Laboratories, Inc – Orlando
ETT Environmental, Inc.
EPA Region IV
Florida Department of Environmental Protection
Meritech, Inc.
NC Division of Water Resources Chemistry Laboratory
NCSU Center for Applied Aquatic Ecology
NOAA Center for Coastal Fisheries and Habitat Research
Raleigh, E. M. Johnson Water Plant
Research & Analytical Laboratories
Santee Cooper Public Service Authority
St. Johns River Water Management District
UNC Institute of Marine Sciences
UNCW Center for Marine Sciences – Aquatic Ecology Lab
US Geological Survey National Water Quality Laboratory
US Geological Survey Oregon Water Science Center

NC DWR appreciates the time and cooperation of each participating laboratory.

Chlorophyll *a* Round-Robin Analysis Details
Answers from Participant's Questionnaires

Lab ID	Method Used	Date Samples Received	Temperature of Samples at Time Received	pH of Samples at Time Received	Temperature at which Samples were Stored Prior to Filtering	Date Samples were Filtered
A	U.S. EPA method 445.0	7/31/2019	1.14 Degrees Celsius	Not measured	5 degrees Celsius	7/31/2019
C	EPA 445.0 Revision 1.3	8/1/2019	1.6° C	Not Measured	Packed in Ice	8/1/2019
E	SM10200H	1-Aug-19	1.2	5.1 - 6.0	1.2	1-Aug-19
F	EPA Method 445.0 modified	7/19/2018	4C	not measured	not stored, filtered immediately	7/31/2019
H	EPA 445.0, Rev 1.2 (Fluorometric)	7/31/2019	8.5°C (no temp blank- checked sample 753)	ranged from 7.70 to 8.90	3.4°C	7/31/2019
I	Standard Methods 10200H (2011)	7/31/2019	<10.0°C	>6.0	Not Stored	7/31/2019
J	EPA 445.0 Rev. 1.2	7/31/2019	1.7 Deg. C	>6 for all samples	N/A, filtered shortly after receipt	7/31/2019
K	SM100200H	7/31/2019	3.1C	7.03 to 7.37	<4C	7/31/2019
L	EPA 445.0 Rev. 2.0-1997, modified option (Invitro Determination of Chlorophyll a)	7/31/2019	14.8 Celsius	CRR281=7.69, CRR933=7.61, CRR615=7.49, CRR840=7.47, CRR828=7.46, CRR753=7.40, CRR917=9.01, CRR435=7.82	23 Celsius	7/31/2019
M	EPA 445.0	7/31/2019	5.8 deg C	7.09 - 9.15	4 deg C	7/31/2019
O	EPA 445.0 Rev. 1.2	7/31/2019	1.3°C	pH of Samples at Time Received > pH 6.0	0.1-4.4° C	8/1/2019
P	SM10200H Spectrophotometer	8.1.19	23C	6.61-8.30	22.8C	8.1.19
R	SM10200H	8/1/2019	3.0°C	7	0-6 degrees C	8/1/2019
S	SM 10200H-2011	8/1/2019	0.9	Not measured	25°C	8/2/2019
T	SM 22nd Ed 10200H Chlorophyll	8/1/2019	4.2 C	We do not test pH on chlorophyll samples as part of our SOP	< 6 C	7/31/2019
U	EPA Method 445.0	8/1/2019	2.4°C	not measured	4.0°C	8/1/2019
W	EPA 445.0 Rev 1.2 (Fluorometric) (Aqueous)	7/31/2019	0.8°C	CRR281=7.40, CRR933=7.54, CRR615=7.21, CRR840=7.14 CRR828=7.25, CRR753=7.20 CRR917=9.04, CRR435=7.56	4.0°C	7/31/2019
X	EPA 445.0	7/31/2019	on ice	N/A	on ice	8/1/2019
Y	Fluorometric (non-acidification)	8/1/2019	5 degrees C	n/a	4C	8/5/2019

