The 2008 North Atlantic Bloom Experiment

Calibration Report #6

Laboratory analysis report

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Abstract:

The Laboratory Analysis Report describes the methods used to acquire and analyze discrete water samples collected from Niskin bottles during the 2008 North Atlantic Bloom experiment aboard the *R/V Bjarni Saemundsson* and *R/V Knorr*. This report describes the methods used to measure the following parameters: particulate organic carbon (POC, Subsection 1), nutrients (Subsection 2), extracted chlorophyll and pheopigments (Subsection 3), high pressure liquid chromatography (HPLC) pigments (Subsection 3), and particulate absorption coefficients (Subsection 4).

I. Particulate organic carbon (POC) analysis report

A. Filter and labware preparation

Whatman GF/F filters (25 mm) were placed in individual, heavy-duty aluminum foil packets; groups of 12 packets were placed inside larger foil packets and were baked at 400º C for 4 h in a muffle furnace to combust organic carbon. The cooled packets were transferred to zip-lock bags that were stored in plastic Tupperware containers cleaned with diluted RBS-35 solution (Thermo Scientific). Filters were stored for up to 3 mo before use.

All plastic-ware was washed with diluted RBS-35 solution before each cruise; when not actively used for sample collection, all plastic-ware was stored in RBS-35 cleaned containers or zip-lock bags to prevent contamination. Nitrile gloves were worn throughout the entire preparation, sampling and analysis process.

B. Sample collection, filtration and storage

Water samples were collected from Niskin bottles directly into clear, Nalgene polycarbonate bottles using clean clean silicone tubing connected to a sampling bell (Thermo Scientific Nalgene, # DS0390-0070) to cover the bottle opening to prevent contamination from extraneous particles during water collection. Bottles were completely filled and capped; standard bottle volume was 1.10 L, but additional 0.63-L and 2.40-L bottles were used for water collection, primarily for the triple-volume intercept blank determination (see *blank nomenclature*, below).

Samples were filtered immediately after the collection of water samples. Bottles were fitted with cleaned two-way T type bottle caps from Biochem Fluidics with two tubing ports. One port was fitted with 1/16" PEEK tubing to vent the bottle. The other port was connected to silicone tubing and a Millipore Swinnex 25 in-line filter holder that was connected to a vacuum line. Two pre-combusted G/FF filters were placed in the in-line filter holder, with the second filter serving as an adsorbed dissolved organic carbon (DOC) blank (double filter blank). The sample bottle was inverted in a rack and low vacuum (5 mm Hg) applied below the in-line filter. When filtration was completed, the in-line filter was opened while still maintaining a vacuum. The O-ring removed with forceps and rinsed with a small volume of filtered seawater to remove any particles that had adhered to the O-ring; the rinse water was directly collected on the filter. Each filter was folded in half and placed individually in a combusted foil packet. Forceps were cleaned with RBS-35 and rinsed with Milli-Q water before each use. A total of 32 pre-combusted, unused filters were stored with the frozen sample and blank filters as cruise dry-filter blanks. All filters from a single station were placed into larger, pre-combusted aluminum foil packets that were stored in zip-lock bags at -20º C until analysis.

C. Blank Nomenclature

Several different types of blanks are referred to in this document.

1) empty tin-cup blank: the average of the last five blanks for each day's run was automatically subtracted from all readings by on-board instrument data processing;
2) lab-filter blanks: G/FF filters that were pre-combusted several days prior to analysis on the CHN analyze;
3) cruise dry-filter blanks: filters that were pre-combusted with the cruise samples and included with every 6 samples and sample blanks;
4) adsorbed DOC blanks: two types of sample blanks were taken during the cruise.
    a. double filter blanks: 2 GF/F filters were placed in the in-line filter holder, with the second filter serving as the blank. Each filters was individually stored in a separate foil packet.
    b. triple-volume intercept blanks: three water samples were taken from the same Niskin bottle: 0.63, 1.10 and 2.40 L. The Y intercept of POC vs. volume was used to determine the intercept blank. Note: 0.63 L and 1.1 L samples also had double filter blanks.

D. Sample Analysis

Samples were stored for up to 5 mo at -20º C. Before analysis, samples were dried in an oven at 50º C for 4 h, fumed with hydrochloric acid (HCl, 11.65 N) for 12 h, and stored in a desiccator for up to 12 h. Filters were rolled and placed in pre-combusted tin cups shortly before analysis on a Perkin Elmer 2400CHN analyzer.

The analyzer was left on overnight on gas saver mode to minimize warm up time. Empty, pre-combusted tin cups were run until readings stabilized; corn flour (Leco Corporation, No. 501-563) and National Research Council Canada Marine Sediment Reference Material, BCSS-1, were run as 'conditioning' standards. When the conditioning standard readings stabilized to the known value, 3 acetanilide standards in the 0.25 – 1 mg range were run to obtain instrument K-factors for calibration; tin cup blanks were run between each standard. After all standards and tin cup blanks gave stable reproducible readings, lab-filter blanks were run until readings were stable and reproducible (e.g., 4 to 10 filters). Only after the analyzer was considered stable were cruise samples analyzed; lab-filter blanks were run every 12th sample to detect instrument drift.

E. Data Analysis

A daily averaged tin-cup blank was automatically subtracted from all other blanks, samples and standards as part of the on-board instrument data processing.

Distributions of lab dry-filter blanks and cruise dry-filter blanks are shown in Fig. 1.1. The mean cruise dry-filter blank was 8.12 µg carbon and standard deviation was 2.07 µg carbon; the higher values of the cruise dry-filter blanks are likely associated with storage. Neither of these values was subtracted from cruise samples.

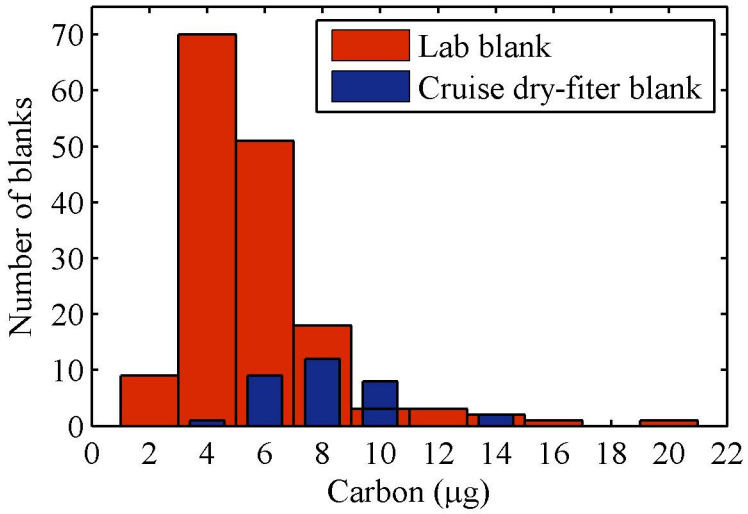

Figure 1.1. The equivalent carbon values of all pre-combusted blank GF/F filters; lab-filter blanks (blue) and cruise dry-filter blanks (red). The mean cruise dry-filter blank was 8.12µg carbon and standard deviation was 2.07 µg carbon.

Two methods were used for determination of sample blanks. A total of 232 double-filter blanks was collected during KN193-03. The mean double-filter blank is 20.96 µg carbon with a standard deviation of 9.37 µg carbon, median of 19.09 µg carbon and mode of 13.00 µg carbon. This histogram is skewed, with more values above the mode, perhaps due to contamination from the overlying sample filter (Fig. 1.2). There was a slight, non-significant trend with depth (Fig. 1.3 A). No trend with chlorophyll was observed (Fig. 1.3 B).

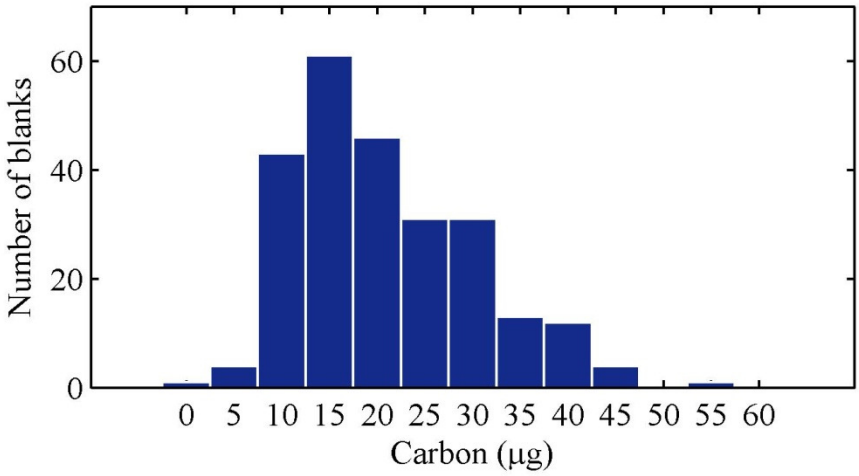

Figure 1.2. Double-filter blanks distribution for KN193-03. The mean double-filter blank is 20.96 µg carbon with a standard deviation of 9.37 µg carbon, the median is 19.09 and the mode is 13.00 µg carbon. The histogram is skewed, with more values above the mode, perhaps due to contamination from the overlying sample filter.

