

## **EFFECTIVELY USING A HANDHELD FLUOROMETER TO PREDICT LABORATORY EXTRACTED CHLOROPHYLL-A MEASUREMENTS IN LAKE WATER SAMPLES**

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### **ABSTRACT**

Chlorophyll-a concentration is one of the most valuable measurements of lake water quality. It is generally known that handheld fluorometers are available which conveniently measure chlorophyll-a *in vivo*, but the trade-off for this convenience is that these yield chlorophyll-a measurements in total fluorescence rather than the conventional micrograms per liter. The purpose of this paper is to propose a method for accurately predicting laboratory extracted chlorophyll-a concentration measurements in micrograms per liter from these *in vivo* total fluorescence measurements. The simplicity, accuracy and cost-effectiveness of this method make it ideal for implementation in volunteer lake monitoring programs.

### **KEYWORDS**

Chlorophyll-a, *in vivo*, volunteer monitoring, fluorometer, lakes, regression

### **INTRODUCTION**

Chlorophyll-a concentration is recognized as one of the most important indicators of lake water quality, in particular its trophic status (Carlson, 1977). Volunteers in lake monitoring organizations frequently take other more convenient measurements, most commonly Secchi disk transparency depth, instead of measuring chlorophyll-a concentration directly; see, for instance, (Clean Lakes Program, 2001). It is known, however, that Secchi disk transparency depth can be significantly confounded with other factors, and consequently regression models of chlorophyll-a concentration on secchi disk transparency depth suffer model assumption violations, primarily homoscedasticity (Carlson and Simpson, 1996). It is therefore of interest to investigate other indirect measurements which might accurately predict laboratory extracted chlorophyll-a concentration.

It is generally known that handheld fluorometers are available which conveniently measure chlorophyll-a *in vivo*. The trade-off for this convenience is that these *in vivo* chlorophyll-a measurements are in total fluorescence and not micrograms per liter, and hence are an indirect measurement of chlorophyll-a concentration. The purpose of this paper is to propose a method that will provide *in vivo* total fluorescence measurements that can accurately predict chlorophyll-

a concentration in micrograms per liter. The simplicity, accuracy and cost-effectiveness of this method make it ideal for implementation in volunteer lake monitoring programs.

### **The 2007 Grace College Water Quality Project**

During the summer of 2007, Grace College took up an initiative to come alongside various lake monitoring organizations to study the lake water quality in Kosciusko County, Indiana. In this initiative, a study was designed in which samples would be taken from several hundred sampling locations on lakes in the county over several months. One of the desired measurements for each sampling location in this study was laboratory extracted chlorophyll-a concentration. However, the planned number of sampling locations in the study made this measurement both inconvenient and cost-prohibitive. As a compromise, it was decided that a handheld fluorometer would be utilized to take in vivo chlorophyll-a measurements; the accepted trade-off for this compromise was that measurements would be in total fluorescence and not in micrograms per liter.

As Grace College carried out its study, fourteen water samples were selected from which to develop a linear regression model for converting the in vivo chlorophyll-a measurements into predicted micrograms per liter measurements that a laboratory extracted chlorophyll-a analysis would have yielded. Careful attention was given to both the measurements and the methodology of how measurements were taken. At each of these fourteen sampling locations a column of water was obtained with an integrated pipe sampler (Clean Lakes Program, 2001). An in vivo chlorophyll-a measurement was taken on this sample, and then the sample was filtered and sent to measure laboratory extracted chlorophyll-a concentration. A regression model of laboratory extracted chlorophyll-a concentration on in vivo total fluorescence was successfully obtained and utilized for converting in vivo total fluorescence measurements into predicted micrograms per liter measurements.

In this paper we will discuss the analysis of this data, which provides evidence that the in vivo measurement of chlorophyll-a is highly correlated with the laboratory extracted chlorophyll-a measurement when the in vivo measurement is taken on a column of water with an integrated pipe sampler.

### **METHODOLOGY**

Fourteen different lakes in Kosciusko County, Indiana were selected for the purpose of developing a regression model of laboratory extracted chlorophyll-a concentration on in vivo total fluorescence. These lakes were selected based upon their wide range of trophic status estimates. One random sampling location was selected over deep water on each of these lakes. All data presented here was gathered between August 6 and August 8, 2007.

