Response to interactive comment of anonymous referee #1

By Hedy M. Aardema in agreement with co-authors.

Reviewer*: The manuscript by Aardema and co-authors investigates high resolution in in situ measurements of phytoplankton photosynthetic activity and abundance in the Dutch North Sea. The main topic of this study is relevant and provides useful information, particularly when considering monitoring requirements and in defining sampling/monitoring strategies. This study is also a very good example of integrated sampling and outputs from different instruments (i.e. fRRF, flow cytometer, FerryBox).*

Response: We really appreciate the elaborate and helpful comments on the manuscript. This detailed and insightful review has allowed us to improve the manuscript considerably.

General comments

Reviewer: *The introduction is focused on primary productivity (PP) but the main part of the paper investigates the photophysiological variables and phytoplankton groups with limited mention of productivity. I would suggest emphasizing more the estimates of PP throughout the ms.*

Response: Although the primary productivity is a very interesting parameter to calculate, the aim of the paper is to give a broader view of the phytoplankton community. Therefore, we shortened the part on primary productivity in the introduction, but did give it more attention in the results and discussion sections.

Reviewer: *Collinearity between variables: flow cytometer (FCM) phytoplankton groups were considered in the analysis even if showing collinearity (VIF>6). Statistical principles should be applied consistently across the analysis and to all the variables. If not, this should be explained clearly.*

Response: This is a good point. We re-ran the PCA and spatial clustering and excluded variabiles with the VIF>6. The Multiple Linear Regression was removed from the manuscript, because of the lack of information derived from it together with the abundance of literature already addressing the predictors of primary productivity.

Reviewer: *Spatial autocorrelation: transect data with high frequency sampling is likely to be spatially autocorrelated – has this been considered? If spatial autocorrelation is not considered to be a problem in this dataset, please explain why. Alternatively, presence of spatial autocorrelation could be investigated with the use of variograms.*

Response: As the reviewer expected, most parameters were spatially autocorrelated. We tested the spatial autocorrelation with Moran's I. This is indeed a problem for the multiple linear regression, but as mentioned previously, we removed this analysis from the manuscript. For the spectral classification clustering and PCA analysis, spatial parameters (latitude, longitude) were not included in the analysis. Without time and space in the calculation we only consider features of the data, so spatial autocorrelation does not influence the results (Demsar et al., 2013, Rousseeuw et al., 2015). Because the similarity between neighbouring points is of interest, we plotted of the spectral clusters on maps to visualize the spatial heterogeneity present.

Reviewer: *Diurnal changes in some of the photophysiological variables: the authors clearly show that the diurnal cycle affect the clustering of observations (e.g. Page 25), so the clusters identified were not only based on changes in phytoplankton community but also in sampling activity (i.e. day vs*

night). As stated in the ms, it is difficult to separate the temporal from spatial variability; however, the effect of spatial variability could be investigated, for example, using measurements collected around specific time of day or night (e.g. 12:00+/4 hours) and rerunning the cluster analysis on this sub-dataset and comparing the outcome with the current clusters. In this way it would also be possible to test the suggestion in line 30-31 (page 27) that spatial patterns are more important than temporal.

Response: We performed the suggested analysis for the month of August by clustering only the measurements that fall into the 12+/-4 h timeframe (see Fig. R1b). In this timeframe the southern coastal zone is distinct from the rest of the Dutch North Sea and corresponds to cluster 10 in the analysis of the complete dataset (Fig. R1a), so this cluster is defined by spatial variability. Cluster 12 and 13 are grouped together in the 12+/-4h timeframe as cluster 1. Cluster 11 is only encountered outside the 12+/-4h timeframe, so is a temporal rather than a spatial cluster. We added this information to the text and added the figure below to the supplementary material.

a).

b).
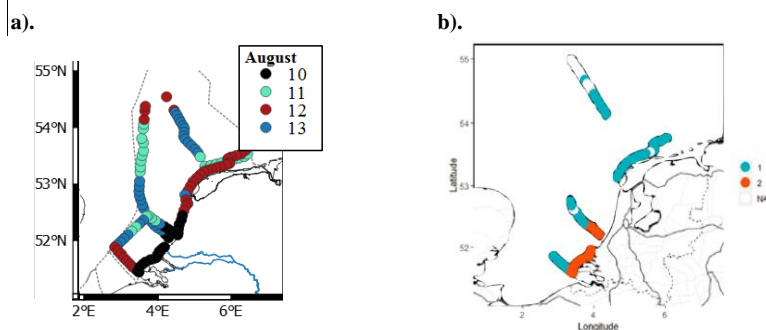
Fig. R1: : Maps of clusters as defined by spectral clustering of the whole dataset (left) and only the measurements at 8h around noon (8:00h to 16:00h). Based on the FCM-based five described phytoplankton groups (Table 2) and non-collinear FRRf-parameters on photophysiology (F_v/F_m, $1/\tau$, [RCII], σ_{PSII}, α, E_k).

Specific comments

Reviewer: *Title – phytoplankton photosynthesis does not provide a clear idea of the content of the paper that covers different photophysiological variables as well as measurements of PP. I would suggest to being more specific.*

Response: We prefer to stay with the chosen title. The main purpose of this study was to provide an example of high-resolution methods that could serve in a phytoplankton monitoring program. Based on the results of these methods further calculation can provide an estimate of the PP or can serve in identification of distinct biogeographical regions, of which we gave examples.

Reviewer: *Data analysis: it would be useful if the authors could explain why clusters, stepwise regressions and PCA have been used as chosen statistical analysis and what they are you aiming to explain with these techniques?*

Response: The main aim of the data analysis was to aid in the interpretation and visualization of the multitude of parameters derived with the high-resolution measurements. The PCA reduces the amount of parameters (or dimensions) and gives an impression on the relationship between parameters. The cluster analysis was chosen to test for spatial heterogeneity; when clusters would contain measurements randomly distributed over the study area, no spatial heterogeneity is present. When clustering shows spatial structure, it is. The stepwise regression was at first used to identify drivers for

primary productivity, but will be removed after realization that the dataset of this study does not add to existing knowledge on this topic.

Reviewer: *Data analysis: Biomass vs chl a – repeatedly in the ms the authors refer to 'biomass', as synonymous of chl a (from validate fluorescence). Although chl a is often used as a proxy for phytoplankton biomass, they are not the same and this should clearly be stated at the start of the ms. Confusion arises from figures and tables referring to 'abundance', 'fluorescence', 'chl a', while the text refers to 'biomass'; please check for consistency. In addition, the implications of a variable Chl-a : C ratio should also be considered and discussed. If the main interest is on biomass the authors could consider calculating it from the FCM measurements (for example, see DOI: 10.1016/j.dsr2.2006.05.004).*

Response: The authors are aware of this issue and tried to address this problem in the results section '3.2 phytoplankton parameters' where we state: "*Both parameters can yield contrasting results due to the wide range of phytoplankton cell sizes and species-specific Chl a content per cell (Falkowski and Kiefer, 1985; Kruskopf and Flynn, 2005).*" This is repeated in the discussion where we write: „*Chlorophyll a concentration is often used as an estimate for biomass, although the Carbon:Chl a ratio is dependent on abiotic conditions and species-specific phenotypic plasticity (Flynn, 1991, 2005; Geider et al., 1997; Alvarez-Fernandez and Riegman, 2014; Halsey and Jones. 2014)*". So we think we clearly stated this. However, to further improve on this point, the term biomass was deleted in the manuscript. Although this is a very interesting parameter, and we are working on a method to calculate biomass based on scattering measured by the FCM. We already found good agreement between our biovolume and images obtained by the Image in Flow of the FCM (unpublished). However, this relationship seems to be taxon specific, which we want to study more in depth and is beyond the scope of the current study. The method to calculate biomass of Tarran et al. (2006) assumes all cells have a spherical shape and a constant C content per biovolume. Because this is an oversimplification, we prefer to use cell counts and fluorescence in the current paper. We did include our view on biomass calculation from flowcytometer data in the discussion.

Reviewer: *UHMM and cluster identification – it is not clear whether the clusters between the different months (Figure 5) are the same or not – in other words, is cluster 1 in April characterized (defined) by the same variables as cluster 1 in May? If not, then it may be better to separate the clusters e.g. with different numbers and/or colours in the figures.*

Response: we adjusted the figure as suggested.

Reviewer: *Discussion of results: results of the analysis of the photophysiological variables and of PP appear discussed separately. Outcomes from these two parts of the study should be brought (and discussed) together, where possible.*

Response: In the result section, primary productivity and Photophysiology are now both under an own header.

Reviewer: *Conclusions – I would suggest to highlight the importance of this study for monitoring program. Also, a bit more considerations on combining low and high resolution measurements would be useful.*

Response: We rewrote the conclusions accordingly:
"A good monitoring program monitors the presence of nuisance phytoplankton, the carrying capacity of the ecosystem and changes in biogeochemical cycling. The objective of this study was to evaluate the use of FRR fluorometry and flowcytometry for monitoring purposes. The four conducted cruises spread over 5 months offered a wide variety of environmental conditions and phytoplankton community states, which the utilized methods were able to visualize.
Inclusion of high-resolution methods in monitoring programs allows for analysis of finer scale events. Furthermore, it allows for analysis of living phytoplankton and is thereby able to measure rates and avoid effects of preservation and storage of samples. Another advantage is that high-resolution

methods allows for easier comparison between countries, once common protocols have been established. Nevertheless, low resolution methods remain a necessity for more detailed taxonomic analysis, information on vertical heterogeneity, to calibrate and to correct for blanks. Data analysis might be the biggest bottleneck of the implementation of these high-resolution methods. The cluster analysis of flowcytometric data has high potential for improvement to increase the informative value of the method. Especially identification of phytoplankton clusters with a functional quality, such as nitrogen fixers, calcifiers or DMS-producers, would be helpful for interpretation of ecosystem dynamics and biogeochemical fluxes. Regarding the FRRf, the main challenge is converting electron transport rate to gross primary productivity in carbon units. Further research in these topics would benefit implementation of these methods into monitoring protocols. Furthermore, it is important to account for diurnal patterns in monitoring set-up to be able to distinguish between diurnal and spatial variability. Possibly the diurnal variability could be modelled, but more studies with a Langragian based approach would be needed for a better understanding of the impact of diurnal variability in the data. Overall, the in this study presented high-resolution measurement set-up has large potential to improve phytoplankton monitoring in supplement to existing low-resolution monitoring programs."

