# The sampling error of organic carbon field measurements in Lake Superior.

A THESIS

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# Dedication

*It is imperfect.*
*So are all things trapped in time.*
Lois McMaster Bujold

To my husband and sister.

## Abstract:

In June 2013, a test of sample processing steps was undertaken for limnological/oceanographic sampling of total organic carbon (TOC), particulate organic carbon (POC), dissolved organic carbon (DOC), and colored dissolved organic matter (CDOM). This was to determine the magnitude and sources of errors in the sampling of these organic carbon field measurements. Replicate Niskin rosette casts in Lake Superior (with a difference of 0.79 km in site location and 1 hour 6 min in sampling time) were found to be significantly different at both $\alpha$= 0.05 and 0.10 for DOC, TOC, e2/e3, and CDOM, but not POC. The TOC, DOC and POC average concentration and the standard deviation of all replicates of the two casts was 2.3 +/- 0.2, 2.3 +/- 0.3, and 0.14 +/- 0.02 mg/L respectively. The variation due to sample handling within one cast for DOC and TOC is 0.1 mg/L or 5% relative standard deviation. For POC, the variation is 0.02 mg/L or 14%. Use of different Niskins from the same cast did not cause significant effects.

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## Introduction

Organic matter suspended or dissolved in water is a complex mix of microscopic organisms, detritus, and chemical compounds that flow in from rivers and precipitation (allochthonous) or is produced by algae and other organisms within the lake (autochthonous). Allochthonous organic matter is generally considered to consist mostly of biologically refractory molecules from the biological decay of plant matter called humic substances. Autochthonous organic matter can be any organic compounds that are dissolved into the water by living, feeding, dying, and decaying processes of algae, zooplankton, or other organisms in Lake Superior.

Due in part to its heterogeneity in aquatic systems, natural organic matter is defined not by specific chemical composition, but by its separation and/or detection method. Particulate organic matter (POM) that can be removed by filtration by a filter with pore sizes between 0.1-1.0 um is called particulate organic carbon (POC) because it is usually measured by elemental analysis. While this choice of filter traps most living organisms (except viruses and smaller bacteria) and allows most macromolecules to pass through, trying to define a large spectrum of substances into two clear categories is a simplification. The dissolved organic matter (DOM) that is not removed by a filter is defined by the method of detection. Colored dissolved organic matter (CDOM) is detected by UV-Visible absorption analysis. Dissolved organic carbon (DOC) is measured as the carbon released by high temperature catalytic oxidation (this study) or wet

chemical oxidation (e.g., Minor and Stephens, 2008). Purgeable organic carbon consists of dissolved hydrocarbon gases, small esters and alcohols, halocarbons, and other volatile species removed by sparging the water sample with carbon free air. Non-purgeable organic carbon remains in solution.

Recognizing organic matter sources and predicting their reactivity is made more complex by degradation processes. Organic molecules are both biologically degraded (e.g., by metabolism by bacteria), as well as photochemically degraded by sunlight. Humic substances often have aromatic components that absorb light in the UV-vis spectrum. A change in absorbance can indicate a change in the type of dissolved organic matter, terrestrial or aquatic, or a change due to photochemical bleaching. The e2/e3 ratio is the ratio of the absorbance coefficients at 250 nm and 365 nm. An increase in this ratio correlates to a decrease in aromaticity and molecular size (Peuravouri and Pihlaja 1997). Identifying the specific changes due to each of these processes is an area of recent research (Minor et al., 2007, Minor and Stephens 2008, Mopper et al., 1991).

CDOM, by definition, absorbs light in the UV-vis spectrum. It is capable of absorbing both ultraviolet light (e.g., Dalzell et al., 2009) and Photosynthetically Active Radiation (PAR) and can reduce the amount of PAR available for algae growth. Minor et al.(2014) measured an increase in the concentration of PAR-absorbing CDOM in the surface waters of Lake Superior after a 500 year flood; this CDOM was able to suppress algae growth despite a concurrent flood-caused increase in the lake's concentration of growth limiting nutrients. The sampling

and measurements during the post-flood sampling (Minor et al. 2014) of the western arm of Lake Superior inspired this study. Organic carbon measurements were taken weekly to observe changes, but as is typical for limnological studies, only one container of water for each measurement was sampled at each site. Since there have been few if any replicate samples, it is difficult to be certain if variation is due to biogeochemical trends over space and time, or to sampling error. While much effort has been expended on understanding the precision and accuracy of high-temperature combustion TOC analyses and elemental analysis of POC, there is little information currently available on how the sampling process itself imparts variability into the measurements.

Consider the process of collecting an eight liter water sample at five meters depth. Deploying the CTD (*Figure 1*), lowering it down through the water column, and trapping water at a specific depth on the return to the surface is called a cast. Depending on the depth of the water, the cast may take several minutes to several hours. There is variation in space and time that could change the composition of that water sample. Lake Superior shows summer stratification from late June or early July to December. A deep chlorophyll max develops with stratification just below the thermocline. There is a low level of chlorophyll deep in the lake because of the loss of photosynthetically active radiation (PAR) with depth. Because of the high amount of PAR at the surface, high chlorophyll concentrations would be expected, however, too much radiation can cause bleaching of the chlorophyll so the surface has lower concentration than at

slightly greater depths.  Even small changes in depth can mean large changes in chlorophyll and CDOM if the sample trapped is near the thermocline.

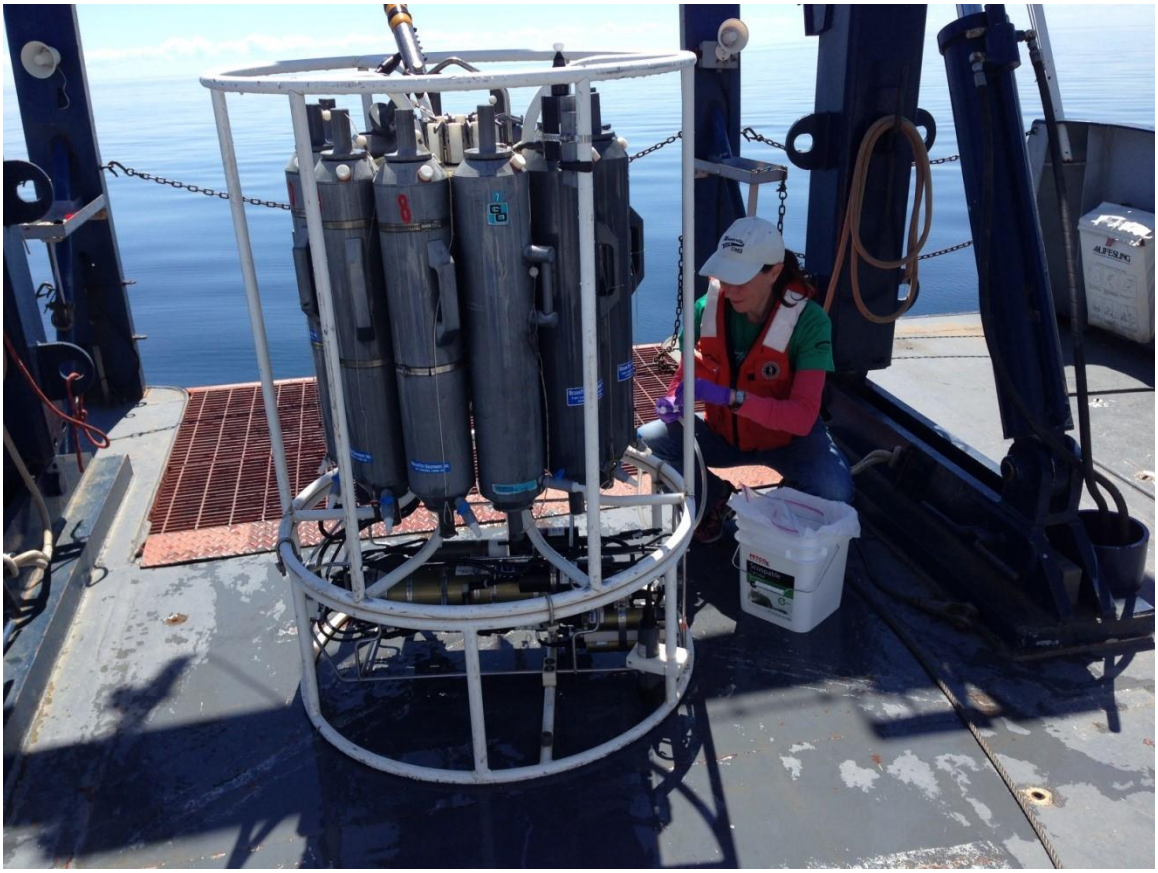


*Figure 1. A CTD (Conductivity-Temperature-Depth) Sensor with 12 Niskin bottles (8 L each) on a rosette was used to record the water column profile and collect water at a depth of 5 meters.  The CTD measures chlorophyll fluorescence and colored dissolved organic matter (CDOM); also, transmittance, pH, conductivity, temperature, and dissolved oxygen in real time as it is lowered through the water column.*

Plumes of sediment and nutrient rich water from river outlets can have sharp outlines.  Sampling near the edge of a plume means that a small change in boat position can cause a large change in water composition.  A plume of muddy

water from the south shore of Wisconsin in July 2014 had a depth component as well. Two meters of warm muddy water was floating on top of the colder clear lake water. Sampling at five meters gives a completely different result than sampling at one meter.

Over time, wind and currents mean that one site on the lake located precisely by GPS has different water composition as time passes. Even with still water the photosynthetic activity and movement of organisms over the diel cycle changes the organic carbon composition of the water over time and space. Even if the water is not changing under the boat, the boat often drifts from the precise site due to currents and wind during a cast and between two casts at the same site.

Not only does the water composition from cast to cast affect the composition and amount of organic carbon, the sampling and filtering procedure provide variation as well. The 12 Niskin bottles are fired sequentially when the target depth is reached. There are at least 12 seconds between the first bottle firing and the last. This is a time difference that lake currents could cause to be significant. The CTD causes a small upward current around itself as it is pulled to the surface. As the CTD stops at the target depth, could the first Niskin bottle fired be different from the last because of this current? Four different 25 L steel pressurized beverage canisters were used to store and pressurize water samples for filtration. Contamination of the canisters and failing to shake the canisters to resuspend particulates before filtration can cause variation. The two different

sets of tubing and stainless steel filter rigs could also have contamination, leakage or other variation.

While all of these factors may cause variation, is it a variation that is significant? Since many of these limnological measurements require large amounts of water, it is difficult to take replicate samples. Knowing the sampling variation would help limnologists collect single samples more knowingly, aware of the possible variation.

Measurements in Lake Superior of DOC vary from 1.0 to 6.0 mg/L, POC from 0.03 to 0.2 mg/L according to Zigah et al. (2011) and Peterson et al. (supporting data, 2012). With such low expected concentrations, the measurement requires careful blanks and clean methods. The high temperature oxidation method for TOC/DOC is used widely now, but was subject to much debate and required a community effort to clarify the blank issue (Sharp, 1995). Further analysis of accuracy of high temperature combustion is found in (Peltzer et al., 1996), (Sharp et al., 2002).

In this study, TOC (from unfiltered samples) and DOC (from filtered samples) was measured by a high temperature combustion process. In this process, the water sample is acidified and sparged with $CO_2$ free air to remove inorganic carbon and other purgeable organic carbon gases. The sample is then injected into a high temperature combustion column with catalytic beads where all carbon is combusted into carbon dioxide gas. A carrier gas stream of $CO_2$ free air carries the combustion gases past an IR source and detector which

measures the absorbance of the carbon dioxide as an integrable peak. A calibration curve is used to correlate peak area with carbon concentration.

POC (the material trapped onto a glass fiber filter) is separated from the other elements and quantified by an elemental analyzer. The filters are combusted in a catalytic furnace sealed to the atmosphere. The analyte gases flow through a gas chromatography column. The analytes from the elemental analysis are measured by an isotope ratio mass spectrometer (IRMS). The filters holding the POC must go through several processing steps before entering the elemental analyzer; these can introduce contamination and variation. Before analysis, the filters are ground up, measured into silver boats, acidified to remove inorganic carbon, allowed to release the HCl gases then sealed in tin boats to better regulate the temperature of combustion.

A spectrophotometer measures the absorbance of UV and visible light by the samples. A light source that produces UV and visible light shines on a diffraction grating which separates the wavelengths of light. Only the small bandwidth that passes through a slit passes through the sample. A motor on the diffraction grating changes the wavelength of the light that passes through the slit, stepping through the UV visible spectrum over time. The monochromatic light passes through a reference of a cuvette with DI water. Then the light is passed through a cuvette with the sample water. The intensity of the transmitted light at each wavelength step is measured. The light that is transmitted through the sample and reference is detected by a photodiode and the ratio of the intensities of the sample and the reference is the transmittance of the sample.

The absorbance at each wavelength is calculated as the negative log of the transmittance.

In summary, the goal of this thesis is to understand and attempt to quantify the limitation of sampling methods for water-column organic carbon.

# Methods

## Sites

The four sampling sites were the Duluth Entry (DE, 3.5 km from the outlet of the Aerial Lift Bridge), the Wisconsin Entry (WE, 2.3 km from the outlet of the Superior Pier), Off Shore (OS, 9 km from Park Point), and a far off shore site in the middle of the western arm called M1 (see Table 1 and Figure 2 ).

To determine the range of error introduced by the sampling and filtering procedure, a cruise on the anniversary of the Duluth-Superior area 2012 flood, June 21, 2013, collected the same suite of samples as the flood cruises. The same sampling and processing procedure was used as in 2012, however, at site OS multiple replicates were sampled for each step in the sampling and filtering process. Two replicates of the sampling site were taken to test for variation in sampling location and time between samples. The boat is positioned for sampling by GPS, but wind and currents can push the boat off-station during sampling. A drift of 0.42 km is common; the boat is usually repositioned over this threshold. Two different casts at Site OS were taken, but Cast 1 was 0.8 km northeast and 1 hour and 6 minutes earlier than Cast 2, see Figure 2.

## Samples

Replicates were sampled to test other sampling variables as well. See Figure 3 and Figure 4. For example: One canister was filled with water from the CTD taken from the first two Niskin bottles deployed (Niskin 1 and 2, but called Niskin 1), another canister was filled from the last two Niskins deployed (11 and 12, but called Niskin 12). The two different canisters were filtered using two different filter-rigs. The sample collection at OS Cast 1 and Cast 2 is shown in Figure 3 and Figure 4 respectively.

## CTD

At each site, a Seabird model 911 plus CTD (Conductivity,Temperature, and Depth) Sensor with 12 Niskin bottles (8 L each) on a rosette was used to record the water column profile and collect water at a depth of 5 meters. The CTD's multiple sensors profile chlorophyll fluorescence and colored dissolved organic matter (CDOM) fluorescence, transmittance, pH, conductivity, temperature, and dissolved oxygen of the water column.

Table 1. Flood Response Site Locations. All samples were collected at a depth of 5 meters unless specified otherwise.

| Site name | site initials | Water column Depth (m) | Latitude | Longitude |
|---|---|---|---|---|
| Duluth Entry | DE | 22 | 46 $^{o}$ 47.0 ' | 92 $^{o}$ 2.9 ' |
| Wisconsin Entry | WE | 20 | 46 $^{o}$ 43.3 ' | 91 $^{o}$ 59.8 ' |
| Off shore | OS | 30 | 46 $^{o}$ 47.3 ' | 91 $^{o}$ 57.8 ' |
| Middle western arm | M1 | 132 | 46 $^{o}$ 55.6 ' | 91 $^{o}$ 27.9 ' |


*Figure 2. Flood anniversary sampling sites on June 21, 2013. Note the time and space between cast 1 and cast 2.*

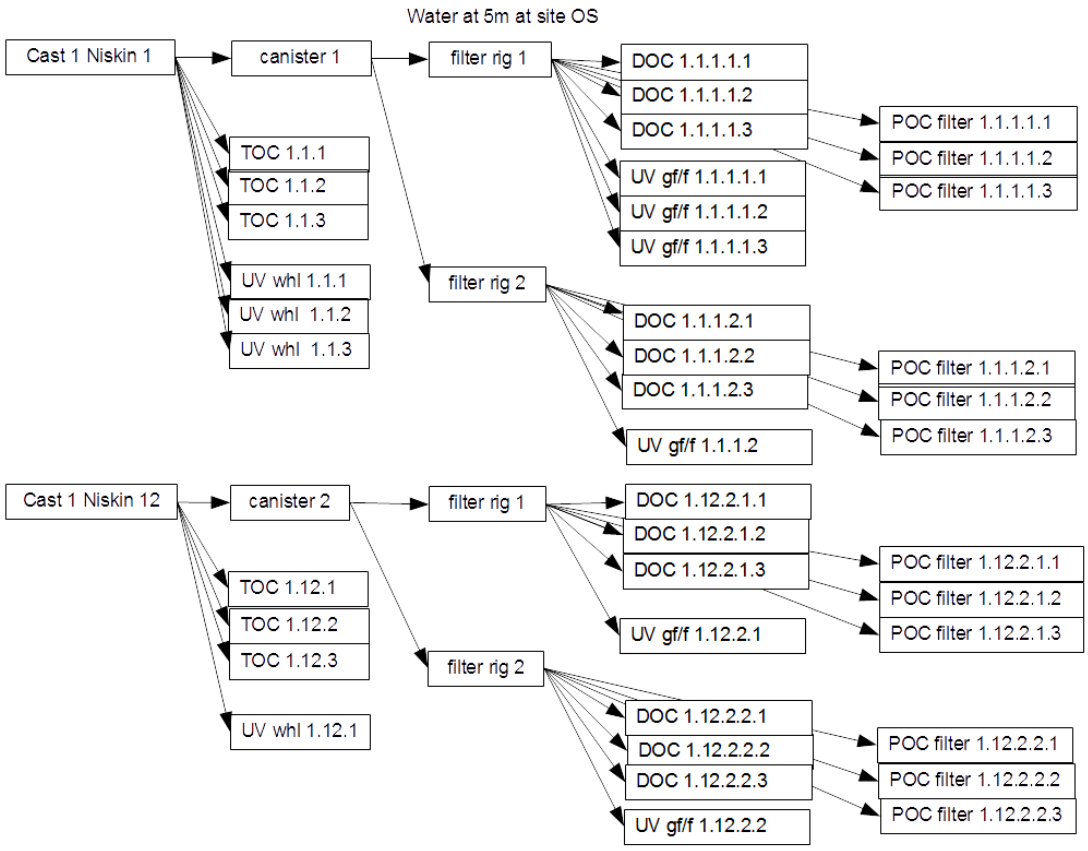



*Figure 3. Propagation of error sampling summary at the OS site, CTD cast number 1 on June 21, 2013. The replicate numbers are in the format: cast.Niskin.replicate for whole water and cast.Niskin.canister.filter.replicate for filtered water. A whole water sample for UV-vis analysis is indicated by "UV whl". A GF/F filtered water sample for UV-vis analysis is "UV gf/f".*

