Prioritizing Safety, Advancing Efficiency:
Developing a New Total Phosphorous Microwave Digestion Method for Sediment Core Nutrient Analysis

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I. Executive Summary

Perchloric acid hot plate digestion is an accepted method for total phosphorous digestion of soil and sediment samples. This method was the standard Tahoe Environmental Research Center (TERC) method for total phosphorous digestion in the long-term project analyzing total phosphorous concentrations in Clear Lake sediment cores. A shift from conducting the analysis on the UCD main campus to the TERC lab at Tahoe precipitated a review of the method and development of a safer alternative.

We found that a microwave assisted digestion method is a suitable method to replace the historic perchloric acid hot plate method. Although the microwave method yields slightly lower returns than the perchloric method, as validated through direct comparison using colorimetric and ICP-MS approaches, the two methods are highly correlated. An ordinary-least-squares linear regression model provides a robust statistically significant conversion from the microwave method to the perchloric method, allowing future data quantified using the microwave method to be compared with the historic perchloric method.

Our major findings are:

1. The microwave and perchloric acid methods were highly correlated but significantly different in pairwise comparisons of the colorimetric and ICP-MS data, with perchloric acid yields slightly higher. Likewise, the colorimetric and ICP-MS methods of measurement differed significantly in pairwise comparisons using both the perchloric acid and microwave datasets, with ICP-MS measurements slightly higher.
2. An ordinary-least-squares linear regression model provides a robust method to convert data between the microwave to perchloric acid methods, providing a tool for maintaining continuity with the historic dataset:
   TP using Perchloric (ug/g) = 52.96 + 0.9812 * TP using Microwave (ug/g)
3. The microwave and perchloric methods both produce consistent and acceptable recoveries (>90%) using a standard reference material (SRM).

II. Introduction and Background

Phosphorus is an essential nutrient for biological function and often the most limiting nutrient in freshwater ecosystems. Understanding how phosphorus cycles through a biogeochemical system is crucial to ascertaining how the system itself functions. Sediment and soil are common reserves for environmental phosphorus within limnological systems, which stores phosphorus in a variety of different forms (Hieltjes and Lijklema 1980). Determining the concentration of various phosphorus fractions in soils and sediments can provide valuable insight as to how phosphorus cycles through a particular limnological system (Goldman and Horne 1994).

Lake County Water Resources Department (LCWRD), in collaboration with University of California, Davis (UC Davis, TERC), has been working for over a decade to better understand nutrient cycling in Clear Lake and within the Clear Lake watershed. By analyzing total phosphorus (TP) concentrations from sediment core samples from multiple sites in the lake, the agency aims to form a more mechanistic understanding of the long-term phosphorus cycling in Clear Lake.

Analyzing TP concentrations in each sediment sample first requires the nutrient to be extracted from the sample. Extracting phosphorus from sediment samples can be accomplished using chemical assisted dissolution. The exact method of extraction can vary. The historic method developed by UC Davis and carried out most recently by lab chemist Tina Hammell for Clear Lake sediment samples used a perchloric acid - hot plate digestion method (Perchloric method; Olsen and Sommers 1982) followed by the ascorbic acid quantification method using colorimetric analysis (Standard Methods 4500-P; Hieltjes and Lijklema 1980). The perchloric acid - ascorbic acid colorimetric method was deemed to provide reliable, accurate results and was used by Hammell until her retirement in 2023.

Several factors precipitated the need to develop a new method after Hammel's retirement. The perchloric method requires the use of concentrated perchloric acid which acts as a potent oxidizer when heated, and therefore requires a specialized chemical fume hood to use safely. Simultaneously, the responsibility of performing the analysis passed to the chemistry team at the UC Davis Tahoe Environmental Research Center (TERC), located in Incline Village, Nevada. As the Tahoe lab was not equipped with the proper ventilation system to work with perchloric acid, the shift in locations was used as an opportunity to develop a safer, faster alternative method utilizing microwave-assisted nitric acid and hydrogen peroxide (HNO_3/H_2O_2) digestion (Microwave method).

III. Method Development

UC Davis historically used the perchloric method for TP determination. The method involves adding concentrated nitric (HNO_3) and perchloric acid ($HClO_4$) to dry sediment samples and digesting each sample on a hot plate. An abbreviated standard operating procedure for the perchloric method is detailed below (see "SOP-Perchloric" in the appendix for unabridged SOP):

A. Perchloric Digestion SOP

1. Sediment samples are dried at 110 ^oC for 24 hours.
2. Once dry, sediment is ground to a fine powder and placed in individual scintillation vials stored in a desiccator.
3. A 0.05 - 0.08 g subsample of dried sediment is added to an Erlenmeyer flask, then 15 mL of concentrated nitric acid and 5 mL of concentrated perchloric acid are added.
4. Boiling chips are added to the flask and digested on a hot plate in a perchloric acid fume hood for approximately 10 minutes.
5. Flask are removed from hot plate and left to cool.
6. Digestate in flasks is then pH adjusted with both concentrated sodium hydroxide (NaOH) or concentrated hydrochloric acid (HCl).
7. Digestate is decanted into clean 50 mL tubes before adding deionized water to bring to a total volume of 50 mL at 20 ^oC.

After a careful review of soil/sediment digestion methods, a microwave assisted digestion was chosen to replace the perchloric method (USEPA 1994), and a new CEM Microwave MARS 6 was purchased. Microwave assisted digestion functions by breaking apart and releasing sediment-bound phosphorus via chemically assisted digestion under high pressure and temperature conditions. In this case, concentrated nitic acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) were selected as the most appropriate chemical reagents.

An abbreviated standard operating procedure (SOP) for the Microwave method is detailed below (see "SOP-Microwave" for unabridged SOP).):

B. Microwave Digestion SOP

1. Sediment samples are dried at 110 ^oC for 24 hours.
2. Once dry, sediment is ground to a fine powder and placed in individual scintillation vials stored in a desiccator.
3. A 0.250 - 0.300 g subsample of dried sediment is added to a microwave vessel, then 9 mL of concentrated nitric acid and 2 mL of 30% hydrogen peroxide are added.
4. Vessels rest in the fume hood for 30 minutes to predigest.

5. Vessels are sealed and vortexed before being placed in the microwave carousel.
6. The microwave digestion program has three primary stages: ramp, hold, and cool down. The samples are brought up to a specified temperature in the ramp stage and then held at that same temperature in the hold stage. The final cool-down stage gradually brings the vessel temperature down, at which point the vessels can be safely removed from the microwave. For the sediment core samples, the microwave digestion program is set to the following parameters:
    1. Temperature: 190 ^{o}C
    2. Hold Time: 15 minutes
    3. Ramp Time: 20-30 minutes
7. Vessels are left to cool for 30 minutes before removing from the microwave, then placed in an ice bath inside a chemical fume hood to cool completely.
8. Vessels are carefully uncapped and decanted into clean 50-mL tubes before adding deionized water to bring to a total volume of 50 mL at 20^{o}C.

