Response to Referee 1

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:

- 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.
- 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.

We thank the reviewer for their time and constructive feedback. We agree that much more detail is needed for the HPLC methods, and we have responded to the reviewer's more specific comment about this below. We also agree that the flow cytometry data requires further explanation and have made several revisions to do so. Specifically, we now:

- State in the Introduction on lines 122-123 "Within cyanobacteria, flow cytometry was also used to measure *Prochloroccocus* and *Synechococcus* cell abundances."
- Describe in the beginning of the Flow Cytometry section in Materials and Methods on lines 292-293: "To measure *Prochloroccus* and *Synechococcus* cell abundances with flow cytometry, providing an additional metric of comparison…"

We also agree that the discussion and the conclusions require more connection to the satellite applications. In response we now state:

- Results and Discussion, lines 487-491: "As phytoplankton pigments directly impact remote sensing reflectance spectra, these results further support that potential models for phytoplankton pigment concentrations via remote sensing may be able to provide comparable global estimates of PCC (Kramer et al., 2022). While HPLC pigments are used validating remote-sensing algorithms, these results also suggest that the absolute

abundances of DNA or RNA may be useful metrics to validate for model development of PCC as well."

- Results and Discussion, Section 3.2.2 Monitoring and forecasting harmful algal blooms, lines 601-605: "In the California Current region, *Pseudo-nitzschia* abundances and DA production are forecasted by the California Harmful Algae Risk Mapping (C-HARM) system, which uses satellite remote-sensing data and a regional ocean circulation model. Specifically, the remote-sensing data used as inputs are chlorophyll *a* concentrations and reflectance at 488 and 555 nm from the S-NPP NOAA VIIRS instrument. As Fuco offers greater specificity for diatoms, substituting Fuco for chlorophyll a may improve model predictions, particularly if *Pseudo-nitzschia* is a dominant diatom overall."
- Conclusion, lines 685-691: "By integrating phytoplankton pigments with quantitative abundances of 18S rRNA genes and total mRNA via metabarcoding and metatranscriptomics respectively, we demonstrate that diagnostic pigments for specific eukaryotic phytoplankton groups correlate with both their DNA- and RNA-based abundances. Although there are inherent biases associated with each of these measurements, their relationships suggest that they are comparable and may all individually be useful for validating potential models of PCC from hyperspectral remote sensing reflectance with satellites such as PACE. These relationships also suggest that the potential development of models for remotely sensed pigment concentrations will provide reasonable estimates for the abundances of different phytoplankton groups (Kramer et al., 2022)."

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.

> Thank you.

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.

> We have now expanded the methods text to describe the additional information requested by reviewer, including the sample extraction, analytical procedure, pigments measured that were used in this study, and estimates of uncertainty. We also provide citations that describe the Horn Point Laboratory (HPL) method, which was the method used here, in detail (Hooker, 2005; Van Heukelem and Thomas, 2001).

With respect to describing the diagnostic pigments, they are first mentioned in the introduction on lines 60-62: “certain accessory pigments can be used as proxies, or diagnostic pigments, to determine the abundances of specific groups, even though some of these pigments are shared among groups (Jeffrey et al., 2011; Kramer and Siegel, 2019).

We also now clarify the use of diagnostic pigments in the Materials and Methods on lines 191-192: As described later in the results and discussion, several of these pigments are diagnostic pigments for certain phytoplankton lineages.“

We also list the diagnostic pigments, which taxa they are diagnostic for, and their abbreviations on lines 357-361.

As suggested, we have also modified the text to now use the pigment abbreviations more uniformly throughout the text, except in a small number of specific circumstances where we feel that it would be clear to use the full pigment name.

The description of the HPLC method references the Phytoclass (line 165), but its relevance to the article is no 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.

We have now modified this methods text to describe the relevance of also analyzing the pigments with phytoclass on lines 193-195: “In addition to use diagnostic pigments directly, taxon-specific contributions to TChla concentrations based on the aforementioned pigments were determined with phytoclass v2.0.0.”

With respect to CHEMTAX, it is only mentioned to describe the results of another study where it was used (lines 371-373). We mention that is another chemotaxonomic approach; therefore, we do not believe that it is necessary to further describe.

As suggested, we also now provide rationale for solely examining diagnostic pigments on lines 192-193: “Although other pigments are measured with HPLC, they do not provide as much specificity as the diagnostic pigments used here; therefore, they were not included in the analysis (Kramer and Siegel, 2019).”

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).

We now clarify this methods text by introducing the section with the following (lines 292-293): “To measure *Prochloroccus* and *Synechococcus* cell abundances with flow cytometry as an additional metric of comparison…”

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

Our intention in mentioning that these samples were collected among different seasons is to describe that potential seasonal variability is accounted for within our primary objective of comparing HPLC pigments to DNA- and RNA-based abundances. Our goal in this manuscript is not to examine potential seasonal patterns in phytoplankton pigments or groups in the region, and we

believe that such an analysis is beyond the scope of this manuscript. As a result, we have not made additional revisions in response to this comment.

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.

Please see our response above to the reviewer's general comment about strengthening the connections to satellite applications. In particular, we describe revisions made that further describe how models of pigment concentrations can aid understanding phytoplankton community structure and harmful algal blooms.

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

We agree with the reviewer that the conclusion could be restructured and revised for clarity, and we done so to make these improvements. Please see the conclusions text in the revised manuscript.

Technical corrections

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

We have rephrased this text to avoid repeated use of derivates of capture (lines 120-134).

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

We have removed the unabbreviated text (line 180).

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

Although the abbreviations for fucoxanthin and 19'-hexanoyloxyfucoxanthin were previously introduced, we feel that reintroducing them here aids the reader since the context of the sentence is to list all the pigments used in this study, their abbreviations, and their assignment as diagnostic for certain taxonomic groups.

Line 321 "correlation", consider to specify Pearson correlation

We now specify that the correlations are Pearson correlations here (line 364).

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.

> We now state that “reduced light availability may lead to cellular increases in **all** accessory pigments **examined here**” on lines 407-408.

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

> Yes, panels A-D show total chlorophyll *a* concentrations whereas panels E-J show taxon-specific chlorophyll *a* concentrations estimated from phytoclass. To make this clearer, we have modified the x-axis of panels E-J to now state “Taxon-specific Chl *a*,” and the caption now species that the panels are showing “diversity of individual phytoplankton groups against their taxon-specific chlorophyll *a* concentrations estimated with phytoclass.”

References

Hooker, S. B.: The second SeaWiFS HPLC analysis round-robin experiment (SeaHARRE-2), National Aeronautics and Space Administration, Goddard Space Flight Center2005.

Kramer, S. J. and Siegel, D. A.: How Can Phytoplankton Pigments Be Best Used to Characterize Surface Ocean Phytoplankton Groups for Ocean Color Remote Sensing Algorithms?, J Geophys Res Oceans, 124, 7557–7574, https://doi.org/10.1029/2019JC015604, 2019.

Kramer, S. J., Siegel, D. A., Maritorena, S., and Catlett, D.: Modeling surface ocean phytoplankton pigments from hyperspectral remote sensing reflectance on global scales, Remote Sens Environ, 270, 112879, https://doi.org/10.1016/j.rse.2021.112879, 2022.

Van Heukelem, L. and Thomas, C. S.: Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments, Journal of Chromatography A, 910, 31–49, 2001.