INVESTIGATION INTO THE NECESSITY OF DISSOLVED ORTHOPHOSPHATE-PHOSPHORUS FIELD FILTRATION

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The preparation of this report was financed through grants from the Texas Commission on Environmental Quality.

July, 2007

Study Design

Texas Commission on Environmental Quality requires that dissolved orthophosphate (OP) be field filtered within fifteen minutes of collection. Previous protocols permitted transport to the laboratory for filtering. Questions have arisen concerning the necessity of field filtration, given that it is more time consuming and costly than laboratory filtration. Presumably field filtration is more accurate due to avoiding artifacts that can arise during sample holding, such as uptake by microorganisms or exchange with abiotic particulates. Possibly, field sampling is less precise, due to variations in filtering implementation under field conditions.

To study the difference between field and lab filtered dissolved OP, the Trinity River Authority (TRA) partnered with Tarrant Regional Water District (TRWD), the Texas Commission on Environmental Quality (TCEQ), and Dr. James Grover of the University of Texas at Arlington (UTA). In an attempt to make the study relevant statewide, the entities determined that four sites should be chosen with the criterion that each represents a different water body type. The following sites were chosen based on a combination of location, water body characteristics, and historical dissolved OP results. The following four sites best fit the design parameters:

Station 1 (10937) - Trinity River at Mockingbird (relatively low turbidity stream)
Station 2 (10919) - Trinity River near Oakwood (high turbidity stream)
Station 3 (15151) - Benbrook Lake surface (epilimnion in a fairly eutrophic reservoir)
Station 4 (15151) - Benbrook Lake bottom (hypolimnion in a fairly eutrophic reservoir)

To increase the confidence of the results, total phosphorous (TP) was collected in duplicate and both field and lab filtered dissolved OP were collected in triplicate. An additional suite of parameters, total suspended solids (TSS), volatile suspended solids (VSS), total dissolved solids (TDS), and Chlorophyll-a (Chl-a), was collected to determine if any organic and/or inorganic processes demonstrated any affect on measured OP concentrations. All samples were collected, preserved, and handled as outlined in the approved TCEQ Surface Water Quality Monitoring Procedures and/or Standard Methods.

Beginning March 2006, TRA Clean Rivers staff collected twelve monthly samples at Station 1 and 2 and TRWD collected samples at stations 3 and 4. After triple rinsing all equipment with deionized water, grab samples were composited into a 5 gallon bucket in order to ensure that all samples at each site were taken from the same parcel of water. Cubitainers for TSS, VSS, TDS, and Chl-a were filled directly from the composite sample. Duplicate TP and triplicate lab filtered OP samples were split into cubitainers from the composite bucket. Field filtered OP samples were filtered from the composite bucket through a 0.45 um in-line capsule filter into a single one gallon cubitainer, then the filtrate was split into three 1 liter cubitainers.

Except for the triplicate reservoir samples for OP, all laboratory analysis was completed at the TRA Central Regional Wastewater System (CRWS) laboratory in Grand Prairie, Texas. Because the CRWS laboratory is unable to reach a reporting limit below 0.04 mg/L, the triplicate reservoir OP samples were analyzed by the TRWD TRAC laboratory which has an RL of 0.005 mg/L. To maximize any biological and inorganic processes occurring within the unfiltered samples that may affect OP values, all field and laboratory filtered OP samples were held for a minimum forty of the maximum forty-eight hour hold time.

All field and laboratory data were compiled and quality assured by TRA staff and delivered to Dr. James Grover for analysis. Errors in collection or laboratory methods were noted and Dr. Grover determined if the error resulted in data being excluded from the analysis.

Results

At Station 10937 (Trinity River at Mockingbird), OP usually ranged from 0.2 to 2.0 mg / liter, generally being lowest in late summer (Fig. 1A), apart from one sample (March 14, 2006) for which two of three field-filtered replicates were analyzed beyond the 48-hour holding time and variance between replicates was unusually large. Because this indicates a substantive methodological error, results from this sampling event were not further analyzed. Aside from this event, variation among replicate determinations was very small for both filtration techniques, and there was a negligible difference between filtration techniques.

At Station 10919 (Trinity River near Oakwood), OP was in a similar range, from 0.2 to 1.4 mg / liter, and was generally highest in summer (Fig. 1B). For both filtration techniques, variation among replicate determinations was very small for both filtration techniques, and there was a negligible difference between filtration techniques.