Lab ID	Pressure at which Samples were Filtered (if not measured, please state that)	Volume of sample filtered (if not same for all samples, please state range)	Describe Technique for Homogenization of Samples Prior to Filtering	Type of Filter Used	How long were samples filtered? (if not same for all samples, please state range)
A	Not measured	200mL to 400mL	The sample bottles were inverted numerous times for 15 seconds prior to filtering.	Glass Fiber Filter (47 mm)	Between 2-10 minutes.
C	5 in Hg	30 mL	Placed on magnetic stir plate with small stir bar, gently stirred for one minute before pulling sample with pipet.	Glass Microfiber, Diameter 25 mm, CAT No. 1825-025	Roughly 30 seconds.
E	24 mm Hg	208 - 535	shaking	nitrocellulose 0.45 u	10 minutes for all except 5 minutes for CRR 435
F	6 in Hg	50 mL	briskly inverted bottle ~10 times	25 mm GF/F	1-5 minutes
H	<5 in Hg	ranged from 62 to 385mL	Samples gently inverted 10 times	GF/C 42.5mm	5-10 sec
I	600 to 700 mbar	200ml	Gently invert sample 4 to 6 times	Glass Fiber	1 to 2 minutes
J	5 in. Hg	150ml for all samples	shake vigorously several times	GF/F Glass Microfiber filters 47mm	32 - 48 seconds
K	4-6 inches of mercury	250mls	shaken	A/E glass fiber 47mm	<5 mins.
L	Less than 20 KPA	CRR281(150mL) CRR933(150mL) CRR615(100mL) CRR840(150mL) CRR828(150mL) CRR753(100mL) CRR917(100mL) CRR435(150mL)	Gently shake bottle several times	'Whatman® GF/F, lot#16827495	all samples are filtered for less than 10min. If it takes longer a smaller volume is used.
M	< 3 in Hg	100 mL	Gently inverted the bottle several times	GF-75, 47 mm	1-2 minutes
O	<6 in Hg	200 mL	Sample bottle inverted by hand for 5-10 seconds	47mm Glass Fiber GF/F	< 10 minutes
P	Not measured	180mL-450mL	Sample bottle is shaken vigorously by hand before filtration.	binder-free glass fiber filters	2m23s - 8m19s
R	N/A	150-350mL	Bottles inverted at least three times	0.7um glass fiber	~1-2min
S	50 cm Hg	100 to 480mL	Samples are shaken well before filtering.	1 micron	7 to 21 mins
T	We do not measure pressure at which samples are filtered	200-500	mix sample volume by inversion of sample container 7 times.	GF/C 42.5 mm Glass Microfiber Filters	4-28 minutes
U	≤6 in Hg	50-100 mL	shaking bottles several times prior to each aliquot measured	glass fiber	about 30-60 seconds per sample
W	-5 kPa	50-100 ml	Sample inverted 4-5 times	GF/F	approximately 3 minutes
X	<6 mm Hg	10-30 mL	Gentle shaking for 10 secs	glass fiber	10-30 seconds
Y	Not measured	180-450 mL	Sample bottle is shaken vigorously by hand before filtration	GF/F (glass fiber) 25mm circles	2m23s - 8m19s

Lab ID	Describe filtering technique (how were sample volumes measured, were sides rinsed, etc.)
A	A cleaned graduated cylinder was rinsed with native water three times before measuring the samples.
C	Samples were measured and pulled using disposable volumetric pipets. Funnel and screen on manifold were rinsed with distilled water and wiped clean with Kimwipes between each sample. The fiberglass filter was placed on the screen, then the funnel was attached, the vacuum valve opened, then 30mL aliquots were pipetted into each funnel.
E	sample volumes measured by graduated cylinder; sides rinsed
F	duplicate aliquots of 50 ml were measured using a 50 ml graduated cylinder, sides of filter towers were not rinsed (we typically measure estuarine samples and do not rinse due to possible osmotic shock and cell lysis)
H	Samples are vacuum filtered. The filter flask is connected with Tygon tubing to a manifold. The manifold is connected with Tygon tubing to the vacuum pump. After mixing, the sample is poured into a 100mL glass graduated cylinder and volume is recorded. After pouring sample into filter funnel, the sides of graduated cylinder are rinsed twice and poured in funnel. The inside of the funnel is rinsed as a last step. Filter is folded in half and wrapped in opaque cover. Samples are stored in the freezer.
I	Samples were measured using a polypropylene graduated cylinder, rinsed cylinder twice with MgCO3 solution
J	Volume measured with graduated cylinder. Funnel was not rinsed.
K	measured with a 250 ml graduated cylinder, vacuum filtered, cylinder & funnel rinsed between uses
L	Sited filter by adding about 5.0 mL , Reagent water , volume measured using class A 250 mL graduated plastic cylinder, Cylinder is rinsed sides , sides of filter funnel are rinsed.
M	Measured volume in a graduated cylinder. Filtered with a hand pump. Rinsed sides of filtration unit and graduated cylinder with DI water.
O	Measured in a class A graduated cylinder, sides not rinsed
P	After mixing, the sample is poured into a 500mL Class to Deliver graduated cylinder to be measured for filtration. Then, the sample is vacuum filtered as quickly as possible. When the filtration is nearing its end, 1-2mL of saturated $MgCO_3$ solution is added. The funnel is then rinsed thoroughly with DI water. The filters are then folded into quarters and wrapped in foil and frozen. The cylinders are thoroughly rinsed with DI water in between samples.
R	Measured with 500mL Class A graduated cylinder. Cylinder and filter flask rinsed three times with DI water.
S	Sample volumes were measured in class A volumetric cylinder and poured slowly into the filter funnel. Cylinder and funnel are rinsed well.
T	500 mL of sample measured in class A graduated cylinder. Sample poured slowly to determine greatest volume that will filter within 30 minutes. If less than 500 mL filtered, volume calculated by subtracting amount remaining in cylinder. Sides of filtration cups not rinsed.
U	measured in 100 mL glass graduated cylinder; poured into plastic filter tower; cylinder and filter towers sides both rinsed with DI water
W	50 ml aliquots filtered in a graduated cylinder. When filtration slows, final volume recorded and sides of cylinder rinsed 3 times with type 1 DI water.
X	Volume measured in a graduated cylinder, filter funnel sides not rinsed down
Y	volumes measured with graduated cylinder, sides of filter funnels were rinsed with deionized water

