

## Near-infrared spectrometry (NIRS) for the analysis of seston carbon, nitrogen, and phosphorus from diverse sources

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### Abstract

Seston carbon (C), nitrogen (N), and phosphorus (P) concentrations are routinely measured by limnologists and oceanographers. Unfortunately, common methods for the determination of seston C, N, and P are expensive and labor intensive and result in sample destruction. It has been suggested that near-infrared spectroscopy (NIRS) may be preferable to primary chemical analyses because NIRS is rapid and nondestructive. The ability of NIRS to measure seston element concentrations has been demonstrated, but an impediment to widespread adaptation of this technique relates to the generality of prediction (i.e., standard) equations. For diverse environmental samples, the ability to develop one global predictive equation is important, since the calibration process involves a considerable investment. It is unclear how often during routine use an investigator must standardize and calibrate NIRS equations. Here we explore the use of NIRS for analysis of seston C, N, and P in diverse aquatic samples, including those from monocultures, Lake Superior, and two sets of numerous lakes and ponds. Predictive equations were developed for specific datasets and for all the datasets combined (global equation). In most cases dataset-specific equations and the global equation accurately predicted ( $R^2 > 0.90$ ) concentrations of seston C and N but not P. Prediction error varied among seston types and increased when equations were tested on novel datasets. Our results indicate that NIRS analysis is an effective alternative to primary C and N chemistry, particularly for large aquatic monitoring programs. However, care must be taken during calibration and routine use, as accuracy depends on the types of seston in the calibration dataset.

### Introduction

Analysis of elemental concentrations in aquatic particulate matter (seston) has been an important component of many limnological and oceanographic studies. Seston carbon (C), nitrogen (N), and phosphorus (P) levels, along with corresponding ratios, have been used to investigate nutrient dynamics and food web interactions (Redfield 1958, Sterner and Elser 2002). Seston is a heterogeneous mixture of inorganic matter, algae, bacteria, detritus, and microzooplankton. The C and N concentration of seston is typically measured using commercial CHN analyzers with high temperature combustion followed by GC/thermal conductivity detection of CO<sub>2</sub> and N<sub>2</sub>. For particulate phosphorus, persulfate digestion at high temperature followed by measurement of SRP is also a long-standing common technique (Strickland and Parsons 1968). A major shortcoming of both of

these procedures is sample destruction. CHN analysis entails a high cost of consumables (on the order of \$3 to \$5 per sample). Moreover, both CHN and digestion procedures for phosphorus are time-consuming. These factors impose a practical limit on the number of seston samples that can be collected for any given study. Alternative procedures that preserve sample integrity and reduce both financial and personnel costs are desirable, especially if accuracy and precision are similar to the traditional methods.

One of the most promising methods for seston elemental analysis is near-infrared reflectance spectroscopy (NIRS). In contrast to traditional methods, NIRS is nondestructive to samples; it is also fast and requires no consumables. The theory and application of NIRS were thoroughly reviewed by Williams and Norris (2001a). Briefly, NIRS instruments detect reflectance spectra in the near-infrared (780–2500 nm), which result from vibrations in covalent bonds among lighter elements such as oxygen, carbon, nitrogen, and phosphorus. Near-infrared spectra are information-rich, although one cannot associate particular spectral features (such as individual peak height) to any individual chemical element. Instead, multivariate data analysis or multiple linear regression is used to develop predictive equations that link spectral characteristics with the elemental

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**Table 1.** Summary information for each calibration dataset (mean  $\pm$  1 SD).

Sample	Location	Particle size	C mean ( $\mu\text{g}$ )	N mean ( $\mu\text{g}$ )	C range ( $\mu\text{g}$ )	N range ( $\mu\text{g}$ )
Global	Composite: 13 samples per dataset	See below	99.5 $\pm$ 103.9	14.5 $\pm$ 14.8	0.0-411.1	0.0-59.0
<i>Scenedesmus</i> (N-limited)	Monoculture (C:P = 123)	> GF/F	283.0 $\pm$ 27.5	35.9 $\pm$ 13.9	200.3-356.6	0.0-77.5
<i>Scenedesmus</i> (P-limited)	Monoculture (C:P = 944)	> GF/F	347.0 $\pm$ 97.4	49.0 $\pm$ 15.9	54.8-639.2	1.4-96.6
L. Calhoun	Minneapolis, MN	< 80 $\mu\text{m}$	121.6 $\pm$ 91.3	20.5 $\pm$ 17.2	0.0-395.6	0.0-72.0
L. Superior 2000	Lake Superior	< 3 and 80 $\mu\text{m}$	41.8 $\pm$ 11.3	4.8 $\pm$ 1.6	7.9-75.8	0.1-9.6
L. Superior 2001	Lake Superior	< 3 and 80 $\mu\text{m}$	53.6 $\pm$ 30.9	8.6 $\pm$ 3.7	5.4-150.2	2.3-16.5
Central MN lakes	10 Central MN lakes (sampled monthly)	< 80 $\mu\text{m}$	139.0 $\pm$ 86.4	26.2 $\pm$ 14.6	0.0-398.2	0.0-70.0
MN and IA lakes	42 MN and IA lakes (sampled once)	< 1, 35, and 80 $\mu\text{m}$	48.7 $\pm$ 28.1	6.2 $\pm$ 3.4	0.0-133.2	0.0-16.4
MI and SD lakes	37 MI and SD lakes (sampled once)	< 1, 35, and 80 $\mu\text{m}$	134.2 $\pm$ 131.6	22.3 $\pm$ 28.1	0.0-529.0	0.0-106.5

