2017 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 eleventh chlorophyll *a* round-robin. Twenty laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh-area waterbodies.

Methodology

Sample Collection

On July 26, 2017, 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 twenty 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
ALS Environmental – Rochester
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	Standard Methods 10200H (2011)	7/26/2017	on ice	Not Stored	7/26/2017
B	SM 10200H-2001	7/27/2017	3.7°C	25°C	7/27/2017
C	Standard Methods 10200H 2b - 2011	7-27-17	1.7	1.7	7/27/2017
D	EPA 445.0	7/26/2017	on ice	on ice	7/26/2017
F	SM10200H-2011	7/26/2017	3.1C	<4C (@2hrs.)	7/26/2017
G	SM 10200H	7/27/2017	3.4 C	Not stored	7/27/2017
H	Fluorometric (non-acidification)	7/26/2017	4 degrees C	4 C	7/27/2017
J	US EPA method 445 (fluorescence with acid correction)	7/28/2017	Temperature was not measured, but there was still plenty of ice in the cooler when they arrived	Samples were filtered immediately.	7/28/2017
K	EPA 445.0, Rev. 1.2 (Fluorometric)	7/26/2017	7.6°C	2.6°C	7/26/2017
L	EPA 445.0	7/26/2017	varied (see below)	4 deg C	7/26/2017
P	EPA 445.0	7/26/2017	4.6°C	filtered immediately following receipt	7/26/2017
Q	EPA Method 445.0	7/27/2017	1.7 °C	4.0°C	7/27/2017
R	EPA Method 445.0 modified	7/26/2017	6 C	not stored, filtered immediately	7/26/2017
S	SM10200H Spectrophotometer	7.27.17	1.9° C	Room temperature	7.27.17
T	EPA 445.0 Rev. 1.2	7/26/2017	2.5 Deg. C	Samples were filtered immediately upon receipt	7/26/2017
U	SM10200H	7/27/2017	2.3°C	0-6 degrees C	7/27/2017
W	EPA 445.0 Rev. 1.2	7/26/2017	5.7° C	0.1-4.4° C	7/26/2017
X	SM 21st Ed 10200H Chlorophyll	7/26/2017	5.0C	< 6 C	7/26/2017
Y	EPA 445.0 Revision 1.3	7/27/2017	1.0° C	N/A	7/27/2017
Z	EPA 445.0 modified option (invitro Determination of Chlorophyll a)	7/26/2017	14 degrees C	analyst reported that temp. were high at receiving (warm about 14 Celsius) Likely temp elevated during samples processing as it has been received at 12:07 on WSC&S form.	7/26/2017

Lab ID	Type of filters used	Brand of filters used	Pressure at which filtered	Volume of sample filtered
A	Glass Fiber	Whatman GF/F-0.7µm porosity, 47mm diameter	600 to 700 mbar	150 to 200 ml
B	1 micron	Whatman	50 cm Hg	100 to 480mL
C	HAWP 4700 Membrane	Millipore	not measured	120-390 mL
D	Glass Fiber	Millipore	<6 mm Hg	25 mL
F	A/E glass fiber 47mm	Millipore	4-6 inches of mercury	Samples CRR 671, 201, & 966 250 mls. Samples CRR 298, 322, 902, 133, 565 150 mls
G	47 mm Diameter 0.70 µm Pore size	Whatman GF/F Part number 1825047	Not Measured	100mL
H	GF/F (glass fiber) 25mm circles	Whatman	5 inches Hg	50mL (attached on data sheet next tab)
J	47-mm glass fiber filter (GF/F)	Whatman	Not measured	100-500 mL
K	GF/C 42.5 mm	Whatman	<5 in Hg	42-189 mL
L	GF-75, 47 mm	Advantec	< 3 in Hg	100 mL
P	Glass Fiber	Whatman GF/F	-5 kPa	100 ml
Q	glass fiber	Whatman (GF/F)	≤6 in Hg	50-200 mL
R	25 mm GF/F	Whatman	6 in Hg	50 mL
S	GF/C	Whatman	Not Measured	190-500 mL
T	GF/F Glass Microfiber filters 47mm	Whatman	4.5 in. Hg	150ml
U	61631	Pall	N/A	100-500mL
W	47mm Glass Fiber GF/F	Whatman	<6 in Hg	100 mL – 200 mL filtered
X	GF/C 42.5 mm Glass Microfiber Filters	Whatman	We do not measure pressure at which samples are filtered	150-500
Y	Glass Microfiber, Diameter 25 mm, CAT No. 1825-025	Whatman	5 in Hg	20 mL
Z	glass microfiber	Whatman	Less than 20 KPA	AC41508 and AC41510 filtered 70ml. All others at 150ml