At station 15151 (Benbrook Lake), OP in both surface and bottom samples was usually undetectable, a result coded as 0.005 mg / liter (Fig. 1C, D). Data from four sampling events at station 15151 had substantive methodological errors and were not further analyzed. Both surface and bottom samples taken October 10, 2006 were analyzed beyond the 48-hour holding time (OP was undetectable in both). On two occasions holes or loose caps were found in sample bottles suggesting possible contamination. For surface samples taken on March 15, 2006, OP was undetectable. For bottom samples taken May 17, 2006, some of the field-filtered sample bottles appeared to be contaminated, since this was the only occasion on which there was substantial variation among replicates, and also the only occasion where there was a large difference between filtration types or between surface and bottom samples.

In summary, OP concentrations were high and seasonally variable at the two river stations, and low and usually undetectable at the two reservoir stations. Except for occasions when substantive methodological errors were noted, variance among replicates was low, and differences between filtration types were very small. After removing errors, differences between surface and bottom samples in Lake Benbrook were also very small. For further statistical analysis, sample events with errors were not considered, and the distinction between surface and bottom samples from Lake Benbrook was neglected.

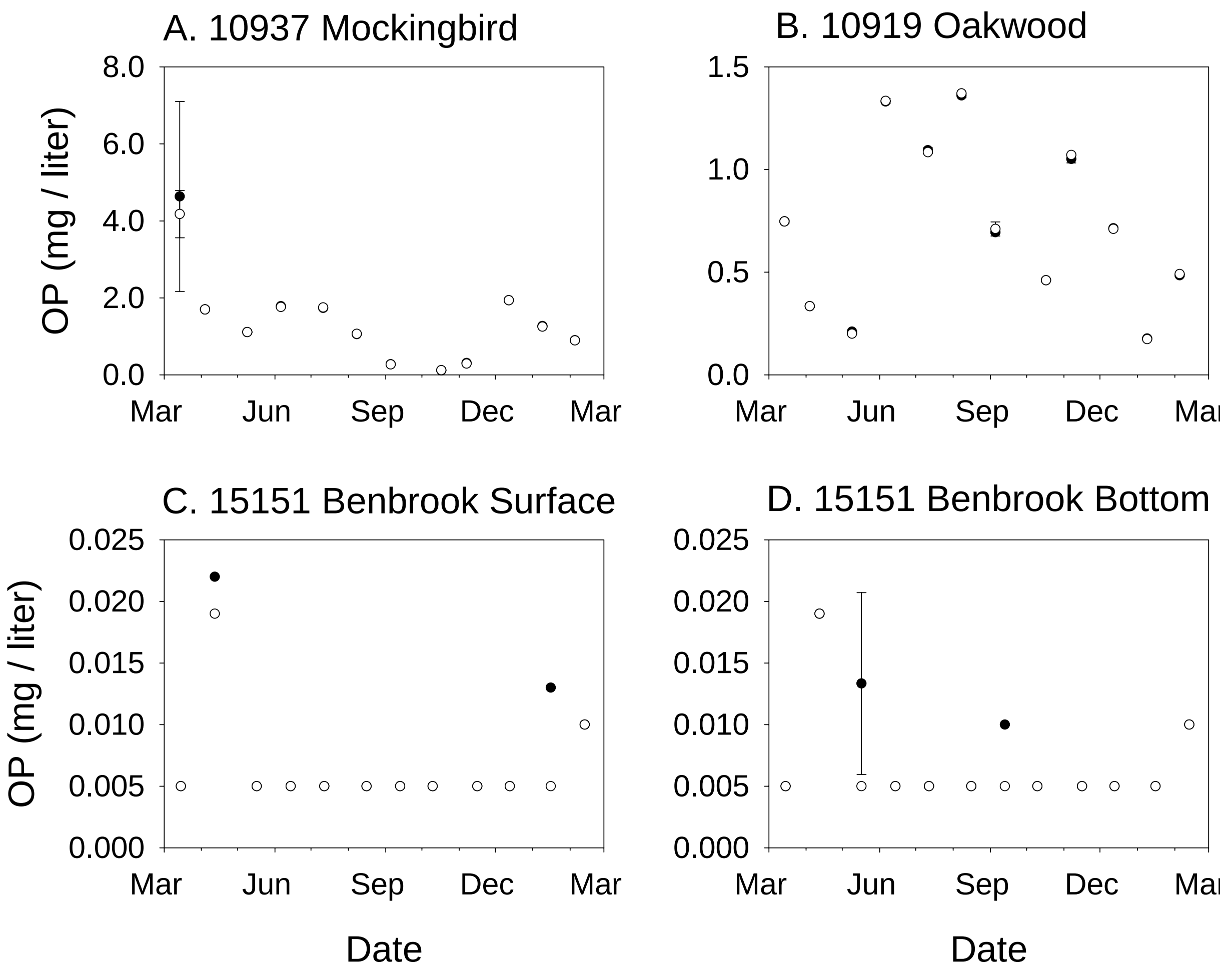

Fig. 1. OP at the four sites studied. Average and standard deviation of three replicate determinations are shown for field-filtered (●) and lab-filtered (○) samples. Note that in most cases data overlap due to the very close agreement of the two techniques, and that in most cases error bars are smaller than the data symbols, indicating very low variance among replicates. A. Station 10937 Trinity River at Mockingbird; B. Station 10919 Trinity River near Oakwood; C. Station 15151 Lake Benbrook Surface; D. Station 15151 Lake Benbrook Bottom.

Difference between Filtration Types

To analyze the difference between OP determined by field- or lab-filtration, a paired sample approach was taken. For each sampling event (excluding those with errors), replicate determinations were averaged for each filtration type, and the difference between filtration types was computed as the average field-filtered OP minus the average lab-filtered OP. The average difference and its standard error (SE) were used to test the null hypothesis of a zero difference using the *t*-statistic. This test was done for the full data set and for data excluding samples where OP was undetectable (i.e. many of the samples from Lake Benbrook).

For both the full data, and data restricted to detectable measurements of OP, the difference between filtration types was small (< 0.02 mg / liter, Table 1). The positive value for the average difference indicates a tendency for field-filtered samples to have higher OP than lab-filtered samples. However, this difference was not significant for either data set ($P > 0.05$, Table 1).

Table 1. Analysis of difference between filtration types

	All Data	Undetectable Data Excluded
Average Difference (mg / liter)	0.0115	0.0179
SD of Difference (mg / liter)	0.0704	0.0889
SE of Difference (mg / liter)	0.0107	0.0171
N	43	27
t	1.074	1.046
P	0.144	0.153

Variation among Replicates