C. Approach for comparing methods

To compare the efficacy of the new microwave method to the historic perchloric method, three sets of Clear Lake sediment core samples (n=54) were each digested with both methods, resulting in six sets of digestates. Then, each set of digested samples was analyzed using colorimetric analysis, as done using the standard method. The analysis followed the same procedure for both digestate types, except the microwave method digestate required specific matrix-matching standards.

Finally, several quality control steps were taken to ensure the accuracy of each dataset. Internal sample spikes, blank spikes, sample duplicates, and standard reference materials (SRM) were included in the digestion process. Laboratory duplicates and laboratory spikes were included during colorimetric analysis.

Internal sample spikes were created by adding 200 µg of a 500,000 ppb-P monosodium phosphate (NaH_2PO_4) spike solution to a sample, for a predicted 100 ppb-P increase in the sample concentration compared to the non-spiked sample. Blank spikes were created in the same way, except no sample material was added. Sample duplicates were included by weighing out two separate aliquots from the same sediment sample.

An SRM was used to assess TP recovery rates for both the perchloric method and microwave method. Montana II Soil, known as NIST 2711a, was selected as the SRM for this determination. Produced by the National Institute of Standards and Technology (NIST), NIST 2711a is a homogenous soil with a known TP value of ~842 µg/g (range = 831 to 853 µg/g). It is important to note that the SRM sample was selected as the closest geologic material available to represent the Clear Lake sediment samples. The

primary function of the SRM is to act as a proxy for each sample, with a goal of 90% or greater recovery rate designated to validate each digestion run.

We further assessed the accuracy of the colorimetric analysis by comparing the same set of digests to quantification by ICP-MS, the industry standard for quantifying TP concentrations. Statistical analyses were done using JMP (SASS). We used paired t-tests and means comparisons to assess and quantify statistical differences between the two methods. Pearson correlation coefficients and ordinary-least-squares (OLS) linear regressions were used to assess the relatability of the two methods and to develop a model for converting data using the microwave method to a comparable value from the perchloric acid method. Outlier sample pairs were identified and excluded from the dataset using the quantile analysis method: the difference between sample pairs was calculated and the lower 25^{th} and upper 75^{th} quantiles were computed. The inter quantile range (IRQ, Q3-Q1) was used to define the lower ($Q1-1.5\times IQR$) and upper bound ($Q3+1.5\times IQR$) outlier bounds. A total of 4 outliers were identified in the colorimetric dataset, 5 outliers in the ICP-MS dataset, and outliers in the combined colorimetric dataset used to develop the inter-method conversion model, representing <7% of data.

IV. Method Validation

A. Comparison of Perchloric Digestion and Microwave Digestion Samples

The method validation process involved digesting Clear Lake sediment samples with two separate digestion methods, then analyzing digestate samples by ICPMS and colorimetric analysis. The samples used were from three sampling events for a total of (n=50) individual sediment samples for colorimetric analysis and (n=48) individual sediment samples for ICPMS analysis (Table 1).

Table 1. Method Comparison Dataset

Perchloric Digestion		**Microwave Digestion**	
ICPMS	Colorimetric	ICPMS	Colorimetric
February 2023 (n =17)	February 2023 (n=17)	February 2023 (n=17)	February 2023 (n=17)
May 2023 (n = 15)	May 2023 (n=18)	May 2023 (n=15)	May 2023 (n=18)
June 2023 (n = 16)	June 2023 (n=15)	June 2023 (n=16)	June 2023 (n=15)

As part of the outlier analysis, a normality analysis was conducted on the data (Appendix Figure 1a, 1b). Both colorimetric datasets failed to pass the Shapiro-Wilks test because of positive skew in the distribution, and mean values that were higher than medians. However, with outliers removed, the effect of skew was comparatively small, and while still statistically significant, distributions fell within a normal quantile plot, suggesting the statistical significance is the result of small sample size rather than truly indicating the data are from non-normal distributions.

The microwave and perchloric acid results were highly correlated using both colorimetric (Pearsons: 0.9459, $p<0.001$; Spearman: 0.9033, $p<0.001$) and ICP-MS (Pearsons: 0.8743, $p<0.001$; Spearman: 0.8799, $p<0.001$) methods of quantification. The microwave method had a significantly lower mean compared to the perchloric method using both colorimetric (mean difference -48.2, t-Ratio -6.67, DF=49, $p<0.0001$) and ICPMS (mean difference –64.1, t-Ratio -6.04, DF=47, $p<0.0001$) quantification.

The mean TP concentrations quantified by ICP-MS was 61 µg/g higher for the perchloric method (900 µg/g) versus microwave (839 µg/g) method. In contrast, the difference in TP concentrations quantified by colorimetric method was 47 µg/g, with the perchloric method (818 µg/g) again slightly higher than the microwave (771 µg/g) method. Hence, the higher TP values determined by the perchloric acid digestion was not due to

interference issues associated with the colorimetric analysis. The overall higher TP concentrations determined by the ICP-MS detection method likely result from quantification of organic and colloidal P forms present in the digestate.

The colorimetric and ICP-MS methods of measurement differed significantly in pairwise comparisons using both the perchloric acid (mean difference 116.7, t-Ratio 8.12, DF=36, $p<0.0001$) and microwave (mean difference 88.6, t-Ratio 8.23, DF=36, $p<0.0001$) datasets, with ICP-MS measurements slightly higher in both cases.

Finally, the two digestion methods were evaluated for their TP recovery from the NIST SRM (~842 µg/g) using colorimetric analysis. Recoveries were consistently >90% (Table 2, 90% is considered acceptable for geologic samples), with the perchloric (97.30%) and microwave (97.75%) digestion methods both providing a robust recovery from the NIST SRM.

Table 2. Summary of NIST values and Recovery Rates

	Colorimetric Analysis			
	Perchloric µg/g	Recovery Rate (%)	µg/g	Recovery Rate (%)
Feb. 2023	766.1	91	833.2	99
Feb. 2023	847.0	101	830.3	99
Feb. 2023	858.6	102	818.6	97
May 2023	766.1	91	833.2	99
May 2023	847.0	101	830.3	99
May 2023	858.6	102	818.6	97
June 2023	808.8	96	815.1	97
June 2023	808.0	96	805.8	96
Average	819.2	97.3	823. 1	97.8

B. Regression model to convert microwave to perchloric results

Future TP values produced with the Microwave method for Clear Lake sediment cores will be converted to the historic data frame of perchloric acid digestion using the OLS linear regression model described below.

