2018 NC DWR Chlorophyll *a* Round-Robin

Currently, 38 miles and 107,221 acres of surface waters in North Carolina are impaired due to elevated levels of chlorophyll *a*, a chemical parameter used to assess algal productivity (2016 Draft 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 twelfth 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 19, 2018, 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's questions and the data from the study are found on pages 3 through 9 and 12. Statistical analyses and results of the data are presented graphically on pages 10 and 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 samples received	Temperature samples stored prior to filtering	Date samples were filtered
A	SM 10200H	7-20-18	1.2°C	1.2°C	7-20-18
B	EPA 445.0 Rev. 2.0-1997, modified option (Invitro Determination of Chlorophyll a)	7/19/2018	10 Celsius	Less than6.0 Celsius	7/20/2018
C	SM 10200H-2011	7/20/2018	0.0°C	25°C	7/20/2018
D	EPA 445.0	7/19/2018	1.4°C	4°C	7/19/2018
F	SM10200H	7/20/2018	4.3°C	0-6 degrees C	7/20/2018
H	SM 21st Ed 10200H Chlorophyll	7/20/2018	4.2 C	< 6 C	7/20/2018
J	EPA 445.0 Revision 1.3	7/20/2018	0.6	N/A	7/20/2018
K	EPA 445.0 Rev. 1.2	7/19/2018	2.0 Deg. C	4.0 Deg. C	7/19/2018
L	EPA 445.0 Rev. 1.2	7/19/2018	CRR-281 2.9°C CRR-840 2.4°C CRR-917 2.4°C CRR-435 2.9°C CRR-828 4.4°C CRR-753 4.5°C CRR-933 4.9°C CRR-615 1.3°C	0.1-4.4° C	7/20/2018
P	EPA 445.0	7/19/2018	on ice	on ice	7/19/2018
Q	EPA Method 445.0 modified	7/19/2018	5.4C	not stored, filtered immediately	7/19/2018
R	fluorometric(non-acidification)	7/19/2018	5 degrees C	4C	7/23/2018
S	US EPA method 445 (fluorescence with acid correction)	7/19/2018	2-3 degrees Celsius (Estimated)	5 degrees Celsius	7/20/2018
T	EPA 445.0, Rev 1.2 (Fluorometric)	7/19/2018	7.0°C	3.2°C	7/19/2018
U	SM 10200 H, Rev. 2011	7/19/2018	3.1C	4C Filtered Immediately	7/19/2018
W	EPA 445.0	7/19/2018	varied (see below)	4 deg C	7/26/2018
X	SM10200H Spectrophotometer	7.20.18	2.4°C	Room Temperature	7.20.18
Y	EPA Method 445.0	7/20/2018	0.3°C	4.0°C	7/20/2018
Z	Standard Methods 10200H (2011)	7/19/2018	6.4˚C	Not Stored	7/19/2018

Lab ID	Type of filters used	Brand of filters used	Pressure at which filtered	Volume of sample filtered
A	Nitrocellulose 0.45u HAWP04700	Millipore	23 in Hg	145 - 300 mL
B	Glass Microfiber	Whatman	Less than20 KPA	150 mL
C	1 micron	Whatman	50 cm Hg	100 to 480mL
D	Glass Fiber	Whatman GF/F	-5 kPa	50-100 ml
F	61631	Pall	N/A	250-300mL
H	GF/C 42.5 mm Glass Microfiber Filters	Whatman	We do not measure pressure at which samples are filtered	200-350
J	Glass Microfiber, Diameter 25 mm, CAT No. 1825-025	Whatman	5 in Hg	30 mL
K	GF/F Glass Microfiber filters 47mm	Whatman	4.0 in. Hg	150ml for all samples
L	47mm Glass Fiber GF/F	Whatman	<6 in Hg	150 - 200 mL
P	Glass Fiber	Millipore	<6 mm Hg	20 mL
Q	25 mm GF/F	Whatman	6 in Hg	50 mL
R	GF/F (glass fiber) 25mm circles	Whatman	5 in Hg	50mL
S	Glass Fiber Filter (47 mm)	Advantec	Not measured	200mL to 500mL
T	GF/C 42.5mm	Whatman	<5 in Hg	60 - 82 mL
U	A/E glass fiber 47mm	Millipore	4-6 inches of mercury	250mls
W	GF-75, 47 mm	Advantec	< 3 in Hg	100 mL
X	GF/C 47mm Glass Fiber Filters	Whatman	Not measured	250mL-500mL
Y	glass fiber	Whatman (GF/F)	≤6 in Hg	50-100 mL
Z	Glass Fiber	Whatman GF/F-0.7µm porosity, 47mm diameter	600 to 700 mbar	200ml

