

Review of " Decadal changes in phytoplankton functional composition in the Eastern English Channel: evidence of upcoming major effects of climate change?"

by

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GENERAL COMMENTS:

This paper deals with phytoplankton functional groups (PFGs) evolution over a pluri-annual monitoring program assessed by automated 'pulse shape-recording' flow cytometry (CytoSenses) in the French waters of the Eastern English Channel along an inshore-offshore transect. Seasonal, decadal and spatial dynamics are explored in relation to environmental variables (mostly temperature and nutrients) from 2012 to 2022. The whole phytoplankton size-range (from 0.1 to 800 µm width) is assessed by those flow cytometers and *in vivo* discrimination of six main PFGs : OraPicoProk, RedPico, RedNano, HsNano, OraNano, and RedMicro as described in Thyssen et al 2022. Over the studied period, a general increase of surface temperatures is accompanied by a significant increase of total abundances with a higher contribution of small cells (picoeukariotes and picoprokariotes) and a significant decrease of microphytoplankton.

This work presents the originality of being the first pluri-annual study of the whole size-range of the phytoplankton community characterized by one unique method. The advantages of these devices are the uniqueness of the protocols, and therefore the consistency of the results, and the speed with which they can be obtained. However, these techniques are particularly valuable when the results are coupled with optical identification of the species present, such as those traditionally used in observation networks.

However, the manuscript needs some additional revision, overall, the discussion section needs to be reworked and re-structured in order to clearly address the main questions of the study and to discuss their own results in relation with previous studies.

Hence, given the ecological and methodological interests of the study, I would not reject the paper but I suggest the authors to make some major revisions in the paper.

SPECIFIC COMMENTS:

1- Introduction, p.3, l. 53: it seems to me that Holland et al. (2023b) showed an increase in diatoms and dinoflagellates, not only the small ones.

2- Materials and Methods, p.4, l. 89: nutrient samples were kept frozen before analysis. Freezing may cause polymerization of part of the dissolved Si and therefore underestimate silicic acid concentrations in the samples. If needed (very low concentrations), the authors might comment on this in the paper.

3- Materials and Methods, p.4, l. 100. Is there a homogeneous analysis of data (same Cytosense, same detector, time of analysis, operator, etc...) from 2012 to 2022? When looking at Fig.9 (Results, p. 15), it seems like there are some shifts... are those due to real abundances shifts or to any methodological bias?

4- Materials and Methods, p. 6, l. 130: Was linear time series interpolation performed only in ONE station (“this station”) for filling gaps in the time series data set, or in all stations? Please modify if necessary.

5- Materials and Methods. Si:N:P diagrams have been already used in the literature to identify potential nutrient limitations of phytoplankton. Please refer to some original or previous papers using these representations.

6- The environmental parameters considered by the authors in the papers are temperature, salinity and nutrient concentrations. What about light availability (either irradiance or underwater PAR measurements if available)? Solar radiation should be the same everywhere for the whole transect for one given sampling date but it will vary with seasons, and, moreover, with years.

Furthermore, seawater turbidity at each station will modify light availability and therefore constrain phytoplankton community structure. If such data (PAR or turbidity) is not available, the authors should consider this important limiting factor and at least discuss this point.

Specially, in the Results section (p. 10, paragraph 3.3.1), the decadal evolution of solar radiation would be an important point to be considered.

7- On the same line of thinking, water column vertical structure (pycnocline, thermocline...) should be considered in the discussion since this will structure the whole phytoplankton community including the surface.

8- Results. A general and important comment on how results are interpreted in terms of “trends”. The authors use cumulative sums as well as Mann-Kendall tests to highlight trends. If Mann-Kendall tests are not significant, therefore positive or negative trends (from cumulative sums) are not to be considered and therefore not interpreted. I would suggest to use Mann-Kendall tests for long term trends over the whole studied period, and cumulative sums for a more short-term tendency (seasonal trends) and to evidence eventual shifts. The combination of both methods is not clearly explained.

9- Table 3, p. 12. What is a significant trend of 0.000 (R3, phosphate)? Furthermore, the Table caption says “The figures indicate the magnitude of the trend”. Do the authors mean “the values”?

10- Page 12, l. 257-259: “The combined analysis of raw data, cumulative sums and trend tests facilitated the identification of trends and periods of change in physico-chemical variables”. Could you explain how those periods (and shifts?) are identified.

11- Figure 7, p. 13: Are all data points (sampling dates) plotted on the figure? If this is the case, the authors should not talk about potential limitation during winter periods when nutrients are very high and phytoplankton production and biomass are low. If all dates are plotted, average nutrients are biased with winter values and potential limitations for productive periods are hidden.

12- Page 13, l. 268-272 (about figure 7): Indeed, figure in Appendix A1 shows those nutrient ratios for each season, and it is clear here that winter nutrient ratios are different from the other seasons (although marker colors do not allow to see the chronology).

The authors also state that “winter has moved from a potential phosphate-limiting situation towards a slight nitrogen-limiting system”: it would be more accurate to say that “winter has moved from a N excess to a P excess period”.

They also state that “These seasonal variations are well reflected in the annual nutrient ratio”: I do not agree with this statement, since it seems to me that seasonal variations are not reflected in the annual average.

