Overview of SeaHARRE-5 Analysis Results

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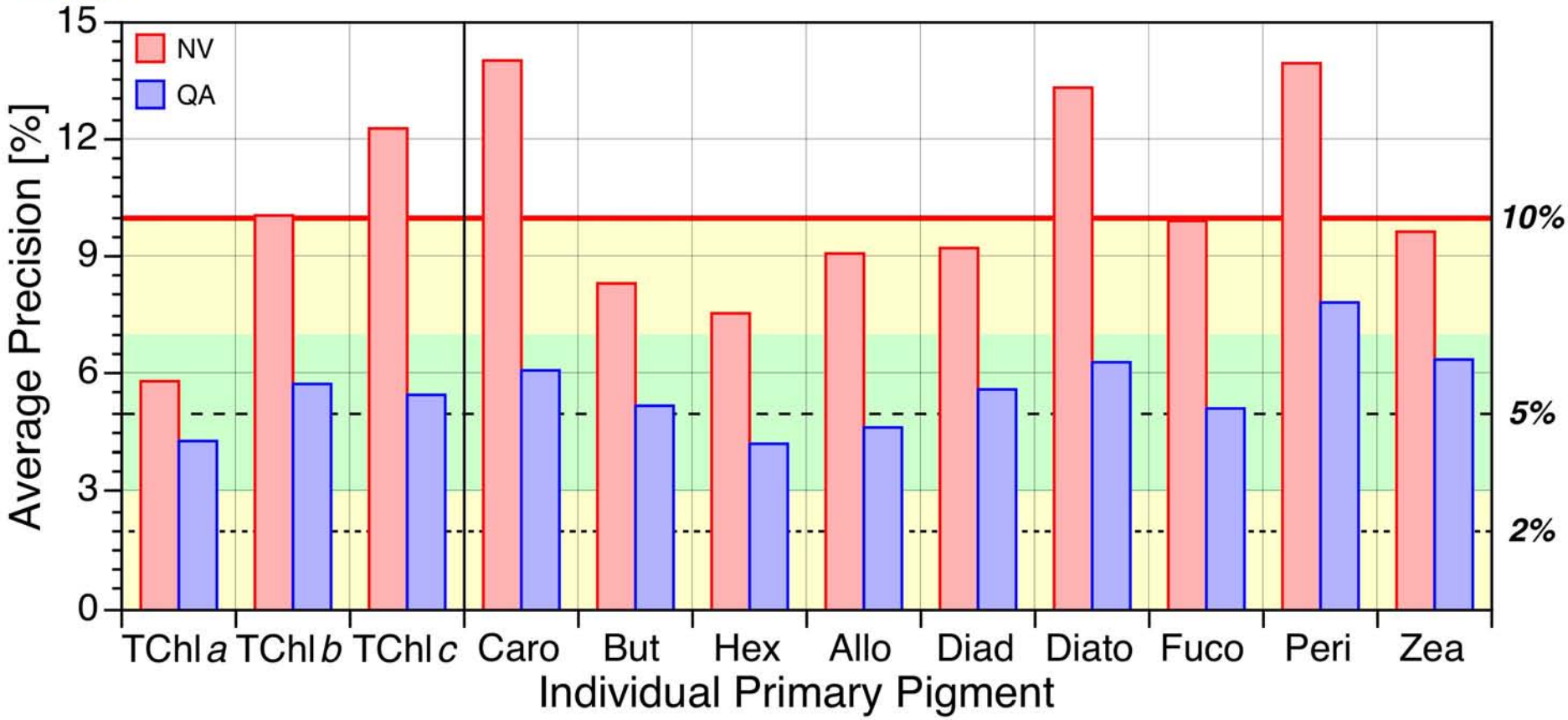