Lab ID	Describe lighting conditions during filtering	List the extraction solvent, its purity and the volume used	Length of time samples were stored after filtering	Length of steeping time?
A	Well-lit laboratory room.	10mL of 90% acetone	24 days	Less than 24 hours
C	Dimmed, no outside light	90% Acetone, 10mL	4 days	4 hours
E	low amber light	90% acetone; 10 mL	28 hrs	28 hrs
F	lights turned off, blinds closed	90% reagent grade acetone	28 days	24h
H	All overhead lights off, two small lamps with 25 watt green bulbs.	90% acetone, Fisher Scientific Certified ACS, 14mL	20 days	21 hours
I	lights off, blinds down and closed, door closed	90:10 Acetone: Saturated	15 Days	22.5 hours
J	dark room with green lights	90% Acetone, Baker analyzed-ACS reagent grade, 25ml	6 days	22 hours
K	Darkroom w/ green light	90% acetone 10% deionized water, Purity = 99.7% @ 10 mls used	ground immediately	18hours
L	Analysis takes place in a dark room with green lighting.	25 mL of 90% Acetone	21 hours	2:45 hours
M	Overhead fluorescent lights	90 % Acetone/ 10 % Water Solution	19 Days	Approx: 20 hrs
O	Dark room with subdued green LED lighting	90% Acetone, Optima grade, 25 mL	5 Days and 21 Hours	20 hours and 9 minutes
P	Filtration is done under regular overhead lighting. (Intensity Range 20-30 ft-candles)	90% HPLC grade acetone & 10% filtered MgCO3 solution extracted up to 12mL	5 days 17hrs	4hrs 12 min
R	Fluorescent lighting; filter funnel covered with foil	90/10 Acetone/MgCO3. The acetone is chromatography grade and the MgCO3 is reagent grade and filtered through a 0.45um filter. 10mL of Acetone/MgCO3 solvent was used to extract the sample.	19 days	48hrs
S	Adequate and bright room lighting	90% Acetone	12 days	17 hours
T	normal/ subdued lighting of room	5 mL of 90% acetone (HPLC grade)/ 10% saturated MgCo3	~9 days	~3
U	dimmed fluorescent (25% of full lab lighting)	90% HPLC-grade acetone, 10 mL	13 days	20 hours
W	Darkened room w/ Red Light	90% Acetone, Type 1 DI water	20 Days	20.75 hours
X	low ambient light from windows	Acetone,90%, 10 mL	20 days	3 hrs
Y	ambient light from window	90% Acetone : 10% water 7.5mL for each sample	1 day	24 hours