Lab ID	Describe filtering technique (how were sample volumes measured? were sides rinsed? etc.)
A	Samples were measured using a polypropylene graduated cylinder, rinsed cylinder twice with MgCO3 solution
B	Sample volumes were measured in class A volumetric cylinder and poured slowly into the filter funnel. Cylinder and funnel are rinsed well.
C	volume measured by graduated cylinder, sides on filtration apparatus rinsed
D	Volume measured in a graduated cylinder, filter funnel sides not rinsed down
F	measured with 250 ml graduated cylinder, vacuum filtered, cylinder and funnel rinsed between uses
G	Sample volume measured in graduated cylinder. Poured onto filter. Grad cylinder rinsed and poured onto filter. After filtration, filter folded in half twice and wrapped in aluminum foil and placed in plastic bag with label prior to storage.
H	volumes measured with graduated cylinder, sides of filter funnels were rinsed with deionized water
J	Sample volume was measured with a graduated cylinder, rinsed with Organic blank water
K	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.
L	Measured volume in a graduated cylinder. Filtered with a hand pump. Rinsed sides of filtration unit and graduated cylinder with DI water.
P	50mL aliquots filtered in graduated cylinder. When filtration slows, final volume recorded and sides of cylinder rinsed 3x with DI water
Q	measured in 100 mL glass graduated cylinder; poured into plastic filter tower; cylinder and filter towers sides both rinsed with DI water
R	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)
S	After being mixed, sample is poured into a 500 mL Class A graduated cylinder to be measured before filtration. Sample is vacuum filtered as quickly as possible. When filtration is nearing the end, 1-2 mL saturated MgCO3 solution is added. Funnel is rinsed thoroughly with DI Water. Filters are folded and wrapped in aluminum foil. Cylinder is thoroughly rinsed after each sample with DI water.
T	Volume measured with graduated cylinder. Funnel was not rinsed.
U	Measured with 500mL measuring cylinder. Cylinder and filter flask rinsed three times with DI water.
W	Measured in a class A graduated cylinder, sides not rinsed
X	500 mL of sample measured in class A graduated cylinder. Sample poured slowly to determine greatest volume that will filter within 20 minutes. If less than 500 mL filtered, volume calculated by subtracting amount remaining in cylinder. Sides of filtration cups not rinsed.
Y	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.
Z	volume measured using class A graduated cylinder, and poured through a filter using a vacuumed. Cylinder is rinsed, sides of filter funnel are rinsed.

Lab ID	Homogenization technique prior to filtering	How long were samples filtered?	Lighting conditions during filtering
A	Gently invert sample 4 to 6 times	1 to 2 minutes	Lights off, blinds down and closed, door closed
B	Samples are shaken well before filtering.	7 to 21 mins	Adequate and bright room lighting
C	shaking	10 min	low level amber light
D	Gentle shaking for 10 secs	10-30 seconds	low ambient light from windows
F	Shaken	Samples CRR 671, 201, & 966 @ 8 mins. Samples CRR 298, 322, 902, 133, & 565 <5 mins	Darkroom w/green light
G	Shaking sample bottle	~30second	Ambient lab lighting, in hood with light off.
H	n/a	3 to 7 minutes	Sunlight through the windows, lab lights were turned off
J	Bottles were gently inverted numerous times	Most samples took from 2-10 minutes	Under fluorescent lighting in the laboratory
K	Samples gently inverted 10 times	5 – 10 sec	All overhead lights off, two small lamps with 25 watt green bulbs.
L	Gently inverted the bottle several times	1-2 minutes	Overhead fluorescent lights
P	Samples inverted 4 times	Up to 6 minutes	Darkened Room with Red light
Q	shaking bottles several times prior to each aliquot measured	about 30-60 seconds per sample	dimmed fluorescent (25% of full lab lighting)
R	briskly inverted bottle ~10 times	1-5 minutes	lights turned off, blinds closed
S	Sample bottle is vigorously shaken by hand before filtration.	02'48"-11'08"	Filtration is done with regular overhead lighting. (Intensity Range 20-30 ft-candles)
T	shake vigorously several times	1 – 4 minutes	Dark room with green lights
U	Shaken	~1min	Fluorescent lighting
W	Sample bottle inverted by hand for 5-10 seconds	1-8 minutes	Dark room with subdued green LED lighting
X	mix sample volume by inversion of sample container 7 times	4-25 minutes	normal/ subdued lighting of room
Y	Gently inverted 3-4 times, placed on magnetic stir plate with small stir bar, stirred for 30-45 seconds before pulling sample with pipet	30 sec	Dimmed, no outside light
Z	shake bottle several times before measuring volume	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