An ordinary-least-squares linear regression model was developed from multiple Clear Lake Sediment core datasets which were digested with both methods and analyzed via colorimetric analysis. The data in the conversion factor regression included the three 2023 datasets (February, May, June) used for method validation, and two additional datasets from 2023 (March and April) (Table 3). Ultimately, increasing the total number of samples (n=86) ensures that the correction factor is as robust as possible. These two additional datasets were not validated via ICMPS, but the validity of the datasets is sound owing to the inclusion of the NIST- SRM in each dataset and robust QA/QC protocols.

This process involves the inclusion of sample dupes, spikes, and SRMs during analysis, followed by a multi-step data validation process to ensure that the data were accurate. First, data were entered into an Excel spreadsheet; the data were always entered by one chemist and then later double-checked by a second chemist to ensure that all values were entered correctly. Second, the slope and correlation coefficient were checked to ensure they fall within an acceptable range based on historic, averaged slope values for the specific analysis. Sample dupes, spikes, and SRMs recovery were also assessed based on acceptable recovery rates for that specific analysis. Finally, the data were validated to confirm that all values were accurate and entered in the correct location.

Table 3. Conversion Factor Dataset

Perchloric Digestion	**Microwave Digestion**
Colorimetric analysis	
February 2023 (n=17)	February 2023 (n=17)
March 2023 (n=18)	March 2023 (n=18)
April 2023 (n=18)	April 2023(n=18)
May 2023 (n=18)	May 2023 (n=18)
June 2023 (n=15)	June 2023 (n=15)

The regression model relating the microwave and perchloric acid methods was strong and statistically significant, reflecting the high correlation between the two TP methods (R^2= 0.911, F(1,84)=855.34, p<0.0001) (Appendix Figure 2). The conversion model (parameter estimates and standard error) was:

Perchloric (µg/g) = 52.96 + 0.9812 * Microwave (µg/g)

The robust regression had parameter estimates and standard errors (43.45±27.71; 0.994±0.0351) nearly identical to the OLS regression. Analysis of residuals from the OLS regression model found no violation of linear regression assumptions. Residuals from the OLS regression were normally distributed (Shapiro-Wilks Goodness of Fit test statistic W = 0.9817, p=0.2641) and visual inspection of residual plots (Appendix Figure 3) found no heteroscedasticity or autocorrelation.

V. Conclusions

After completing all the above regression analyses, the Microwave method proved to be a suitable method to replace the perchloric method. The official switch to the microwave occurred in July 2023 at the Wickson Lab at UC Davis, using a CEM Microwave Model MARS 6 230/60 (model #910900). TERC took over responsibility for TP digestion of sediment core samples starting in August 2023 using a CEM Microwave MARS 6 (model #910900).

VI. Acknowledgments

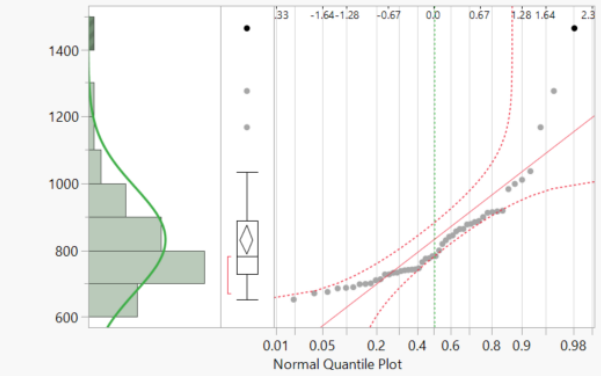
The authors of this paper would like to thank everyone who contributed to this multi-year process of method development and constructing this white paper, specifically: Alicia Cortez-Cortez, Alexander Forrest, Angela DePalma-Dow, and Randy Dahlgren.

VII. Appendices

A. Tables and Figures

Distributions

Perchloric (ug/g)



Compare Distributions

Show	Distribution	AICc	BIC	-2*LogLikelihood
☑	Normal	651.00637	654.57509	646.75105

Quantiles

100.0%	maximum	1463.81
99.5%		1463.81
97.5%		1412.209
90.0%		1009.184
75.0%	quartile	890.19
50.0%	median	782.105
25.0%	quartile	727.495
10.0%		687.494
2.5%		657.41125
0.5%		652.31
0.0%	minimum	652.31

Summary Statistics

Mean	831.2372
Std Dev	157.36563
Std Err Mean	22.254861
Upper 95% Mean	875.96002
Lower 95% Mean	786.51438
N	50
N Missing	36

Fitted Normal Distribution

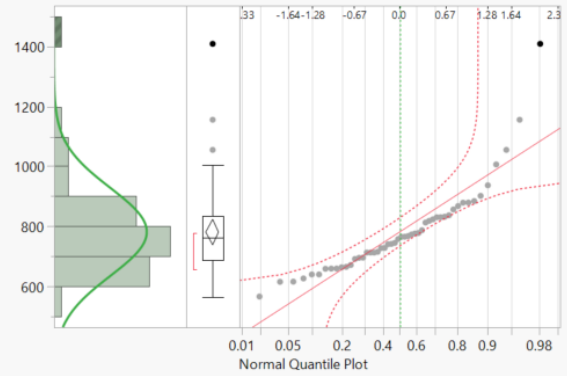
Parameter		Estimate	Std Error	Lower 95%	Upper 95%
Location	μ	831.2372	22.254861	786.51438	875.96002
Dispersion	σ	157.36563	15.978059	131.45286	196.09856

Measures	
-2*LogLikelihood	646.75105
AICc	651.00637
BIC	654.57509

Goodness-of-Fit Test

	W	Prob<W
Shapiro-Wilk	0.8200811	<.0001*

Microwave (ug/g)



Compare Distributions

Show	Distribution	AICc	BIC	-2*LogLikelihood
☑	Normal	643.86195	647.43068	639.60663

Quantiles

100.0%	maximum	1408.04
99.5%		1408.04
97.5%		1338.6383
90.0%		934.298
75.0%	quartile	834.52
50.0%	median	762.81
25.0%	quartile	687.8925
10.0%		641.466
2.5%		581.465
0.5%		567.99
0.0%	minimum	567.99

Summary Statistics

Mean	783.0068
Std Dev	146.515
Std Err Mean	20.720349
Upper 95% Mean	824.6459
Lower 95% Mean	741.3677
N	50
N Missing	36

Fitted Normal Distribution

Parameter		Estimate	Std Error	Lower 95%	Upper 95%
Location	μ	783.0068	20.720349	741.3677	824.6459
Dispersion	σ	146.515	14.876344	122.38896	182.57722