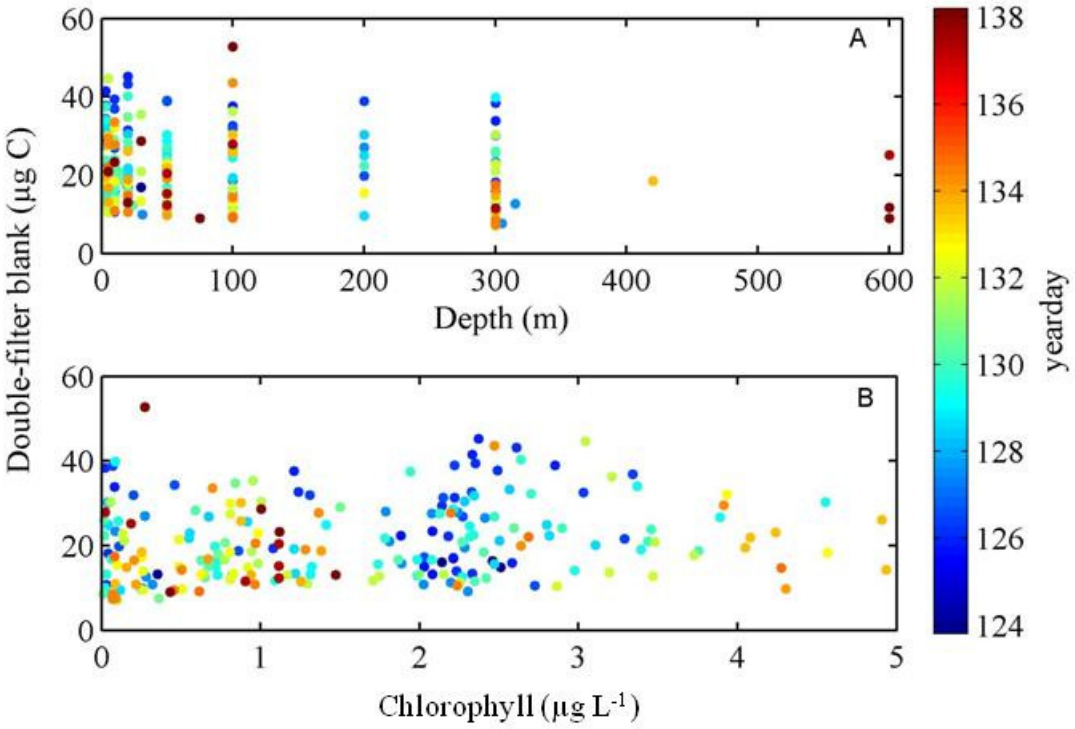

Figure 1.3. Double-filter blanks collected during KN193-03 vs. sampling depth (Panel A), and double-filter blanks vs. chlorophyll concentration (Panel B). Both panels are color-coded by yearday.

A total of 24 triple-volume intercept blanks were collected during KN193-03 (Fig. 1.4). For all triple-volume intercept blank sets, there was a strong linear correlation between POC and volume filtered (r^2 >0.99, Fig 1.4 A). Average triple -volume intercept blanks was 18.86 µg carbon with a standard deviation of 10.61 µg carbon, and a median of 18.89 µg carbon (Fig. 1.4 B). There was no clear trend in three-volume intercept blanks with sampling depth or chlorophyll concentration (Fig. 1.5).

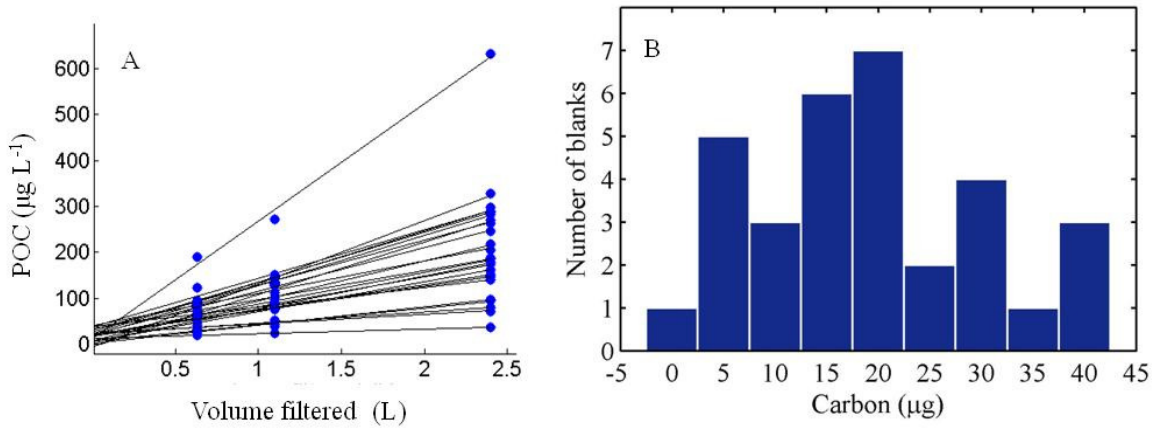

Figure 1.4. POC vs. filtered volume for 24 three-volume intercept blanks sets collected during KN193-03, blue points, with regressions (black lines, Panel A). Distribution of triple-volume blanks (panel B).

The median double filter blank (19.1 µg carbon) was subtracted from all the POC samples since:

- the standard deviation of the double filter blank was smaller than standard deviation found for three-volume intercept blank,
- median values for the double filter and the three-volume blanks differed minimally (0.2 µg carbon).

The range in double filter blanks values was 8.4 - 40.5 µg carbon (95% confidence limits) and this range should be considered for in context of the few negative POC concentrations, primarily for samples collected at the deeper depths.

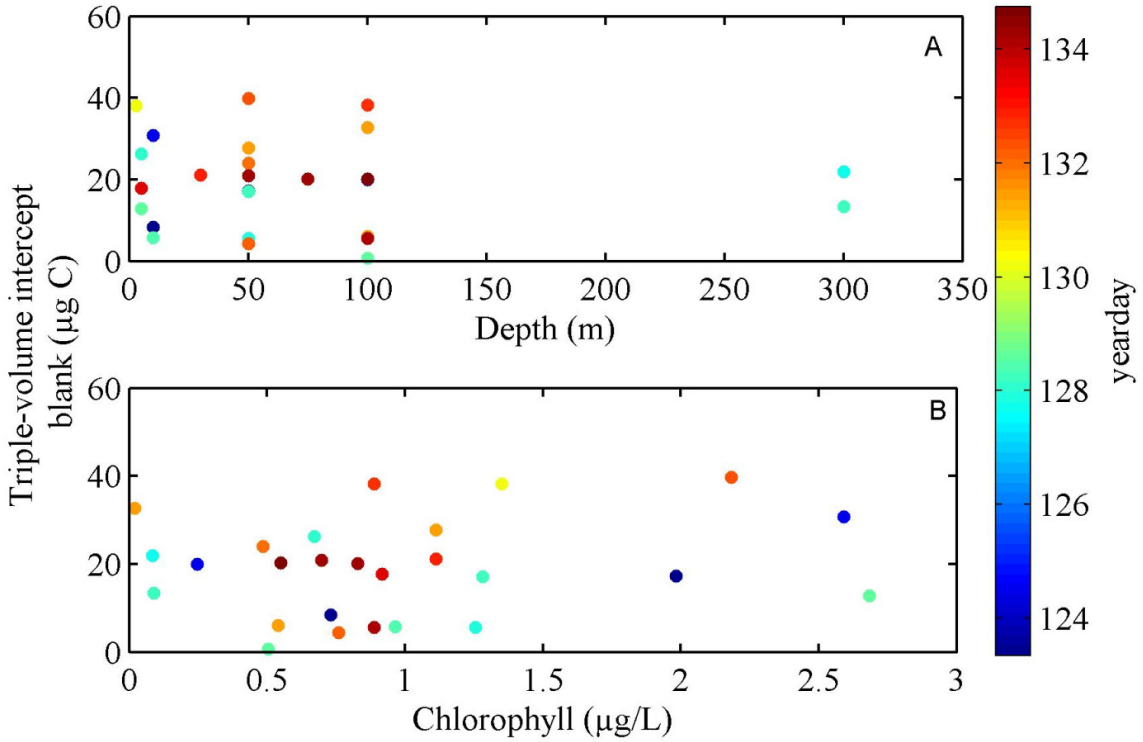

Figure 1.5. Triple -volume intercept blanks for KN193-03 vs. depth (panel A), triple-volume intercept blanks vs. chlorophyll (panel B). Both panels are color-coded by yearday.