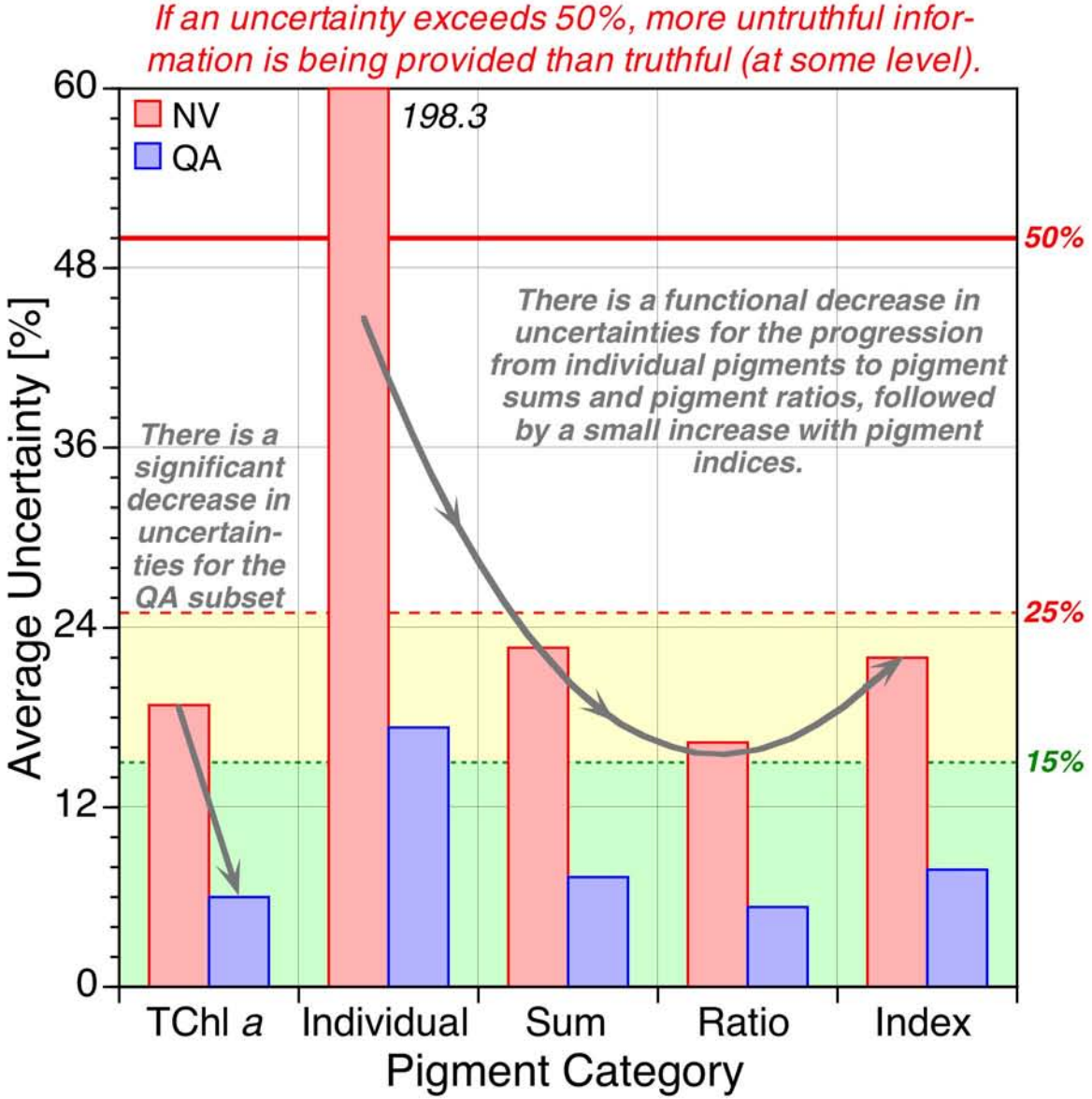
Field Sample Precision: Quality-Assured (QA) Methods versus Not Validated (NV) at the QA Level