Lab ID	Describe filtering technique (how were sample volumes measured? were sides rinsed? etc.)
A	measured with graduated cylinder; rinsed
B	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.
C	Sample volumes were measured in class A volumetric cylinder and poured slowly into the filter funnel. Cylinder and funnel are rinsed well.
D	50mL aliquots filtered in graduated cylinder. When filtration slows, final volume recorded and sides of cylinder rinsed 3x with DI water
F	Measured with 500mL measuring cylinder. Cylinder and filter flask rinsed three times with DI water.
H	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.
J	Samples were measured and pulled using disposable volumetric pipets. Funnel and screen were rinsed with distilled water and wiped clean with Kimwipes between each sample. Filter was placed on screen, then the funnel was attached and filled with each sample before opening the vacuum valve. 20 mL produced a sufficient green stain for each sample.
K	Volume measured with graduated cylinder. Funnel was not rinsed.
L	Measured in a class A graduated cylinder, sides not rinsed
P	Volume measured in a graduated cylinder, filter funnel sides not rinsed down
Q	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)
R	volumes measured with graduated cylinder, sides of filter funnels were rinsed with deionized water
S	A cleaned graduated cylinder was rinsed with native water three times before measuring the samples.
T	Samples are vacuum filtered. The vacuum pump is connected to a manifold and the filter flask is connected to the manifold. After mixing, 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.
U	measured with a 250 ml graduated cylinder, vacuum filtered & funnel rinsed between uses
W	Measured volume in a graduated cylinder. Filtered with a hand pump. Rinsed sides of filtration unit and graduated cylinder with DI water.
X	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.
Y	measured in 100 mL glass graduated cylinder; poured into plastic filter tower; cylinder and filter towers sides both rinsed with DI water
Z	Samples were measured using a polypropylene graduated cylinder, rinsed cylinder twice with MgCO3 solution

Lab ID	Homogenization technique prior to filtering	How long were samples filtered?	Lighting conditions during filtering
A	container shaken	10 minutes	low amber light
B	Gently shake bottle several times	all samples are filtered for less than 10min. If it takes longer a smaller volume is used.	Analysis takes place in a dark room with green lighting.
C	Samples are shaken well before filtering.	7 to 21 mins	Adequate and bright room lighting
D	Samples inverted 4 times	Up to 6 minutes	Darkened Room with Red Light
F	Shaken	~1min	Fluorescent lighting
H	mix sample volume by inversion of sample container 7 times.	4-28 minutes	normal/ subdued lighting of room
J	Placed on magnetic stir plate with small stir bar, stirred for two minutes before pulling sample with pipet.	30 sec	Dimmed, no outside light
K	shake vigorously several times	1 - 3 minutes	dark room with green lights
L	Sample bottle inverted by hand for 5-10 seconds	< 10 minutes	Dark room with subdued green LED lighting
P	Gentle shaking for 10 secs	10-30 seconds	low ambient light from windows
Q	briskly inverted bottle ~10 times	1-5 minutes	lights turned off, blinds closed
R	n/a	3-5 minutes	ambient light from window
S	The sample bottles were gently inverted numerous times for 15 seconds prior to filtering.	Between 2-10 minutes.	Under fluorescent lighting in the laboratory
T	Samples gently inverted 10 times	5 - 10 sec	All overhead lights off, two small lamps with 25 watt green bulbs.
U	Shaken	approx. 5 mins	Darkroom w/ green light
W	Gently inverted the bottle several times	1-2 minutes	Overhead fluorescent lights
X	Sample bottle is shaken vigorously by hand before filtration.	2min 27sec - 4min 7sec	Filtration is done under regular overhead lighting. (Intensity Range 20-30 ft-candles)
Y	shaking bottles several times prior to each aliquot measured	about 30-60 seconds per sample	dimmed fluorescent (25% of full lab lighting)
Z	Gently invert sample 4 to 6 times	1 to 2 minutes	lights off, blinds down and closed, door closed

