Assessment of bbe Fluoroprobe for algal taxonomic discrimination in Lake Winnipeg

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Executive summary

This paper reports on an investigation of the capability of the bbe Moldaenke Fluoroprobe instrument for distinguishing major algal taxa. It employs a Department of Fisheries and Oceans database of Fluoroprobe data collected simultaneously with water samples later analyzed for chlorophyll fluorescence, various water quality parameters and algal taxonomy, between August and September/October 2003.

The Fluoroprobe measures fluorescence at six excitation wavelengths and records both raw fluorescence intensity and estimated chlorophyll biomass concentrations of four major algal taxa. Multiple regression of the fluorescence data explains 63% of variance in chlorophyll a in samples, and predicts chlorophyll a with a root mean square error of 7% of the range of concentrations characteristic of Lake Winnipeg.

Bacillariophytes and cyanophytes dominate the algal community in Lake Winnipeg, the former characteristically dominant in spring and late autumn, and the latter from mid-summer through to early autumn. Multiple fluorescence successfully distinguishes and predicts bacillariophyte and cyanophyte biomass with similar success, i.e. $r^2 = 0.68$ in both cases, although with a larger relative RMSE, at best 10% and 19% of the range in Lake Winnipeg, respectively. It is only a weak predictor of cryptophyte biomass, a sub-dominant group in Lake Winnipeg except at very low concentrations, and a weaker predictor of chlorophyte biomass, also a sub-dominant group in the lake. Nonetheless, by use of multiple regressions developed from the paired data set, we were able to successfully reproduce the seasonal patterns determined by microscope counts, of both dominant and subdominant groups through a mid-summer to late autumn period in 2003.

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Introduction

This report is in fulfillment of a contract with the Department of Fisheries and Oceans (Freshwater Institute, Winnipeg) to use a matched *in situ* chlorophyll fluorescence, water quality and algal taxonomy database collected in Lake Winnipeg between August and September/October 2003 to assess the accuracy of the Fluoroprobe instrument in distinguishing major algal taxa. The proximate reason for this study is to validate the Fluoroprobe as a tool for algal taxonomic discrimination in the course of remote sensing studies on Lake Winnipeg, where the longer term goal is to improve remote sensing algorithms for chlorophyll and to develop algorithms for discrimination of cyanophyte blooms.

Fluorescence line height in cyanophyte and bacillariophyte spectra

Previous work towards developing chlorophyll algorithms from Lake Winnipeg appear to have been limited by differences in the reflectance spectra of cyanophytes compared to other algal groups in Lake Winnipeg, and by the highly variable vertical stratification of cyanophytes compared to these other groups (McCullough 2006). This problem was investigated more thoroughly in a subsequent study (McCullough 2007) of the comparative capabilities of two satellite-borne water sensors with multispectral water colour analysis capabilities, MODIS (NASA's MODerate resolution Imaging Spectroradiometer) and MERIS (European Space Agency's MEdium Resolution Imaging Spectrometer). In the latter report it was demonstrated that the fluorescence line height (FLH) calculable using data from either sensor and used for chlorophyll determination, responds differently in the presence of cyanophyte than in the presence of other major algal assemblages in Lake Winnipeg. The following is a brief summary of that discussion.

The three bands defining the FLH peak and baseline are designed to measure the chlorophyll-a fluorescence peak at about 685 nm that is commonly used in oceanographic/limnologic fluorometers designed to estimate phytoplankton concentration. In marine waters, fluorescing radiation has been shown to be superimposed on surface reflectance spectra as a small peak measurable at 681 nm (MERIS FLH wavelength) or 678 nm (MODIS FLH wavelength) (e.g. Gower et al., 1999). Fluorescence is measured as a deviation from the local slope in the reflectance spectrum, where in MERIS and MODIS the slope is defined by reflectance measured in narrow bands on either side of the fluorescence peak.

Unfortunately, reflectance spectra characteristic of one of the dominant algal groups in Lake Winnipeg, the cyanophytes, exhibits a minor peak at 650 nm and a strong minimum at 675 nm that together dominate the spectral pattern in the region examined by the FLH technique. This problem is of particular concern on Lake Winnipeg, where a second dominant group, the bacillariophytes (usually in combination with chlorophytes and crytophytes) do appear to conform to the superimposed fluorescence peak pattern.

Figures 1 and 2 show reflectance spectra recorded over a near monoculture of *Aphanizomenon flos-aquae* compared with reflectance spectra recorded over mixed bacillariophyte-cryptophyte populations. In neither case of spectra did the algae form surface mats. For *Aphanizomenon flos-aquae*, the spectral pattern between 665 and 709 nm shows a local minimum at 675-680 nm determined by the two reflectance peaks to either side, at roughly 650 nm and again at 700-705 nm. The latter is well known and explained by locally increasing (with increasing wavelength) chlorophyll reflectance eventually overcome by locally increasing water absorption (e.g. Gower et al. 2005).

As illustrated in Figures 1 and 2, the MERIS peak at 681 nm with baseline definition at 665 and 709 nm returns positive FLH for bacillariophyte-cryptophyte but negative FLH *Aphanizomenon*. For *Aphanizomenon*, FLH is smaller (more negative) at the higher chlorophyll concentration (31.2 mg m^{-3} compared to 7.3 mg m^{-3}, Tables 1 and 2). Increasingly negative FLH with increasing cyanophyte concentration is readily explained by the position of the FLH band between near the phycoeritherin absorption peak characteristic of absorption spectra of both *Aphanizomenon flos-aquae* and *Anabaena* sp.; local fluorescence is overwhelmed by phenomena determining the general reflectance spectrum (McCullough 2007). This inverse relationship does not appear to carry through to the bacillariophyte-cryptophyte assemblages. Since these communities dominate the algal community in the lake until as late as early July, regression slopes dominated by the more intensely concentrated mid- to late summer cyanophyte assemblages are unlikely to predict the spring bacillariophyte-cryptophyte concentrations particularly well.

