Phytoplankton coastal-offshore monitoring by the Strait of Dover at high spatial resolution: the DYPHYRAD surveys

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Reply to Referee Report

Zéline Hubert, Aurélie Libeau, Clémentine Gallot, Vincent Cornille, Muriel Crouvoisier, Éric Lécuyer, Luis Felipe Artigas

First of all, we would like to thank Referee 1 (R1) and Referee 2 (R2) for their constructive remarks and insightful comments. In order to best respond to this feedback, the response letter combines R1 and R2's comments in two separate sections: section 1 deals with R1's comments, section 2 covers R2's comments. To make these responses easier to read, the letter was structured as follows: the referees' comments are shown in blue, the authors' responses in black, and the changes made in the manuscript, in green.

1 Referee 1 comments (RC1)

1.1 Technical changes

Line 9: A missing word in here: "Weekly sampling resolution allowed to address. . . ."
Thank you for pointing this out. We have revised the sentence to read: *"Weekly sampling resolution allowed us to address. . . "* (line 9, page 1) to correct the grammatical error and improve clarity.

Figure 1: the black station labels are difficult to see in the deep depths in blue. Can you change the color of the sampling stations?
Thank you for your suggestion. We have modified the color of the station labels in Figure 1 to enhance visibility against the background, especially in the deeper (blue) areas. The figure has been updated and replaced in the revised manuscript (Fig. A, page 5).

Figure 2: It is not that easy to discern the differences in the sizes of the circles to indicate the number of stations. If the authors feel this is an important point to make, perhaps they could use different colors to indicate the number of stations visited.
Thank you for your helpful comment. We agree that the differences in circle sizes were not easily distinguishable. To improve clarity, we have revised Figure 2 by using a color gradient to represent the number of stations visited, in addition to adjusting circle sizes for better visibility. The updated figure was replaced in the revised manuscript (Fig. B, page 6).

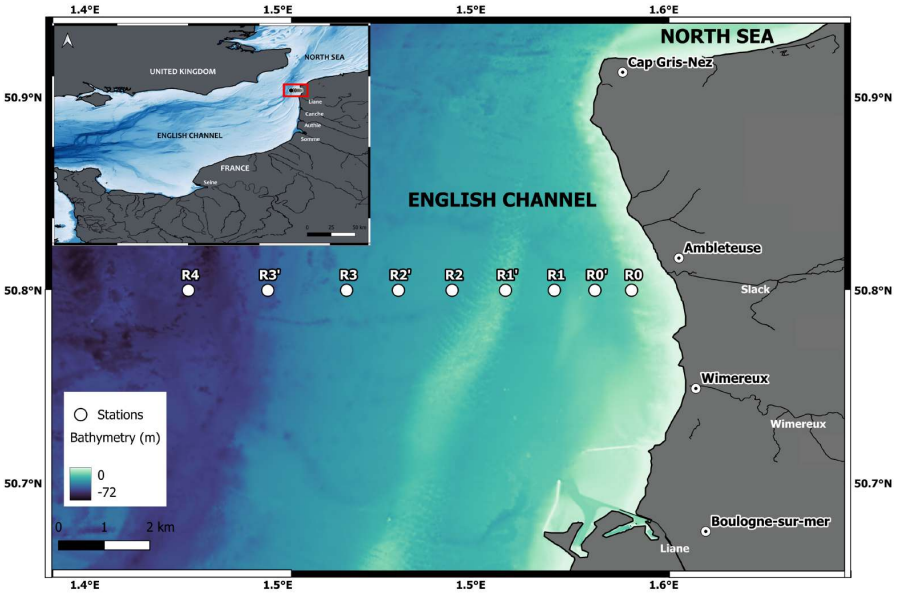

Figure A: Map of the study area (indicated by the red square on the high scale map) and location of the DYPHYRAD stations. The bathymetry data was retrieved from SHOM 2015 and 2016 calculations (`https://diffusion.shom.fr/donnees/bathymerie/mnt-cotier-pas-de-calais.html`).

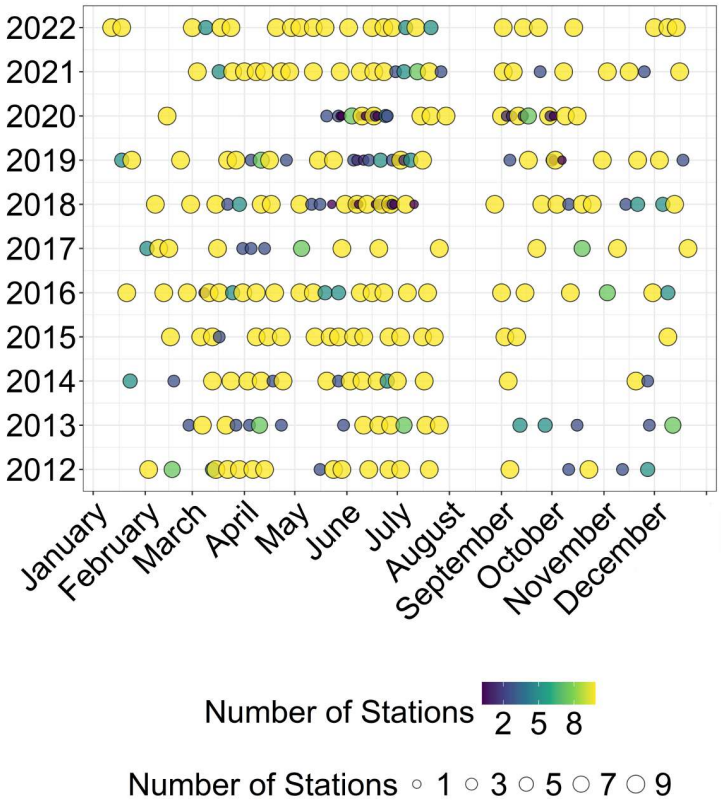

Figure B: Distribution of DYPHYRAD sampling over years (2012-2022). The dot size represent the number of monitored stations for each discrete sampling.

Line 111: The N species have superscripted numbers, but they should be subscripts

Thank you for pointing this out. We have corrected the formatting so that the numbers appear as subscripts rather than superscripts : NO_2^-, NO_3^-.

Line 125: Perhaps the authors could include here how long the samples were typically held before being analyzed in the laboratory.

Thank you for this valuable suggestion. We have now included information on the typical holding time of the samples before laboratory analysis in the Methods section (line 126). On average, samples were held for a maximum of 4 hours, cooled in the dark by placing them in an icebox with ice packs prior to analysis. The following sentence was added (line 152, page 7): Upon returning to the laboratory, *after a maximum of 4 hours of dark conservation in a cooler, in vivo* fluorescence was measured immediately on 50 mL water samples using a Turner Designs benchtop fluorometer (10-AU Field Fluorometer, Turner Designs Ltd, USA).

