A Parametric Correction Scheme for Anomalous HPLC Results

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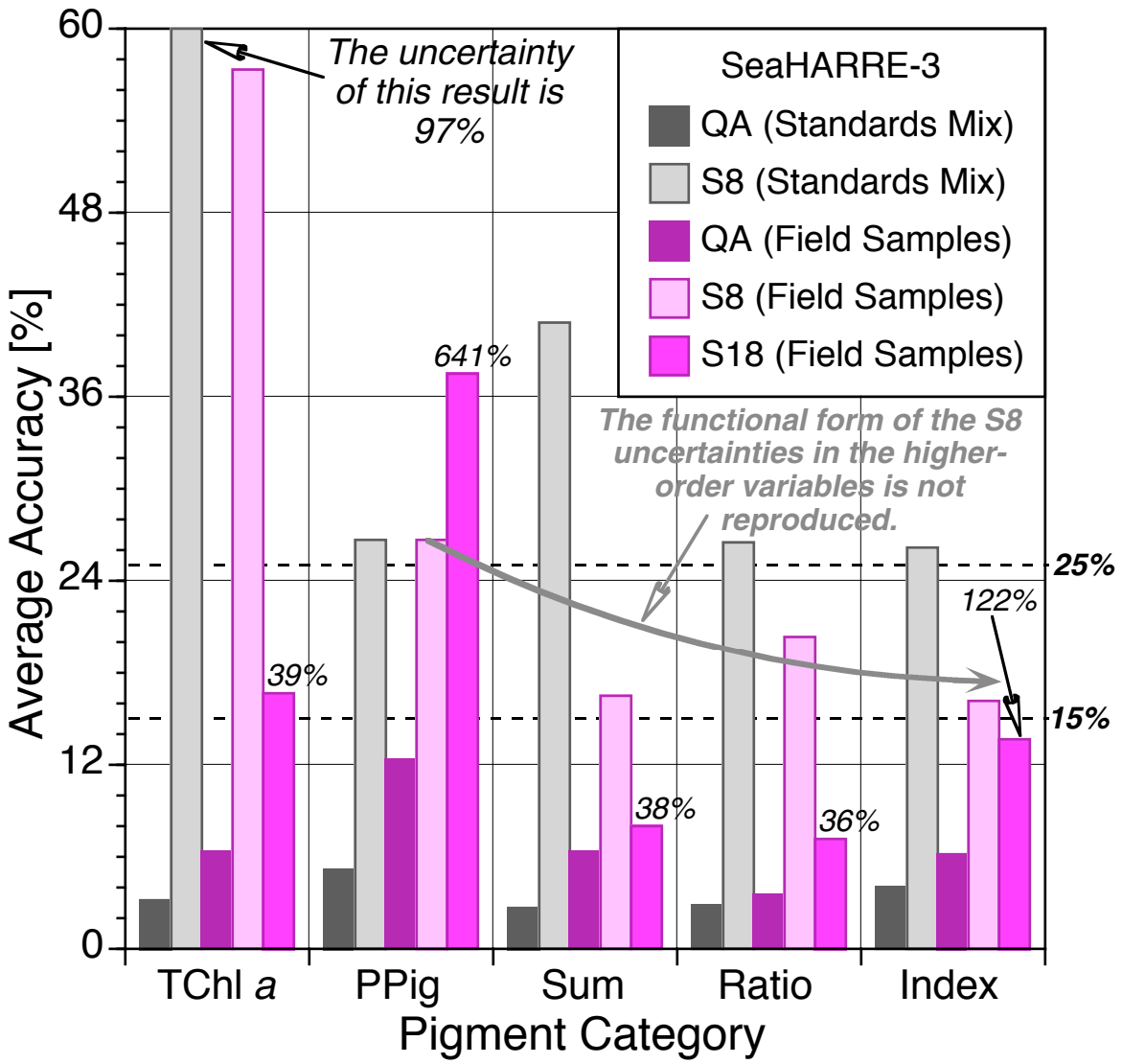
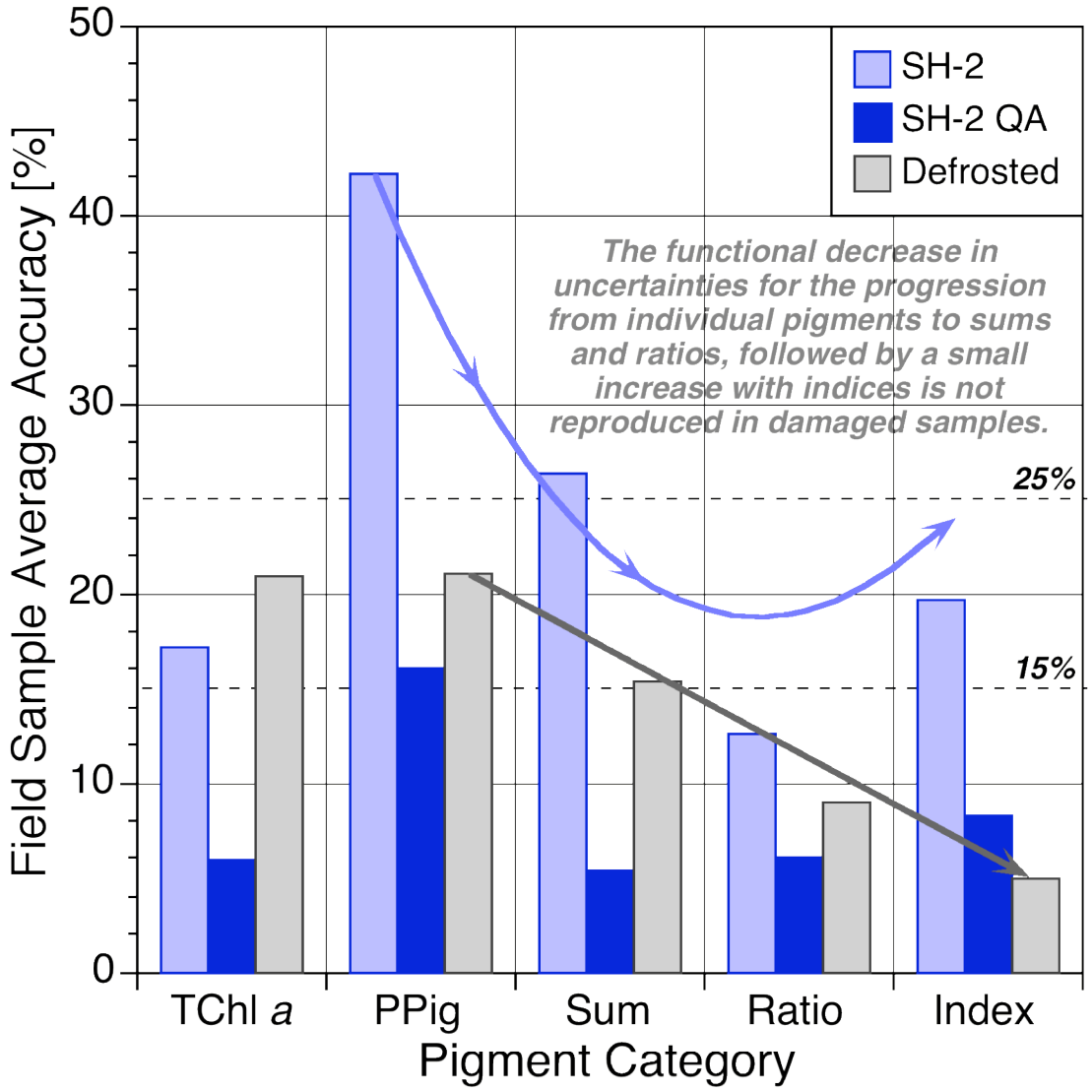
A Summary of the CHORS Results in SeaHARRE-3 (For TChl *a* Spanning 0.02–1.37 mg m^{-3})