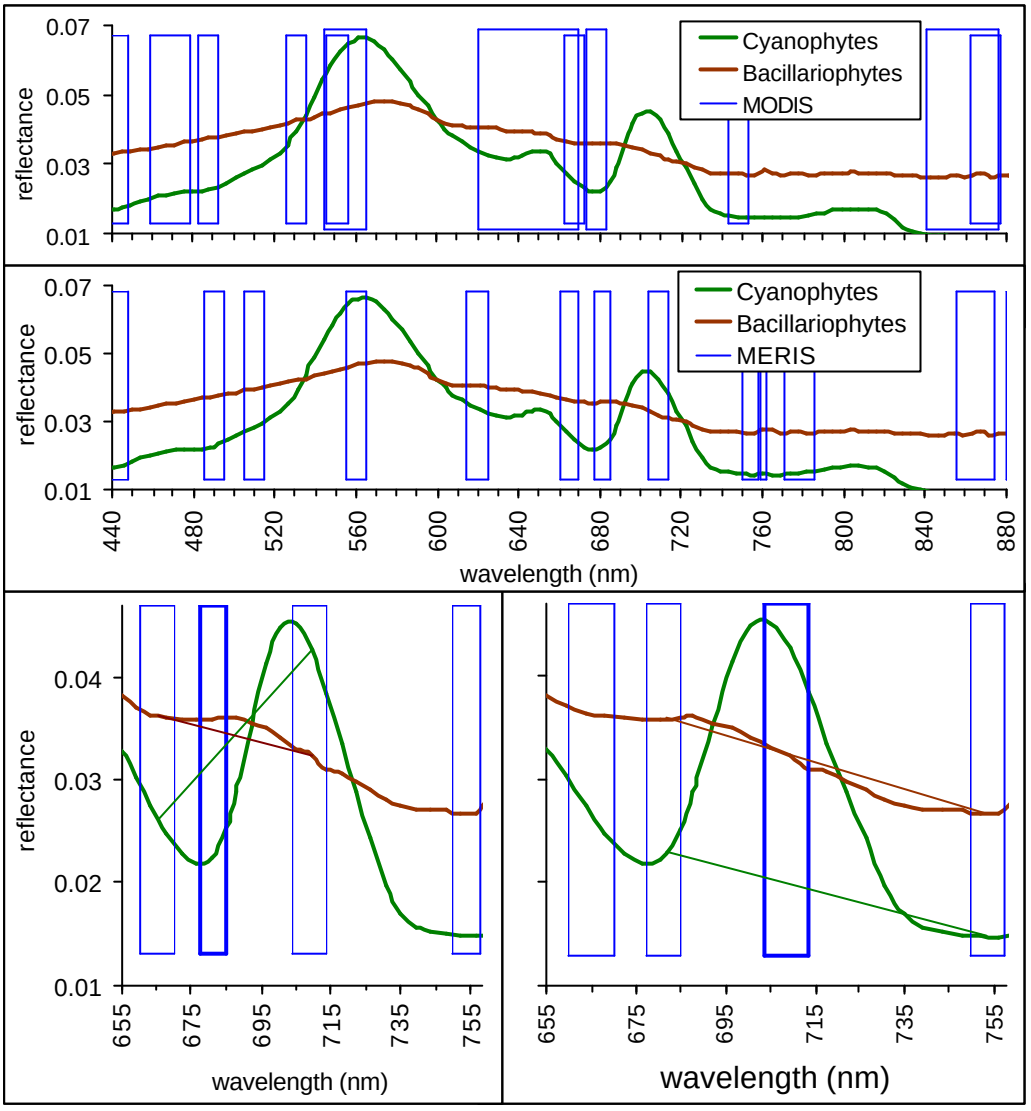

Figure 1. Spectra showing typical shapes for cyanophyte-dominant and bacillariophyte-dominant algal communities (95% *Aphanizomenon flos-aquae* on 8 August 2003, and 85% bacillariophytes – predominantly *Stephanodiscus niagarae* – with 5% cryptophytes, 4% chlorophytes on 23 September 2003). MODIS and MERIS spectral bands indicated on upper and middle panel respectively. MERIS FLH baselines indicated in lower left panel; MCI baselines shown in lower right.

Table 1. In situ water quality data associated with the two spectra in Figure 1, above. (DOC, chl and HPLC chl a in mg m^{-3}, SSC and tripton in g m^{-3}).

Time	DOC	Chl	HPLC chl a	SSC	Tripton
2003 Aug 08 12:00	679	31.2	29.4	1	0.5
2003 Sep 23 15:00		12.0	8.6	4	0.5

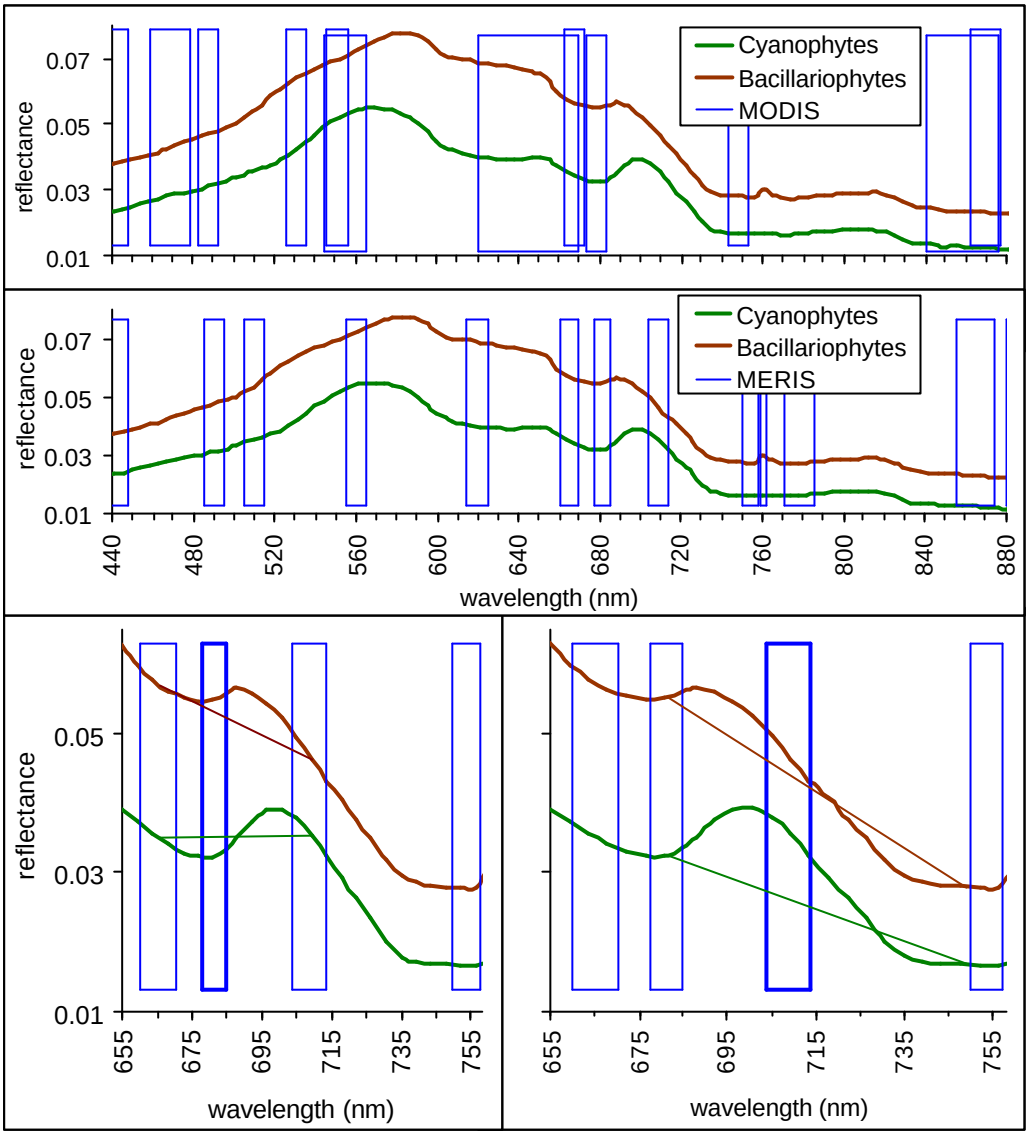

Figure 2. Spectra showing typical shapes for cyanophyte-dominant and bacillariophyte-dominant algal communities (99% cyanophytes – predominantly *Aphanizomenon flos-aquae* with minor *Pseudanabaena limnetica* and *Anabaena* spp. – on 6 August 2003, and 82% bacillariophytes – predominantly *Aulacoseira islandica* and *Aulaoseira ambigua* – with 14% cryptophytes – predominantly *Cryptomonas* spp. – on 11 June 2003). MODIS and MERIS spectral bands indicated on upper and middle panel respectively. MERIS FLH baselines indicated in lower left panel; MCI baselines shown in lower right.

Table 2. In situ water quality data associated with the two spectra in Figure 2, above. (DOC, chl and HPLC chl a in mg m^{-3}, SSC and tripton in g m^{-3}).

Time	DOC	Chl	HPLC chl a	SSC	Tripton
2003 Aug 06 11:23	773	7.3	5.1	4	0.5
2003 Jun 11 18:21	788	8.3	4.5	5	1

Geographic features of the study region

Data analyzed in this report were collected in Lake Winnipeg, Manitoba. Figure 3 shows the major basins of the lake and place names mentioned in the text of this report. Lake Winnipeg has a diverse optical geography. The South Basin and Narrows region and sometimes the east shore of the North Basin – regions with maximum depths under 12 m – are often turbid, with suspended solids concentrations (SSC) ranging from 20-60 g m^{-3} (Figure 4). In the remainder of the North Basin, inorganic suspended solids (tripton) only occasionally exceed 5 g m^{-3}. In this central and western part of the North Basin, where light is less likely to be limiting to algal productivity (at least in the absence of cyanophyte blooms) widespread plankton blooms have historically developed (e.g. bright green in Figure 4). Water near the mouths of tributary rivers draining from the Precambrian Shield, to the east, is often coloured a rich red-brown, with dissolved organic carbon concentrations (DOC) of 1000-1200 mg m^{-3} (Figure 4). Away from the river mouths, DOC of 400-600 mg m^{-3} is typical.