At each of the fourteen sampling locations, a personally constructed integrated pipe sampler, as depicted in the Indiana Volunteer Lake Monitoring Program Expanded Monitoring Handbook (Clean Lakes Program, 2001), was used to obtain a column sample of water. A Turner Designs Aquafluor handheld fluorometer (Turner Designs; Sunnyvale, California) was utilized to take all in vivo total fluorescence measurements. Specifically, a column of water was sampled and emptied into a pitcher, and the in vivo total fluorescence measurement was taken on water in this

pitcher. In order to check the measurement accuracy of the fluorometer, a reading on the same sample of water was taken five times. Specifically, with the same water in the sample chamber, the fluorometer was turned off, then back on, and the measurement was re-read; these five readings are given in Table 1. Water from the pitcher was then filtered per laboratory specifications. Specifically, we added one pipet (approximately 1mL) of magnesium carbonate ( $MgCO_3$ ) to the sample for preservation, then filtered a specified amount, as specified in the Clean Lakes Program (2001), through a  $0.45\mu m$  membrane filter. The filter was then folded in half, sealed in aluminum foil to keep out light, and frozen solid. The filter was then sent to the laboratory frozen; the laboratory was told how much water had been filtered. All laboratory chlorophyll-a concentration measurements were done by the Great Lakes Environmental Center laboratory in Traverse City, Michigan; the laboratory analyses were done using Standard Method 10200 H (APHA, 1998). Other data that were gathered at each sampling location included Secchi disk transparency depth in meters using a standard 8 inch Secchi disk and an in vivo total fluorescence measurement on an arm's depth sample of water taken from the sampling location, commonly referred to as a grab sample. All sample measurements for each sampling location are given in Table 2; note that TF in Table 2 is the average of the five readings shown in Table 1.

**Table 1. Five Fluorometer Readings for Each Sampling Location**

(TF Readings 1 – 5, total fluorescence readings with the same water in the test chamber of the fluorometer taken from the column sample)

Lake	TF Reading 1	TF Reading 2	TF Reading 3	TF Reading 4	TF Reading 5
Beaver Dam	152.6	159.7	152.1	163.9	146.7
Big Barbee	150.8	138.8	139.2	126.4	140.8
Diamond	227.4	248	232.5	243.4	227.4
McClures	376.3	375.2	378.4	369.5	370.2
Palestine	557.6	550	576.7	573.8	586.1
Pike	294.9	270.7	275.9	281.4	293.3
Ridinger	95.76	99.55	93.34	89.1	90.76
Sellers	622.7	558	521.5	510.5	513.3
Silver	231.2	244.2	225.9	238.4	232.3
Spear	175.4	130.1	150	161.5	132.9
Syracuse	46.82	46.21	44.32	47.09	43.47
Tippecanoe	100.3	105.8	105.3	103.6	103.3
Waubee	66.57	60.29	58.47	57.23	61.07
Wawasee	49.06	53.37	49.13	50.19	45.13

**Table 2. Data from Fourteen Sampling Locations**

(SD, Secchi disk transparency depth; Chl, laboratory extracted chlorophyll-a concentration on the column sample of water; TF, total fluorescence, the average of TF Readings in table 1; TFGS, total fluorescence on the grab sample of water)