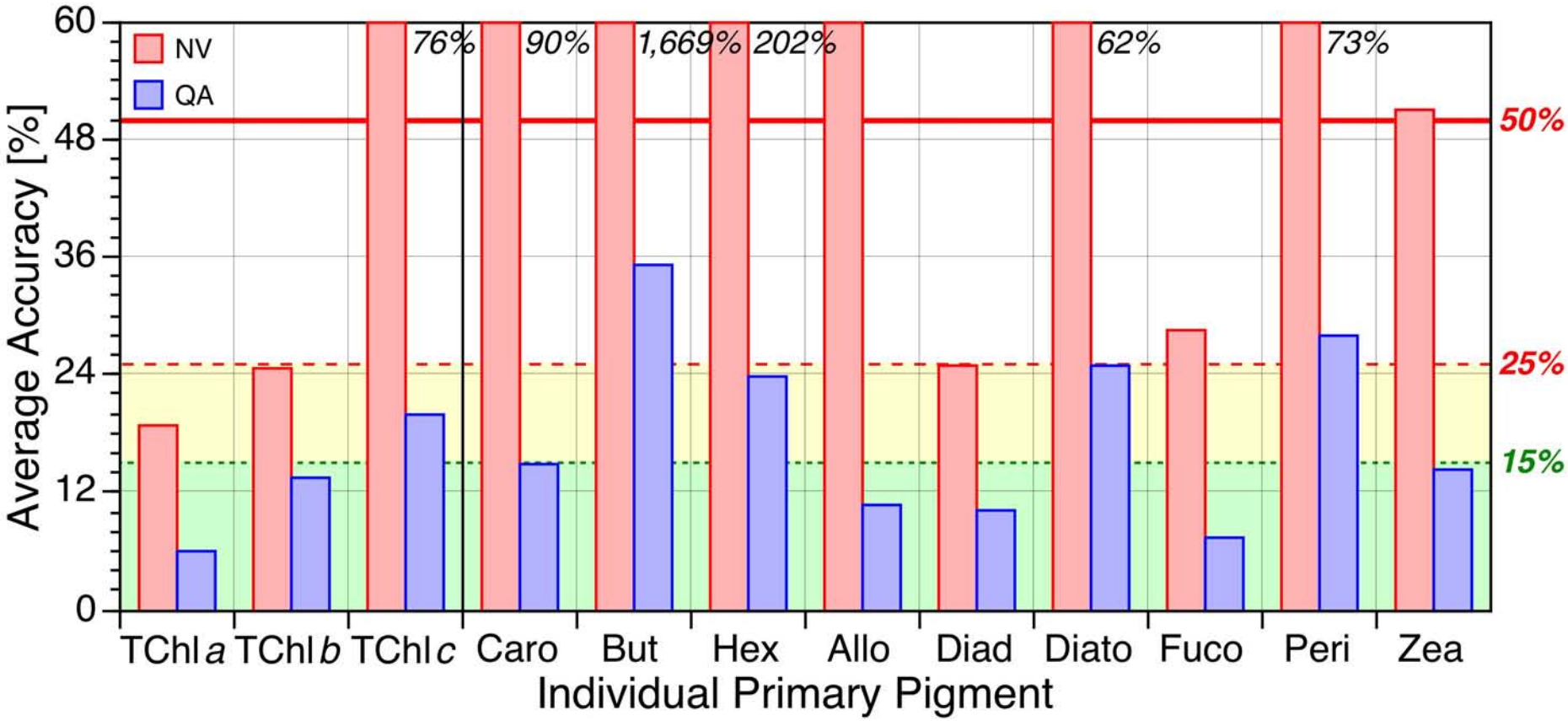
Precision is the starting point in the uncertainty budget. There is a significant difference between QA (blue) and NV (red) methods: the average individual PPig precision for all five SeaHARRE activities is 5.6% and 10.3%, respectively. To control uncertainties, precision warning limits should be set at approximately 3 and 7% (the green band), and control limts at 2 and 10% (dotted and solid red lines).