The SeaHARRE-3 results are divided into methods that were properly validated or *quality assured* (dark bars) and those that were not (light bars). For the latter, the worst-case average result is shown above the bar. CHORS executed two methods based on a C_8 and a C_{18} column, denoted S8 and S18, respectively, and both have significant problems: *the new S8 method has poor TChl a and nearly adequate PPig results, while the old S18 method has the opposite*. Higher-order data products are not as notably degraded, but the functional form of the S8 uncertainties is corrupted.



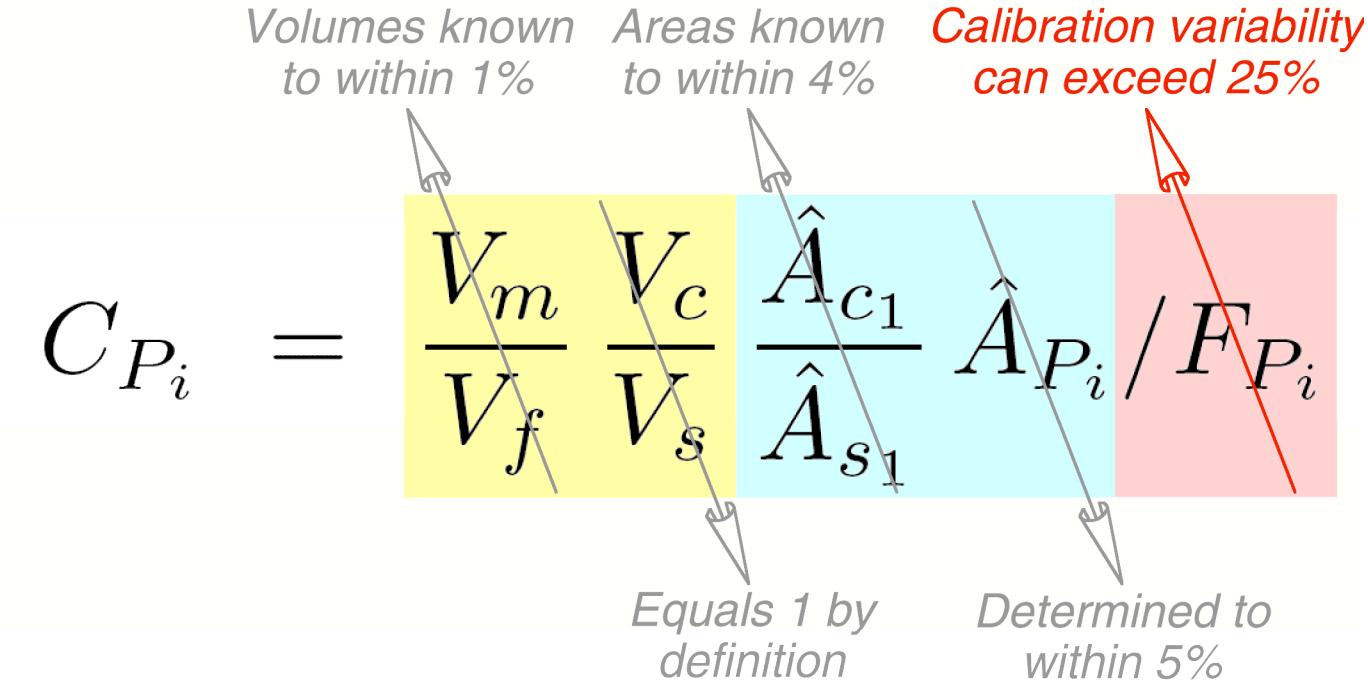
An Important Clue from an Unequivocally Damaged Set of Samples

During SeaHARRE-2, one of the QA laboratories analyzed a set of unequivocally damaged samples (they were defrosted during shipping). The results showed a QA laboratory analyzing bad samples was superior to a method lacking a proper QA scheme and analyzing good samples. This was confirmed by the precision data as well. An important aspect of the results, however, was that the functional form of the higher-order variables was not properly reproduced in the damaged samples. Consequently, this is now a test to look for aberrant data or analyses.