F. Error Analysis

The accuracy of the POC method is 1%, expressed as mean percent difference between the analytical determination (from stable readings) and the known carbon content of BCSS-1 (2.19%). The precision of BCSS-1 carbon estimate was within 1.2 % (expressed as coefficient of variance, CV).

Only one station, 100.05, had true replicates with identical volumes and processing. The standard deviation (SD) for the triplicate sample was 2.72 µg carbon L^{-1}, with CV of 3.8%. Triple-volume intercept sets were collected from the same Niskin bottle, and although different volume they can be used as an additional measure of precision. The median SD for that set of triplicates was 7.1 µg carbon L^{-1}, and CV of 11%.

II. Nutrient analysis report

A. Sample collection

Unfiltered samples were collected directly from Niskin bottles into acid-cleaned 60-mL low density polyethylene plastic bottles. Bottles were rinsed with three aliquots of sample; samples were immediately frozen. All bottles from each station were aggregated and stored in sealed zip-lock bags for up to 8 mo at -20 ºC. A total of 1189 nutrient samples were collected and analyzed, 978 from the *R/V Knorr* (KN193-03) and 211 from the three *R/V Bjarni Saemundsson* cruises (B4-2008, B9-2008 and B10-2008).

B. Analysis

The samples were analyzed for phosphate (PO_4^{-3}), silicic acid ($Si(OH)_4$) and nitrate + nitrite (NO_3^-+ NO_2^-) with a Lachat Quickchem 8000 Flow Injection Analysis System using standard absorptiometric techniques (Lachat 1996; Lachat 1998; Lachat 1999)

Samples were slowly thawed in the dark at room temperature for 24 h to allow depolymerization of any polymerized silicic acid (although all samples had much lower silicate concentration than the minimal polymerization threshold; Gordon et al. 1992) and vortexed vigorously before analysis.

Primary standards, made in saline solution (Milli-Q water with 20.76 g NaCl/L), contained 25 µM N as KNO_3, 25 µM Si as K_2SiF_6, and 0.92 µM (125 µg/L) P as KH_2PO_4. Primary standards were made monthly and stored in refrigerator. Additional working standards were made up daily by diluting primary standards in saline solution to concentrations of 1.25, 2.5, 5 and 10 µM for nitrate and silicic acid, and 0.08, 0.16, 0.33 and 0.66 µM for phosphate. Saline solution, the base for working standards, was used as a blank.

Before each day's run, the Lachat was flushed for 20 min with Milli-Q water while the heaters warmed and stabilized. Standards and a saline solution blank were run every 30 samples; the lines were flushed with Milli-Q water between each sample, blank and standard. The reduction column for nitrate + nitrite was changed about every 200 samples. Pump tubing was changed after 24 h of use. The samples were re-frozen after analysis.

C. Quality control

1. QC of spectra

After analysis, all Lachat spectra were twice examined visually for quality control; spectra with air bubble spikes or an unstable baseline were considered unacceptable. Forty samples were

eliminated from the KN193-03 nitrate + nitrite and silicic acid dataset, and 28 samples from the B4-2008, B9-2008 and B10-2008 datasets were eliminated.

Most of the phosphate spectra exhibited unstable baseline, probably due to the malfunctioning of the phosphate detector. Hence, phosphate data were considered to be of poor quality and were not submitted to BCO-DMO.

Figure 2.1 shows the linear relationship between silicic acid and nitrate + nitrite for the entire QC data set and for samples shallower than 50 m. Nitrate + nitrite concentrations never fall below 3 µM, while silicic acid became depleted in May.

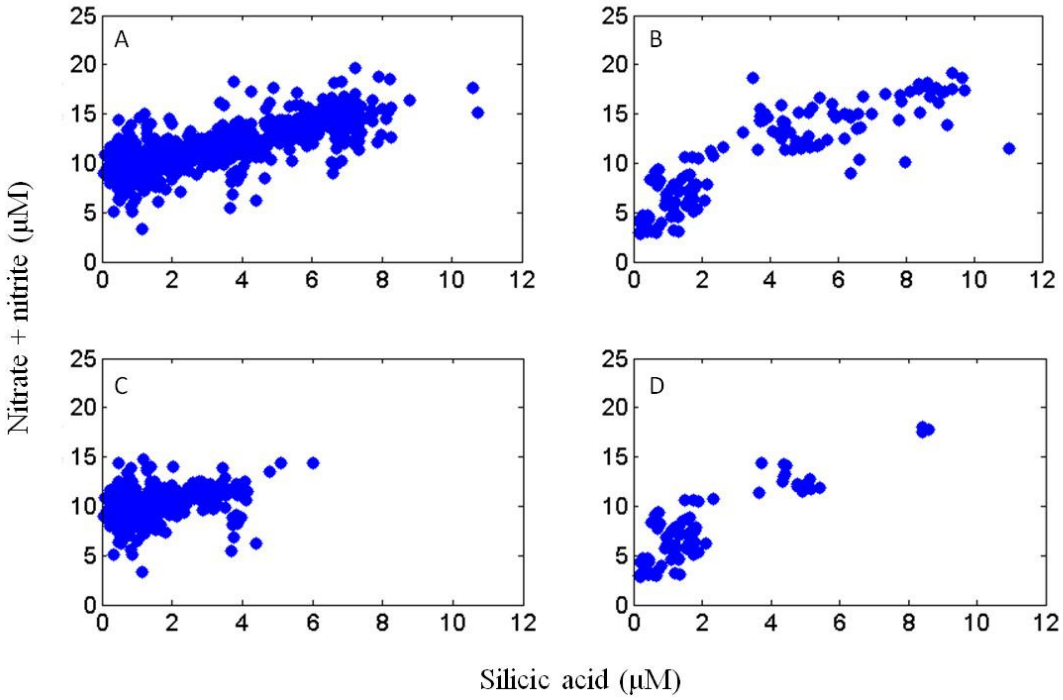

Figure 2.1. Nitrate + nitrite vs. silicic acid for all data from KN193-03 (May 2008) in panel A (top 50 m subset in panel C) and all data from *R/V Bjarni Saemundsson* cruises (B4-2008, B9-2008 and B10-2008) in panel B (top 50 m subset in panel D). A linear fit best explained nitrate + nitrite vs. silicate relationship for both datasets; for *R/V Knorr* cruise KN193-03 Nitrate + nitrite = 0.830 * Silicic Acid + 8.80 (r^2 = 0.61, n = 909); for *R/V Bjarni Saemundsson* cruises Nitrate= 1.35* Silicic acid + 5.38 (r^2 of 0.73, n=146.)

2. QC of profiles

Visual inspection of profiles of silicic acid and nitrate + nitrite concentrations for all stations was used as an additional quality control parameter following the recommendation of the IODE

workshop on quality control of chemical oceanographic data (IOC, 2010). Concentrations that were clearly out of expected/observed range and not associated with temperature or salinity intrusions were rejected, resulting in removal of an additional 17 silicic acid and 28 nitrate + nitrite data points for KN193-03 and 1 nitrate + nitrite data point from B10-2008 data.

D. Error analysis

Accuracy of this method was determined via residual analysis of the calibration curves. 95% of the residuals fall within 11 % (nitrate) and 21% (silicic acid) of expected value.

During B4-2008, all samples were collected in duplicate, while during KN193-03, duplicate samples were collected for most of 10-m samples. The precision is expressed as the standard deviation (SD) and coefficient of variation (CV) of duplicate samples. For silicic acid, SD was on average 0.075 µM, with an average CV of 8%. For nitrate + nitrite, average SD was 0.41 µM, with an average CV of 4%.

III. Chlorophyll *a* and pigment analysis

A. Chlorophyll *a* – standard fluorometric analysis

Procedures were based on Parsons et al. (1984) and JGOFS Protocols (UNESCO 1994), with modifications as noted. A single sample of seawater (V=280 mL; for high concentrations of chlorophyll, V=140 mL) was collected from all Niskin bottles, with the exception of the 10-m depth from which triplicate samples were collected. The water was filtered within 10 min of collection onto Whatman GF/F filters. Filters were extracted in 5 ml of 90% acetone in 10-ml screw-cap tubes in a -20° C freezer for 24 h. After extraction, the tubes were shaken and the filters were removed from the tubes. The tubes were centrifuged for 5 min to ensure that cell debris and remnants of the filters were sedimented to the bottom of the tubes; samples were read in the tube.