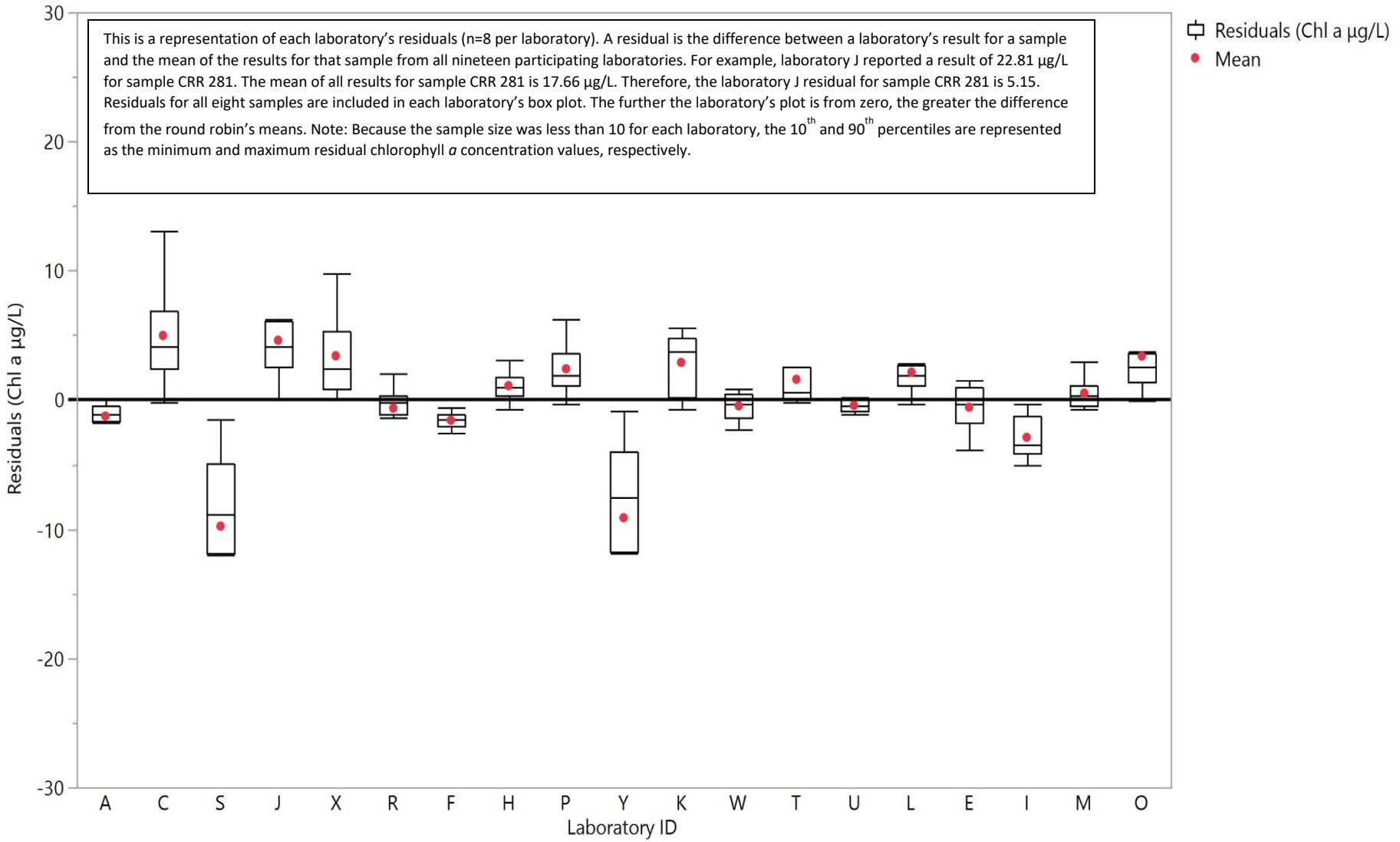
Lab ID	Description of grinding setup
A	A clean and dry Teflon pestle is placed into the grinding tube. This Teflon pestle is connected to a drill with a base for stability.
C	Glass centrifuge tube, tissue grinder; Teflon pestle with grooves in the tip with 1/4 stainless steel rod long enough to chuck on to a IKA RW 20 Overhead Stirrer, spun at 1170 rpm. Rm temp controlled by thermostat: 19.6°C
E	high speed micromixer
F	Teflon (PTFE) tissue grinder, temperature was not controlled however grinding time was very short ~ 15 seconds per sample to prevent heating of the acetone/ filter slurry
H	Pro Scientific homogenizer with stainless steel saw tooth generator, 5mL glass grinding vessel, temperature was not controlled, transferred to 15mL graduated centrifuge tube to steep in refrigerator overnight.
I	Tear filters into quarters and place into grinding tube (Kimble #886000-0023) with PTFE grooved tip; samples macerated using electric lab mixer (Arrow engineering - Model 1750); temperature not controlled but grinding time was short to avoid heating of the sample
J	Teflon pestle with radial serrations on tip; temperature controlled by touch/feel
K	Arrow 850 motor 1/10hp kontes tissue grinder, pestle SZ24 matching tube No temperature control
L	Tissue grinder tip on a drill press.
M	Drill with a Teflon® pestle with grooves, 50-mL polypropylene conical tubes
O	Ground in a glass mortar. Pestle has round, serrated Teflon pestle. Unit powered by an electric drive motor. Temperature monitored by feel, sample was not allowed to heat.
P	Filter is rolled up and placed in a 30 mL glass tube that is kept on ice between samples (to minimize heat from friction). An Eberbach power unit with a Wheaton Tissue grinder is used to grind sample down with solvent. The slurry is added to a centrifuge tube. The 30 mL grinding tube is rinsed with solvent until clean and added to the centrifuge tube. The grinder is rinsed with solvent a second time and added to the centrifuge tube. If slurry in centrifuge tube is less than 8 mL, the volume is brought up to 8 mL with solvent. If the slurry in the centrifuge tube is greater than 8 mL, solvent is used to bring the volume up to the nearest whole number up to 14 mL. Samples are steeped in refrigerator.
R	Drill press with a Teflon grinding tip. Not temperature controlled, ambient temp.
S	Samples are ground using a Teflon tip in a glass test tube for 1 minute with 3mL of 90% Acetone solution. Then sample was transferred into a 25mL screw top centrifuge tube with an additional 7mL of 90% Acetone. Grinding occurs at room temp and was not temperature controlled.
T	Tissue grinder with stainless steel tip, in 10 mL vial, not temperature controlled
U	Teflon grinding pestle attached to rotor; glass test tube; temperature controlled by feel
W	Tissue Grinder, sample in plastic centrifuge tube. Temperature controlled to prevent evaporation.
X	A Teflon tip tissue grinder is attached to a motor and the filter is ground in a 50 mL centrifuge tube till completely macerated. Temperature does not rise significantly as felt when holding the tube.
Y	samples were poured into 100mL graduated cylinder and then into filter manifold. The cylinder and manifold was rinsed with deionized water between each sample