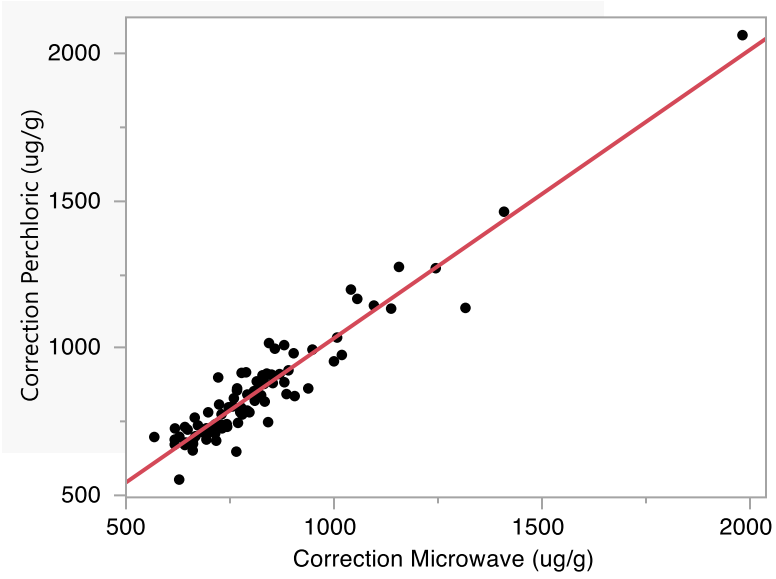
Measures	
-2*LogLikelihood	639.60663
AICc	643.86195
BIC	647.43068

Goodness-of-Fit Test

	W	Prob<W
Shapiro-Wilk	0.8473289	<.0001*

Figure 1. Results of normality test for Perchloric method and Microwave method colorimetric datasets (1a) and ICPMS datasets (1b).

Bivariate Fit of Correction Perchloric (µg/g) By Correction Microwave (µg/g)



Linear Fit

Correction Perchloric (µg/g) = 52.96 + 0.981*Correction Microwave (µg/g)

Summary of Fit

RSquare	0.910576
RSquare Adj	0.909511
Root Mean Square Error	62.75379
Mean of Response	855.6936
Observations (or Sum Wgts)	86

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3368381.5	3368382	855.3449
Error	84	330795.3	3938	**Prob > F**
C. Total	85	3699176.8		<.0001*

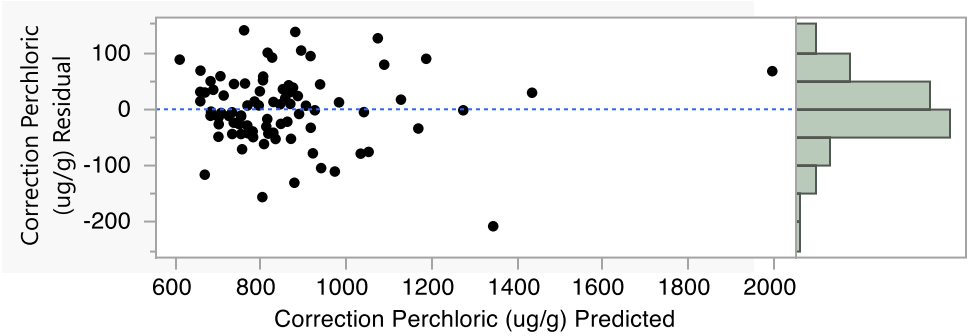
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	52.964346	28.26909	1.87	0.0645
Correction Microwave (ug/g)	0.9812195	0.03355	29.25	<.0001*

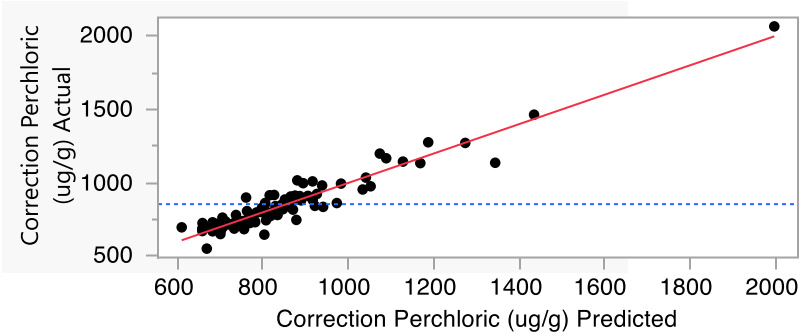
Figure 2. OLS regression for converting microwave colorimetric method to perchloric acid colorimetric method. Shaded area is the prediction interval based on RMSE.

Diagnostics Plots

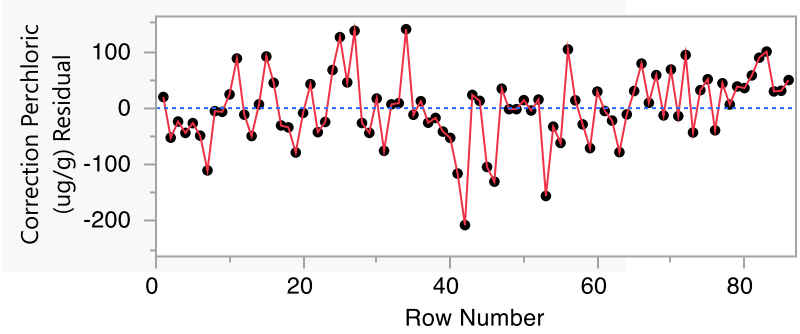
Residual by Predicted Plot



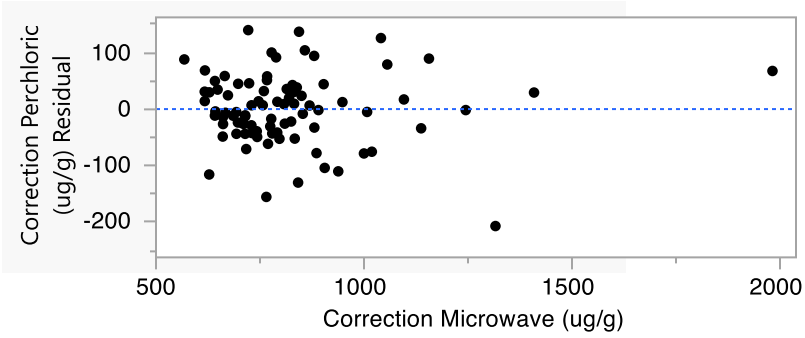
Actual by Predicted Plot



Residual by Row Plot



Residual by X Plot



Residual Normal Quantile Plot

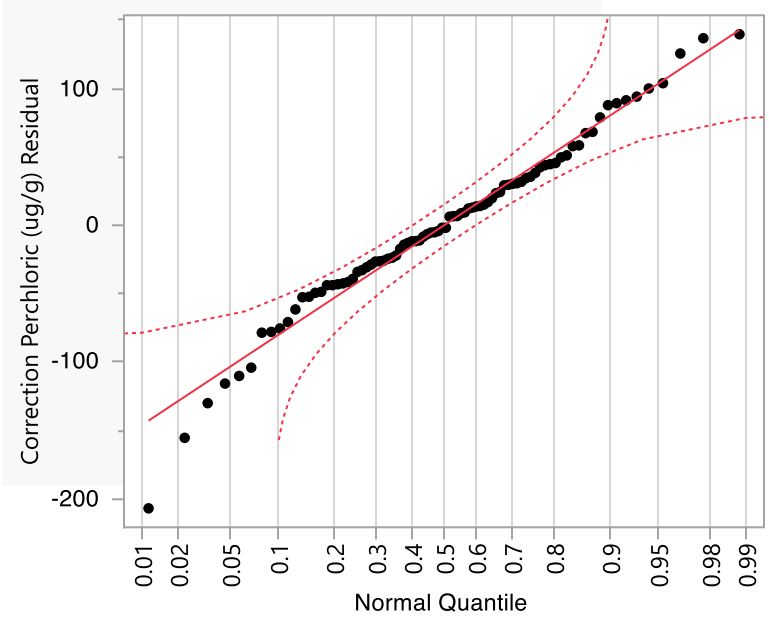


Figure 3. Summary output table and residual analysis plots for statistical regression analysis relating the perchloric acid and microwave methods.

