# HPLC Phytoplankton Pigment Measurements: Monovinyl and Divinyl Chls *a* and Chl *b* Biases

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# HPLC $C_{18}$ and $C_8$ History

SIMBIOS Funding – $C_{18}$ Method (Wright *et al.* 1991)

- Processed 7,582 samples in 3 years.
- One of 4 (Group A) with the lowest uncertainties under SeaHARRE-2 (Hooker *et al.* 2005).

MODIS Funding – $C_8$ Method (Van Heukelem & Thomas 2001)

- Main difference is the physical separation of mono- and divinyl Chls *a* on the HPLC column.
- Method also requires that the HPLC system pick up the buffer and sample in "Segmented Train" (no mixing prior to injection). This requirement was not know until comparative analyses had been performed.

# $C_8$ Method Changes for CHORS HPLC

The length of time the buffer is contact with the sample reduces pigment concentration and increases uncertainties (solution; minimize mixing time).

The used on Vitamin E acetate as an internal standard did not work (solution; change to *trans*-β-apo-8'-carotenal, Dr. Ray Barlow's suggestion ).

Even with these changes we could not duplicate the low uncertainties found by Van Heukelem and Thomas, but felt that the $C_8$ method, which was the current method of choice by the oceanographic community, would still produce high quality HPLC pigment data on the CHORS HPLC System.

# SeaHARRE-3 Intercalibration Exercise (Summer 2005)

Because of the problems with the $C_8$ method and our HPLC, we decided to analyze side-by-side pigment & std samples from SeaHARRE-3 on both methods.

Preliminary results showed that there was a difference (for only divinyl chl *a*, monovinyl chl *a* and chl *b*). Since we did not have the SH3 results, it could not be determined which method was in error.

Since SH3 field samples had a limited concentration range (0.02–1.4 mg $m^{-3}$) and only included 24 triplicate samples, we decided to continue running both methods on some existing MODIS samples (546 samples from a variety of areas and depths; D. Clark, D. McGillicuddy, G. Mitchell and M. Moline).

# SeaHARRE-3 Preliminary Results

A poster was presented (Ocean Sciences Meeting in Feb 06) showing that the two methods produced different results for the 3 compounds in question and that the differences were log-linear.

In Mar 06 the SH3 results were made available, thus showing that the new $C_8$ method used on the CHORS HPLC system had a bias for monovinyl chl *a*, divinyl chl *a* and chl *b*.

# Log-Linear Relationship Between Methods for Monovinyl Chl *a*

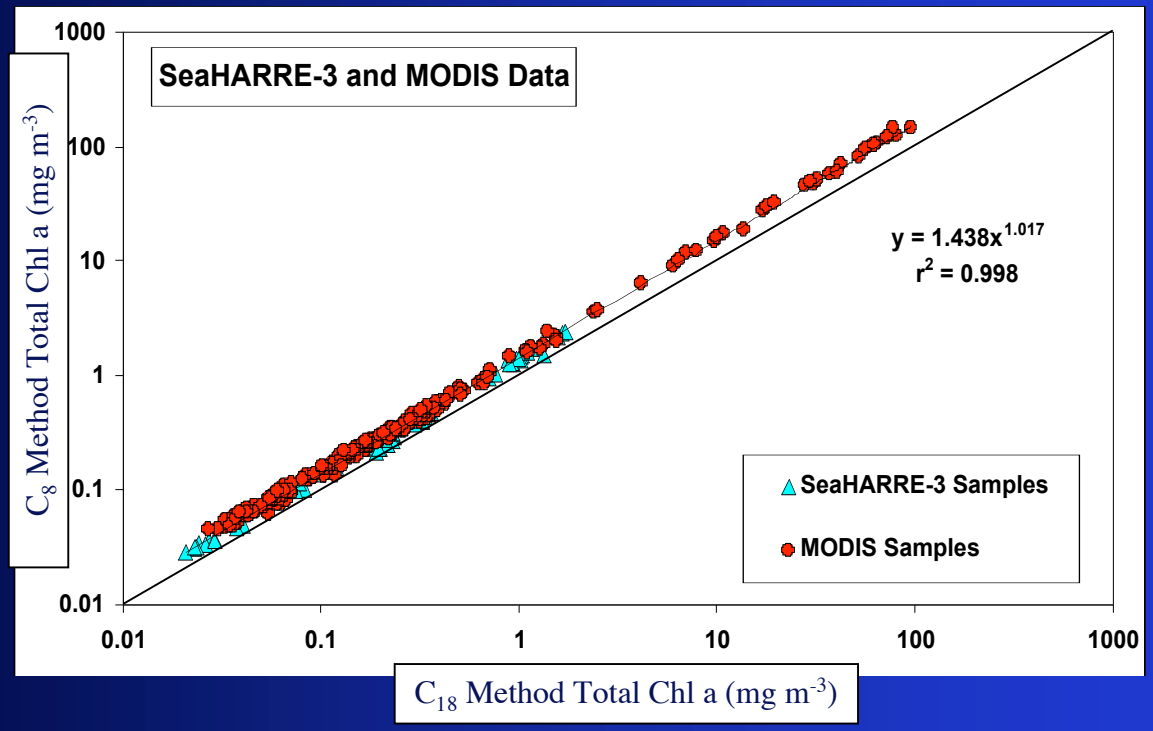



*Fig 1. Log-linear relationship between $C_{18}$-measured total chlorophyll a and $C_8$-measured total chlorophyll a for SeaHARRE-3 (turquoise filled triangles) and MODIS (red filled circles) samples.*