Lab ID	Were samples acidified? If so, what type, concentration, and volume?	Type of calibration standard and source
A	0.1 N HCL	Concentrated solutions (8-22 mg/L chlorophyll-a) in 90% acetone, produced at the USGS National Water Quality Laboratory using Sigma concentrated pigment
C	No	Commercially Prepared Chlorophyll a Std Turner Designs
E	yes, 10uL of 1N HCl	Turner Designs
F	No	Primary standard was pure chl-a extracted from Anacystis nidulans (Sigma) dissolved in HPLC grade 90% acetone. This standard was used to determine chl-a equivalent of Turner Designs solid secondary standard which is used to account for instrument drift (should be minimal with LED excitation source). Solid standard is run immediately before measurement of environmental chl-a extracts.
H	No	Chlorophyll a from Anacystis nidulans, Sigma C6144
I	Yes, 0.1N HCL used 0.1 ml per 3ml of extract	Turner Designs P/N 10-950 (20ml ampule of known concentration)
J	No	Fluorometric Chlorophyll standard, Turner Designs
K	No	N/A
L	not acidified	Make a calibration curve standards from a stock solution
M	0.1 N HCl solution, 90uL to 3.0ml of sample	Chlorophyll a free of chlorophyll b Neat, Sigma
O	Not Applicable	Turner Designs Fluorometric Chlorophyll Standard
P	Samples are acidified with 100 uL of 0.1 N HCl, mixed with a mixing paddle, and timed for 90 seconds.	200ug/L chl-a from spinach
R	0.1mL of 0.1N HCL	N/A
S	Yes, with 0.1mL of 0.1N HCl	N/A
T	Yes, samples acidified with 100uL 0.1 M HCl	quarterly check: PerkinElmer Secondary Spectrometric Calibration Standards daily check: Chlorophyll a std made using Anacystis nidulans algae (Sigma-Aldrich)
U	no	chlorophyll a from Anacystis (Sigma C6144); chlorophyll a from spinach (Sigma C5753)
W	no	Turner Designs Chlorophyll A & B Standard
X	no	Pure Chla in 90% acetone, from Anacystis, Sigma Chemical
Y	no	chla pigment standard (sigma aldrich)

