The Evaluation of Laboratory and Field Methods to Quantify Chlorophyll-a

- 2026 Surface Water Quality Assessment Advisory Work Group Meeting

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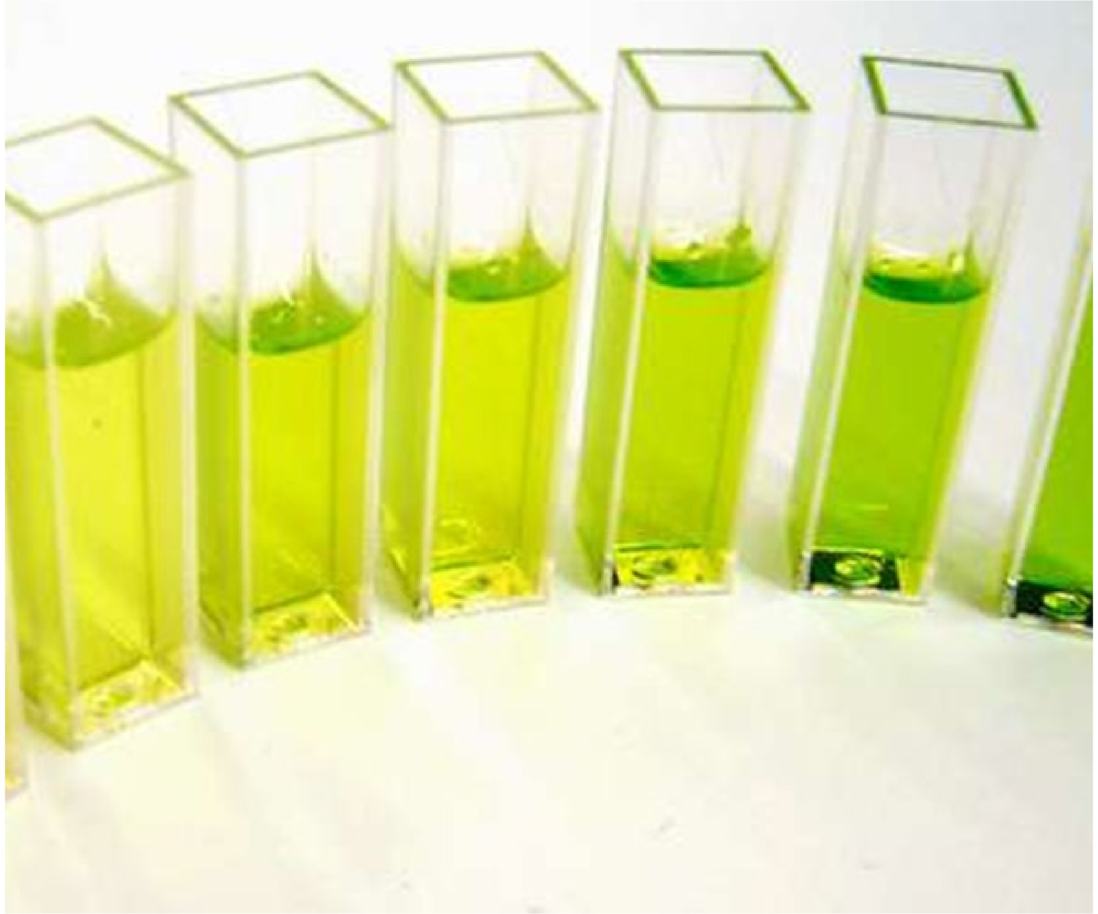
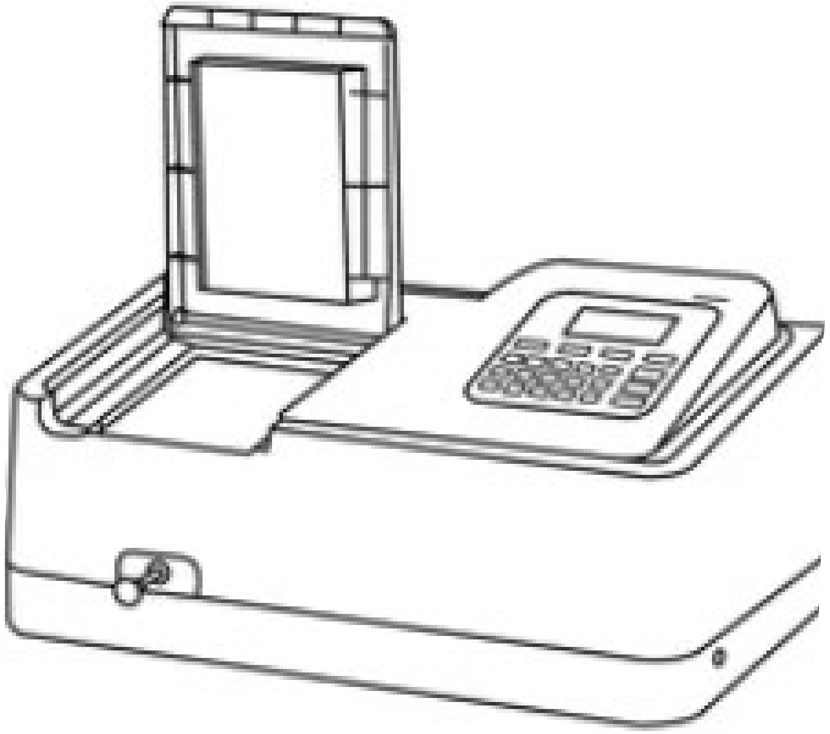
Background