Line 139: "manufacture's" should be "manufacturer's"

Thank you for catching this typo. We have corrected "manufacture's" to *manufacturer's* at line 165 (page 7).

Figure 11: The words and labels on this figure are probably too small

Thank you for your comment. Figure 11 showing the spatial variability of phytoplankton biomass over time along the DYPHYRAD transect was moved to the appendix of the manuscript according to the reviewer's 2 comment.

Line 283: I am not sure what "relaying on chlorophyll a concentrations" means

Thank you for pointing out the unclear phrasing. We have now clarified the sentence at line 283, which has been revised to: *Particularly, intense spring blooms were observed in 2013 and 2021, as indicated by chlorophyll a concentrations.*

Line 298: This first sentence needs to be edited. "enables to differentiate" is incorrect grammar.

Thank you for this helpful comment. We have corrected the sentence at line 298 to improve grammar and clarity. It now reads (line 382, page 19): *Multi-spectral fluorescence allows differentiation of up to four spectral (pigmentary) groups.*

Figure 14 caption. For the last sentence, I would say "All surface stations were grouped by season." (also add a period at the end of the sentence.

Thank you for this suggestion. We have revised the last sentence of the caption for Figure 14 as recommended. It now reads: "*All surface stations sampled from 2012–2022 were grouped by season.*". It was also done for Figure 7, 8, 9, 10, 11 and Appendix D1.

Line 337: make it "coast-offshore" (i.e., delete the space). But later (line 338), it is "coastal-offshore". Be consistent.

Thank you for pointing out this inconsistency. We have revised the text to use "*coastal-offshore*" consistently throughout the manuscript for clarity and consistency (for exemple, line 424, page 20).

Figure 16: The words and labels on this figure are probably too small

Thank you for this helpful comment. Figure 16 showing the spatial variability of phytoplankton abundance over time along the DYPHYRAD was moved to the appendix of the manuscript according to

the reviewer's 2 comment.

2 Referee 2 comments (RC2)

2.1 Major Comments

I recommend rejecting the paper for now, for two main reasons which are linked: - Too much overlap with another published paper - Not enough value beyond the data repository The manuscript's primary issue is that it presents itself as an abbreviated or "light" version of the scientific analysis already published in Ocean Science (Hubert et al., 2025; https://doi.org/10.5194/os-21-679-2025). The Ocean Science paper tells the full scientific story: it provides the scientific context, analyzes long-term trends in detail, and offers an ecological interpretation. While this ESSD manuscript avoids extended interpretation, it mirrors the Ocean Science paper in scope and narrative, and therefore fails to stand on its own. This lack of an independent purpose became particularly evident during my review. Most of the scientific questions I initially had regarding data patterns and potential interpretations were already fully addressed in the Ocean Science paper. This highlights the core problem: the current manuscript raises scientific questions that it cannot explore in depth, because that work is already published. As a result, it feels redundant. Also, the manuscript must cite the Ocean Science paper. Not doing so is a major problem. This is not just about giving credit; it's essential so that other scientists can understand the full context. It lets people reading the data paper see how the data has been used, and it helps people reading the science paper find the detailed documentation they need.

Thank you for your detailed and thoughtful comments. We understand your concerns regarding overlap with the Ocean Science paper (Hubert et al., 2025) and the need for the ESSD manuscript to clearly stand on its own. We fully agree that the Ocean Science article must be cited, and we have added this citation in the revised version. We acknowledge that the initial manuscript did not sufficiently highlight the distinct purpose of an ESSD data paper. In the revision, we have removed or condensed elements that could be seen as analytical or narrative, and we now explicitly emphasize that the scientific interpretation is presented only in the Ocean Science paper except for what concerns spectral groups variability from multispectral fluorometry measurments that were not included in the Ocean Science paper. The ESSD manuscript is refocused on documenting the dataset, its construction, processing steps, uncertainties, and accessibility, rather than discussing ecological or long-term trends. In response to your comments, we strengthened the unique contribution of the data paper. We now provide expanded documentation that is not included in the Ocean Science article: detailed descriptions of data sources, quality control procedures, file structures, data formats, and guidance for reuse. We hope these changes address your concerns and demonstrate the independent value and purpose of the ESSD manuscript.

The paper does not give enough of the deep methodological details, technical validation, and data descriptions that are the key parts of a true data paper. The purpose of ESSD is to publish papers on datasets so they can be easily found, understood, and reused. This requires much more detail than what you find in a simple data repository. As it is now, the manuscript is missing this detail. For example: Methods: It says that different Cytosense instruments were used over the 11 years but gives no information on how they were compared or intercalibrated. This is absolutely critical to know if

we are to trust a long-term time series. Data Processing: There is no description of the steps taken to process the data, the quality control flags used, how uncertainty was estimated, or the specific gating strategies for the flow cytometry data. Validation: There is no technical validation comparing different measurement approaches (e.g., Fluoroprobe chlorophyll estimates vs. extracted pigments, flow cytometry abundance vs. microscopy counts, or sensor performance across different environmental conditions). Without this information, it is very difficult for another researcher to trust and reuse the data. The paper basically just says "this dataset exists," but the SEANOE repository already tells us that. While the dataset is excellent, this manuscript doesn't justify being a separate paper yet. I believe that with a major rethinking and rewriting, it could become a suitable submission. This requires a shift in perspective: from "how we used this data" to "how you can use this data." I see two possible paths for the authors.

We agree that these aspects are essential for an ESSD data paper, and we have substantially revised the manuscript to address these points. We acknowledge that the initial submission did not include sufficient details on instrument intercomparison. In the revised version, we now provide a full description of the different Cytosense instruments used over the 11-year period, the intercalibration procedures performed during instrument replacements or maintenance. We have added comprehensive information on the processing pipeline applied to raw data, quality control steps and flagging criteria, the gating strategies and parameter definitions used for flow cytometry. These details go significantly beyond what is provided in the SEANOE repository and are now fully documented in the manuscript. Following your recommendation, the manuscript now includes dedicated validation analyses, including comparisons between Fluoroprobe and extracted pigment chlorophyll measurements. This section aims to strengthen user confidence in the dataset and explicitly supports its reusability. We appreciate your guidance on shifting the focus from "how we used this data" to "how you can use this data." The manuscript was reorganized accordingly: descriptions are now centered on transparency, reproducibility, and practical reuse, scientific interpretation was minimized and the dataset's structure, strengths, and limitations are more clearly articulated for future users. We believe these major revisions now provide the level of detail expected for an ESSD submission and demonstrate the dataset's long-term value and reliability.