All seston samples were collected on a GF/F filter. The global dataset includes 13 randomly selected samples from each of the eight datasets. See Table 2 for sample sizes.

content of known samples. Once the predictive equation is developed, it can be used to predict additional samples, greatly reducing the need for traditional techniques. Thus, once an acceptable predictive equation is constructed, NIRS can potentially be used to measure hundreds to thousands of samples rapidly at comparatively low cost. However, 5% to 20% of samples must be analyzed with primary chemistry for quality assurance and to ensure that unanticipated sources of variability have not crept into the samples.

NIRS has been widely used in the pharmaceutical, food, and agricultural industries for many years; it is only recently seeing adoption for aquatic samples. Some of the earliest aquatic applications were for determination of seston elemental content (Malley et al. 1993, 1996) and in paleolimnological studies of lake sediments (Malley et al. 2000). Malley et al. (1993, 1996) developed NIRS predictive equations for seston C, N, and P concentrations from a broad variety of Precambrian Shield lakes, but they did not examine how seston variety affects NIRS predictive equations. Application of NIRS to aquatic ecology depends critically on the robustness of predictive equations. Because seston samples are by nature highly heterogeneous and NIRS does not directly measure specific constituents, one must have a good understanding of how widely applicable any particular predictive equation may be. For example, in lake seston work, is a separate predictive equation needed for each lake, and for each season or depth within a lake? Or does a single global lake seston equation apply for all samples that may be taken in a broad survey?

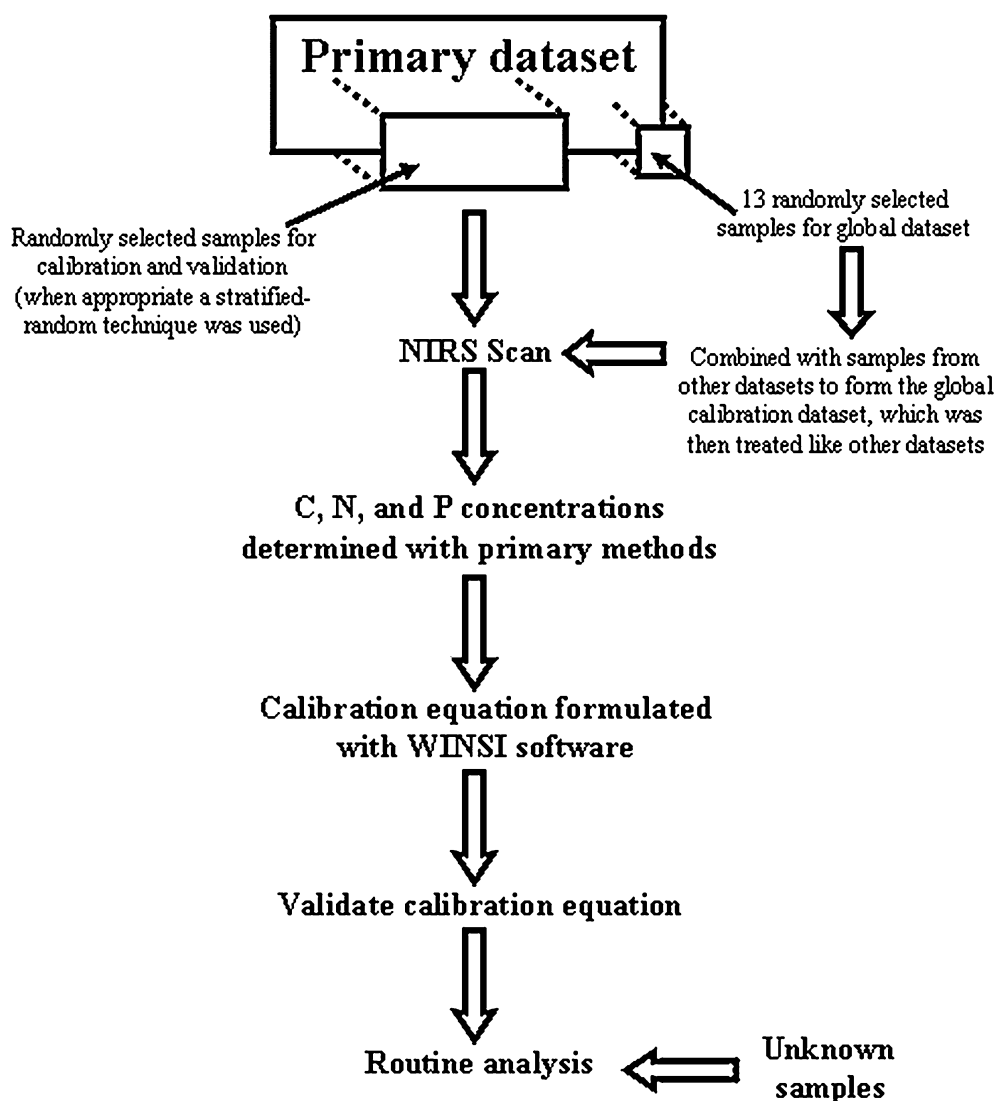
Here we examine the robustness of NIRS prediction equations by cross-comparing analytical results from nine diverse

seston datasets, including monocultures, multiple samples from single lakes, and three large surveys of numerous lakes. We used these datasets to analyze the effect of underrepresented and unrepresented samples on the precision and accuracy of NIRS equations. In addition, we examined whether the precision and accuracy of NIRS equations varied predictably with common limnological parameters.