B. SOP – Perchloric

Digestion Procedure for Total Phosphorus:

1. Using a Sharpie, mark the bottoms of empty aluminum weigh pan with the sample site information and date of the sample. There should be 21 per sample date including 3 dups and 3 spikes.
2 Weigh empty aluminum weigh pan and record weight on designated worksheet.
2. Scoop wet soil into the aluminum weigh pan for a total weight of 3.5-5.0 g and record weight (soil in pan will be the approximate size of a silver dollar).
3. Weigh wet soil + aluminum tin and record weight.

4. Place all aluminum pans in drying oven at 110°C for 24-48 hours.
5. Weigh the dried sample in the aluminum pan and record weight (weight of pan + dried soil) on worksheet.
6. Using a Sharpie or label maker, label empty 50-mL glass scintillation vials & lids with the sample site information and date of the sample.
7. Using a mortar and pestle individually grind each soil sample into a fine powder or granular substance.
8. Transfer the ground soil into the designated 50-mL scintillation vial using the lip of the bowl and a spatula (you will not be able to transfer every particle). Store sediment sample in desiccator.
9. Wipe mortar and pestle with Kimwipes between samples.

Reagents (all chemicals are reagent grade):

1. Phenolphthalein
2. NaOH (6.0 M), made by adding 60 grams of sodium hydroxide pellets to a 250-mL volumetric flask filling DI water to mark.
3. 12 N Hydrochloric Acid
4. Nitric Acid, concentrated
5. Perchloric Acid, concentrated
6. 500,000 ppb spike solution, NaH_2PO_4

Procedure:** *The following procedure MUST be performed in a certified perchloric acid fume hood* **

1. Add 0.05-0.08 g oven-dried sample from scintillation vial to a labelled (Sharpie) 125-mL Erlenmeyer flask. Tare Erlenmeyer flask and tap out soil sample from scintillation vial. No spatula.
2. Add 400 µl of Standard Reference Solution (SRS), NaH_2PO_4 500 ppm, to designated spike samples and swirl flask to mix into soil.
3. Add 15 mL concentrated HNO_3 by graduated cylinder to soils, swirl to mix.
4. Add 5 mL concentrated $HClO_4$ using pipettor and swirl to mix.
5. Add about 10-12 boiling chips (Teflon chips).
6. Place flask on a pre-heated hotplate in the hood. Evaporate gently until dense white fumes of $HClO_4$ appear. Digest through fumes for 10 minutes, the sample will clear or becomes colorless. The final sample volume will be about 1-2 mL in total. If solution has not cleared, add 5-10 mL HNO_3 by pipettor (this is rare) to complete digestion.
7. Using tongs remove Erlenmeyer flask off the hotplate and put on foil (to protect the fume hood surface from scorching) in the hood to cool. You know that it's cool by touching the bottom of the flask with your gloved hand to make sure it's not warm. About 10 minutes. Cover with Parafilm. This is not urgent to go on to the next step immediately.

8. *pH adjustment:* Cool digested solution, place on white lab bench paper. Neutralize using 1 drop of phenolphthalein indicator.
9. Add 15-20 drops of 6.0 N NaOH (solution should turn slightly orange to grapefruit in color, will often also appear cloudy, use a white piece of paper under the flask to aid in color change identification as it is subtle).
10. Discharge the orange/grapefruit color with a few drops of concentrated HCl (4-5 drops initially and you will notice the color change to yellow, then add 2 more drops to ensure the sample is acidic) to bring the solution back to a slightly acidic state (color should be bright yellow). *Note: Concentrations of Phosphorus differ between samples. All color changes will not necessarily be distinct and uniform.*
11. Transfer solution to a 50-mL plastic conical tube and bring to 50-mL mark with DI water. Rinse the Erlenmeyer flask by first pouring what is in the flask directly to the conical tube, then rinse the flask with DI water 2 times and pour DI rinse into conical rube. Let settle, then analyze extract as described in the section titled "Colorimetric Analysis of Phosphorus".

C. SOP – Microwave

Step One: Drying and Grinding Soil Samples

In the first step of the Total Phosphorous digestion, soils samples are weighed before and after drying to calculate the percent soil and percent moisture of each sample. Then, each sample is manually ground into a fine powder for the digestion process.

Day #1:

1. Using a pen, write on the bottoms of empty aluminum weigh pan with the sample site information and date of the sample. For Clear Lake Sediment Cores (CLSC), there should be 21 samples for each sample date including 3 dups and 3 spikes.
2 Weigh empty aluminum weigh pan and record weight on designated worksheet.
3 Massage bag of soil for approximately 10 seconds to homogenize before you scoop.
2. Scoop wet soil into the aluminum weigh pan for a total weight of 5.0-7.0 g and record weight (soil in pan will be the approximate size of a silver dollar).
3. Wipe spatula inside bag.
4. Clean spatula with a Kimwipe in between samples.
5. Weigh wet soil + aluminum tin and record weight.
6. Place all aluminum pans in drying oven at 110^{o}C for 24-48 hours.
7. Acid wash the following equipment for Day #2:
    a. Mortar and pestle
    b. Scintillation vials and caps
    c. Spatula,
    d. Microwave tubes, caps, and stoppers

Day #2:

1. After 24-48 hours, quickly take the aluminum tins out of the oven and place them in a desiccator for 30 minutes.
2. Remove sample from the desiccator and weigh the dried sample in the aluminum pan and record weight (weight of pan + dried soil) on worksheet.
3. Using a Sharpie or label maker, label empty 50-mL glass scintillation vials and lids with the sample site information and date of the sample.
4. Using a mortar and pestle, individually grind each soil sample into a fine powder or granular substance.
5. Transfer the ground soil into the designated 50-mL scintillation vial using the lip of the bowl and a spatula (you will not be able to transfer every particle).
6. Transfer soil over a Kimwipe so you can recover soil in case of a spill.
7. Wipe mortar and pestle and spatula with a fresh Kimwipes between samples.