Turner Designs Model 10-AU Digital fluorometer with standard filters (Daylight White lamp; excitation filter # 10-050R color specification 5-60, 340-500 nm band; emission filter # 10-051R color specification 2-64, >665 nm) was calibrated with pure chlorophyll *a* (Turner Designs Liquid Primary Chlorophyll a Standard # 10-850) prior to the cruise (November 2007) and after the cruise (July 2008). The difference in calibration factors between two calibrations was minimal (Fig. 3.1).

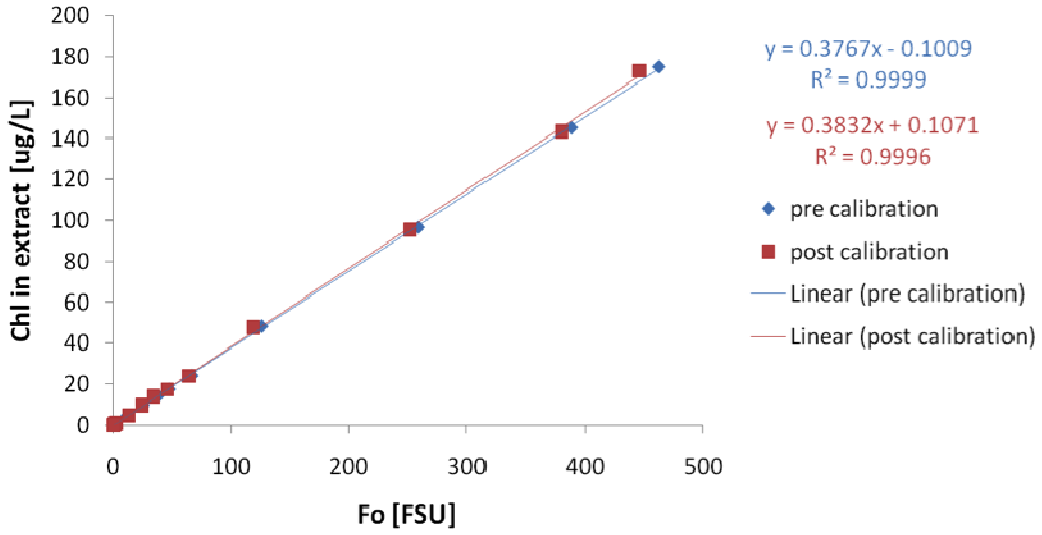

Figure 3.1. Comparison of pre (blue) and post (post) cruise calibrations for Turner 10AU fluorometer with standard chlorophyll filters according to Parsons et al. (1984).

Before each set of samples was read, a solid fluorescence standard (10-AU-904) and 90% acetone blank were read and recorded; the blank was subtracted from all samples. Extracted pigments were measured before (F_o) and after (F_a) the addition of two drops of 10% HCl.

Chlorophyll *a* and pheopigment (pheophytin) concentrations were calculated as described in the JGOFS protocols (UNESCO, 1994):

Chl (µg L^{-1}) = (Fm/(Fm-1)) * (F_o-F_a) *F_s * (vol $_{ex}$/vol $_{filt}$)

Pheo (µg L^{-1}) = (Fm/(Fm-1)) * ([F_m* F_a] – F_o) * F_s * (vol $_{ex}$/vol $_{filt}$)

where

Fm = acidification coefficient (Fo/Fa) for pure Chl *a,* defined during the calibration

F_o = reading before acidification

F_a = reading after acidification

F_s = response factor for the sensitivity setting, defined during the calibration

vol $_{ex}$ = extraction volume

vol $_{filt}$ = sample volume

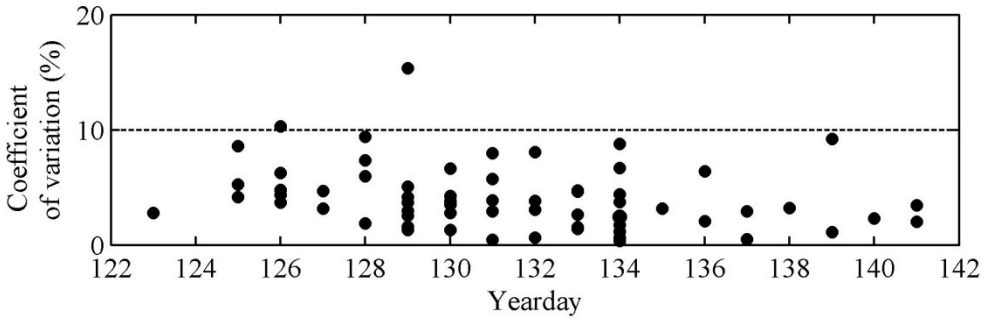

Figure 3.2. Statistics for triplicate 10-m samples collected during KN193-03 following the Parsons et al. (1984) method.

The accuracy of this method was determined by residual analysis of the calibration curve; resultant calibration residuals were within 10% of the true value. Throughout KN193-03, triplicate samples were collected from 10-m bottles. Coefficient of variation, measure of triplicate filter precision, was within the 10% for most of the samples (Fig 3.2).

B. Chlorophyll – fluorometric analysis using Welschmeyer (1994) filter set

Discrete water samples from Niskin bottles were collected from two depths (10 and 30 m) using 2-L brown Nalgene bottles (4 L total from each depth). Two liters were immediately filtered through GE Water Systems GE Nylon 20-µm pore-size filters (#R22SP04700) to remove >20 µm fraction. Filters were changed after each ~500 mL aliquot of seawater to avoid clogging of

the filter. The filtrates were stored in rinsed 2-L brown Nalgene bottles in a ~4.4˚ C (~40˚ F) refrigerator until all samples were filtered f was completed (0.5-1 h).

For fluorometric chlorophyll analysis, unfiltered water (total water sample) and <20-µm filtrate were filtered in triplicate onto Whatman GF/F filters and stored immediately in liquid N_2 until analysis 1-2 mo later. Volumes filtered varied based on the filter loading; for total water samples, volume ranged from 180 to 800 mL, and for <20 µm samples, from 450 to 1000 mL.

Prior to the fluorometric analysis, filters were removed from the liquid nitrogen and placed into a centrifuge tube filled with 10 ml of 90% acetone. Tubes were shaken, vortexed for 15 s and placed in the -20°C freezer for a 24-h extraction. After extraction, tubes were shaken, vortexed for 5 seconds, and centrifuged for 5 min. Samples were decanted to a clean vial for analysis.

Chlorophyll *a* and pheopigments were determined fluorometrically using a bench top Turner 10-AU benchtop fluorometer that was calibrated with chlorophyll *b*-free pure chlorophyll *a* (Sigma-Aldrich #C5753). The fluorometer was equipped with a Blue Mercury Vapor Lamp and narrow band interference filters that allows only chlorophyll a specific excitation (436 nm) and emission (680 nm) wavelengths to pass (http://www.turnerdesigns.com/t2/doc/appnotes/S-0013.pdf). Before each set of the samples was read, a 90% acetone blank was read and recorded; the blank was subtracted from all samples. Extracted pigments were measured before (F_o) and after (F_a) the addition of two drops of 10% HCl. Chlorophyll and pheopigment concentration were calculated following Welschmeyer (1994):

$$\textbf{Chl } (\mu g\ L^{-1}) = M * (Fo - Fa) * (vol_{ex}/vol_{filt})$$

$$\textbf{Pheo } (\mu g\ L^{-1}) = M * ([A * Fa] - Fo) * (vol_{ex}/vol_{filt})$$

where:

A = acidification coefficient (Fo/Fa) for pure Chl *a,* defined during the calibration

$\mathbf{F_o}$ = reading before acidification

$\mathbf{F_a}$ = reading after acidification

M= slope factor [mg chl/flo], defined during the calibration

$\mathbf{vol_{ex}}$ = extraction volume

$\mathbf{vol_{filt}}$ = sample volume

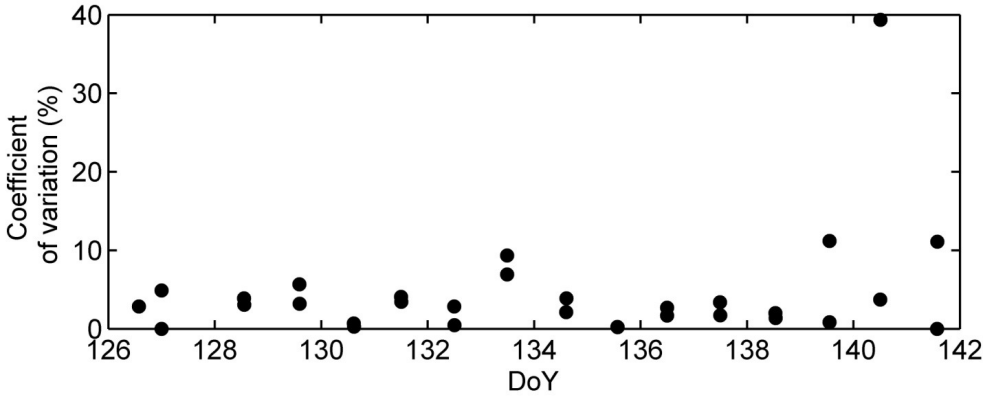

Figure 3.3. Coefficient of variation for triplicate samples collected during the cruise for extracted chlorophyll measured by the Welschmeyer (1994) method.