# Log-Linear Relationship Between Methods for Monovinyl Chl *a*

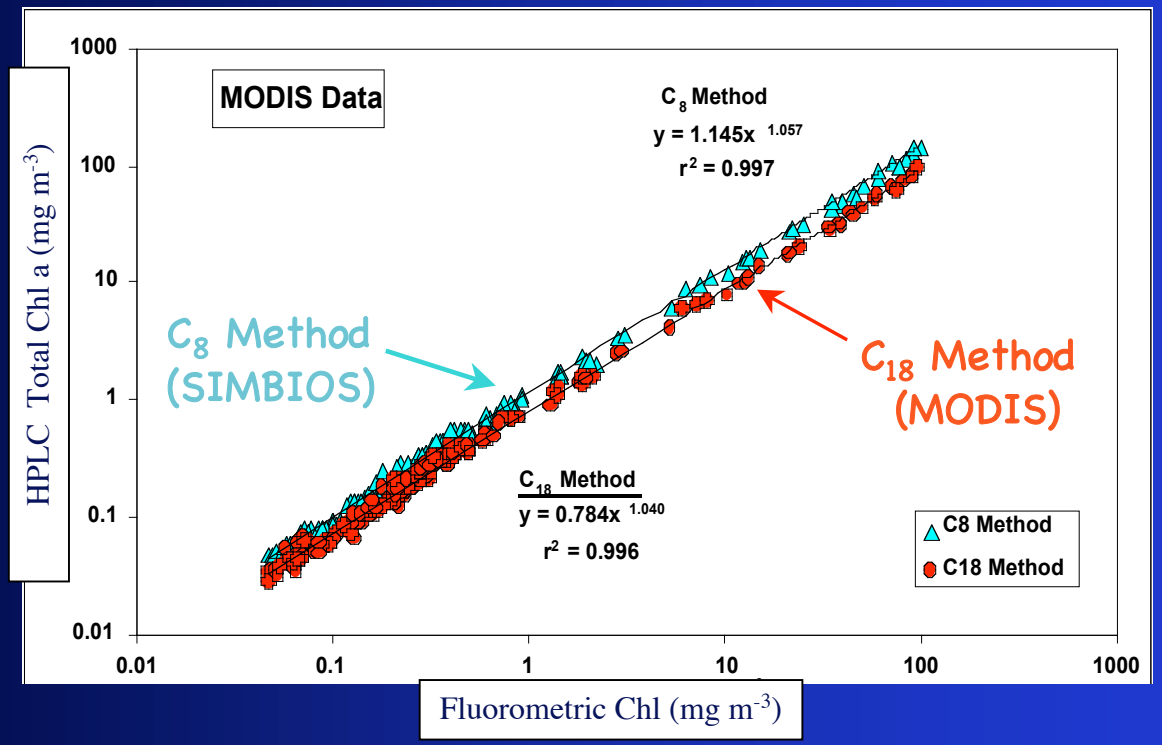



*Fig. 2. Log-linear relationship between HPLC total chl a and fluorometric chl for MODIS samples ($C_8$-turquoise filled triangles; $C_{18}$-red filled circles).*

# Pigment Bias for $C_8$ Method on CHORS HPLC System

| Pigment | Chl a | DV a | Chl b | Chl c2 | Diad | Fuco | Hex | Zea |
|---|---|---|---|---|---|---|---|---|
| Group A **Ave** | **7.436** | **1.123** | **2.850** | **0.322** | **0.621** | **0.301** | **0.514** | **0.568** |
| Group A **Stdev** | 0.380 | 0.035 | 0.168 | 0.147 | 0.037 | 0.013 | 0.016 | 0.019 |
| Group A **CV** | 5.1 | 3.1 | 5.9 | 45.7 | 5.9 | 4.4 | 3.1 | 3.3 |
| CHORS **Ave** | **15.252** | **1.799** | **4.963** | **0.307** | **0.645** | **0.256** | **0.460** | **0.544** |
| **A:CHORS** | **0.488** | **0.624** | **0.574** | **1.051** | **0.964** | **1.175** | **1.117** | **1.045** |

Table 1. Comparison of CHORS $C_8$ method for SH3 pigment standards (SH3 field samples were not included because of the variability between replicates).

Only monovinyl (Chl *a*), divinyl (DV *a*) Chls *a* and Chl *b* showed statistically significant biases.

