Precision Assessment of the HPLC Phytoplankton Pigment Dataset Analyzed by NASA to Quantify Global Variability in Support of Ocean Color Remote Sensing

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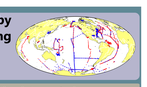

Abstract

The ability to generate chlorophyll *a* (Chl *a*) assessments from ocean color orbital sensors, such as VIIRS and MODIS, that satisfy the requirements to be climate-quality data record (CDR) quality is contingent in part on the quality of the *in situ* ground or sea truth observations that serve as datasets for vicarious calibration and algorithm validation activities. NASA has a mandate to collect, analyze, and distribute *in situ* data of the highest possible quality with documented uncertainties and in keeping with established performance metrics. Using a dataset of over 18,000 HPLC phytoplankton pigment samples representing water collected in all major ocean basins analyzed a central laboratory (Field Support Group (FSG) of the Ocean Ecology Laboratory (OEL) at NASA Goddard Space Flight Center (GSFC)), we performed an assessment of the global precision among sample replicates of Chl *a* as well as major accessory pigments. We investigated the impacts of filtration volume, water basin, collection technique, pigment concentration, and different filtration volumes for replicate filters on replicate filter precision, as well as investigating any pigment-specific differences. Our results quantify sample variability with the goal of understanding any systemic biases or biogeographic influences.

Dataset/Background

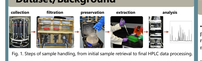

Fig. 1. Steps of sample handling, from initial sample retrieval to final HPLC data processing.

The quality of any chlorophyll *a* climate-quality data record (CDR) from ocean color orbital sensors is contingent on the quality of the *in situ* validation datasets. Meeting remote sensing accuracy requirements is more likely when pigments measurements are based on replicate, not single observations, particularly for pigments that exhibit poor precision[1]. Our laboratory follows a rigorous QA/QC program, which renders highly precise and accurate results.

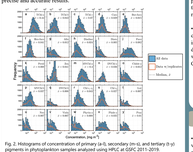

Fig. 2. Histograms of concentration of primary (a-l), secondary (m-s), and tertiary (t-z) pigments in phytoplankton samples analyzed using HPLC at GSFC 2011-2018.

The data used for this analysis represents:

- Type I and II water, from multiple photic zone depths
- 8 years of analysis
- 36 Principal Investigators
- 4873 replicate filters (blue dots on map in title banner) from 12,594 unique samples (all dots on map in title banner)

Abbreviations/terms

Primary Pigments [1,2] (%Pig ≥ 1 in at Chlorophyll-A containing samples) used in phytoplankton analysis

TChl *a*	Total Chlorophyll *a*
TChl *c*	Total Chlorophyll *c*
TChl *b*	Total Chlorophyll *b*
Caro	Carotenoids
Lut	(Lu and Lz) Lutein
But-fuco	19' Butanoyloxyfucoxanthin
Hex-fuco	19' Hexanoyloxyfucoxanthin
Allo	Alloxanthin
Diato	Diatoxanthin
Perid	Peridinin
Fucox	Fucoxanthin
Zea	Zeaxanthin

Secondary pigments [1,2] (pigments that factor into calculation of PPg products)

MVChl *a*	Monovinyl Chlorophyll *a*
DVChl *a*	Divinyl Chlorophyll *a*
Chlide *a*	Chlorophyllide *a*
MVChl *b*	Monovinyl Chlorophyll *b*
DVChl *b*	Divinyl Chlorophyll *b*
Chl *c*3	Chlorophyll *c*3
Chl *c*2	Chlorophyll *c*2 + MGDVP
Chl *c*1	MV chlorophyll *c*1 + chlorophyll *c*1

Tertiary pigments [1,2] (pigments less frequently used in analysis, usually present in smaller amounts)

Lut	Lutein
Neo	Neoxanthin
Viola	Violaxanthin
Phytin *a*	Phytylrhodin *a*
Phytol *a*	Phytol *a*
Pras	Prasoxanthin

Pigment Sums [1]

TChl	total chlorophylls
PPg	phytoplankton pigments
PPC	phytoplankton carotenoids
PSP	phytoxanthin pigments
PSC	phytosterol carotenoids
Tacc	total accessory pigments
Tdp	total diagnostic chlorophyll pigments

CI = confidence interval
CV% = coefficient of variation
LOD = limit of detection
LOESS = locally estimated scatterplot smoothing

Precision: Probability Distribution

- Mean precision for almost half of the pigments is < 5%, and only Chlide *a* and Phide *a* have mean precisions > 10%.
- The probability of observing a set of replicates with precision >10% is less than 4% for all pigments, with the exception of Chlide *a* and Phide *a*.