For the series of triplicate samples collected throughout the cruise, CV was < 10% except for one sample, on day 141 (Fig. 3.3). This dataset with the Welschmeyer (1994) method is not included in the bottle file, but was used for comparison.

C. HPLC pigment analysis

Samples for HPLC pigment analysis varied in volume from 400 mL to 2330 mL, depending on the group who collected and filtered the samples (Mary Jane Perry lab, Michael Sauer, or Toby Westberry/OSU). Upon collection, water was filtered onto a Whatmann GF/F filter and stored in liquid nitrogen until the analysis. A subset of the Mike Sauer's HPLC samples were pre-filtered through 20-µm pore size filters as part of his size fractionation primary productivity experiments, following the procedure explained above.

Two sets of samples were shipped to Horn Point laboratories for HPLC analysis in liquid nitrogen: samples from April and May cruises arrived in good condition. Samples from the late June cruise arrived at Horn Point with no liquid nitrogen present in the shipping Dewar, although samples were cool. HPLC chromatograms of the June samples were additionally checked for signs of any degradation products that could have formed due to the improper shipping, but none were found (Crystal Thomas, Horn Point Laboratories, pers. comm.).

Some of the filters from the May cruise were reported to have some blue and black ink present on the filter, colored fibers on the edge of the filter (Perry and Sauer samples). Black ink can interfere with quantification of the chlorophyll *c*3, and sometimes it can interfere with the quantification of chlorophyll *b*. Only three samples were noticed to have unstable baseline due to the presence of the black marker (08-1864, 08-1865 and 08-1850); accurate chlorophyll *c*3 concentration couldn't be derived so the value of 0.001 was assigned to those samples. Additionally, chlorophyll *b* concentrations for these samples might be underestimates of the real

in situ concentration (0.006, 0.002 and 0.001 µg L^{-1} respectively), although the reported concentration of chlorophyll *b* throughout the cruise was low (0.030 ± 0.019 µg L^{-1}).

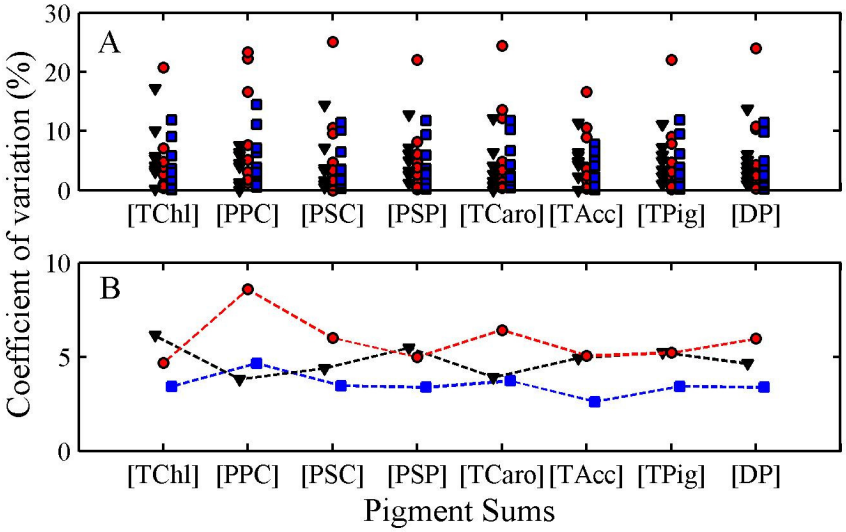

Figure 3.4. Duplicate filter precision expressed as CV (%) for pigment groups (Claustre et al. 2004); for all duplicate samples (panel A), and mean CV for each of different processing groups (panel B). Symbols present results for different groups: Perry (black triangles), Sauer (blue squares) and Westberry (red circles).

HPLC analysis performed by Horn Point laboratories followed standardized procedures in Van Heukelem and Thomas (2001), NASA technical reports (Hooker et al. 2005; Hooker et al. 2009), and Claustre et al. (2004). Pigment analysis was done using methanol-based reversed-phase gradient C8 chromatography column system. This method uses simple linear gradient and elevated column temperatures to obtain quantitative information up to 25 pigments, and offers qualitative information for additional pigments (Hooker et al., 2009). Concentration of pigments was determined using pigment-specific spectrophotometric response, taking into consideration the calibration of HPLC system and response of the internal standard and errors associated. Calibration of the HPLC system was done prior to the sample analysis using standard pigments isolated in HP (Van Heukelem and Thomas 2001). Reported accuracy of Horn Point HPLC method varies across pigment types and concentrations, with average accuracy of 10.9% for primary pigments[1] and 5.5 % for total chlorophyll *a* (see Table 15 and 16 in Hooker et al., 2009)

Analytical precision of the method was accessed by repeated measurement of single sample. Analytical precision for total chorophyll *a* ranged from 0.25 to 0.77 %, while analytical precision

[1] Primary pigments, as defined by Hooker et al. 2009 are total chlorophylls and carotenoids most commonly used in open and coastal ocean studies. Namely, these are total chlorophyll *a*, total chlorophyll *b*, total chlolorphyll *c*, carotenes, alloxanthin, 19'-butanoyloxyfucoxanthin, diatoxanthin, diadinoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin, peridinin and zeaxanthin.

for primary pigments ranged from 1.42-3.94 %. Duplicate filter precision for primary pigment and pigment sums was on average below 10% (Fig. 3.4, bottom panel), on average less than 7% for total chlorophyll *a*, although some replicates were found to have CV higher than 20% (Fig. 3.4, upper panel) probably due to the presence of large aggregates and diatom chains (heterogeneity in the water column). Average observed CV are comparable with previously published values (Claustre et al., 2004; Hooker et al., 2000).

IV. Filter Pad Absorption

A. Sample collection, filtration and storage

For each profile that was sampled, water was collected from two depths. Two 1.1-L polycarbonate bottles were filled from a single Niskin bottle from one depth and a single 1.1-L polycarbonate bottle was filled from the second depth. Samples were filtered through a 25-mm Whatman GF/F filter within 30 min of collection by inverting the entire bottles into the filter cups. Filtration was done under low vacuum (5 mm Hg). Note that the quality of GF/F filters has recently changed for the worse (blistering of filters); the filters used for these measurements were from a 5-y old, better quality lot that was found to be acceptable for the Quantitative Filter Method (Mitchell 1990).

Upon filtration, filters were placed into a Petri dish on top of moistened Kimwipes, covered with aluminum foil and kept in the dark until they were analyzed (no more than 15 min after filtration).

B. Blanks

The same volume of Milli-Q water (1.1L) was filtered through 25-nm Whatman GF/F filters for the blank; the resulting scans were applied as the blank to all the samples collected during the cruise. The stability of the blank spectrum was verified with blanks prior to and after the cruise (Fig. 4.1).

C. Filter analysis

1. Spectral scans

Filters were scanned at sea on a Varian Cary 50 UV-Visible Spectrophotometer with a xenon flash lamp and a 1.5 nm slit width. Each filter was scanned a minimum of three times at 0.5 nm s^{-1}; filters were rotated between scans to sample different areas of the filter. Filters were scanned before (a_{part}) and after the Kishino methanol treatment (a_{det}) (Fig 4.2).

2. Kishino et al. (1985) methanol extraction method for determining a_{det}

The filter was placed on a filter rack under a hood. Hot 100% methanol was carefully added to the filter and vacuum applied for a few seconds. About 10 mL of hot methanol was added to each filter cup, refreshing methanol as needed for a period of ~15 min. The filter was then rinsed with filtered seawater, including the flanges, to remove all residual methanol. Filters were rescanned three times as described above.