### Materials and procedures

*Seston datasets*—We worked with previously unpublished datasets (Table 1). These datasets were purposely chosen to represent a broad range of chemical heterogeneity. Sampling methods varied somewhat among datasets based on the initial purpose of the study, but all samples were collected on Whatman GF/F filters and dried at 60 °C. In some datasets, sestons were fractionated by size. Seston samples were grouped into datasets first by study and then by the sampling protocol if necessary.

We grouped our seston samples into eight datasets (Table 1). The first two datasets are composed of samples from two types of *Scenedesmus* cultures which differ in their state of nutrient limitation. *Scenedesmus* were grown in chemostats in a modified COMBO medium (Kilham et al. 1998) under two different N:P loading rates to produce N-limited algae (C:P = 123  $\pm$  78.3; mean  $\pm$  1 SD) and P-limited algae (C:P = 944.5  $\pm$  113.2). Culturing methods were described in detail by Villar-Argaiz and Sterner (2002). The third dataset is composed of replicate samples taken from a single depth of Lake Calhoun, MN, on a single date. The fourth and fifth datasets contain seston samples collected from the western basin of Lake Superior during 2000 and 2001, respectively. Both of these datasets contain seston



**Fig. 1.** Flow chart of analytical procedure.

samples from multiple depths and dates. The sixth dataset contains samples from a set of 10 shallow central Minnesota lakes (average depth 2 m). For this project, three randomly selected samples were taken monthly from each lake at approximately 1 m of water depth.

The seventh and eighth datasets come from a large survey study of Midwestern lakes. Because of differences in analytical procedures, the seston samples from this project were split into two datasets: 1) lakes in Minnesota and Iowa visited once during the summer of 2000 (hereafter MN/IA dataset) and 2) lakes in South Dakota and Michigan visited once in 2001 (hereafter SD/MI dataset). Both datasets include stratified and unstratified lakes. From the stratified lakes we took two seston samples each from the epilimnion, metalimnion, and hypolimnion. In the shallow, unstratified lakes we took two samples from two depths spread evenly through the water

column. These two datasets include a wide variety of seston size fractions, which other datasets do not (Table 1). Eighty-one Minnesota and Iowa lakes were sampled in 2000. Minnesota lakes were congregated in three regions: Ely, St. Paul, and Itasca County, Minnesota. Iowa lakes are located in the north-central region of the state. Thirty-four South Dakota and Michigan lakes were visited in 2001. South Dakota lakes are located in the eastern region of the state. Michigan samples are from lakes in the southwestern region of the state. South Dakota and Michigan seston samples were exposed to concentrated HCl fumes for 24 h to remove carbonates (Hedges and Stern 1984).

*Determination of particulate C, N, and P with NIRS*—The protocol for the development of predictive equations for routine estimation of seston C, N, and P concentrations is outlined in Figure 1. All seston filters were scanned in a 5-cm cell with a

**Table 2.** Summary data for the eight C and N predictive equations, the global equation, and for examples of unsuccessful (\*) and successful P equations.