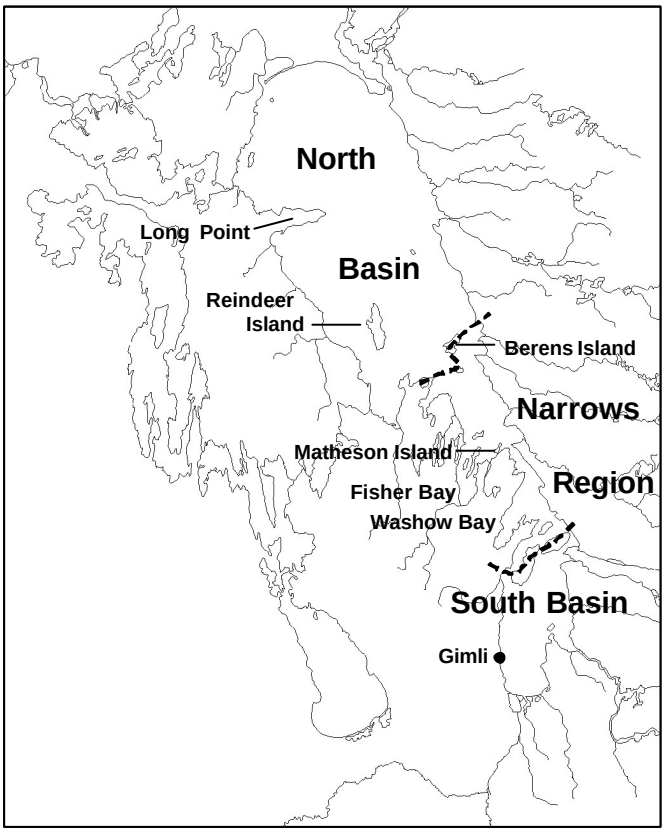

Figure 3. Base map of Lake Winnipeg and region, showing locations mentioned in this report.

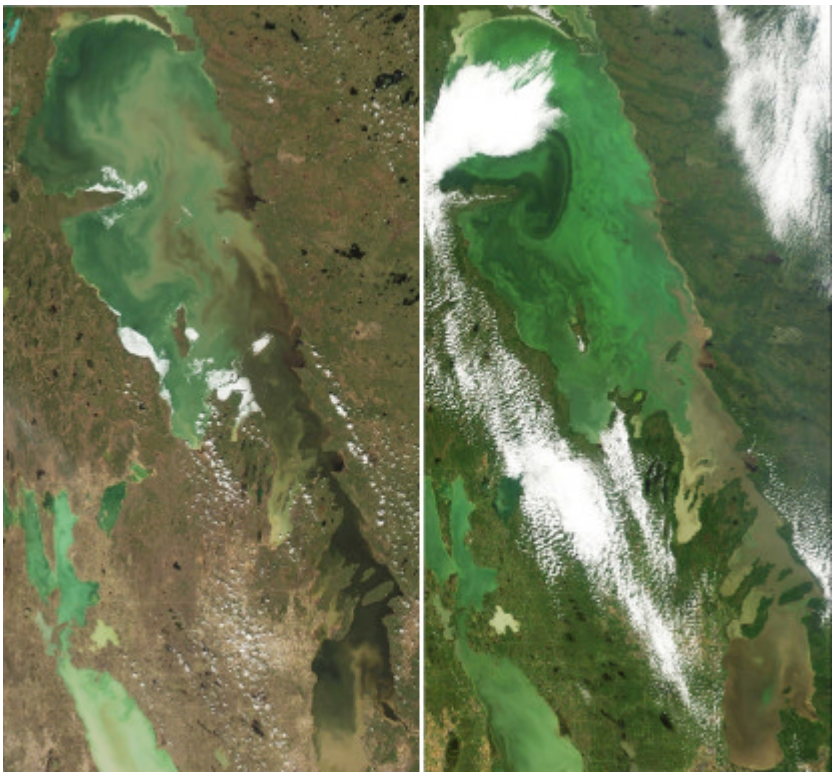

Figure 4. True-colour rendition of MODIS (RGB=1,4,3) data for Lake Winnipeg recorded at 14:25 16 May 2005 (left) and 12:35 CDT 29 August 2005 (right) showing regions of relatively clear or algae-rich waters (dark and green, respectively), more turbid waters (lightest yellowish-brown indicating highest mineral suspended solids concentrations) and local regions with high dissolved organic carbon concentrations (dark reddish brown, mostly near river mouths along the east shore). Source: MODIS Rapid Response System, U. Maryland.

Algal community distributions

The Lake Winnipeg algal assemblage is marked by seasonal succession. In the analysis conducted for this study it has become apparent that differences among the spectral signatures of different algal assemblages complicate the determination of chlorophyll biomass by MERIS and MODIS. Consequently, the succession is described here – in general for 2002-2004, and in particular for 2003, from which year much of the data for this study will be selected.

June populations are typically dominated by bacillariophytes and cryptophytes, with subordinate populations of chrysophytes and chlorophytes. Cyanophytes are rare in June, but they frequently dominate the phytoplankton population by mid-July to early August, when they are found in association with cryptophytes and chlorophytes, In warm summers, they often attain near-monoculture status. Bacillariophyte populations may increase again when the lake begins to cool in autumn, although cyanophytes may remain

dominant through into late autumn in parts of the lake. Chlorophytes tend to persist into autumn as the main sub-dominant group.

In the spring of 2003, individual samples were variously dominated by bacillariophytes, cryptophytes and chrysophytes, and in one sample, by cyanophytes, with no distinct differences between the major basins of the lake. By August, different distributions had developed between the basins. In the South Basin and central part of the lake in August 2003, in a few samples cyanophytes comprised over 90% of the biomass, but other samples were 70-90% cryptophytes and/or chlorophytes. In the North Basin in the same mid-summer sample set, most samples were near-monocultures of cyanophytes, chiefly *Aphanizomenon flos-acquae.* By autumn, bacillariophytes dominated the North Basin, and comprised as much as 90% of biomass in some samples. However, in the South Basin, although cyanophytes still dominated most samples, other taxa, in particular chlorophytes, were well represented.

Methods

In situ data used in this analysis was collected in the course of research funded by the Canadian Space Agency under a Canadian Space Plan Proposal to the Earth and Environment Applications Program, 2002/03 to 2004/05 CSP Application Area: 1.3.5 Marine Environment: Inshore and Coastal. The data collection program and field and laboratory analytical methods employed are described in the report "Chlorophyll Mapping using MODIS/MERIS imagery over Case 2 Waters, Lake Winnipeg", submitted to the Canadian Department of Fisheries and Oceans (McCullough, 2006; submitted to M. Stainton, Freshwater Institute, Winnipeg, Canada). These methods are described in brief below.

In situ data were collected from on board the Canadian Coast Guard Ship Namao on Lake Winnipeg during a series of whole lake surveys from 2002 to 2004. The Namao was used to complete numerous short missions in the South Basin of Lake Winnipeg, operating out of Gimli harbour, and on three whole Lake Winnipeg surveys each year, in May/June, July/August and October/November. Data specifically for the Canadian Space Agency project were collected in conjunction with broader limnological surveys undertaken by the Department of Fisheries and Oceans, Environment Canada, Manitoba Water Stewardship and the University of Manitoba (Department of Biology, and the Centre for Earth Observations Science, Department of Environment and Geography, University of Manitoba).

Locations of the standard limnological stations and the track of a typical whole lake cruise are shown in Figure 5.

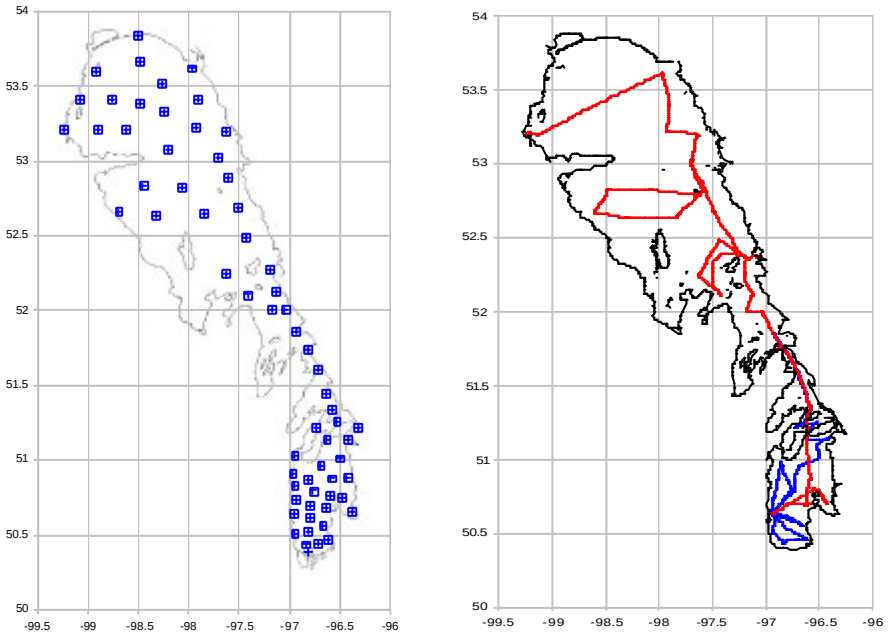

Figure 5. Distribution of standard stations (left). Sample CCGS Namao cruise track (31 July-17 August 2003). Blue: continuous chlorophyll fluorescence, turbidity and temperature data. Red: also with optical spectral reflectance data and samples for TSS, tripton, DOC, chlorophyll, HPLC analysis at 20 min intervals when under clear sky.