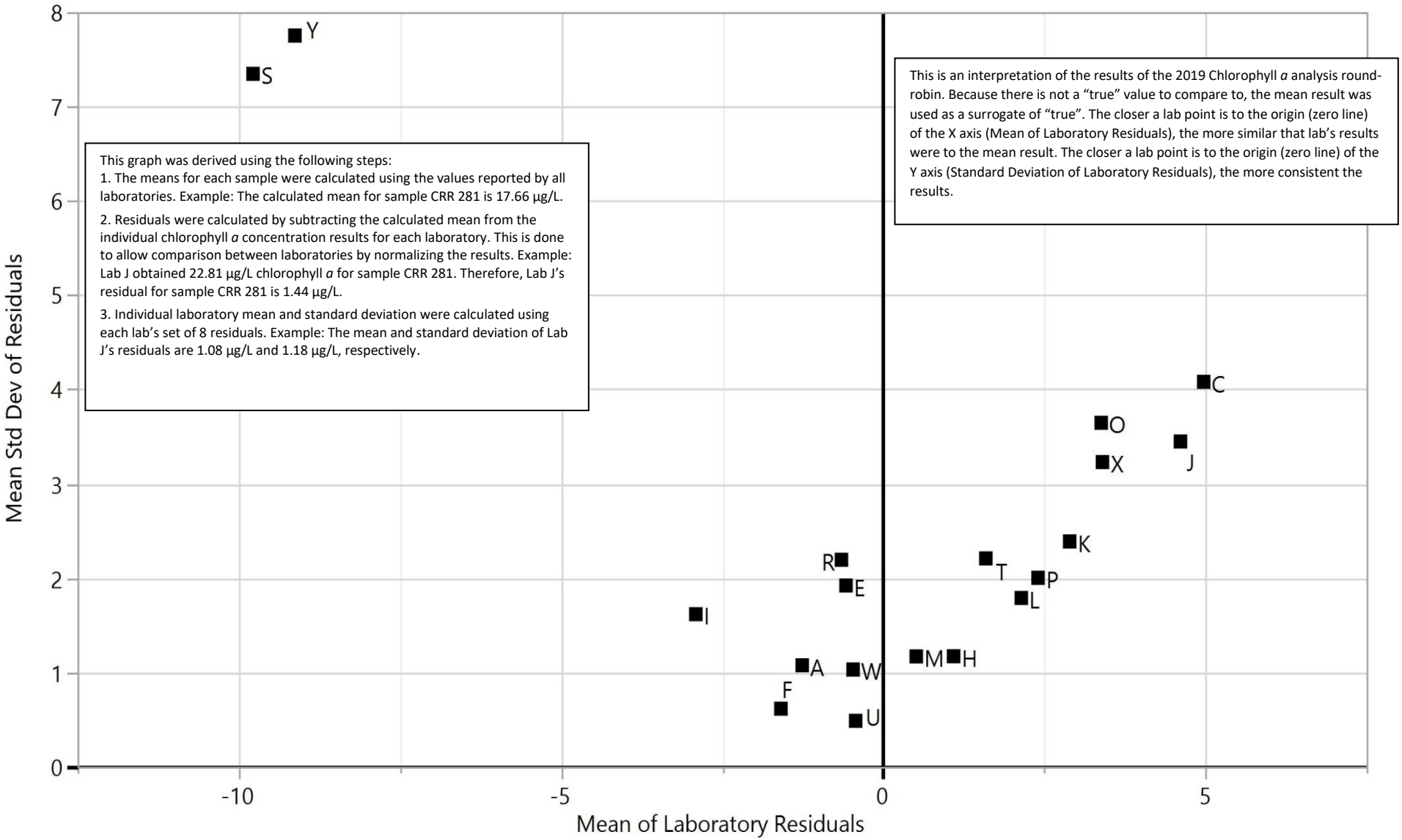
Notes:

1. Answers to the questionnaire are entered as the laboratory presented them unmodified, except for spacing and spelling.
2. Additional information obtained from participating laboratories: time samples were filtered, brand of filters, length of filtering time, make and model of instrument, instrument bandwidth(s) and wavelength(s), time between acidification and analysis by instrument, and notable differences between samples.