Variation among replicates within a given sampling event and filtration type was generally very low, with many instances of zero variance (when all replicates yielded the same measurement). Instance of zero variance were not limited to undetectable results (where it is an artifact of data coding). To express variation among replicates, pooled standard deviations were calculated from the within-sample variation, for both filtration types, and for both the full data set and with undetectable results removed (Table 2).

Table 2. Pooled within-sample standard deviations (mg / liter)

	Filtration Type	
	Field	Lab
All Data	0.000577	0.000773
Undetectable Data Excluded	0.000729	0.000975

For both filtration types, pooled within-sample standard deviations were < 0.001 mg / liter, regardless of whether undetectable data were excluded. Thus variation among replicate determinations is very small compared to the detection limit of the method, and it is also small compared to typical seasonal and between-station variations for the data presented here.

Nevertheless, the data also indicate a consistent tendency for higher variation among replicates when filtration is done in the lab rather than in the field. To test the significance of this trend, the F_{max} statistic was used. This statistic is the ratio of the highest to the lowest variance in a set of two or more variances. The pooled standard deviations in Table 2 were squared, and the F_{max} statistic was calculated for the full data set, and the data with undetectable values excluded. Available tables of critical values for F_{max} were inadequate given the degrees of freedom involved, so approximate critical values for $\alpha = 0.05$ were computed using nonlinear interpolation. For the full data set, the F_{max} ratio of lab- to field-filtered samples was 1.79, compared to an approximate critical value of 1.23. For data excluding undetectable values, the F_{max} ratio of lab- to field-filtered samples was again 1.79, compared to an approximate critical value of 1.75. Both of these results indicate significantly higher variance for lab-filtered samples, compared to field-filtered samples, at a significance level of $P < 0.05$. However, this must be regarded as a weak result. The F_{max} ratios do not greatly exceed the critical values, which are themselves approximate, so that even if the true P-value is less than 0.05, it is not much lower, and statistically the result should be regarded as "barely significant". Given some underlying problems with the F_{max} statistic, many statisticians advise caution when interpreting such a result. In any case, the variation among replicate determinations for either filtration technique is very low, and well within the limits required for a practically useful technique.

Correlates of Methodological Variations

For the results presented here, differences between filtration types were not statistically significant, and within-sample variations were generally small. Nevertheless, the additional data collected during this study permit an exploration of several extraneous variables that might affect determinations of OP. These variables can be broken down into meteorological variables (air temperature, days since rain), geophysical variables (Secchi depth, flow, flow severity, water temperature, lake elevation), solute variables (pH, conductance, dissolved oxygen, TDS), particulates (VSS, TSS, Chl-a, TP), and methodological variables (delivery temperature, holding time). Summary statistics for these variables are presented in the Appendix. Correlations were computed for all these variables with (1) the difference between field- and lab-filtered determinations, (2) the within-sample standard deviation of field-filtered samples, and (3) the within-sample standard deviation of lab-filtered samples.