SeaHARRE Summary of Field Sample Accuracy for HPLC TChl *a* Spanning 0.020 – 42.704 mg m^{-3}

The accuracy of the methods are primarily distinguished by the pigment categories and whether or not the methods were properly quality assured (blue bars) or not (red bars). For the latter, the worst-case average result is shown at the top of the bar (individual samples can be worse). The QA methods have the lowest uncertainties; they always meet the 25% validation requirement and almost always satisfy the 15% refinement objective. Furthermore, there is a functional decrease in the uncertainties for the progression from the primary pigments to the sums and ratios, followed by a small increase with the indices.



Field Sample Accuracy Also Establishes Method Differences and the Problematic Pigments



There is a significant difference between QA (blue) and NV (red) methods: the average individual primary pigment (PPig) precision for all five SeaHARRE activities is 5.6% and 10.3%, respectively, and the former is always better than the latter. For QA results, warning limits should be set at approximately 3 and 7% (the green band) and control limts at 2 and 10% (dotted and solid red lines).

Field Sampling for SeaHARRE-5

The emphasis for SeaHARRE-5 was on coastal waters. Two different sample sets were collected in the rivers, estuaries, and bays of New Hampshire (USA) and Tasmania (Australia). The former were anticipated to be the most complicated and likely to contain confounding compounds, whereas the latter were expected to be the cleanest. All samples were collected in triplicate and distributed in late 2008 and early 2009.

Establishing the Quality-Assured (QA) Subset to Ensure a Proper Referencing System

The first step in the analysis of the SeaHARRE data is to establish the QA (A') subset. This is initially based on the precision obtained with the field samples.

A method not reporting all primary pigments for all samples is excluded (C and I).

A laboratory with an average precision not satisfying the metric for *semiquantitative* analysis—more than 8% plus 2% for field sample variability—is excluded (E and O).

In addition, a laboratory with three or more primary pigments with a precision worse than 10% is *considered for exclusion* (K and N).

Table 3	[TChla]	[TChlb]	[TChlc]	[Caro]	[But]	[Hex]	[Allo]	[Diad]	[Diato]	[Fuco]	[Peri]	[Zea]	Avg.
C	6.0	5.7	5.9	5.8	2.1	3.7	5.1	7.1	5.3	6.7	4.1	4.2	5.1
D	4.6	4.5	6.3	7.1	5.4	5.3	7.2	8.0	13.8	5.2	9.6	10.6	7.3
E	7.8	14.8	34.3	13.7	5.1	3.3	10.1	18.1	19.3	8.0	19.1	11.2	13.7
G	5.8	6.4	6.3	7.3	2.2	3.1	5.5	6.5	9.2	5.1	9.7	6.1	6.1
H	4.5	4.4	4.9	4.6	3.7	4.9	4.7	5.6	14.1	4.9	8.3	5.6	5.8
I	12.6	35.1	25.7	73.0	23.1	18.3	6.0	23.7		44.1	35.3	28.1	29.5
K	6.3	14.5	11.1	8.0	10.6	2.9	5.4	6.0	20.7	5.2	7.0	9.3	8.9
L	4.1	4.3	5.5	7.2	3.6	2.3	4.8	6.4	10.7	4.5	8.5	6.1	5.7
N	5.6	6.0	7.4	7.7	6.0	8.2	5.6	10.1	11.6	5.3	11.8	6.3	7.6
O	8.8	7.5	10.5	44.0	2.3	7.0	23.0	8.1	6.7	13.0	11.5	17.1	13.3
T8	5.8	6.1	4.4	4.5	1.4	1.9	4.5	6.7	5.2	5.1	5.5	4.9	4.7
T18	8.4	8.5	6.5	5.6	1.2	2.6	4.4	6.6	7.7	5.6	6.5	4.3	5.7
A' Avg	4.7	4.9	5.7	6.6	3.7	3.9	5.6	6.6	12.0	4.9	9.0	7.1	6.2
A^{+} Avg	7.9	13.2	14.3	22.4	7.1	6.3	8.4	11.3	11.9	12.3	13.8	11.6	11.7

Additional Consistency Tests for Establishing the QA Subset

Consistency tests are applied to all the candidate methods for the QA subset. If a method significantly fails one of these tests, it is excluded.