Chlorophyll *a* Round-Robin Box Plots of Laboratory Residuals



2019 Chlorophyll *a* Round-Robin Laboratory Residual Mean vs. Standard Deviation



This graph was derived using the following steps:

1. The means for each sample were calculated using the values reported by all laboratories. Example: The calculated mean for sample CRR 281 is 17.66 µg/L.
2. Residuals were calculated by subtracting the calculated mean from the individual chlorophyll *a* concentration results for each laboratory. This is done to allow comparison between laboratories by normalizing the results. Example: Lab J obtained 22.81 µg/L chlorophyll *a* for sample CRR 281. Therefore, Lab J's residual for sample CRR 281 is 1.44 µg/L.
3. Individual laboratory mean and standard deviation were calculated using each lab's set of 8 residuals. Example: The mean and standard deviation of Lab J's residuals are 1.08 µg/L and 1.18 µg/L, respectively.

This is an interpretation of the results of the 2019 Chlorophyll *a* analysis round-robin. Because there is not a "true" value to compare to, the mean result was used as a surrogate of "true". The closer a lab point is to the origin (zero line) of the X axis (Mean of Laboratory Residuals), the more similar that lab's results were to the mean result. The closer a lab point is to the origin (zero line) of the Y axis (Standard Deviation of Laboratory Residuals), the more consistent the results.

2019 Chlorophyll *a* Round-Robin Results

Values reported by laboratories participating in the Round-Robin, as well as the mean, median, and standard deviation (Std Dev) for each sample, are displayed in the table below, in µg/L. Acceptance ranges (PT Min and PT Max) were calculated using NELAC Proficiency Testing (PT) methods* for microbiological parameters in non-potable water.

2019 Lab ID	281	435	615	753	828	840	917	933
A	15.9	1.0	6.1	6.4	7.3	14.5	38.7	17.8
C	23.300	1.757	9.767	9.967	10.300	22.733	55.333	24.400
E	18.5	3.5	8.4	8.3	5.8	14.0	40.7	13.9
F	15.64	1.39	5.86	6.45	6.05	13.75	39.71	16.23
H	19.1	1.3	7.8	7.7	8.1	17.5	45.4	19.6
I	13.4	1.0	5.3	4.0	4.0	10.7	38.7	17.4
J	22.81	2.09	10.08	9.81	10.71	21.17	53.88	24.07
K	23.2	6.6	12.2	11.5	10.9	17.5	42.0	17.0
L	19.582	1.693	8.478	8.425	9.546	18.563	48.108	20.473
M	18.832	1.481	6.563	7.649	8.428	16.116	42.084	20.725
O	21.2	1.9	9.1	8.6	9.4	19.0	54.1	21.5
P	19.9099	1.6308	8.8089	8.4349	9.2671	19.6424	48.5485	20.7037
R	17.9	4.0	7.7	6.9	7.6	15.4	36.7	16.4
S	5.7	0.51	2.6	2.0	1.5	4.3	17	5.9
T	17.5	2.4	7.3	7.9	8.2	18.3	48.7	20.1
U	17	1.0	7.0	6.8	6.5	16	42	18
W	17.4	1.6	6.8	7.2	8.4	13.5	42.9	16.2
X	23	2	8	9	9	21	52	21
Y	5.8	1.2	1.7	3.1	3.7	6.3	16.5	6.4
Mean	18.50	1.63	7.70	7.70	8.17	16.12	42.08	18.00
Median	17.66	2.00	7.35	7.38	7.62	15.79	42.27	17.78
Std Dev	5.01	1.40	2.46	2.34	2.52	4.78	10.63	4.91
PT Min	4.98	0.31	1.68	1.91	1.67	4.24	14.83	5.34
PT Warning Low	7.44	0.54	2.68	2.93	2.69	6.43	20.72	7.83
PT Warning High	37.19	5.29	17.18	16.17	18.28	34.04	79.05	36.19
PT Max	55.61	9.35	27.34	24.78	29.52	51.64	110.47	53.06

Note: Data values are shown with significant figures as reported by laboratories.

* EPA/600/R-04/003, table available at http://nelac-institute.org/fopt.php, full document available at http://nelac-institute.org/docs/2003nelacstandard.pdf