The Parameters Describing the CHORS Quantitation Equation

The quantitation of pigments using the CHORS methodology involves the use of volumes, peak areas, and an inverse response factor (the calibration term). The experiments and analysis conducted at CHORS from 22–25 June (inclusive) showed the following: a) the volumetric terms *not involved with calibration* are known to within 1%; b) the peak areas associated with the natural samples and the internal standard, appear to be known to within 4%, and c) the calibration process is inadequate for calibration and validation activities and has calibration-to-calibration variability exceeding 25%.



The Parametric Correction Scheme

Because of the collection of duplicate seawater samples by A. Mannino and M. Russ (MR) as part of coastal investigations with S. Hooker (MODIS investigator), as well as the participation of CHORS in the SeaHARRE activity (SH-3), three data sets could be assembled wherein CHORS results could be compared to the corresponding analyses by HPL. Using the HPL results as truth (whose accuracies are within the current state of the art), the original CHORS calibration can be replaced with a *parametric* calibration derived from the HPL results. The HPL calibration uncertainty is to within 3% (95% confidence) over an 18-month time period.

The correction process involves matching the quantitated values from CHORS and HPL on a pigment-by-pigment and sample-by-sample basis. In each instance, the original CHORS calibration is removed and a new calibration (inverse response) factor is computed by assuming the HPL data are correct.

The new CHORS calibration (inverse response factor)

The original CHORS quantitation (partially correct)

The corresponding HPL quantitation (completely correct)

$$F_{P_i}^{\text{new}} = C_{P_i}^{S} F_{P_i}^{\text{old}} / C_{P_i}^{H}$$

Removal of the original CHORS calibration (the primary source of inaccuracy)

The Final C_8 Inverse Response Factors Derived Using the Parametric Correction Scheme

The final inverse RFs were derived using the following steps:

- Individual inverse RFs were computed from the three duplicate data sets for all pigments and all samples for which the concentration of the pigment was at least 0.05 mg m^{-3}.
- An average inverse RF was computed for each pigment from those duplicate data sets having an average concentration of at least 0.05 mg m^{-3} for the pigment.

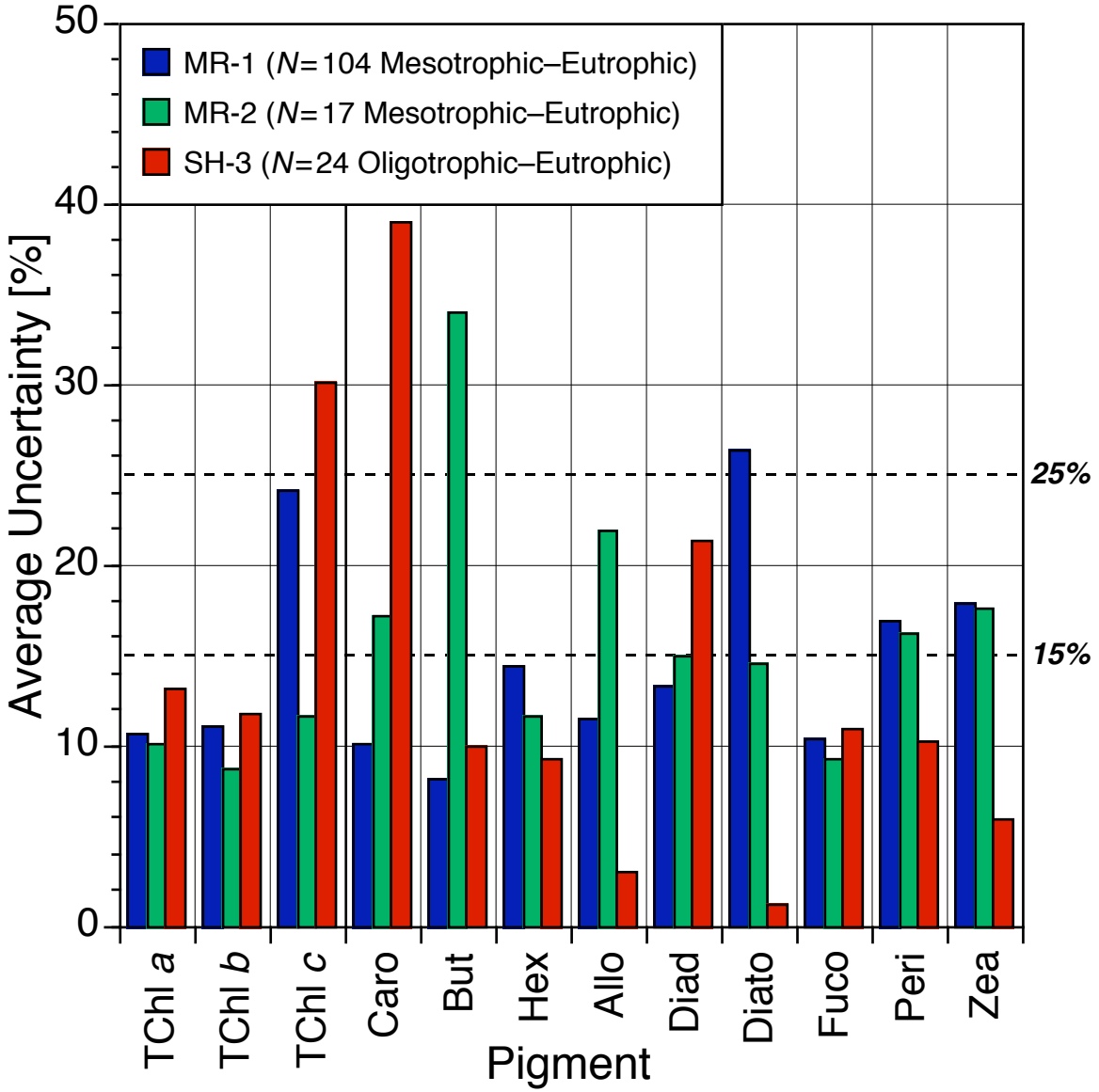
If an inverse RF could not be computed from two or three of the duplicate data sets, because of an excessive amount of data at low concentrations, the inverse RF was also computed by applying the parametric correction scheme to the data from the DHI Mix distributed during SeaHARRE-3. The pigments in the mix were all at concentrations ensuring large, well-formed peaks, but the mix did not contain all the pigments found in a natural sample, because it was formulated using phytoplankton cultures. The inverse RF was then computed by analyzing the potential relative RFs of the pigment from the various available data sets, and selecting the result with the best overall RRF value. For some pigments (Caro, But, Diato, DVChl *a*, Neo, Phide *a*, Pras, and Viola), the final inverse RF was computed using only the parametric correction of the DHI Mix results .