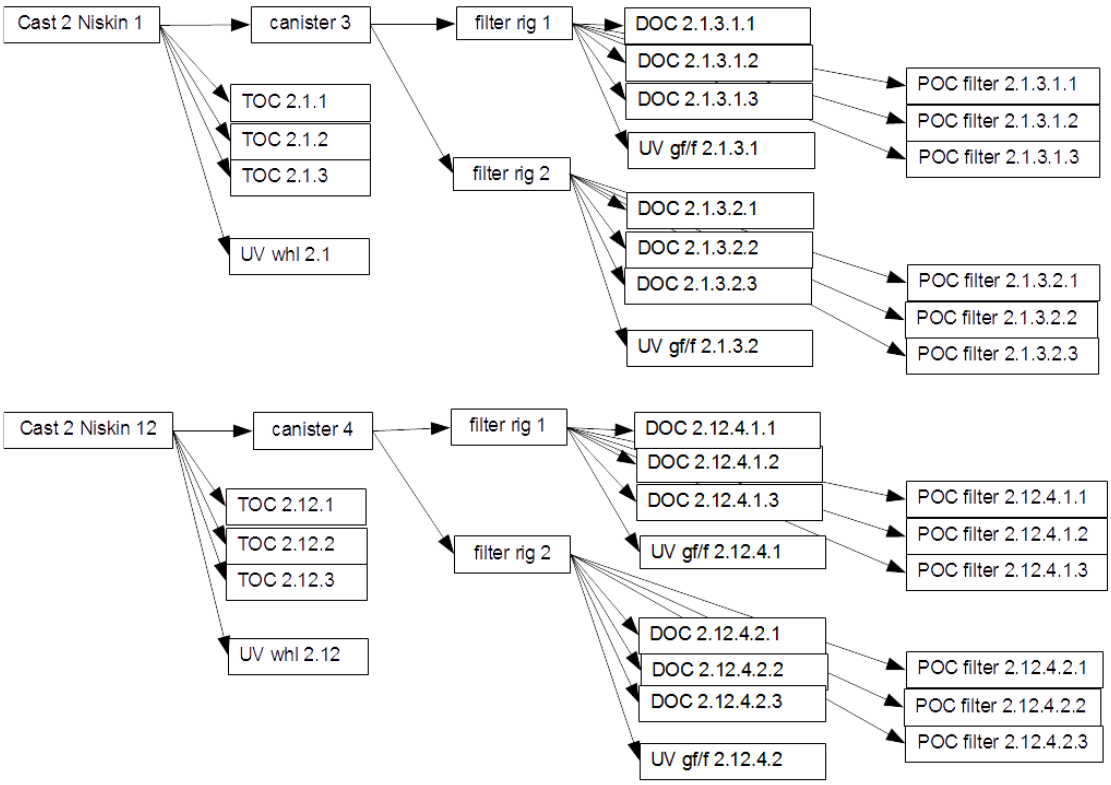



*Figure 4. Propagation of error sampling summary at the OS site, CTD cast number 2 on June 21, 2013. The replicate numbers are in the format: cast.Niskin.replicate for whole water and cast.Niskin.canister.filter.replicate for filtered water. A whole water sample for UV-vis analysis is indicated by "UV whl". A GF/F filtered water sample for UV-vis analysis is "UV gf/f".*

## Sample collection, filtering, and preservation

Water samples were collected whole or placed in a stainless steel canister for filtering. Silicone tubing (acid washed and rinsed with deionized water prior to each cruise) was used to transfer all sample from the Niskin bottles. Whole water samples were collected in 40 mL amber glass vials sealed with silicone septa for measurement of total organic carbon (TOC) and UV-vis analysis. Prior to sample collection these vials were acid-cleaned (10% HCl), rinsed with deionized (DI) water, combusted for at least 4 hours at 450°C, and thrice rinsed with sample. To filter the water, 25 L steel pressurized beverage canisters were rinsed with sample water three times, then filled. A nitrogen tank was used to pressurize the canisters. A stainless steel filter rig held a combusted glass fiber filter (GF/F, nominally 0.7 um). The filter rig was connected to the canister with reinforced PVC tubing and a Ball Lock connector. The filter rig (without filter) and connective tubing was rinsed with sample water under pressure for 10 seconds, before installing the glass fiber filter. The sample water was forced through the tubing and the filter.

Filtered samples were collected in 40 mL amber glass vials sealed with silicone septa for measurement of dissolved organic carbon (DOC) and UV-vis analysis. These vials were acid-cleaned, DI-water rinsed, combusted, and rinsed with sample three times prior to the sample collection.

All UV-vis samples were refrigerated within 1 hour of collection. The glass fiber filters, removed with forceps, were folded in half, wrapped in combusted aluminum foil, and (within 1 hour) were frozen in plastic bags for later

measurement of particulate organic carbon (POC). All DOC and TOC samples were acidified with 40 uL of 6N HCl added to each vial to bring the pH to between 2.0 and 2.5 within 3 hours of collection. This acidification served two purposes: to preserve the samples by inhibiting biological processes and to get rid of inorganic carbon.

After each cruise, the beverage canisters, the filter rig, and tubing were rinsed with DI water three times and allowed to air dry. The silicone tubing was rinsed with DI water, soaked in 10% HCl, and rinsed again with DI water.

On May 5, 2012 and August 5, 2014, milliQ water was run through the canister and filter-rig to obtain collection-method-blanks. The results are shown in Table 2.

*Table 2. Filtering method blanks results. MilliQ water through the canister and filter rig.*

| MilliQ water sample date | Average C concentration mg/L | Standard Deviation mg/L | Number of replicate vials |
|---|---|---|---|
| May 5, 2012 | 0.13 | 0.16 | 3 |
| August 5, 2014 | -0.10 | 0.02 | 6 |

## Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC)

The TOC and DOC samples were measured by a Shimadzu TOC-Vcsh analyzer. The TOC analyzer uses catalytic oxidation at 680$^{o}$C and measurement of $CO_2$ gas by peak area of IR absorption, similar to (Peltzer and Hayward, 1995). TOC and DOC samples were randomized and analyzed with at least a

six-point calibration curve and five known check standards, and nine blanks throughout the sample run. Potassium hydrogen phthalate was massed and dissolved in MilliQ water (from a MilliQ system (18.0 M W or better) and acidified like the samples to create the calibration curve and check standards. Blanks were acidified MilliQ water. At least 7 blanks were run before the calibration curve in order to condition the catalyst by removing residual carbon on the catalyst (Sharp et al., 1994). Average concentrations were calculated from 3-5 replicate measurements of each standard, sample, and blank. Six "sample storage blanks" were collected the same day as sampling to determine storage contamination and changes. Milli-Q water was placed in 40 mL sample vials and stored in the same bag as the samples.

**Possible errors and attempted corrections**

Several possible errors occurred in the measurement of TOC and DOC. First, a Shimadzu service representative suggested waiting at least an hour after starting the TOC-Vcsh to allow for stabilization of the combustion tube and IR lamp. The wait time was not recorded and may have been less than this recommended time. Second, the calibration curve was analyzed in order from lowest to highest concentration. Randomizing the calibration curve order is recommended to reduce the effects of possible instrument drift on the calibration curve.

Third, and most importantly, the KHP sample was not dried in an oven before use, nor kept in a dessicator, nor kept in an air tight bottle. Water absorption by the KHP was significant. All calibration curve and check standards

had actual carbon concentrations significantly lower than expected measurements and are not valid. Two vials of Deep Sea Consensus Reference Material (CRM) (Hansell Lab, Miami Florida batch 11 lot 03-11 Florida Straight at 700 m, concentration 0.49-0.51 mgC/L) were analyzed with samples on January 29, 2014. The Deep Sea CRM was measured with the invalid calibration curve as 1.7+/-0.5 mg/L. More recent TOC runs using KHP from an air-tight bottle have given reasonable results for the Deep Sea CRM. Major points in dealing with this error are enumerated below:

1. The KHP solid was not dry. How much did this affect the concentration?

The Deep Sea CRM measured 3 times higher than expected.

2. Is there a constant amount of water added? Can a constant be subtracted from each concentration measurement to correct for the water?

No. The amount of water error decreases at lower concentrations. See Figure 5. Two calibration curves were compared. See Table 3 for more information about the TOC runs referenced below. The not-dried KHP calibration curve (run on 7/29/15) and the dry KHP (run on 08/06/15) have a difference in slope that shows the linearity of the KHP water absorption error with concentration. The error, or the difference between the assumed (not-dried) concentration and the actual (dry) concentration is not constant and is much larger as the concentration of the standard increases. The linear decrease in water absorption error at lower concentration makes sense since the concentration of the original solution was lower than expected and as it was diluted into the calibration samples, the lower concentrations would have a

smaller concentration difference from the expected value than the higher concentration samples.

3. Can a recent calibration curve using dry KHP be applied to an older TOC run without excessive error?

No. The difference of concentration measurements of identical sample vials measured almost two months apart is over 25%. These residuals are shown in Figure 6. Sample peaks measured one year ago would be even less reliable because of the maintenance done on the TOC analyzer between the runs.

4. What trustworthy data can be used to determine the slope of the correction?

The blanks and the Deep Sea CRM are unaffected by the KHP moisture problem.

5. Does a 2 point calibration curve made of the blanks and the Deep Sea CRM approximate an air-tight KHP 5-point calibration curve? If yes, we will call this 2-point curve the Deep Sea Blank correction or DSB correction.

Yes. To less than 16% relative percent deviation. The slopes of the measured concentrations of the KHP standards and the DSB correction line are similar (Figure 7). The difference between the measured concentrations of the KHP standards and the concentration calculated by the DSB correction line for that same peak area are shown in Figure 8 . Notice that the residuals reach a maximum of almost 0.4 mg/L and do become greater with greater concentration but they are not much greater than the random variation of about 0.3 mg/L.

6. Only one (1/29) of the three Flood anniversary runs had Deep Sea CRM vials in it. However, all three used the same calibration curve vials. Can we assume all three calibration curves are similar enough to use the same DSB curve to calculate concentrations of all three runs.

See in the appendix comparing the peak areas of all three runs for the same calibration standard vials to see reproducibility the peak area. Comparing the calculated concentrations of the standards from the 01/27 and 01/29 run shows a percent average deviation range of 0.1% at 1.3 mg/L to 3.3% at 0.5 mg/L. The deviation is greater for 01/22, however, only M1 samples from this run were used in this study. M1 has a concentration of around 1 mg/L. At concentrations near 1 mg/L (0.6 to1.5) the percent average deviation from 01/29 standards is 7% to 13%. The same DSB curve was used for all three runs.

*Table 3. Relevant TOC analyses for correcting KHP calibration curve.*

| Analysis date | Samples | Deep Sea Std | Calibration curve | Concerns |
|---|---|---|---|---|
| 01/22/14 | Flood anniversary Only M1 samples | none | 01/22/14 | NOT air-tight KHP beaker |
| 01/27/14 | Flood anniversary ½ of all OS, DE, WE samples | none | 01/22/14 | NOT air-tight KHP beaker |
| 01/29/14 | Flood anniversary ½ of all OS, DE, WE samples | 2 vials | 01/22/14 | NOT air-tight KHP beaker |
| 06/10/14 | LCCMR1 | 1 vial | 06/09/14 | NOT air-tight KHP beaker |
| 07/29/14 | LCCMR3 | 1 vial | 07/29/14 | NOT air-tight KHP beaker |
| 08/06/14 | LCCMR1 | 1 vial | 08/05/14 | Air-tight KHP bottle. |
| 08/07/14 | LCCMR3 | none | 08/05/14 | Air-tight KHP bottle. |

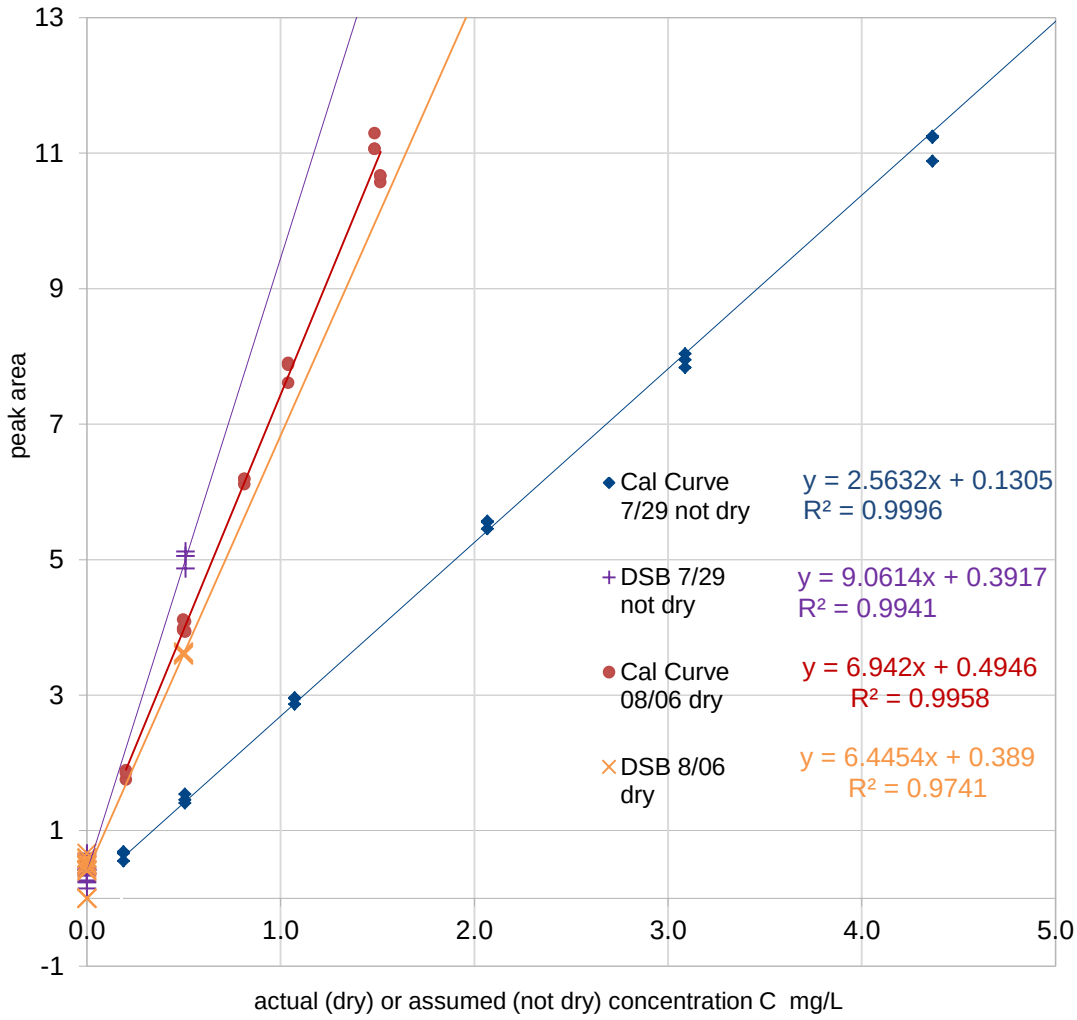



*Figure 5. Comparison of calibration curves from not-dried KHP (run on 7/29/15) to dry KHP (run on 08/06/15). The difference in slope between these two lines shows the linearity of the KHP water absorption error with concentration. The error, or the difference between the assumed (not-dried) concentration and the actual (dry) concentration is not constant and is much larger as the concentration of the standard increases. Trendlines for the Deep Sea CRM and blanks for both runs are also shown as "DSB". Notice that these are both similar to the dry KHP calibration curve.*

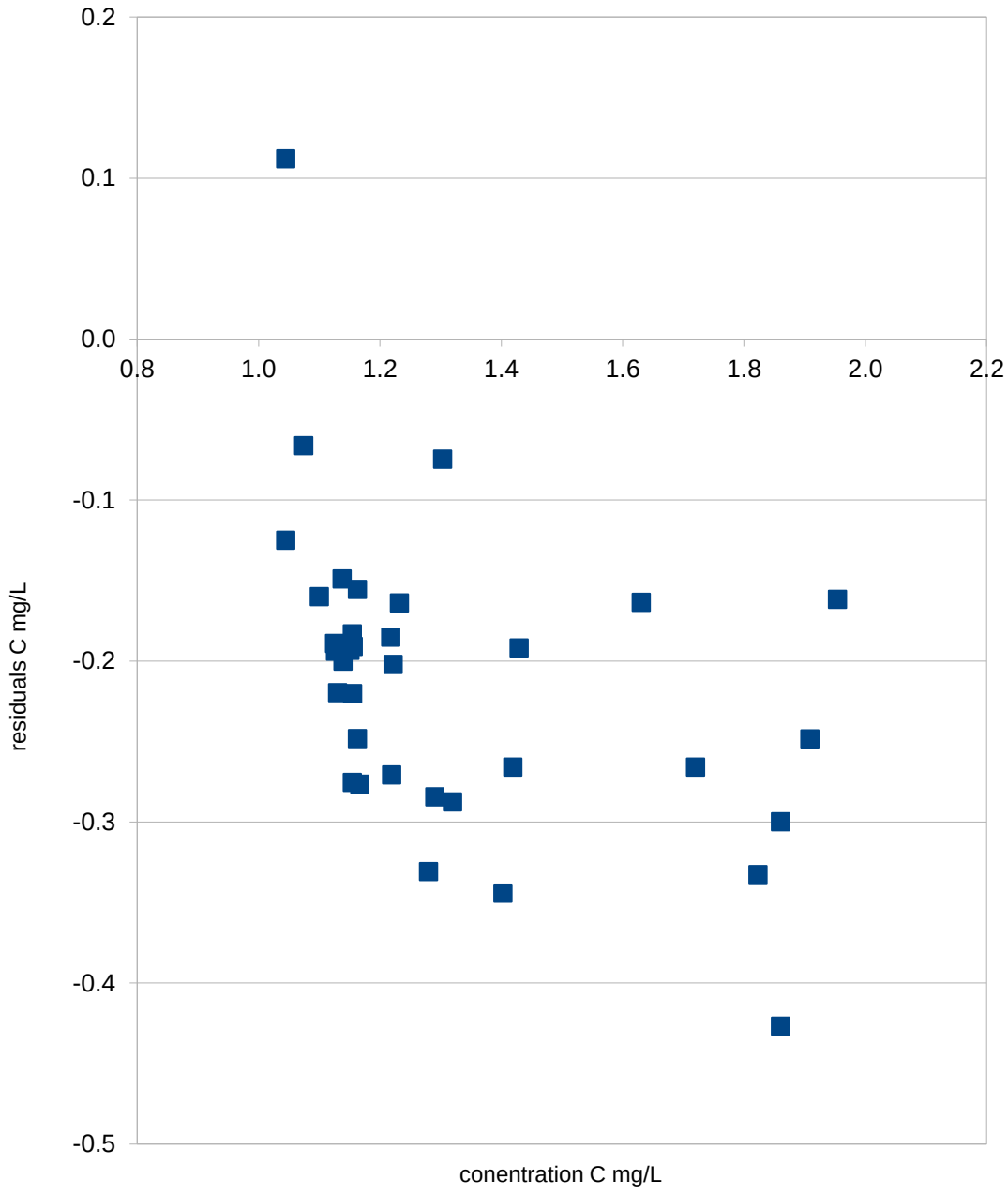



*Figure 6. Residuals of OC concentration for identical sample vials analyzed on both 6/10 (not-dried) and 8/06 (dried). Both used the 8/06 calibration curve formula to calculate C concentration.*