Reviewer: *Supplementary information – need to be linked (and referred to) in the main text of the ms, otherwise it may be difficult for the reader to know that this info is available.*

Response: Done.

Technical corrections

Reviewer: *Page 1: 23-26 – rewording is needed*

Response: Rephrased to: "One of the major concerns when using these methods for monitoring purposes is the presence of a diurnal cycle concurrent to the spatial variation, especially in photophysiological parameters. This concurrent presence of spatial and temporal patterns needs to be taken into account when designing a monitoring program. Nevertheless, the richness of additional information provided by high-resolution methods, such as the FCM and FRRf, can supplement low-resolution monitoring to attain a better understanding of the phytoplankton community."

Reviewer: *Page 1 30 -keywords, consider adding primary productivity*
Response: Added.

Reviewer: *Page 2: 10-12 – this sentence would fit better at the start of the paragraph. It also requires references*
Response: Moved to beginning of the paragraph.

Reviewer: Page 3: 5 – 'a sum': consider replacing with 'a combination'
Response: Done.

Reviewer: Page 3: 23 – 'pigment ratio' slightly incorrect as the ratio considered is of fluorescence
Response: Agreed and adopted.

Reviewer: Page 3: 24-25 – Aims – this statement about key driver of PP is very general and can be misinterpreted as the ms focuses on only 4 months during the growing season of a particular year. Time frame of this study should be specified
Response: reformulated

Reviewer: Page 4: 3-5 – not clear, needs rewording
Response: Rephrased to: "The Dutch North Sea is a shallow tidal shelf sea in the southern part of the North Sea. The main water flow is Northward flowing Atlantic water that enters the North Sea in the

south through the Channel. The Atlantic water flowing around Scotland enters the North Sea and meets the Channel water and the freshwater from the rivers forming the Frisian Front."

Reviewer: Page 5: 1- would be useful to have the exact dates of the surveys.
Response: Added.

Reviewer: Page 5: 6 – more details on the temporal frequency indicated as 'low resolution' should be provided (e.g. how many samples per station? How many a day? How many depths?)
Response: Added.

Reviewer: Page 5: 27-32 – please provide more details of the methods or a published reference (for people not being able to access the internal protocols).
Response: Added.

Reviewer: Page 6: 16 & 18 – acronyms (e.g. NPQ and F0') should be explained when used the first time
Response: Added.

Reviewer: *Page 8: 12-13 – formula 8 is missing*
Response: It was removed. We changed formula 9 to formula 8.

Reviewer: *Page 8: 17 – need rewording*
Response: Rephrased as: "Volumetric P_{max} and α were derived by fitting JV_{PII} in μmol photons m^{-3} h^{-1} to equation 1 (the exponential model of Webb et al., 1974) and used to integrate productivity over depth. The light availability in the water column was estimated as […] with $E(z)$ being the irradiance at depth z, $E_{surface}$ the incoming surface irradiance and K_d the light extinction coefficient."

Reviewer*: Page 8: 20-21 – it is not clear how surface irradiance was calculated; please reword this section*
Response: We adjusted the text to the following explanation: "To avoid effects of changing incident surface irradiance ($E_{surface}$) on the spatial pattern and to be able to compare GPP between regions we used monthly average surface irradiances ($E_{surface}$) in our calculations of primary productivity. From 2010-2016 irradiance (400-700 nm) was measured at the roof of the NIOZ building in Yerseke using a LI-190 quantum PAR sensor and hourly averages stored using a LI1000 datalogger. $E_{surface}$ was then calculated by averaging all irradiance data from the years 2010-2016 for the respective month."

Reviewer: *Page 9: 17 – was the clustering carried out by the FCM software or was it done by expert judgment manually? Also, was data cleaned from potential presence of air bubbles etc? Please provide details on these points,*
Response: The chosen cluster criteria were based on expert judgement. The clustering was done by the software Easyclus 1.26 (ThomasRuttenProjects) according to these criteria. Noise, air bubbles and other potential outliers were removed after the clustering.

Reviewer*: Page 10: 2 – outliers –specify which analysis you are referring to (e.g. outliers from the fRRF?)*
Response: All data, rephrased in manuscript.

Reviewer: *Page 10: 5 – provide a reference for the value of 0.65*
Response: Added; Kolber, Z. and P. G. Falkowski. 1993. Use of active fluorescence to estimate phytoplankton photosynthesis in situ. Limnology and Oceanography. 38:1646-1665.

Reviewer: Page 10: 12 – please specify which are the photophysiological variables considered

Response: We added the following sentences to the data analysis section: "Phytoplankton parameters were first tested for collinearity and predictors with a variance inflation factor (VIF) over 6 were removed (Zuur et al., 2009). This left for the cluster analysis FCM-parameters Pico-red, Nano-red, Micro-red and *Synechococcus* and the FRRf-parameters σ_{PSII}, F_v/F_m, a_{LHII}, $1/\tau$, E_k."

Reviewer: *Page 10: 13 – acronyms (VIF) should be defined here*

Response: Added.

Reviewer: *Page 11: 20 – 'nitrate': should this be 'DIN'?*

Response: Yes.

Reviewer*: Page 11: 27-28 – please explain the evidence for P and Si-limitation (i.e. discuss the ratios vs expected limiting ratios in literature). Also, please specify the value of Redfield Ratio and reference.*

Response: We removed the nutrient ratios from the results. The paper only reports the nutrient values as additional background information to understand phytoplankton dynamics. A detailed analysis of concentration vs ratio is past the subject of this paper, but in the discussion nutrient limitation is now discussed.

Reviewer: *Table 3 legend – 'not completely comparable': this expression doesn't have a clear statistical meaning. Please specify briefly in the legend which month had a different sampling route and station so for the reader to understand in which month the study area is not fully covered.*

Response: True. We removed the term 'not completely comparable' from the legend and added a short explanation of the differences between months. Also, we moved the table to the supplementary information and replaced it with the nutrient concentration table.

Reviewer: *Figure 2 provide equations of linear regressions with R2 and significance*

Response: The R^2 and significance are now added to the legend. The linear regressions are irrelevant because the unit of the x-axis is in relative fluorescence units (RFU) and instruments will require separate calibration.

Reviewer*: Page 14: 27 – 'suggesting physiological stress', please provide reference*

Response: Suggett et al., 2009.

Reviewer: *Page 16: 9 – it is not clear to which phytoplankton group the % are referring to.*

Response: The nanophytoplankton. Rephrased.

Reviewer: *Page 16: 14 – please specify which are 'these regions'*

Response: Rephrased.

Reviewer: *Page 16: 15-16 – this paragraph should be moved to the discussion so to allow the concept to be developed further.*
Page 16: 17 – please explain why low sigmaPSII may reflect Rhine River waters.

Response: Moved to discussion.

Reviewer: *Page 17 – Figure 4 – I appreciate the different scaling was necessary to 'visualize the spatial heterogeneity' however it makes very hard the comparison between figures. In fact, the reader needs to keep checking the legend, which is printed in very small characters difficult to see. I would suggest reconsidering the use of a uniform scale (at least for some of the variables, if possible).*

Response: We adjusted the figure to a uniform scaling.

Reviewer*: Page 18: 17 – there is limited or no comments on the results of some of the photophysiological variables such as alpha, Pmax, effective absorption cross section.*
Response: We expanded the result section on the Photophysiology.

Reviewer: *Page 18: 25 – 'sake of completeness'. See general comment about collinearity, please explain why statistical principle of VIF>6 was not applied consistently to all variables*
Response: We agree that this might not have been the best choice, we preferred to include all the phytoplankton groups. As mentioned before, we now deleted the collinear variables with VIF>6.

Reviewer: *Page 18: 28-29 – table should be provided (for example in the additional info) showing the contribution of each variable to the PC1 and PC2 for the 4 months, and total variance explained.*
Response: We added this table to the manuscript, in combination with figure 6:

	April		May		June		August	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Sigma	0.8	**28.8**	0.1	**36.7**	0.0	9.3	12.1	9.9
F_v/F_m	13.7	0.6	0.8	**14.5**	**27.6**	0.1	0.0	**17.5**
a_{LHII}	**18.7**	3.4	17.5	6.7	**20.9**	8.7	**21.0**	3.3
[RCII]	**17.1**	6.6	**20.4**	1.6	**28.0**	2.6	**25.8**	0.0
$1/\tau$	9.8	**22.7**	4.4	7.5	0.4	1.7	0.2	**20.6**
E_k	3.9	13.8	0.7	**26.3**	3.7	3.9	0.7	**16.8**
Pico-red	4.2	**15.1**	**18.5**	0.4	6.1	**26.9**	0.3	11.8
Nano-red	**16.9**	0.0	**21.1**	0.6	2.9	**16.9**	15.3	3.1
Micro-red	10.5	4.5	16.4	1.4	6.3	2.9	**22.9**	0.4
Synechococcus	4.3	4.4	0.0	4.3	4.2	**27.0**	1.8	16.7
Variance explained	45.6 %	19.3 %	42.5 %	18.9 %	29.1 %	18.7 %	33.9 %	25.7 %

Reviewer: *Page 19: 1 – alpha is defined as Light utilisation efficiency (Table 1) but then in the text is referred to as 'affinity'. please check for consistency.*
Response: Changed in table. The value for alpha is the slope of the FLC, and is a measure for photosynthetic affinity for incoming light.