Step Two: Microwave Digestion:

The second part of the process involves weighing 0.250-0.300 g of each sample into microwave digestion vessels before adding reagent to each tube and microwave digesting the sample set for an hour. The following reagents and spike solution are required for this step:

1. Nitric Acid, concentrated (9 mL per sample)
2. 30% Peroxide solution (2 mL per sample)
3. 500,000 ppb spike solution, NaH_2PO_4 (400 µl per sample)**PLACE IN 20 ^oC WATERBATH FOR AT LEAST 1 HOUR BEFORE WEIGHING**

Procedure:

1. Dry NIST 2711a and ground samples for at least 2 hours @110 degrees C (0.250-0.300 grams per tube). After you take it out of the drying oven, put it in a desiccator to cool down before weighing.
2. Create a worksheet for the TP microwave digestion:
    a. Digestion vessel number
    b. Sample site
    c. Date of sample
    d. Mass of sample in grams
    e. There should be 21 soil samples for CLSC. For each date, include:
        i. 3 dups (CL-01, CL-03, CL-04)
        ii. 3 spikes (internal standard) for CL-01, CL-03, CL-04
        iii. At least 2 SRMs (NIST 2711a, for example)
        iv. At least one DI blank
        v. At least on blank spike (400µl of 500,000 ppb NaH2PO4)

1. Acid wash and 6X DI rinse digestion tubes (should be washed the night before, ideally the vessels are completely dry at the time of weighing).
2. Use a Sharpie and number each digestion vessel. This will coincide with the number on your worksheet.
3. To weigh each sample:
    a. Tare the clean and dry digestion vessel.
    b. Place weighing paper into vessel and do not tare (paper should weigh around 0.500 g)
    c. Add weighing paper weight to sample weight range to get modified weight range to aim for when weighing (ex. 0.500 g + 0.250 g = 0.750 g and 0.500 g + 0.300 g = 0.800 g. Weight range = 0.750 -0.800 g).
    d. Weigh dried and crushed soil by using a using a curled-up weigh paper as a funnel for each digestion vessel for pouring the soil. Weigh enough soil to hit calculated weight range. Record mass on designated worksheet.

4. Also weigh approximately 0.250-0.300 g of NIST 2711a SRM into 2-3 digestion tubes using the same method described above.
5. Carry all tubes in the racks to the fume hood for the next steps. Add all chemicals into the fume hood.
6. Highlight in yellow on your spreadsheet the tubes that need to be spiked. Using a 5-mL tip on a repeater pipettor, dial to 4. Add 400 µl of 500,000 ppb spike solution (NaH_2PO_4) directly to the dried spike soil in the digestion vessel. Place stopper, but not the cap, on each vessel after adding spike.
7. Add 400 µl of the 500,000 ppb to the Blank Spike digestion vessel. Stopper and cap each vessel when you have completed a spike.
8. Add 9 mL of concentrated Nitric Acid, followed by 2 mL of 30% Hydrogen Peroxide solution to each tube.
9. Use the designated repeater pipettor and set the volume to 9 mL. Add a little nitric acid into the designated beaker and swirl to prime the beaker. Discard the nitric acid into the designated waste container. Also prime the repeater pipettor tip with nitric acid and discard in waste container.
10. Dispense 9 mL from the repeater pipettor into all digestion vessels. You can only pipet 5 tubes at a time. At the end of each of a set of 5 tubes, evacuate the repeater pipettor tip of nitric acid back into the beaker before filling again.
11. Use the designated repeater pipettor and set the volume to 2 mL. Add a little hydrogen peroxide into the designated beaker and swirl to prime the beaker. Discard the hydrogen peroxide into the designated waste container. Also prime the repeater pipettor tip with hydrogen peroxide and discard in waste container.
12. Dispense 2 mL of hydrogen peroxide into all digestion vessels. You can pipet 24 tubes at a time before refilling. Work quickly because this reagent drips.
13. After adding both reagents to each vessel, place the stoppers on each vessel, but not the caps.
14. Let the samples predigest for 30 minutes. While you wait, turn on the microwave and put all reagents and spike solution back into their appropriate storage areas.
15. Once the samples have finished predigesting, screw on a threaded cap digestion tube threaded cap. Be certain the cap is sealing the tube securely. Use the digestion tube tightening tool to securely tighten each cap, turn the cap with the tool clockwise until you hear a click (this will ensure the lid will remain sealed under pressure during the digestion). Be sure this is done under the fume hood, not outside the fume hood.
16. You can carry the stoppered and capped tubes back to the microwave. Set them on the counter to the right of the carousel which is to the right of the microwave.

17. Before you load the digestion vessels to the carousel, have an exact plan where you will place each test tube. Refer to the vessel diagram to ensure even heating throughout the microwave process.
18. Transfer digestion vessels to the designated positions on the carousel. The numbers on the test tube rack may or may not match the numbers on the carousel. Always start loading digestion vessel #1 on the inner circle which will either be #1 or the position indicated on the diagram after #1. On the outer circle, the first space is #17, so the same will hold on the outer circle. Each run may have a different configuration depending on the number of digestion vessels.
19. Load the carousel into the microwave by aligning position #1 with the front of the instrument. The turntable must be seated down on the drive lug.
20. On the front of the microwave, you will see a screen that has 2 choices: One Touch and _____.
21. Press ONE TOUCH. Then select pre-programmed ".190 soil" method (with a bust of a person icon). This method is:
    a. Temperature is 190 degrees C
    b. Hold time is 15 minutes
    c. Ramp time is 20-30 minutes
22. Press start and set a timer for 1 hour. This allows for digestion and cooling.
23. Remove the digestion vessels from the carousel and carefully place in the designated positions on the original test tube rack.
24. If you plan to decant the samples on the same day of digestion, place the rack in a bin and fill the bin with ice and cold water to make an ice bath. Then, place the vessels in the fume to cool for 30 to 45 minutes.
25. If you plan to decant the samples at a different time, you can leave the samples overnight in the microwave to cool.
26. Under the fume hood, remove the cap using the digestion tube tightening tool, turn counterclockwise. Keep your gloved hands to the sides, not directly over the top of the tubes AND point the top of the tube to the back of the fume hood. While it's unlikely that the vessels will be under pressure, it's still crucial to proceed with caution.
27. Pour the digestate from the digestion vessel into the corresponding 50 mL conical tube. Then using a squirt bottle; rinse the digestion tube with DI water, vortex the tube to mix the DI water, and pour into the conical tube. Repeat this step two to three times.

28. The 50-mL conical tubes will likely be slightly warm after decanting and rinsing with DI. Place the tubes in a 20 ^{o}C water bath for 30 minutes to let the samples come to temperature.
29. After the temperature of the samples is brought down to 20 ^{o}C, bring the volume of the conical tube up to the 50-mL line with DI. Screw the cap of the conical tube on securely.
30. Vortex each sample for 10 seconds on high after bringing them to 50-mL mark.
31. Centrifuge each sample for 20 minutes.
32. The samples are now ready to be analyzed colorimetrically using the ascorbic acid method.