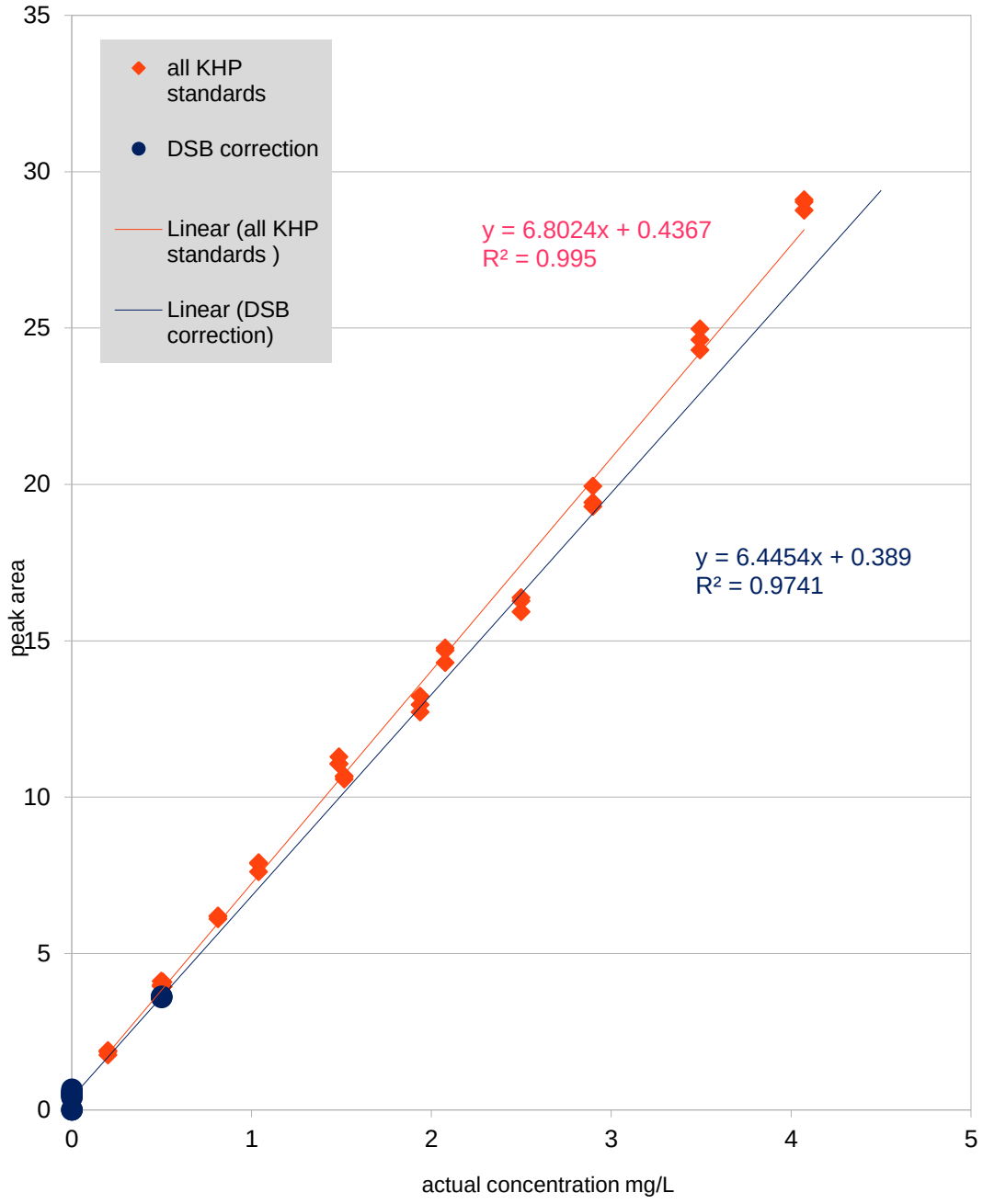



*Figure 7. Calibration curve for Run 8/06 (airtight KHP) of all KHP standards in red compared with the DSB correction applied to the same run in blue.*

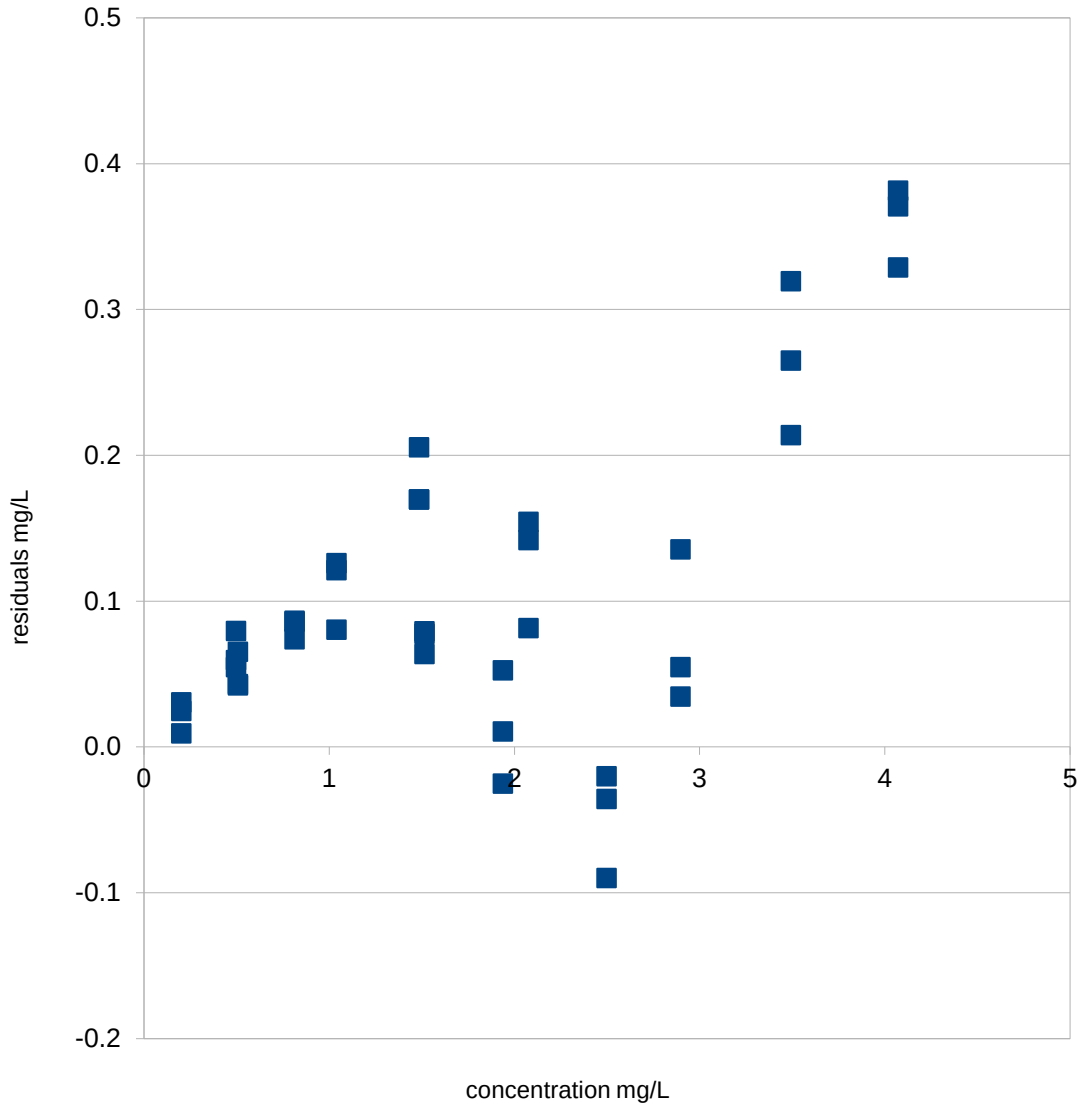



*Figure 8. Residuals from Figure 7, comparing the measured concentration of KHP standards of Run 8/06 to the DSB correction line applied to that run.*

One difference in sample collection and processing for the anniversary cruise and the flood samples was the diameter of the GF/F filters used. The flood anniversary replicates were filtered with 42.5 mm GF/F filters instead of the 47 mm diameter filters used for all other flood work. In order to be sure that variation in DOC values from 2012 and 2013 were not from particles leaking past the smaller filter, water sampled from the flood anniversary cruise was also brought back to the lab and filtered using 47 mm GF/F filters for comparison with the 42.5 mm filter processing. Two replicates each of water from sites M1, WE, and OS were filtered the same day upon return from the flood anniversary cruise. The sample water from Site OS for both size filters was from cast 1, Niskin 12, and canister 2.

The other major difference from flood procedure is that the flood anniversary TOC and DOC replicates were processed on January 22-29 of 2013, seven months after collection. This is not suggested as sample integrity can be compromised by storage, usually contamination from volatile organic compounds in the storage environment. In order to assess possible storage contamination, six Sample Storage Blanks were stored with the samples throughout the storage period.

Many Sample Storage Blanks had a low concentration similar to standard milliQ blanks, however a few were consistently high. Could contamination of the vial caps by poor rinsing or contamination during storage cause a few Sample Storage Blanks to be high? In order to test this hypothesis forty blanks were made. Twenty were rinsed three times before filling, twenty were only filled, not

rinsed. All forty were placed in the sample fridge for 3 months on their side to expose water to the cap.

## Particulate Organic Carbon (POC)

POC filters were oven dried to a constant weight at 60$^{o}$C, homogenized with mortar and pestle, massed and loaded into silver capsules, fumigated with 12 M HCl (ACS Plus grade) overnight to remove inorganic carbon, dried again, and cooled in a desiccator. They were then loaded into tin capsules and analyzed (as in Zigah et al., 2011). They were measured by CHN analysis in a Costech ECS 4010 elemental analyzer and then through a Finnigan Delta Plus XP isotope ratio mass spectrometer for isotopic analysis. Four acid fumigation method blanks were made by dripping MilliQ water onto a blank GF/F filter, and allowing them to dry in the oven with the other filters then be subjected to all the following procedures. The MilliQ water did not go through the filtering process, so these are not complete method blanks

Thirty-five of the sixty-two filters from the 2013 sampling season were lost in a freezer power outage accident. Only seven of the thirty-seven flood anniversary filters were lost in the same accident. Unfortunately, all of the 47 mm filters were lost. Five samples had tin capsules that cracked as they were being rolled. A second tin capsule was added to contain the capsule in these cases. This change did not result in a significant change in POC.

## Scanning UV-VIS Spectrometry (UV-VIS)

The UV-vis whole and filtered samples were scanned from 800 to 200 nm by a Genesys 6 scanning spectrophotometer (Thermo Electron Corp.) using a 1 cm quartz cuvette within three days of sampling. Samples were removed from the refrigerator one hour before analysis and were allowed to reach room temperature. A blank of deionized (DI) water from a MilliQ system (18.0 MΩ or better) was run at least every 5 samples. Absorbance spectra were blank corrected, backscatter-corrected using the mean absorbance from 700 to 800 nm (Green and Blough, 1994), and converted to the Naperian absorbance coefficient, *a*, using the relationship:

$$a_{(\lambda)} = 2.303 * A_{(\lambda)} / l$$

where A is the corrected absorbance, $\lambda$ is the wavelength and $l$ the path length in meters. Absorbance coefficients were used to calculate the CDOM proxies. The e2/e3 proxy is the absorbance coefficient at 250 nm divided by the absorbance coefficient at 365 nm. CDOM is calculated as the sum of all the absorbance coefficients from 250 to 400 nm. Note: samples from M1 were removed from UV-vis analysis because the low concentration of CDOM caused a low signal to blank ratio at 400nm.

## Statistical methods

Confidence intervals using a Student's t distribution were calculated for all replicates with $n$ greater than 3 using the "CONFIDENCE.T(alpha,std_dev,size)" function in Microsoft Excel then adding and subtracting the result from the average. Alpha was adjusted from 0.1, and 0.05, to 0.001 to find the lowest

alpha that does not show overlap of the confidence intervals (rejects the null hypothesis that the difference between the means is zero). The 95% confidence intervals for TOC and DOC replicates were also calculated (and compared to the Excel formula result) using the formula:

$$\text{sample mean} +/- \; t * ( s / \text{sqrt} (n) )$$

where the value of *t* depends on the degrees of freedom, and *s* is the standard deviation of the replicates, and *n* is the number of replicates.

The TOC and DOC replicates were analyzed for the effect of individual factors (Miller and Miller, 1988). First ANOVA was performed by using the formulas in the table below:

| **Source of variation** | **Sum of squares** | **Degrees of freedom** | **Mean square** |
|---|---|---|---|
| Between-sample | $n \sum_i (\bar{x}_i - \bar{x})^2$ | $h$-1 | Sum of squares / degrees of freedom |
| Within-sample | $\sum_i \sum_j (x_{ij} - \bar{x}_i)^2$ | $h$-($n$-1) | Sum of squares / degrees of freedom |
| Total | $\sum_i \sum_j (x_{ij} - \bar{x})^2$ | $hn$-1 | |

The F-statistic was calculated as the ratio of the between-sample mean square and the within-sample mean square. The p-value was calculated using the FDIST(F_statistic,degree_of_freedom_between,degree_of_freedom_within) function in Microsoft Excel. This p-value result was checked against a Table of Critical values of F for a two-tailed test (Table A.3, Miller and Miller, 1988)

The mean organic carbon concentration of replicates which have all sampling factors the same are calculated. The difference of replicate means that

have only one factor different are summed to show the total possible effect of individual factors, then divided by the number of differences to find the average effect for a typical limnological/oceanographic sample. The largest standard deviation of the set of replicates of that factor is used to show significance.

*Table 4. An example of the average of effect of cast 1 to cast 2*

| type | site | **cast** | nisk | rig | $N_i$ | **mean** | **Difference** |
|---|---|---|---|---|---|---|---|
| DOC | OS | **1** | 1 | 1 | 9 | 2.165 | |
| DOC | OS | **2** | 1 | 1 | 9 | 2.386 | 0.221 |
| DOC | OS | **1** | 1 | 2 | 9 | 2.189 | |
| DOC | OS | **2** | 1 | 2 | 9 | 2.569 | 0.380 |
| DOC | OS | **1** | 12 | 1 | 9 | 1.950 | |
| DOC | OS | **2** | 12 | 1 | 9 | 2.553 | 0.603 |
| DOC | OS | **1** | 12 | 2 | 9 | 1.975 | |
| DOC | OS | **2** | 12 | 2 | 9 | 2.596 | 0.621 |
| | | | | | | **total difference** | 1.824 |
| | | | | | | **Ave diff cast 1 to 2** | **0.456** |

To determine if the differences between sampling factors were significant, the least significant difference was calculated (Miller and Miller, 1988). This is calculated by the formula below where s is the square root of the within-sample mean square, t is the t value for the h(n-1) degrees of freedom of this estimate, and n is the number of samples.

$$\text{Least significant difference} = s^* \sqrt{2/n} * t_{h(n-1)}$$

POC and UV-Vis samples were not tested with ANOVA. Sets of samples were compared. First an F-test to determine the similarity of the variance between each of the sets was done. If the variance was not significantly different at $\alpha$=0.05, a t-test using the pooled mean was calculated using the following formula:

t-statistic = (AVE1- AVE2)/(POOLED_STDEV*(SQRT((1/N1)+(1/N2))))

If the variance was significantly different this formula was used:

t-statistic = (AVE1-AVE2)/(SQRT((STDEV1^2/N1)+(STDEV2^2/N2)))

The results above were compared to the t-critical in a standard two-tailed t-table. These results were also compared to the p-value that was reported by the Microsoft Excel formula:

=T.TEST(ARRAY1,ARRAY2,2,3)

The 2 refers to a two-tailed test. The 3 represents significantly different variance. The 3 was used when the F-test found significant difference, a 2 was entered when the variance was similar. Although it was found that when the variance was similar, there was not much difference between using 3 and 2.

# Results

## Flood anniversary TOC and DOC Replicates

The use of different diameter GF/F filters for the two data sets could have added systematic error. Figure 9 compares three different canisters of water sampled on June 21, 2013 and filtered with both 42.5mm and 47mm GF/F filters. The difference in DOC in the filtrate does not appear significant at $\alpha = 0.05$ at WE and OS. The M1 filters are significantly different, however only one sample vial showed the dramatic difference and there is no trend across all sites. For all other measurements it is assumed not significant.

The other concern is the long storage time of the flood anniversary TOC/DOC samples. The Sample Storage Blanks shown in Figure 10 show conflicting results. While most of the blanks remain low indicating there was not much addition of carbon from storage, two blanks show high readings despite being stored in the same manner. We speculated that this increase could be due to contamination from caps. The plastic caps on the vials are acid soaked and rinsed with DI water, however, samples get rinsed with sample water 3 times before filling and storing. In the past, the blank bottles have not been rinsed with MQ water 3 times before filling. Could dust or other contamination have increased these two blanks? Further testing, where 40 caps and vials were sampled in a Sample Storage Blank study, does not indicate that the rinsing step causes a significant difference in concentration from non-rinsed vials. See Figure 11. The few high outliers could be a sign of other contamination that is not removed by rinsing, like fingerprints or other oily substances. Clean caps are

stored in gallon ziptop bags. There are a large number of caps in each bag. If even gloved hands reach in each time to remove caps, there are many chances for contamination. More careful removal of caps from storage bags might be recommended or smaller bags of caps to reduce the number of times the bag is disturbed.

The error due to weighing and diluting the known solutions was calculated using propagation of error; at 21 mg/L this error is 0.51% of the measured concentration and at 0.21 mg/L the error is 1.3%. The error due to the line fit of the calibration curve was 21.2+/- 0.9 mg/L or 4.3% and at a low concentration of 0.21 +/- 0.9 or 400%. These errors are now insignificant due to the deep sea blank correction.

TOC concentrations at all sites measured on the flood anniversary are shown in Figure 12 and shown in Table 6. While the range of TOC measurements at site OS is large because of the large number of replicates, it is significantly different from TOC amounts at sites WE, DE, and M1. Spatial difference in TOC is significant at the 99% confidence level.

The reproducibility of the TOC measurements of the site OS replicates is shown in Figure 13. The 95% confidence intervals appear to support that there is a difference in cast. The F-statistic shown in the ANOVA results in Table 5, strongly supports that there is a significant difference between the treatments, but, which treatments? It appears that, as expected, the greatest difference is due to cast, and not Niskin bottle. The cast 2 replicates of both Niskin bottle 1 and 12 appear higher than the cast 1 replicates. From this graph, it also appears

that the Niskin 12 of both cast 1 and 2 is lower. Is it significantly lower than Niskin 1? The least significant difference for TOC at $\alpha$=0.05 is 0.098 mg/L, at $\alpha$=0.10 it is 0.082 mg/L. The change from cast 1 to cast 2 is significant at both 90% and 95%. The difference from cast 2 Niskin 1 to cast 2 Niskin 12 is also significant at both $\alpha$ values. The difference between cast 1 Niskin 1 and cast 1 Niskin 12 is significant at 90% but not at 95%. The average effect of the spatial and time difference in sampling between cast 1 and cast 2 causes an increase in TOC of 0.34 mg/L. Using Niskin 12 instead of Niskin 1 led to an average decrease of 0.1 mg/L. Both differences are larger than the least significant difference, however, only cast 1 to cast 2 gave a difference larger than the standard deviation of the within-sample replicates.