Reviewer: *Page 21: 8-13 – consider whether to move this text in additional info (or to remove it?). It breaks the flow of the results and the addition of clusters 'manually' appears to not be meaningful and/or significant (as it doesn't adopt the same statistical robust principle).*
Response: It is true that it does not adopt the same statistical robust principle. However, there is spatial heterogeneity in the flowcytomer data, that are not visualized with the UHMM and this is what we wanted to explore. We do agree that the manual increase of amount of clusters might not the best way to go forward with this, so we deleted this section from the manuscript.

Reviewer: *Page 22: 6 – 'abiotic' and 'salinity' misspelled. Page 22: 9 – as for previous PCA, please provide variables used and information on their contribution towards variance explained.*
Response: this paragraph and figure were removed from the manuscript because the PCA does not provide useful insights or new information on the phytoplankton community or Dutch North Sea.

Reviewer*: Page 23: 6-7 – this paragraph is not clear particularly what is meant with 'opposite'*
Response: rephrased.

Figure 7 legend – Size of the open circles is a bit confusing and misleading as the reader may assume the size of the bubble refers to the amount of PP. Consider simplifying the figures and only plot productivity

Response: The figure was simplified as suggested.

Reviewer: *Page 24: 15 – please indicate how much of the variability in PP is explained by the stepwise regression (e.g. R2?).*

Response: because information on the nutrient availability was only available on a low-resolution spatial scale, the information provided by high resolution methods are not effectively used. To study the drivers of primary productivity another study design should have been chosen. Therefore, this analysis was deleted from the manuscript.

Reviewer: Page 25: 4 – reword please.

Response: rephrased

Reviewer: Page 26: 2-5 – require rewording particularly the need to clarify and be more specific on the work done in this study.

Response: removed from manuscript.

Reviewer: Page 26: 5 – this sentence may be misleading. The authors calculated PP along the sampling transects but did not provide an estimate for the wider Dutch North Sea as it may appear here.

Response: removed from manuscript.

Reviewer: *Page 26: 8 & 11 – timing of the bloom is discussed in this section however it would not be possible to define the start of the bloom based on a 4-day sampling per month. Continuous observations throughout the year by an instrument buoy or remote sensing would allow to 'contextualise' the measurements within the growing season (i.e. determine when sampling was carried out within the phytoplankton growing season).*

Response: Agreed and removed from manuscript.

Reviewer: *Page 26: 24-25 – please reword*

Response: rephrased

Reviewer: *Page 27: 8-9 – repetition of method; should be deleted.*

Response: Rephrased.

Reviewer: *Page 29: Figure 10 legend, possibly just my issue, I don't see the similarity between the two figures.*

Response: We do see a basic similarity, with the separation between the different water masses being reflected in our results. However, the similarity might not be striking enough to include the figure and therefore we leave it out of the manuscript.

Reviewer: *Page 30: 13 – 'low resolution': should this be 'high-resolution'?*

Response: no, we meant to say low-resolution. We rephrased to make it easier to follow: "Extra low-resolution sampling points in clearly deviating areas would be useful, because only low-resolution offer the level of detail which is required to identify toxic, keystone or invasive species."

References

Demšar, U., Harris, P., Brunsdon, C., Fotheringham, A. S., & McLoone, S. (2013). Principal Component Analysis on Spatial Data: An Overview. Annals of the Association of American Geographers, 103(1), 106–128. https://doi.org/10.1080/00045608.2012.689236

Rousseeuw, K., Poison Caillault, E., Lefebvre, A., & Hamad, D. (2015). Achimer Hybrid hidden Markov model for marine environment monitoring. IEEE JOURNAL OF SELECTED TOPICS IN APPLIED EARTH OBSERVATIONS AND REMOTE SENSING, 8(1), 204–213. https://doi.org/http://dx.doi.org/10.1109/JSTARS.2014.2341219

Suggett, D. J., C. M. Moore, A. E. Hickman, and R. J. Geider. (2009b). Interpretation of fast repetition rate (FRR) fluorescence: signatures of phytoplankton community structure versus physiological state. Marine Ecology-Progress Series **376**:1-19.

Response to interactive comment of anonymous referee #2

By Hedy M. Aardema in agreement with co-authors.

Reviewer: *This paper analyses spatial and temporal patterns in cruise data with 3 high-resolution monitoring methods: FRRF, Flow-cytometry and Ferrybox. Correlations between the observed variables are also analysed. The large dataset, including many phytoplankton and environmental variables observed together enables the authors to understand the patterns in the various phytoplankton variables. The results could guide the optimal application of such novel monitoring methods in operational monitoring for a.o. MSFD.*

Response: We thank the reviewer for the helpful and critical comments. We rewrote and restructured the manuscript extensively based on these comments. We are happy to hear that the reviewer sees the potential of our applied methods.

General comments

Reviewer: *The paper lacks a clearly stated research question or hypothesis to be tested. Therefore, it is unclear what is the purpose of the various analyses performed and what we can learn from the results. Based on the conclusion that this type of "high-resolution is a very useful supplement to current monitoring", I would expect a hypothesis such as "combined high-resolution monitoring of many phytoplankton variables along with environmental variables allows us to quantify seasonal and meso-scale patterns in phytoplankton biomass, species composition and primary production. The concurrent measurement of different phytoplankton variables allows us to understand the effect of phytoplankton species composition and physiological adaptation processes on the observed patterns in phytoplankton biomass and production". Then the analysis should show how the variables should be combined to provide the most reliable estimates of phytoplankton biomass and primary production.*

Response: Because of the exploratory nature of our research, a hypothesis was not defined. The addition of the suggested sentences does help in making the manuscript easier to follow. We therefore adopted part of the sentences and added of the following sentences to the introduction: "The aim of this study is to test the suitability of these two high-resolution methods to be developed as novel phytoplankton monitoring method. The two high-resolution methods, a flowcytometer and a FRR fluorometer, were deployed concurrently on four 4-day cruises in April, May, June and August to

meet a wide range of environmental conditions and phytoplankton community states. These measurements allow for quantification of seasonal and mesoscale spatial patterns in phytoplankton abundance, photophysiology and gross primary production. In this paper we provide an overview of the acquired results, use a spectral cluster analysis to visualize spatial heterogeneity and evaluate the potential of these methods to optimize current monitoring programs."

Reviewer: *There are many observed variables, which are not consistently named in the text, figures and tables. Therefore, it is easy to get lost in the description of patterns for all individual variables. A clear definition of variables that is consistently used throughout the text would help the reader to understand the storyline. Some of the variables observed by the FRRF seem to be very similar. Which of the variables should be used as indicator and which are redundant to answer the research questions?*

Response: We corrected the inconsistent naming. The variables of the FRRf might seem similar under some conditions. However, because these variables vary depending on community composition and environmental conditions, they might deviate when conditions change (Sugget et al., 2009; Kromkamp and Forster, 2003). Therefore, care must be taken into choosing the parameters. For the current study the main interest is on monitoring the phytoplankton community, therefore we are interested in parameters that are informative on physiological adaptation or characteristic for phytoplankton taxons. Additionally, we focus on high resolution measurements, so limit the parameters to the ones attainable at high-resolution. Based on these considerations we decided to include the current parameters, which give us a broad overview of the photophysiological status of the phytoplankton community.

Reviewer: *In the conclusions section a recommendation on next steps would be much appreciated: what would be required to use the high-resolution methods in scope to provide reliable estimates of phytoplankton biomass, production and species composition for long term monitoring? In the introduction and conclusion the species composition is defined in functional types such as nitrogen fixers, calcifiers or DMS-producers, but these do not correspond to the phytoplankton clusters used in this paper.*

Response: the conclusions were rewritten:
"A good monitoring program monitors the presence of functional types of phytoplankton, including the harmful taxons, the carrying capacity of the ecosystem and changes in biogeochemical cycling. The objective of this study was to evaluate the use of FRR fluorometry and flowcytometry for such monitoring purposes. The four conducted cruises spread over 5 months offered a wide variety of environmental conditions and phytoplankton community states, which the utilized methods were able to visualize. Inclusion of high-resolution methods in monitoring programs allows for analysis of finer scale events. Furthermore, it allows for analysis of living phytoplankton and is thereby able to measure rates and avoid effects of preservation and storage of samples. Another advantage is that high-resolution methods allows for easier comparison between countries, once common protocols have been established. Nevertheless, low resolution methods remain a necessity for more detailed taxonomic analysis, information on vertical heterogeneity, to calibrate and to correct for blanks. Data analysis might be the biggest bottleneck of the implementation of these high-resolution methods. The cluster analysis of flowcytometric data has high potential for improvement to increase the informative value of the method. Especially identification of phytoplankton clusters with a functional quality, such as nitrogen fixers, calcifiers or DMS-producers, would be helpful for interpretation of ecosystem dynamics and biogeochemical fluxes. Regarding the FRRf, the main challenge is converting electron transport rate to gross primary productivity in carbon units. Further research in these topics would benefit implementation of these methods into monitoring protocols. Furthermore, it is important to account for diurnal patterns in monitoring set-up to be able to distinguish between diurnal and spatial variability. Possibly the diurnal variability could be modelled, but more studies with a Langragian based approach would be needed for a better understanding of the impact of diurnal variability in the data. Overall, the in this study presented high-resolution measurement set-up has large potential to improve phytoplankton monitoring in supplement to existing low-resolution monitoring programs."

<u>Specific comments</u>

Reviewer: *Sentences are often long: consider breaking up in multiple sentences to improve readability.*

Response: We apologize for the difficulties and hope to have improved the readability in the new manuscript.