- Chlorophyll-a (Chl-a) can be used to identify changes in water quality conditions due to nutrient enrichment and as a measure of eutrophication in lakes and reservoirs
- Chl-a is part of the SWQM Program routine monitoring
- Historically, TCEQ has received Chl-a data from several laboratories who use either spectrophotometric or fluorometric methods
  - Fluorometric considered to be more sensitive = lower detection limits
  - Spectrophotometric less sensitive, but considered to be more accurate at higher concentrations



Phase 1 – Laboratory Methods



Spectrophotometric method with acidification

- Chl-a, corrected for pheophytin
- EPA 446.0 or SM 10200 H.2.b.

Conventional fluorometric method with acidification

- Chl-a, corrected for pheophytin
- EPA 445.0 or SM 10200 H.3.

Modified fluorometric method

- Chl-a, free of pheophytin
- Using special, narrow-bandpass filters to eliminate spectral interference from pheophytin and chlorophyll-b
- EPA 445.0

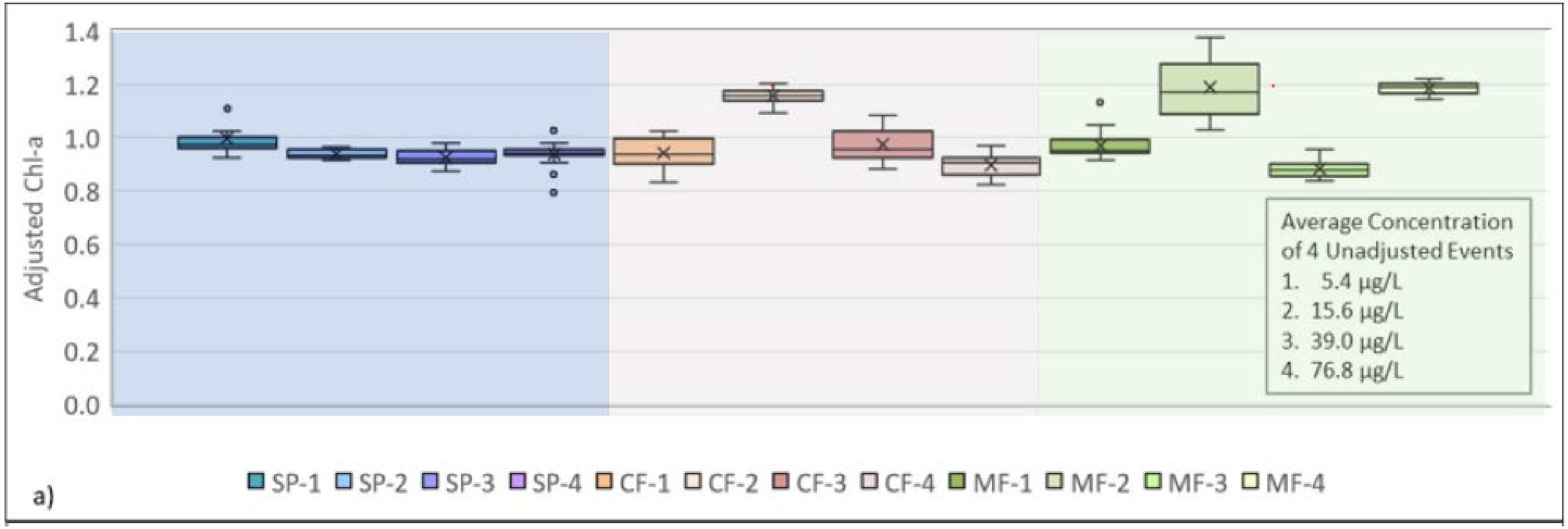
Phase 1 – Evaluation of Methods

- Laboratory-prepared samples and ambient water samples representing a range of Chl-a concentrations were analyzed by 7 participating Texas environmental laboratories.
- Both intra- and interlaboratory variation was examined