The Estimated Accuracy of the C_8 Individual Primary Pigments (PPig) After Final Parametric Correction

The final inverse RF values are evaluated by using them to compute new CHORS data products and comparing them to the corresponding HPL data (i.e., the HPL data are the reference data in the uncertainty computations):

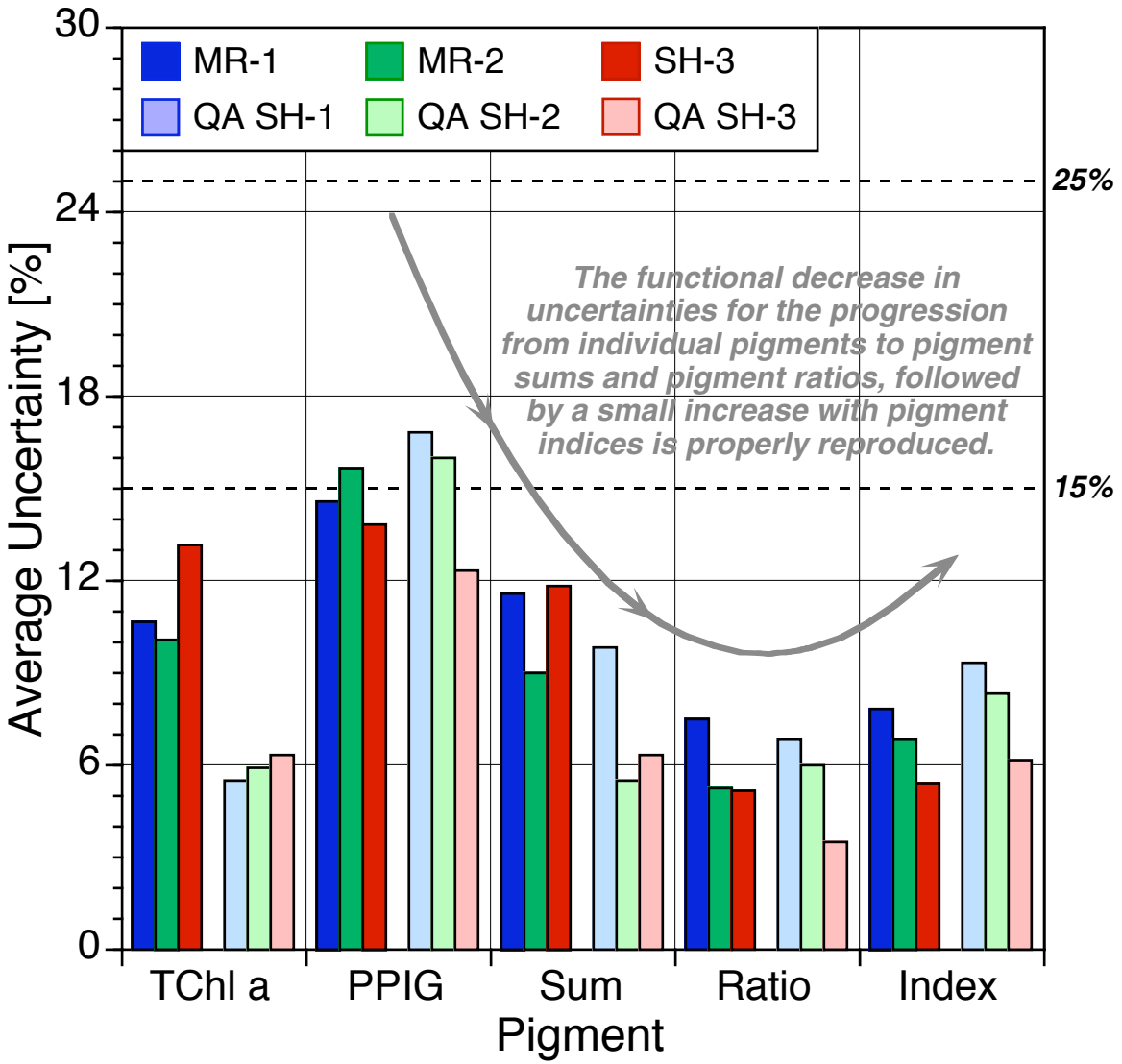
$$\psi_{P_i} = 100 \frac{|C^S_{P_i} - C^H_{P_i}|}{C^H_{P_i}}$$

Absolute values are used to prevent variance cancellation when computing averages (shown in the figure). The calibration and validation objective is an average uncertainty below 25% (less than 15% is desired for algorithm refinement activities).



The Estimated Accuracy of the C_8 Corrected Data Products (For TChl *a* Spanning 0.02–21.06 mg m^{-3})

One of the important tests of the efficacy of the parametric correction is whether or not the functional form of the uncertainties in the higher-order variables is reproduced (recalling that the CHORS SeaHARRE-3 results had aberrant relationships). The plot to the right shows the correct functional form for the corrected data and it also shows the average uncertainties are very nearly the same as the uncertainties obtained for the quality-assured laboratories for all three SeaHARRE activities. Some uncertainties are actually lower, which is an artifact of forcing agreement with HPL (which usually had the best results).