*Table 5. TOC concentration statistics for all TOC replicate vials sampled on July 21, 2013. All TOC concentrations are in mg/L.*

| site | cast | nisk | repl vial | ave conc of rep inj | std dev of rep inj | AVE nisk | SD nisk | AVE cast | SD cast |
|---|---|---|---|---|---|---|---|---|---|
| OS | 1 | 1 | 1 | 2.37 | 0.04 | 2.20 | 0.13 | 2.15 | 0.11 |
| OS | 1 | 1 | 2 | 2.12 | 0.01 | | | | |
| OS | 1 | 1 | 3 | 2.12 | 0.03 | | | | |
| OS | 1 | 12 | 1 | 2.03 | 0.04 | 2.11 | 0.07 | | |
| OS | 1 | 12 | 2 | 2.16 | 0.04 | | | | |
| OS | 1 | 12 | 3 | 2.13 | 0.02 | | | | |
| OS | 2 | 1 | 1 | 2.64 | 0.04 | 2.54 | 0.08 | 2.49 | 0.11 |
| OS | 2 | 1 | 2 | 2.48 | 0.03 | | | | |
| OS | 2 | 1 | 3 | 2.51 | 0.02 | | | | |
| OS | 2 | 12 | 1 | 2.52 | 0.04 | 2.44 | 0.13 | | |
| OS | 2 | 12 | 2 | 2.28 | 0.02 | | | | |
| OS | 2 | 12 | 3 | 2.52 | 0.05 | | | | |
| WE | | | | 5.22 | 0.06 | | | | |
| DE | | | | 4.64 | 0.08 | | | | |
| M1 | | | | 1.10 | 0.02 | | | | |

*Table 6. ANOVA results for all TOC samples at site OS on July 21,2013. All replicates of the same treatment (same cast and same niskin) are considered a sample.*

| source of var | sum of squares | degrees of freedom | mean square | RESULTS | |
|---|---|---|---|---|---|
| btwn sample | 1.110 | 3 | 0.370 | F – statistic | 35.366 |
| within sample | 0.335 | 32 | 0.010 | Critical value | 2.134 |
| Total | 1.445 | 35 | | p-value | 2.8E-10 |

A similar analysis was done for DOC. The filtering process for DOC introduces more opportunities for variation. In addition to cast and Niskin bottle, the variants now include different canisters and filter rigs. The canister numbers were recorded, but since they were not independently varied from Niskin bottle, the affect is unknown. I have also included in this data the different GF/F filter sizes, though as shown in Figure 9, these did not lead to a significant difference in DOC values.

As with TOC, the difference in DOC between sites is significant. In Figure 15, the two casts at site OS are significantly different from sites DE, WE, and M1 even between the 99% confidence intervals. The standard deviation of each of the sites ranges from 0.05 at DE to 0.25 mg/L at M1. M1 was analyzed in a separate TOC run and is less precise (see discussion on p. 18) The standard deviations of the eight DOC replicate groups range from 0.02 to 0.11 mg/L (Table 8).

Figure 16 seems to suggest a difference in within-cast DOC variations between casts. In Cast 1 there appears to be a difference (between the 99% confidence intervals) for data from Niskin 1 and Niskin 12, but this difference is

not as clear, and the between-Niskin trend is actually reversed, in Cast 2. This difference might depend on the timing of filling the canisters. The order of filling was not recorded to confirm this. According to the ANOVA results in Table 7, there are strongly significant differences between the treatments. The least significant difference will show which treatments are significant.

*Table 7. ANOVA results for all DOC samples at site OS on July 21,2013. All replicates of the same treatment (same cast, niskin, filter rig) are considered a sample.*

| **source of var** | **sum of squares** | **degrees of freedom** | **mean square** | **RESULTS** | |
|---|---|---|---|---|---|
| btwn sample | 4.410 | 7 | 0.630 | F – statistic | 122.649 |
| within sample | 0.329 | 64 | 0.005 | Critical value | 2.134 |
| Total | 4.738 | 71 | | p-value | 1.4E-34 |

The average values of the 8 replicates are shown in Figure 17. The least significant difference between these sets of replicates at $\alpha$ =0.05 is 0.067 mg/L and at $\alpha$ =0.10 is 0.056 mg/L. According to this, filter rig number should not be significant as all have overlap (this is true even at $\alpha$ =0.10). The difference between cast 1 and cast 2 is significant at both probabilities. The difference between Niskin 1 and Niskin 12 is more difficult to tell. It is significant for cast 1, but gives mixed results in cast 2. Figure 18 more easily shows the average affect each of these differences has on the DOC measurement. The concentration of Cast 2 is significantly higher (by 0.46 mg/L) than Cast 1. This is similar to the TOC measurement increase. Niskin bottle 12 has an average effect of being lower than Niskin 1 by 0.06 mg/L; this is larger than the significant difference at

$\alpha$ =0.10 but not at $\alpha$ =0.05. It is also not larger than the standard deviation of the within sample replicates. Filter rig 2 has an average effect of being 0.07 mg/L higher than filter rig 1; this is larger than the significant difference at both probabilities, but not larger than the standard deviation of the within sample replicates.

*Table 8. DOC concentration statistics for all DOC replicate vials sampled on July 21, 2013. All DOC concentrations are in mg/L.*

| site | cast | nisk | can | rig | rep vial | ave conc rep inj | std dev rep inj | AVE rig | SD rig | AVE nisk | SD nisk | AVE cast | SD cast |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OS | 1 | 1 | 1 | 1 | 1 | 2.12 | 0.01 | 2.17 | 0.05 | 2.18 | 0.08 | 2.07 | 0.13 |
| OS | 1 | 1 | 1 | 1 | 2 | 2.20 | 0.04 | | | | | | |
| OS | 1 | 1 | 1 | 1 | 3 | 2.18 | 0.04 | | | | | | |
| OS | 1 | 1 | 1 | 2 | 1 | 2.09 | 0.04 | 2.19 | 0.11 | | | | |
| OS | 1 | 1 | 1 | 2 | 2 | 2.16 | 0.04 | | | | | | |
| OS | 1 | 1 | 1 | 2 | 3 | 2.32 | 0.04 | | | | | | |
| OS | 1 | 12 | 2 | 1 | 1 | 2.00 | 0.04 | 1.95 | 0.06 | 1.96 | 0.06 | | |
| OS | 1 | 12 | 2 | 1 | 2 | 1.96 | 0.01 | | | | | | |
| OS | 1 | 12 | 2 | 1 | 3 | 1.89 | 0.03 | | | | | | |
| OS | 1 | 12 | 2 | 2 | 1 | 2.01 | 0.03 | 1.97 | 0.05 | | | | |
| OS | 1 | 12 | 2 | 2 | 2 | 1.91 | 0.03 | | | | | | |
| OS | 1 | 12 | 2 | 2 | 3 | 2.00 | 0.03 | | | | | | |
| OS | 2 | 1 | 3 | 1 | 1 | 2.37 | 0.00 | 2.39 | 0.02 | 2.48 | 0.12 | 2.53 | 0.11 |
| OS | 2 | 1 | 3 | 1 | 2 | 2.38 | 0.01 | | | | | | |
| OS | 2 | 1 | 3 | 1 | 3 | 2.40 | 0.04 | | | | | | |
| OS | 2 | 1 | 3 | 2 | 1 | 2.54 | 0.02 | 2.57 | 0.11 | | | | |
| OS | 2 | 1 | 3 | 2 | 2 | 2.47 | 0.01 | | | | | | |
| OS | 2 | 1 | 3 | 2 | 3 | 2.70 | 0.01 | | | | | | |
| OS | 2 | 12 | 4 | 1 | 1 | 2.50 | 0.05 | 2.55 | 0.08 | 2.57 | 0.07 | | |
| OS | 2 | 12 | 4 | 1 | 2 | 2.64 | 0.03 | | | | | | |
| OS | 2 | 12 | 4 | 1 | 3 | 2.51 | 0.04 | | | | | | |
| OS | 2 | 12 | 4 | 2 | 1 | 2.56 | 0.05 | 2.60 | 0.06 | | | | |
| OS | 2 | 12 | 4 | 2 | 2 | 2.58 | 0.04 | | | | | | |
| OS | 2 | 12 | 4 | 2 | 3 | 2.64 | 0.07 | | | | | | |
| OS | 1 | 12 | 2 | 47 | 1 | 1.89 | 0.04 | 1.91 | 0.04 | | | | |
| OS | 1 | 12 | 2 | 47 | 2 | 1.93 | 0.03 | | | | | | |
| WE | | | | | | 4.48 | 0.05 | | | | | | |
| WE | | | | 47 | 1 | 4.78 | 0.04 | | | | | | |
| WE | | | | 47 | 2 | 4.52 | 0.04 | | | | | | |
| DE | | | | | | 4.22 | 0.05 | | | | | | |
| M1 | | | | | | 1.54 | 0.03 | | | | | | |
| M1 | | | | 47 | 1 | 1.17 | 0.04 | | | | | | |
| M1 | | | | 47 | 2 | 0.98 | 0.06 | | | | | | |

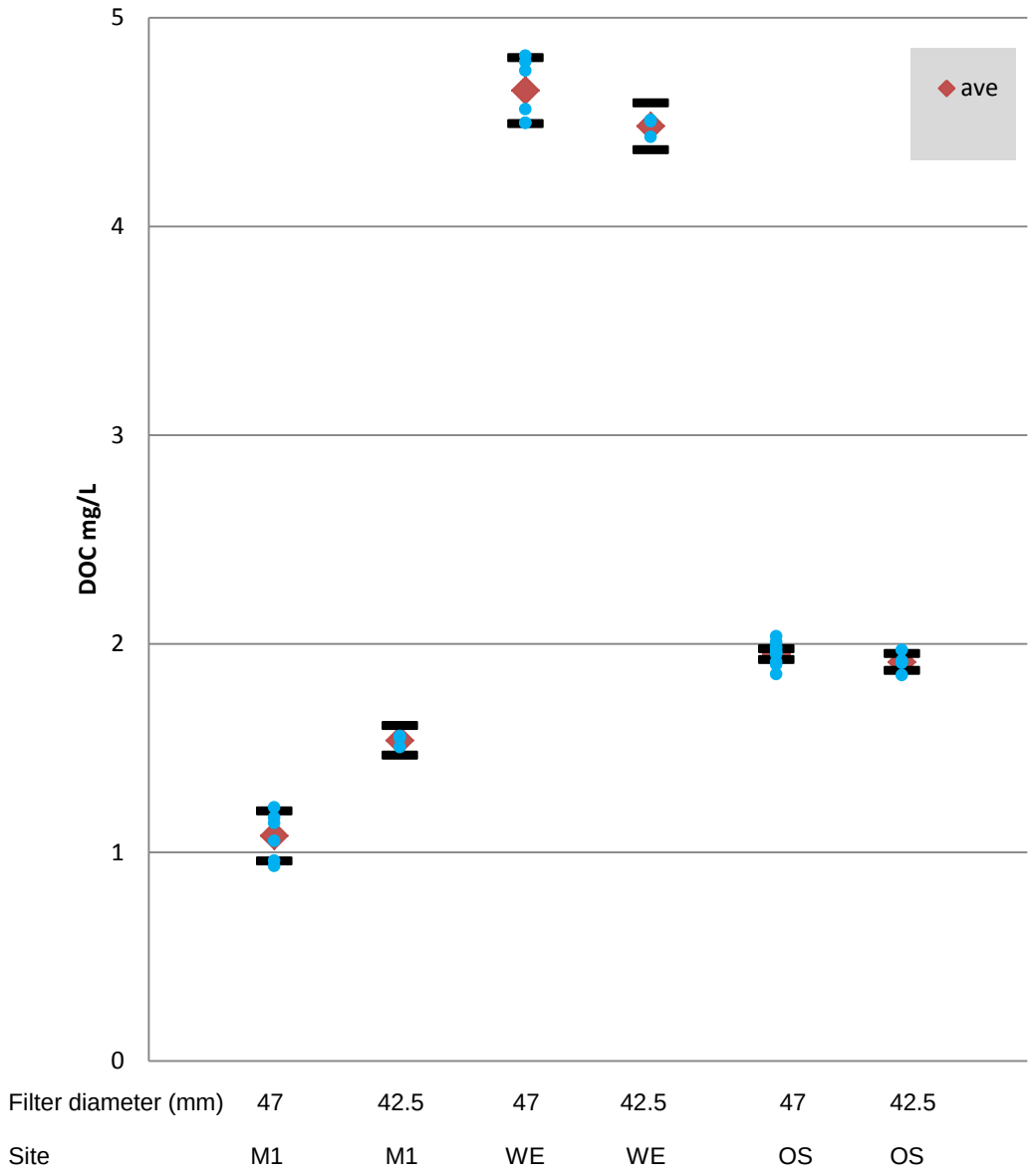



*Figure 9. Size of filter. DOC measurements of water filtered by both 42.5 and 47 mm GF/F filters. Black bars are the 95% confidence interval of the replicate injections of all vials of each treatment shown.*

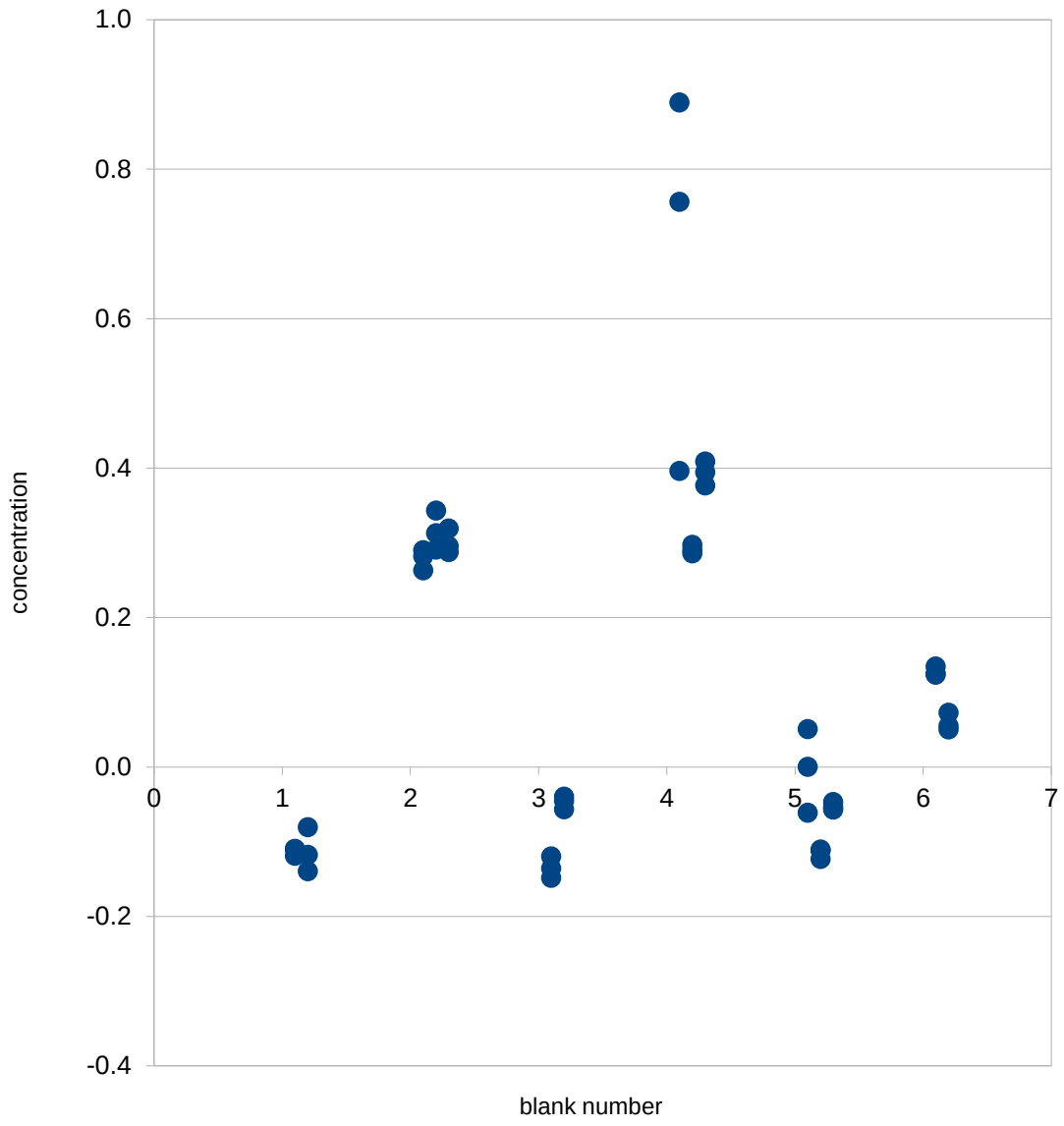



*Figure 10. Sample Storage Blank replicates for June 21, 2013 samples.*

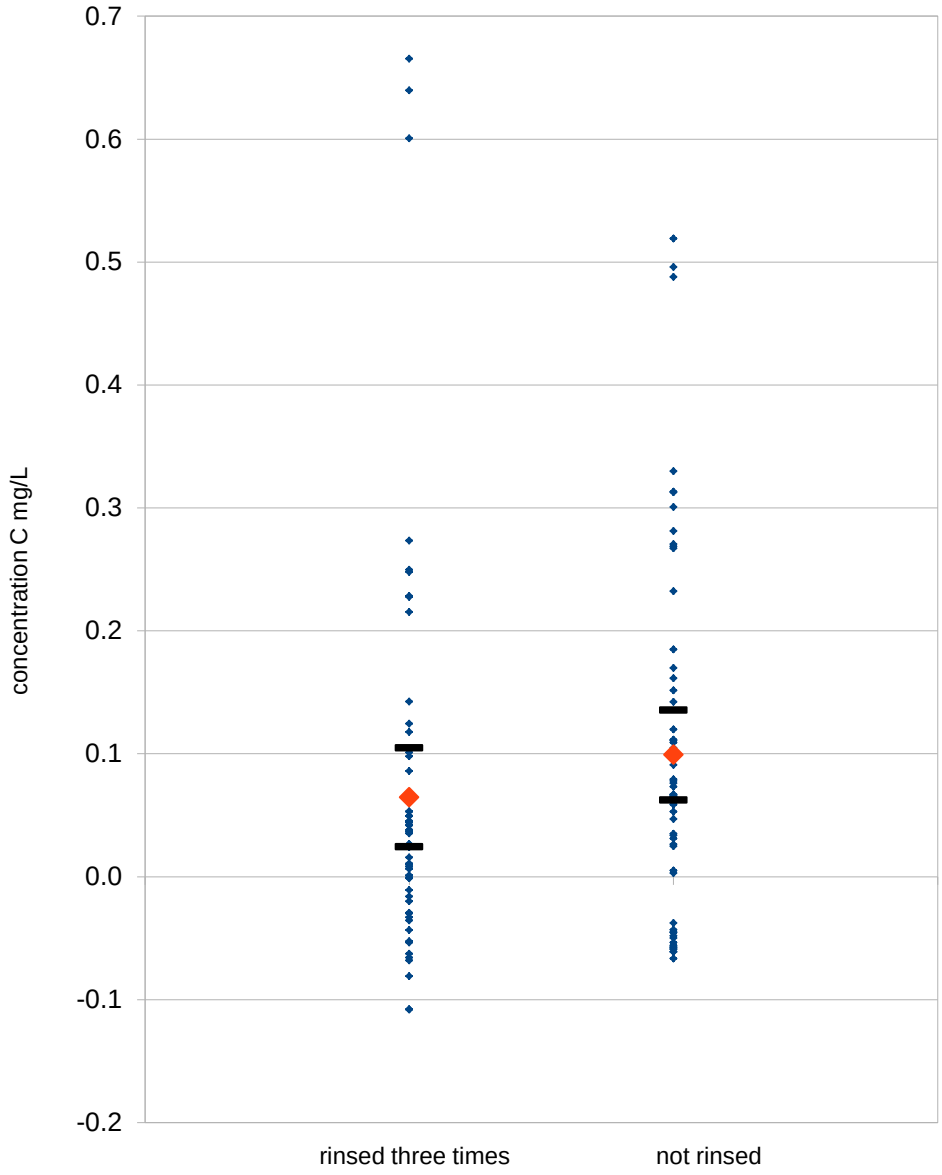



*Figure 11. Sample Storage Blank Study. 40 caps in the fridge for 3 months. The black bars are the 95% confidence interval. The difference between rinsed caps and not rinsed caps is insignificant.*