Option 1: Reformat as a "Pure" Data Paper The manuscript must be expanded to include the precise, practical information a future user would need to assess quality, understand limitations, and trust the data for their own projects. This includes: Expand the Methods and Data Description: Sampling: Station coordinates, sampling protocols, depth sampling... Instrumentation: a detailed report on the intercalibration between the different instruments used (i.e., CytoSense). Data Processing: A complete workflow from the raw files to the final data, including software used, code/scripts (if possible), the QC steps, and a full description of the quality flags applied to the data. Add a "Data and Potential Applications" section: Instead of a scientific discussion, this section should briefly mention the results of Hubert et al. (2025) as one example of how the data can be used. Then, it can suggest other potential uses (e.g., for satellite algorithm validation, data assimilation in models, or studies of short-term events). Reference Hubert et al. (2025): The authors must cite their Ocean Science paper in the introduction as the first scientific paper to come from this dataset. ESSD exemplars you can follow:

- Acri et al. (2020): A long-term (1965–2015) ecological marine database from the LTER-Italy

Northern Adriatic Sea site: plankton and oceanographic observations; 5194/essd-12-215-2020

- Lefebvre and Devreker (2023): How to learn more about hydrological conditions and phytoplankton dynamics and diversity in the eastern English Channel and the Southern Bight of the North Sea: the Suivi Régional des Nutriments data set (1992–2021); 10.5194/essd-15-1077-2023
- Sosinski et al. (2025): Long-term monitoring of hydrological dynamics and phytoplankton biomass indicator in three shellfish ecosystems of the English channel (2000–2024); 10.5194/essd-17-4737-2025

Option 2: Create a Different "Hybrid" Paper If the authors want to keep a scientific story, it must be completely different from the one in Ocean Science. It would need a new scientific question and a different analysis. For example, they could focus on a methodological study, like a detailed comparison of how the high-frequency sensors performed in this turbid coastal water, or an analysis of short-term (daily, tidal) changes, which is not what they did in the Ocean Science paper. Conclusion: I recommend rejection of the manuscript in its current form because its identity as a "light version" of existing research does not align with the core mission of ESSD. However, the dataset is of high quality and importance. I would strongly encourage the authors to undertake a significant reconceptualization. By transforming this manuscript into a pure, technically detailed data descriptor that complements rather than condenses their Ocean Science paper, they could make a valuable and welcome contribution to the data community. I would be very supportive of a resubmission that follows this path.

Thank you for outlining the possible directions for improvement. We agree with your recommendation to follow Option 1 and have fully restructured the manuscript as a "pure" data paper. We have expanded the Methods and Data Description (sampling protocols, station details, depth sampling, Cytosense intercalibration), added a complete processing and QC workflow with detailed quality flags, and incorporated a technical validation section. We also added a new "Data and Potential Applications" section that cites Hubert et al. (2025) as the first scientific use of most of the dataset and highlights additional potential uses. These revisions follow the structure of the ESSD exemplars you suggested and aim to meet the journal's expectations for transparency and reusability. We thank you for your constructive guidance and believe the revised manuscript now aligns with the mission of ESSD.

2.2 Specific Comments

I really appreciate the authors' transparency about using different instruments over the years. This is very important information for anyone who wants to use the data.
We appreciate the positive feedback from the reviewer and are glad that the transparency regarding the instrumentation was valuable.

Phaeocystis globosa is a very important species in this region. It would be really useful if the authors could add a short section that focuses specifically on its dynamics using this dataset.
We thank the reviewer for this valuable suggestion. *Phaeocystis globosa Scheffrel* is indeed a key species in this region, and we agree that highlighting its dynamics would strengthen the manuscript. Following the reviewer's recommendation, we have now added a short dedicated section describing the temporal patterns and interannual variability of *P. globosa Scheffrel* within our dataset (see Section

4.3.1, lines 453–479, pages 22–24). This additional analysis emphasizes its seasonal development, interannual fluctuations, and potential environmental drivers.

The statistical reporting in the text is inconsistent. Sometimes the mean is reported (often without a measure of variance like SD, SE, or CI), other times the mean with min/max, and elsewhere the median. A consistent statistical reporting style (e.g., median [min – max or IQR] or mean ± CI) must be adopted throughout the manuscript. See this article https://www.nature.com/articles/nmeth.2659
Thank you for this comment. We have revised the manuscript to ensure consistent statistical reporting throughout. All values are now presented as Mean ± SD, following a uniform style across the text.

It needs to be much clearer that all the data is from the sub-surface This is a critical detail. I suggest mentioning this in the Abstract and making it very clear in the Methods section.
This clarification has been added to the Abstract : "*... were sampled sub-surface off the Slack estuary...*" (line 4, page 1) and described in the Methods section : "*...consisting of nine sub-surface (1-2m) sampling stations...*" (line 95, page 4).

All the figures are too small, and it is very hard to read the axis labels. Please make them much bigger.
We have increased the size of all the figures presented.

Please consider using different color palettes for different variables to make the plots easier to understand.
Thank you for your comment, however, we do not believe that changing the colors based on variables makes the figures more understandable.

For the plots showing the transect, please add a label on the axis to show which side is "inshore" and which is "offshore."
Following your comment regarding the repetition of graphs representing transects and time series, we have decided to move these figures to the appendix and have added "Inshore" and "Offshore" to the figures for clarity.

I find that showing both the time-series plots (like Fig. 7) and the boxplots (like Fig. 8) for the same data is a bit repetitive. Maybe you could choose just one format to make the paper clearer?
We fully understand that presenting both time series (e.g., Fig. 7) and box plots (e.g., Fig. 8) may seem redundant. However, these two graphical representations had distinct and complementary objectives.

Box plots summarize the temporal evolution of variables by calculating the average of all stations, thus providing an overview of large-scale temporal dynamics. In contrast, transect graphs highlight small-scale spatial variations along the studied gradient, allowing for a more detailed examination of local patterns and variability.

These two representations are therefore complementary, but in light of your comment, we have chosen to prioritize boxplots to visualize long-term dynamics and have placed the other figures in the appendix.

L18: "Berglund et al., 2007" Please consider citing a more general, foundational reference for this statement, such as a paper by Falkowski Falkowski, P. G. and Raven, J. A. (2007) Aquatic Photosynthesis. Princeton University Press, Princeton.
Thank you for the suggestion. We have added the foundational reference to Falkowski and Raven (2007) (line 18, page 1).