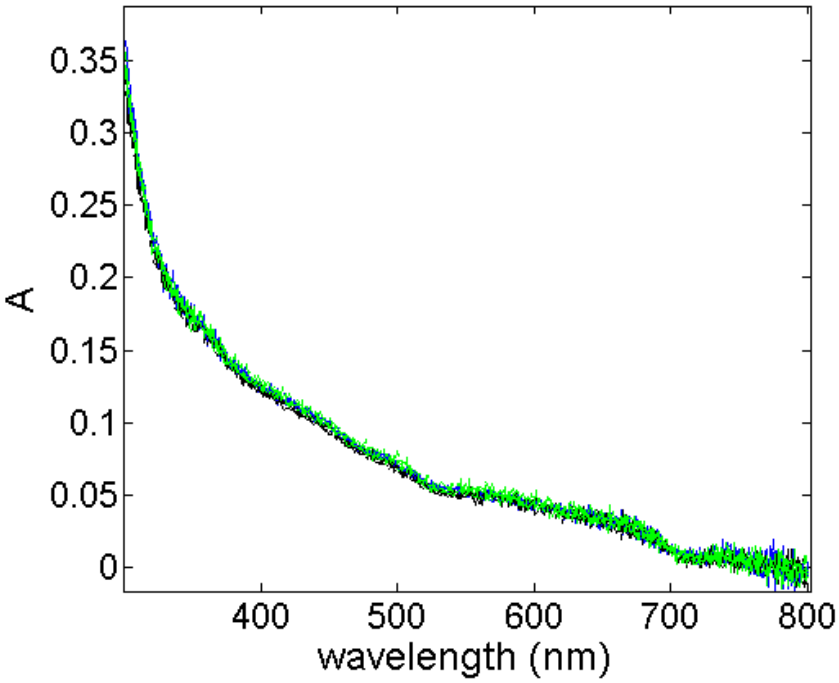

Figure 4.1. Milli-Q water blanks. Uncorrected scans from the cruise (green) and scans from after the cruise (blue). Filters treated with methanol are shown in black.

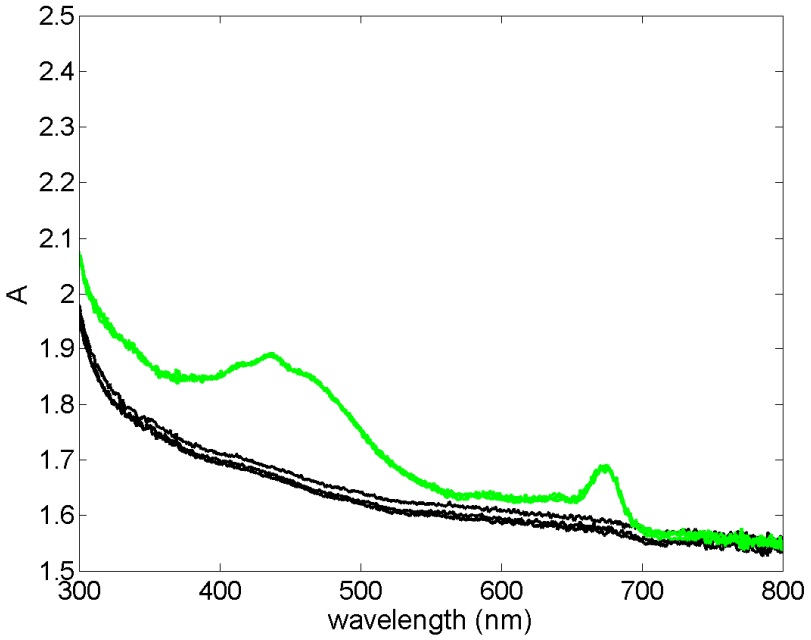

Figure 4.2. Uncorrected absorbance for Station 141a from 20 m; triplicate spectra for total particulate absorbance (green) and particulate detrital (black) absorbance.

D. Data processing

The standard assumption is that particulate absorption above 750 nm is negligible, and that any measured absorbance in that portion of the spectra is due to the scattering (Babin and Stramski, 2002, and references within). An averaged baseline (750 – 800 nm) was subtracted from all spectra (300 – 750 nm) for all samples and blanks (Fig 4.3). The baseline corrected blank (filter through which Milli-Q was filtered) was subtracted from the samples (Fig 4.4). A 10-point mean smoothing filter was applied to the raw data before the baseline was calculated.

The figures (Figs. 4.1-4.6) show the sequence in processing, from uncorrected absorbance spectra to fully correction absorption coefficients. Note that Y-axis scale changes among figures; units for the Y axis are either absorbance (A, unitless) or absorption coefficient (a, m^{-1}). Absorption coefficients, $a(\lambda)$, were calculated as follows:

$$a(\lambda) = 2.303 \frac{A(\lambda)}{\beta L}$$

where L is the geometric pathlength (volume filtered/filter area) and optical pathlength correction factor β= 2 (Roesler, 1998).

Phytoplankton absorption, a_{phyt}, was calculated as:

$$a_{phyt}(\lambda) = a_{part}(\lambda) - a_{det}(\lambda).$$

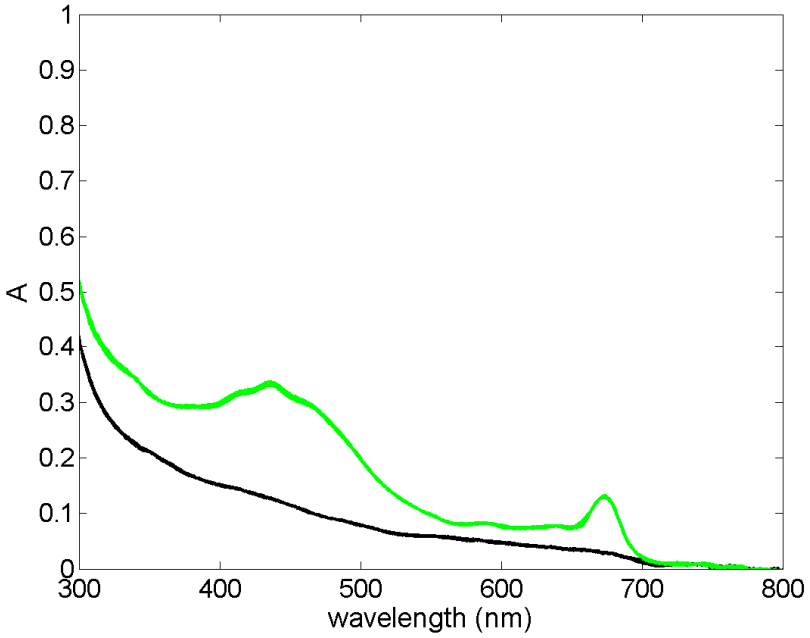

Figure 4.3. Baseline corrected absorbance for Station 141a from 20 m; triplicate spectra (green) for total particulate absorbance and particulate detrital (black) absorbance.

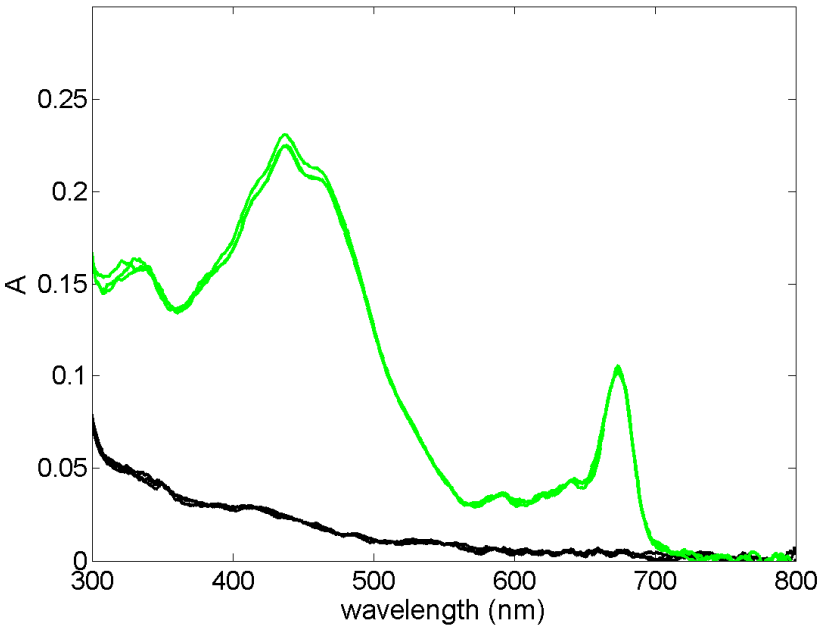

Figure 4.4. Milli-Q water blank and baseline corrected absorbance for Station 141a from 20 m; triplicate spectra (green) for total particulate absorbance and particulate detrital (black) absorbance.