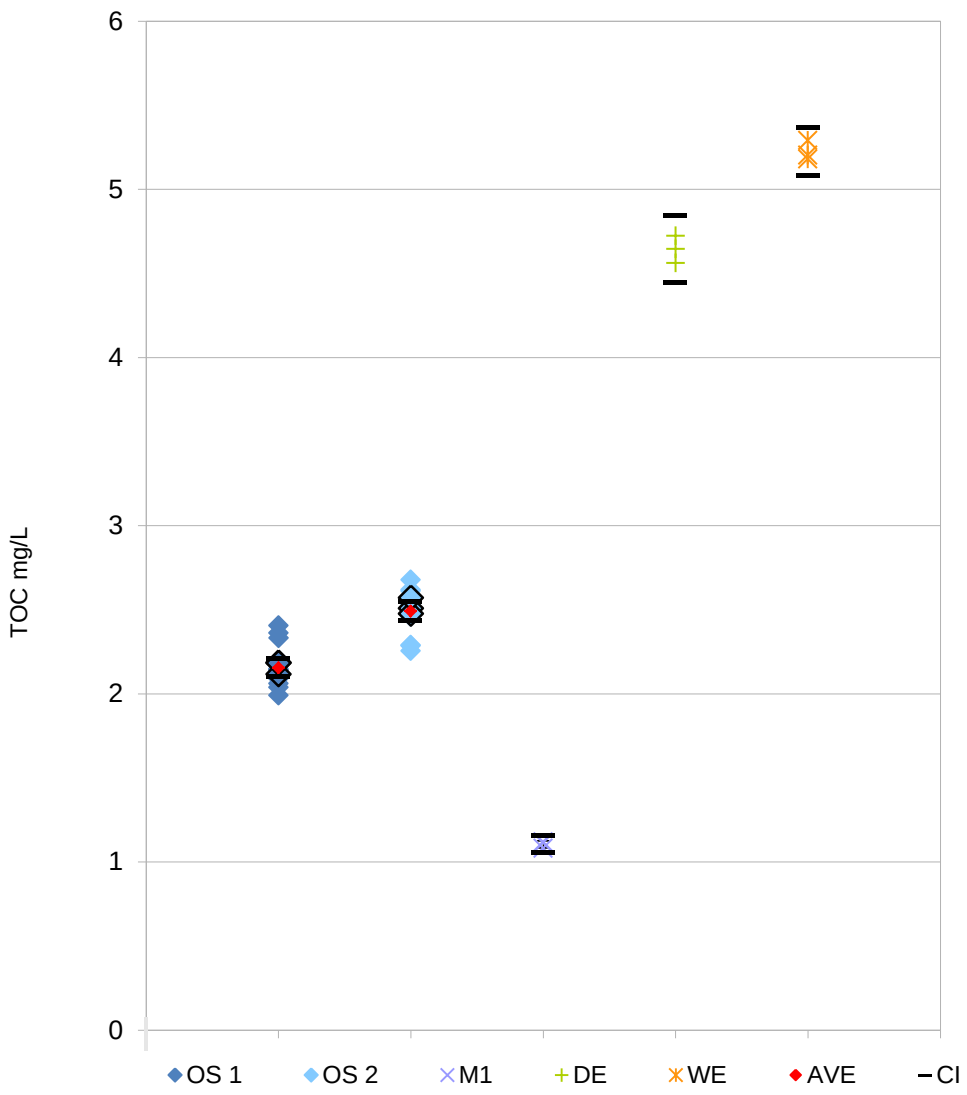



*Figure 12. Total Organic Carbon at all sites June 21, 2013. Black bars show the 99% confidence interval of all the replicate injections of all vials collected at a site. Only one sample vial was measured at M1, DE, and WE. Replicate injections of the vial in each OS cast with the highest standard deviation are outlined in black.*

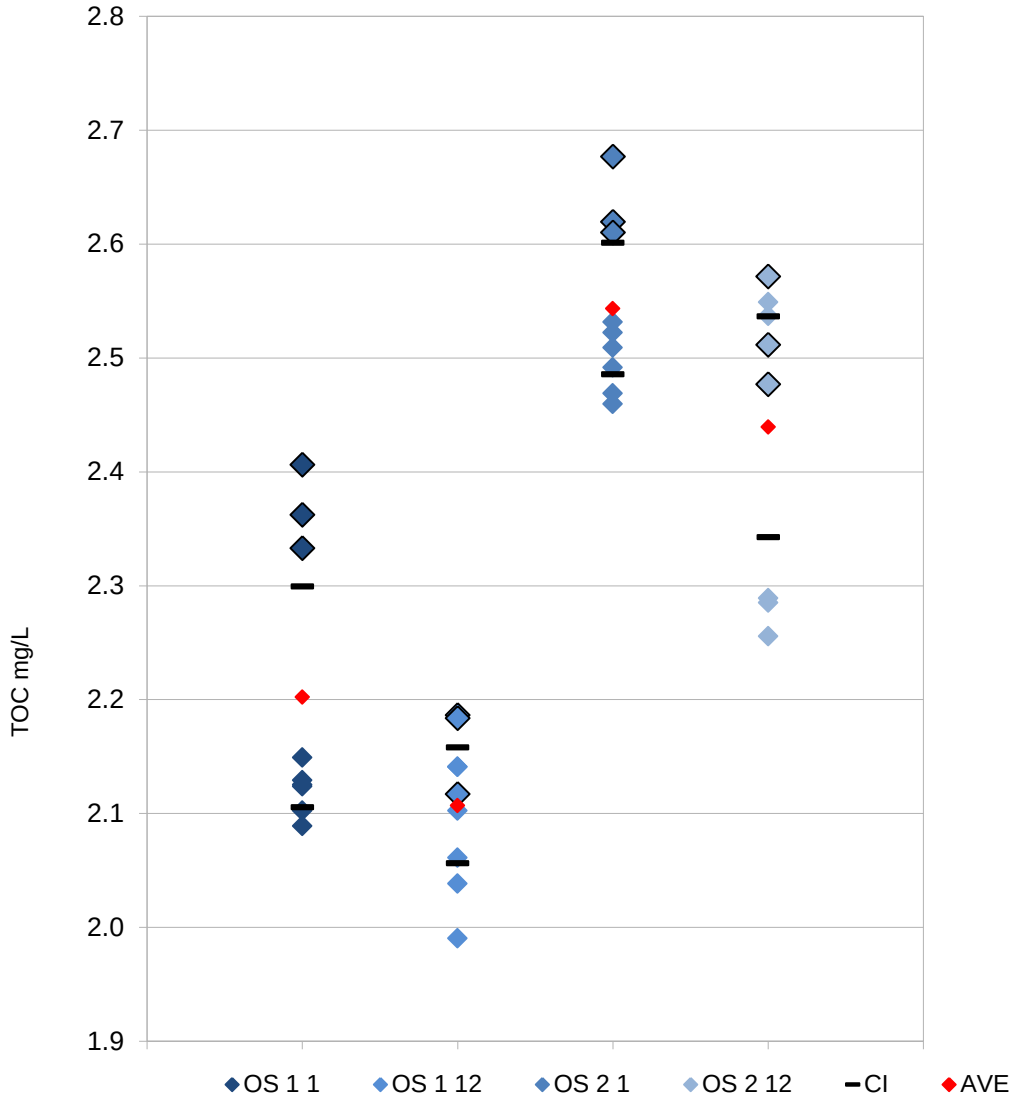



*Figure 13. All replicates of Total Organic Carbon at site OS June 21 2013 by cast, Niskin. Black bars are 95% confidence interval of all replicate injections of all vials collected at each treatment. Replicate injections of the vial in each treatment with the highest standard deviation are outlined in black.*

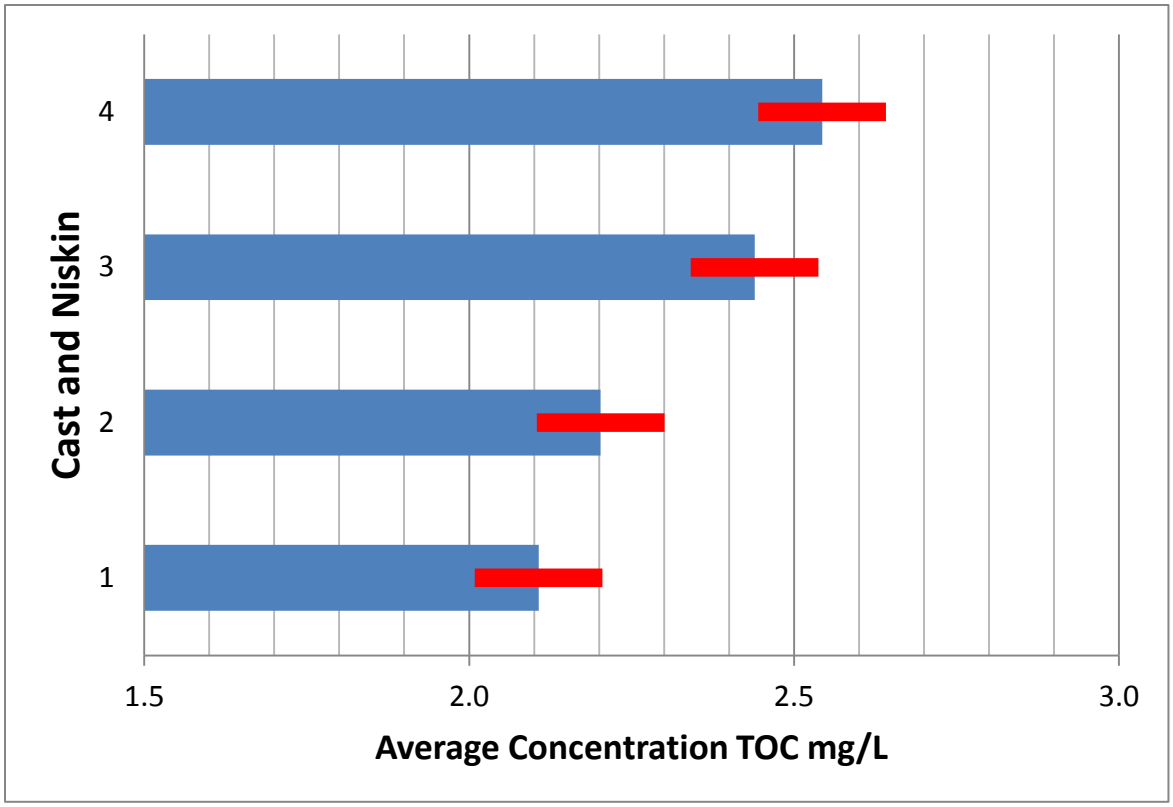



*Figure 14. Least Significant Difference (α= 0.05) from cast and Niskin. The least significant difference is shown as a red bar. The difference from cast 1 to cast 2 is clearly significant. The difference from cast 2 Niskin 1 and cast 2 Niskin 12 is also significant.*

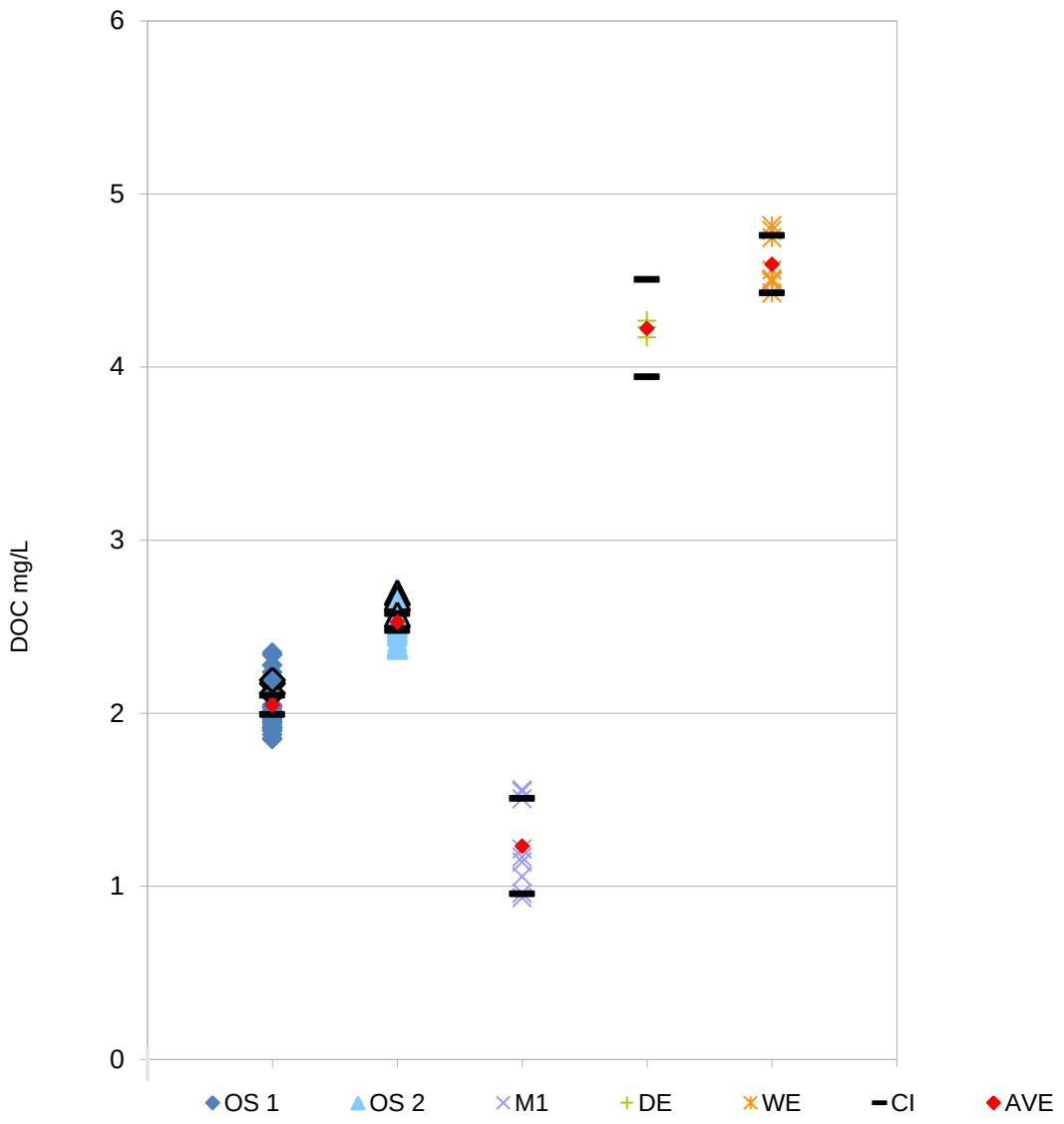



*Figure 15. Dissolved Organic Carbon at all sites June 21 2013. Black bars are the 99% confidence interval for all replicate injections for all vials collected at a site. Only one sample vial was measured at DE. M1 was measured during a less precise run on 1/22/14. See notes in appendix. Replicate injections of the vial in each treatment with the highest standard deviation are outlined in black.*

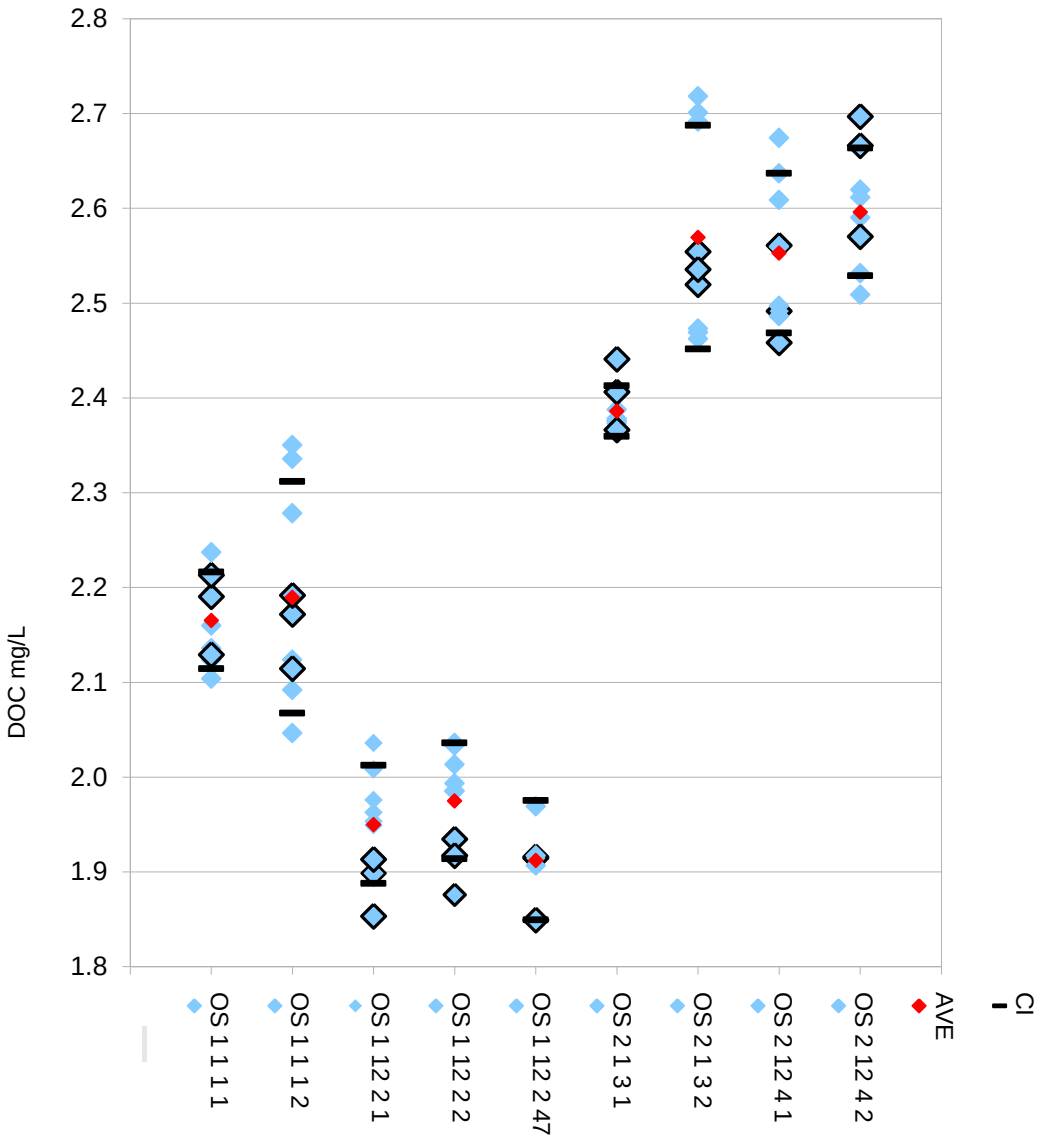



*Figure 16. All samples of dissolved organic carbon at site OS June 21 2013 by cast, Niskin, canister, filter rig. 99% confidence intervals of replicates injections of all vials in each treatment are shown as black bars. Note that the 47 mm GF/F filter replicates are included in this graph. Replicate injections of the vial in each treatment with the highest standard deviation are outlined in black.*