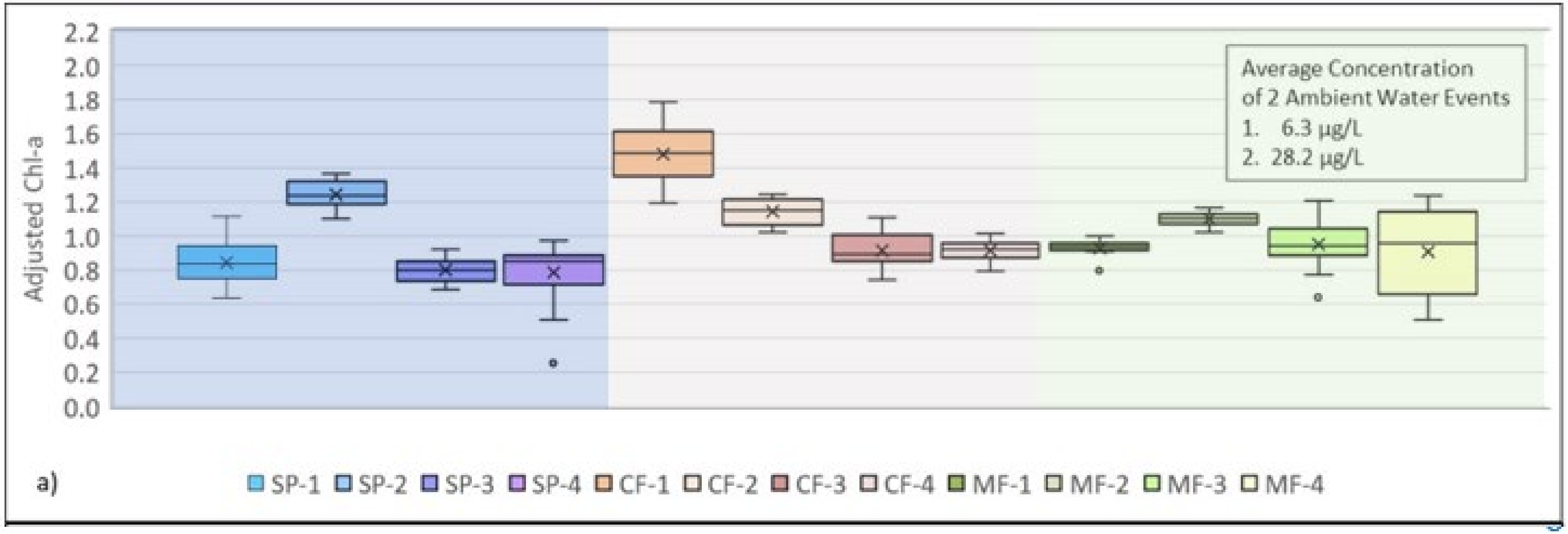
Chlorophyll-a Concentration		
Range	**Lab Prepared Samples (ug/L)**	**Ambient Water Samples (ug/L)**
Low	5.0 ± 2.0	3 to 10
Mid-Low	15.0 ± 3.0	11 to 25
Mid-High	35.0 ± 5.0	26 to 40
High	70.0 ± 10.0	> 40

Phase 1 – Summary of Results

Laboratory Prepared Samples



Ambient Samples



Phase 1 – Summary of Results

- 1,534 samples analyzed
  - 480 lab prepared samples / 1,056 ambient samples

- Variability in Chl-a measurements was observed between methods and laboratories
- Laboratory-prepared samples showed lower variability compared to ambient water samples