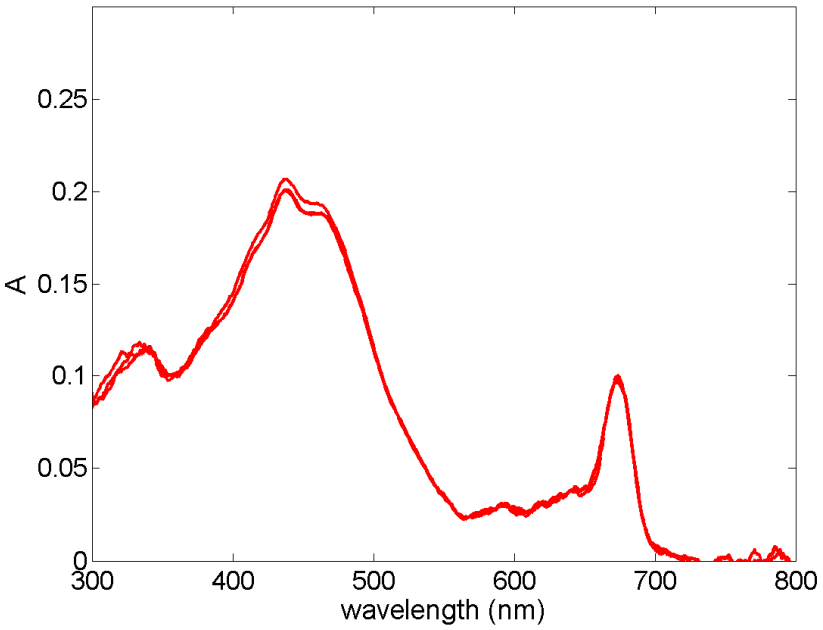

Figure 4.5. Phytoplankton absorbance for Station 141a from 20 m was calculated as the difference spectrum between total particulate absorbance spectrum and the mean detrital absorbance spectrum.

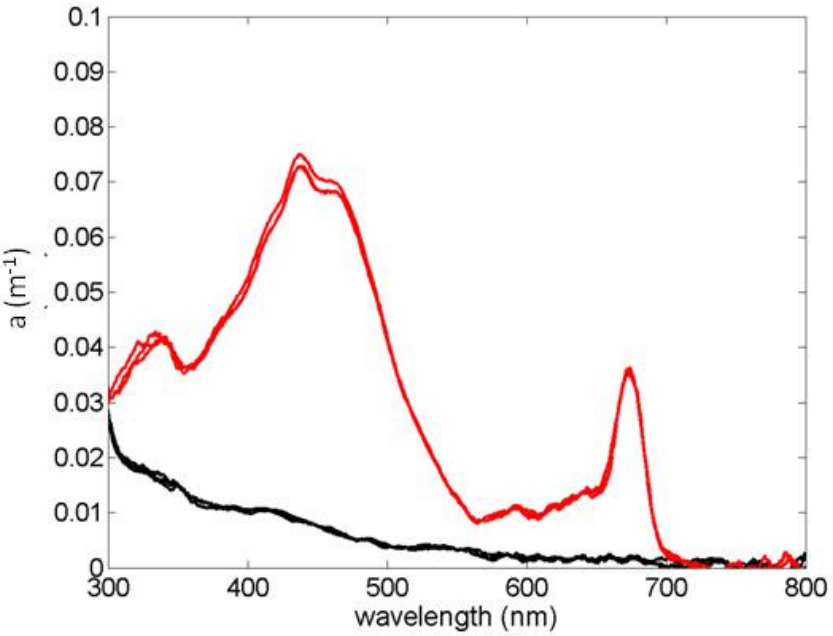

Figure 4.6. Phytoplankton absorption (red) and detrital absorption (black) coefficients for Station 141a from 20 m calculated according to Roesler (1998) using β = 2.

E. Error Analysis

1. Intra-filter variability

A minimum of three replicate scans was done for each filter pad. Intra-filter variability changed with time, as a function of phytoplankton cell size (Fig 4. 7). Early in May, during diatom domination, variability was high (Fig. 4.8), but during picoeukaryotic phytoplankton domination, in late May, inter-filter variability was low (e.g., Fig. 4.3). The high abundances of diatom chains caused non-homogeneous distribution of particulate absorbing material on the filter pad, which was reflected in high variability among triplicate scans of a single filter. Variability was larger at shorter wavelengths, even for absorbances of the same magnitude.

The CV of a_{phyt} (330, 440, 676 nm), calculated to assess the error associated with the non-homogeneous distribution of particles, can be regarded as a measure of inter-filter precision (Table 4.1). The peak at 330 nm is associated with absorption of mycosporin-like amino acids (MAA), when present; 440 nm is associated with chlorophyll *a* and phytoplankton accessory pigments; and 676 nm is associated primarily with chlorophyll *a* absorption.

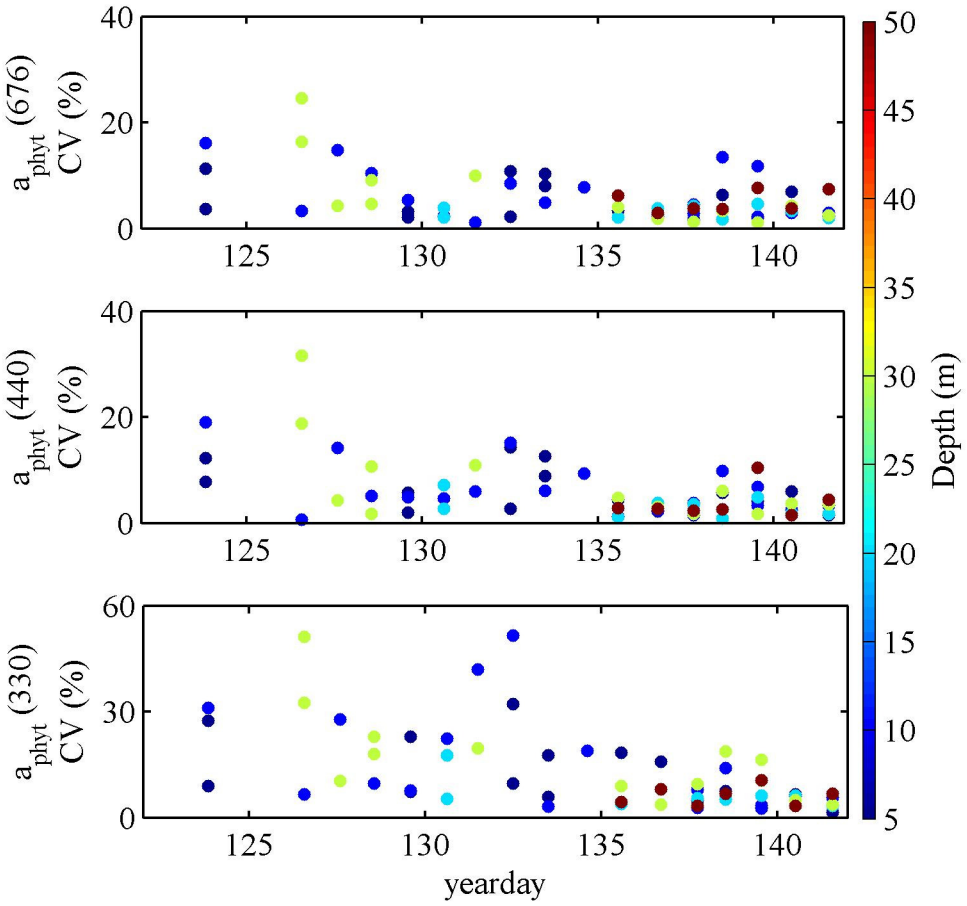

Figure 4.7. Coefficient of variation (%) for replicate scans of the same filter pad for all samples collected during the May 2008 cruise. Note the decrease in CV after yearday 134. The color bar is the same for all plots; Y-axis scales vary.

Table 4.1 Intra-filter variability and precision estimates for a_{phyt}.