None of the extraneous variables was significantly correlated with the difference between field- and lab-filtered determinations (Table 3). Two indicators of overall solute concentration were positively correlated with the standard deviations of both field- and lab-filtered samples: conductance and TDS (Table 3, Fig. 2). Replicate determinates for both filtration types were more variable when conductance and TDS were high, suggesting that high solute concentration contributes to variance in OP determinations. TP was also positively correlated with the standard deviations of both field- and lab-filtered samples (Table 2, Fig 3A, B). In many cases, OP was a substantial portion of TP, so this result implies that the variance of replicate OP determinations increases with the level of this parameter, as is expected since the OP data were skewed. Secchi depth was negatively correlated with the standard deviations of both field- and lab-filtered samples (Table 2, Fig 3C, D). When Secchi depth is low, variance in replicate OP determinations increases, possibly because low Secchi depths are related to high concentrations of P-reactive particles such as clays and microbial cells. However, no other indicators of particulates were significantly correlated with variation in OP determinations.

Two methodological variables were significantly correlated with the standard deviations of field- or lab-filtered samples: delivery temperature and holding time (Table 3, Fig. 4). Samples at higher temperatures upon delivery had higher variance for both field- and lab-filtered determinations of OP. Many of these samples were from the Trinity River Mockingbird site. These samples were transported to the lab on ice, but the transport time for these samples was very short (30-45 min), which might not have allowed complete cooling. Samples from other sites were transported for 2-5 hr, and thus reached lower temperatures. Therefore, the higher variance of samples with warmer delivery temperatures does not represent deviation from study protocols, and probably does not imply storage artifacts. Rather, it is likely that the Mockingbird site simply has higher variance for OP than other sites. The OP data analyzed here are skewed, and since the Mockingbird site has the highest average OP, it is expected also to have the highest variance.

The correlation of holding time with variance of OP determinations was negative, though significant only for field-filtered samples. This is both surprising and difficult to interpret. Storage artifacts would be expected to contribute to greater variance as holding time increased. Protocols for this study required holding samples until near the end of the maximum 48-hour holding time. However, the two laboratories performing analyses achieved this protocol to differing extents. Samples analyzed at CRWS were held for an average of 41.6 hours, while those analyzed at TRAC were held for an average of 47.1 hours. Thus the different variances associated with different holding times could have resulted primarily from differences in procedures at the two laboratories. In any case, the within-sample variances of replicate OP determinations found in this study for both filtration methods were very low. While these variances do appear to be associated with certain environmental and methodological parameters, they are well within the limits required for a practically useful technique.

Table 3. Correlations of extraneous variables with methodological variations

Variable	Field-Lab Difference	Field-filtered Std. Deviation	Lab-filtered Std. Deviation
Air Temperature	-0.088	0.152	0.270
Days Since Rain	0.097	-0.196	-0.061
Secchi Depth	0.055	-0.509*	-0.355*
Flow	0.122	-0.265	-0.189
Flow Severity	0.333	-0.249	-0.189
Water Temperature	-0.065	0.049	0.253
Lake Elevation	-0.011	0.120	†
pH	-0.122	-0.145	-0.048
Conductance	-0.089	0.695*	0.465*
DO	-0.055	0.207	-0.014
TDS	-0.101	0.716*	0.469*
VSS	0.129	-0.005	0.009
TSS	0.154	0.027	0.061
Chl-a	-0.007	-0.247	-0.002
TP	0.032	0.648*	0.476*
Delivery Temperature	0.273	0.398*	0.364*
Holding Time	-0.158	-0.518*	-0.232

*Significant at $P < 0.05$

†This correlation was not computed due to lack of variation in reservoir samples where elevation data were also available.