Water quality parameters

Water samples at standard stations were taken from the upper metre of the water column using a van Doorn sample bottle. Water samples taken while underway were pumped to the deck for an intake located just foreward of the ship's bow. The intake was held roughly 0.1-0.3 m below the surface of the lake. The actual depth varied with wave conditions. Pumped water samples were collected at roughly 20 min. intervals when we enjoyed clear-sky conditions, and at irregular intervals when sampling under less optically ideal conditions. At a typical cruising speed of 20 $km h^{-1}$, under ideal conditions, water quality samples were collected at 6-7 km intervals.

Analytical methods for laboratory determinations of chlorophyll by gross fluorescence method (chl), chlorophyll-a by HPLC method (chl a), suspended solids concentration (SSC), tripton and dissolved organic carbon (DOC) are described by Stainton *et al.,* 1977.

Algal taxonomy

Algal taxonomic data was provided by Hedy Kling of Algal Taxonomy and Ecology, Limited, of Winnipeg Manitoba. Microscope counts were in part funded by the Canadian Space Agency under the project mentioned above. Ms. Kling has kindly provided additional taxonomic data for this study.

Fluoroprobe data

In 2003, the Department of Fisheries and Oceans operated a Fluoroprobe instrument (manufacturer: Bbe Moldaenke GmbH, Kiel-Kronshagen, Germany) during summer and autumn whole-lake cruise. Water was supplied to the Fluoroprobe via a line from the intake used to retrieve water samples while underway (described above). Observations were recorded at 5 min intervals. Water quality and/or algal taxonomic data paired with these Fluoroprobe observations were collected from the same line within no more than 5 min of each other.

Software provided with the Fluoroprobe outputs of total chlorophyll biomass and separate chlorophyll biomasses of four major taxa: bacillariophytes, cyanophytes, cryptophytes and chlorophytes. This default output is estimated by multiple regressions on fluorescence of laboratory cultures using five excitation wavelengths: 470, 525, 570, 590 and 610 nm. Sample excitation fluorescence spectra supplied by the manufacturer are shown in Figure 6. (The Fluoroprobe records fluorescence emitted at a single fixed

wavelength of 680 nm.) Notably, the greatest contrast is between bacillariophytes and cyanophytes, two dominant taxa in Lake Winnipeg. Bacillariophytes fluoresce most strongly in the response to blue and green light and less strongly in response to red light; cyanophytes show the opposite pattern (Figure 6).

In this study, I compare both the chlorophyll biomass output based on the manufacturer's calibration and biomass estimates that I have generated by regression with Lake Winnipeg *in situ* data with chlorophyll biomass by laboratory analysis and with algal biomass estimated by microscope counts. As a co-estimator of biomass of major taxa, in addition to fluorescence intensity at the five excitation wavelengths described above, I also incorporate the fluorescence intensity at an excitation wavelength of 370 nm, used internally in the Fluoroprobe for estimate the concentration of coloured dissolved organic matter (CDOM). Dissolved organic carbon varies in Lake Winnipeg over a range exceeding 400 - 1200 mg m^{-3}, and therefore CDOM may be expected to vary considerably as well. Incorporation of fluorescence due to excitation at 370 nm improves correlation marginally in some regressions described in this study. Fluoroprobe output also includes a measure of light transmission at each excitation wavelength. Because turbidity also varies greatly in Lake Winnipeg (suspended solids concentrations range from < 1 g m^{-3} to more than 60 g m^{-3}) I also tested transmission as one of the suite of independent variables for estimation of biomass of major taxa. In none of these early tests did transmission contribute to correlation coefficients, so that it is not further discussed below.

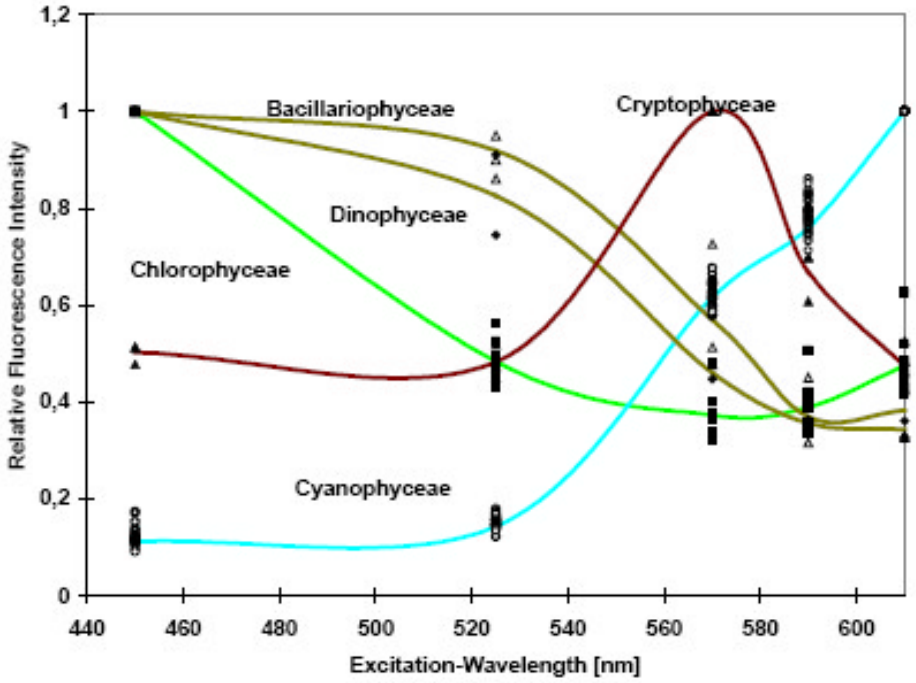

Figure 6. Excitation fluorescence spectra for several algal taxa. After Figure 2 in bbe Moldaenke (Undated).

Results and discussion

Distributions of major taxa in sample

The distribution of major taxa in the August – October 2003 sample of 58 observations are described in Table xxx. Observations in the sample were dominated by bacillariophytes, cyanophytes, chlorophytes and cryptophytes, which were present in over 90% of observations and comprised as much as half of some observations. Bacillariophytes and cyanophytes comprised more than 90% of 8 and 12 observations respectively. Chrysophytes were present in 81% of observations, but comprised no more than 3% of the biomass in any given observation. Xanthophytes comprised 33% of one sample, but were present in less than 20% of samples and averaged only 2% of the biomass in these. Of the remaining taxa occurring in the sample, dinoflagellates, euglenophytes, haptophytes and peridineae and none comprised more than 10% of any individual observation, and each was present in less than a third of the observations. None of the latter are further considered in this study. Only the four dominant taxa, plus chrysophytes, were tested for prediction by Fluoroprobe data.

Table 3. Descriptive statistics for biomass of major taxa by microscope counts in the August – October 2003 observations used for Fluoroprobe validation, where units are mg m^{-3}. Present = per cent of observations in which at least one representative of applicable taxon was observed. n = 58.

	Bacillario-phytes	Chloro phytes-	Chryso-phytes	Crypto-phytes	Cyano-phytes	Dinofla-gellates	Eugleno-phytes	Hapto-phytes	Peridineae	Xantho-phytes
median	721	60	7	23	787	0	0	0	0	0
mean	1944	152	12	206	3785	32	2	1	19	84
s.d.	3262	221	16	847	7293	135	8	7	36	441
min.	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
max. (mg m^{-3})	16032	1066	51	4709	31512	720	16	2	125	2440
max. (% of biomass)	97	52	3	64	100	2	0.5	0.2	9	33
present (% of obs.)	98	100	81	98	93	16	17	10	31	19

Estimation of algal biomass by Fluoroprobe

In the discussion below, chl and chl a refer specifically to chlorophyll and chlorophyll a biomass by the gross fluorescence method and by high performance liquid chromatography respectively. Chl and chl a refer to totals for an individual samples and cannot be differentiated by taxa. Biomass determined by microscope counts is estimated using size statistics and assumed densities for cells counted under the microscope, and may refer to totals for individual taxon, or be summed arithmetically to refer to total biomass. In either case, biomass by microscope counts is reported as total biomass, as opposed to chlorophyll biomass, and may be two orders of magnitude or more greater than chlorophyll biomass for individual taxa or individual samples.