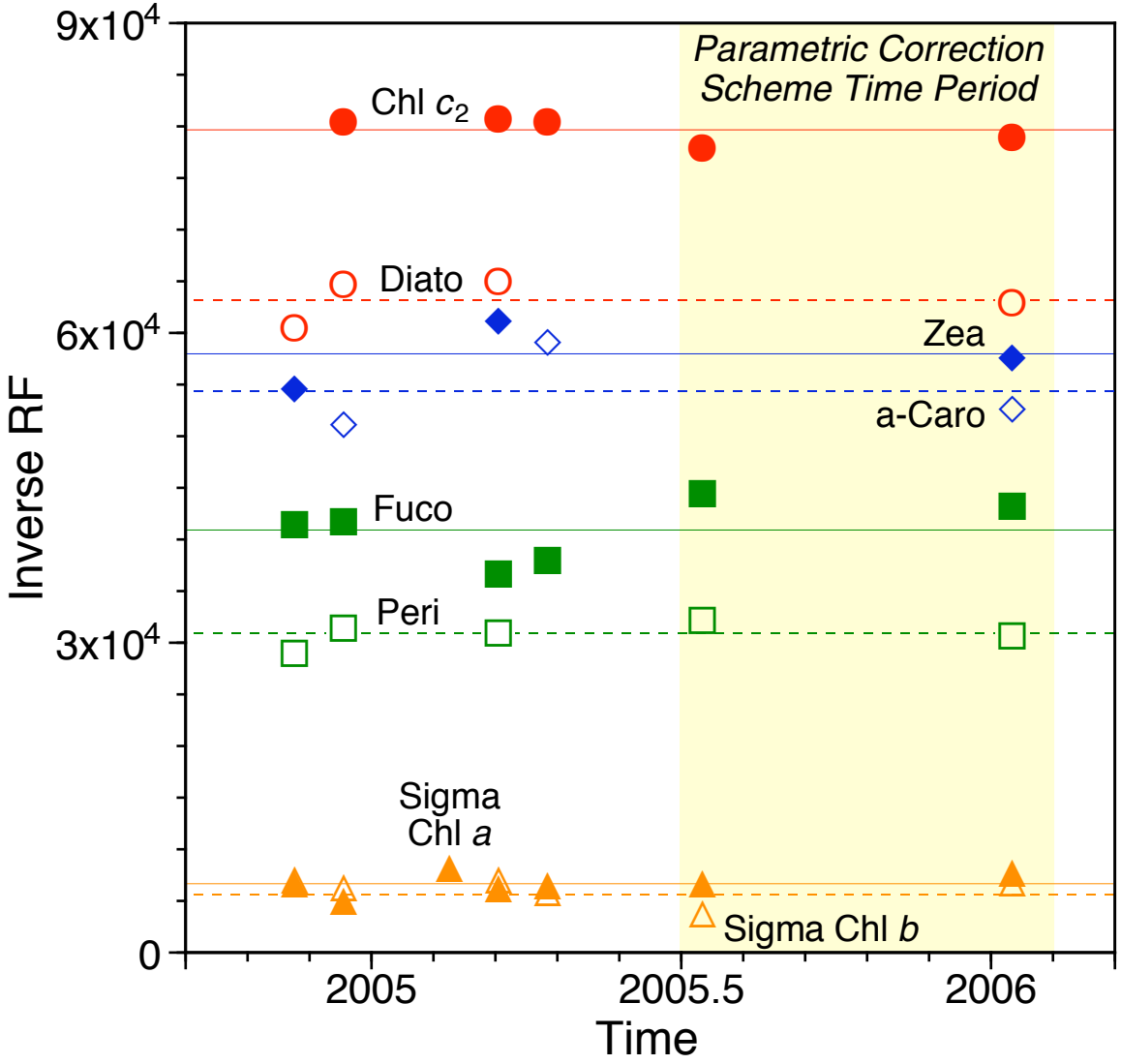
A Summary of Issues Identified During the First Investigation of CHORS C_8 Quantitation Problems

The first investigation of CHORS C_8 quantitation problems established a) the parametric correction scheme and showed it could correct the data to within calibration and validation requirements (always to within an average uncertainty of 25% and with few exceptions to within 15%); b) the CHORS calibration working range was inadequate and contributed significantly to calibration variability (at as much as the 50% level); c) some of the other CHORS calibration procedures were less than optimal (e.g., substandard pipette choices and the use of unscored vial caps) and degraded precision; and d) the inverse response factors for new calibration curves (executed using recommended improvements) agreed to within 1% of the response factors obtained from the parametric correction scheme.

- The original and the new calibration curves were not linear and there was a difference between the chlorophylls and the carotenoids. The nonlinearity was also seen in the C_{18} calibrations.
- The data used in the parametric correction scheme were from July 2005 to January 2006, but the entire CHORS HPLC analysis time period spanned September 2004 to January 2006, so there is a need to demonstrate the system and data quality were unchanging over the whole time period.
- The red-to-blue detector ratio during calibrations was not constant and was evident for both the C_8 and C_{18} methods.

