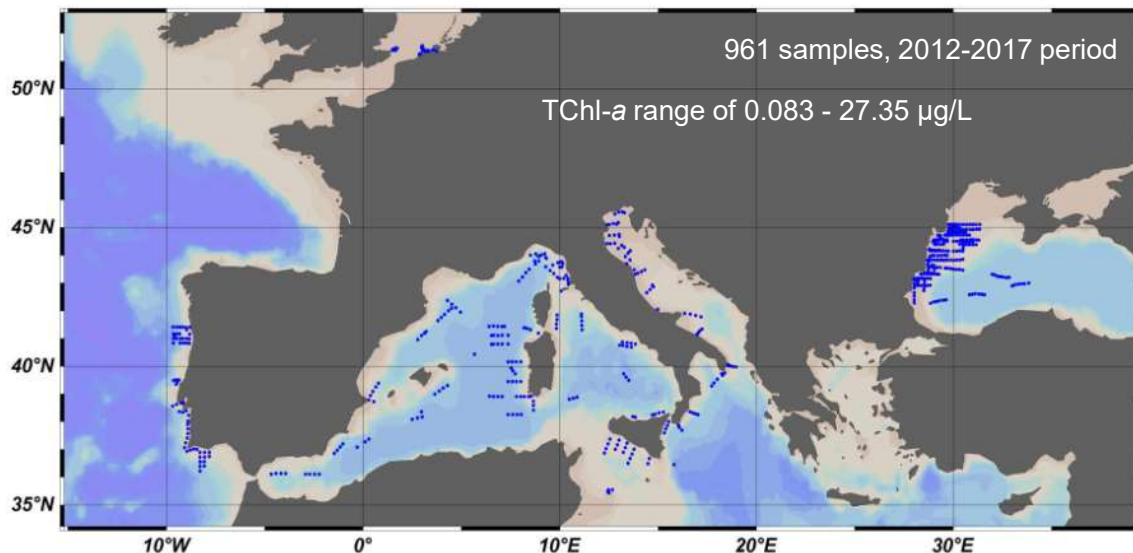


# HPLC Analysis of Phytoplankton Pigments: An Inter-laboratory Comparison

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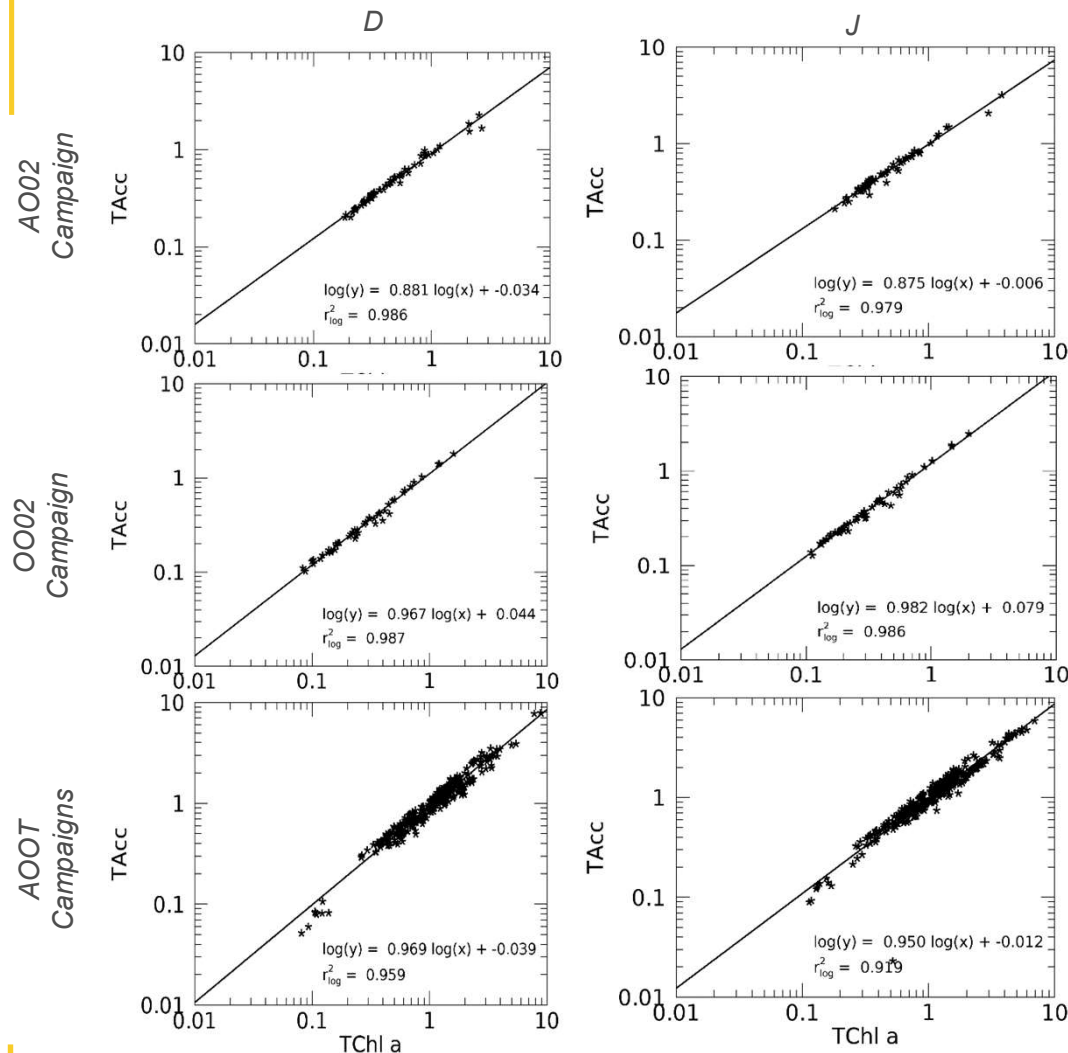
**Introduction:** Photosynthetic Pigments data are fundamental in ecological studies. The development of bio-optical algorithms, the study of trophic transfer for primary producer and the validation of satellite data products require availability of high quality in-situ measurements. The current exercise aims to investigate the uncertainties associated with **phytoplankton pigments** quantification