T18	Diato	Peri
A	80.6	98.3
B	74.5	11.0
C	81.8	14.8
D	60.1	98.2
E	38.0	98.9
F	50.2	97.9
G	43.4	10.2
H	18.5	97.9
I	56.5	99.4
J	65.8	99.6
K	36.1	22.1
L	36.6	99.0
AA	93.7	6.4
AB	96.5	0.4
AC	95.5	1.5
AD	93.1	5.2
AE	98.1	0.6
AF	86.8	36.6
AG	67.6	14.2
AH	97.8	0.6
AI	90.3	0.7
AJ	94.7	0.2
AK	98.7	99.9
AM	97.9	8.5

T8	Diato	Peri
A	80.6	98.3
B	538.3	56.3
C	81.8	30.4
D	22.3	98.2
E	22.9	98.9
F	36.4	11.7
G	91.9	3.2
H	20.3	97.9
I	3.0	99.4
J	30.5	99.6
K	20.5	23.0
L	96.9	99.0
AA	93.7	15.9
AB	96.5	8.7
AC	95.5	10.2
AD	9.4	16.8
AE	98.1	10.3
AF	86.8	44.2
AG	67.6	4.1
AH	18.0	11.5
AI	517.5	12.4
AJ	33.1	10.3
AK	3.3	99.9
AM	4.5	20.4

Large number of false negatives.

N	Zea	Zea+Lut
A	107.6	16.0
B	94.7	98.4
C	190.0	31.1
D	63.9	43.0
E	32.3	19.7
F	49.4	36.7
G	89.4	17.5
H	49.7	23.4
I	65.9	30.9
J	43.1	12.0
K	48.2	14.7
L	120.9	26.9
AA	43.3	26.2
AB	94.8	95.2
AC	77.2	47.2
AD	20.2	5.4
AE	43.5	6.1
AF	85.7	10.2
AG	66.6	10.9
AH	19.2	14.5
AI	19.0	12.5
AJ	30.7	25.4
AK	82.1	2.2
AM	177.6	15.7

Coelution evidence.

Lab.	Lut	Neo	Neo+Vio	Viola	Zea+Lut
D	19.0	18.2	16.3	16.2	11.5
G	36.5	9.2	12.1	16.5	11.4
H	25.2	15.6	13.7	17.9	8.1
L	27.4	15.1	12.8	17.4	11.8
K	176.9	44.4	34.2	34.1	48.8

Significant inconsistency with respect to the emerging or established QA subset.

A primary goal of these tests is to remove as many sources of bias as possible—for as many pigments as possible—in the proxies for truth. This does not mean every pigment in the QA subset is necessarily unbiased.