Phase 2 – Sources of Variablity

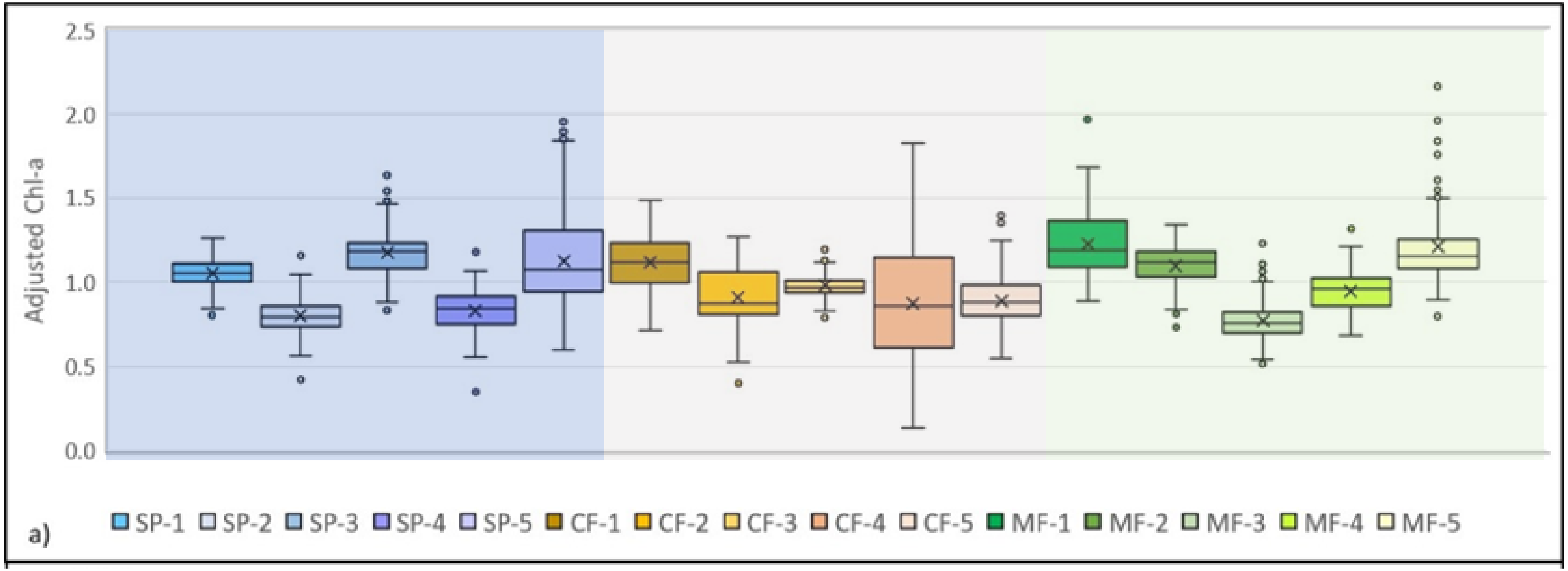
- Goals
  - Identify potential sources creating variability
  - Identify best practices in both field sample collection procedures and sample processing prior to lab analysis

Goal - Identify potential sources creating variability

- Continue evaluating 3 analytical methods on ambient water
  - Intra- and interlaboratory analysis
  - Include pheophytin and TOC for spectrophotometric and conventional fluorometric methods
  - Examine for effects of seasonal variation

Phase 2 – Interlaboratory Results Summary

Ambient
Samples



Goal - Identify Best Practices

- Review laboratory SOPs and identify key differences, determine if the variations affect analytical results.
    - Perform intralaboratory analysis on laboratory prepared and ambient samples

Phase 2 – SOP Variations

- SOPs from Phase 1 and Phase 2 laboratories were reviewed for differences in sample prep processes that might contribute to variability
  - Filter vs centrifuge to clarify extract
  - Addition of $MgCO_3$ during filtering vs no addition
  - Macerating filter vs shaking to extract Chl-a from filter
  - Filter pore size
  - Sample volume
  - Steeping time
  - Temperature of extract*

    *Laboratory prepared sample used
  - Field vs Lab filtering

Phase 2 – Summary of SOP Variation Results

Summary of Evaluation of SOP Variations Compared to Positive Control

Variation	Description of Variation	Description of Positive Control	Concentration	Within Method Variability	Between Method Variability
A	Centrifuge to clarify	Nylon filter to clarify	Decrease	Increase	Increase
B	No $MgCO_3$ addition	$MgCO_3$ addition	Increase	Slight increase	Slight increase
C	Shake filter in acetone	Macerate filter	Decrease	Increase	Increase
D	0.45 μm filter	1.0 μm filter	Increase	Slight increase	Decrease
E	Filter volume = 0.1 L	Filter volume up to 1 L	Same	Same	Same
F	2 hrs steeping time	24 hrs steeping time	Same	Slight decrease	Decrease
G	Sample cool temperature	Sample warm temperature	Slight increase	Slight decrease	Slight increase
H & I	Field filter with $MgCO_3$	Lab filter with $MgCO_3$	Increase	Slight decrease	Increase
H & I	Field filter no $MgCO_3$	Lab filter with $MgCO_3$	Increase	Same	Increase
H & I	Lab filter no $MgCO_3$	Lab filter with $MgCO_3$	Same	Increase	Slight increase