Fluoroprobe default outputs are estimates of chlorophyll biomass whether for the total or for individual taxa. Factory settings report chlorophyll biomass for bacillariophytes, chlorophytes, cryptophytes and cyanophytes, as well as the total. The latter output data are simply the arithmetic total of the four major taxa. That is, the Fluoroprobe default output record "Total algae" is not determined using a separate, independent regression.

Results of regression analysis of chlorophyll and total biomass, and of individual taxon biomasses versus fluorescence at multiple excitation wavelengths are summarized in Tables 2 – 5 and Figures 7 - 10, below. For both for total biomass of individual taxa and for the chlorophyll and total biomass of whole samples, stepwise regression analysis indicates that in each case some wavelengths may be dropped from the multiple regression without significant loss of information. However, in this study, results are reported for regression on fluorescence intensity at all six Fluoroprobe excitation wavelengths (referred to below as "F6").

All regression results reported below are for log-transformed dependent and independent variables. Where zero algal biomass was reported for a particular taxon, 0.1 mg m^{-3} was substituted as the value for the dependent variable prior to log transformation. Regression residuals for log-transformed data were graphically inspected and found to be normally distributed. In every case except chrysophytes, chlorophyll and/or total algal biomass by independent estimates are correlated with F6 at greater than 99% confidence (Tables 3 and 5).

F6 predicts both chl and chl a with coefficients of determination (r^2) of 0.57 and 0.63 respectively (Table 3). The root mean square error of prediction (estimated minus observed values) is 7 – 8% of the range of chl and chl a in Lake Winnipeg. Multiple regression on F6 explains only a slightly smaller fraction of total biomass by microscope count, with $r^2 = 0.51$, although it returns a larger relative RMSE, 17% of the range in Lake Winnipeg. The Fluoroprobe default output of total chlorophyll biomass (which derives from the manufacturer's calibrations for chlorophyll biomass of each of the four major taxa) with $r^2 = 0.47$ and RMSE = 18% of range, is essentially as good a predictor of total biomass by microscope count as is F6 (where the regression is calculated on Lake Winnipeg *in situ* data).

Examination of Figures 7 and 8 indicates that multiple regression on F6 tends to under-predict both chlorophyll and total biomass at high concentrations (> 10 000 mg m^{-3}) and to over-predict at low concentrations (< 2000 mg m^{-3}). The pattern is the same whether using regression developed on *in situ* fluorescence data or using regression on the manufacturer's estimate of total chlorophyll biomass.

Table 4. Coefficients of multiple regression of chlorophyll and total algal biomass on fluorescence intensity at 6 excitation wavelengths with dependent and independent variables log-transformed. "Chl" indicates chlorophyll biomass by gross fluorescence method; "Chl a" indicates chlorophyll a biomass by HPLC method. Total biomass is estimated by microscope counts.

	Chl	Chl a	Total biomass
Intercept	-0.42738	-1.08238	5.66625
f2 (525 nm)	1.63604	1.59967	2.49257
f3 (570 nm)	-0.11062	-0.24213	-0.83301
f4 (610 nm)	-0.37523	-0.12551	0.54199
f5 (590 nm)	0.78908	0.97534	-3.21041
f6 (470 nm)	0.44031	0.29130	0.94779
f7 (370 nm)	-1.74183	-1.64433	-0.09375

Table 5. Statistics for multiple regression of chlorophyll and total algal biomass by microscope counts on Fluoroprobe fluorescence intensity, using paired data from summer and autumn, 2003 cruises. "Chl" indicates chlorophyll biomass by gross fluorescence method; "Chl a" indicates chlorophyll a biomass by HPLC method. Total biomass is estimated by microscope counts. n = 261, 244, 58 and 58 for each row respectively.

	r^2	F	RMSE	min.	max.	RMSE/range
Chl	0.57	0.000	16	2	199	8%
Chl a	0.63	0.000	14	2	191	7%
Total biomass	0.51	0.000	5505	539	33357	17%
Total biomass*	0.47	0.000	5753	539	33357	18%

***Regression with Fluoroprobe default output of chlorophyll biomass substituted as the independent variable.**

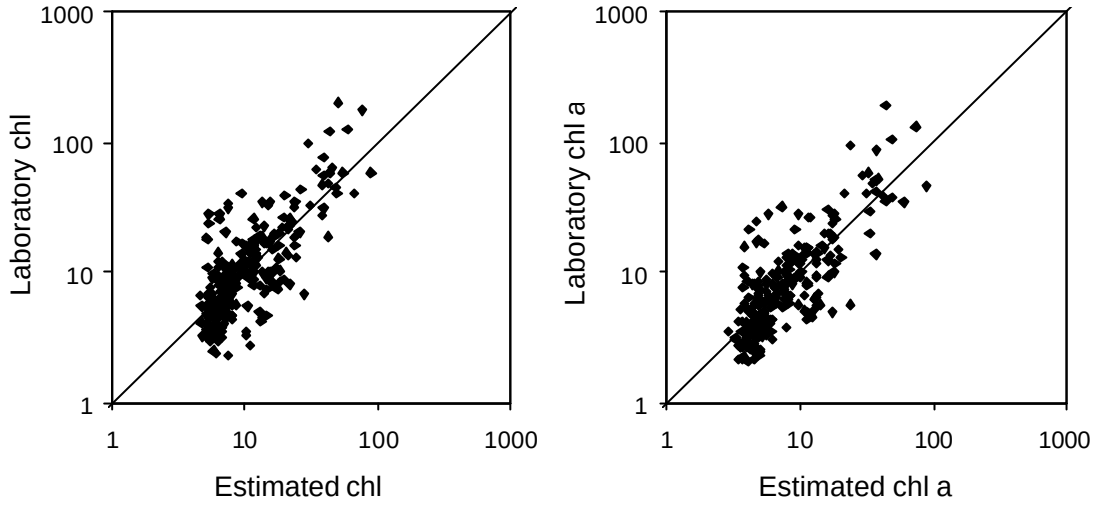

Figure 7. Total chlorophyll biomass determined by gross fluorescence method (chl, left, n = 260) and by HPLC method (chl a, right, n = 245) plotted against chlorophyll biomass estimated by multiple regression on F6 (left). Two outliers (same 2 samples in both sets) have been removed from these data sets.

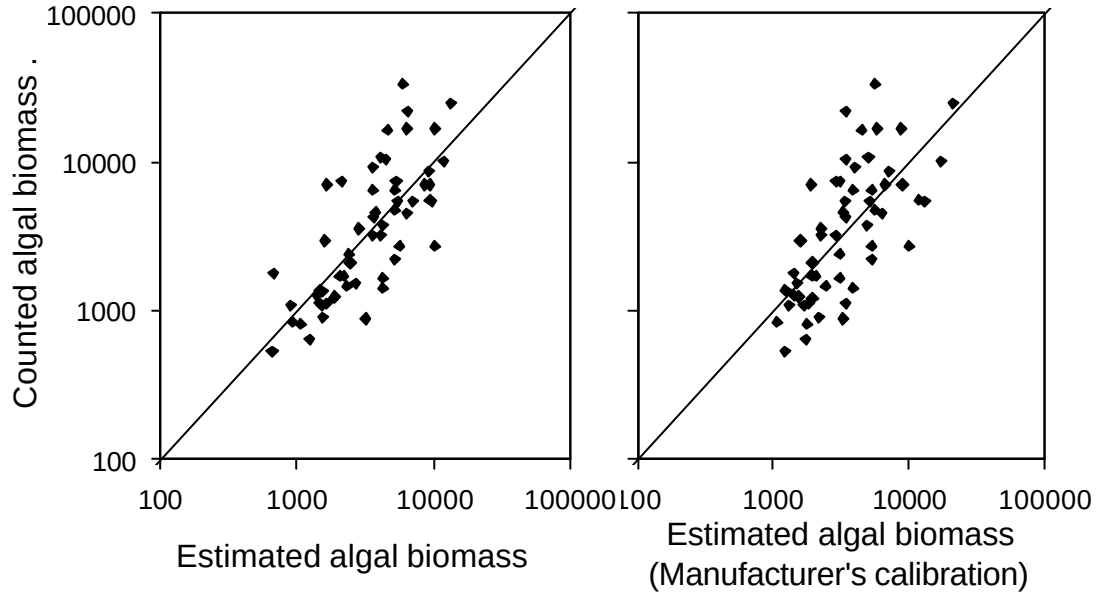

Figure 8. Total algal biomass estimated by microscope counts plotted against biomass estimated by multiple regression on F6 (left) and by regression on manufacturer's calibration for total chlorophyll biomass (right).