Details of the PPig Uncertainties for the Methods in the QA Subset

Table 13	[TChla]	[TChlb]	[TChlc]	[Caro]	[But]	[Hex]	[Allo]	[Diad]	[Diato]	[Fuco]	[Peri]	[Zea]	Avg.
A	4.1	9.8	19.0	8.0	101.4	0.0	3.8	6.5	80.6	3.9	23.6	7.7	22.4
B	4.3	2.2	18.2	7.8	0.0	49.3	6.5	4.7	111.7	7.2	19.5	5.3	19.7
C	3.7	2.4	19.1	7.3	98.6	64.3	4.6	5.6	81.8	4.0	23.0	9.6	27.0
D	5.7	9.5	9.8	10.1	15.0	7.4	4.5	2.6	5.1	8.4	33.6	8.2	10.0
E	4.0	7.5	9.8	6.5	6.4	4.7	3.9	3.6	8.6	4.9	25.2	6.5	7.7
F	4.7	8.2	12.1	7.1	10.3	4.9	4.5	4.9	17.1	6.0	20.8	21.1	10.1
G	4.6	9.1	13.2	7.2	11.3	9.7	7.9	7.7	49.7	6.8	10.0	7.1	12.0
H	3.3	5.6	10.5	6.0	5.6	4.6	4.8	5.7	13.2	3.0	7.5	5.4	6.3
I	4.1	5.9	8.5	6.2	4.3	4.4	5.8	5.7	5.2	3.7	7.5	6.6	5.7
J	3.6	7.3	9.3	7.3	49.5	3.6	5.5	5.6	10.0	2.7	12.3	8.0	10.4
K	2.9	7.6	9.8	6.5	12.3	4.3	6.3	6.4	13.9	1.3	5.5	6.6	7.0
L	4.8	5.9	20.9	7.8	48.2	9.5	9.4	9.3	48.5	2.8	21.4	4.2	16.0
AA	7.9	7.5	10.2	35.3	0.0	18.2	7.5	14.5	12.3	6.0	10.8	14.1	12.0
AB	7.5	15.7	8.4	36.9	0.0	0.0	8.4	10.4	55.1	5.8	5.2	13.9	13.9
AC	5.2	13.1	11.5	43.5	0.0	0.0	13.9	18.3	12.0	8.0	9.9	28.7	13.7
AD	3.3	9.4	8.3	28.8	43.6	12.0	12.1	22.5	23.7	5.7	9.8	7.0	15.5
AE	5.3	12.9	8.6	26.3	0.0	23.4	13.6	12.6	14.4	7.4	10.0	7.3	11.8
AF	13.4	3.7	30.7	23.2	0.0	103.6	9.6	27.1	57.7	5.2	28.8	5.9	25.8
AG	7.6	3.1	28.6	13.9	0.0	0.0	11.8	14.2	101.4	5.1	24.8	6.2	18.0
AH	6.1	20.4	9.1	38.2	13.6	13.8	7.4	17.8	10.0	8.6	10.0	10.6	13.8
AI	4.8	7.1	15.0	23.3	22.7	9.0	9.3	18.3	21.7	6.9	11.8	8.0	13.2
AJ	5.9	8.7	9.9	32.6	18.4	9.0	4.1	19.3	8.9	5.4	9.3	10.0	11.8
AK	5.6	10.1	23.4	15.7	49.5	137.4	13.6	12.5	49.3	5.8	24.9	11.5	30.0
AM	7.6	7.0	22.5	23.9	0.0	55.5	24.3	34.5	35.0	18.1	13.3	18.3	21.7
A' Avg.	5.4	8.3	14.4	17.9	21.3	22.9	8.5	12.1	35.3	5.9	15.8	9.9	14.8
A' US	4.2	6.7	13.4	7.3	30.2	13.9	5.6	5.7	37.1	4.6	17.5	8.0	12.9
A' Aus.	6.7	9.9	15.5	28.5	12.3	31.8	11.3	18.5	33.4	7.3	14.1	11.8	16.8