Reviewer: *Figure 1: please show only the stations (with names/ abbreviations) used in this study (see table S1) and the areas used in the text (such as Dogger Bank, Wadden, Den Helder, Rhine outflow) so the description of spatial patterns can also be understood by people that are not Dutch.*

Response: We updated the figure to the following:
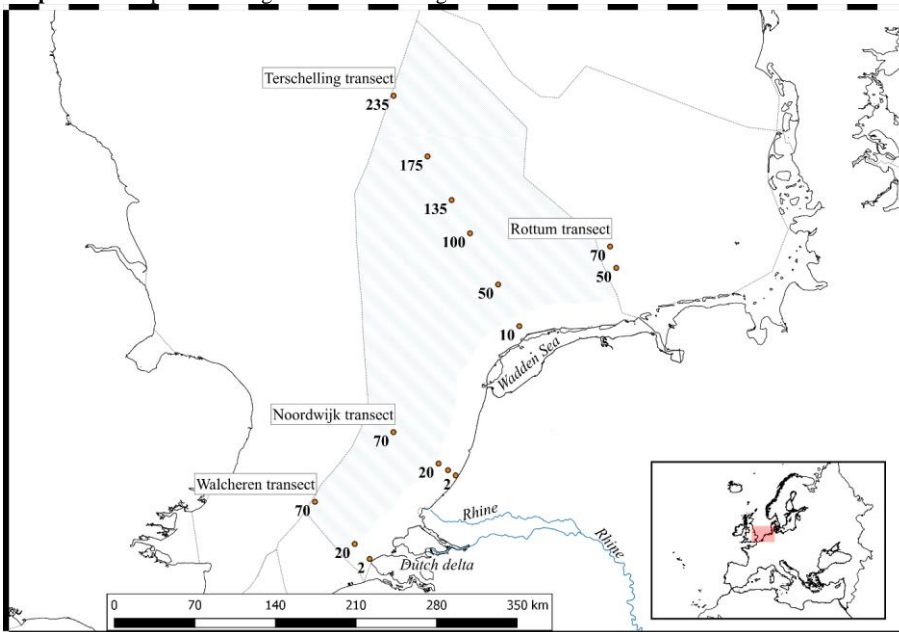
Reviewer: *Section 2.2: please refer to international protocols/methods rather than internal protocols.*
Response: We added a more detailed description to the method section.

Reviewer: Table 1: it would help to have an additional column stating the interpretation / meaning of this variable, such as total biomass, nutrient stress, maximum growth rate, efficiency of light uptake etc. Then later in the text you can use these 'meaningful' names instead of codes, to facilitate understanding of observed patterns. Also a figure illustrating the meaning of the different variables (alfa,Ek, F', Fm' etc.) could prevent getting lost in all abbreviations.

Response: Unfortunately, the meaning of the different variables is usually not straightforward and dependent on multiple predictors (species, nutrient concentration, light availability etc.; Suggett et al., 2009). Nonetheless, we tried to make the table more information easier to understand.

Reviewer: *Equation 9: why did you use monthly averaged irradiance if you are looking at high-resolution patterns. Why did you not use irradiances measured during the cruise?*

Response: Unfortunately, we were unable to collect reliable irradiance data for all cruises. Clearly, it is preferable to have irradiance (PAR) continuously measured in parallel to the FRRF measurements when aiming to monitor current primary productivity.

Reviewer: *Table 2: Since you use both Length_FWS and O/R ratio as criteria to distinguish the phytoplankton groups, it would be logical to include a column for O/R ratio with the applied criteria.*

Response: Good idea, we added the O/R-ratio to the table.

Reviewer: *It is not entirely clear whether pico-red includes pico-Synecho or not. On page 14, line 30 it says: "Both groups of picophytoplankton (Synechococcus and total)", whereas table 2 and figure 3 suggest the two groups are exclusive.*

Response: Pico-red and Pico-synecho are two different groups, as correctly understood from table 2 and figure 3. We rephrased the sentence to: "Both groups of picophytoplankton (Synechococcus and Pico-red)", and scanned the manuscript for other mixing up.

Reviewer: *Section 2.4: please state with every type of analysis what is the purpose / research questions for that analysis. For example: what are you trying to predict from what and why?*

Response: We added the following the sentences to section 2.4: "To find regions with similar phytoplankton communities, data was spectrally clustered using the uHMM R package (Poisson-caillault and Ternynck, 2016) in the statistical software R (version 3.4.1, R Core Team, 2017)." and "Principal Component Analyses (PCA) were performed to find which variables contributed most to the cluster results."

Reviewer: *Section 3.1: I don't see the value of comparing averages over whole transects (with large spatial variability, which is the subject of this paper), that are not even the same, between months. The only thing you see is seasonal patterns that are well-known from other studies and that can be summarized in section 2.1 in a description of the study area. Most of this section describes the data in table S1. I would replace table 3 with table S1 and remove table S2. N/P ratios address that same question as table S1, but with an indicator that is controversial.*

Response: For the authors the table helped to visualize the seasonal patterns, but we agree on the comment that this table does not add to the already existing knowledge on seasonal patterns. We therefore adopted the suggestion to replace the table with the table S1 from the supplementary material.

Reviewer: *The text in this section (and subsequent sections) is sometimes hard to follow as it is not clearly structured in time and space and variable. We go back and forth in time. Section 3.2 describes first figure 2, then figure 3 and then again figure 2 and then figure 3. I suggest to make one section about phytoplankton biomass (figure 2) and then one section on species composition (figure 3).*

Response: Rewritten

Reviewer: *Page 16, line 14: the southern coastal stations are more strongly affected by the Rhine outflow than the Scheldt outflows (see for example: Lacroix, G., Ruddick, K., Ozer, J., & Lancelot, C. (2004). Modelling the impact of the Scheldt and Rhine/Meuse plumes on the salinity distribution in Belgian waters (southern North Sea). Journal of Sea Research, 52(3), 149-163.).*

Response: We reformulated to Rhine and Scheldt river outflow.

Reviewer: *Figure 4: Please use consistent legends for the same variable between different months, with the same colour scheme and symbols (squares vs. circles) and with blue indicating low values and red indicating high values, so the high values stand out, more than the low values. Also captions in the table per line (red fluorescence, O/R ratio etc.) and per column (april, may etc.) would help to easier understand the figure.*

Response: We remade the figures, see manuscript.

Reviewer: *Section 3.5: I don't see the added value of this analysis. What does it tell us?*

Response: Agreed. We aimed to get a better understanding of the drivers of primary productivity in the Dutch North Sea. However, we realize now that the dataset is not very well suited for this and we therefore removed the analysis.

Reviewer: *Page 24: I suggest to mention in the table all the variables that were included in the analysis and note coefficients or 'ns' for not significant and the p values per explanatory variable. Then readers don't need to reconstruct the overview from the text. Actually, the significance test is likely not valid due to strong spatial autocorrelation in the data.*

Response: The Multiple Linear Regression was removed from the manuscript, because of the lack of information derived from it together with the abundance of literature already addressing the predictors of primary productivity.

Reviewer: *Discussion: Here I would expect to get some advice: How to best estimate phytoplankton biomass from these data? Should we use total red fluorescence (best R2) or F0 (least affected by NPQ)? Is there a way to combine both (with other available variables) to get an even better estimate?*

Response: We added the following paragraph to the discussion:
"Biomass might be one of the most important parameters to understand phytoplankton dynamics, but its direct measurement is not possible using high-resolution methods. Chlorophyll a concentration is often used as an estimate for biomass, although the Carbon:Chl a ratio is dependent on abiotic conditions and species-specific phenotypic plasticity (Flynn, 1991, 2005; Geider et al., 1997; Alvarez-Fernandez and Riegman, 2014; Halsey and Jones, 2015). Red fluorescence gave a good estimate of chlorophyll a concentration, both using the FRRf (adjusted R^2= 0.66) and FCM (adjusted R^2=0.90). Both the FRRf and the flowcytometer estimate the chlorophyll a concentration based upon the fluorescence in the red spectrum after excitation in the blue spectrum. There are some slight differences in the optics, the FRRf excites with a 450 nm LED and measures the fluorescence at 682 ± 30 nm, while the FCM excites at 488 nm and filters the red fluorescence over a longpass 650 nm filter towards the red fluorescence detector. The smaller detection range of the FRRf detector is optimized around the maximum emission of PSII and limits contamination by PSI (Franck et al., 2002; Oxborough et al., 2012). The second difference is the fluorescent state of the photosystems, the strong laser of the flowcytometer can only measure the maximum fluorescence (Fm), which is a parameter more prone to quenching than the minimum fluorescence measured by the FRRf. Yet, the biggest difference concerns the method; where the flowcytometer measures the fluorescence per particle, the FRRf does only a bulk measurement. In a bulk measurement other particles in solution scatter the excitation and emission photons, plus the emitted fluorescence of the phytoplankton is subject to reabsorption, especially at higher biomass densities. The latter seems to have the most impact on chlorophyll a concentrations, as the fit of the flowcytometer derived red fluorescence is a better than the FRRf minimum fluorescence. Other studies that use the FCM to estimate chlorophyll a concentrations also showed good relationships, but find better fits using the bulk measurements using a fluorimeter (Thyssen et al., 2015; Marrec et al., 2018). The conversion to biomass may also be done from cell abundances. Some studies use the oversimplified assumption that all cells have a spherical shape and a constant C content per biovolume (Tarran et al., 2006). With the scanning flowcytometer it is also possible to estimate biovolume based on scattering properties of the cell, but this relationship appears to be taxon specific (Rijkeboer, pers. comm.). This relationship will be further explored by comparing the calculated biovolume based on the Image in Flow pictures and the flowcytometric properties of these phytoplankters."