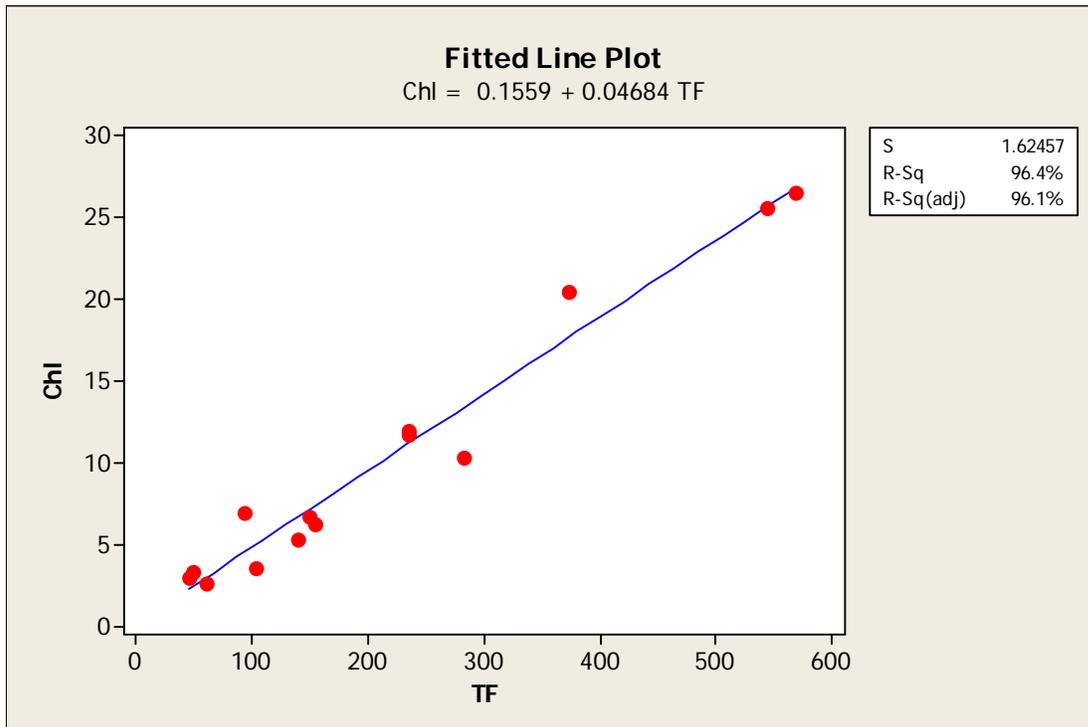
Lake	SD, (m)	Chl, (µg/L)	TF	TFGS
Beaver Dam	1.402	6.22	155	98.89
Big Barbee	1.645	5.28	139.2	110.5
Diamond	0.96	11.99	235.74	141.9
McClures	0.472	20.51	373.92	222.3
Palestine	0.563	26.51	568.84	592
Pike	1.051	10.31	283.24	326.1
Ridinger	1.005	6.97	93.702	80.36
Sellers	0.685	25.62	545.2	282.6
Silver	0.944	11.76	234.4	156.4
Spear	2.697	6.77	149.98	98.93
Syracuse	1.935	2.95	45.582	47.15
Tippecanoe	1.432	3.58	103.66	112
Waubee	2.392	2.68	60.726	67.62
Wawasee	1.63	3.36	49.376	52.46

## RESULTS

All statistical analyses were carried out with Minitab<sup>®</sup>, Release 14 (Minitab Statistical Software, State College, PA).

Using the data from Table 2, a simple linear least squares regression model of Chl on TF yields

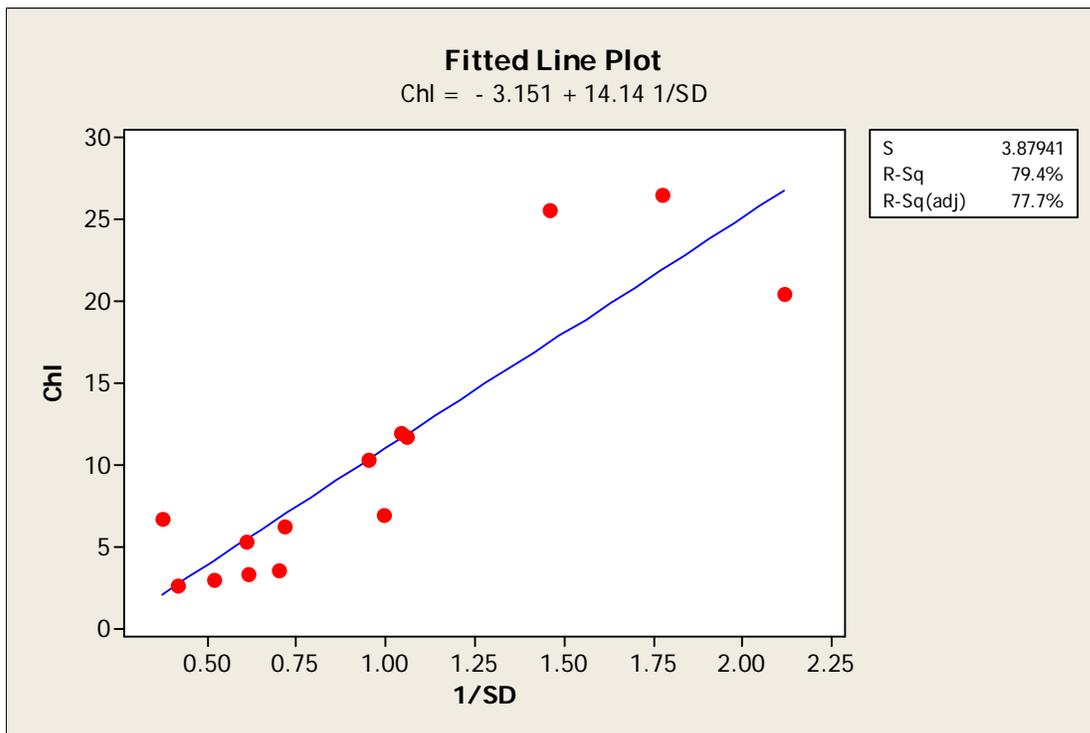
$$\text{Chl} = 0.1559 + 0.04684 * \text{TF}$$