Details of the PPig Uncertainties for the Methods Not in the QA Subset

Table 20	[TChla]	[TChlb]	[TChlc]	[Caro]	[But]	[Hex]	[Allo]	[Diad]	[Diato]	[Fuco]	[Peri]	[Zea]	Avg.
A	22.3	64.8	87.0	67.2	67.6	211.1	26.7	61.8	80.6	40.6	146.4	174.4	87.5
B	17.2	26.0	49.6	46.0	42.9	79.3	11.7	29.8	210.8	8.1	52.4	179.3	62.8
C	17.6	29.0	61.5	41.5	65.7	42.9	24.3	29.3	81.8	6.4	52.7	128.7	48.5
D	16.3	34.9	30.1	36.6	22.4	19.1	12.2	25.9	44.8	14.1	100.1	67.7	35.3
E	13.3	23.7	18.7	43.1	15.0	15.7	15.7	28.0	39.9	12.1	74.0	42.8	28.5
F	16.0	20.2	21.7	40.8	34.6	15.3	19.0	31.5	43.0	11.4	56.0	53.8	30.3
G	11.7	67.0	28.6	35.0	83.2	23.2	17.1	19.8	55.0	11.4	10.9	136.3	41.6
H	13.6	20.2	19.2	40.5	43.4	14.4	23.7	19.4	42.2	11.8	87.5	69.4	33.8
I	12.2	21.7	21.8	40.7	14.8	13.4	17.4	23.9	39.4	11.5	54.3	57.4	27.4
J	12.0	17.3	21.6	34.7	85.2	12.7	13.5	24.3	42.9	8.3	66.7	56.2	33.0
K	11.8	21.6	18.0	39.4	26.9	16.0	13.4	23.0	23.6	14.0	23.9	46.9	23.2
L	14.5	9.6	46.6	50.0	85.4	38.9	25.9	33.1	73.9	9.9	89.4	87.7	47.1
AA	19.7	23.3	33.6	40.4	0.0	103.0	48.0	18.3	71.7	10.2	10.9	76.0	37.9
AB	24.3	24.3	35.5	40.2	0.0	0.0	39.6	15.0	109.6	13.0	7.4	92.8	33.5
AC	15.2	44.0	32.1	40.3	0.0	0.0	24.8	19.7	71.1	12.6	11.9	87.9	30.0
AD	11.6	20.7	35.9	48.5	75.8	29.8	26.0	22.5	53.4	12.3	12.3	65.5	34.5
AE	22.3	29.0	26.0	34.5	127.3	77.2	27.5	16.4	59.5	19.1	10.0	99.3	45.7
AF	23.1	20.6	35.4	45.6	0.0	92.2	24.1	28.1	73.5	10.3	22.3	75.5	37.5
AG	14.2	22.6	47.5	48.0	0.0	360.2	25.4	48.5	67.6	17.6	60.2	60.4	64.3
AH	25.5	34.5	28.6	48.8	74.3	31.0	38.1	22.4	46.5	16.6	10.3	83.0	38.3
AI	9.7	21.7	32.9	47.3	69.2	56.3	29.0	36.2	139.1	13.1	21.3	85.9	46.8
AJ	22.7	24.8	39.8	49.3	80.6	63.8	23.5	30.0	67.4	12.0	11.6	84.5	42.5
AK	15.6	27.8	28.1	33.8	90.5	112.1	32.6	14.0	71.3	20.7	38.6	94.2	48.3
AM	17.0	15.5	35.1	47.1	281.9	148.3	41.0	36.5	61.1	27.3	17.7	78.6	67.3
A^+ Avg.	16.6	27.7	34.8	43.3	57.8	65.7	25.0	27.4	69.6	14.3	43.7	86.8	42.7
A^+ US	14.9	29.7	35.4	43.0	48.9	41.8	18.4	29.2	64.8	13.3	67.9	91.7	41.6
A^+ Aus.	18.4	25.7	34.2	43.6	66.6	89.5	31.6	25.6	74.3	15.4	19.6	82.0	43.9

Summaries of the PPig Uncertainties for all the Methods

Table 24	[TChla]	[TChlb]	[TChlc]	[Caro]	[But]	[Hex]	[Allo]	[Diad]	[Diato]	[Fuco]	[Peri]	[Zea]	Avg.
D	3.3	6.0	9.5	14.8	17.8	28.3	7.8	8.7	34.0	8.8	26.8	12.9	14.9
G	5.9	5.0	7.4	11.1	21.5	27.4	7.5	8.0	31.7	4.3	12.0	8.5	12.5
H	8.6	10.2	23.1	29.0	28.5	19.0	10.2	16.8	48.2	7.0	15.2	6.4	18.5
L	3.9	12.1	17.8	16.7	17.4	16.8	8.4	14.9	27.3	3.6	9.1	11.8	13.3
C	33.6	32.5	22.0	44.3	44.6	40.5	41.6	42.2	62.7	30.4	45.3	45.8	40.5
E	28.0	67.8	48.9	61.5	43.9	26.7	22.2	29.9	79.3	15.2	41.0	89.0	46.1
I	30.4	49.8	57.6	82.5	64.2	44.9	71.5	58.4		38.4	77.2	92.0	60.6
K	8.2	14.5	26.4	12.7	30.4	29.7	10.8	11.4	44.8	3.5	27.6	52.7	22.7
N	4.7	13.8	13.4	9.5	139.0	251.0	7.6	30.6	55.3	12.0	16.3	71.5	52.0
O	18.5	10.6	30.7	87.6	44.5	47.1	27.1	35.9	74.6	17.2	56.2	229.8	56.6
T8	12.7	23.8	15.6	21.0	38.4	29.5	20.2	8.0	90.4	6.5	45.0	36.6	29.0
T18	14.0	13.5	51.1	28.2	43.9	30.8	15.6	17.6	73.0	7.5	42.6	36.4	31.2
A' Avg	5.4	8.3	14.4	17.9	21.3	22.9	8.5	12.1	35.3	5.9	15.8	9.9	14.8
A^{+} Avg	16.6	27.7	34.8	43.3	57.8	65.7	25.0	27.4	69.6	14.3	43.7	86.8	42.7