D. Raw datasets for Method Comparison and Conversion Factor Calculation

Table 4. Perchloric Method vs. Microwave Method Colorimetric Data

Date:	Station:	Depth:	Perchloric (µg/g)	Microwave (µg/g)
2/2/23	CL-01	1-2cm	876.91	819.58
2/2/23	CL-01	3-4cm	818.4	833.29
2/2/23	CL-01	5-6cm	712.94	696.73
2/2/23	CL-01	5-6 cm (dup)	709.96	714.15
2/2/23	CL-01	7-8cm	675.15	661.08
2/2/23	CL-01	9-10cm	652.31	660.33
2/2/23	CL-03	1-2cm	863.12	937.81
2/2/23	CL-03	3-4cm	727.58	692.92
2/2/23	CL-03	5-6cm	700.74	666.81
2/2/23	CL-03	5-6 cm (dup)	737.47	672.81
2/2/23	CL-03	7-8cm	698.07	567.99
2/2/23	CL-03	9-10cm	**620.12***	**887.88***
2/2/23	CL-04	1-2cm	670.86	641.46
2/2/23	CL-04	3-4cm	732.77	743.04
2/2/23	CL-04	5-6cm	775.4	729.45
2/2/23	CL-04	5-6 cm (dup)	917.95	788.44
2/2/23	CL-04	7-8cm	781.99	697.41
2/2/23	CL-04	9-10cm	782.22	774.42
5/16/23	CL01	1-2 cm	884.12	880.38
5/16/23	CL01	3-4 cm	746.1	769.02
5/16/23	CL01	5-6 cm	998.24	857.54
5/16/23	CL01	5-6 cm (dup)	799.07	746.34
5/16/23	CL01	7-8 cm	739.76	729.17
5/16/23	CL01	9-10 cm	685.54	716.71
5/16/23	CL03	1-2 cm	1463.81	1408.04
5/16/23	CL03	3-4 cm	1036.1	1007.09
5/16/23	CL03	5-6 cm	840.12	824.63
5/16/23	CL03	5-6 cm (dup)	844.01	885.51
5/16/23	CL03	7-8 cm	742.77	714.23
5/16/23	CL03	9-10 cm	899.16	831.48
5/16/23	CL04	1-2 cm	1167.52	1055.55
5/16/23	CL04	3-4 cm	878.49	831.69
5/16/23	CL04	5-6 cm	764.01	665.1
5/16/23	CL04	5-6 cm (dup)	740.9	714.23
5/16/23	CL04	7-8 cm	727.24	617.56
5/16/23	CL04	9-10 cm	687.31	660.85
6/7/23	CL01	1-2 cm	1010.4	879.93
6/7/23	CL01	3-4 cm	774.48	779.22
6/7/23	CL01	5-6 cm	829.59	759.11
6/7/23	CL01	5-6 cm (dup)	856.21	766.51
6/7/23	CL01	7-8 cm	741.87	742

6/7/23	CL01	9-10 cm	**738.45***	**1463.82***
6/7/23	CL03	1-2 cm	**1317.1***	**825.09***
6/7/23	CL03	3-4 cm	982.68	902.69
6/7/23	CL03	5-6 cm	911.88	868.89
6/7/23	CL03	5-6 cm (dup)	913.72	838.21
6/7/23	CL03	7-8 cm	887.2	814.01
6/7/23	CL03	9-10 cm	863.39	766.94
6/7/23	CL04	1-2 cm	1276.17	1155.67
6/7/23	CL04	3-4 cm	915.98	777.65
6/7/23	CL04	5-6 cm	698.93	628.16
6/7/23	CL04	5-6 cm (dup)	689.15	616.99
6/7/23	CL04	7-8 cm	732.1	641.52
6/7/23	CL04	9-10 cm	**345.96***	**680.15***

*Bolded values are outliers and were excluded from statistical analyses.

Table 5. Perchloric method vs. Microwave method ICPMS Data

Date:	Station:	Depth:	Perchloric (µg/g)	Microwave (µg/g)
2/2/23	CL-01	1-2cm	950.98	960.85
2/2/23	CL-01	3-4cm	943.02	887.18
2/2/23	CL-01	5-6cm	827.22	757.53
2/2/23	CL-01	5-6 cm (dup)	856.51	774.25
2/2/23	CL-01	7-8cm	760.08	699.87
2/2/23	CL-01	9-10cm	750.76	708.69
2/2/23	CL-03	1-2cm	1003.05	982.35
2/2/23	CL-03	5-6cm	778.37	735.54
2/2/23	CL-03	5-6 cm (dup)	817.09	744.81
2/2/23	CL-03	7-8cm	792.51	782.05
2/2/23	CL-03	9-10cm	777.1	785.72
2/2/23	CL-04	1-2cm	771.63	731.51
2/2/23	CL-04	3-4cm	813.3	822.97
2/2/23	CL-04	5-6cm	855.56	813.56
2/2/23	CL-04	5-6 cm (dup)	991.46	895.1
2/2/23	CL-04	7-8cm	863.8	850.67
2/2/23	CL-04	9-10cm	839.67	872.91
5/16/23	CL01	1-2 cm	1186.7	1015.31
5/16/23	CL01	3-4 cm	992.37	878.81
5/16/23	CL01	5-6 cm	959.43	941.08
5/16/23	CL01	5-6 cm (dup)	835.18	885.32
5/16/23	CL01	7-8 cm	921.95	968.99
5/16/23	CL01	9-10 cm	810.6	705.11
5/16/23	CL03	1-2 cm	**2359.95***	**1067.36***
5/16/23	CL03	3-4 cm	1223.68	1072.57
5/16/23	CL03	5-6 cm	1063.03	847.45
5/16/23	CL03	5-6 cm (dup)	912.1	1040.99
5/16/23	CL03	7-8 cm	1172.39	973.19
5/16/23	CL03	9-10 cm	**583.76***	**801.8***

5/16/23	CL04	1-2 cm	**4499.23***	**1017.27***
5/16/23	CL04	3-4 cm	1087.62	1059.63
5/16/23	CL04	5-6 cm	775.24	730.81
5/16/23	CL04	5-6 cm (dup)	792.46	705.64
5/16/23	CL04	7-8 cm	784.85	730.72
5/16/23	CL04	9-10 cm	859.43	845.62
6/7/23	CL01	1-2 cm	1093.3	937.79
6/7/23	CL01	3-4 cm	839.73	826.9
6/7/23	CL01	5-6 cm	908.28	818.41
6/7/23	CL01	5-6 cm (dup)	899.71	833.31
6/7/23	CL01	7-8 cm	807.73	799.57
6/7/23	CL01	9-10 cm	**766.38***	**1579.88***
6/7/23	CL03	1-2 cm	**1519.72***	**881.74***
6/7/23	CL03	3-4 cm	1075.14	951.74
6/7/23	CL03	5-6 cm	961.93	884.08
6/7/23	CL03	5-6 cm (dup)	962.24	878.1
6/7/23	CL03	7-8 cm	1071.2	836.36
6/7/23	CL03	9-10 cm	904.78	778.54
6/7/23	CL04	1-2 cm	1460.42	1242.68
6/7/23	CL04	3-4 cm	971.02	861.85
6/7/23	CL04	5-6 cm	769.28	678.92
6/7/23	CL04	5-6 cm (dup)	762.05	690.53
6/7/23	CL04	7-8 cm	762.31	704.85
6/7/23	CL04	9-10 cm	729.71	738.45

*Bolded values are outliers and were excluded from statistical analyses.