Phase 2 – Summary of Results

- No environmental or laboratory variables appeared to be a significant source of variability in Chl-a results from intra- or interlaboratory analysis
- SOP variations potentially add to the uncertainty or variability to the Chl-a data

Phase 3 – SOP Variations

- Primary objective - Identify sample collection and laboratory analysis practices that may reduce variability in Chl-a data
  - Expanded review of SOP variations of the 3 analytical methods
  - Conduct both intra- and interlaboratory analyses on SOP modifications

Phase 3 – SOP Variations

- Evaluate SOPs from 20 different laboratories
- Areas of focus:
    - Processing ambient water samples
    - Processing the filter

Phase 3 – SOP Variations

Sample processing:

Field and laboratory filtration and preservation with and without the addition of $MgCO_3$

Volume of ambient water filtered

Filter pore size

Filter processing/ Chl-a extraction

Centrifuge vs vacuum filtration to remove turbidity

Room temp vs chilled extract

Addition of $MgCO_3$ vs no addition

Steeping filter only vs shaking

Filter steep time

Macerate filter with glass rod vs gently grinding

Summary of Intralaboratory SOP Modifications Evaluated

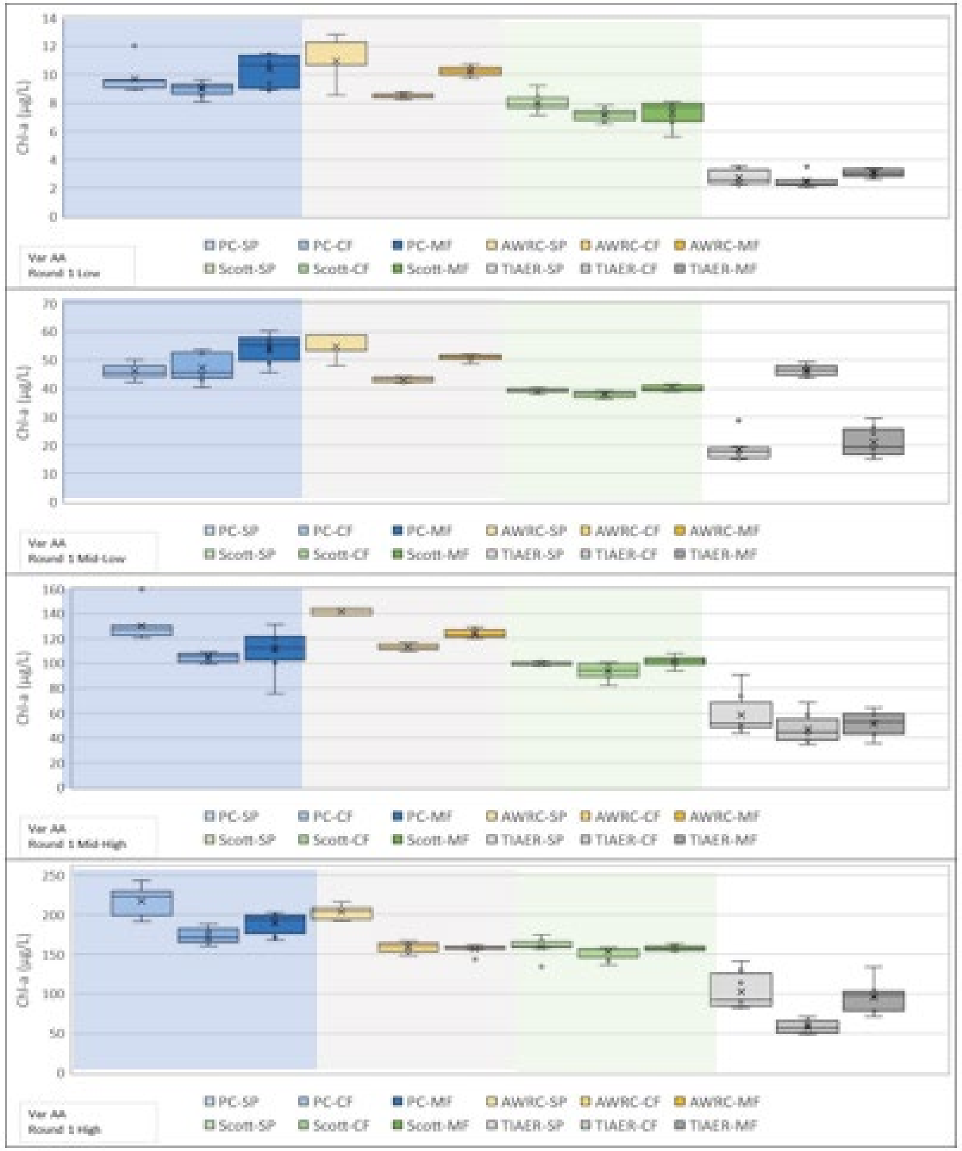
Medium	Description	# of Events
Water Sample	Lab filter, add $MgCO_3$ in field	8
Water Sample	Field filter, add $MgCO_3$ in field	8
Filter	Centrifuge to clarify	2
Filter	No $MgCO_3$ added to acetone	2
Filter	Shake, no grinding	2
Filter	Shake, no grinding, <6"Hg	2
Filter	Centrifuge to clarify	2
Water Sample	20 in Hg vacuum	2
Water Sample	Filter 1/2 of reference volume	2
Water Sample	Filter 1/4 of reference volume	2
Water Sample	Filter 1/8 of reference volume	2
Water Sample	Filter 1/16 of reference volume	2
Filter	Shake filter no maceration	4
Filter	Glass rod to macerate	4
Filter	Steep 24 hr, no shaking	4