	Method	Treatment	n	Slope	Bias	SEP	r <sup>2</sup>	RPD
Carbon								
Global	Mod PLS	1,4,4,1	104	0.999	1.124	20.479	0.981	6.78
Scenedesmus (N-limited)	PLS	1,4,4,1	35	1.075	-0.964	7.629	0.958	3.58
Scenedesmus (P-limited)	Mod PLS	1,4,4,1	51	0.991	-1.049	23.603	0.941	4.13
L. Superior 2000	PLS	0,0,1,1	65	0.951	0.035	4.498	0.907	2.51
L. Superior 2001	Mod PLS	0,4,10,1	66	1.003	-0.245	5.790	0.964	5.34
L. Calhoun	Mod PLS	0,1,4,1	42	0.992	5.041	5.000	0.998	18.26
Central MN lakes	Mod PLS	0,0,1,1	87	1.006	0.632	6.580	0.995	13.13
MN and IA lakes	Mod PLS	1,15,15,1	187	0.981	-0.016	11.042	0.909	2.55
MN and IA epilimnion	Mod PLS	1,4,2,1	115	1.050	1.000	10.051	0.940	3.86
MN and IA metalimnion	Mod PLS	1,5,5,1	110	1.022	0	5.387	0.965	5.34
MN and IA hypolimnion	Mod PLS	1,3,3,1	88	0.965	0.763	7.807	0.911	3.36
MI and SD lakes	Mod PLS	1,4,4,1	97	0.990	0.387	10.935	0.998	13.03
Nitrogen								
Global	Mod PLS	1,4,4,1	104	1.030	0.122	2.238	0.874	2.10
Scenedesmus (N-limited)	PLS	1,4,4,1	36	0.999	0	5.183	0.891	3.08
Scenedesmus (P-limited)	Mod PLS	1,4,4,1	50	1.018	0.515	4.350	0.924	3.65
L. Superior 2000	PLS	0,1,1,1	64	0.590	-0.080	1.464	0.543	1.09
L. Superior 2001	Mod PLS	0,4,10,1	66	1.002	-0.032	0.852	0.948	36.27
L. Calhoun	Mod PLS	0,1,4,1	44	1.039	-0.319	2.084	0.982	8.25
Central MN lakes	Mod PLS	0,0,1,1	87	0.967	-0.220	1.826	0.990	8.00
MN and IA lakes	Mod PLS	1,15,15,1	193	0.986	0.146	1.977	0.867	1.72
MN and IA epilimnion	Mod PLS	1,4,2,1	114	1.017	0.184	1.457	0.888	2.99
MN and IA metalimnion	Mod PLS	1,3,3,1	105	1.006	0.239	1.568	0.829	2.28
MN and IA hypolimnion	Mod PLS	1,3,3,1	86	0.963	0.023	1.733	0.713	1.85
MI and SD lakes	Mod PLS	1,4,4,1	95	0.999	0.336	2.184	0.996	12.87
Phosphorus								
Scenedesmus (N-limited)	Mod PLS	1,4,4,1	50	0.977	-0.004	0.049	0.963	5.25
Scenedesmus (P-limited)	Mod PLS	1,2,2,1	77	1.002	0.000	0.010	0.906	3.30
L. Superior 2001*	Mod PLS	0,0,1,1	96	1.000	0.000	1.014	0.755	2.12
MN and IA lakes*	PLS	0,15,15,1	113	2.405	6.079	32.681	0.298	0.26

Also shown are three equations developed by splitting the MN and IA dataset into depth categories: epilimnion, metalimnion, and hypolimnion. In the treatment column the first number is the derivative, the second the gap, the third a smooth function, and the fourth a second smooth function (all settings used in the WINSI software). Either a modified partial least squares (mod PLS) or a partial least squares (PLS) was used to develop prediction equations. The number of samples in the calibration dataset (*n*) is also shown.

Foss NIRSystems NIRS spectrometer over the wavelengths 1100 to 2500 nm. Scans were corrected for a blank filter scanned alternately with the sample filter. The blank was prepared by filtering nanopure water and was desiccated following the same procedure as for the sample filters. For each dataset, calibration equations were developed on a randomly selected sample of filters. Equations were developed for all eight primary datasets, for subsets of the MN/IA dataset, and for all of the datasets combined (hereafter the global dataset) using WINISI software (WINISI 1999). For the MN/IA and SD/MI datasets, we stratified calibration samples by depth category (e.g., epilimnion, metalimnion, hypolimnion, and unstratified) and by state. Calibration datasets contained between 35 and 187 samples (Table 2). For the central Minnesota dataset we stratified by

lake then randomly selected calibration samples. The global calibration equation was developed by randomly selecting 13 samples from each of the 8 datasets. An additional set of randomly selected samples, approximately 30% to 50% of each calibration dataset, was analyzed with primary methods to validate calibration equations.

Calibration equations were developed using partial least squares (PLS) and modified PLS incorporated in the WINISI software (Table 2). During the calibration process several common mathematical treatments were used to standardize spectra and reduce noise (WINISI 1999). Calibration equations used either no derivative or the first derivative and several combinations of data smoothing and gap options specified in the WINISI software (Table 2). The WINISI software also provides

**Table 3.** Means and standard deviations for the analysis of Lake Calhoun seston using reference and NIRS methods.

Volume filtered (mL)	Sample	n	CHN mean (mg/L)	NIRS mean (mg/L)	CHN SD ( $\mu\text{g/L}$ )	NIRS SD ( $\mu\text{g/L}$ )	CHN CV	NIRS CV
500 mL	C	17	282.3	262.4	25.4	25.0	9.0	9.5
	N	17	45.9	45.6	3.8	5.5	8.3	12.1
1000 mL	C	10	256.6	247.5	12.7	11.5	4.9	4.6
	N	10	46.7	44.2	1.9	2.8	4.1	6.3

several mathematical techniques for diminishing scatter, a nonlinear function that distorts the relationship between the NIR spectrum and the reference value. These techniques were used in a number of our calibration equations. For each dataset we selected the calibration equations that, when used to predict samples in the validation dataset, gave the highest  $r^2$ , slope closest to 1, bias closest to 0, and highest RPD (RPD = SD/SEP) (Malley et al. 1993) for the linear regression relationship between predicted values and reference (chemical) values. The RPD statistic is used to evaluate the efficiency of NIRS calibrations (Williams and Norris 2001b). RPDs higher than 3.0 are acceptable and RPDs higher than 5.0 are required for the most accurate analysis (Malley et al. 1993).