13- Discussion, p. 14, l. 298. Please recall here the results, *i.e.* which groups of “smaller cells” have significant increasing trends, and refer to table 4. Pico- but also Nano- phytoplankton are both smaller than micro-phytoplankton.

14- Page 15, l. 300-301: I fail to see the relevance of this comment. Is it a conclusion of the « Results » section ? Is it really essential?

15- Page 16, l. 321-322: How are explained the decrease of river flowrates and the increase in nutrient concentrations (P near the coast and Si in open sea)? Which rivers are the most relevant to be considered in this ecosystem? What about rainfall?

16- Page 16, l. 326: The authors say that “The trend analysis showed an increase in the monthly mean of nitrogen concentrations in the transect”: I don't understand what increase is being referred to? Is it on a spatial gradient? Table 3 shows a decreasing trend in N during the period.

Other statements are also contradictory with Table 3 trends.

17- Page 16, 4.2 Subsection “Change in nutrients concentrations and ratio”: I fail to see the final goal of the nutrient discussion. I fully understand a discussion on trends for the overall period (own data presented in the paper) in relation to possible causes of those trends (from previous literature) and more specifically consequences on the observed FPG trends (own data presented in

the paper). The messages might be blurred because global and seasonal trends are all discussed together. I would suggest the authors to clearly identify the main messages to be drawn from their own data from this study and then re-organize the discussion section according to this.

18- Page 17, l. 357-359: "...while the SOMLIT (National Observation Service of the Research Infrastructure ILICO) coastal station (South of Boulogne-sur-Mer and further offshore) showed a decrease in all N:P, Si:P and Si:N ratios." Is there any publication showing such decrease of ratios? No references are cited.

In addition, citing SOMLIT as a national observation network in the text is necessary but its belonging to the ILICO infrastructure should be placed in the acknowledgment section.

19- Page 18, l. 361-383: This subsection is entitled "Changes in phytoplankton biomass and abundances". The whole subsection, and those first twenty lines in particular, are much more discussing nutrients in relation to species identified in other papers. Echoing my comment n°17, in my opinion, a single subsection bringing together the discussion about phytoplankton and nutrients would be more appropriate, and focusing on the authors own results on FPG observations and trends from the present study.

20- Page 18, l. 379. I do not find any "positive correlation between nitrogen availability, particularly during winter, and chlorophyll a concentration" in the paper. Have correlations been calculated? If so, a table with the corresponding results would be necessary.

21- Page 19, l. 410. The authors state that their study "has also highlighted the importance of bottom-up control" but I don't see what they are exactly referring to. Bottom-up control is very large including light, nutrients, temperature, hydrodynamics... The only controlling factor, which, moreover, has already been widely discussed in the paper, is nutrient availability.

22- Page 19, "General discussion, limitations and perspectives". I agree that it is important to highlight the great additional data that this local monitoring survey brings to phytoplankton dynamics understanding, in addition to regular national monitoring services that are related to phytoplankton. Is weekly sampling a general rule (and therefore I agree with the higher sampling frequency)? However, COAST-HF network does not acquire phytoplankton data and therefore I do not think that this would be a limitation.

In addition to the advantages of this technique, I would suggest to mention that the major limitation is the lack of identification at a more precise taxonomical level and therefore the need of coupling those Cytosense measurements with optical identifications.

TECHNICAL CORRECTIONS:

- 1- At different places in the paper (abstract, fig. 7 and fig. A1, page 13) the authors make reference to Redfield ratios. Please add when absent the Brzezinski reference (only done in page 7).
- 2- Page 2, l. 24: replace “higher food webs” by “through the food web” or “to higher trophic levels”.
- 3- Page 4, l. 87: please replace SiO_2 by $\text{Si}(\text{OH})_4$ when talking about silicic acid (or dissolved silicon). SiO_2 is silica, which is particulate silicon (biogenic or opal, or lithogenic). Also, although much more widely accepted in the literature, it would be more accurate to use “dissolved Si” or “silicic acid” or just “Si” instead of “silicate” (Fig. 3 and 6, pages 8, 10, 11, 13, 16, 17, 20, Table 3).
- 4- Page 8, l. 199: prefer the words “coastal” or “nearshore” to “littoral”.
- 5- Figure 7, p. 13.
 - ➔ Please explain what “>” or “<” signs mean (“more limiting than” or “higher concentrations”?)
 - ➔ It would help understanding if colors used for years are chosen from clear to dark, or any other color gradient to be able to follow the chronology. Same for Appendix figure.
 - ➔ The horizontal black line is not visible (N:P = 16:1)
- 6- Page 16, l. 260 (and elsewhere). Use “molar ratios” or “nutrient ratios” instead of “Redfield ratios”.
- 7- Page 15, 4.1 section title “Changes in physical parameters”: The only physical parameter of the study is temperature. Salinity is a chemical parameter. The authors may use “Physical and chemical parameters” and then discuss salinity and nutrients in the same section.
- 8- Page 16, l. 323, 4.2 subsection title: Please add “s” (plural) to “ratio” (“Change in nutrients concentrations and ratios”)