Regression results for prediction of algal biomass of individual taxa are reported in Tables 4 and 5 and Figures 9 and 10. Multiple regression on F6 is a reasonably good predictor of bacillariophyte and cyanophyte biomass, with $r^2 = 0.68$ and 0.66 respectively, but only a weak predictor of chlorophyte and cryptophyte biomass – $r^2 = 0.21$ and 0.32 respectively. Chrysophyte biomass is not predicted at a statistically significant level.

Graphical analysis confirms that overall, multiple regression predicts bacillariophyte and cyanophyte biomass, and perhaps also cryptophyte biomass, reasonably well. For bacillariophytes the regression determined using *in situ* data appears to outperform the Fluoroprobe default output at very low algal biomass (< 10 mg m^{-3}, Figure 9); otherwise the two estimates are roughly equal in precision. For cyanophytes, the Fluoroprobe default output predicts algal biomass with less scatter (RMSE = 19% of range compared to 34% of range using F6 and *in situ* data) than does regression based on *in situ* data. By graphical analysis, this can be seen as a slightly tighter fit of most estimated values about the line of 1:1 prediction using multiple regression on F6, compared to regression on the Fluoroprobe default output for cyanophytes, with a few points in relatively outlying positions (Figure 9).

Also by graphical analysis, chrysophytes are clearly not predicted by Fluoroprobe data. Chlorophytes are underestimated at higher values (> 50 mg $^{-3}$) and overestimated at lower values. Only cryptophytes are well-distributed about the 1:1 line at all values, indicating consistent overall prediction, although with high scatter. Note that the Fluoroprobe default output of cryptophyte chlorophyll biomass is not a significant predictor of cryptophyte biomass by microscope counts (Table 5) and indeed produces almost meaningless total biomass estimates within a narrow range of 14 – 32 mg m^{-3} (14 mg m^{-3} being the regression output at a Fluoroprobe default cryptophyte chlorophyll biomass of zero; Figure 10).

Table 6. Coefficients of multiple regression of biomass of five major taxa on fluorescence intensity at 6 excitation wavelengths with dependent and independent variables log-transformed.

	Bacillariophytes	Cyanophytes	Chlorophytes	Chrysophytes	Cryptophytes
Intercept	-0.37594	4.67543	-1.94507	-103.25486	15.68702
f2 (525 nm)	-1.13125	-0.94453	-4.27244	78.48107	-6.58221
f3 (570 nm)	-2.11414	-1.50292	0.37628	-0.39595	3.85978
f4 (610 nm)	-1.67286	10.82535	1.32306	24.17881	-2.76002
f5 (590 nm)	-0.38700	-6.42132	3.06506	-29.76607	-10.64779
f6 (470 nm)	2.09255	-5.95432	-2.15714	42.01856	4.88227
f7 (370 nm)	5.14983	4.38431	2.80274	-62.48974	9.63549

Table 7. Statistics for multiple regression of biomass of major taxa by microscope counts on Fluoroprobe fluorescence intensity, using paired data from summer and autumn, 2003 cruises. n = 58 for all regressions.

	r^2	P(reg'n)	RMSE	min.	max.	RMSE/range
Total biomass	0.51	0.000	5505	539	33357	17%
Total biomass*	0.47	0.000	5753	539	33357	18%
Bacillariophytes	0.68	0.000	1555	0.0	16032	10%
Bacillariophytes*	0.52	0.000	2011	0.0	16032	13%
Cyanophytes	0.66	0.000	10782	0.1	31512	34%
Cyanophytes*	0.68	0.000	6038	0.1	31512	19%
Chlorophytes	0.21	0.006	189	1.4	1066	18%
Chrysophytes	0.08	0.120	15	0.1	51	30%
Cryptophytes	0.32	0.000	601	0.1	4709	13%
Cryptophytes*	0.00	0.280	627	0.1	4709	13%

***Regression with Fluoroprobe default output of chlorophyll biomass substituted as the independent variable.**

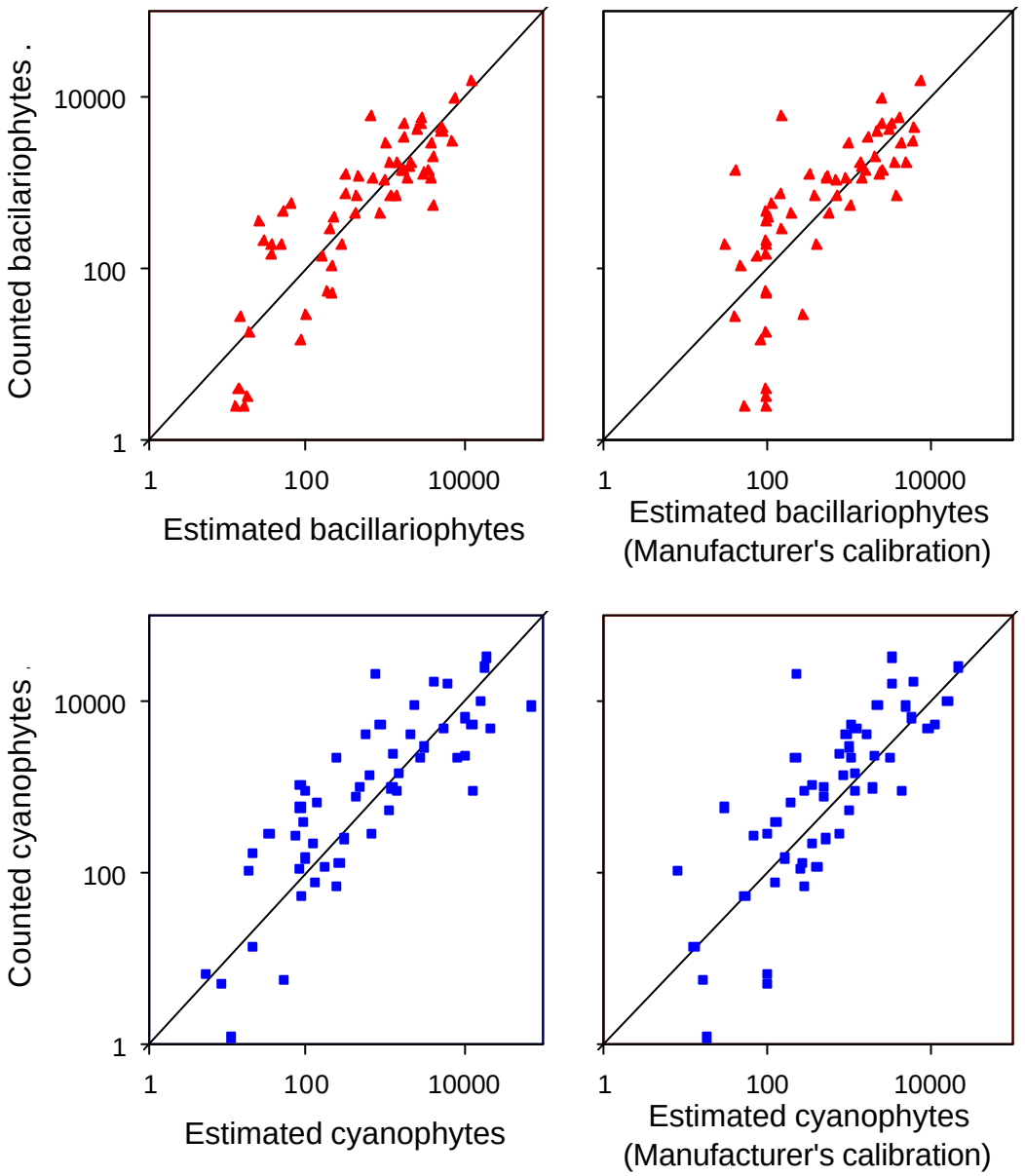

Figure 9. Bacillariophyte and cyanophyte total biomass plotted against biomass estimated by multiple regressions on F6 (left) and by regression on Fluoroprobe default output for bacillariophyte and cyanophyte chlorophyll biomass (right). Target data are biomass in mg m^{-3} determined by microscope counts.