Summary of Interlaboratory SOP Modifications Evaluated

SOP Variation AA	SOP Variation BB	SOP Variation CC
Each lab used its normal SOP through the filtration of the ambient water sample Chl-a extracted from the filter using a 90 percent acetone solution Grinding filter for 1 minute at 500 rpm Steep sample between 2 and 24 hours Centrifuge sample for 5 minutes at 1000 g	Each laboratory used its normal SOP with one exception: Add $MgCO_3$ at the end of the ambient water filtration process	Each lab used its normal SOP up to the steps for extracting the Chl-a from the filter. Chl-a extracted from the filter using a 90 percent acetone solution(1 lab adds $MgCO_3$ during this step) Grinding filter for 1 minute at 500 rpm Filter to remove turbidity in supernatant

Phase 3 – Interlaboratory SOP Variation Results

Var AA box plots of Chl-a results by concentration category



SOP Variation AA

Each lab used its normal SOP through the filtration of the ambient water sample

Chl-a extracted from the filter using a 90 percent acetone solution

Grinding filter for 1 minute at 500 rpm

Steep sample between 2 and 24 hours

Centrifuge sample for 5 minutes at 1000 g

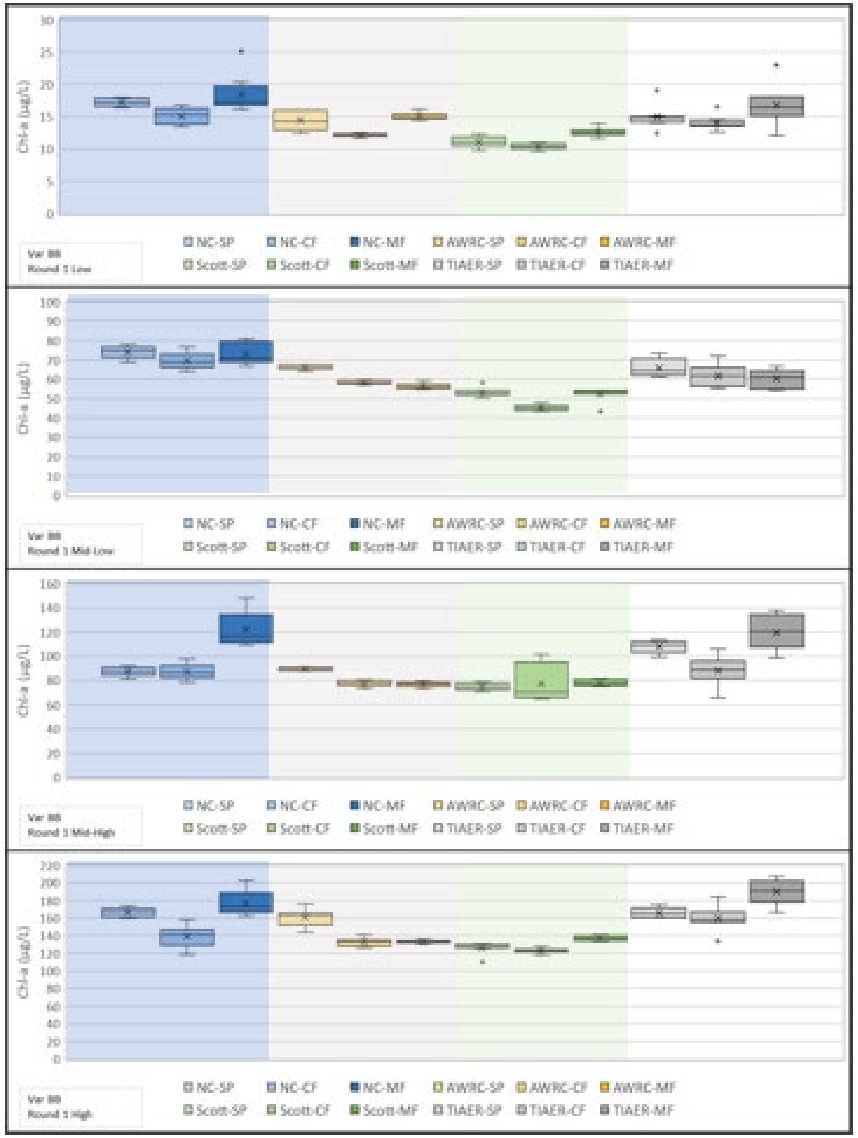
Phase 3 – Interlaboratory SOP Variation Results

Var BB box plots of Chl-a results by concentration category

SOP Variation BB

Each laboratory used its normal SOP with one exception:

Add MgCO3 at the end of the ambient water filtration process



Phase 3 – Interlaboratory SOP Variation Results

Var CC box plots of Chl-a results by concentration category

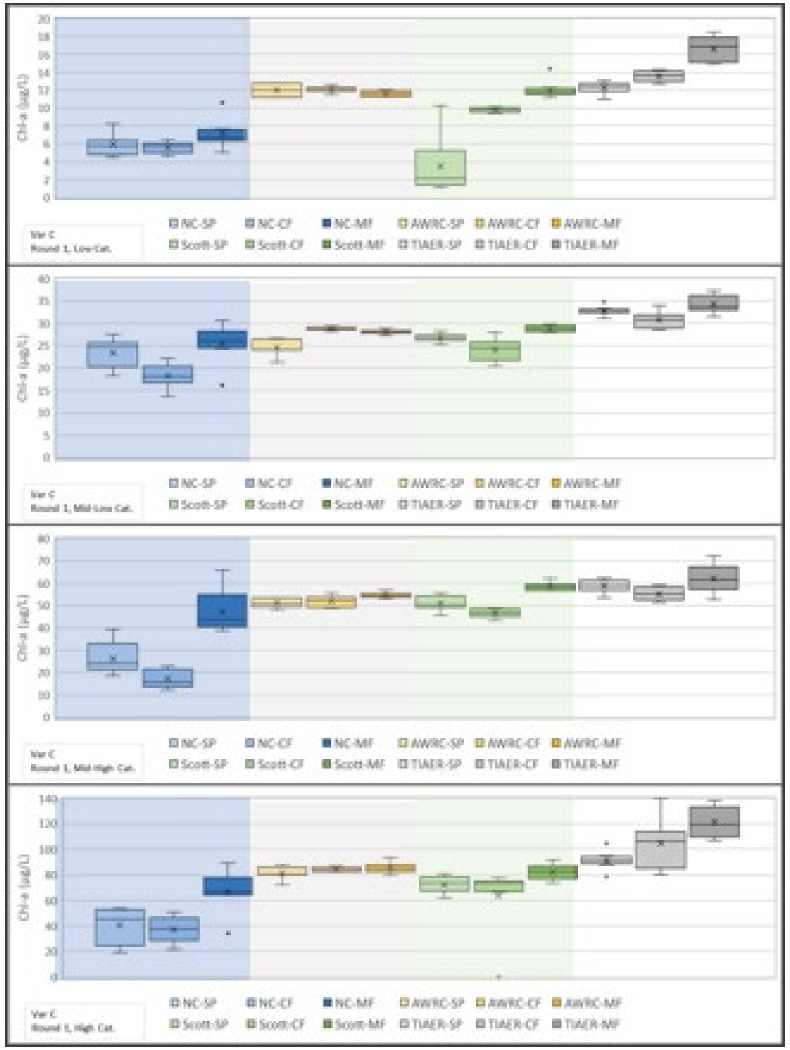
SOP Variation CC

Each lab used its normal SOP up to the steps for extracting the Chl-a from the filter.