Wavelength (nm)	a_{phyt} (m^{-1}) range	CV (%) range	CV (%) mean/median
330	0.0005 - 0.3432	5.4 - 51.2	18.9/17.7
440	0.0042 - 0.0867	1.7 - 31.2	9.5/7.7
676	0.0045 - 0.0506	1.3 - 23.5	7.4/6.2

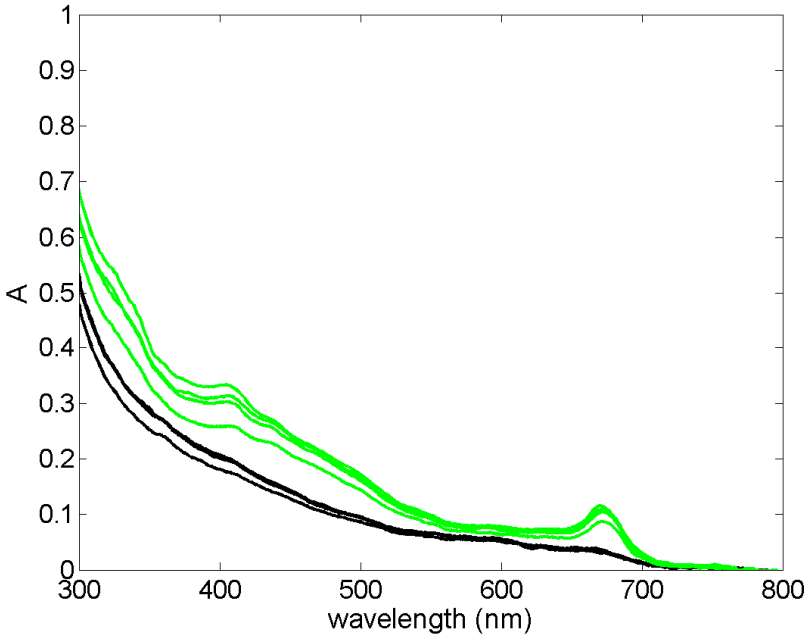

Figure 4.8. Baseline corrected absorbance for Station 1c at10 m during the diatom bloom; triplicate spectra (green) for total particulate absorbance and particulate detrital (black) absorbance.

2. Inter-filter variability

At eight stations, single depths, two separate replicate water samples were taken from the same Niskin bottle. These eight duplicate samples were taken in the first half of the cruise (before year day 140). Each of the replicate samples had minimum of four scans. At some stations, there was little difference between absorption coefficients measured on the two filters (Fig 4.9, station 63.02). For other stations, especially the ones with high absorption, both inter- and intra-filter variability was high, most likely due to the presence of diatom chains (e.g. Fig 4.9, station 9.03). Inter-filter a_{phyt} (330, 440, 676 nm) variability is reported in Table 4.2. The final number reported in the bottle file is the median of all scans from both of the filters, shown in Figure 4.9 as a black line.

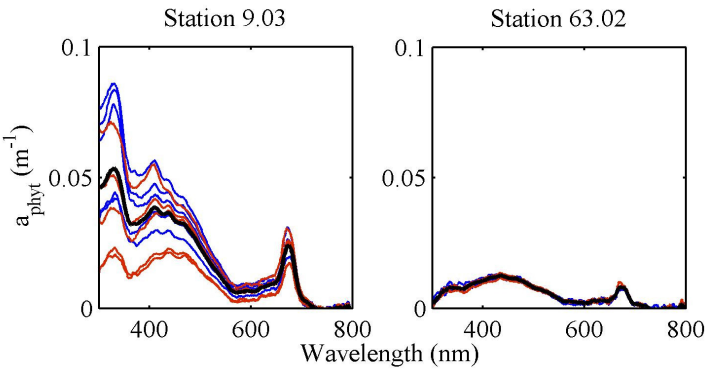

Figure 4.9. Replicate phytoplankton absorption measurements; quadruple scans of the first filter (first sample) are shown in red, and scans of the second filter in blue. The black line represents the median phytoplankton absorption calculated using all scans from both of the filters.

Table 4.2 Inter-filter variability and precision estimates for a_{phyt}.

Wavelenght (nm)	a_{phyt} (m^{-1}) range	CV (%) range	CV (%) mean/median
330	0.0005 - 0.3432	12.2 - 45.4	24.2/20.5
440	0.0042 - 0.0867	5.4 - 25.1	10.4/8.7
676	0.0045 - 0.0506	3.1 - 18.8	8.3/7.8

F. Mycosporine-like amino acid (MAA) absorption

MAA specific absorption peak was present in some of the a_{phyt} samples collected during R/V Knorr KN193-03 cruise, with variable peak height and wavelength (Fig. 4.10). For the samples with $a_{phyt}(676)$ higher than 0.02 m^{-1}, we defined MAA peak to be present if the ratio of a_{phyt}(MAA peak λ) and $a_{phyt}(676)$ was higher than 1.5 (samples with $a_{phyt}(676) < 0.02$ m^{-1} were not part of MAA analysis).

MAA peak wavelength shifted during the cruise, from 332 nm to 312 nm. With the decrease in peak wavelength, MAA absorption coefficients (peak height) increased (Fig 4.11). The highest MAA absorption peaks were also found concurrent with highest concentrations of chlorophyll *a*.

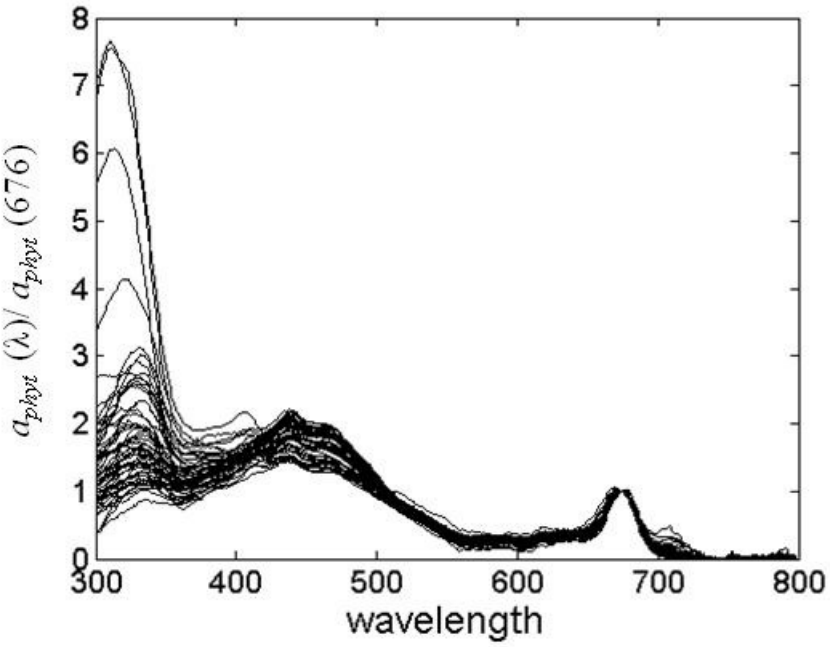

Figure 4.10. Phytoplankton absorption coefficients, normalized to $a_{phyt}(676)$, for all data collected during May 2008. Note the variability in 300-350 nm region, associated with MAA absorption.

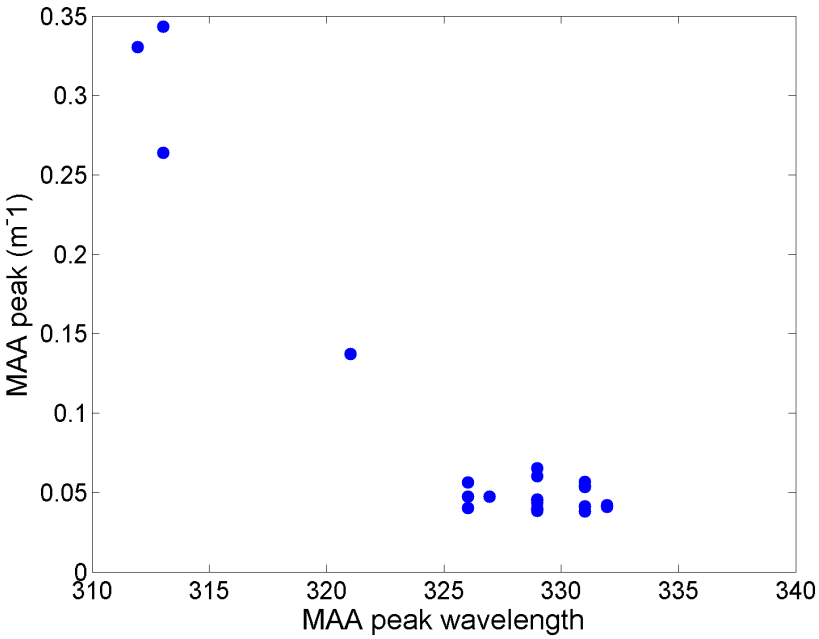

Figure 4.11. Relationship between peak MAA absorption wavelength and absorption coefficient for the peak wavelength.

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