Table 6. Perchloric Method vs. Microwave Method Conversion Factor Data

Date:	Station:	Depth:	Perchloric (µg/g)	Microwave (µg/g)
2/2/23	CL-01	1-2cm	876.91	819.58
2/2/23	CL-01	3-4cm	818.4	833.29
2/2/23	CL-01	5-6cm	712.94	696.73
2/2/23	CL-01	5-6 cm (dup)	709.96	714.15
2/2/23	CL-01	7-8cm	675.15	661.08
2/2/23	CL-01	9-10cm	652.31	660.33
2/2/23	CL-03	1-2cm	863.12	937.81
2/2/23	CL-03	3-4cm	727.58	692.92
2/2/23	CL-03	5-6cm	700.74	666.81
2/2/23	CL-03	5-6 cm (dup)	737.47	672.81
2/2/23	CL-03	7-8cm	698.07	567.99
2/2/23	CL-03	9-10cm	**620.12***	**887.88***
2/2/23	CL-04	1-2cm	670.86	641.46
2/2/23	CL-04	3-4cm	732.77	743.04
2/2/23	CL-04	5-6cm	775.4	729.45
2/2/23	CL-04	5-6 cm (dup)	917.95	788.44
2/2/23	CL-04	7-8cm	781.99	697.41
2/2/23	CL-04	9-10cm	782.22	774.42

3/8/23	CL-01	1-2cm	1134.46	1136.85
3/8/23	CL-01	3-4cm	955.25	999.49
3/8/23	CL-01	5-6cm	881.28	852.62
3/8/23	CL-01	5-6 cm (dup)	907.67	827.8
3/8/23	CL-01	7-8cm	727.43	730.62
3/8/23	CL-01	9-10cm	720.78	705.44
3/8/23	CL-03	1-2cm	2063.88	1980.76
3/8/23	CL-03	3-4cm	1199.25	1040.31
3/8/23	CL-03	5-6cm	808.57	723.66
3/8/23	CL-03	5-6 cm (dup)	1017.32	843.5
3/8/23	CL-03	7-8cm	723.83	710.47
3/8/23	CL-03	9-10cm	689.39	693.16
3/8/23	CL-04	1-2cm	1144.89	1095.48
3/8/23	CL-04	3-4cm	976.91	1018.44
3/8/23	CL-04	5-6cm	801.99	756.46
3/8/23	CL-04	5-6 cm (dup)	853.92	807.32
3/8/23	CL-04	7-8cm	900.48	721.48
3/8/23	CL-04	9-10cm	713.59	685.19
4/5/23	CL-01	1-2cm	995.28	947.81
4/5/23	CL-01	3-4cm	821.31	809.3
4/5/23	CL-01	5-6cm	797.89	776.76
4/5/23	CL-01	5-6 cm (dup)	787.94	791.27
4/5/23	CL-01	7-8cm	782.04	796.55
4/5/23	CL-01	9-10cm	553.36	627.79
4/5/23	CL-03	1-2cm	1136.91	1315.4
4/5/23	CL-03	3-4cm	910.14	849.68
4/5/23	CL-03	5-6cm	842.47	791.44
4/5/23	CL-03	5-6 cm (dup)	837.18	905.19
4/5/23	CL-03	7-8cm	748.61	841.35
4/5/23	CL-03	9-10cm	723.19	647.99
4/5/23	CL-04	1-2cm	1271.83	1244
4/5/23	CL-04	3-4cm	924.69	890.22
4/5/23	CL-04	5-6cm	672.65	617.13
4/5/23	CL-04	5-6 cm (dup)	679.37	642.61
4/5/23	CL-04	7-8cm	673.66	617.13
4/5/23	CL-04	9-10cm	648.38	765.03
5/16/23	CL01	1-2 cm	884.12	880.38
5/16/23	CL01	3-4 cm	746.1	769.02
5/16/23	CL01	5-6 cm	998.24	857.54
5/16/23	CL01	5-6 cm (dup)	799.07	746.34
5/16/23	CL01	7-8 cm	739.76	729.17
5/16/23	CL01	9-10 cm	685.54	716.71
5/16/23	CL03	1-2 cm	1463.81	1408.04
5/16/23	CL03	3-4 cm	1036.1	1007.09
5/16/23	CL03	5-6 cm	840.12	824.63
5/16/23	CL03	5-6 cm (dup)	844.01	885.51
5/16/23	CL03	7-8 cm	742.77	714.23
5/16/23	CL03	9-10 cm	899.16	831.48

5/16/23	CL04	1-2 cm	1167.52	1055.55
5/16/23	CL04	3-4 cm	878.49	831.69
5/16/23	CL04	5-6 cm	764.01	665.1
5/16/23	CL04	5-6 cm (dup)	740.9	714.23
5/16/23	CL04	7-8 cm	727.24	617.56
5/16/23	CL04	9-10 cm	687.31	660.85
6/7/23	CL01	1-2 cm	1010.4	879.93
6/7/23	CL01	3-4 cm	774.48	779.22
6/7/23	CL01	5-6 cm	829.59	759.11
6/7/23	CL01	5-6 cm (dup)	856.21	766.51
6/7/23	CL01	7-8 cm	741.87	742
6/7/23	CL01	9-10 cm	**738.45***	**1463.82***
6/7/23	CL03	1-2 cm	**1317.1***	**825.09***
6/7/23	CL03	3-4 cm	982.68	902.69
6/7/23	CL03	5-6 cm	911.88	868.89
6/7/23	CL03	5-6 cm (dup)	913.72	838.21
6/7/23	CL03	7-8 cm	887.2	814.01
6/7/23	CL03	9-10 cm	863.39	766.94
6/7/23	CL04	1-2 cm	1276.17	1155.67
6/7/23	CL04	3-4 cm	915.98	777.65
6/7/23	CL04	5-6 cm	698.93	628.16
6/7/23	CL04	5-6 cm (dup)	689.15	616.99
6/7/23	CL04	7-8 cm	732.1	641.52
6/7/23	CL04	9-10 cm	**345.96***	**680.15***

*Bolded values are outliers and were excluded from statistical analyses.

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