Lab ID	Extraction solvent, purity, and volume used	Length of time samples were stored after filtering	Steeping time	Was grinding used?
A	90% acetone 10 mL	96 hr	96 hr	yes
B	25 mL of 90% Acetone	6 days	22:30	Yes
C	90% Acetone	12 days	17 hours	Yes
D	90% Acetone, Type 1 Water	13 days	18.5 hours	Yes
F	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.	7 days	2.5hrs	yes
H	5 mL of 90% acetone (HPLC grade)/ 10% saturated MgCo3	~4 days	~3.5 hours	yes
J	90% Acetone, 10mL	4 days	20 hrs 20 minutes	Yes
K	90% Acetone, Baker analyzed-ACS reagent grade, 25ml	N/A; samples were extracted immediately after filtering	23 hrs.	Yes
L	90% Acetone, Optima grade, 25 mL	5 days	22 hours	Yes
P	Acetone,90%, 10 mL	13 days	3.5 hrs	yes
Q	90% reagent grade acetone	40 days	24h	yes
R	90% Acetone: 10% water 7.5mL for each sample	1 day	24 hours	yes
S	10 mL of 90% acetone are used for extraction	24 days	Less than 24 hours	Yes
T	90% acetone, Fisher Scientific Certified ACS, 14mL	18 days	21 hours	yes
U	90%acetone 10% deionized water Purity = 99.7% @ 10 mls used	N/A	Overnight 2pm to 1:30pm	yes
W	90 % Acetone/ 10 % Water Solution	11 Days	Approx. 22 hrs	Yes
X	90% HPLC Grade acetone and 10% $MgCO_3$ solution. Extract has a final volume between 8 and 14 mL.	6 days 2 hrs 30 mins	17 hrs 10mins	yes
Y	90% HPLC-grade acetone, 10 mL	13 days	20 hours	yes
Z	90:10 Acetone: Saturated	14 Days	20 hours	yes