Lab ID	Extraction solvent, purity, and volume used	Length of time samples were stored after filtering	Steeping time	Was grinding used?
A	90:10 Acetone: Saturated	13 Days	3 hours	yes
B	90% Acetone	12 days	17 hours	Yes
C	90% acetone; 10 mL	6 hr	6 hr	yes
D	Acetone,90%, 10 mL	12 days	5 hrs 40 min	yes
F	90% Acetone 10% deionized water. Purity =99.7% @ 10 mls used	Overnight @ 15.5 hours	Overnight 9AM 07/27/17 to Noon 07/28/17	yes
G	acetone:DI (90:10)	14-15 days	2 or more hours	yes
H	90% Acetone : 10% water 7.5mL for each sample	3 days	24 hours	yes
J	10 mL of 90% acetone are used for extraction	20 days	~16 hours	yes
K	90% acetone, Fisher Scientific Certified ACS, 14mL	8 days	21 hours	yes
L	90 % Acetone/ 10 % Water Solution		Approx: 22 hrs	Yes
P	90% Acetone, Type 1 Water	7 days	19.7 hours	Yes
Q	90% HPLC-grade acetone, 25 mL	21 days	20 hours	yes
R	90% reagent grade acetone	33 days	24h	yes
S	90% Acetone with 10% $MgCO_3$ solution. Extract has a final total volume of 8 mL.	5 days 14 hrs 10 mins	4 hrs 25 mins	Yes
T	90% Acetone, Baker analyzed-ACS reagent grade, 25ml	6 days	22 hrs.	Yes
U	90/10 Acetone/$MgCO_3$. The acetone is chromatography grade and the $MgCO_3$ is reagent grade and filtered through a 0.45um filter. 10mL of Acetone/$MgCO_3$ solvent was used to extract the sample.	4 days	3hrs	yes
W	90% Acetone, Optima grade, 25 mL	7 days	Approximately 15 hours, 19 minutes	Yes
X	5 mL of 90% acetone (HPLC grade)/ 10% saturated $MgCO_3$	~13 days	~13 hours	yes
Y	90% Acetone, 10mL	12 days	3 hrs 5 minutes	Yes
Z	25ml of 90% acetone	6 days	18:15	yes