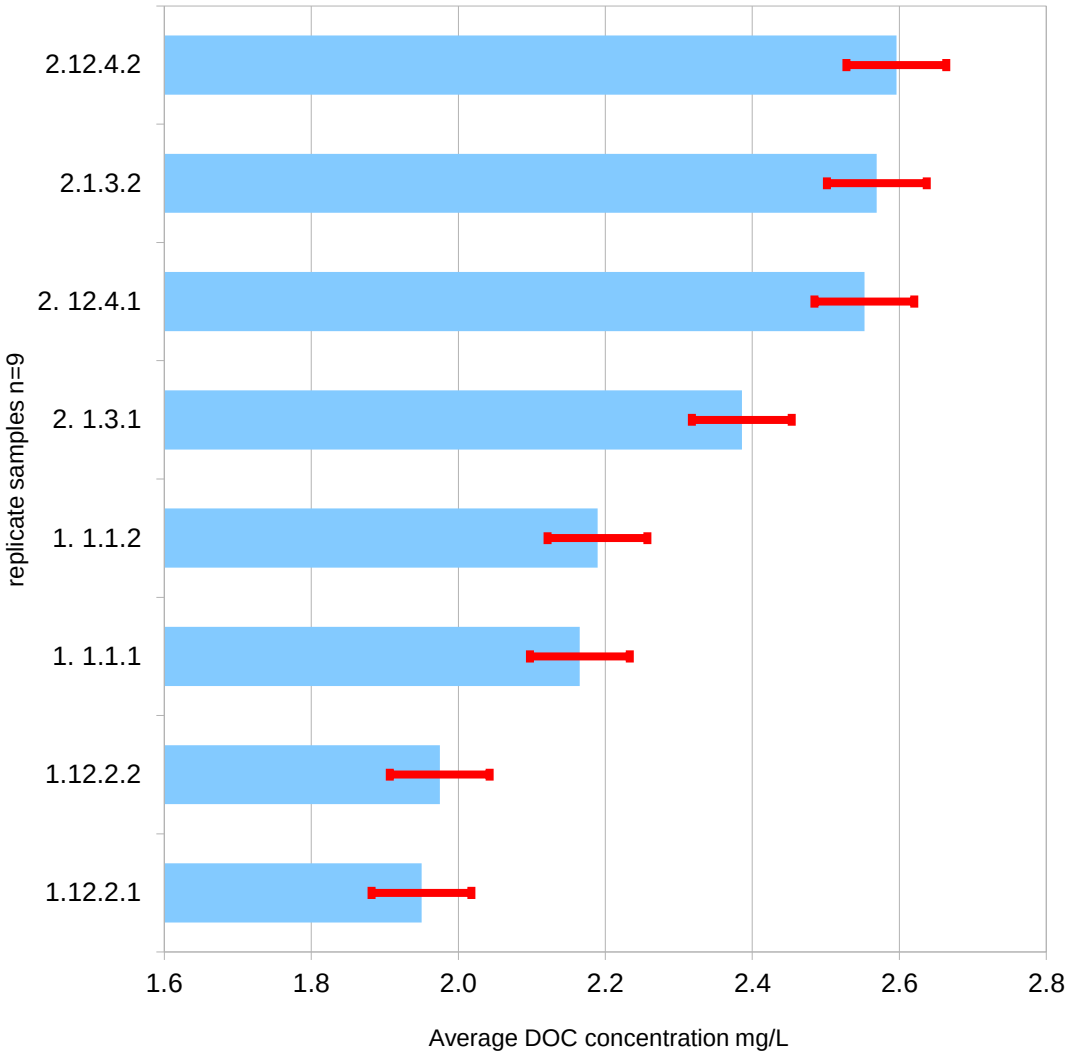



*Figure 17. Least Significant Difference ($\alpha$= 0.05) from cast.Niskin.canister.filter rig (as shown on y axis). The least significant difference is shown as a red bar*

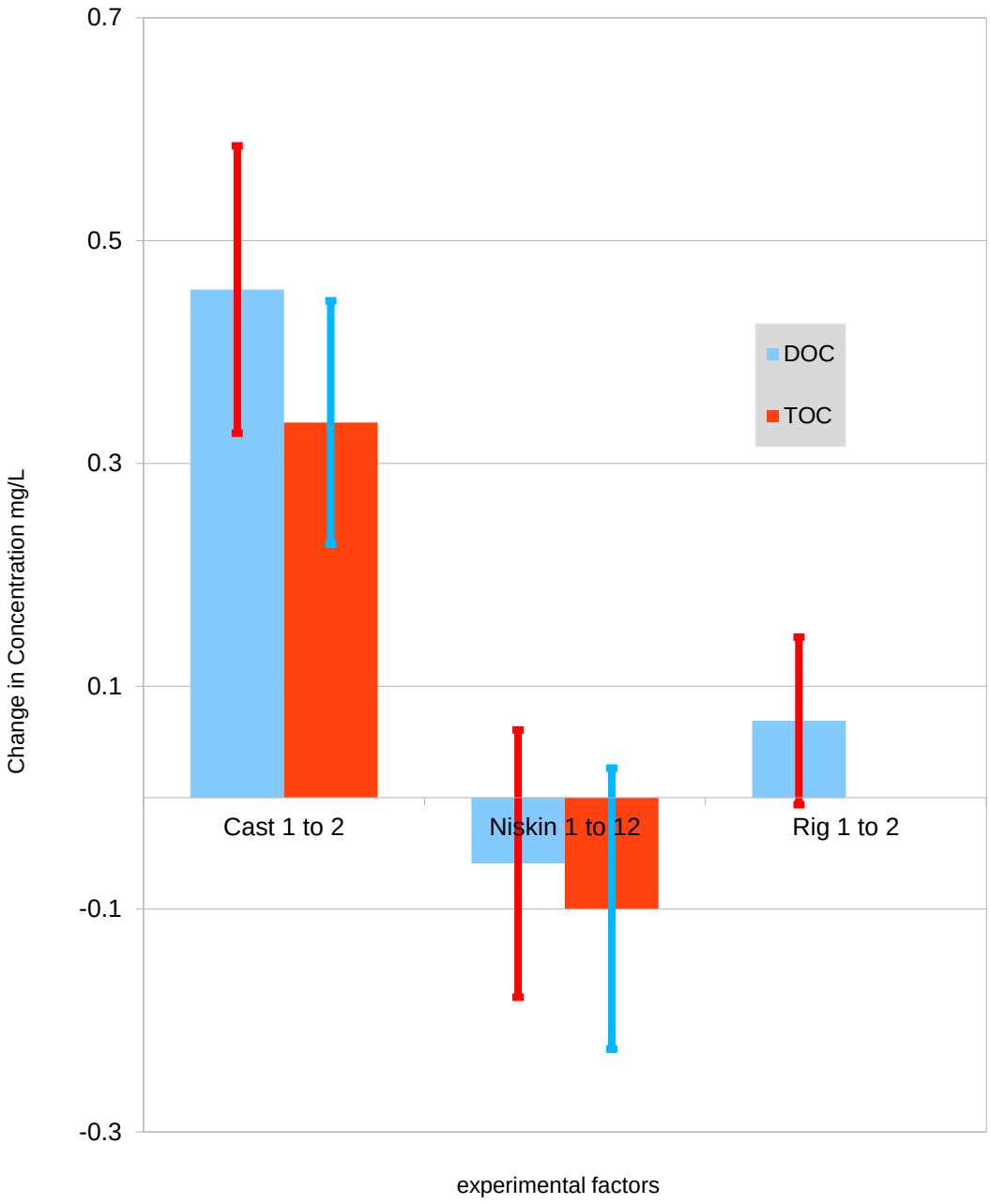



*Figure 18. Average effect of sampling factors on TOC and DOC. The error bars are standard deviation of the replicates of each factor.*

## Flood anniversary POC replicates

All samples were analyzed in two separate IRMS runs (run 1 was analyzed on July 2, 2014, run 2 on August 22, 2014). The variation between the two runs (Figure 19 and Table 10) does not appear significant although there were more technical errors (popped foil caps that needed to another tin cap to contain them) in the first run because of inexperience. The errors caused more variation, but did not appear to cause a general increase or decrease as shown in Figure 20. Two acid fumigation method blanks were analyzed in each run. The average mass carbon on each blank filter was 0.035 +/- 0.009 mg. All concentrations have been blank corrected. Similar to TOC and DOC, the sites appear different (Figure 21). However, because site DE only has two data points, the 95% confidence interval is extremely large. Sites WE and M1 have only 1 data point, therefore, no measurement of variance or significance is possible. To determine if the difference is significant, more data would be needed. POC does not show significant differences between casts. T-tests support a significant difference between Niskin 1 and Niskin 12 in cast 2 at $\alpha$=0.10 but not at $\alpha$ =0.05. T-tests do not support a significant difference between the cast 1 and cast 2, nor any other factor.

*Table 9. T-test results for flood anniversary POC samples at site OS.*

| cast | nisk | can | rig | **compare to** | cast | nisk | can | rig | sig. diff. of var | T.TEST p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | | | | 1 | 12 | | | no | 0.71 |
| 1 | | | | | 2 | | | | no | 0.19 |
| 2 | 1 | | | | 2 | 12 | | | yes | **0.05** |
| 1 | 1 | 1 | 2 | | 1 | 12 | 2 | 2 | no | 0.97 |
| 1 | 12 | 2 | 2 | | 2 | 1 | 2 | 2 | no | 0.49 |
| 1 | 1 | 1 | 2 | | 2 | 1 | 3 | 2 | no | 0.47 |

*Table 10. POC statistics summary for Figures 18-20. All concentrations are in micromoles C per Liter.*

| **Replicate set** | **Average** | **Std dev** | **number** |
|---|---|---|---|
| Site OS IRMS run 1 | 12.2 | 2.1 | 8 |
| Site OS IRMS run 2 | 11.8 | 1.5 | 10 |
| No packing errors | 11.9 | 1.4 | 14 |
| Packing error | 12.4 | 3.0 | 4 |
| Site OS cast 1 | 11.4 | 1.5 | 9 |
| Site OS cast 2 | 12.6 | 1.9 | 9 |
| Site DE | 20.1 | Ave dev = 1.8. | 2 |
| OS 1.1.1.2 reps | 11.4 | 1.4 | 3 |
| OS 1.12.2.2 reps | 11.4 | 2.4 | 3 |
| OS 2.1.3.2 reps | 11.4 | 1.3 | 3 |

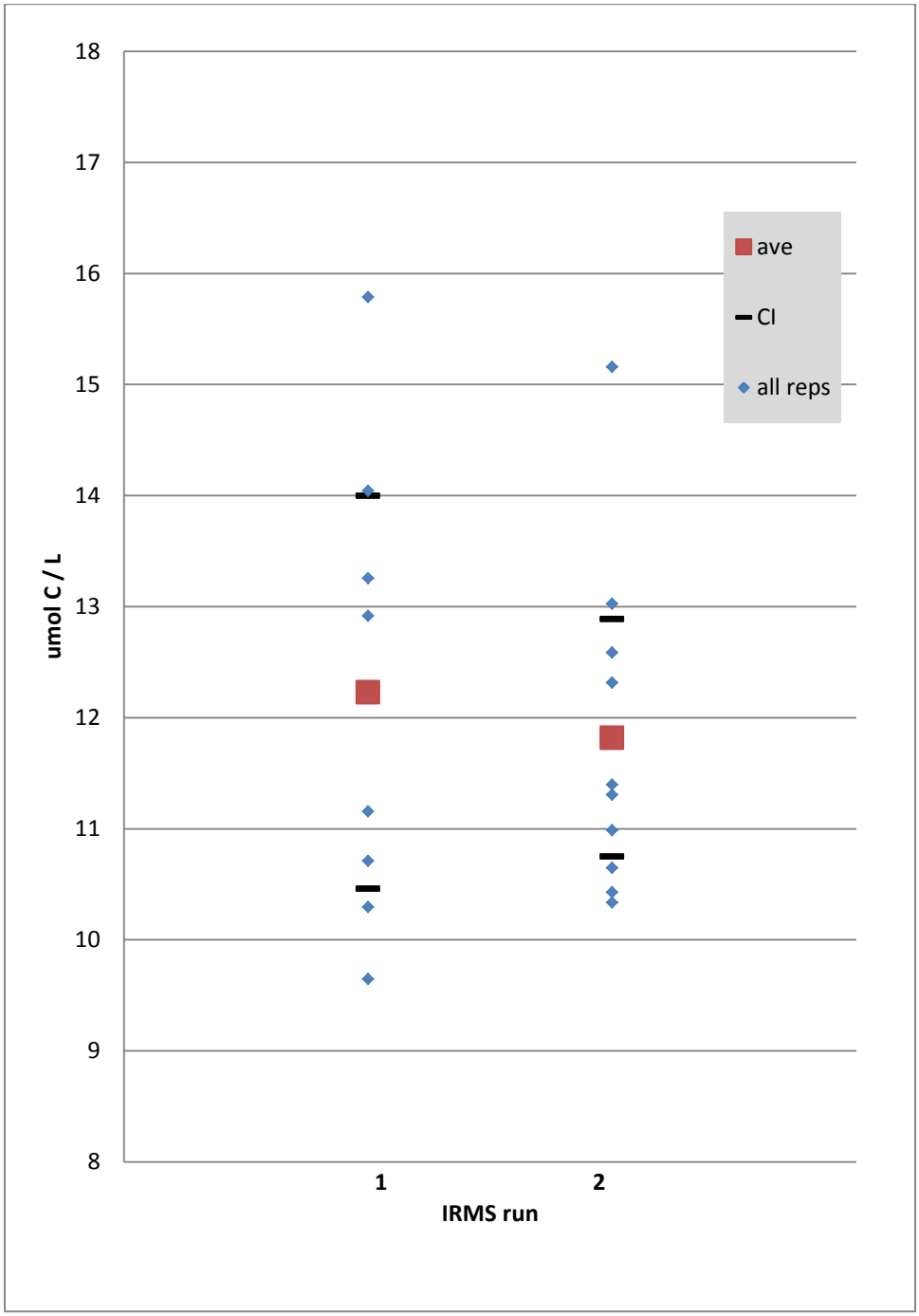



*Figure 19. The two different IRMS analyses of Flood anniversary POC samples. Black bars are 95% confidence intervals of all samples in each run.*

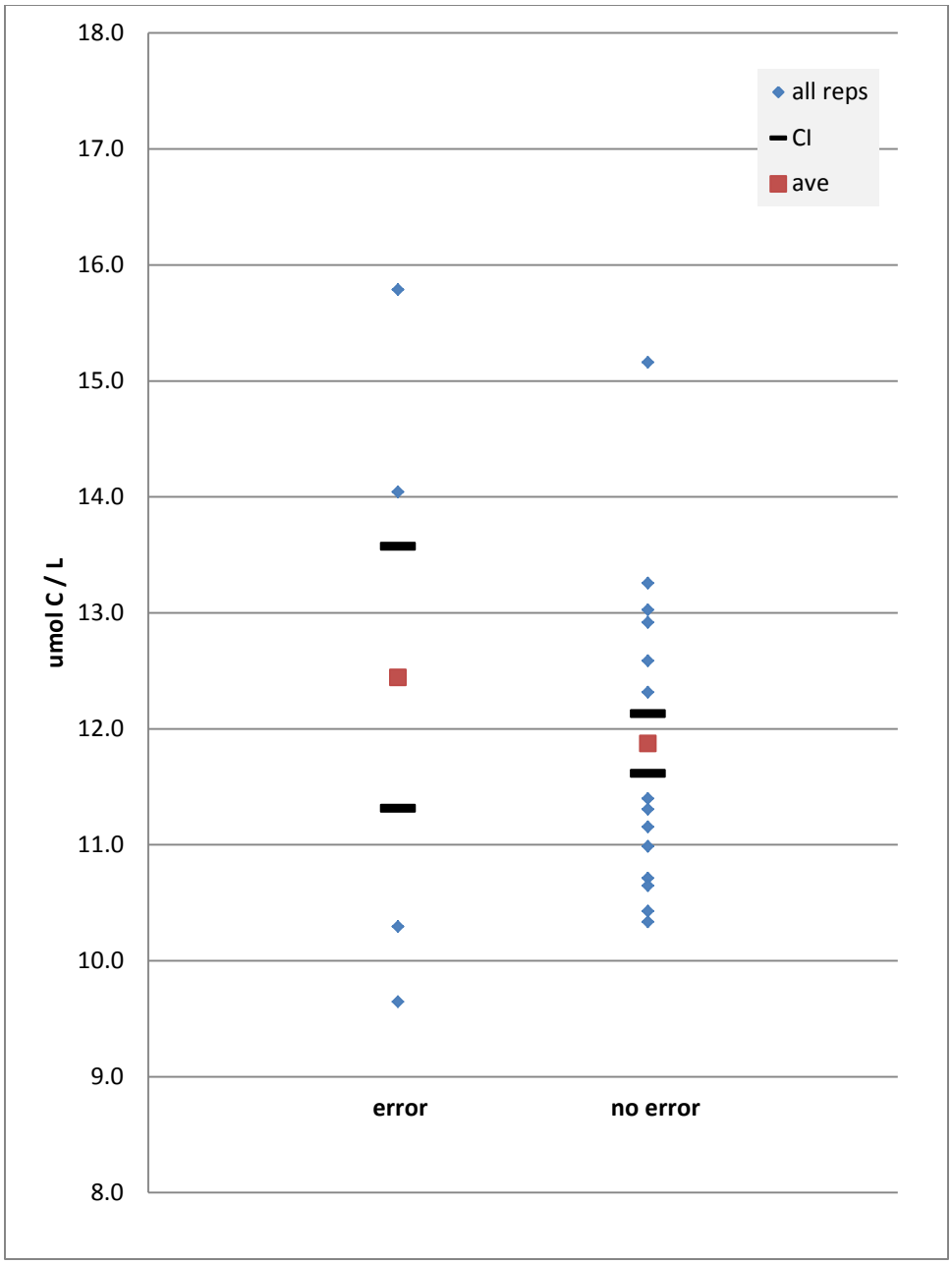



*Figure 20. Packing tin boats errors. Caps breaking open and needing to be contained in a second foil cap are in the error column. Notice that they cause increased variation, but no overall trend. Black bars are 95% confidence intervals of all samples within each treatment.*