The slope parameter 0.04684 is significant with a p-value less than .001 and the coefficient of determination for the model is 96.4%. There is no evidence of regression model assumption violations. In particular, there are no outlying residuals, the residuals have an Anderson-Darling normality test p-value of .610, and the spread of residuals about the fitted line show no symptoms of heteroscedasticity.

Chl is often regressed on 1/SD (Carlson and Simpson, 1996), and we do so here to contrast this model with our model of Chl regressed on TF. Using the data from Table 2, the simple linear least squares regression model of Chl on 1/SD yields

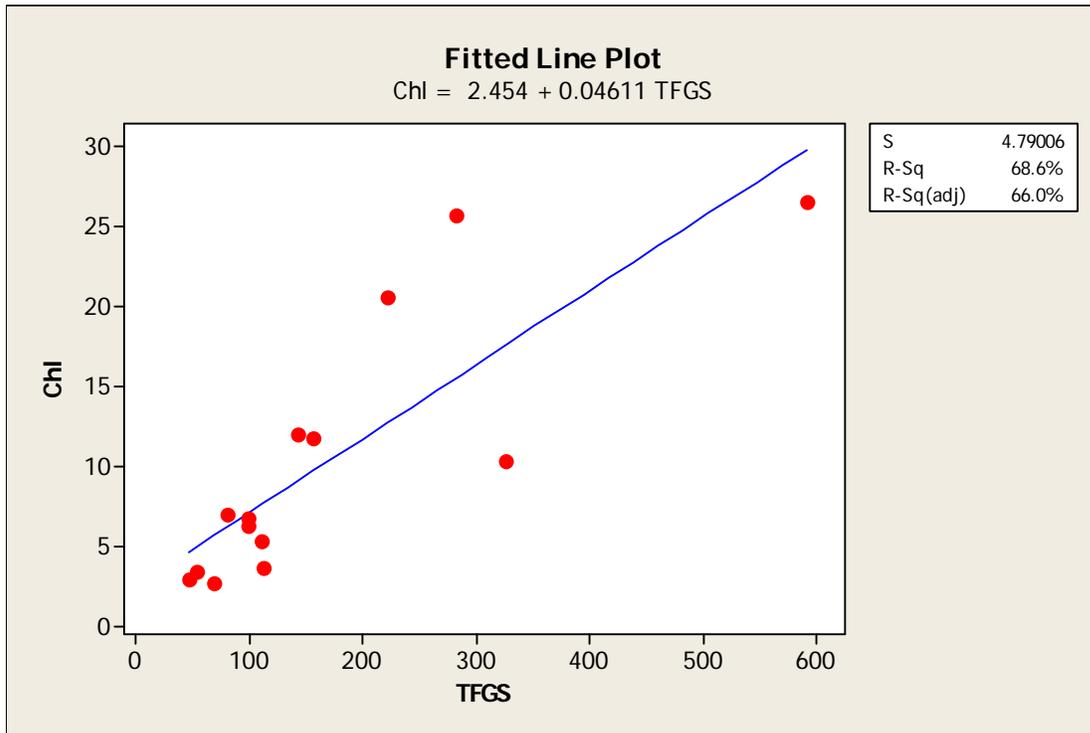
$$\text{Chl} = - 3.151 + 14.14 * 1/\text{SD}$$



The slope parameter 14.14 is significant with a p-value less than .001. The coefficient of determination for the model is 79.4%. There is evidence that at least one observation is outlying, and that one observation, namely McClures Lake, has significant influence on the model; the leverage, Cook’s distance, and DFFITS values for this point were all significantly large. There is some visual evidence of heteroscedasticity. An Anderson-Darling normality test on residuals yields a p-value of .143.