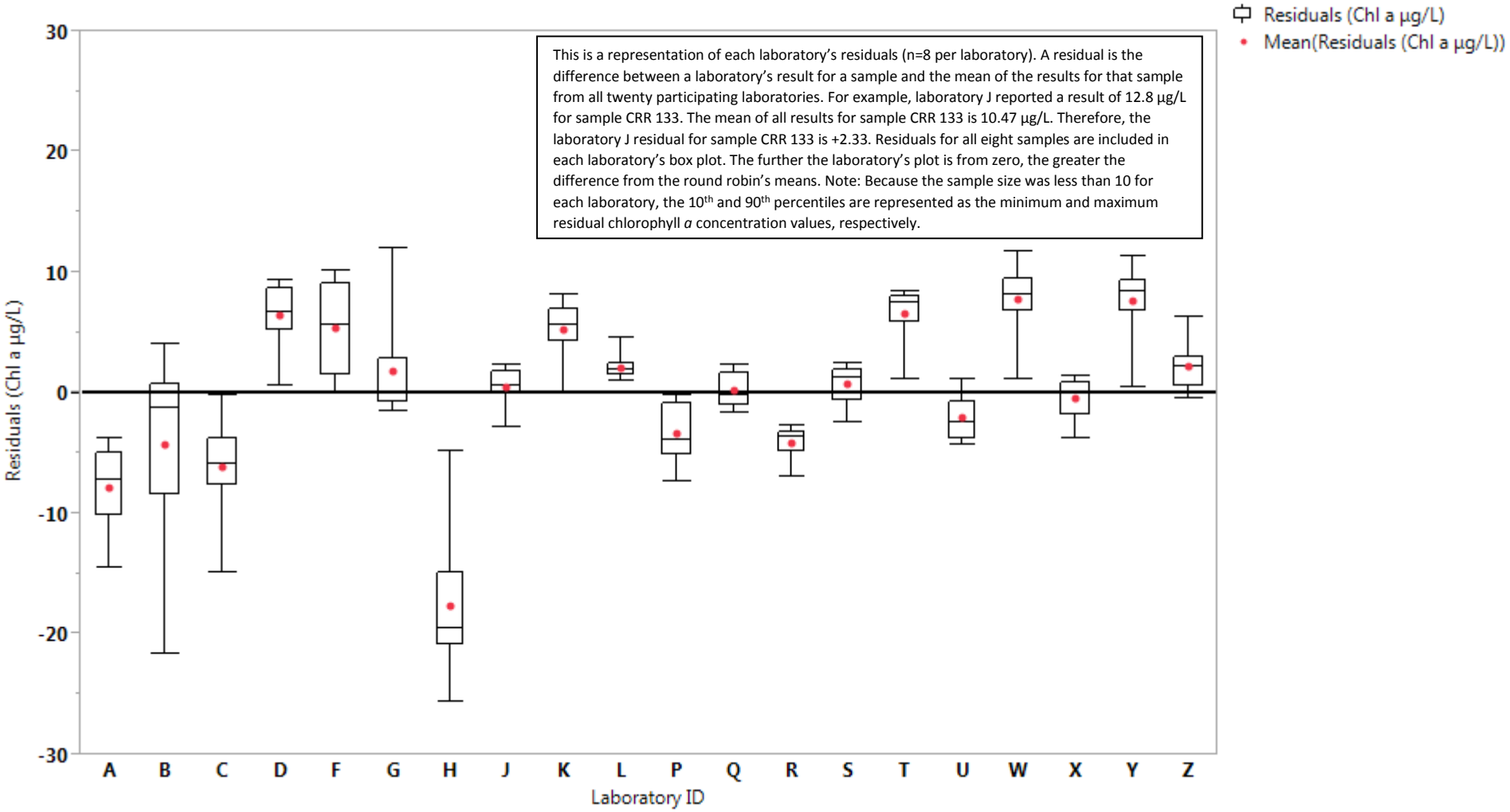
Lab ID	Description of grinding setup
A	Tear filters into quarters and place into grinding tube (Kimble #886000-0023) with PTFE grooved tip; samples macerated using electric lab mixer (Arrow engineering - Model 1750); temperature not controlled but grinding time was short to avoid heating of the sample
B	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.
C	high speed tissue grinder in 1 inch diameter test tube
D	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.
F	Arrow 850 motor 1/10 hp Kontes tissue grinder pestle SZ24 and matching tube. No temperature control.
G	Tissue Grinder with Teflon tip in glass vessel (wrapped in foil). Temperature not controlled. Slurry transferred to centrifuge tube.
H	samples were poured into 100mL graduated cylinder and then into filter manifold. The cylinder and manifold was rinsed with deionized water between each sample
J	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.
K	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
L	Drill with a Teflon® pestle with grooves, 50-mL polypropylene conical tubes
P	Tissue Grinder. Sample in plastic centrifuge tube. Temperature controlled to prevent evaporation
Q	teflon grinding pestle attached to rotor; glass test tube; temperature controlled by feel
R	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
S	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. Samples are steeped in refrigerator.
T	Teflon pestle with radial serrations on tip; temperature controlled by touch/feel
U	Drill press with a teflon grinding tip. Not temperature controlled, ambient temp.
W	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.
X	Tissue grinder with stainless steel tip, in 15 mL vial, not temperature controlled
Y	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
Z	tissue grinder tip on a drill press.