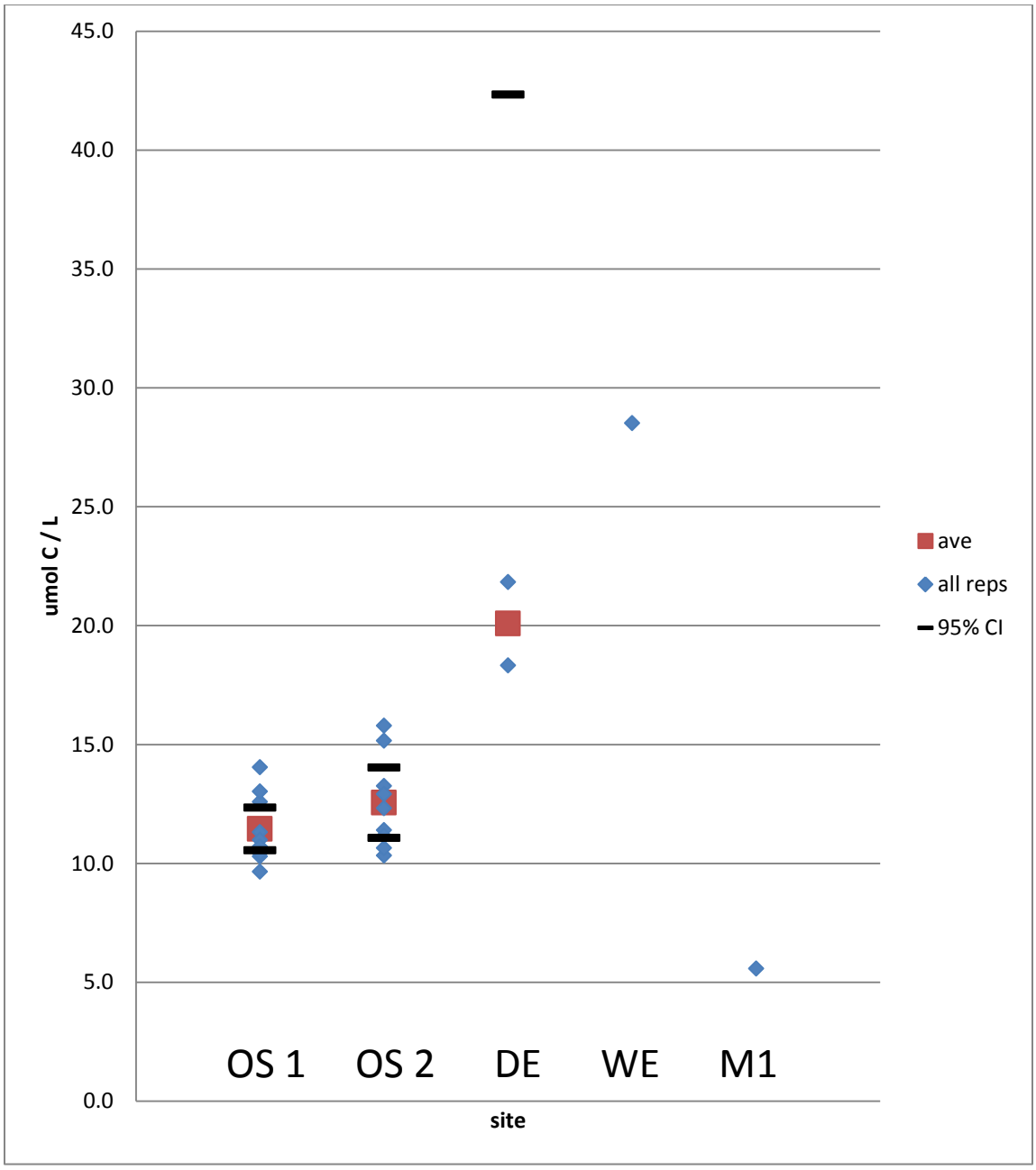



*Figure 21. POC variation at all sites June 21 2013. OS 1 is Site OS cast 1. Black bars are the 95% confidence interval for all replicate samples collected at a site.*

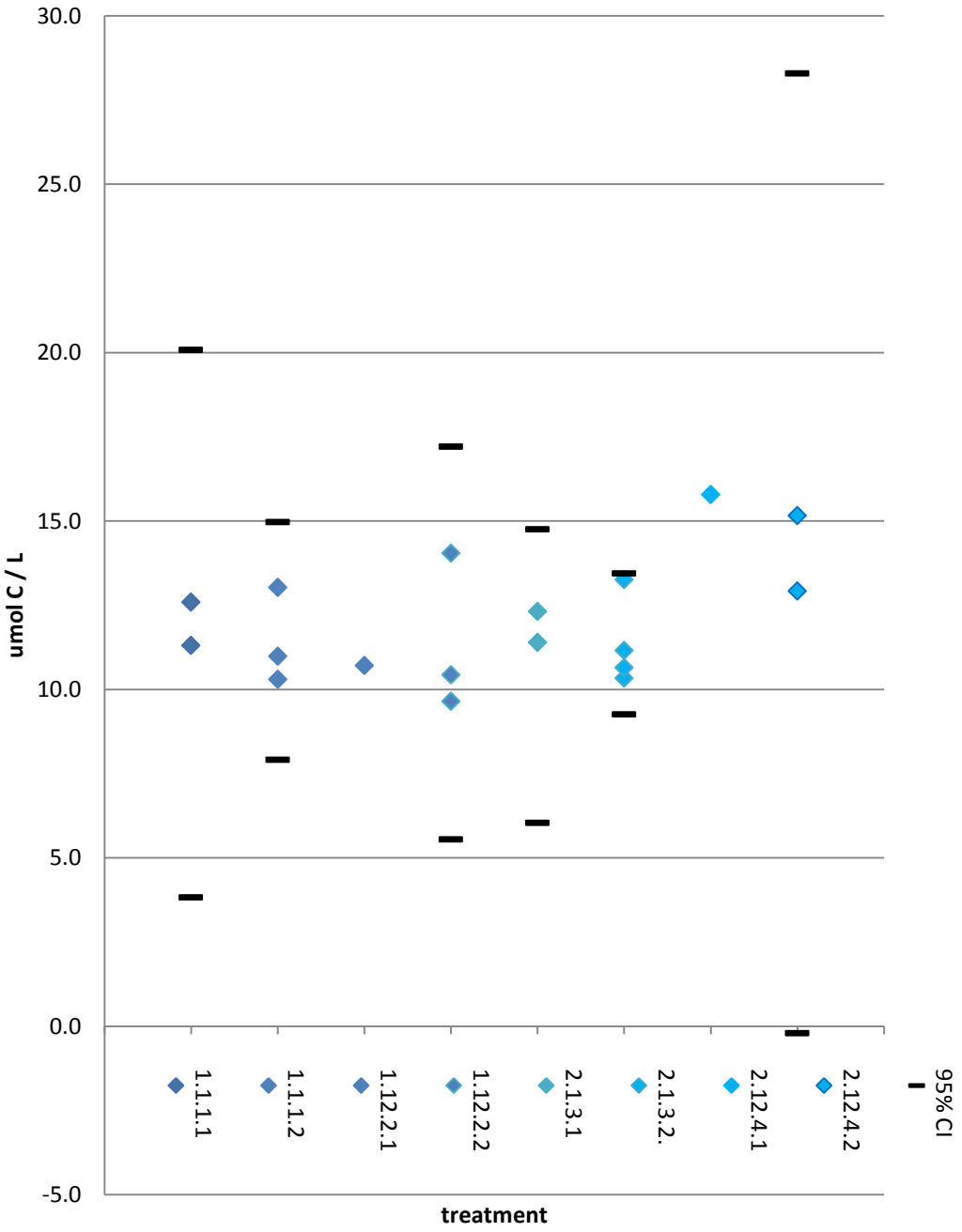



*Figure 22. Variation of POC measurement at site OS June 21 2013. The numbers are in the format: cast.Niskin.canister.filter. 95% confidence intervals of all replicate samples in each treatment are shown as black bars.*

## Flood anniversary UV-Vis

The UV-Vis replicate samples are shown in Table 11. There are fewer UV-Vis replicates (and even fewer for whole water samples) than the DOC and TOC samples, yet some comparisons are possible. Table 12 shows a significant difference at $\alpha = 0.05$ between cast 1 and cast 2 in filtered samples in both e2/e3 and CDOM. These differences can be seen in the confidence intervals of Figure 23 and Figure 24. Only one sample was taken at each site other than OS, so it is not possible to statistically determine difference, but the measurements at WE and DE lie outside of the 95% confidence interval of OS replicates for both e2/e3 and CDOM. The OS replicates are shown in more detail in Figure 25 and Figure 26. CDOM shows significant differences even at $\alpha = 0.01$. While other e2/e3 comparisons were not significantly different, CDOM shows significant differences between different Niskin bottles at $\alpha = 0.01$ for cast 1 and at $\alpha = 0.05$ for cast 2. Despite these differences, the percent standard deviation for all filtered samples (casts 1 and 2) at site OS is only 3% for e2/e3 and 12% for CDOM.

*Table 11. UV-Vis statistics summary for replicate sample sets at site OS on July 21, 2013. See Figure 3 and Figure 4 for summary of samples collected.*

| Filtered | Cast | Niskin | Number of sample vials | | | | Average e2/e3 | Std dev e2/e3 | Average CDOM | Std dev CDOM |
|---|---|---|---|---|---|---|---|---|---|---|
| GF/F | 1 | 1 | 3 | | | | 5.62 | 0.01 | 1559 | 3 |
| GF/F | 1 | 1 | 3+1 | 4 | | | 5.62 | 0.01 | 1560 | 3 |
| GF/F | 1 | 12 | | 2 | | | 5.71 | 0.03 | 1400 | 0.1 |
| GF/F | 1 | 1 & 12 | | 4+2 | 6 | | 5.65 | 0.05 | 1506 | 82 |
| GF/F | 2 | 1 | | 2 | | | 5.31 | 0.04 | 1879 | 0.2 |
| GF/F | 2 | 12 | | 2 | | | 5.38 | 0.13 | 1862 | 5 |
| GF/F | 2 | 1 & 12 | | 2+2 | 4 | | 5.35 | 0.09 | 1870 | 10 |
| GF/F | 1 & 2 | 1 &12 | | | 6+4 | 10 | 5.53 | 0.17 | 1652 | 198 |
| whole | 1 | 1 | | 3 | | | 4.84 | 0.18 | 1779 | 43 |
| whole | 1 & 2 | 1 & 12 | | 3+3 | 6 | | 4.87 | 0.12 | 1838 | 190 |

*Table 12. t-test results for flood anniversary UV vis samples at site OS. Significant differences in mean at $\alpha = 0.05$ are in bold.*

| | | | | | | e2/e3 | | CDOM | |
|---|---|---|---|---|---|---|---|---|---|
| filt | cast | nisk | **compare to** | cast | nisk | sig. diff. of var | T.TEST p-value | sig. diff. of var | T.TEST p-value |
| GF/F | 1 | 1 | | 1 | 12 | yes | 0.153 | yes | **0.0000** |
| GF/F | 1 | | | 2 | | no | **0.003** | yes | **0.0001** |
| GF/F | 2 | 1 | | 2 | 12 | no | 0.546 | no | **0.0339** |
| whole | 1 | | | 2 | | no | 0.928 | no | **0.0148** |

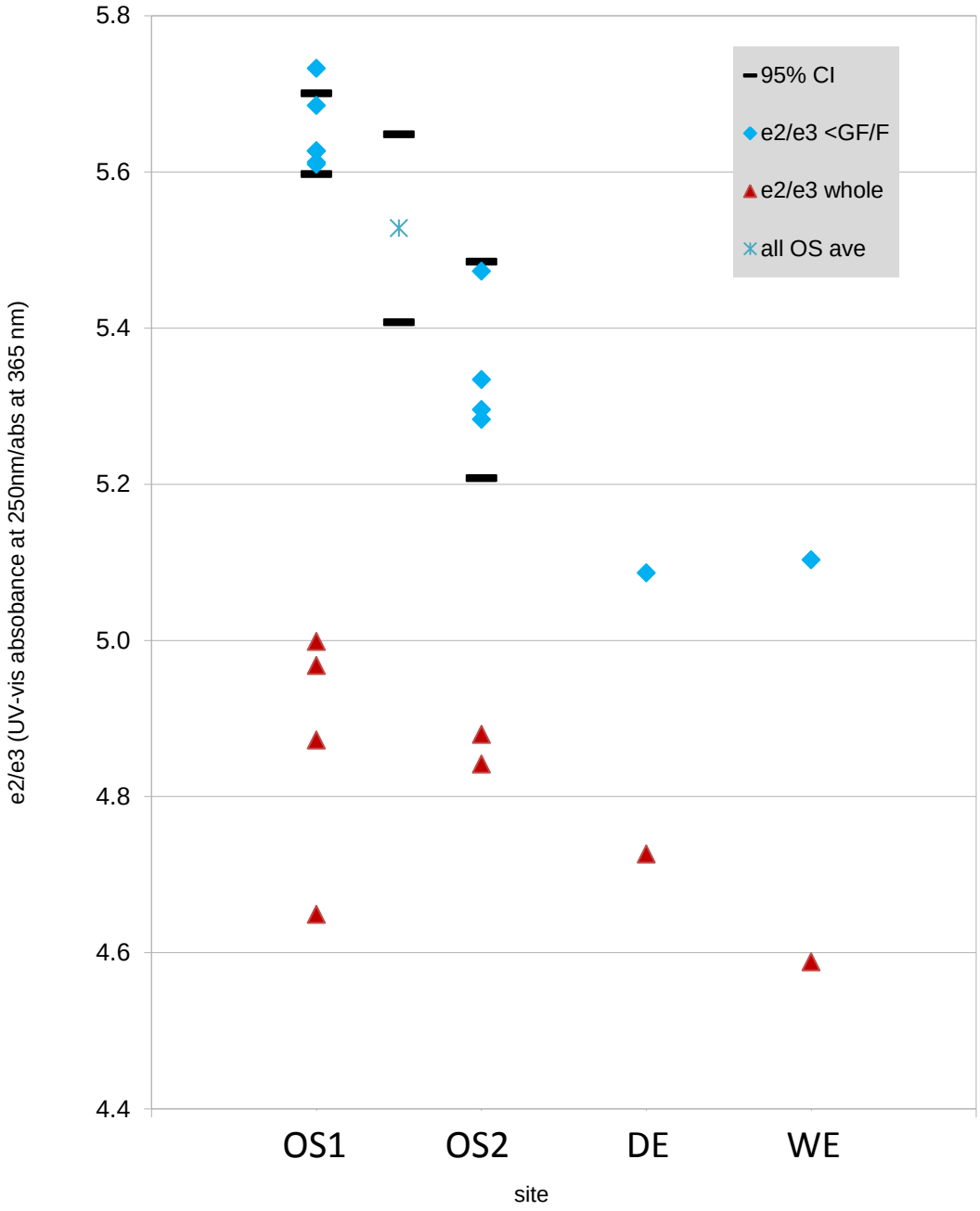



*Figure 23. e2/e3 (UV-vis absorbance at 250nm/abs at 365nm) measurements for all flood anniversary samples at all sites (except M1). Statistics are only for <GF/F samples.*

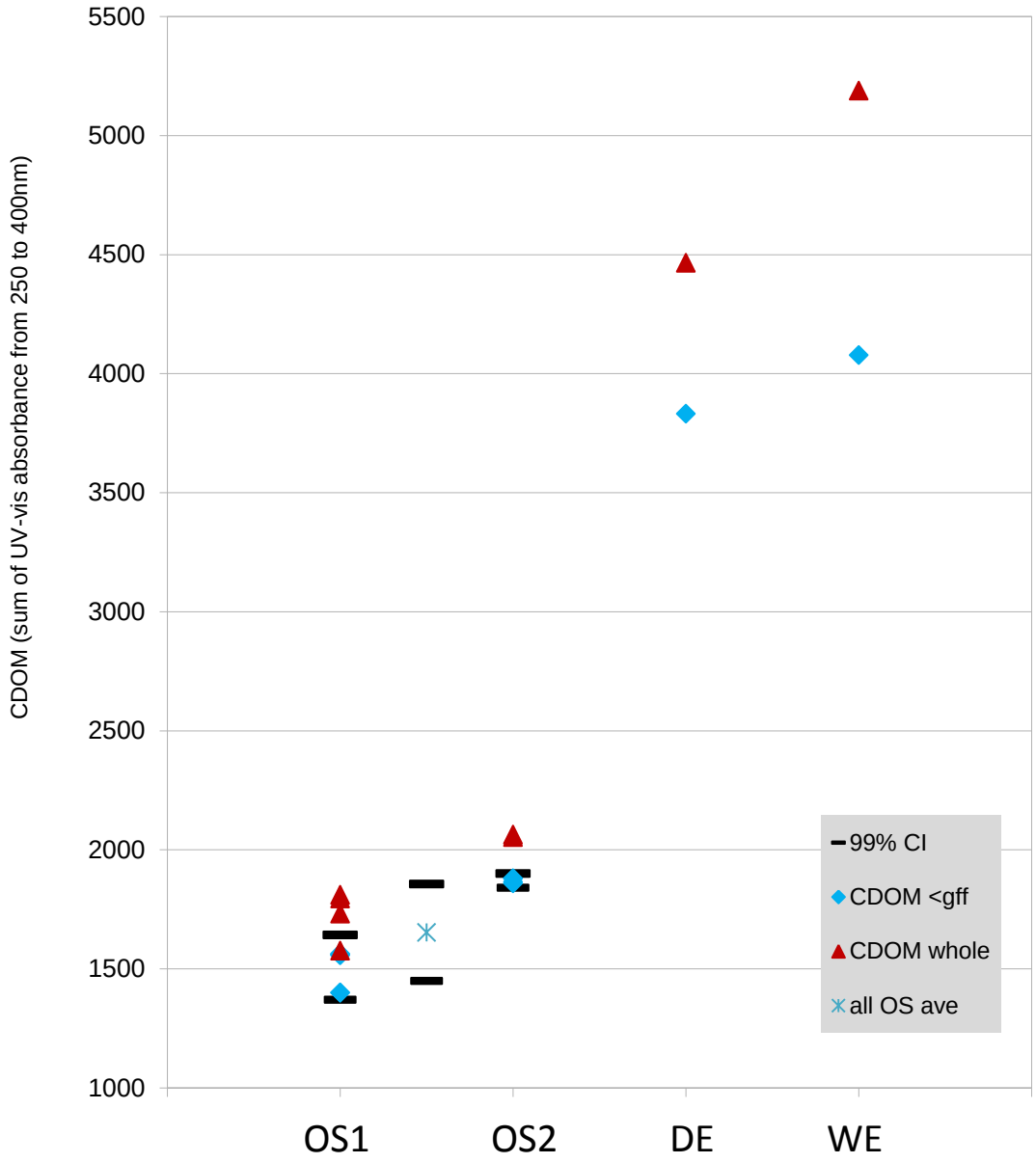



*Figure 24. CDOM (sum of UV-vis absorbances from 250 to 400nm) measurements for all flood anniversary samples at all sites (except M1). Statistics are only for <GF/F samples.*