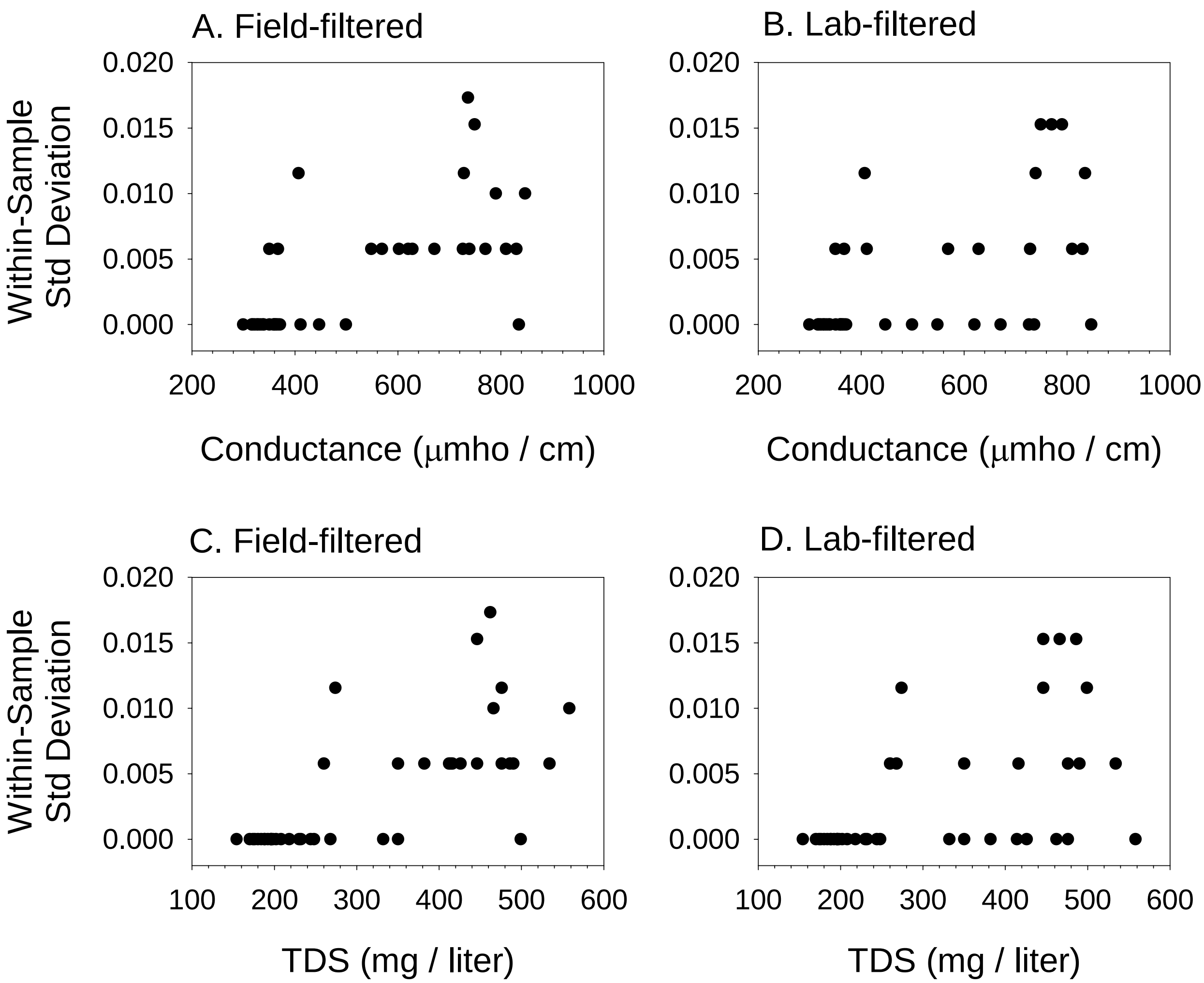

Fig. 2. Within-sample variation of OP determinations versus measures of total solute concentration.