It is simpler to obtain grab samples at arms depth than it is to use an integrated pipe sampler to obtain a column sample of water. We were interested in the correlation between total fluorescence of such grab samples and the laboratory extracted chlorophyll-a concentration from a column sample of water, so we regressed Chl on TFGS. The simple linear least squares regression model of Chl on TFGS yields

$$\text{Chl} = 2.454 + 0.04611 * \text{TFGS}$$



The slope parameter .04611 is significant with a p-value less than .001. The coefficient of determination for the model is 68.6%. There is evidence that two or more observations are outlying or have significant influence on the model, and most notably, there is evidence of severe heteroscedasticity. An Anderson-Darling normality test on residuals yields a p-value of .174.

It is known that Secchi disk transparency depth is confounded with other factors as a predictor of chlorophyll-a concentration. We were interested in whether or not total fluorescence of a grab sample used in conjunction with Secchi disk depth would adequately adjust for these confounders. The linear least squares regression model of Chl on both 1/SD and TFGS yields the following output.

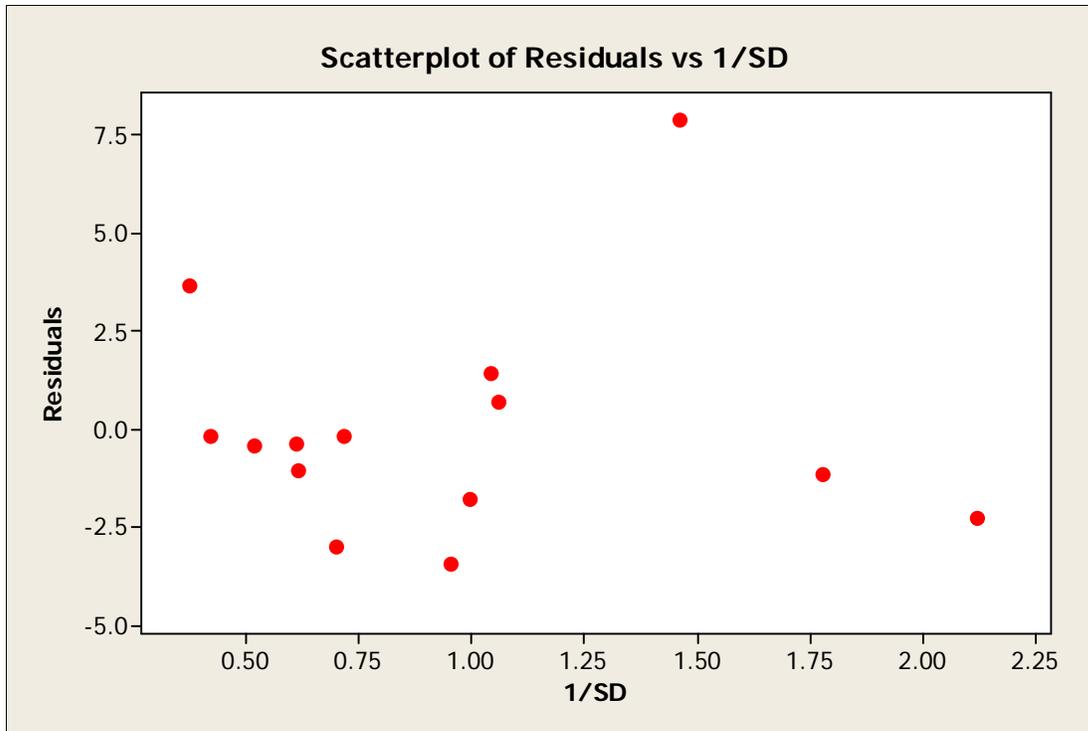
$$\text{Chl} = - 2.695 + 9.687 * 1/\text{SD} + 0.0222 * \text{TFGS}$$