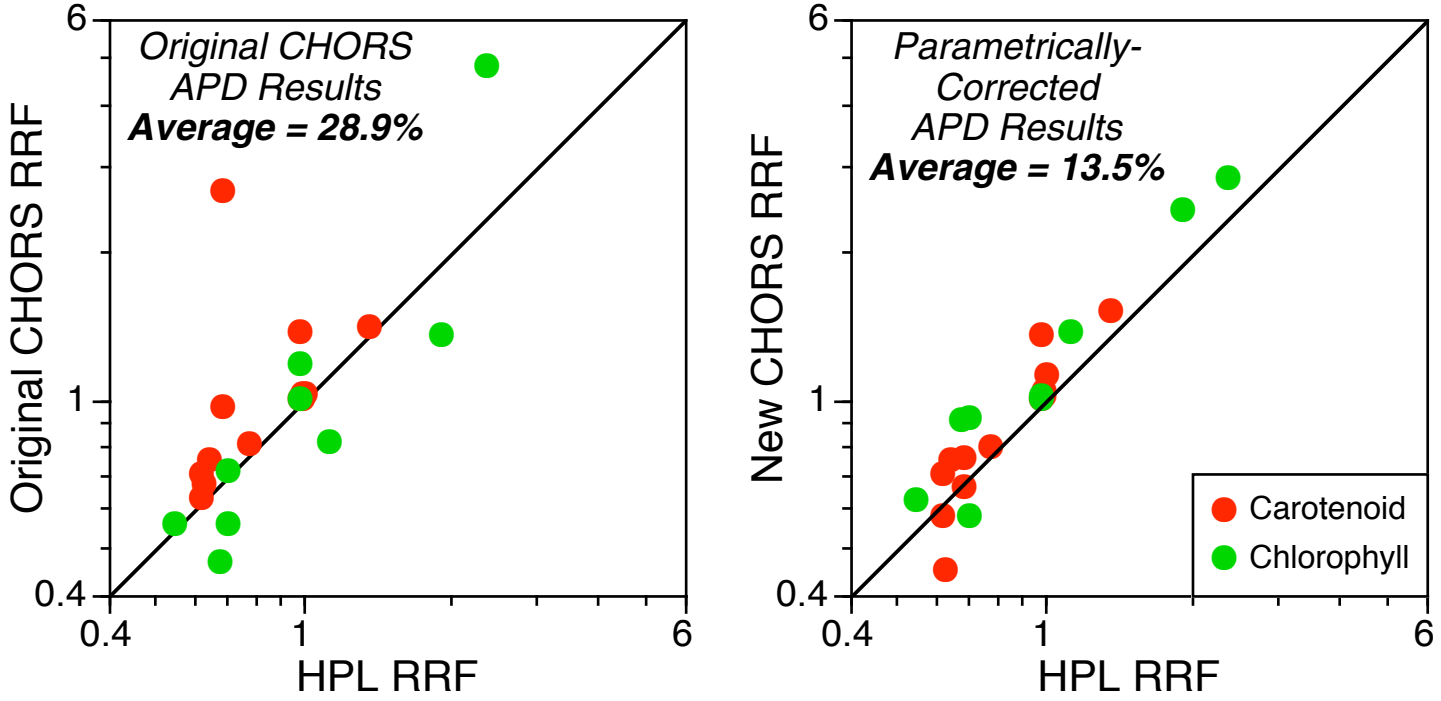
The Temporal Stability of the CHORS C_8 HPLC System: Inverse RFs at 10–15 ng inj^{-1}

The DHI calibrations provide an extensive set of actual calibrations across the entire C_8 analysis time period. By selecting those data with a 10–15 ng inj^{-1} concentration, the high variability seen in CHORS calibrations can be avoided. These data show an average stability (CV) of approximately 1.5–8.0% across the analysis time period, except for the Chl *a* and Chl *b* calibrations, which have a stability of 16.6–28.5%. This higher variability was also seen in the more frequent Sigma calibrations, which had a stability of 16.3–20.6%. The reasons for this instability are explored later on.



Convergence and Self Consistency of the C_8 Inverse Response Factors from the Parametric Correction

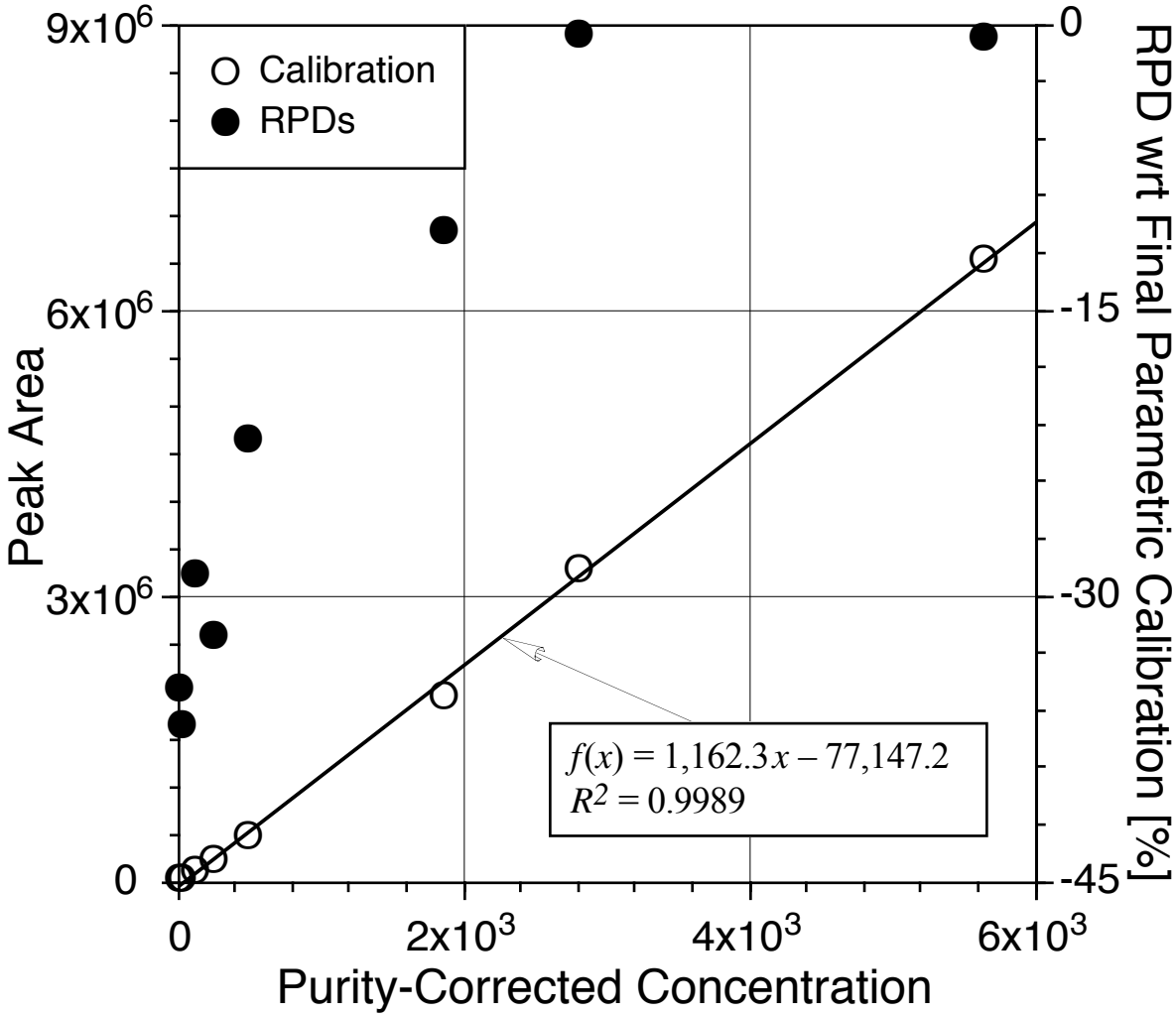
The relative response factor of the CHORS calibrations provides an important indicator of whether or not the inverse response factors from the parametric correction properly converged and are self consistent. For this inquiry, Fuco and MVChl *a* were chosen as the normalizing pigments. The original CHORS RRFs exhibit a large amount of scatter and an average APD (with respect to the HPL RRF values) of 28.9%. The new RRF values from the parametric correction scheme have have less scatter and an average APD of 13.5%. (A log-log plot is used, so the individual points are easier to discern with respect to one another.)