L28: "Phaeocystis globosa" Please add the authority to the species name: Phaeocystis globosa Scherffel.
Thank you for your suggestion, we have added the authority to the species name as you suggested.

L30: " (Lancelot et al., 1994, 1998; Medlin and Zingone, 2007; Li et al., 2022)." To improve conciseness, consider replacing these multiple citations with a single, comprehensive recent review, such as Phaeocystis: A Global Enigma; 10.1146/annurev-marine-022223-025031.
Thank you for the recommendation. We have replaced the multiple citations with the suggested comprehensive review (Smith and Trimborn, 2024) to improve conciseness (line 31, page 2).

L30-33: ...these blooms are considered undesirable due to their tendency to form dense gelatinous colonies... It would be relevant to mention that this species is listed as a Harmful Algal Bloom (HAB) species in the IOC UNESCO database (`https://www.marinespecies.org/hab/aphia.php?p=taxdetails&id=160538`).
Thank you for this important comment. We have added this clarification to the text: "While non-toxic in the Eastern English Channel and the North Sea, *they are classified as harmful algae blooms (HAB) by UNESCO (Lundholm et al., 2025) because* these blooms are considered undesirable due to their tendency to form dense gelatinous colonies and foam layers..." (lines 32–33, page 2) and added the corresponding reference (Lundholm et al., 2025).

L34: " (Peperzak and vanWezel, 2023)." Please consider starting a new paragraph here.
It is true that the paragraph is easier to understand when a line is skipped. Thank you for pointing this out.

L85: "Sampling Strategy" The 'Sampling Strategy' section lacks information on the vertical dimension. Figure 2 suggests some variability in sampling depth, which could lead to vertical variability in the data. This needs to be addressed.
Thank you for the comment. We clarified in the revised manuscript that all samples were collected in the subsurface layer only. The apparent variability in Figure 2 reflects the number of stations sampled, not variations in depth.

L91: "sub-surface sampling" Please define 'sub-surface sampling' more precisely (e.g., specific depth or depth range).
Following the previous comment on the need to clarify what the sub-surface area is, we have provided further details in the Methods section (lines 88, 95 and 100, page 4).

L96: "from surface water samples" Does 'surface water' correspond to a specific depth (e.g., 1 m)? Given the riverine inputs, some stratification is expected. Please clarify how depth variations

were handled.
Surface samples are taken at a depth of approximately 1.5 m. This is not a precise measurement to the nearest centimeter. This precision has been added to the sampling strategy in the Methods section: "Seawater samples were collected using Niskin bottles at approximately 1.5 m from the surface."

L99-101: "high-frequency dynamics during periods of particular interest". Please specify what made these "periods of particular interest." For example, were they during expected blooms?
We have provided details regarding the periods chosen line 105–108, page 4: "Between 2018 and 2020, extra daily sampling cruises were conducted at three key stations (R1, R2, and R4) to capture high-frequency dynamics during periods of particular interest *in June-July and September-October in order to capture rapidly changing phytoplankton dynamics. The post-bloom period was chosen because of the high bacterial activity following Phaeocystis globosa Scheffrel blooms (Lamy et al., 2009) and and sporadic low-intensity blooms may occur in automn (Breton, 2000)*".

L106: "Sub-surface values were calculated as the average of measurements taken between 1 and 2 meters deep". By averaging data from 1-2m, do you risk catching only the influence of river plumes, especially in inshore areas?
Although this depth generally represents the upper mixed layer in the study area, we acknowledge that in coastal zones and during significant river plume events, some influence from freshwater inputs cannot be entirely excluded. However, subsurface values from the CTD were averaged between 1 and 2m to match water samples, which are typically collected around 1.5m depth, thus ensuring methodological consistency. A sentence was added in Material and Method section to clarify this point (line 100, page 4).

L111: nutrients (NO_2^-, NO_3^-, SiO2 and H3PO4) I agree with Reviewer 1's comment on this.
We have corrected this remark following the RC1 comment.

L111: the sub-surface Again, does 'sub-surface' always mean 1m? Was sampling always at the same depth? This needs to be clarified.
We have clarified this remark thanks to the comments you made earlier.

L112-113: The samples were transferred to sterile 50 mL PVC flasks, then analyzed... Were the nutrient samples filtered? If so, what was the filter type and pore size? If not, the data could be influenced by intracellular nutrients, especially during blooms. Please clarify this critical step. Also, were the analyses performed in triplicate?
We thank the reviewer for this important point. In our study, nutrient samples were not filtered prior to analysis. Therefore, the measured concentrations represent total nutrients, including both dissolved and any intracellular nutrients present in the samples. We acknowledge that during phytoplankton blooms, intracellular nutrients could contribute to the measured values.

Regarding replication, the nutrient analyses were performed on single samples due to logistical constraints.

We have clarified these points in the Methods section to ensure transparency about the sample processing and analytical approach: "The samples were transferred to sterile 50 mL PVC flasks, without pre-filtration, ..." (line 121, page 5) and "The analyses followed the SOMLIT protocol, based on

the methodology described by Aminot2007, and were performed on single (non-replicated) samples" (line 125, page 5).

L140-141: The instrument can also deliver total chlorophyll a estimates... Was an inter-comparison performed to check the agreement between the different methods of estimating total Chl-a?

Yes, we performed an inter-comparison between the different methods used to estimate total chlorophyll *a* (Chl-*a*). The results are shown in Figure C (Appendix B1 in the manuscript).

Panel A compares the *in vivo* chlorophyll *a* estimates obtained from the FLP with those from the Turner fluorometer ($R = 0.84$, $p < 2.2 \times 10^{-16}$), showing a strong and highly significant linear relationship. Panel B compares the FLP estimates with extracted Chl-*a* concentrations determined by extracted fluorometry ($R = 0.55$, $p < 2.2 \times 10^{-16}$), indicating moderate agreement but higher dispersion, as expected from methodological differences between optical *in vivo* and extraction-based techniques *in vitro*. Panel C compares Turner *in vivo* Chl-*a* fluorescence with extracted Chl-*a* ($R = 0.67$, $p < 2.2 \times 10^{-16}$), showing a significant and positive correlation.

Overall, these comparisons confirm that all three methods provide consistent trends in total Chl-*a* estimates, with the strongest agreement between the two *in vivo* fluorometers (FLP and Turner). The differences in slope and scatter between methods mainly reflect differences in calibration and measurement principles (optical signal vs. pigment extraction).