*Primary determination of particulate C, N, and P*—To both calibrate and validate NIRS equations, randomly selected samples were measured using reference methods. Primary determination of particulate C and N was conducted with a Perkin Elmer series II CHN analyzer using acetanilide as a standard. Phosphorus samples were digested in a 5% potassium persulfate solution and autoclaved for 30 min. Liberated soluble reactive phosphorus was analyzed with the molybdate-ascorbic acid method (Strickland and Parsons 1968).

### Assessment

*Prediction equations*—C and N in each dataset could be accurately measured by NIRS with a local prediction equation, regardless of the degree of chemical homogeneity or heterogeneity of the samples. Each carbon equation yielded  $r^2 > 0.9$  (Table 2). Excluding the Lake Superior 2000 equation, N equations for the eight primary datasets had  $r^2$  values  $> 0.85$  (Table 2). Slopes were all close to 1. The  $r^2$  values of validation datasets were within 0.05 of the  $r^2$  for the calibration dataset. For some datasets we could not develop predictive equations with RPDs greater than 3 (Table 2). Our results suggest that NIRS is equally applicable to C and N measurement for a broad variety of seston types from chemically homogeneous to highly heterogeneous, when prediction equations are developed from the set of samples being measured.

In contrast, the application of NIRS technology to the measurement of seston P appears to be limited. We were not able to develop successful P prediction equations for all datasets (Table 2). Unsuccessful attempts yielded P equations with  $r^2 < 0.7$ . In fact, successful equations were developed only for the

two most homogeneous datasets, the N- and P-limited *Scenedesmus*. Thus, NIRS is not broadly applicable to the measurement of seston P, although under some circumstances it may be useful.

*Precision of NIRS analysis*—Deviations from perfect agreement between primary chemistry and NIRS might come from several sources, including errors associated with the primary chemistry or the NIRS measurement, as well as the extent to which there are sufficiently strong NIR absorbers that correlate with the reference data for the constituent. NIRS might even theoretically yield a more accurate measure than the primary chemistry itself if most of the variation is associated with measurement error in the primary chemistry. To compare precisions of NIRS versus primary chemistry, we filtered replicate seston samples taken from Lake Calhoun (Minneapolis, MN). Samples with two different sample loads (0.5 L and 1.0 L) were compared. Successful C and N equations were developed, but we were not able to produce an accurate NIRS equation for P.

Our comparison of NIRS and CHN precision indicates that these two methods have similar precision (Table 3). NIRS N estimates were less precise than CHN estimates; however, this difference was not substantial. Perhaps the difference between C and N precision is a reflection of the somewhat less accurate Lake Calhoun N equation (Table 2). From this comparison of precisions, we conclude that to a first approximation, NIRS and CHN chemistry can yield measurements of similar quality.

*Prediction of NIRS error*—We wondered if the accuracy of any individual NIRS measurement of seston chemistry was potentially related to gross limnological characteristics of that sample or site. We thus related NIRS-CHN residuals from the MN/IA dataset to common limnological variables (Table 4). None of these variables were strongly related to prediction equation residuals, although the slopes of several relationships were significantly different from zero, indicating a weak but significant relationship between prediction error and limnological characteristic (Table 4). This analysis suggests that NIRS C and N measurements are not particularly sensitive to any of the limnological characteristics we measured.

*Equation robustness*—Another approach to studying the applicability of NIRS calibration equations is to examine whether prediction equations developed for individual datasets can accurately be used for specific subsets of those data. We therefore analyzed the robustness of NIRS equations