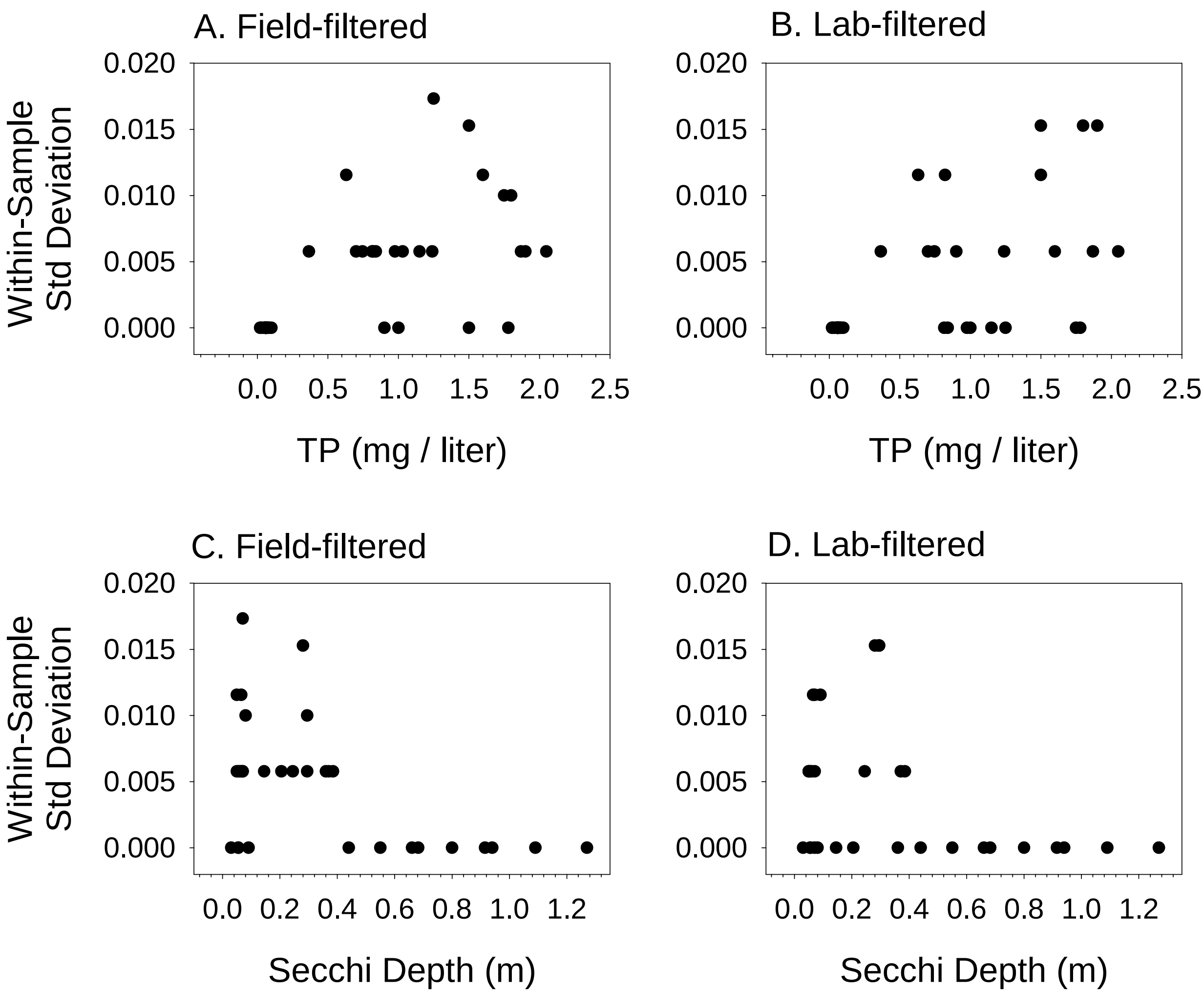

Fig. 3. Within-sample variation of OP determinations versus TP and Secchi depth.