# $C_8$ Bias Consistency

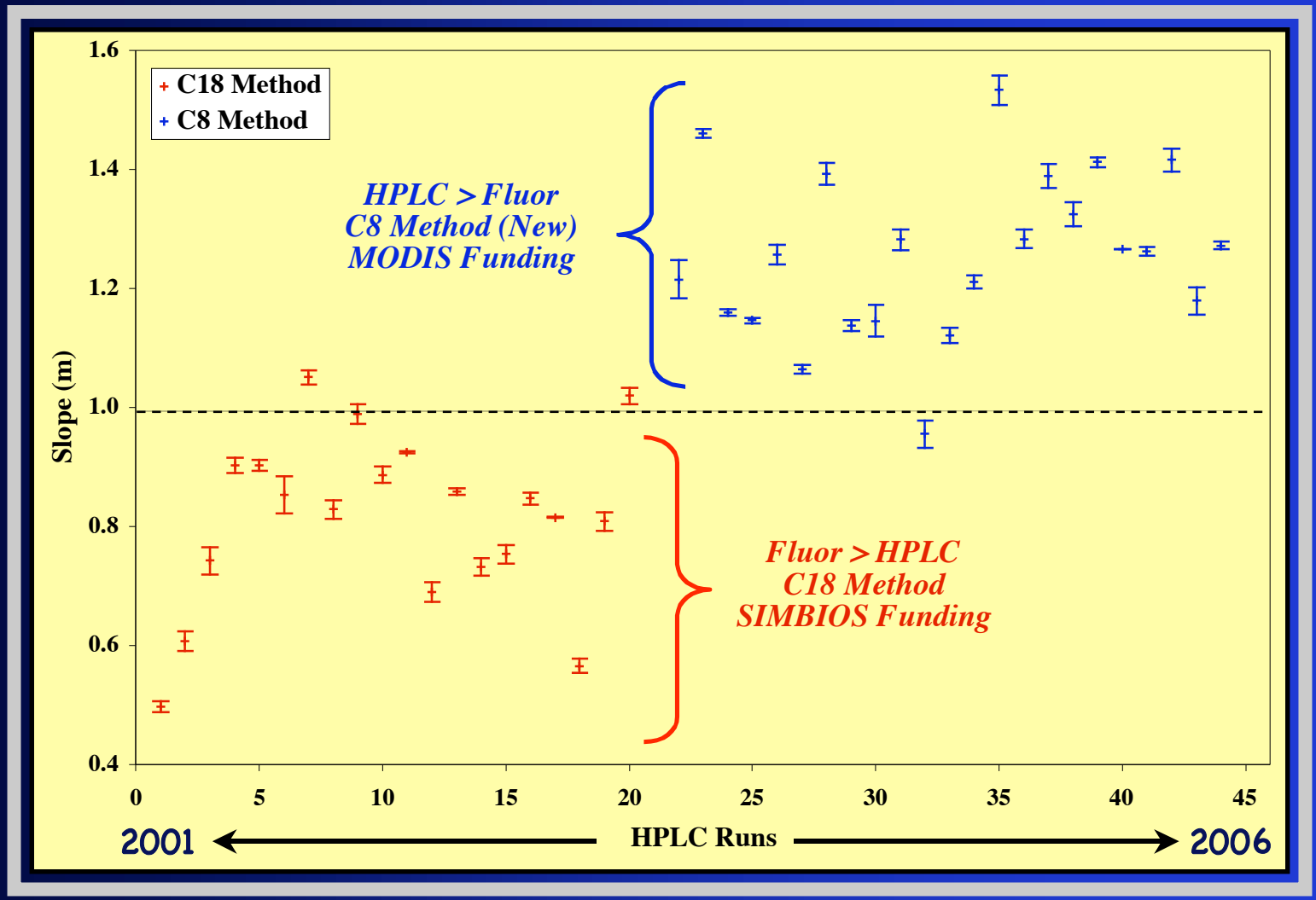
SH3 samples and standards were analyzed in Aug-Sep 05, along with the 546 MODIS samples. The best estimate of the bias is obtained with the pigment standards (CHORS vs SH3 Averages).

The SH3 field samples showed a similar bias, except that the variance in the estimate was larger because the comparison was between replicate filtered samples.

The question to be asked is, "Has the bias been consistent throughout the processing period (Dec 04-Jan 06) for the MODIS pigment samples?"

# HPLC and Fluorometric Comparison

## (HPLC Chl a = m Fluor Chl + b)



# Conclusions

The uncertainty in the results for the $C_8$ method was not random but followed an log-linear bias.

The problem with the $C_8$ method occurred throughout the MODIS pigment data processing period.

The pigment bias for the 3 compounds affected by $C_8$ error can be corrected for by scaling the 3 compounds by the coefficients listed in Table 1.

# Future Analyses

1. Comparison of duplicate HPLC samples processed by Horn Point that can be used to evaluate the consistency of the bias in other waters and depths, as well as during a different analysis periods for CHORS. Data provided by A. Mannino. Currently, we have only the fluor data to compare the consistency of the bias.

2. An independent review of the possible reasons for the bias, as well as verification of the approach proposed by CHORS to determine bias consistency and correction factors for the 3 compounds in question (monovinyl chl *a*, divinyl chl *a* and chl *b*).