Reviewer: *Can we trust GPP from FRRF as a reliable estimate of primary production or is more work needed to achieve that goal? If so, what needs to be done?*

Response: We added the following paragraph to the discussion:
"The reliability of variable fluorescence as estimate of gross primary productivity is depending on many cell processes from the photon absorbance to carbon assimilation. The variable fluorescence reflects the first step of photosynthesis; the efficiency of which photons are captured and electrons

produced and transferred. However, to interpret gross primary productivity in an ecological or biogeochemical meaningful way, the FRR units of electrons per unit time need to be converted to carbon units. Gross photosynthesis correlates well with photosynthetic oxygen evolution (Suggett et al., 2003), and multiple studies have shown good correlation between 14C-derived estimates of primary productivity and FRRf-derived estimates using a constant conversion factor (Melrose et al., 2006; Kromkamp et al., 2008). However, in reality this parameter is not a constant, as along the pathway from electron to carbon atom electrons are consumed by other cell processes (Flameling and Kromkamp, 1998; Halsey and Jones, 2015; Schuback et al., 2016). Therefore, a reliable GPP estimate in carbon units from FRR fluorometry requires more research and estimates provide relative rather than qualitative values. Despite its limitations the fact that the method can measure in situ, with relatively little phytoplankton manipulation before measurement, makes the method promising. Calibration with other methods, such as concurrent C14 of C13 incubations, could help to better understand the processes from electron excitation to carbon fixation. However, it should be recognized that these types of measurements come with their own problems, and measure something in between net and gross primary productivity depending on the incubation time and growth rate of the phytoplankton (Halsey and Jones, 2015). So it remains a question which method is measuring the 'real' primary productivity. Attempts to calculate primary productivity from flowcytometer data have also been made, which is actually based on the diurnal cycle in cell size caused by cell division (Marrec et al., 2018). Despite the limitations of GPP estimates by variable fluorescence, our results clearly show large spatial variability in gross primary production concurrent to the expected strong variability during the growth season. This spatial heterogeneity is not fully captured by sampling at the standard low-resolution monitoring stations, showing the added value of our approach. Primary productivity was highest in April, and relatively large values were also observed offshore, indicating that a low phytoplankton biomass does not necessarily means that primary production is low. Our GPP rates were based on the same electron requirement for C-fixation ($\Phi e,C$). However, this is a likely oversimplification as $\Phi e,C$ is known to vary with abiotic conditions (Lawrenz et al., 2013) and the changes in nutrient conditions and temperature during the growth season are likely to affect GPP. This will be the topic of a future publication and we expect that the detection of several biogeographic regions will help us in predicting $\Phi e,C$."

Reviewer: *It is not really clear whether the diurnal variability in the FRRF variables is a problem that needs to be solved.*

Response: It is not so much a problem that needs to be solved, but it does need to be taken into account when setting up a monitoring program including FRRF variables. It is important to realize that measurements taken at different times of the day, might not be comparable. To be able to include FRRF variables in a long-term monitoring program, the included sampling points should be sampled at the same time of the day.

Reviewer: *Are the clusters in the FCM analysis the relevant ones to provide 'useful' information to science & society? Should we / Can we move on to other clusters that are mentioned in the conclusions?*

We added the following text to the discussion:
"To understand the role of the phytoplankton in biogeochemical cyckes, the FCM clusters would ideally reflect taxonomic or functional groups, as calcifiers, silicifiers, DMS producers (such as Phaeocystis) and nitrogen fixers (le Quéré et al., 2005). The lack of identification of distinct clusters makes this sofar impossible. Other studies manually separate up to 10 phytoplankton groups with the same instrument (Marrec et al., 2018). These groups included Prochlorococcus, which is at the absolute limit of resolving capacity of the FCM because of their small size and low fluorescence. They furthermore distinghuished the Pico-red in three groups based on FLO/FLR-ratio. Nano-cryptophytes group in high and low orange fluorescence and included a micro-eukaryotes group with a size from 10 to 20 uM. But these groups are still made up of many taxonomic genera and, apart from size, won't allow much for further interpretation of their role in the ecosystem or biogeochemical cycles. The same accounts for detection of nuisance phytoplankton; distinct clusters of toxic phytoplankton species are lacking. Although this will remain a challenge because toxicity in

phytoplankton can differ within morphotypes and sometimes even differ per strain within a species (Tillman and Rick, 2003). But potentially, further research in flowcytometry can result in suspicious clusters to be flagged and further inspected by a specialist using microscopy. The potential is certainly there, as much of the information retrieved by the FCM is still unexplored; the clustering is performed on totals (area under the peak) instead of the pulse-shape. This in combination with more advanced camera options will need to further distinguish between groups in the future."

Reviewer*: Do the FCM data help to better understand the FRRF data (and vice versa)? For example, do we see diatoms under light limited conditions (high F'/Fm', high alfa, low Ek) and picoplankton under nutrient limited conditions (low F'/Fm')? Other ecological niches that we know from literature? Different conditions promoting Synechococcus compared to other picoplankton?*

Response: We tried to incorporate the link between the methods better, we added the following sentences to the manuscript:
"In this study a large part of the Dutch North Sea shifted from nutrient sufficiency to nutrient limitation between April and May, which was reflected in the low efficiency of PSII (Fv/Fm; Fig. 4). The Fv/Fm recovered between May and June, which suggest that the phytoplankton adapted to nutrient limiting conditions (Kruskopf and Flynn, 2005). However, photophysiological parameters are also varying per taxonomic group; smaller taxa typically have lower Fv/Fm values and higher σPSII values (Kolber et al., 1988; Suggett et al., 2009b). Indeed, by flowcytometry we find that the biggest shift in community composition took place between May and June from a nanophytoplankton dominated community to a picophytoplankton dominated community. These findings demonstrate how flowcytometry and fast repetition rate fluorometry can supplementary improve ecosystem understanding."

Technical comments

Reviewer:
- Collinear should be spelled with 2 ll's throughout the whole text.
- Page 9, line 4 & 5: I guess um means micrometers?
- Page 18, line 4: middle-right, please refer to the label C4 a-x.
- Figure 5: The figure would be easier to read if the colours per group are consistent between the cluster analysis on the right and the map on the left. Labels (A-D for April to August panels) would also help.
- Figure 9: Please add the hours of the day on the x-axis.
- Page 25, line 3: the word influenced is repeated too many times and therefor should get an e in the end.
- Page 28, line 11: estimates are.
- Line 13: parameter without s.
- Page 31, line 8: Jerico-next, without h.

Response: We adopted the suggested technical improvements.

References
Franck, F., Juneau, P., Popovic, R. (2002). Resolution of the Photosystem I and Photosystem II contributions to chlorophyll fluorescence of intact leaves at room temperature. Biochimica et Biophysica Acta - Bioenergetics, 1556(2–3), 239–246. https://doi.org/10.1016/S0005-2728(02)00366-3

Suggett, D. J., Moore, C. M., Hickman, A. E., & Geider, R. J. (2009). Interpretation of fast repetition rate (FRR) fluorescence: Signatures of phytoplankton community structure versus physiological state. Marine Ecology Progress Series, 376, 1–19. https://doi.org/10.3354/meps07830

Response to minor revisions 'suggestions to authors'

By Hedy M. Aardema in agreement with co-authors.

<u>Title</u>

Editor: *As suggested by referee 1, the present title is probably not optimal relative to the content of the manuscript. I have seen your answer on this specific point but still suggest that the title could be rephrased to better suit the objective listed at the end of the introduction.*

Response: Rephrased as: << High-resolution underway measurements of phytoplankton photosynthesis and abundance as innovative addition to water quality monitoring programs >>

<u>Abstract</u>

Editor: L14 remove « in time and space »

L15 and 16 (see also L27): Abbreviations ae generally not used in an abstract

L22 « do » should become « does »

L22 Remove the first « Still »

Response: Corrected

<u>Introduction</u>

Editor: P2, L11 I would suggest to modify the order temperature, sea level, acidification

Response: Corrected

Editor: P2, L24 Could be interesting to provide information on the time frequency as well. I know that this is provided later in the manuscript but is neverthelees missing at this level

Response: Included << Monitoring cruises take samples in the Dutch North Sea between March and October every two or four weeks. >>

Editor: P2 L30 Could be interesting to discuss of possible consequences associated with the reduction in resolution in the assessment of community composition as well

Response: Added to the introduction: << Underway measurements are not able to replace some more detailed low-resolution measurements, but their higher spatial and temporal resolutions provide the possibility to identify short-lived events, detect small-scale changes in phytoplankton dynamics, evaluate consequences of possible (spatial) undersampling, and act as an early warning system. >>

Materials and Methods

Editor: P5 L8 « was situated » should become « is situated » or remove « On the RW… »

Response: Removed << On the RV Zirfaea >> and added <<of the underway system >>

Editor: P5 L23 freezing temperature??

Response: -18°C. Added to text.

Editor: P5 L26 Unless I am wrong « RWS » has not been defined before

Response: Rijkswaterstaat. Added to text.

Editor: P5 L29 ammonium, calcium, magnesium

P6 Fluorescence Light Curve

P6 L29 Non-Photochemical Quenching

Response: corrected

Editor: P8 L12 Reaction Centres. What is the signification of H in RCH ?

Response: The H is actually two II's. The two II's refer to photosystem II, which was added to the text: << The amount of reaction centres of PSII per cubic metre ([RCII]) was calculated as >>

Editor: P9 L7 Light Scatter, Sideward Light Scatter

Response: corrected

Editor: P9 L22-23 « having an angle of inclination of almost 1 ». Not clear. What do you mean : slope ?. If yes you s should also consider the intercept (close to 0 ?)

Response: Replaced by:

<< The acceleration of the particles in the sheath fluid positions the cells along their long axis, which allows for size estimation based on the FWS pulse shape. A linear relation was found between Length FWS and measured length of diverse phytoplankton species (Length FWS = 0.92*Measured length – 1.57; R^2=0.98; Rijkeboer, 2018). Size estimation is limited by the width of the laser beam (5 μm) so estimations of cell sizes smaller than 5 μm is not possible based on the FWS. >>

Editor: P10 L2-5. May be too qualitative. Precision on the different thresholds could be provided

Response: Inlcuded into Methods following description:

<< For the FRRf data, quality control of the FLC fits was done based on the quality ratio of the induction curve fit per FLC light step and the r^2 of the FLC fit. The quality ratio of the induction curve fit was calculated as the ratio of Fv or Fv' to the standard error (SE) of the linear regression of the saturation phase. FLC fits with an r^2 < 0.75, or with over 30% of the data points with a quality ratio below 6, were visually inspected and removed based on expert judgement. This led to removal of 1% to 7% of the FLC fits per month>>

Editor: P10 L18 « scaled » do you mean centered and reduced as is the usual prdure for PCA ?