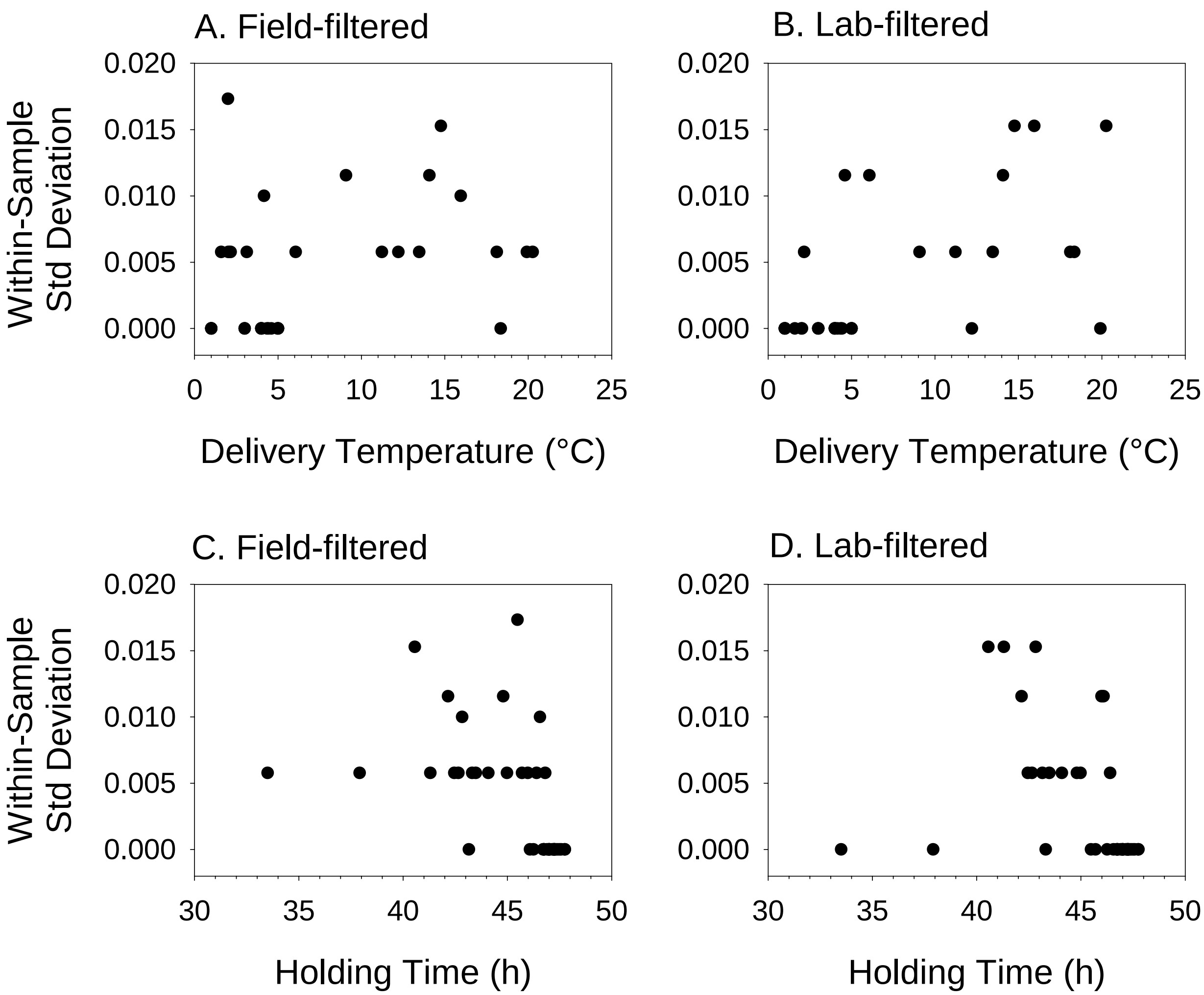

Fig. 4. Within-sample variation of OP determinations versus delivery temperature and holding time.

Conclusions and Recommendations

Conclusion 1: There is no evidence for a statistically significant or practically important difference between field- and lab-filtered OP determinations. The magnitude of the difference between filtration methods for samples collected during the same event is small relative to the variation in OP between stations used in this study, and is small relative to the seasonal variation in OP observed at two of the four stations.

Conclusion 2: Within-sample variances of replicate OP determinations are very small for either method. For both methods, there was high agreement among replicate determinations of OP on the same sample, filtered by the same method. The within-sample variations were small relative to the variation in OP between stations used in this study, and were small relative to the seasonal variation in OP observed at two of the four stations.

Conclusion 3: There is suggestive evidence that the lab-filtration method has somewhat higher within-sample variance than the field-filtration method. This conclusion is only suggestive. There are statistical problems in the analysis that compares the variances of the two filtration methods. Moreover, the within sample variance is small for both filtration methods, as concluded above. In particular, the small increase in variance for lab-filtration (if indeed it is real) does not seem to outweigh its large advantages in materials cost and labor.

Conclusion 4: There is suggestive evidence that the within sample variance of OP determinations is sensitive to storage conditions and holding time. This conclusion is supported by the correlations found for the variances of both filtration techniques with delivery temperature and holding time. This conclusion is only suggestive, and these patterns apparently result from subtle differences in procedures for transportation and storage.

Conclusion 5: There is suggestive evidence that the within sample variance of OP determinations increases with solute concentrations, sample phosphorus concentration (TP and OP), and the concentration of P-reactive particles. This conclusion is supported by the correlations found for the variances of both filtration techniques with certain environmental parameters determined for the same samples.

Recommendation 1: Filtration of OP samples at the laboratory after transportation from the field should be permitted.

Recommendation 2: Storage and transportation of OP samples should be standardized as much as possible.