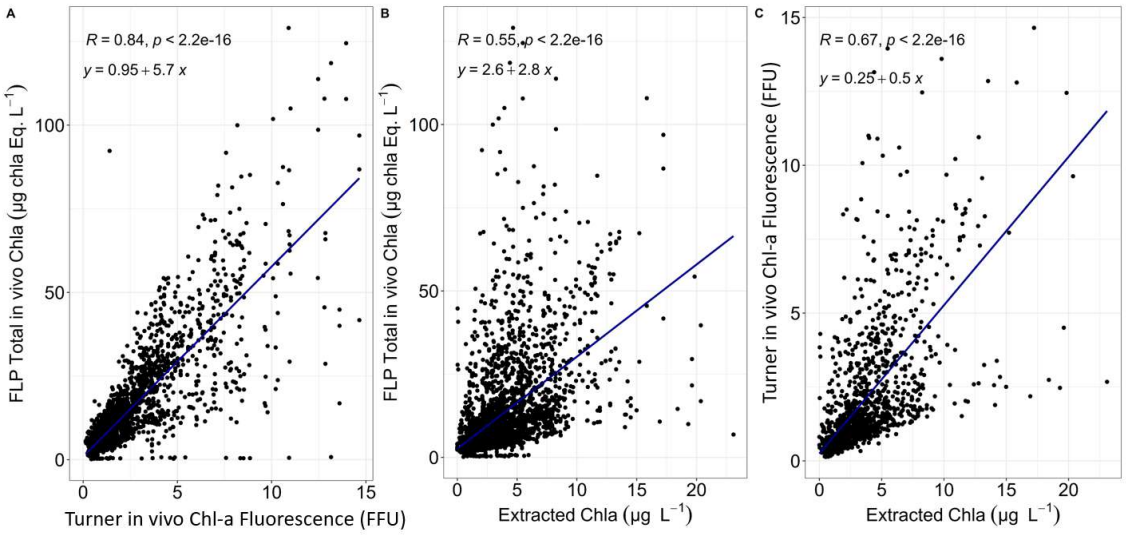

Figure C: Inter-method comparison of three approaches for estimating chlorophyll-*a* concentration

L142: Only four spectral groups can be addressed at a time. Why is this? Is it a limitation of the instrument? Please clarify.

We have added information explaining why only four spectral groups can be analyzed at a time. This is a limitation of the device due to the number of lasers used. "*This instrument can only address four spectral groups at a time, this limitation being imposed by the limited number (five) of excitation wavelengths available. The fluorescence signal is decomposed from five independent spectral channels, which allows for the resolution of only four distinct components at most.* (lines 172–174, page 8).

L169-170: (FWS and SWS). These acronyms are not defined.

This is an oversight, thank you for pointing it out. The acronyms have been defined. "*...Forward*

Scatter - FWS and Sideward Scatter - SWS..." (line 202, page 8)

L177-180: OraPicoProk or Synechococcus-like cells, RedPico or picoeukaryotes... These acronyms are a bit difficult to follow. Please consider using more intuitive names if possible.

Thank you for the comment. The acronyms we use (e.g., OraPicoProk, RedPico) follow the standardized nomenclature proposed in Thyssen et al. (2022) for flow cytometry, which facilitates comparison across studies. To improve clarity, we have added a table (Table 1) summarizing these groups, axis combination used and their likely corresponding species based on the literature, allowing readers to better interpret the acronyms (page 11).

Table 1: Combinations used to highlight different Phytoplankton Functional Groups (PFGs)

Functional Group	**Axis Combination Used**	**Comments**
OraPicoProk (Synechococcus-like)	Max SWS vs Max FLY; Max FLR vs Max FLO; Max FLR/Max FLY vs Length FWS	Prokaryotes containing phycoerythrin (Fragoso et al., 2019)
OraNano (Cryptophytes-like)	Max FLR vs Max FLO; Total FLY/Total FLR vs Total FLO/Total FLR	Eukaryotes containing phycoerythrin (Fragoso et al., 2019; Hubert et al., 2025)
RedPico (Picoeukaryotes)	Max SWS vs Max FLY; Total FWS vs Total FLR; Max FLR vs Max FLO	Small red-fluorescent eukaryotes (chlorophytes, prasinophytes) (Thyssen et al., 2014; Hubert et al., 2025)
HsNano (Coccolithophorids-like)	Max SWS vs Max FLY; Total FWS vs Total FLR	SWS saturation for high sensitivity: coccoliths generate high SWS signal (Von Dassow et al., 2012)
RedNano	Total FWS vs Total FLR; Max FLR vs Max FLO	Autotrophic nano-flagellates as haptophytes (and mainly different life stages of *Phaeocystis globosa* in the English Channel and North Sea (Rutten et al., 2005; Guiselin, 2010))
RedMicro	Total FWS vs Max FLR; Total FWS vs Max FWS	Single-cell and chain-forming diatoms (Bonato et al., 2015), also possible detection of dinoflagellates and *Phaeocystis* colonies only if abundant
RedMicro-2 (Pennate-like)	Length FWS vs Total FLR; Max FLR/FLY vs Length FWS	Two chloroplasts giving two peaks in red fluorescence; thin forms of *Nitzschia* and *Pseudo-nitzschia* spp.

L187-188: ...the following four instruments were used... Were any intercalibration exercises performed to ensure data comparability between the four different Cytosense instruments?

Thank you for raising this important point. Yes, intercalibration exercises were performed to ensure comparability between the four Cytosense instruments. These intercomparisons were conducted on shared samples to directly compare cell counts and optical signals. We have now added a detailed description in the Methods section (lines 222–229, page 11): *To ensure data comparability between instruments, intercalibration exercises were systematically performed at each machine changeover.*

Specifically, a set of common samples, consisting of both natural assemblages collected during routine sampling and calibration bead standards, were analyzed on the outgoing and incoming instruments after acquisition settings parametrization according Gallot et al. (2025). This procedure allowed us to verify the stability of key optical parameters (e.g., forward scatter, sideward scatter, red fluorescence, particle size proxies). When necessary, minor adjustments to the PMT voltage settings and trigger levels were applied.

L190: 3.5 Quality control This section is too brief. The manuscript is missing details on data processing. Please specify the software used (e.g., R, Python) and consider making the analysis scripts available in the data repository to ensure full reproducibility.

Thank you for the comment. We have expanded the Quality Control section to provide a clear overview of data processing and reproducibility. All samples were analyzed within a few hours of collection to preserve fluorescence, with careful homogenization and temperature monitoring. Regular instrument maintenance and calibration—including laser alignment, core alignment, pump calibration, and the use of fluorescent and silica beads—ensured measurement consistency. Measurement protocols were optimized to avoid under- or over-estimation of particle abundances, and low-quality measurements (e.g., insufficient volume, low particle counts, or anomalous files) were excluded from the dataset. All analyses were performed in R, and an experienced operator manually clustered cytograms to maintain consistent classification across the dataset.