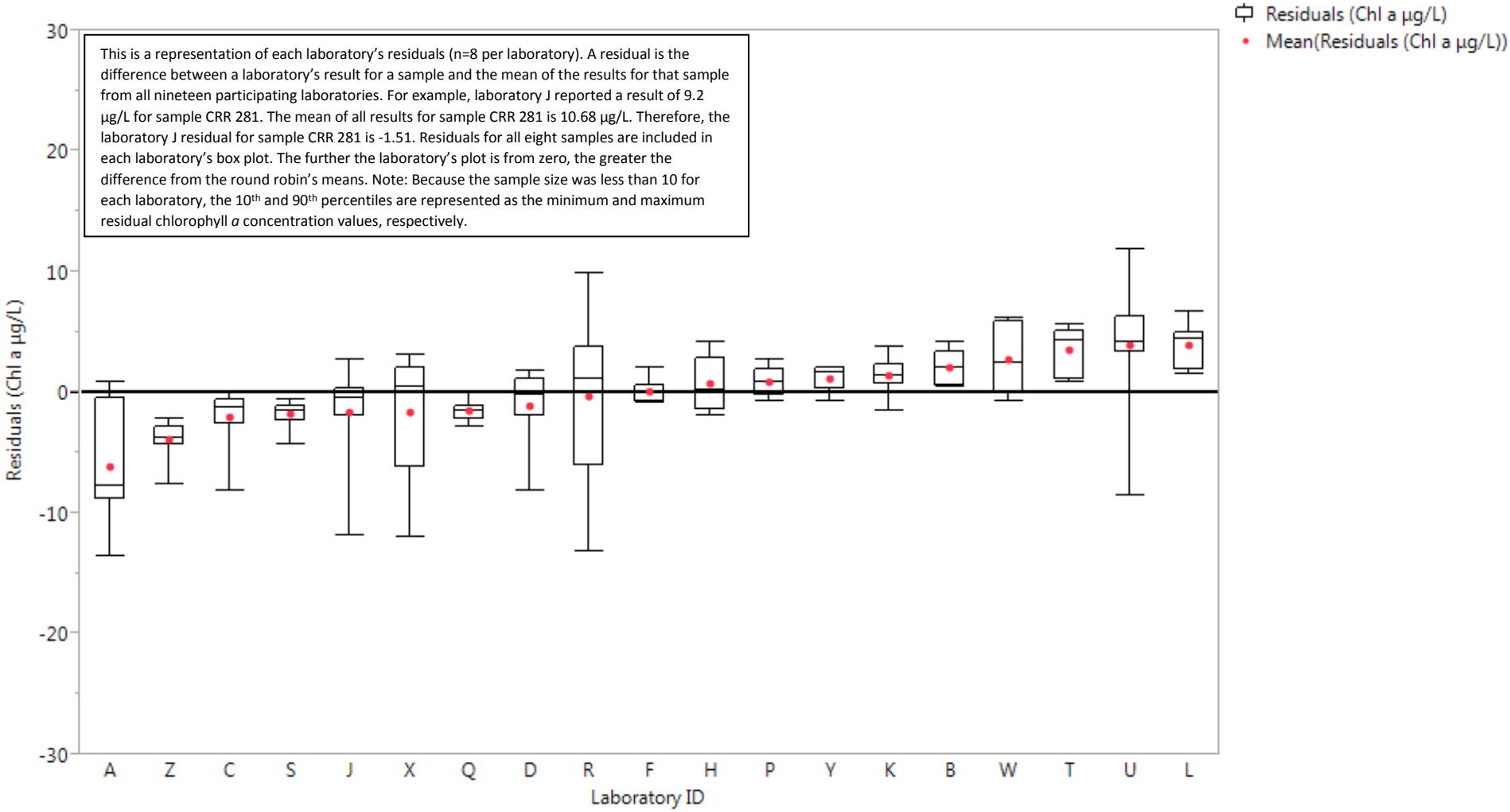
Lab ID	Description of grinding setup
A	high speed rotating glass macerator
B	Tissue grinder tip on a drill press.
C	Samples are ground using a teflon tip in a glass test tube for 1 minute with 3mL of 90% Acetone solution. Then sample was transferred into a 25mL screw top centrifuge tube with an additional 7mL of 90% Acetone. Grinding occurs at room temp and was not temperature controlled.
D	Tissue Grinder. Sample in plastic centrifuge tube. Temperature controlled to prevent evaporation
F	Drill press with a teflon grinding tip. Not temperature controlled, ambient temp.
H	Tissue grinder with stainless steel tip, in 15 mL vial, not temperature controlled
J	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: 21.6 C
K	Teflon pestle with radial serrations on tip; temperature controlled by touch/feel
L	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	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.
Q	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
R	samples were poured into 100mL graduated cylinder and then into filter manifold. The cylinder and manifold was rinsed with deionized water between each sample
S	Grinding is done with a T Line Laboratory Stirrer (model 104) and a serrated PTFE pestle. Temperature was not controlled. Grinding is performed quickly to minimize warming.
T	Pro Scientific homogenizer with stainless steel saw tooth generator, glass grinding vessel, temperature was not controlled, transferred to 15mL graduated centrifuge tube to steep in refrigerator
U	Arrow 850 motor 1/10hp Kontes tissue grinder pestle SZ24 and matching tube. No temperature control.
W	Drill with a Teflon® pestle with grooves, 50-mL polypropylene conical tubes
X	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.
Y	teflon grinding pestle attached to rotor; glass test tube; temperature controlled by feel
Z	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