by comparing the analyses performed on duplicate sample by two the Joint Research Centre of the European Commission (*J*) and Danish Hydraulic Institute, Denmark (*D*).



## Comparison:

- Two Certified laboratories (JRC - ISO 9001; DHI - ISO/IEC 17025:2005);
- Applying same validated HPLC method (Van Heukelem and Thomas, 2001);
- Already participated in Round Robins or inter-comparison;
- **Object of comparison:** large number of samples collected in different water-kinds

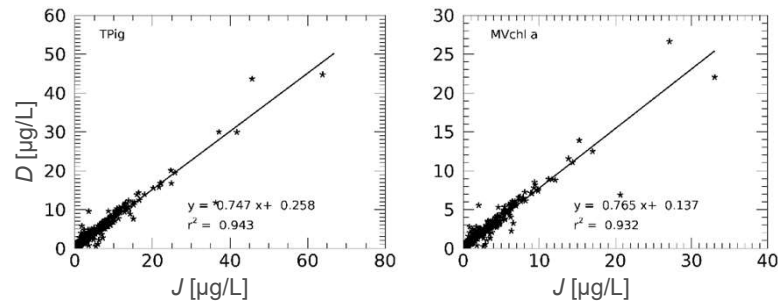
## Dataset Consistency:



Each data set is checked for consistency:

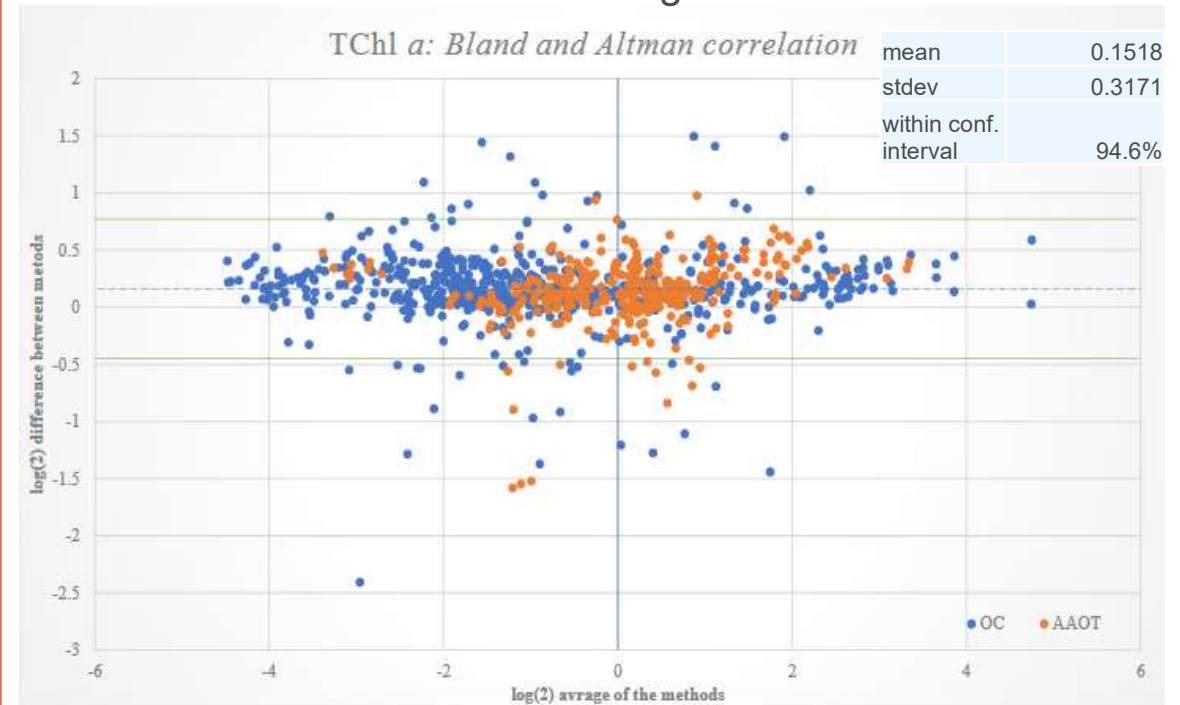
- **Grubbs' test** (IUPAC guidelines) is used for outliers identification;
- Co-variation of Chlorophyll-a (TChl-a) and Total Accessories pigments (TAcc) (**Trees et al.** 2000) is verified independently for each of the 11 oceanographic cruises compared and for the 12 Acqua Alta Oceanographic Tower in Venice (AAOT) field campaigns