L191: Only data with a good quality code What constitutes a "good quality code"? Please explain the criteria.

In the revised manuscript, we clarified that "good quality" data correspond to measurements assigned quality codes 2 and 6, as defined in the Quality Control section (lines 251, page 12).

L191: according to Argo quality control (www.somlit.fr/codes-qualite/; Wong et al., 2019). The linked website is in French. Please provide a brief summary of the quality control procedures in English in the manuscript.

We have added the translated table (Table 2 bellow) in the appendix (Appendix C1).

L201: mean: 8.43 °C, When reporting a mean, please also provide a measure of variance (e.g., SD, SE, or CI). Also, please clarify why the mean is used here while the median is used elsewhere.

Thank you for the comment. We have added the standard deviation alongside the mean to indicate variance.

L211: Over these 11 years, SST have increased, particularly in winter. Is this observation supported by a statistical test (e.g., a linear regression)?

This is an observation of the results; no statistical tests have been performed to prove it. However, we have added in the section "Data and Potential Applications" that this type of test can be performed.

L241: which explains the dynamics "Explains" is a very strong word. It would be more accurate to say "is consistent with."

We have modified the text to make it less assertive: "...decrease during spring bloom until depletion, which *is consistent with* the dynamics observed in this study..." (line 434, page 21)

Table 2: Translation of SOMLIT quality codes

Code	Description
0	Value below the detection limit
1	Sample collected, but measurement not performed (e.g., lost, broken, mislaid, or improperly stored; missing value indicated as 999999 or —)
2	Good measurement, single sample (measurement performed under optimal conditions and appears scientifically valid, but not replicated)
3	Questionable measurement (single value or mean of replicates with some doubt, e.g., analytical issues, calibration problems, value markedly different from typical observations, dispersed replicates without reason; future users are advised to treat with caution)
4	Poor measurement (measurement clearly unreliable; value retained in the database but should not be used)
5	Sample collected, but measurement not yet available (e.g., awaiting analysis; default value 999999 or —)
6	Good measurement, mean of multiple replicates (value averaged from several replicates of the same sample; considered reliable)
7	Good measurement, acquired outside SOMLIT protocols (sample collection and/or measurement performed using a non-SOMLIT protocol; value considered valid; contact the station's scientific manager for details)
8	Unqualified data (value provided but not yet validated)
9	Sample not collected (measurement impossible, e.g., due to sea conditions preventing sampling)

L244-247: During this period, phosphorus (P), silicate (Si), and nitrogen (N) are sequentially depleted... This is a very interesting point and should be expanded. Is this sequence of nutrient depletion (P → Si → N) consistent every year? Changes in this pattern could explain some of the phytoplankton community dynamics. A plot of nutrient ratios (e.g., Si:N vs N:P) could illustrate this very effectively.

We thank the reviewer for this insightful comment. To evaluate whether the sequential nutrient depletion pattern (P → Si → N) is consistent across years, we computed the seasonal stoichiometric ratios N:P, Si:N and Si:P over the full 2012–2022 time series and represented them as barplots (Figure D).

This analysis reveals a coherent and recurrent seasonal pattern that is fully consistent with the proposed depletion sequence: N:P ratio is lowest in winter and increases markedly in spring and summer, indicating that phosphate is the first nutrient to reach limiting concentrations while DIN remains relatively high. Si:N reaches its minimum in spring, reflecting the depletion of silicate following the winter–early spring diatom bloom, while DIN is still available. N:P decreases again from summer to autumn, whereas Si:N increases, which is consistent with DIN becoming limiting later in the productive period, after P and Si have already been drawn down. Si:P follows an opposite pattern, with highest values in winter and lowest values in spring, again supporting the early depletion of P followed by that of Si.

Together, these seasonal trajectories of nutrient ratios provide robust evidence that the P → Si → N depletion sequence is not only present but highly recurrent across the decade-long dataset. This pattern is also fully consistent with the known ecological functioning of the coastal southern North Sea, where phosphate is rapidly exhausted after winter mixing, silicate is consumed during the early spring diatom bloom, and DIN becomes limiting later in the growing season. Importantly, this stoichiometric

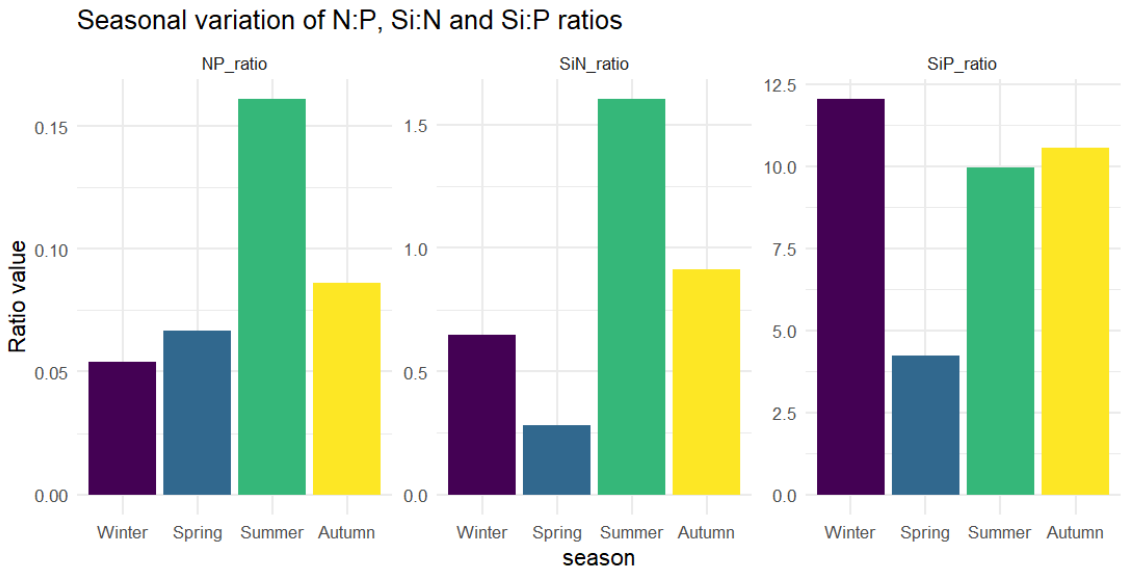

Figure D: N:P:SI limitation during DYPHYRAD time-series.

progression aligns with observed phytoplankton dynamics. The early depletion of phosphate favors taxa capable of exploiting dissolved organic phosphorus (e.g., *Phaeocystis*), while low Si:N in spring reflects diatom limitation. The subsequent decline in nitrogen availability toward summer and early autumn is consistent with the decrease in overall phytoplankton biomass.