Lab ID	Were samples acidified? If so, what type, concentration, and volume?	Type of calibration standard and source
A	yes	Turner Designs
B	not acidified	Make a calibration curve standards from a stock solution
C	Yes, with 0.1mL of 0.1N HCl	N/A
D	no	Turner Designs Chlorophyll A and B Standard
F	0.1mL of 0.1N HCL	N/A
H	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)
J	No	Commercially Prepared Chloropyll a Std Turner Designs
K	No	Fluorometric Chlorophyll standard, Turner Designs
L	Not Applicable	Turner Designs Fluorometric Chlorophyll Standard
P	no	Pure Chla in 90% acetone, from Anacystis, Sigma Chemical
Q	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.
R	no	chla pigment standard (sigma aldrich)
S	Yes: 0.15 mL of 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
T	No	Chlorophyll a from Anacystis nidulans, Sigma C6144
U	N/A	N/A
W	0.1 N HCl solution, 90uL to 3.0ml of sample	Chlorophyll a free of chlorophyll b Neat, Sigma
X	Samples are acidified with 100 uL of 0.1 N HCl, mixed with a mixing paddle, and timed for 90 seconds.	A 0.20 mg/L concentration of chlorophyll-a standard is read at the beginning of each batch. The standard is made from Sigma Chlorophyll-a from spinach 5 mg powder. The standard read at 102% and 105% recovery.
Y	no	chlorophyll a from Anacystis (Sigma C6144); chlorophyll a from spinach (Sigma C5753)
Z	Yes, 0.1N HCL used 0.1 ml per 3ml of extract	Turner Designs P/N 10-950 (20ml ampule of known concentration)

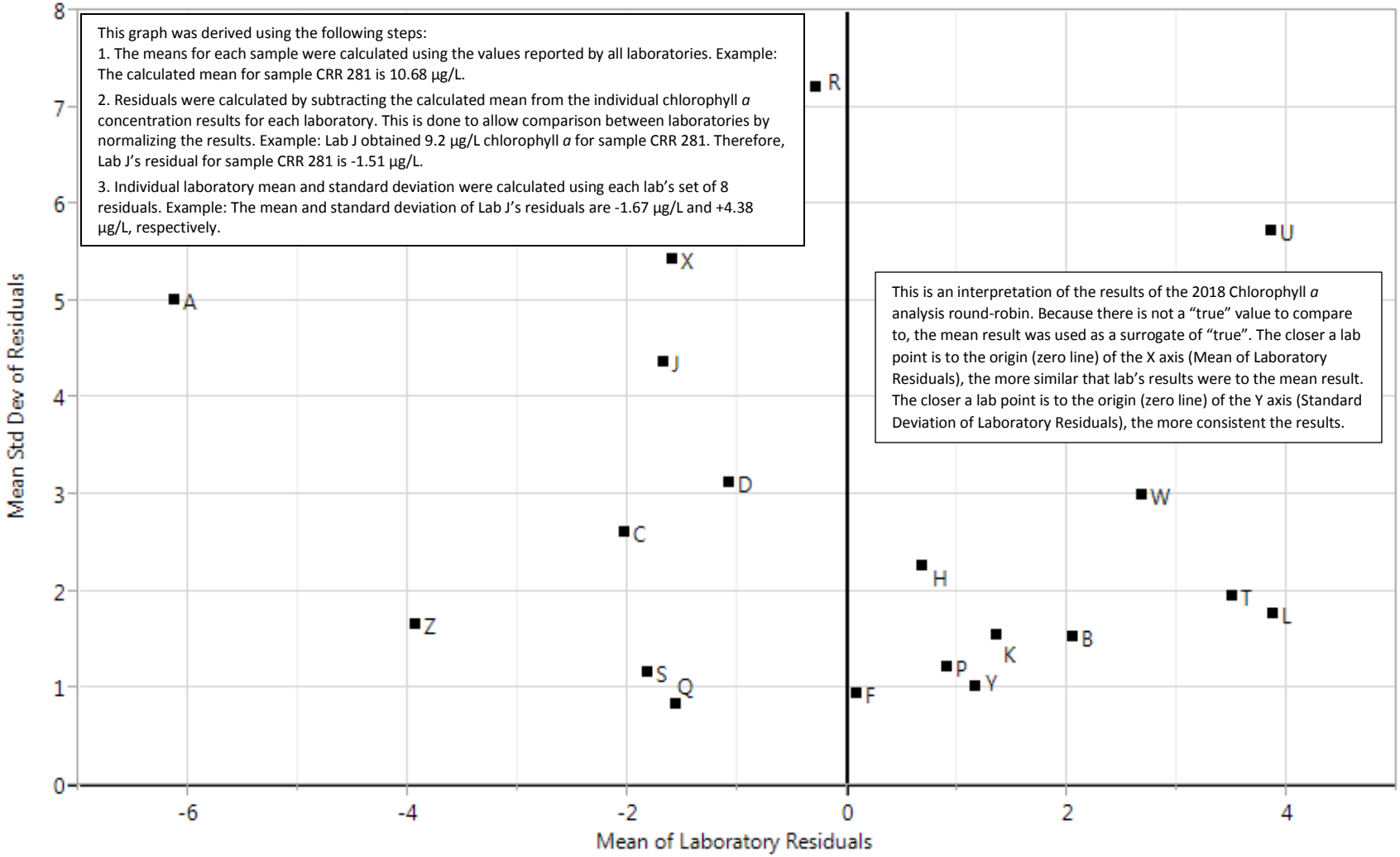
Notes:

1. Answers to the questionnaire are entered as the laboratory presented them unmodified, except for spacing.
2. Additional information obtained from participating laboratories: time samples were filtered, 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



2018 Chlorophyll *a* Round-Robin Laboratory Residual Mean vs. Standard Deviation



2018 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.

Lab ID	CRR 281	CRR 435	CRR 615	CRR 753	CRR 828	CRR 840	CRR 917	CRR 933
A	11.6	11.3	21.1	25.6	28.7	16.0	11.7	20.7
B	11.090	24.380	31.577	37.340	37.947	21.074	11.380	37.360
C	10.1	18.7	28.5	31.0	36.4	19.5	9.08	26.2
D	10.7	22.0	29.8	31.8	28.8	20.1	11.1	32.8
F	10.8	19.4	30.5	34.0	37.7	20.2	10.3	33.5
H	9.78046	22.7071	32.5738	36.918	36.8368	18.8467	8.86474	34.7011
J	9.2	20.6	16.5	31.83	36.0	20	10.77	37.0
K	12.34	21.28	32.22	36.51	35.36	21.58	12.38	34.94
L	12.2	24.7	33.5	38.2	43.6	23.1	12.5	38.9
P	11	23	30	36	38	20	10	35
Q	9.2	20.14	27.16	31.77	34.06	19.42	9.25	32.19
R	10.9	24.3	20.3	20.74	39.6	22.4	10.97	44.2
S	9.0	19.2	27.2	33.3	35.7	18.4	8.4	30.0
T	11.5	25.2	32.4	39.0	42.6	22.6	11.6	38.9
U	14.3	11.7	34.5	37.7	48.8	24.9	17.1	37.6
W	10.597	24.387	27.661	39.071	42.982	21.219	10.679	40.532
X	11	12	31	37	25	21	11	35
Y	11	22	30	36	39	21	10	36
Z	6.7	17.4	24.0	30.1	34.7	16.7	8.0	26.7
Mean	10.68	20.23	28.45	33.89	36.94	20.45	10.79	34.33
Median	10.90	21.28	30.00	36.00	36.84	20.47	10.77	35.00
Std Dev	1.57	4.41	4.84	4.77	5.59	2.12	1.99	5.50
PT Min	6.60	9.16	15.67	20.80	22.75	14.79	6.38	19.94
PT Warning Low	7.72	11.82	19.02	24.39	26.64	16.45	7.57	23.79
PT Warning High	14.45	32.76	41.20	46.06	50.06	25.15	14.96	48.19
PT Max	16.90	42.27	49.98	54.00	58.62	27.97	17.74	57.49

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