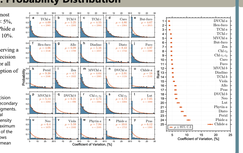

Fig. 3. Histograms of precision (CV%) for primary (a-l), secondary (m-s), and tertiary (t-z) pigments. Red line is the exponential probability distribution density function with maximum-likelihood estimate (mle) of the mean (μ). Right panel shows pigments ranked by mle mean precision.

Precision: Effect of Concentration

- Locally averaged precision for TChl *a* remains below 5% for most of its dynamic range.
- Locally averaged precision for most individual pigments is < 10%.
- Sums of all pigment categories show good precision (CV% < 5) over their entire concentration ranges.
- Precision degrades as concentrations approach instrument limits of detection (~0.001 mg m^{-3}), when considering the CV% > 0 subset.

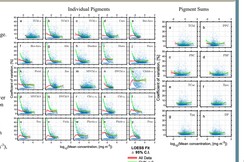

Fig. 4. Precision (CV%) among sample replicate sets versus $\log_{10}$-transformed mean pigment concentration for each set for primary (a-l), secondary (m-s), and tertiary (t-z) pigments and pigment sums (right). Lines depict LOESS fits for all the data (red), and CV% > 0 (green). Shaded areas are the 95% confidence intervals for each fit. The CV% > 0 results ignore the data from replicates that have identical concentration values (i.e., precision = 0%). Separate evaluation of this CV%=0 subset was carried out to explore the possible drivers of that variability, which otherwise was obscured by assessing them in conjuction with replicates for which CV% = 0.

Precision: Effect of Filtered Volume

- Small filtration volumes worsens precision for almost all pigment products, including pigment sums.
- For most pigments, CV% generally remains stable or modestly improves as filtration volume increases. When considering the CV% > 0 subset, some primary (Allo, Diato, and Perid), and a range of tertiary pigments (Lut-Pras), exhibit noticeable degradation in CV% as filtration volume increases. Summing pigments dampens effects of the filtration volume extremes..

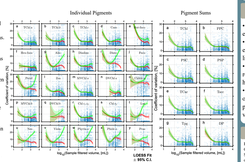

Fig 5. Precision (CV%) among replicate sets versus $\log_{10}$-transformed mean filtered volume of each replicate for primary (a-l), secondary (m-s), and tertiary (t-v) pigments and pigment sums (right). Lines depict LOESS fits for all the data (red), and CV% > 0 (green). Shaded areas are the 95% confidence intervals for each fit.

Multivariate analysis

- Pigment concentration generally ranked as the most important factor affecting precision, particularly when all data were used. However, the direction of the effect changed from positive to negative when the zero data was removed.
- No model explained more than 40% of the variance, with more explaining only between 10-30%, and in some cases < 1%.

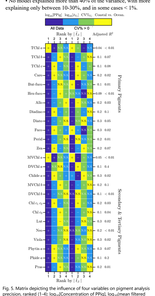

Fig. 5. Matrix depicting the influence of four variables on pigment analysis precision, ranked (1-4). $\log_{10}$(Concentration of PPg), $\log_{10}$(mean filtered volume), precision of filtration volume, and a variable denoting the coastal or oceanic (> 200 km coastline) origin of each set of replicate samples. The rankings were derived from multivariate least-squares regression analyses performed on the data (all data (left), and CV% > 0 subset of the data (right columns). The symbol (+/-) denotes if the effect was positive or negative (i.e., CV% increased or decreased, respectively). NS denotes that the variable had no significant effect on the linear model at p < 0.05 level. The two columns of R^2 on the right correspond to each of the two regressions for each pigment using all the data, and CV% > 0, respectively.

Conclusions/Future directions

- Analysis precision for phytoplankton pigments at GSFC exceeds performance metrics needed for satellite validation.
- Of the variables examined, concentration has the largest effect on precision. However, the effect is modest and generally limited to the range approaching instrument limits of detection.
- Improving precision at low concentrations will require more replicates per sample (n > 2) and improvement in HPLC hardware and methodology.
- Either a different model should be formulated, there are other unexamined factors that have a greater influence on precision, or most of the uncertainty seen is due to natural variability and random errors field and laboratory processing.

Acknowledgements

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