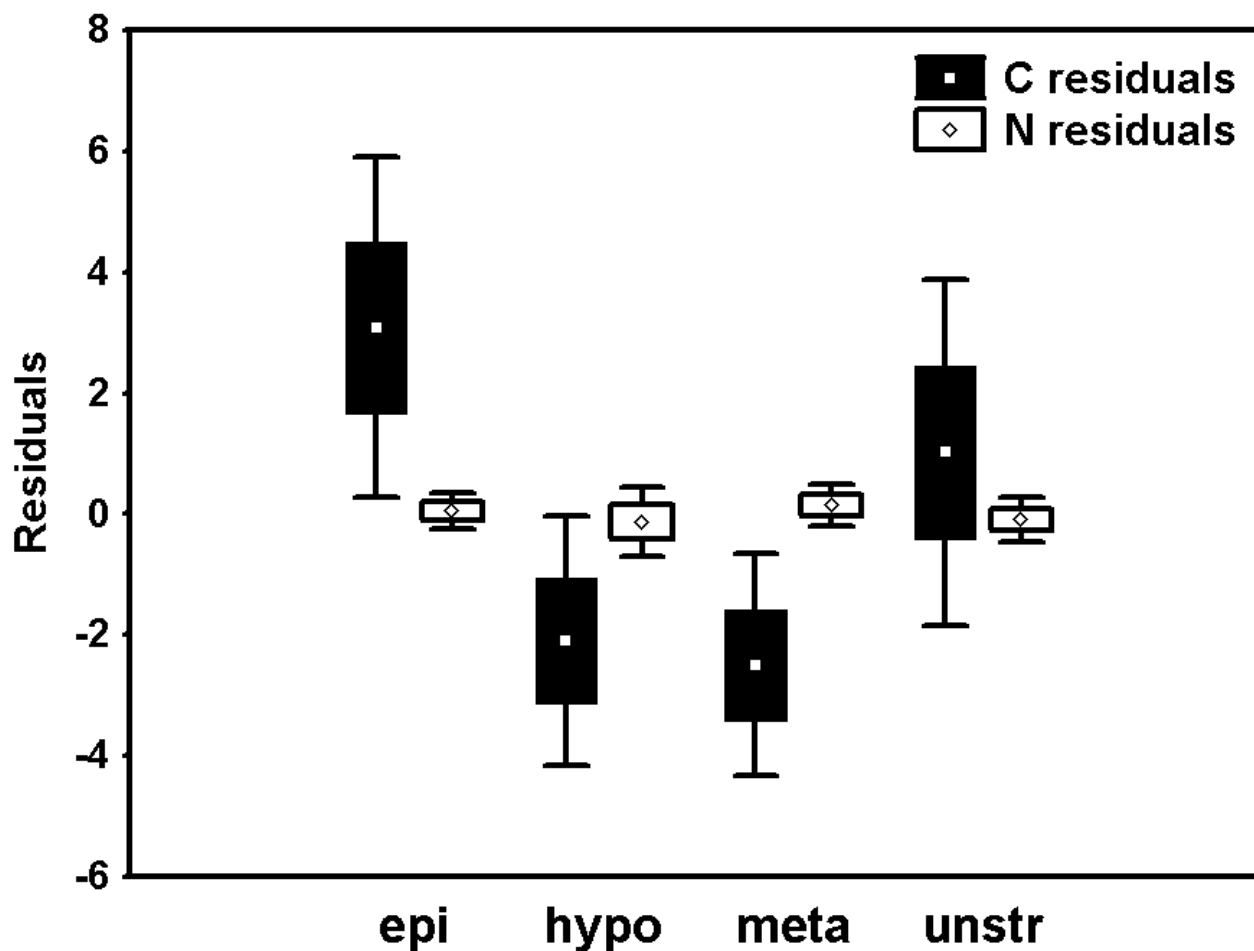
**Table 4.** Predictive power of various major limnological characteristics on the residuals of reference vs. NIRS estimates of C and N for the MN and IA dataset.

Limnological characteristics	Carbon $r^2$	Nitrogen $r^2$
Chlorophyll $a$ ( $\mu\text{g/L}$ )	0.002	0.011*
Secchi (m)	0.034*	0.000
Surface area (ha)	0.007	0.000
$Z_{\text{max}}$ (m)	0.006	0.001
pH	0.016*	0.006
Conductivity ( $\mu\text{S}$ )	0.002	0.000
$\text{NH}_4$ ( $\mu\text{M}$ )	0.000	0.001
$\text{NO}_3$ ( $\mu\text{M}$ )	0.001	0.012*
SRP ( $\mu\text{M}$ )	0.009*	0.003
TDN ( $\mu\text{M}$ )	0.013*	0.013*
TDP ( $\mu\text{M}$ )	0.006	0.001
TOC ( $\mu\text{M}$ )	0.025*	0.000

\*Linear regressions with significant slopes ( $P < 0.05$ ).

to underrepresented and unrepresented seston samples. The effect of underrepresented samples on equation accuracy was examined with the global dataset and the MN/IA dataset (Table 5). Equation accuracy appears to generally decline when used on data subsets (Figure 3). For example, the accuracy of the global equation (although very high when applied to diverse samples) decreases considerably when used on individual datasets, such as the MN/IA datasets (Table 5). In addition, the accuracy of the MN/IA equation declined when used on subsets of this dataset (Table 5).

We asked if the accuracy of NIRS equations differed with sample types (e.g., epilimnion versus hypolimnion seston samples). Errors for C and N were systematic. The MN and IA equation significantly overestimated the C concentration of epilimnion samples and underestimated hypolimnion and metalimnion samples (Figure 2). Region and filter size were also marginally related to both C and N regression residuals (Table 6). Although highly significant, the difference between

**Fig. 2.** Residuals from the regression of NIRS and CHN seston C (closed boxes) and N (open boxes) estimates for the MN and IA dataset grouped by depth category. Points are mean estimates; the box includes the standard error (see Table 2 for  $n$ ), and the whiskers include the 95% confidence intervals.

**Table 5.** Accuracy ( $r^2$  values) of the relationship between reference and NIRS estimates of seston C or N from the MN and IA dataset using both the global and the MN and IA equations.