The details of the PPig uncertainties for all the laboratories show many of the pigments for the laboratories not in the QA subset are within the requirements for calibration and validation activities: 15% for TChl *a* and 25% for all the other pigments. Some of the results for the QA subset exceed the thresholds for calibration and validation (red), but the PPig averages are all to within 25%. The K results also satisfy the 25% PPig threshold even though K was not part of the QA subset; the only anomalous performance is with Zea. Both the T8 and T18 results very nearly satisfy the 25% threshold and with some improvements to just a few pigments, these methods can very likely be brought into the QA subset.

Confirmation of Laboratory Performance Within the Tertiary Pigments

Table 24	[Lut]	[Neo]	[Neo+Viola]	[Phytina]	[Phidea]	[Pras]	[Viola]	[Zea+Lut]	Avg.
D	19.0	18.2	16.3	14.5	59.5	51.3	16.2	11.5	25.8
G	36.5	9.2	12.1	11.6	29.2	77.9	16.5	11.4	25.6
H	25.2	15.6	13.7	9.2	24.1	95.6	17.9	8.1	26.2
L	27.4	15.1	12.8	12.2	39.6	42.0	17.4	11.8	22.3
C	65.2	49.5	46.0	201.3	81.9	51.2	48.1	46.5	73.7
E		36.1	28.9	46.8	95.8	46.1	33.4	37.4	46.4
I		92.0	96.7			83.4		85.8	89.5
K	176.9	44.4	34.2			47.9	34.1	48.8	64.4
N								26.7	
O		77.6	79.7	30.3	39.0	84.6	103.5	126.4	77.3
T8	37.0	25.3	20.2	48.3	69.1	57.9	15.7	38.9	39.1
T18	54.6	33.3	138.9	38.2	63.6	41.8	28.9	40.2	54.9
A' Avg.	27.0	14.5	13.7	11.9	38.1	66.7	17.0	10.7	25.0
A^+ Avg.	89.5	51.4	66.4	40.9	66.9	60.3	43.1	57.7	59.5

The tertiary pigments are usually characterized by poorer accuracy, and for the QA subset the average accuracy is 25.0%. For the methods not in the QA subset, the average accuracy is 63.6%. For both types of data, there are recurring indications of false negatives (uncertainties just below 100%), and false positives (uncertainties above 100%) for methods not in the QA subset.

Given the high uncertainties for some of the important tertiary pigments, even in the QA subset (e.g., Pras), a relevant question is: *How can we improve the quantitation of these pigments?*

Trace Analyses, Accuracy, and Detectability: *Uncertainties as a Function of SNR*

Individual HPL pigment APDs per sample from SeaHARRE-3 exhibit a strong increase once the SNR is less than 50. A subset of carotenoids, which are frequently found in low concentrations are shown. The data are split into two groups: all QA laboratories agree a pigment is present (solid symbols), and one or more laboratories do not report a pigment as present (open symbols). The data show that when all laboratories do not agree a pigment is present (open circles), the HPL SNR is about 50 or less and the average HPL APDs are 29%; but when all QA laboratories agree a pigment is present (solid symbols), the average HPL APDs are 7%.