Response: Not completely. Scaling does include centering, but also standardizes the deviation from the center to equal units, so that relative difference from the mean, rather than absolute deviatons are used for the PCA.

Results

Editor: *General: Better care should be taken with utilization of the present and the preterite. Generally, the preterite is used in results sections. The whole section should be checked an corrected for that.*

Response: corrected.

Editor: *General: The sampling took place between April ad May. This is not enough to refer to seasonal changes. I thus recommend that « seasonal » be replaced by « between cruises ». This should be checked and corrected throughout the whole manuscript*

Response: Corrected.

Editor: *Unless I am wrong, the ANCOVA has not been presented in the M&M section, which should be corrected. Moreover, readers may not be familiar with this procedure which is aiming at testing for significant differences between regression lines (i.e., in the present ases relationships observed during the 4 sampling months). This could be more clearly stated. Moreover, the wording of the results is not clear as it stands. I would like remind that the procedure is a three step process :*

1. Checking for the homogeneity of the residuals between the 4 models (if this condition is not met, then the procedure is not possible)

2. Comparing the slopes (if the slopes are different then the models are different and there is no need/sense to compare the intercepts)

3. Comparing the intercepts if the slopes are not different (if the intercepts are different then the regression models are different although their slopes are not)

I suggest that the paragraph could be rewritten based on this sequence.

Response:

Added to the m&m:

<< To test whether environmental conditions (as present in the different months) had a significant effect on fluorescence as predictor for Chl *a* concentration, an ANCOVA was performed with the month as a factorial predictor.>>

In the methods section replaced the respective chapter by:

<< Before the ANCOVA analysis, natural logarithm transformations were required to correct for inhomogeneity of the residuals and unequal variances between months. Both the FRRf F_0 ($p<0.01$, adjusted $R^2=0.66$) and FCM total red fluorescence ($p<0.01$, adjusted $R^2=0.90$) provided significant predictors of HPLC-derived Chl *a* concentration (Fig. 2). The ANCOVA with the FRRf-derived F_0 as Chl *a* predictor revealed that the slope did not differ per month, but the intercept did ($p<0.01$). The ANCOVA with the FCM-derived TFLR as Chl *a* predictor resulted not only in a significant difference of the Chl *a* concentration per month ($p<0.01$) but also in a significantly different slope ($p<0.05$), suggesting that other predictors that differ per month were influencing the fluorescence per Chl *a* molecule (Fig. 2). >>

Editor: P14 L2-3 Does the first sentence really belongs to A results section ?.

Response: Moved paragraph to discussion.

Editor: P14 L12 Remove « present as »

Response: Corrected.

Editor: P14 L12-13 There is a problem of singular and plurial between the two sentences

Response: These sentences were removed from the method section as response to an earlier comment.

Editor: P16 L12 Usually sentences do no start with an abbreviation « Ek » should thus become « The Ek » as used afterward

Response: Included 'The'

Editor: P18-L17 I suggest to simplify the sentence to « Gross pimary productivity ranged from XX in June to XX in the coastal zone during May »

Response: Agreed and corrected.

Editor: P19 L14 « the identification »

Response: Corrected.

Editor: P19 L15 « seen » should be replaced by « considered »

Response: Corrected.

Discussion

Editor: *General: I suggest to rearrange the discussion by stating at the beginning of each paragraph/section the results from the study that are discussed. This will help to reduce he confusion presently generated by the mixture of general statements and results from the study*

Response: Rearranged discussion.

Editor: *General: For each paragraph/section, specific inputs from the study could/should be better put in evidence and their consequences stated more clearly*

Response: Rewritten discussion.

Editor: *P22 L3 and 5 : There is a confusion here due to the use of singular (L3) and plural (L5) for method. Maybe one way of avoiding that would be to replace « method » by approach L3*

Response:

Editor: P22 L9 « with and » ??

Response: Checked, but can't find.

Editor: P22 L10 replace « in both the » by « during both »

Response: Checked, but can't find.

Editor: P23 L5-27 care should be taken to put the a of chlorophyll or chl in italics. Moreover, a clearer conclusion should emerge of this paragraph.

Response: Corrected the italics of Chl *a*. Added in the paragraph: << The lower sensitivity to environmental conditions implies that the FRRf is better suited to estimate chlorophyll *a* concentration in comparison to the FCM..>> and added to the end of the paragraph: << As long as there is no uncontroversial method to derive phytoplankton biomass, calculation of multiple parameters and critical evaluation remains necessary.>>

References

Editor: General : Please check this whole section for typing mystakes (e.g. p28 L31-35, p29 L2....)

Response: Checked, but can't find.

High-‑resolution *in situ*underway measurements of phytoplankton photosynthesis and abundance ~~in the Dutch North Sea~~as innovative addition to water quality monitoring programs

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Abstract. Marine waters can be highly heterogeneous both on a spatial and temporal scale, yet monitoring programs are currently relying primarily on low-resolution methods. This potentially leads to undersampling. This study explores the potential of two high-resolution methods for monitoring of phytoplankton dynamics; Fast Repetition Rate fluorometry for information on phytoplankton photosynthesis and productivity and automated scanning flowcytometry for information on phytoplankton abundance and community composition. These methods were tested in combination with an underway Ferrybox system during four cruises on the Dutch North Sea in April, May, June, and August of 2017. The high-resolution methods were able to visualize both spatial and temporal variability of the phytoplankton community in the Dutch North Sea. Spectral cluster analysis was applied to objectively interpret the multitude of parameters and visualize potential spatial patterns. This resulted in the identification of biogeographic regions with distinct phytoplankton communities, which varied per cruise. Our results clearly show that the sampling based on fixed stations does not give a good representation of the spatial patterns, showing the added value of underway high-resolution measurements. To fully exploit the potential of the tested high-resolution measurement set-up, methodological constraints need further research. Among these constraints are; accounting for the diurnal cycle in photophysiological parameters concurrent to the spatial variation, better predictions of the electron requirement for carbon fixation to estimate gross primary productivity, and the identification of more flowcytometer clusters with informative value. Nevertheless, the richness of additional information provided by high-resolution

~~regular~~methods can improve existing low-resolution monitoring. programs towards a more precise and ecosystemic ecological assessment of the phytoplankton community and productivity.

KEY WORDS: Fast Repetition Rate fluorometry, flow cytometry, phytoplankton photosynthesis, spatial variability, primary productivity

1 Introduction

~~Due to~~The Dutch North Sea is of major socio-economic importance because of its close proximity to densely populated areas and its intensive utilization for shipping, fishing, sand extraction and development of offshore windmill farms. Due to this high anthropogenic pressure, the North Sea has undergone considerable biogeochemical and biological changes in the past decades (Burson et al., 2016; Capuzzo et al., 2015 and 2017). ~~Nutrient concentrations have shifted from a situation with increased input by agricultural run-off and wastewater to a large imbalance in the~~ For example, nutrient load and stoichiometry were fluctuating substantially due to the inflow of wastewater and agricultural run-off and subsequent mitigation efforts (Burson et al., 2016; Philippart et al., 2000). Additionally, water clarity decreased in large parts of the North Sea ~~decreased~~ during the 20th century (Capuzzo et al., 2015). These abiotic changes affect ~~biology resulting in~~primary productivity and community composition shifts throughout the trophic levels ~~and decrease of primary productivity~~, with large implications for ecosystem ~~structure~~functioning and fisheries production (Capuzzo et al., 2017; Burson et al., 2016). ~~Good biological monitoring of the North Sea is required for good management. A "robust North Sea" in which ecological processes and biodiversity can thrive will ensure sustainable use, which is of major socio-economic importance; the North Sea is in close proximity to densely populated areas with high recreational value, crossed with major shipping lanes, serving as intensive fishing ground, used for sand extraction and is used for the on-going energy transition involving the creation of many offshore windmill farms.~~

~~In the future, large~~Over time, further changes are expected due to the planned energy transition and under the impact of climate change ~~and coinciding ocean acidification, sea level rise, and~~. Anticipated climate change effects include increasing temperatures~~,~~ sea level rise, and ocean acidification. Already, the North Sea is warming more ~~rapid~~rapidly than most other seas (Philippart

et al., 2011). These changing environmental conditions will have a big impact on marine biogeochemistry ~~and thereby on~~, phytoplankton community composition and primary productivity (Sarmiento et al., 2004; Behrenfeld et al., 2006; Marinov et al., 2010~~; Schiebel et al., 2017).~~). Changes in phytoplankton community composition and primary productivity ~~impact~~affect the entire ecosystem and global biogeochemical cycles (Montes-Hugo et al., 2009; Falkowski et al., 1998; Schiebel et al., 2017). Systematic and sufficient monitoring of these changes is of crucial importance to recognize threats, and, once identified as such, develop mitigation actions.~~).~~