The Effects of Using HPL Calibration Practices on Accuracy

HPL dilution procedures were used at CHORS to prepare eight Chl *a* solutions which were injected on the CHORS HPLC using cap septa that facilitate injection volume accuracy. The correlation coefficient (0.9989) is very good and the slope agrees with the parametric value (1,180.4). A better indicator of accuracy are the percent residuals of the data with respect to the final parametric calibration, which show a nonlinear response of the CHORS HPLC system (also seen in the large nonzero *y*-intercept). *If the CHORS working range is used, the slope is 811.8*.

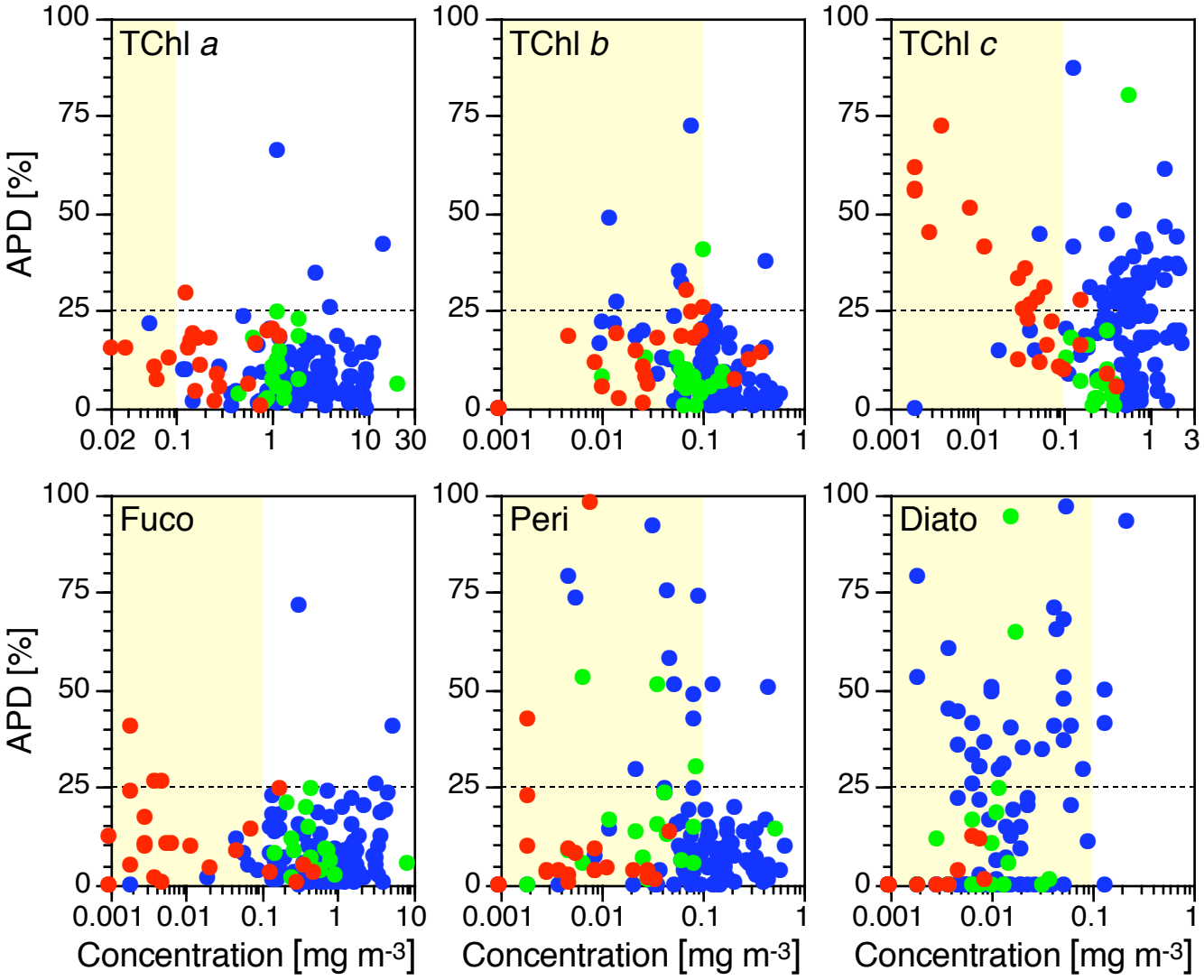
Correlation coefficients exceeding 0.9999 are achievable, as are average percent residuals to within 2%.