Finally, we note that long-term trends in nutrient ratios over the past decade was already examined in Hubert et al., 2025. For this reason, and to avoid repetition, we focus here exclusively on the seasonal coherence of the depletion sequence rather than reanalysing multi-year trends.

L249: The years with the highest median Why switch to the median here, when the mean was used previously? Please be consistent.

There is indeed an error here: What is presented is indeed the mean, not the median. Thank you for pointing this out.

L258-259: Winter silicate concentration decreased until 2017 and then increased again... Is there a reason for this trend? Does it correlate with any changes observed in the phytoplankton community in your dataset?

The decrease in silicate concentrations observed until 2017, followed by an increase, does not appear to influence all phytoplankton communities in the dataset. However, we observed that the dynamics of silicate concentrations appear to have influenced the diatom community in the study area. Indeed, an increase in diatom abundance is noticeable after 2017 (see Figure 12). This pattern can be explained by the essential role of silicates in the formation of the diatom frustule. This trend represents an interesting avenue for future research using these data. A potential link could be explored with changes in salinity, rainfall patterns, mineral runoff, or other climatic events.

L267-269: Stations nearest the coast had highest values. Only years 2013 and 2021 showed a spring bloom that spreads across the entire transect. What might explain this? Do these stations/years have the highest nutrient concentrations?

Yes, as shown in Figure 9, the winters of 2012 and 2020 were characterized by high nutrient concentra-

tions across the entire transect. This high winter nutrient availability explains the spatial extent of the blooms observed along the whole transect during the following spring. In addition, inshore stations show the highest nutrient concentrations, which can be explained by runoff of rainwater enriched in nutrients from the watershed.

L274-276: ...phytoplankton growth is limited by reduced light availability What is the role of temperature in limiting winter growth? This should also be considered.
We agree that temperature could also play an important role in limiting phytoplankton growth during winter. Low temperatures reduce metabolic and enzymatic activity, leading to slower cell division rates and lower overall productivity, even when nutrients are abundant. In our study area, winter temperatures are typically below the optimal growth range for most phytoplankton species, which, combined with reduced light availability, strongly constrains primary production. However, it is possible that winter flowering may occur at these latitudes under favorable conditions, indicating that winter growth is reduced but not completely prevented. Therefore, both light limitation and suboptimal temperatures act synergistically to restrict winter phytoplankton growth.

"In addition, nutrient stocks are relatively high in winter, as phytoplankton growth is limited by reduced light availability *and low temperatures. However, occasional blooms can occur in winter at these latitudes if conditions are favorable.*". (line 355–356, page 17).

L289-291: The timing and intensity of spring blooms can be influenced by limited light availability or competition with diatoms. Your dataset can be used to test this hypothesis. Is there evidence in your PAR data for light limitation? Do your phytoplankton data show that diatoms bloom before Phaeocystis? It would be stronger to support this statement with your own data rather than only a citation.
We thank the reviewer for this important point. Our PAR data indicate that light availability is low during winter and increases in spring (Fig 8), consistent with a seasonal limitation of phytoplankton growth by light. However, inter-annual variations in PAR do not fully explain bloom magnitude: for example, spring 2015 was relatively sunny but did not correspond to particularly high phytoplankton biomass, whereas less sunny periods such as spring 2013 and 2014 and winter 2014 and 2016 still exhibited moderate blooms, indicating that temperature, nutrient availability, and water column dynamics also play important roles (Fig. 7, 8, 12).

Importantly, our phytoplankton data (Fig. 14) show that diatom blooms consistently precede *Phaeocystis* in spring, confirming the classical succession pattern. These observations support the idea that both light availability, inter-specific competition, and the nutrient stocks accumulated during the preceding winter contribute to the timing and intensity of spring blooms, and allow us to base this conclusion on our own dataset rather than solely on literature citations.

We added the following sentence: *Our PAR data indicate that light availability is low during winter and increases in spring (Fig. 8), consistent with seasonal light limitation, and our phytoplankton data show that diatom blooms consistently precede Phaeocystis blooms in spring (Fig. 14), supporting the idea that both light availability and inter-specific competition contribute to bloom timing and intensity and grazing/viral lysis (not studied) to their decay.*". (line 407–410, page 20)

L297: 4.2.2 Phytoplankton spectral (pigmentary) groups... It would be helpful to start this section with a brief overview of the typical phytoplankton community composition in this region.

Thank you for the comment. We have added a brief introduction at the beginning of Section 4.2.2 summarizing the typical phytoplankton community composition in the region, highlighting the dominant groups and seasonal succession patterns, to provide context before presenting the spectral (pigmentary) group analysis. *The phytoplankton community in the Eastern English Channel shows strong seasonal succession. Diatoms generally dominate most of the year, representing up to 85% of total phytoplankton biomass (Breton et al., 2000; Grattepanche et al., 2011; Lefebvre et al., 2011; Hernández Fariñas et al., 2014), except during the spring bloom when the Prymnesiophyte Phaeocystis globosa can exceed 90% of biomass (Brunet et al., 1996; Lamy et al., 2009; Guiselin, 2010; Bonato et al., 2016). Pre- and post-bloom periods are marked by two distinct diatom blooms observed via microscopy, HPLC pigment analysis, multispectral fluorimetry, automated flow cytometry, and environmental DNA (Breton et al., 2000; Schapira et al., 2008; Houliez et al., 2013; Monchy et al., 2012; Christaki et al., 2014; Guiselin, 2010; Bonato et al., 2016). Cyanobacteria, picoeukaryotes, and Phaeocystis globosa dominate abundance patterns, with Synechococcus spp. and picoeukaryotes prevailing in winter and summer, while cryptophytes, coccolithophores, and dinoflagellates occur at lower abundances (Hernández Fariñas et al., 2014; Bonato et al., 2016). Phytoplankton biomass and abundance are influenced by environmental conditions, hydrodynamics, grazing pressure, and microbial seasonal dynamics (Seuront, 2005; Cotonnec et al., 2001; Lamy et al., 2009; Rachik et al., 2018).* (lines 140–150, pages 6–7)

L302-304: Phaeocystis was the main Haptophyte in our study area... Was this confirmed by the parallel microscopy work mentioned in the acknowledgements (i.e., Skouroliakou et al., 2022, 2024)?
Yes, this was confirmed by the parallel microscopy work of Skouroliakou et al. (2024), which showed that *Phaeocystis globosa Scheffrel* dominated the phytoplankton community during spring blooms in our study area. Skouroliakou et al. (2022) further show that diatoms dominated in winter and early spring, consistent with our seasonal observations.