Appendix. Summary Statistics for Additional Data Collected

Days Since Rain					
Station	Mean	N	SD	Minimum	Maximum
10919	3.5	12	2.3	1.0	7.0
10937	3.5	11	2.4	1.0	7.0
15151	5.0	20	2.8	1.0	7.0
All Stations	4.2	43	2.6	1.0	7.0

Air Temperature (°C)					
Station	Mean	N	SD	Minimum	Maximum
10919	24.0	12	7.9	11.0	33.0
10937	26.7	11	7.7	10.0	36.0
15151	18.4	20	11.3	0.0	33.0
All Stations	22.1	43	10.1	0.0	36.0

Secchi Depth (m)					
Station	Mean	N	SD	Minimum	Maximum
10919	0.07	12	0.03	0.03	0.15
10937	0.24	11	0.12	0.06	0.39
15151	0.82	9	0.26	0.44	1.27
All Stations	0.34	32	0.35	0.03	1.27

Flow (cfs)					
Station	Mean	N	SD	Minimum	Maximum
10919	1887	12	1984	703	7430
10937	1610	11	2263	447	8070
15151	Parameter not applicable to reservoirs				
All Stations	1755	23	2078	447	8070

Flow Severity					
Station	Mean	N	SD	Minimum	Maximum
10919	2.8	12	0.87	2	5
10937	3.5	11	0.93	3	5
15151	Parameter not applicable to reservoirs				
All Stations	3.1	23	0.97	0	5

Water Temperature (°C)					
Station	Mean	N	SD	Minimum	Maximum
10919	20.7	12	7.8	8.0	29.8
10937	22.6	11	6.3	12.6	31.0
15151	18.9	20	8.2	7.7	30.7
All Stations	20.4	43	7.6	7.7	31.0

Lake Elevation (ft)					
Station	Mean	N	SD	Minimum	Maximum
10919	Parameter not applicable to streams				
10937					
15151	688	20	3.2	682	692
All Stations	688	20	3.2	682	692

pH					
Station	Mean	N	SD	Minimum	Maximum
10919	7.83	12	0.115	7.66	8.03
10937	7.75	11	0.149	7.61	8.04
15151	7.93	20	0.375	7.48	8.78
All Stations	7.85	43	0.280	7.48	8.78

Conductance (μmhos / cm)					
Station	Mean	N	SD	Minimum	Maximum
10919	628	12	152	367	847
10937	649	11	177	350	830
15151	341	20	21	300	371
All Stations	500	43	190	300	847

Dissolved Oxygen (mg / liter)					
Station	Mean	N	SD	Minimum	Maximum
10919	8.24	12	1.82	6.27	11.44
10937	8.32	10	1.56	5.49	10.83
15151	7.25	20	4.04	0.10	11.16
All Stations	7.79	42	3.04	0.10	11.44

Note – one obvious erroneous value was removed.

Total Dissolved Solids (mg / liter)					
Station	Mean	N	SD	Minimum	Maximum
10919	427	11	70	332	558
10937	412	11	99	260	534
15151	199	20	25	154	248
All Stations	314	42	128	154	558

Volatile Suspended Solids (mg / liter)					
Station	Mean	N	SD	Minimum	Maximum
10919	28.3	12	32.4	2.0	124.0
10937	10.5	11	11.1	2.0	39.0
15151	4.3	18	1.8	2.0	8.0
All Stations	13.0	41	20.6	2.0	124.0

Total Suspended Solids (mg / liter)					
Station	Mean	N	SD	Minimum	Maximum
10919	231.0	12	293.5	25.0	1130.0
10937	86.3	11	130.9	14.0	448.0
15151	9.1	20	3.0	6.0	18.0
All Stations	90.8	43	188.3	6.0	1130.0

Chlorophyll-a (mg / liter)					
Station	Mean	N	SD	Minimum	Maximum
10919	11.5	11	8.1	5.0	28.0
10937	14.2	11	9.7	5.0	33.0
15151	19.4	20	12.6	5.0	41.0
All Stations	16.0	42	11.2	5.0	41.0

Total Phosphorus (mg / liter)					
Station	Mean	N	SD	Minimum	Maximum
10919	1.15	12	0.406	0.70	1.78
10937	1.31	11	0.561	0.37	2.05
15151	0.06	20	0.018	0.02	0.10
All Stations	0.68	43	0.684	0.02	2.05

Delivery Temperature (°C)					
Station	Mean	N	SD	Minimum	Maximum
10919	4.0	11	2.2	1.6	9.1
10937	15.8	10	3.2	11.2	20.3
15151	3.1	16	1.6	1.0	5.0
All Stations	6.8	37	6.0	1.0	20.3

Holding Time (hr)					
Station	Mean	N	SD	Minimum	Maximum
10919	45.4	12	2.5	37.9	47.3
10937	41.8	11	2.9	33.5	44.1
15151	47.1	20	0.4	46.3	47.8
All Stations	45.3	43	2.9	33.5	47.8