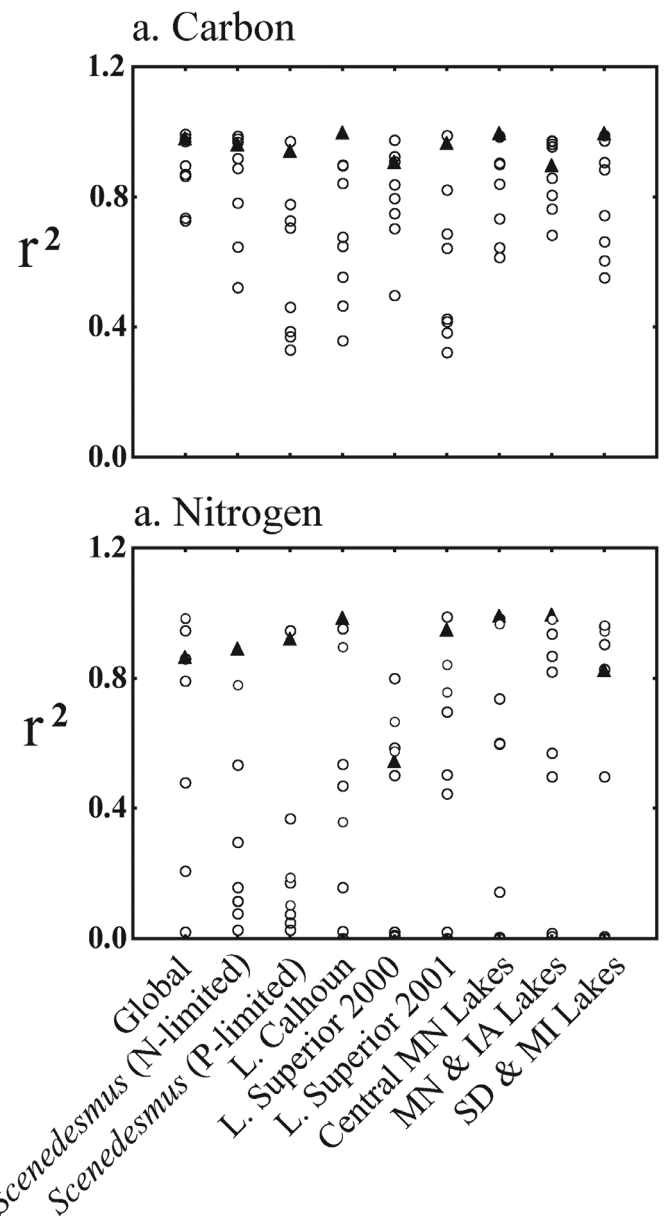
Dataset	Carbon equations		Nitrogen equations	
	Global	MN and IA	Global	MN and IA
Global	0.980		0.870	
MN and IA	0.568	0.895	0.455	0.823
MN and IA epilimnion	0.580	0.809	0.367	0.694
MN and IA metalimnion	0.598	0.897	0.429	0.654
MN and IA hypolimnion	0.659	0.861	0.462	0.648

epilimnion and hypolimnion samples is approximately 5  $\mu\text{g C}$  or only 3.7% of the mean seston C concentration ( $\mu\text{g C}/\text{filter}$ ) for this dataset and might be judged within acceptable error. Nevertheless, for analytical analysis a marginally significant but systematic difference between samples will often be unacceptable. Our recommendation is that caution be exercised when working with data subsets in NIRS measurement of seston, with sufficient validation performed to ensure adequate accuracy is being achieved.

One further way to consider the general applicability of prediction equations is to see how successfully they measure seston chemistry in other datasets with novel seston samples. In other words, can equations developed for one lake or for one set of lakes be used to measure seston nutrients in other sites that were not part of the original dataset? The range in  $r^2$  we observed in our cross-dataset analyses (Figure 3) indicates that the success of such an attempt is highly variable. Equations based on diverse datasets (e.g., global, MN/IA, SD/MI, and central MN) performed best on novel samples. However, in general both C and N predictive equations performed poorly when used to estimate samples from novel datasets (Figure 3). Nitrogen predictive equations were far more sensitive to unrepresented samples than C predictive equations. Finally, since some equations do better on novel datasets than their indigenous dataset, this analysis suggests that some seston types may simply be difficult to predict (e.g., Lake Superior).

Another approach to examining the global applicability of NIRS prediction equations is to ask whether a single relationship developed with maximal sample heterogeneity is broadly applicable across the entire dataset. Our analysis suggests that a global equation with all datasets equally represented is of only marginal use. Although the global equation most accurately predicted the C and N concentrations of novel samples, the accuracy of this equation often was far below analytical standards (Figure 3).

Taken as a whole, our analyses demonstrate that precise and accurate predictive equations can be developed for nearly any set of samples ranging from chemically homogeneous (e.g., cultures) to heterogeneous (e.g., many diverse lakes). However, predictive equations are not transportable between datasets;



**Fig. 3.** Do predictive equations constructed from one dataset accurately measure C and N in other datasets? The predictive ability ( $r^2$ ) of the nine predictive equations for all nine datasets varied widely and often was very poor. The indigenous dataset for each equation is a triangle.

instead, equations must be designed specifically for each dataset and be updated following the addition of novel sample types.

**Discussion**

Near-infrared spectrophotometry is a potentially highly advantageous method for analyzing the C and N concentration of seston samples. Our results confirm earlier reports (Malley et al. 1993, 1996) that NIRS is a rapid, nondestructive, and accurate method. In addition, we show here that NIRS can be used for highly heterogeneous sets of samples with accu-

**Table 6.** One-way ANOVA *P* values from a comparison of grouping characteristics and the residuals of reference versus NIRS estimates from the MN/IA dataset.