L309-310: The start of the bloom for "brown algae" (mainly diatoms here) is in winter, A diatom bloom starting in winter is unusual. Please specify the timing (e.g., early or late winter). Also, the manuscript should explicitly define the months corresponding to each season (winter, spring, etc.).
The identified diatom blooms are indeed observed at the end of winter. This clarification has been added to the text: "The start of the bloom for "brown algae" (mainly diatoms here) is in late winter...". The seasons had already been detailed in Figure 8. For clarity, we have described the seasons in the Methods section rather than in the figure caption. (lines 394, page 20)

L320-323: It represented between 28 and 90 % of the total chlorophyll a... This is a very wide range. Can the authors use their data to explain the conditions that lead to either low or high dominance?
Thank you for this comment. We have addressed this point by detailing the conditions leading to low or high dominance of *Phaeocystis* in the paragraph describing the "Case of *Phaeocystis globosa Scherffel*".

L344-349: ...RedNano dominated the community by almost 90 % corresponded mostly to the well-known P. globosa bloom. Flow cytometry can discriminate its different life stages. but not shown here. Which fraction did RedNano dominate? The assertion that the abundant RedNano group is P. globosa is too strong without direct microscopic or genetic confirmation. Please rephrase this more cautiously (e.g., 'is consistent with' or 'likely represents'). The manuscript states that flow cytometry

can discriminate Phaeocystis life stages but the data is 'not shown here'. This is a major missed opportunity. Presenting this data would significantly increase the impact of the dataset. Could the authors explain why this was omitted or consider including it?
Thank you for the comment. We have revised the text to state that the dominance of RedNano "is consistent with" the *Phaeocystis globosa Scheffrel* bloom. While flow cytometry can discriminate its life stages, these data were not systematically quantified across the long-term dataset and are therefore not shown.

Fig1: I agree with Reviewer 1; please modify the size and color of the points.
These changes were made following the RC1's comment.

Fig2: I agree with Reviewer 1. The differences in point size should be more pronounced. Consider filling the points with a color gradient to better show the differences. The graph should also be wider. Alternatively, using geomtile might be a good way to display this data.
These changes were made following the RC1's comment.

Fig8 and other boxplot plots: For your information, you can easily control the order of the x-axis in R using factor(). This would avoid the need to put numbers in front of the season names.
We are aware that the numbers in the legend can be removed. However, the issue with the boxplot sizes is not due to the season legend itself, but rather to the limited horizontal space available to align the four seasons, since the figures are composed of multiple subplots.

Table A2: In Table A2, the number of NA values for some parameters is very high (e.g., ¿50% for CTD Fluorescence). Could the authors provide a brief explanation for
We acknowledge that a large number of fluorescence values from the CTD probe are missing. This is due to the fluorescence sensor being non-operational from 2014 to 2019, as well as the absence of part of the data in 2021. Consequently, no fluorescence data are available for these periods. For the sake of completeness, we nevertheless decided to include the available fluorescence measurements from the CTD in our database.

3 Other changes

Additional modifications and technical corrections have been incorporated into the manuscript and are detailed below.

3.1 Major changes

Addition of two new sections "Database Structure" and "Potential Applications".

3.2 Minor changes

A brief introductory paragraph was added to the "Automated *in vivo* phytoplankton measurements" sub-section to clarify the links between the different methods presented.

3.3 Technical changes

Line XX, page X: “Etats-Unis” was replaced with “United States” for consistency in English terminology.

References

Bonato, S., Christaki, U., Lefebvre, A., Lizon, F., Thyssen, M. and Artigas, L. F. (2015), ‘High spatial variability of phytoplankton assessed by flow cytometry, in a dynamic productive coastal area, in spring: The eastern english channel’, *Estuarine, Coastal and Shelf Science* **154**, 214–223.

Fragoso, G. M., Poulton, A. J., Pratt, N. J., Johnsen, G. and Purdie, D. A. (2019), ‘Trait-based analysis of subpolar north atlantic phytoplankton and plastidic ciliate communities using automated flow cytometer’, *Limnology and Oceanography* **64**(4), 1763–1778.

Gallot, C., Hubert, Z., Haraguchi, L., Aardema, H., Artigas, L. F., Bellaaj Zouari, A., Cauvin, A., Casotti, R., Créach, V., Dubelaar, G. et al. (2025), ‘Best practices for optimization of phytoplankton analysis in natural waters using cytosense flow cytometers’, *Cytometry Part A* .

Guiselin, N. (2010), ‘Etude de la dynamique des communautés phytoplanctoniques par microscopie et cytométrie en flux, en eaux côtières de la manche orientale’, *ULCO-MREN, Doctorate (Ph. D.) Thesis in Biological Oceanology, University of Littoral Côte d’Opale (ULCO)* **725**.

Hubert, Z., Artigas, L. F., Li, L.-L., Dédécker, C. and Monchy, S. (2025), ‘Exploring the regional diversity of eukaryotic phytoplankton in the english channel by combining high-throughput approaches’, *MicrobiologyOpen* **14**(6), e70097.

Lundholm, N., Bernard, C., Churro, C., Escalera, L., Hoppenrath, M., Iwataki, M., Larsen, J., Mertens, K., Moestrup, Ø., Murray, S., Salas, R., Tillmann, U. and Zingone, A. (2025), ‘IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae’.

Rutten, T. P., Sandee, B. and Hofman, A. R. (2005), ‘Phytoplankton monitoring by high performance flow cytometry: a successful approach?’, *Cytometry Part A: The Journal of the International Society for Analytical Cytology* **64**(1), 16–26.

Thyssen, M., Grégori, G., Créach, V., Lahbib, S., Dugenne, M., Aardema, H. M., Artigas, L.-F., Huang, B., Barani, A., Beaugeard, L. et al. (2022), ‘Interoperable vocabulary for marine microbial flow cytometry’, *Frontiers in Marine Science* **9**, 975877.

Thyssen, M., Grégori, G. J., Grisoni, J.-M., Pedrotti, M. L., Mousseau, L., Artigas, L. F., Marro, S., Garcia, N., Passafiume, O. and Denis, M. J. (2014), ‘Onset of the spring bloom in the northwestern mediterranean sea: influence of environmental pulse events on the in situ hourly-scale dynamics of the phytoplankton community structure’, *Frontiers in microbiology* **5**, 387.

Von Dassow, P., van Den Engh, G., Iglesias-Rodriguez, D. and Gittins, J. R. (2012), ‘Calcification state of coccolithophores can be assessed by light scatter depolarization measurements with flow cytometry’, *Journal of plankton research* **34**(12), 1011–1027.