Although phytoplankton community composition and productivity can be highly variable on a spatial and temporal scale, governmental monitoring still consists mainly of low-resolution measurements (Baretta-Bekker et al., 2009; Kromkamp ~~&~~and van Engeland, 2010; Cloern~~, 1996; Cloern~~ et al., 2014; Rantajarvi et al., 1998). ~~In spite~~Currently, biological monitoring of ~~this,~~ phytoplankton in the ~~amount~~Dutch North Sea is dictated by the requirements set by OSPAR and the EU Marine Strategy Framework Directive (MSFD 2008/56/EC). Samples are taken between March and October with a frequency of ~~low-resolution sampling arrays has been cut back considerably since~~every two or four weeks. The phytoplankton analysis consists of HPLC analysis of Chl *a* concentration and microscopy counts of *Phaeocystis* cells and, at some stations, coccolithophore or toxic dinoflagellate cells. Sampling points were reduced from almost 70 in 1984 ~~(Fig. 1; Baretta-Bekker et al., 2008).~~

to less than 20 today, while strong seasonal patterns, high riverine input, and tidal forces make the Dutch North Sea a region with high spatiotemporal variability. Modern automated flow-~~through-flow~~ underway systems have the potential to be an effective addition to monitoring programs because they offer the opportunity to record ~~phytoplankton composition, abundance and photosynthetic activity~~the surface ocean with high spatial and temporal resolution. ~~This could potentially be an effective addition to current monitoring programs. These methods are~~ Such high-resolution methods are well established in physical oceanography but for biological parameters, the implementation has been lacking. This is mostly due to the complicated interpretation of biological parameters, resulting in high uncertainties in the current global estimates of net primary productivity (Silsbe et al., 2016). Underway measurements are not able to replace some more detailed low-~~-~~resolution measurements ~~such as species identification by microscope~~, but their higher spatial and temporal ~~resolution and potentially shorter analysis time make it easier~~resolutions provide the possibility to identify short-lived events~~and serve as~~, detect small-scale changes in phytoplankton dynamics, evaluate consequences of possible (spatial) undersampling, and act as an early warning system. Additionally, ~~they are able to give extra~~underway measurements acquire information on ~~photophysiology, which can improve understanding of ecosystem dynamics.~~living organisms and samples unaffected by transport, storage or conservation. Two non-invasive, high-resolution ~~instruments that can~~methods with the potential to be ~~used~~implemented in ~~marine ecosystem~~phytoplankton monitoring programs are scanning ~~flowcytometers~~flowcytometry (FCM) for information on phytoplankton abundance and community composition and Fast Repetition Rate ~~fluorometers~~fluorometry (FRRf) to give information on phytoplankton photophysiology. Scanning flowcytometry is a method for counting and pulse-shape recording of phytoplankton cells informative on size, fluorescence and scattering properties per algal cell. Based on these characteristics cluster analysis allows for division into groups of similar pigment characteristics and size classes (Thyssen et al., 2015; Rijkeboer, 2018). The FRRf uses active fluorescence to gain insight into phytoplankton photophysiology. This technique is an alternative to the traditional production-light curves (PE-curves) by estimating the photosynthetic electron transport rate (or gross

photosynthesis) at increasing ambient light levels (Suggett et al., 2009a; Silsbe and Kromkamp, 2012). Electron transport rate per unit volume is estimated based on the fluorescence response to a series of single turnover light flashes that cumulatively close all photosystems (Kromkamp and Forster, 2003; Suggett et al., 2003). This single turnover technique allows for the calculation of the effective absorption cross-section and, in combination with an instrument-specific calibration coefficient, the number of reaction centers per volume (Kolber et al, 1998; Kromkamp and Forster, 2003; Oxborough et al., 2012; Silsbe et al., 2015). Electron transport rate per volume can be used to estimate gross primary productivity (Kromkamp et al., 2008; Smyth et al., 2004; Suggett et al., resulting in a high number of parameters on size, fluorescence and scattering properties per algal cell. These characteristics allow for division into groups based on pigment characteristics and size classes (Thyssen et al., 2015). Ideally these groups reflect functional groups, such as calcifiers, silicifiers, DMS producers (such as *Phaeocystis*) and nitrogen fixers to aid in better functional understanding of ecosystem dynamics (le Quéré et al., 2005). Because2009a). These two methods are supplementary because the interaction of phytoplankton with their environment is always a sum of the community composition and their physiology, inclusion of phytoplankton physiology can improve understanding and interpretation of ecosystem dynamics.. For instance, if waters become more turbid, phytoplankton can acclimate by increasing their effective absorption cross section, but it could also lead to a shift in community composition toward species with higher light use efficiency (Moore et al., 2006). Thererfore, the combination of these two instruments allows for more in-depth analysis and understanding of ecosystem processes.

The aim of this study is to test two high-resolution methods, a pulse shape recording flowcytometer and an FRR fluorometer, on their potential to be developed into a novel phytoplankton monitoring method. The two instruments were deployed concurrently on four 4-day cruises in April, May, June, and August to meet a wide range of environmental conditions and phytoplankton community states. These measurements allow for quantification of temporal and mesoscale spatial patterns in phytoplankton abundance, photophysiology, and gross primary production. In this paper we provide an overview of the acquired results, use spectral cluster analysis to visualize spatial heterogeneity and we evaluate the potential of these methods to optimize current monitoring programs.

The FRRf uses active fluorescence to gain insight into phytoplankton photophysiology. This technique is an alternative to the traditional production-light curves (PE-curves) by measuring the electron transport rate (or gross photosynthesis) at increasing ambient light levels (Suggett et al., 2009; Silsbe et al., 2012). Electron transport rate per unit volume is estimated by a series of single turnover light flashes that cumulatively close all photosystems (Kromkamp et al., 2003; Suggett et al., 2003). This single turnover technique allows for calculation of the effective absorption cross-section and, in combination with an instrument specific calibration coefficient, the absorption coefficient and amount of reaction centres per volume (Kolber et al, 1998; Kromkamp et al., 2003; Oxborough et al., 2012; Silsbe et al., 2015). Electron transport rate per unit volume is used to estimate gross primary productivity (Kromkamp et al., 2008; Smyth et al., 2004; Suggett et al., 2009).

In this study, we provide an example of how high-resolution methods can serve in biological monitoring. During four cruises in different seasons, aiming to find four different stages in the seasonal phytoplankton growth period, continuous measurements were conducted with an FCM, FRRf, and Ferrybox to retrieve a wide range of data. An overview will be given on the information acquired with these measurements. Additionally, we will use a model to visualize spatial

~~heterogeneity and identify regions based on the photophysiological characteristics and presence of five phytoplankton groups separated based on pigment ratio and size. Lastly, gross primary productivity is calculated and compared to high resolution data and low resolution data to get a better understanding on the key drivers for gross primary productivity in the Dutch North Sea.~~

2 Methods

2.1 Study site and sampling

The Dutch North Sea is a shallow tidal shelf sea in the southern part of the North Sea. The main water flow is northward. Atlantic water enters the North Sea ~~in the south~~ from the south via the Channel ~~but~~and from the ~~majority of the Atlantic~~northeast where it curves around Scotland ~~and flows southwards and eastwards, where it meets the Channel water and the freshwater from the rivers~~. Both currents meet north of the Dutch coast forming the Frisian Front. For a detailed description on the North Sea physical oceanography, see ~~Sundermann~~Sündermann and Pohlman (2011). Along the Dutch coast, high river input from especially the Rhine River ~~decrease~~decreases the salinity and loads the coastal zone with high nutrient concentrations (Burson et al., 2016). Anthropogenic pressure is high in the Dutch North Sea resulting in a history of large shifts in nutrient concentrations and water clarity (Capuzzo et al., 2015; Burson et al., 2016).

The monitoring of the Dutch North Sea is performed by the Dutch government (Rijkswaterstaat) in a monitoring program called MWTL (Monitoring Waterstaatkundige Toestand des Lands, freely translated as 'Monitoring of the status of the governmental waters of the country'). The ~~location~~locations of the sampling stations of the program are organized along transects (Fig. 1). ~~Notice the decrease in the number of sampling stations between 1983 (small brown dots) and 2014 (larger blue filled circles).~~ The stations are sampled between March and October with a frequency of every two or four weeks, dependent on the transect.
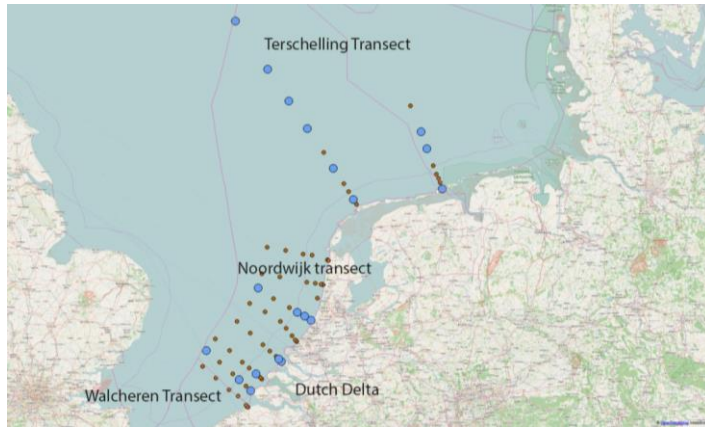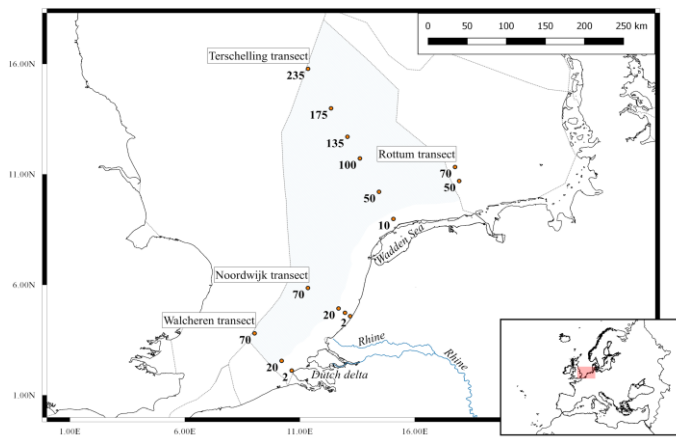
Figure 1: Sampling locations of the MWTL monitoring program referred to in this study. The stations are named according to the transect (Terschelling, Noordwijk and Walcheren), followed by the amount of kilometres from the coast (labels next to sampling points). The boundaries of the Exclusive Economic Zone (EEZ) are indicated by the grey dotted lines and the Dutch EEZ is coloured light blue. The locations of three major infows to the Dutch North Sea are named at the corresponding locations (Rhine river, Dutch Delta and the Wadden Sea). Insertion visualizes the location of the Dutch North Sea in a broader map of Europe.