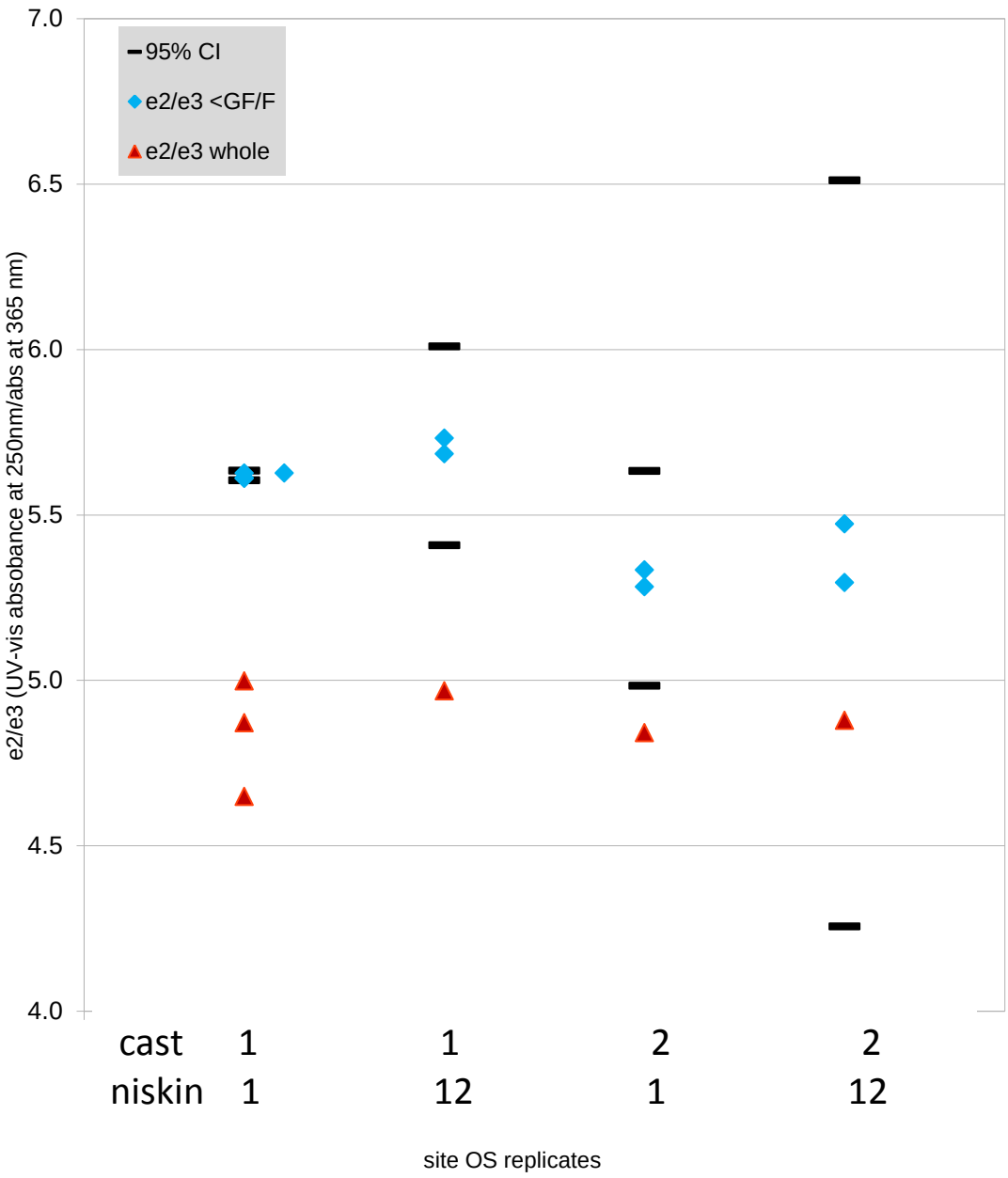



*Figure 25. e2/e3 (UV-vis absorbance at 250nm/abs at 365nm) measurements for all flood anniversary samples at site OS. Confidence intervals are for <GF/F samples.*

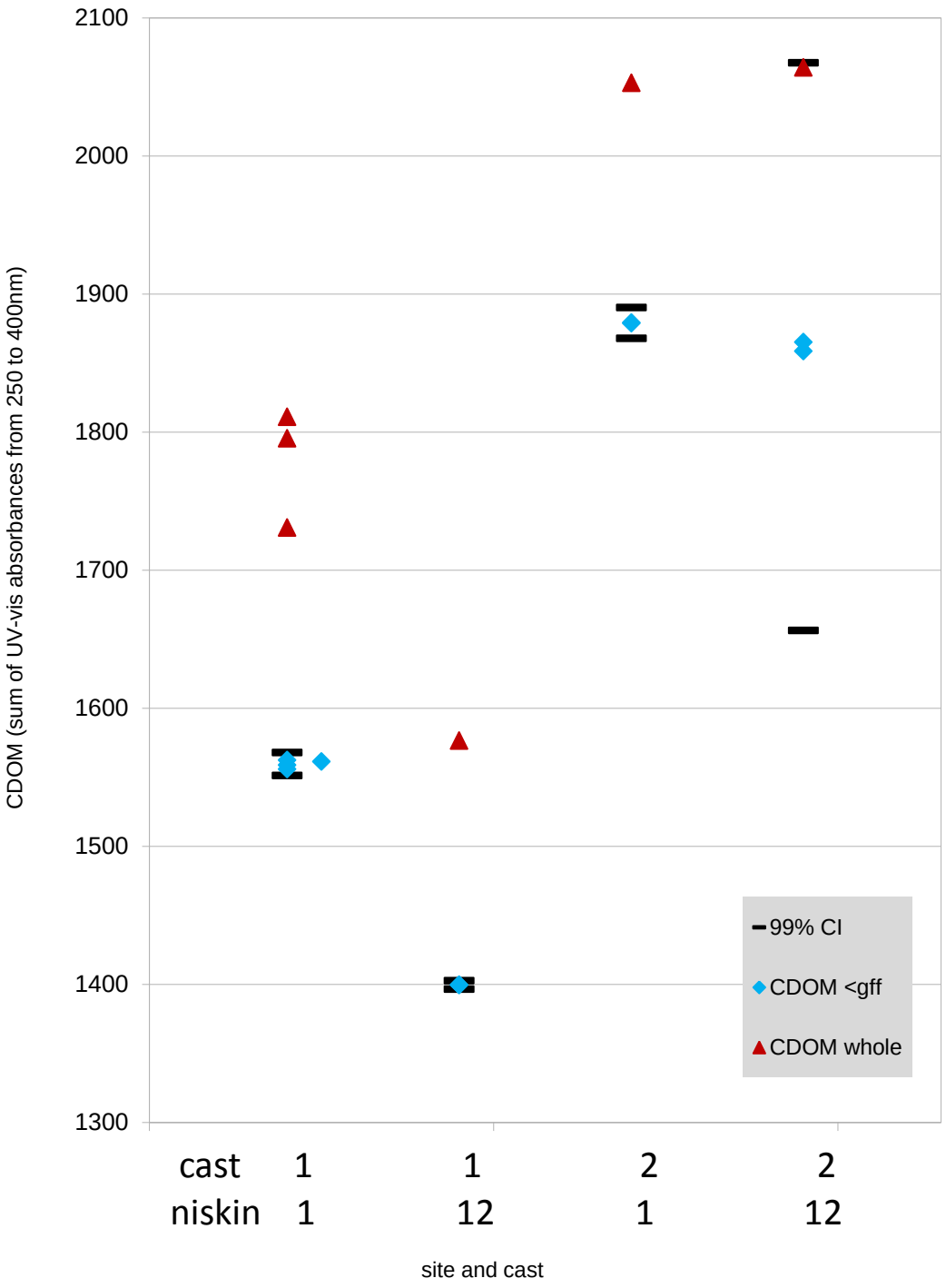



*Figure 26. CDOM (sum of UV-vis absorbances from 250 to 400nm) measurements for all flood anniversary samples at site OS. Confidence intervals are for <GF/F samples.*

## Conclusion

Sampling results at two different casts 0.8 km apart were found to be significantly different at both $\alpha$= 0.05 and 0.10 for DOC, TOC, e2/e3, and CDOM, but not POC. The TOC, DOC and POC average concentration at site OS and variation due to space and time of these two casts was 2.3 +/- 0.2, 2.3 +/- 0.3, and 0.14 +/- 0.02 mg/L respectively. The expected variation due to only sample handling at each cast for DOC and TOC is 0.1 mg/L or 5% relative standard deviation. For POC, the expected variation is 0.02 mg/L or 14% relative standard deviation (Table 13). As an additional check on our data (which investigates possible carbon addition or loss from sample handling) we performed a mass balance calculation. This check that the total organic carbon should equal the dissolved organic carbon plus the particulate organic carbon supports the data presented here.

*Table 13. Summary of all organic carbon measurements for Site OS cast1 and cast 2.*

| | Cast 1 | | Cast 2 | | |
|---|---|---|---|---|---|
| | Average | Std dev | Average | Std dev | units |
| DOC | 2.1 | 0.1 | 2.5 | 0.1 | mg/L |
| POC | 0.14 | 0.02 | 0.15 | 0.02 | mg/L |
| TOC | 2.2 | 0.1 | 2.5 | 0.1 | mg/L |
| DOC+POC = TOC | 2.2 | | 2.7 | | mg/L |
| CDOM filtered | 1506 | 83 | 1870 | 10 | |
| e2/e3 filtered | 5.65 | 0.05 | 5.35 | 0.09 | |

## Works Cited


Dalzell, B. J., E. C. Minor and K. M. Mopper. "Photodegradation of estuarine dissolved organic matter: a multi-method assessment of DOM transformation." *Organic Geochemistry* 40 (2009): 243-257.

Del Vecchio, R and N.V. Blough. "On the origin of optical properties of humic substances." *Environ. Sci. Technol.* 38 (2004).

Green, Sarah A. and Neil V. Blough. "Optical Absorption and Fluorescence Properties of Chromophoric Dissolved Organic Matter in Natural Waters." *Limnology and Oceanography* 39.8 (1994): 1903-1916.

Miller, J. C. and J. N. Miller. *Statistics for Analytical Chemistry*. Chichester, UK: Ellis Horwood Limited, 1988.

Minor, E. C. and B. Stephens. "Dissolved organic matter characteristics within the Lake Superior watershed." *Organic Geochemistry* 39 (2008): 1489-1501.

Minor, E. C., B. B. Forsman and S. J. Guildford. "The effect of a flood pulse on the water column of western Lake Superior, USA." *Journal of Great Lakes Research* 40 (2014): 455-462.

Minor, E. C., et al. "Evaluating the photoalteration of estuarine dissolved organic matter using direct temperature-resolved mass spectrometry and UV-visible spectroscopy." *Aquat. Sci.* 69 (2007): 440-455.

Mopper, K., et al. "Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle." *Nature* 353 (1991): 60-62.

Peltzer, E., et al. "A comparison of methods for the measurement of dissolved organic carbon in natural waters." *Marine Chemistry* 54 (1996): 85-96.

Peterson, B. M., et al. "Spatial and Temporal Distribution of Singlet Oxygen in Lake Superior." *Environ. Sci. Technol.* 46 (2012): 7222-7229.

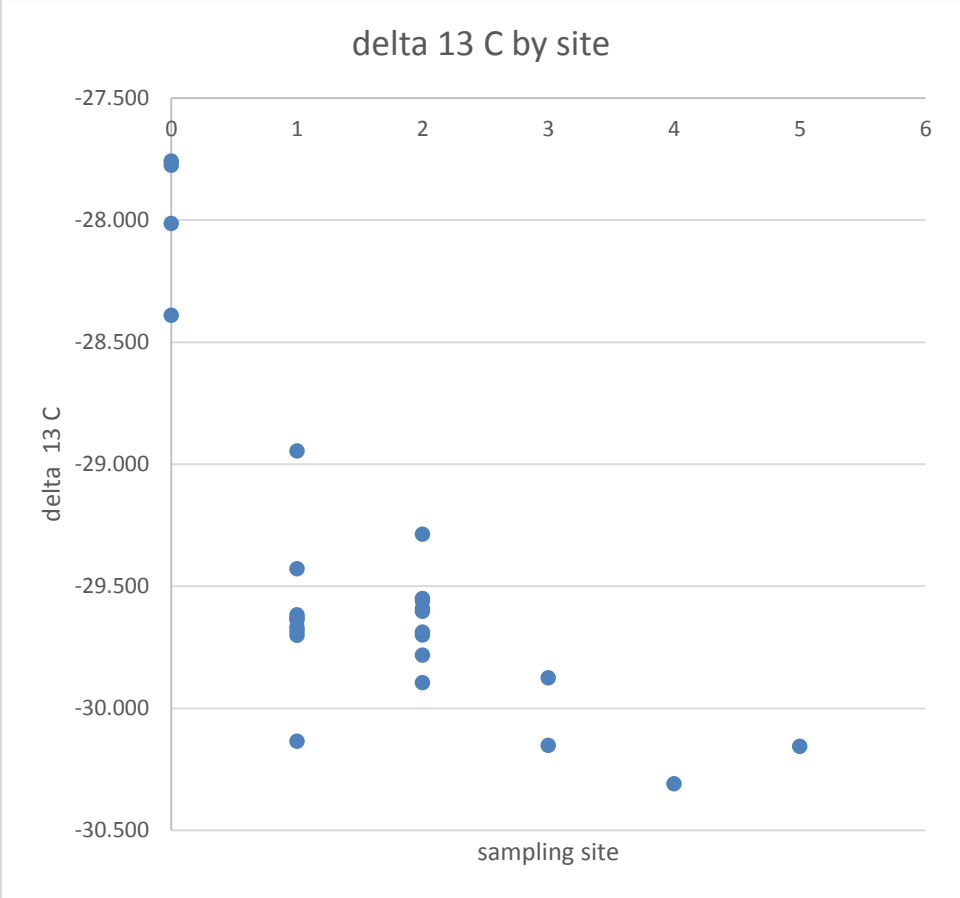
Peuravuori, J. and Pihlaja, K. "Molecular size distribution an spectroscopic properties of aquatic humic substances." *Analytica Chimica Acta* 337 (1997): 133-149.

Sharp, J. H., et al. "Analyses of dissolved organic carbon in seawater: the JGOFS EqPac methods comparison." *Mar. Chem* 48 (1995): 91-108.

—. "Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials." *Marine Chemistry* 77 (2002): 239-253.

Zigah, P. K., et al. "Radiocarbon and stable carbon isotopic insights into provenance and cycling of carbon in Lake Superior." *Limnology and Oceanography* 56 (2011): 867-886.

# Appendix




*Stable carbon variation by site.  0 blank, 1 OS1, 2 OS2, 3 DE, 4 WE, 5 M1.*

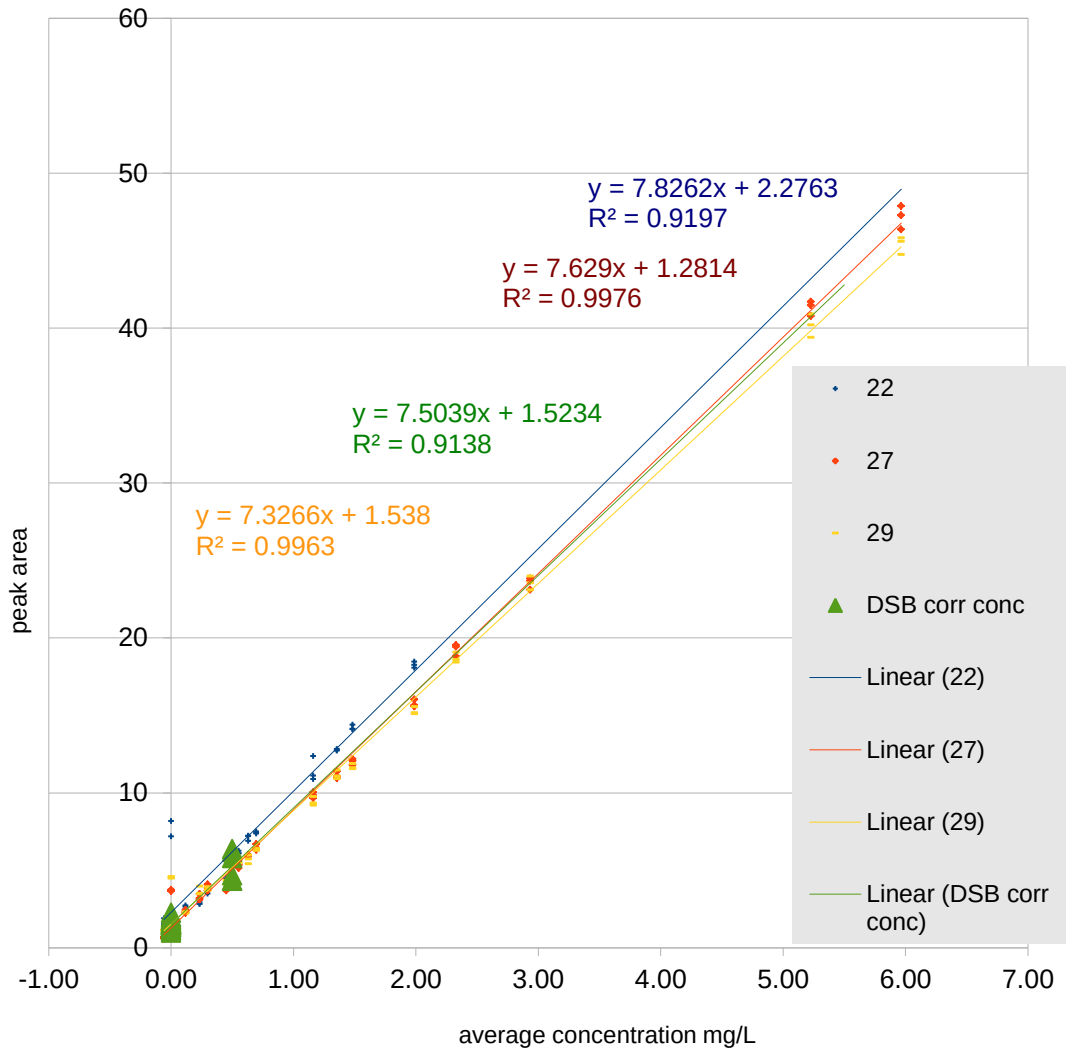



*Figure 27. A comparison of the three calibration curves used for TOC runs of Flood anniversary samples. The y-axis shows the variability of peak area of replicate injections of TOC calibration curve standards used for flood anniversary analysis. The x-axis is the average concentration of each calibration curve standard calculated by DSB correction. This is not a calibration curve. Analysis on 1/22/14 is labeled as 22, 1/27/14 is 27, 1/29/14 is 29.*