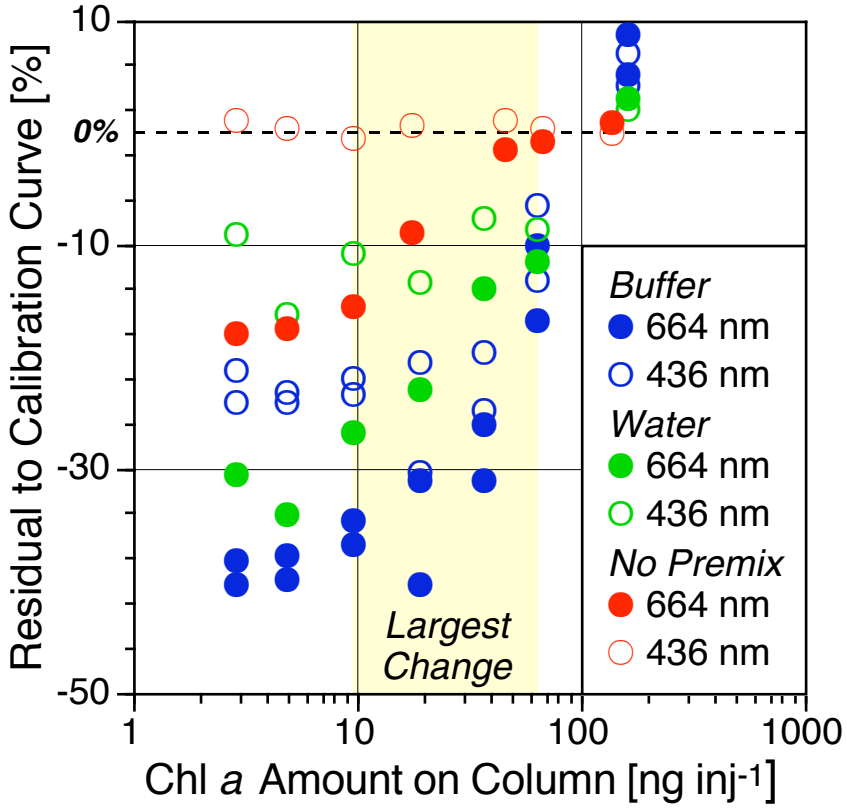
Individual Examples of the Uncertainties in the C_8 Parametric Correction Scheme

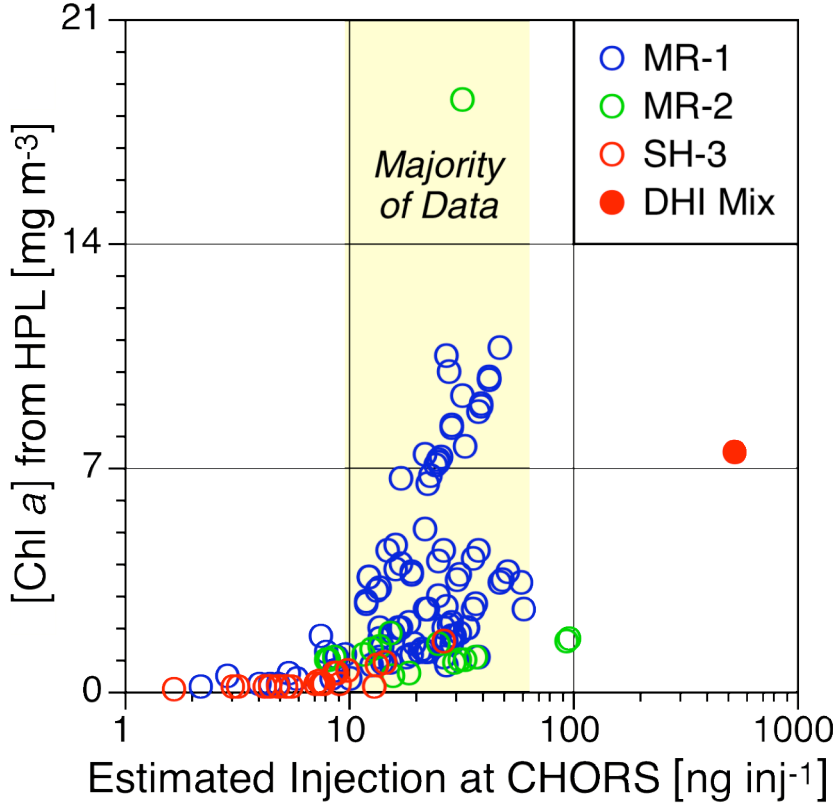
Although the average uncertainties for the primary pigments and the higher-order data products are to within calibration and validation requirements, some individual pigments do not satisfy this criteria for a large number of samples, particularly at lower concentrations. Many of the discrepancies are a direct result of analyst-to-analyst differences in how small peaks are quantitated and are present in all databases (larger uncertainties are normal here).

Low (<0.1) and very low (<0.01) concentrations are shaded yellow.



The Performance of the C_8 Parametric Correction Scheme in Terms of Detector Nonlinearity





The nonlinearity in CHORS calibrations are reduced if the buffer is replaced by water, and is completely removed at 436 nm if there is no premixing (left plot). The reason the parametric correction is not so negatively influenced by the nonlinearity is the majority of the data—*the average response of which almost exclusively influence the parametric correction factors*—correspond to the area of largest change in the expression of the nonlinearity (right plot).

Limitations of the Parametric Correction of the CHORS C_8 HPLC Data

The parametric correction scheme cannot correct all of the CHORS pigments with equal efficacy, because the HPL pigment set is different, and HPL pigment integration procedures were not the same as those used by CHORS—*particularly for pigments at low concentrations or with recurring coelution problems*—which influences both the duplicate data sets used to derive the corrections and the *in situ* samples to be corrected. The combination of method-to-method and natural differences within the overall data set partitions the final data set into five groups, with the uncertainties ranging from lowest to highest, respectively (the marginal pigments are shown in green and the unacceptable pigments shown in red):

- *The higher order pigments*: pigment sums, ratios, and indices (which are all derived from the primary pigments).
- *The primary pigments*: TChl *a*, TChl *b*, TChl *c*, Caro, But, Hex, Allo, Diadino, Diato, Fuco, Peri, and Zea (some of which are derived from the secondary pigments).
- *The secondary pigments*: MVChl *a*, DVChl *a*, Chlide *a*, MVChl *b*, Chl c_1, Chl c_2, and Chl c_3.
- *The tertiary pigments*: Lut, Neo, Phytin *a*, Phide *a*, Pras, and Viola.
- *The uncorrectable pigments*: Gyro-diester, α-Caro, β-Caro, and DVChl *b*.