# Laboratories Comparison: Primary (PPig) and Secondary (Psec) Pigments, Sum and Indices (PSum, PIndex)



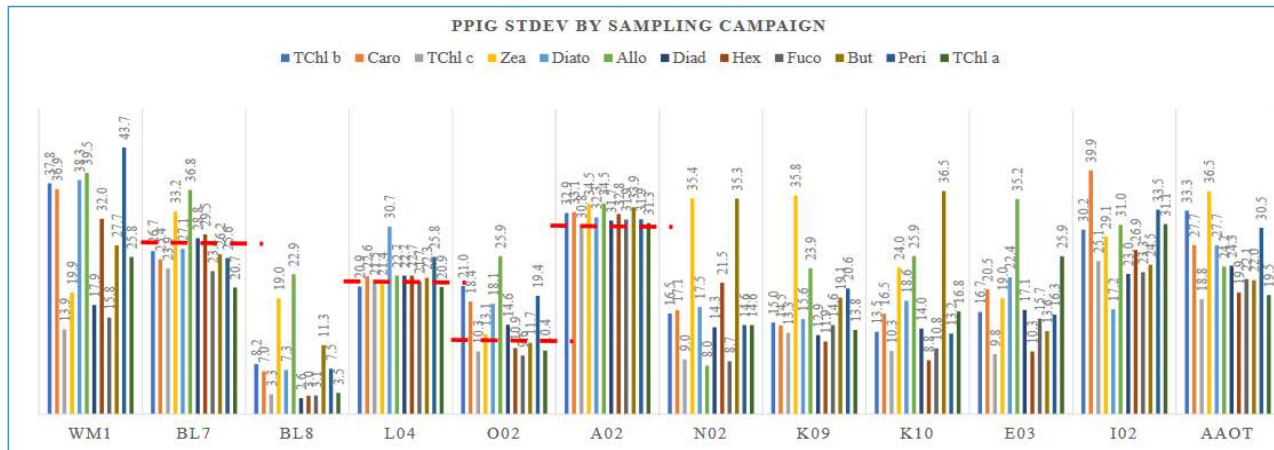
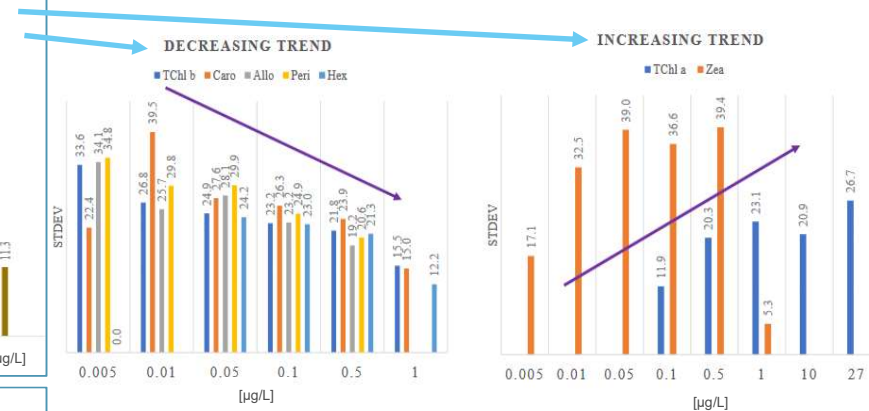
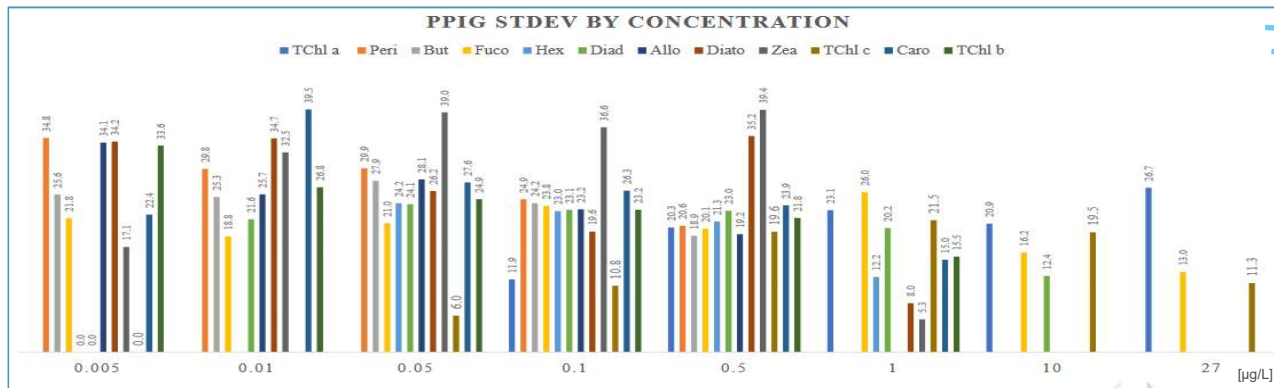
**Determination coefficient (method correlation):** The  $J$  and  $D$  have been compared through scatter plots of PPig, and Psum. Highest correlations ( $R^2 \geq 0.93$ ) are observed for TChl-a, TCaro, Diad, Fuco, for all the Pigment Sum (except PPC), as well as mPF and nPF. Correlation decreases for PPig usually detected at low concentration (i.e. But, Zea) and secondary and tertiary pigments often characterized by values close to the Low Limit of Detection (LOD).

**Bland&Altman:** The limit of agreement is determined when the differences between the data from the two laboratories are normally distributed and the standard deviation and the mean are the same across the entire range of measurements.