Grouping characteristic	Carbon <i>P</i> value	Nitrogen <i>P</i> value
State (MN, IA)	0.108	0.771
Region	0.104	0.149
Stratified (yes/no)	0.364	0.598
Depth (epi-, meta-, hypolimnion)	0.003*	0.740
Particle size	0.151	0.068

The MN/IA equation was used to estimate concentrations of seston C and N. \*Significant *P* values ( $\leq 0.05$ ).

racy indistinguishable from that achieved on very homogeneous samples. Indeed, one equation (MN/IA) successfully predicted seston C and N concentrations for epilimnion, metalimnion, and hypolimnion samples from 42 lakes. In addition, our global equation accurately predicted the C and N concentration of a very diverse seston dataset. However, our analysis also makes it clear that the most accurate equations must be tailored to specific datasets.

We found that NIRS cannot consistently be used to analyze seston P, which contradicts earlier reports (Malley et al. 1993, 1996). We were able to develop accurate seston P equations for the two monoculture chemostats, but generally NIRS did not accurately predict seston P concentrations. It may not be surprising that we were able to develop P equations only for the monoculture datasets, our most homogeneous samples. Perhaps in the lakes we studied there are several forms of particulate P that complicate equation development. Our findings do not exclude the use of NIRS in seston P analysis; they only suggest that NIRS may have limited application and bears continued investigation.

Our results indicate that NIRS analysis of seston C and N is affected by characteristics of the seston. However, it is difficult to determine a priori which seston characteristics might affect NIRS analyses. Our results do demonstrate that common limnological characteristics have only a weak relationship to NIRS accuracy. On the other hand, we also show that both particle size and depth category affected the accuracy of NIRS analyses. In addition, we required separate predictive equations for the two *Scenedesmus* cultures, which differ only in growth rate and cellular C:N:P stoichiometry.

Here, we asked if a global equation, based on a heterogeneous mix of seston samples, could be used for all seston C and N analyses. If the global equation was completely robust to novel samples, there would be no need to develop dataset-specific predictive equations. Such a global equation could save both personnel hours and money. Our results suggest that the answer to this question is scale-dependent. The global equation performed well on random selections of seston samples when evaluated against a heterogeneous compilation of seston samples; however, it performed poorly when

tested on specific datasets or specific types of samples (e.g., epilimnion samples).

### Comments and recommendations

Our results lead to two principle conclusions. First, NIRS is broadly applicable to the analysis of seston C and N as commonly used in limnology even for highly diverse sets of samples. Second, prediction equations are not globally transportable; greatest accuracy is achieved when prediction equations are developed for the particular set of samples under consideration at the time. Thus, since the accuracy of predictive equations is scale-dependent, predictive equations should be developed specifically for each project.

Given the investment necessary to develop and validate prediction equations for particular datasets, our results indicate that NIRS is most applicable to situations where a large number of samples are taken. In such a context NIRS has two important benefits: (1) Because NIRS has a low per-sample cost, NIRS analysis allows for repeated analysis of seston community C and N and construction of datasets of large sample size. Thus, NIRS lends itself to regular monitoring of experiments, lakes, or cultures. However, the large cost of NIR spectrometers can be initially prohibitive. (2) Because NIRS is non-destructive, multiple analyses can be conducted on the same filter. For example, after NIRS analysis, we commonly use traditional analytical methods to analyze seston filters for P. This feature both reduces the overall cost of sampling and allows a researcher to measure C, N, and P on the same seston sample. This alone is a considerable saving.

The apparent sensitivity of NIRS equations to seston types does pose an interesting experimental design issue. The physical and chemical aspects of lake seston that create "novel" samples is not understood. But our analysis suggests that with caution and careful design, accurate and broadly useful C and N equations can be developed. When using NIRS to measure seston C and N, we suggest that initial calibration samples (i.e., for reference chemistry) be stratified over grouping characteristics that might affect sample type. For our datasets, region, depth, and particle size were related to the novelty of samples. Time or season might also be added to this list. Although these precautions constitute a large initial investment of time and money, the use of NIRS to measure seston C and N could save hundreds to thousands of dollars and allow for higher replication or shorter sampling intervals. In our experience, the necessity to continually update calibration equations for novel samples does not limit the functionality of this method.

In summary, NIRS is a rapid, nondestructive, and accurate method for analyzing seston C and N samples. Once the system is set up, it allows for the rapid and inexpensive analysis of a large number of C, N, and in some cases even P samples. This method can be used to determine the C and N concentration of seston samples ranging from monocultures to large lake surveys. In contrast to previous results (Malley et al. 1993, 1996), our larger and more diverse dataset suggests that the applica-

tion of NIR spectrophotometry to P analysis is limited to specific seston types. Finally, care should be taken when developing equations and using equations on novel datasets. As noted by previous authors (Malley et al. 1993, 1996; Williams and Norris 2001a), calibration datasets should include all sources of expected variation and should be amended for novel samples. In spite of these precautions and the system's large initial cost, NIRS remains a useful tool for aquatic ecologists.

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