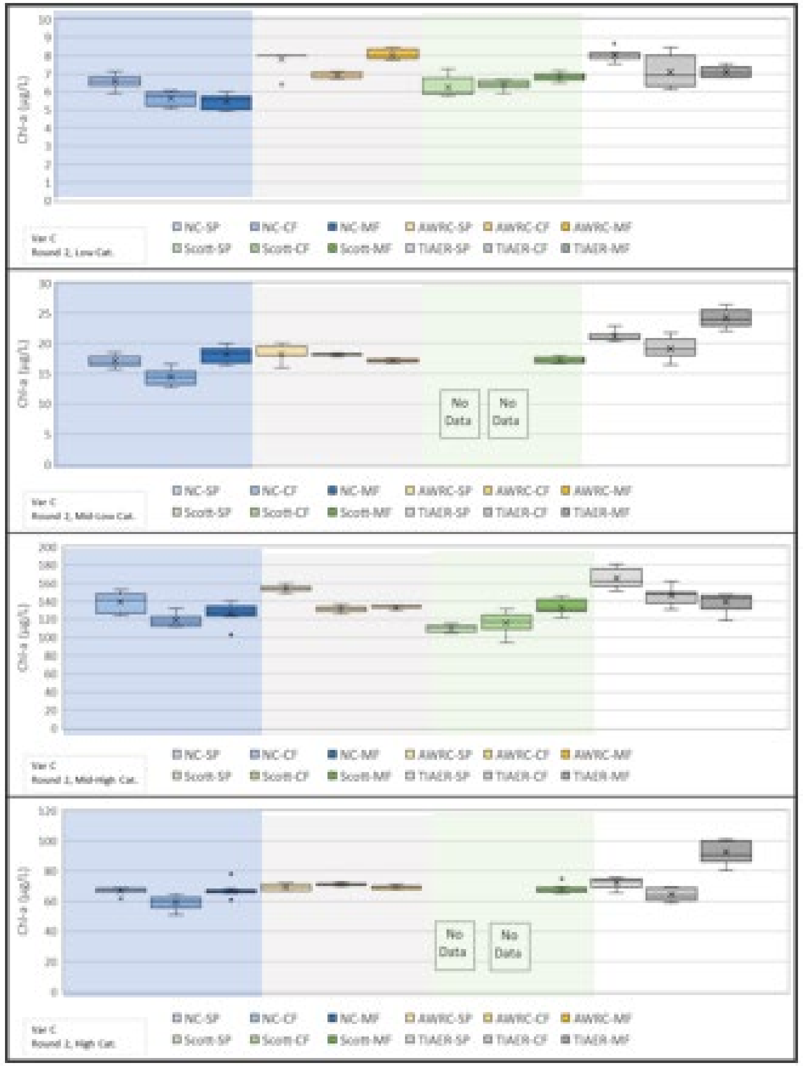
Chl-a extracted from the filter using a 90 percent acetone solution(1 lab adds MgCO3 during this step)

Grinding filter for 1 minute at 500 rpm

Filter to remove turbidity in supernatant



Round 1



Round 2

Phase 3 – Summary of Results

- Results of intralaboratory analyses of SOP modifications did not demonstrate any substantial reductions in variability of data
- Results of interlaboratory analyses did not demonstrate any substantive improvement (reduction) within method, between methods, or between laboratories data variability, even though required SOP modifications forced consistent procedures across laboratories

Project Conclusion

- There was notable variability in data from labs participating in the study
  - Some laboratories have greater variability in their data than others
  - Project sampling and sub-sampling protocols could introduce variability into the laboratory results
- SOP modifications did not reduce within method variability, between method variability, or between laboratory variability
  - This suggests that EPA and SM methods are robust and insensitive to the procedural differences observed in laboratory SOPs
  - No one of the three laboratory methods performed superior to the others in interlaboratory analyses

Questions?