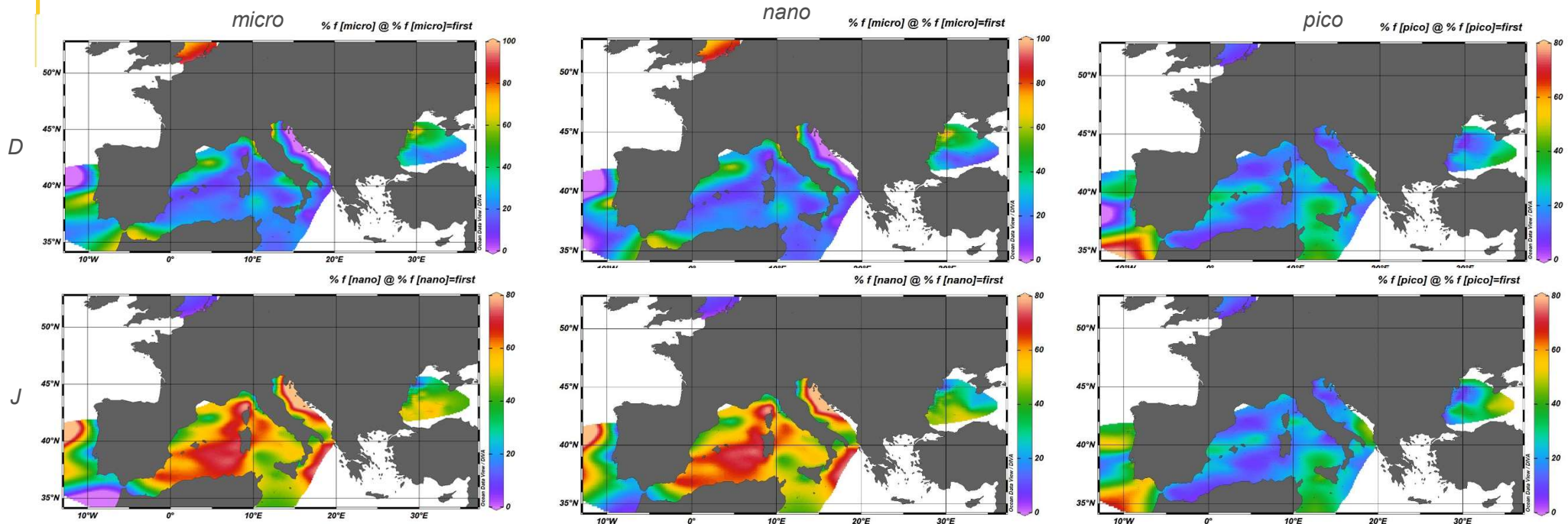
# Laboratory Comparison: Percent Difference (PD) on PPIg and PSum

	TPig	TChl	PPC	PSC	PSP	TAcc	DP	TChl a	Peri	But	Fuco	Hex	Diad	Allo	Diato	Zea	TChl c	Caro	TChl b	After removing the outliers, c.a. 95% of the data are within the interval confidence
$\overline{\psi_p}$	13.7	11.0	16.7	19.0	12.5	16.4	15.4	10.8	25.2	21.0	15.9	20.3	21.9	31.1	48.6	-0.2	9.7	-5.6	1.3	
$s_p$	21.6	22.2	25.9	24.4	21.6	22.9	23.2	22.7	60.7	42.8	27.7	37.5	28.4	50.3	52.0	53.9	26.0	28.3	36.7	
% conf interval	94.7	94.9	95.0	95.2	94.6	95.1	95.5	94.5	94.3	95.8	95.4	95.5	95.3	94.2	95.8	95.1	96.2	95.3	95.7	



Regardless of the pigments concentration, the in-homogeneity of water samples may affect the assumed equivalence of duplicates and consequently the agreement between independent analysis.

**PSC and PFT Algorithm Application:** The two datasets are used independently to estimate phytoplankton size classes (PSC) and phytoplankton functional types (PFT) by the Uitz algorithm (2006).



**Results and Discussion:** A constant bias is found for the *J* values of PPIg and PSum with respect to those from *D*. The observed 10.8% mean PD between the two independent analyses of TChl-*a* fully satisfies the requirement of 15% uncertainties associated to TChl-*a* measurement applicable for the validation of satellite data products. The uncertainties are within 16% for all PSum, with the exception of PPC (19%). The differences are largely explained by the in-homogeneity of duplicate samples.

# Knowledge gaps and priorities

- Investigation of **uncertainties** associated with **phytoplankton pigments** should be extended to longer data series, covering diverse water-kind and trophic condition, when possible;
- **Comparison** for assessing the **uncertainties** should be organized regularly among laboratories that contribute to common data set;
- The **algorithm application** suggested the investigation of an **uncertainty propagation model**.