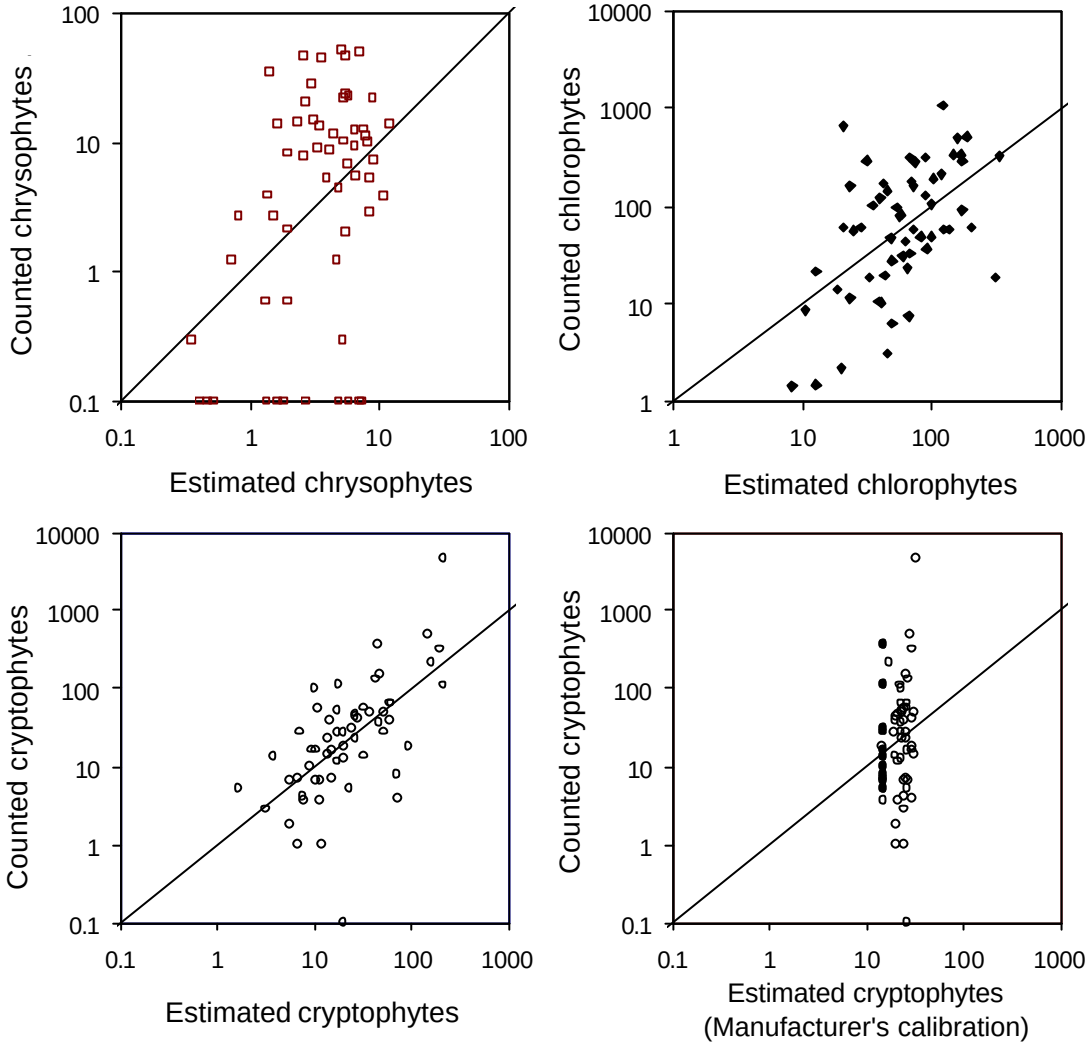

Figure 10. Chrysophyte, chlorophytes and cryptophyte biomass estimated by multiple regression on F6. Lower right: cryptophyte biomass estimated by regression on Fluoroprobe default output for cryptophyte chlorophyll biomass. Diagonal line indicates 1:1 predicti on. Target data are biomass in mg m^{-3} determined by microscope counts.

Seasonal patterns of algal taxonomic distributions

Figures 11 and 12 show seasonal patterns of algal community structure as determined by Fluoroprobe and by microscope counts. The data set is comprised of matched samples where, for each pair, water for the microscope count was drawn from the lake within 5 min of the sample for the associated Fluoroprobe determination. All data shown are from samples drawn during summer and autumn cruises in 2003. Major taxonomic groups not shown rarely comprised more than 10% of the algal biomass.

Fluoroprobe and microscope count data show similar seasonal patterns of dominance by the two major algal groups, bacillariophytes and cyanophytes. Moreover, for these two taxa the seasonal patterns are also similar between the two methods, i.e. the manufacturer's equations for bacillariophyte and cyanophyte biomass (default output), and the equations developed by regression on in situ biomass data (regression output).

In the August sample, cyanophytes comprise 70 - 100% of most samples, whether by Fluoroprobe or by microscope count. The degree of cyanophyte dominance is not much different between the default and the regression output – excepting in one noticeable case on 17 August. In that observation, by the default output cyanophytes dominate the observation as they do by the microscope count, but between the regression output and the microscope count the fractions of cyanophytes and chlorophytes are reversed.

Bacillariophytes are the most frequent sub-dominant taxon in the August observations. They comprise < 30% of biomass both by the default output and by microscope counts. Note, however, that by the default output bacillariophytes are present in only 8 of 18 August observations. By the regression output they are present in 15 observations and range from <1% to 80% of the biomass in each. Thus the frequency of bacillariophyte observations by the regression output is more in keeping with the results by microscope count, where they are present in 13 observations, but the fractions of bacillariophytes by microscope counts appears to be more accurately reproduced by the Fluoroprobe default output.

By microscope count, either chlorophytes or cryptophytes may make up the balance in the August sample – in only 2 of 18 observations comprising more than 10% of the biomass. By the Fluoroprobe default output, cryptophytes make up the balance in almost all observation, and comprise roughly 10% or less of the biomass of most individual observations. (Note where cryptophyte fractions are high, total biomass is generally low. For example, for the two August observations in which cryptophytes comprised 58% and 43% of the biomass respectively, total sample biomasses were only 2.4 mg m^{-3} and 1.0 mg m^{-3} respectively. Figure 11) By the regression output, the balance may be either cryptophytes or chlorophytes – either are also less than 10% of most observations – i.e. compared to the regression output, the default output poorly reproduces the pattern of the microscope count for the minority taxa.

Likewise, in the autumn sample, the fractions of bacillariophytes and cyanophytes are generally similar between Fluoroprobe determinations and microscope counts. In this autumn subsample there is no dramatic difference between the default and regression output in their reproduction of the progression of bacillariophyte and cyanophyte fractions. The bacillariophyte-dominant late-September sub-sample was collected in the North Basin of the lake. The cyanophyte-chlorophyte dominant early-October period was collected in the South Basin. Unlike for the dominant taxa, there is a difference between the two chlorophyte results. For chlorophytes the regression output better reproduces the pattern shown by the microscope count than does the default output. Chlorophyte-dominant assemblages found by microscope count in the early-October, South Basin observations were not at all replicated in the default output, for which cyanophytes and bacillariophytes together comprise over 90% of each observation. By the regression output chlorophytes – not bacillariophytes -- were usually subdominant in early October, and this is consistent with the microscope count results.

For the two dominant taxa, bacillariophytes and cyanophytes, the Fluoroprobe strongly reproduces the seasonal succession and geographic patterns of dominance. It is important to remember that the pattern by regression output *should* reproduce the pattern by microscope count; it is derived by regression on this data set. An independent sample is needed to better evaluate this procedure. Given the weak correlation and modest RMSE reported by regression analysis for two subordinate taxa (chlorophytes and cryptophytes) it is reassuring that seasonal and geographic patterns are well-reproduced for these as well (by the regression output, though not by the default output). The reasonable reproduction of the seasonal pattern of these subordinate taxa as determined by microscope counts gives confidence that in future surveys of Lake Winnipeg, the Fluoroprobe will reasonably describe occurrence and succession for these taxa as well.