Predictor	Coef	SE Coef	T	P
Constant	-2.695	1.832	-1.47	0.169
1/SD	9.687	2.387	4.06	0.002
TFGS	0.0222	0.0084	2.65	0.023

S = 3.16588

R-Sq = 87.4%

R-Sq(adj) = 85.2%



The slope parameters 9.687 for 1/SD and .0222 for TFGS are significant with a p-values .002 and .023, respectively. The adjusted coefficient of determination for the model is 85.2%. There is evidence that two or more observations are outlying or have significant influence on the model. An Anderson-Darling normality test on residuals yields a p-value of .038. The scatterplot of residuals on 1/SD shows evidence that this model experiences less severe heteroscedasticity than the model of Chl regressed on 1/SD alone.

## DISCUSSION

The regression model of Chl on TF shows an excellent fit and coefficient of determination ( $R^2 = 96.4\%$ ), which is evidence that total fluorescence measurements on a column of water are an excellent predictor of the laboratory extracted chlorophyll-a concentration. In contrast, the regression of Chl on 1/SD shows a considerably smaller coefficient of determination ( $R^2 = 79.4\%$ ), and the typical symptoms of heteroscedasticity are exhibited.

Total fluorescence measurements on grab samples are certainly more convenient than total fluorescence measurements on column samples, and volunteer monitoring programs may see them as preferable. However, our regression of Chl on TFGS alone shows a relatively poor fit and relatively weak coefficient of determination ( $R^2 = 68.6\%$ ). It therefore does not seem advisable to take total fluorescence measurements on grab samples in lieu of either taking Secchi disk transparency measurements or doing a laboratory extracted chlorophyll-a concentration analysis. Our regression of Chl on both 1/SD and TFGS shows some potential to be a better model than simply regressing Chl on 1/SD alone; the former model appears to exhibit more homoscedasticity than the latter, and the former has a larger coefficient of determination than the

latter ( $R\text{-Sq}(\text{adj}) = 85.2\%$  as compared with  $R\text{-Sq} = 79.4\%$ ). But questions remain about the general fit of Chl regressed on both  $1/SD$  and TFGS, and models such as this should be investigated further.

Our fluorometer showed less accuracy than we would have preferred. The measurements in Table 1 yield means and standard deviations as follows.

**Table 3. Mean and Standard Deviation of Readings in Table 1**  
(Total fluorescence measurements)

Lake	Mean	Standard Deviation
Beaver Dam	155	6.79
Big Barbee	139.2	8.68
Diamond	235.74	9.47
McClures	373.92	3.9
Palestine	568.84	14.71
Pike	283.24	10.63
Ridinger	93.7	4.14
Sellers	545.2	47.3
Silver	234.4	7.05
Spear	149.98	19.14
Syracuse	45.582	1.601
Tippecanoe	103.66	2.16
Waubee	60.73	3.6
Wawasee	49.38	2.95

The lack of precision exhibited in our fluorometer is disconcerting, but our practice of averaging five readings on the same sample appears to be a good practice for controlling for this imprecision.

## CONCLUSIONS

We propose that a handheld fluorometer can be used to accurately predict laboratory extracted chlorophyll-a concentration with the following method:

1. Use an integrated pipe sampler to obtain a column sample of water at the sampling location.
2. Empty the contents of the integrated pipe sampler into a container and select a sample from this container for total fluorescence measurement.
3. Take multiple total fluorescence measurements (at least five) on the exact same water sample in the instrument chamber.

This appears to be a much more accurate predictor of laboratory extracted chlorophyll-a concentration than Secchi disk transparency depth.

In order to effectively convert in vivo total fluorescence measurements into predicted laboratory extracted chlorophyll-a concentration, it would be important to first develop a regression model for this purpose. Due to the differing types and species of algae in different geographical regions, a customized regression model should be developed based on the lakes that are being monitored.

The handheld fluorometer that was utilized in this project cost approximately \$2,500, and laboratory chlorophyll-a concentration analyses cost approximately \$50 each. For volunteer monitoring organizations, the initial cost of the fluorometer must therefore be considered against continued laboratory costs. If the number of planned laboratory analyses is large, then the method proposed in this paper is potentially a simple, accurate, and cost-effective alternative to the standard laboratory chlorophyll-a analysis.

## **ACKNOWLEDGMENTS**

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