

## General comments

Marine phytoplankton pigments determined via HPLC analysis have been extensively used to develop and validate remote sensing algorithms for determining the specific abundance of phytoplankton groups, becoming a reference metric. The study under review aims to compare phytoplankton pigments with measurements of DNA-based metabarcoding and mRNA abundances via metatranscriptomics. This study seeks to determine to what extent existing datasets of DNA metabarcoding and marine mRNA can be used to develop models of phytoplankton group distributions, supporting the next generation of hyperspectral satellites.

The manuscript appears well-structured and written. The abstract and title reflect the content accurately, and the references are appropriate. This is interesting work by comparing the significant number of samples. Even though such comparisons has been done previously, this cover different geographical locations and explored quantitative methods that express both 18S rDNA and total mRNA as concentrations. This work merits to be published after a series of minor weaknesses that are reported here will have to be addressed. These involve the following:

- 1) The scientific methods and assumptions are clearly outlined regarding DNA, mRNA techniques, primary productivity, and flow cytometry. However, there is a notable lack of detailed description of the HPLC pigment analysis methods, and the relevance of flow cytometry in the context of the presented work is not well explained.
- 2) The results are sufficient to support the interpretations and conclusions, and the description of experiments and calculations is sufficiently complete and precise. However, the discussion and conclusion sections lack a connection to the potential satellite applications of the results presented.

## Specific comments

Introduction:

The Introduction is well-structured, and the database is adequately described and appropriate in terms of its representativeness for the study presented.

Materials and Methods:

In the Methods section, substantial attention is devoted to describing the DNA metabarcoding and mRNA methods and analyzing their results. The cytometry method is adequately described. However, the HPLC method is not described at all: the analytical procedure applied, the pigments measured, and the sample pre-treatment method are not mentioned. Additionally, there is no discussion of the uncertainty associated with the pigment measurements. It is unclear how the composition of pigments, such as chlorophyll *a*, is calculated. Furthermore, diagnostic pigments are only briefly mentioned in line 315. The abbreviation for the pigments are not clarified (i.e., Total Chlorophyll *a*) and uniformly used and need to be revised through all the text.

The description of the HPLC method references the Phytoclass (line 165), but its relevance to the article is not further discussed except using for the fig. 4 and fig. S4. On the other hand, at line 329 the text, there's a reference to CHEMTAX (but no link with the phytoclass). There is also no rationale provided for limiting the analysis to diagnostic pigments alone.

Another point to clarify is when cytometry data is used: an explanation of the added value of this information should be included (e.g., why cytometry is important for the *Prochlorococcus*; lines 305 and 408 but not elsewhere).

The seasonal variation is presented but not further discussed in the follow session

Results and Discussion:

Finally, both the abstract and introduction mention the potential use of this study to support the development and validation of remote sensing products. However, additional explanation on how could be realized should be added. Specifically, potential models of pigment concentrations for remote sensing of harmful algal blooms or phytoplankton community structure are not adequately explored.

Conclusion:

This conclusion is comprehensive, presenting the study's findings effectively and connecting them to broader ecological and methodological implications. However, the text could be improved for clarity, conciseness, and better flow (i.e., moving between themes like biases, PDRs, HABs, and remote sensing lack sometimes of clear transitions) Some ideas, such as the limitations of 18S rRNA gene copy number variability and the importance of quantitative approaches, are repeated multiple times, making the text unnecessarily long. jumping between themes like biases, PDRs, HABs, and remote sensing without clear transitions

### **Technical corrections**

Line 115-120: capture/capturing used 3 times in few sentences: consider to use synonym

Line 160. The HPLC acronym was already introduced in line 57

Line 315 the Fuco, Perid, etc... acronyms were introduced already at line 285

Line 321 "correlation", consider to specify Pearson correlation

Line 364 "example, reduced light availability may lead to cellular increases in accessory pigments" please specify if this included accessory pigments used in the present study.

**fig 4.** Total Chl a in A and D is different from and Chl a of E-J ?