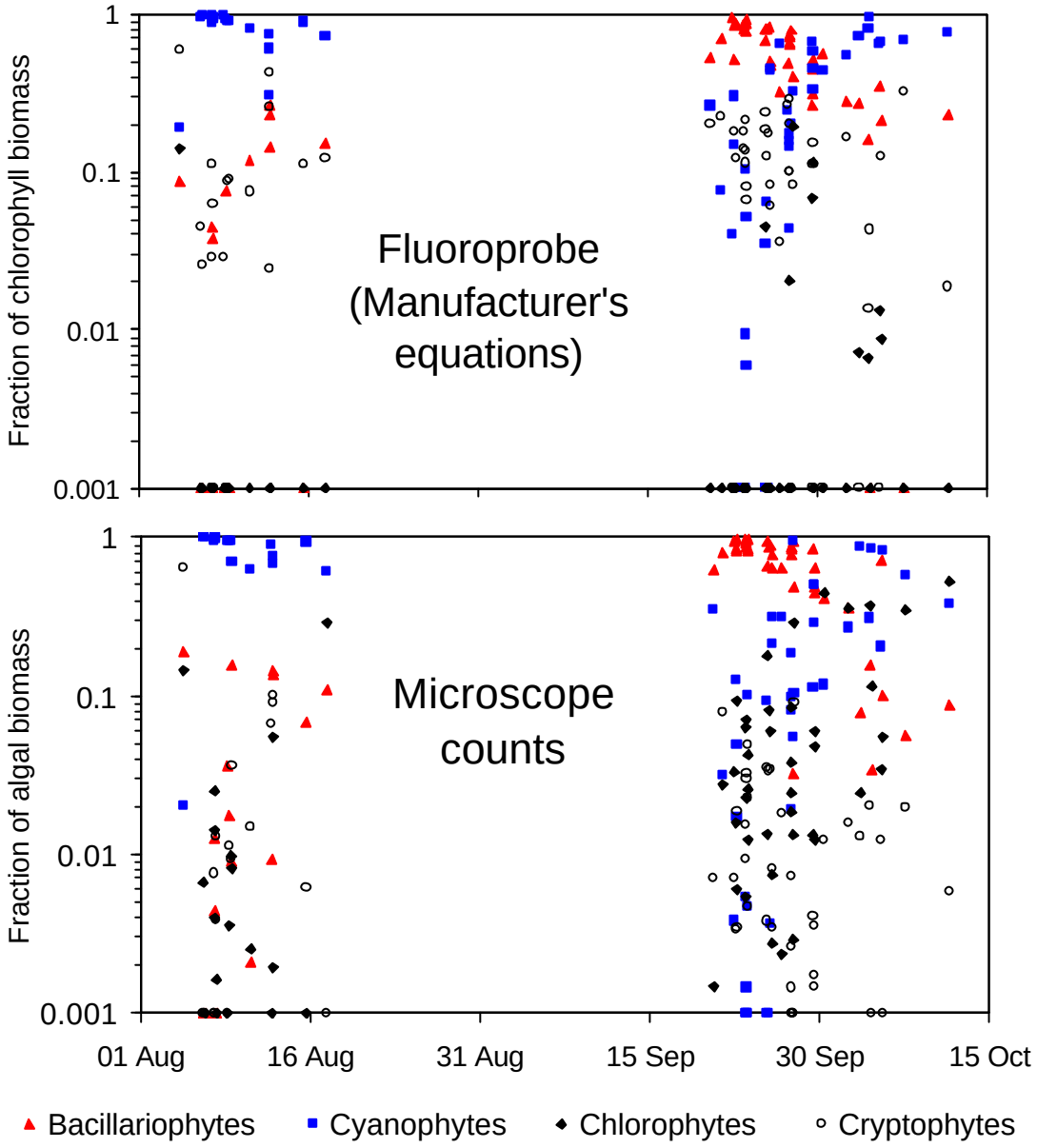

Figure 11. Fractions of major algal phyla estimated by Fluoroprobe and by microscope counts, where Fluoroprobe data are calculated using equations supplied by manufacturer. Data are from summer and autumn cruises in 2003. The data set is comprised of matched samples where, for each pair, water for the microscope count was drawn from the lake within 5 min of the sample for the associated Fluoroprobe determination.

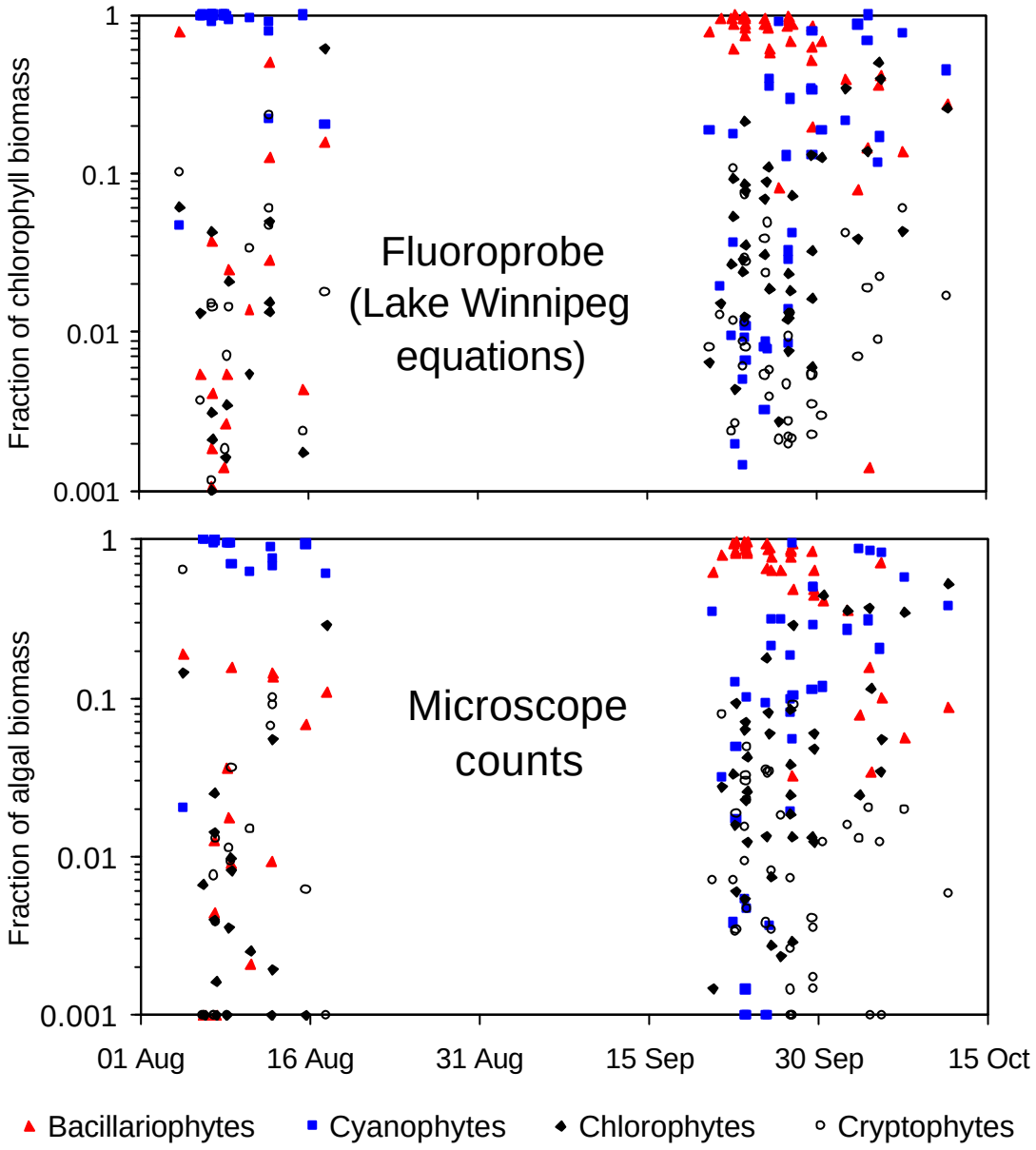

Figure 12. Fractions of major algal phyla estimated by Fluoroprobe and by microscope counts, where Fluoroprobe data are by regressions determined using Lake Winnipeg *in situ* data as described in this report. Data are from summer and autumn cruises in 2003.

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