Lab ID	Were samples acidified? If so, what type, concentration, and volume?	Type of calibration standard and source
A	Yes, 0.1N HCL used 0.1 ml per 3ml of extract	Turner Designs P/N 10-950 (20ml ampule of known concentration)
B	Yes, with 0.1mL of 0.1N HCl	N/A
C	acidified for pheophytin with 10 uL of 1N HCl per 3 mL in cuvette	Turner Designs
D	no	Pure Chla in 90% acetone, from Anacystis, Sigma Chemical
F	No	N/A
G	0.06 mL 0.1N HCl into a 2 mL extract. Measured with and without acidification.	Initial Calibration: Turner Designed foil wrapped sealed ampules at nominal concentrations of 20 and 200 ug/L, diluted as needed for a range of 4-200 ug/L. Daily cal check: Solid Secondary Standard Turner P/N 8000-952
H	no	chla pigment standard (sigma aldrich)
J	Yes: 0.15 mL of 0.1 N HCl	A Solid Secondary standard P/N 8000-952 from Turner Designs is used each time to check the machine performance.
K	No	Chlorophyll a from Anacystis nidulans, Sigma C6144
L	0.1 N HCl solution, 90 uL to 3.0 ml of sample	Chlorophyll a free of chlorophyll b Neat, Sigma
P	no	Turner Designs Chlorophyll A and B Standard
Q	no	chlorophyll a from Anacystis (Sigma C6144); chlorophyll a from spinach (Sigma C5753)
R	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.
S	Samples are acidified with 100 uL of 0.1 N HCl, mixed with a mini-mixer, 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 (Cat# C5753-5MG). The standard read at 102% and 105% recovery.
T	No	Fluorometric Chlorophyll standard, Turner Designs
U	0.1mL of 0.1N HCL	N/A
W	Not Applicable	Turner Designs Fluorometric Chlorophyll Standard
X	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)
Y	No	Commercially Prepared Chlorophyll a Std Turner Designs
Z	not acidified	Make a calibration curve from a stock solution

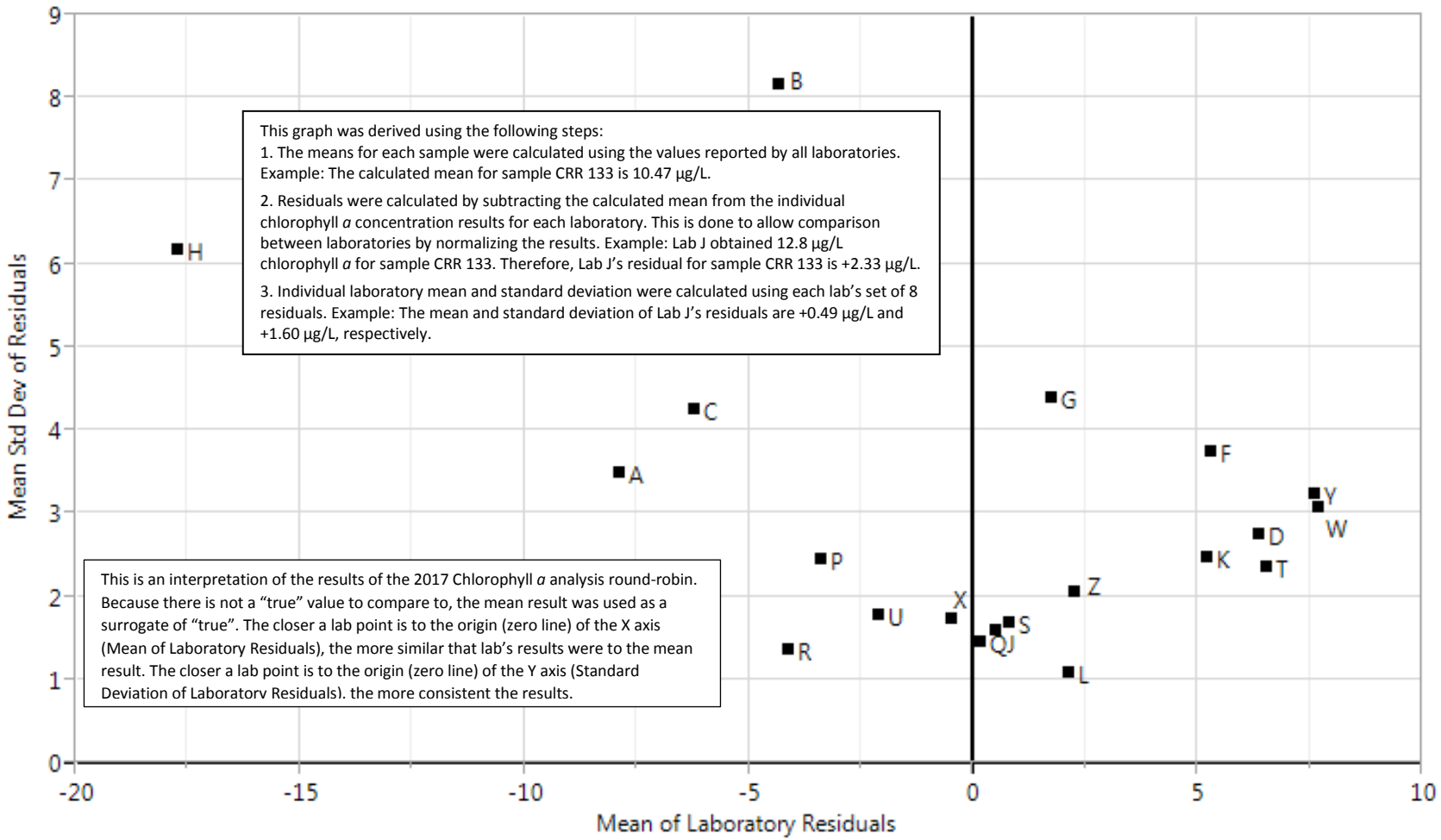
Notes:

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



2017 Chlorophyll *a* Round-Robin Laboratory Residual Mean vs. Standard Deviation



2017 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 133	CRR 201	CRR 298	CRR 322	CRR 565	CRR 671	CRR 902	CRR 966
A	6.7	24.7	29.4	28.0	38.7	21.4	25.4	18.7
B	8.4	33	44	32	26	21	23	34
C	10.3	28.9	32.4	25.0	32.8	22.5	27.9	26.7
D	11	40	49	39	57	34	37	40
F	20.6	32.5	45.8	39.2	57.5	31.6	37.5	33.9
G	11.0	35.7	39.2	31.8	59.6	27.2	30.5	35.1
H	5.6	12.2	19.5	13.7	22	13.4	15.5	12.3
J	12.8	29.7	42.0	33.6	47.4	28.3	32.3	33.9
K	10.4	37.8	47.3	38.2	55.8	33.2	37.7	37.3
L	12.481	35.156	41.522	34.244	52.188	29.232	32.981	35.17
P	9.9	25.2	36.5	28.1	47.4	22.4	30.5	28.9
Q	9.5	34	39	32	46	30	32	35
R	7.77	28.82	33.01	27.88	42.68	24.59	28.52	29.75
S	9.4	35	41	30	49	29	34	35
T	11.60	38.73	47.93	40.52	54.85	33.55	40.43	40.89
U	9.1	29.5	37.7	29.9	43.4	27.2	28.1	34.3
W	11.6	41	47.4	40.4	59.4	34.4	40.5	43.1
X	10.3302	32.5782	36.1812	30.2582	48.4135	29.0998	31.3624	34.0949
Y	10.9	41.4	47.9	41.40	59.0	34	41.50	40.6
Z	10	35	43	34	54	28	34	36
Mean	10.47	32.54	39.98	32.46	47.66	27.72	32.03	33.23
Median	10.32	33.50	41.26	32.00	48.71	28.65	32.15	34.65
Std Dev	2.99	6.79	7.44	6.58	10.88	5.50	6.39	7.32
PT Min	4.61	14.14	20.35	15.17	20.27	13.62	15.78	13.48
PT Max	22.24	70.73	75.47	66.09	105.20	53.96	62.17	76.71

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