In 2017, four 4-day sampling surveys (10-13 April, 15-18 May 12-15 June and 14-17 August), were conducted for the JERICO-NEXT project on board the RV *Zirfaea* during their regular monitoring

cruises on the Dutch North Sea. To assess the heterogeneity of the Dutch North Sea and the benefits associated with high- -resolution monitoring the four cruises were conducted in different months (April, May, June, and August), thereby aiming to cover different seasons and stages of the phytoplankton bloom (Baretta-Bekker et al., ~~2009). During these cruises the high-resolution methods (FRRf, FCM and Ferrybox) were combined with lower resolution methods on nutrient concentrations and vertical light extinction using vertical deployments of a rosette frame equipped with a CTD and Niskin bottles.~~2009).

~~On the RV *Zirfaea* the~~The water inlet of the underway system was situated approximately 3.5 m below sea surface level. From the water inlet the sample water, with a flow rate of approximately 24 ~~litre~~liters per minute, was split towards~~:~~ 1) a flow-through -4H-JENA Ferrybox (-4H- JENA engineering GmbH, Germany) equipped with an FSI Excell® Thermosalinograph (Sea-Bird Scientific, USA) to measure temperature and salinity ~~and a SCUFA™ Submersible Fluorometer (Turner Designs Inc., USA),~~ and 2) ~~at a flow rate of 1 L per minute towards~~ a 230 cm^3 flow through sampling ~~container~~chamber (Cytobuoy BV, the Netherlands) where water was cleared from bubbles and sand~~.~~ (~ flow rate of 1 L per minute). The time from the water inlet to the sampling chamber was approximately 2 minutes. A FastOcean Fast Repetition Rate fluorometer (FRRf) with Act2-based laboratory flow- -through system (Chelsea Technologies Group Ltd, UK) and a Cytosense scanning flowcytometer (Cytobuoy BV, the Netherlands) automatically sampled from the sampling ~~unit~~chamber every 30 minutes. Since the average speed of the ship was 8 knots, the average spatial resolution of FCM and FRRf measurements was on average 7.5 ~~kilometres~~kilometers. The Ferrybox sensors stored data every minute. ~~At discrete~~During the cruises the high-resolution methods (FRRf, FCM, and Ferrybox) were combined with lower resolution methods, consisting of measurements at 13 to 19 stations ~~(10 to 15 per cruise) water~~. At these stations, surface samples were ~~collected~~taken for nutrient ~~analysis with~~ and chlorophyll *a* analyses (see 2.2 chemical analyses) using a rosette sampler~~. Simultaneously, the rosette sampler recorded the irradiance extinction in the water column equipped~~ with a ~~QSP-200L Log Quantum Scalar Irradiance Sensor (Biospherical Instruments Inc., USA). The diffuse attenuation coefficient K$_d$ (m^{-1}) was calculated as the linear regression of the natural logarithm of irradiance corrected for changes in surface irradiance (PAR; 400-700 nm) versus water depth~~CTD and Niskin bottles.

2.2 Chemical analyses

Samples for nutrient ~~analysis~~analyses were filtered over Whatmann GF/F filters and kept frozen (-18 °C) until analyses. The analyses of ammonium (NH$_4^+$), nitrite (NO$_2^-$), nitrate (NO$_3^-$), ~~ortho~~phosphate (PO$_4$) and silicate (Si) concentrations were conducted by the Rijkswaterstaat laboratory (RWS; the Netherlands) according to ~~RWS internal analysis protocol A1.004~~ISO 13395, 15681, 16264 using a San-~~plus++~~ Analyzer (Skalar Analytical B.V., the Netherlands). In the RWS internal protocol, nitrite+nitrate is measured by first reducing nitrate to nitrite using a cadmium/copper column and addition of ammoniumchloride as a buffer. Thereafter, sulphanilamide, α-naphthyl ethylenediamine dihydrochloride and phosphoric acid are added and the extinction at 540 nm compared to a NaNO$_2$ standard. For measurement of ammonium concentrations first EDTA was added to bind calcium and magnesium. Then, sodium salicylate, sodium nitroprusside and sodium hypochlorite were added and the extinction at 630 nm compared to a NH$_4$Cl standard. Phosphate was measured by adding molybdate reagent and ascorbic acid to the sample and led through an oilbath at 37 ± 2 °C. Followed by measuring the extinction at 880 nm and comparing to a standard. Silicate concentration was

measured by subsequent addition of molybdate reagent, oxalic acid and ascorbic acid. The silicate concentration was then determined by measuring the extinction at 810 nm and compared to a silicate standard. The detection limits of the nutrient analyses were NO_3NO_2: 0.7 µM, Si: 0.36 µM and PO_4^{3-}: 0.03 µM.

Chlorophyll *a* concentration (hereafter Chl *a*) was ~~sampled~~determined by filtering over Whatmann GF/C filters and freezing the filter at -80 ºC. ~~Thereafter the~~The Chl *a* was extracted in 20 ml 90% acetone and centrifuged for 15 minutes with glass pearls (1.00-1.05 mm) using a Bullet Blender Tissue homogenizer (Next Advance, Inc., Troy, USA) under cooling of solid CO_2. The extract was analyzed in duplicates using Ultra High Performance Liquid Chromatography (UHPLC). The calibration of the UHPLC system is performed every analysis day by making a 12-point standards calibration curve calculated using quadratic regression with weighting method 1/A to better distinguish smaller peaks (R^2>0.995). The injection volume was 20 µl unless the concentration was below the lowest standard, in which case a second injection of 40 µl was reanalyzed. The analysis was conducted by the MUMM laboratory (Belgium) ~~using High-Performance Liquid Chromatography (HPLC)~~ according to RWS analysis protocol A200. Quality control was performed by the RWS laboratory (The Netherlands).

2.3 High frequency methods

2.3.1 Variable fluorescence

Variable fluorescence was measured with a FastOcean Fast Repetition Rate fluorometer (FRRf) and Act2-based laboratory system (Chelsea Technologies Group Ltd, UK). ~~Temperature~~The temperature was controlled by connecting a Lauda ecoline cooler (LAUDA-Brinkmann, LP., USA) to the water jacket of the Act2 system.

The acquisition protocol consisted of 100 excitation flashes with a flash pitch of 2 µs and 40 relaxation flashes with a flash ~~duration~~pitch of 60 µs. Excitation flashes were performed with the blue LED (450 nm) and strength of the LEDs was automatically adjusted to the phytoplankton concentration by the manufacturer' FAstPro software. A loop of simultaneous blue and green flashes (450 nm+530nm) was performed after the acquisition loop of only blue LEDs in case the blue LEDs were not able to reach saturation (for instance with high cyanobacteria concentrations), but as this was not the case, only the parameters measured by blue LEDs were used for further calculation. The sequence was repeated 20 times with a sequence interval of 100 ms. The sample was refreshed before each ~~fluorescent light curve~~Fluorescent Light Curve (FLC) by flushing for 60 seconds and kept well-mixed by "~~ ~~flushing"~~ ~~ for 200 ms between acquisition loops.

The FLC protocol consisted of 14 light steps of 100 s, of which the light intensity was automatically adjusted to get the optimal FLC shape based on the previous light curve. A pre-illumination step (55 seconds on 12 µmol photons m^{-2} s^{-1}) was included before the FLC to low light acclimate the phytoplankton and to relax Non-Photochemical Quenching (NPQ) of diatoms and other chlorophyll *a* -*c* algae as they stay in the light activated state in the dark~~.~~ (Goss et al., 2006). After each light step, measurements were made in the dark for 18s to retain a value for F_0'~~.~~ (minimal fluorescence in light acclimated state). The data ~~was~~were corrected for the background fluorescence by taking sample

blanks multiple times per day by filtration over a 0.45 µm filter and subtracting the last determined background fluorescence from the sample fluorescence.

An overview of the derived photosynthetic parameters can be found in Table 1. To derive values for the maximum photosynthetic electron transport rate (P_{max}), minimum saturating irradiance (E_k) and the light utilisation efficiency (α) the relative electron transport rate (rETR) of the samples was fitted to the exponential model of (Webb et al. 1974), after normalizing the data to the irradiance as described by (Silsbe and Kromkamp, 2012):

$$F_q'/F_m' = \frac{P_{max}\left(1-\exp\left(\frac{-E}{E_k}\right)\right)}{E} \tag{1}$$

where E is the irradiance in µmol photons m^{-2} s^{-1}, F_q'/F_m' the effective ~~PSII~~ quantum efficiency~~, α~~ of photosystem II (PSII), α is the initial slope of the rETR vs irradiance curve and E_k is the light saturation parameter (in µmol photons m^{-2} s^{-1}). The relative maximum rate of photosynthetic electron transport (P_{max}) was calculated as:

$$P_{max} = E_k \times \alpha \tag{2}$$

The PSII flux in µmol electrons m^{-3} h^{-1} was calculated as the product of the effective PSII efficiency (F_q'/F_m'), the optical absorption cross section of the light harvesting pigments of PSII (a_{LHII}) and the irradiance (E):

$$JV_{PII} ~~= F_q'/F_m'~~ (\text{in µmol electrons (PSII } m^{-3}) \; h^{-1}) = F_q'/F_m' * a_{LHII} * E \tag{3}$$

where

$$F_q'/F_m' = \frac{F_m'-F'}{F_m'} \tag{4}$$

and

$$a_{LHII} \; (in \; m^{-1}) = \frac{F_0 * F_m}{F_m - F_0} * K_a \tag{5}$$

	Description	unit
Parameters derived from fluorescence induction curve		

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F_0	Minimum fluorescence, measured at zero[th] flashlet of an FRRf single turnover measurement when all PSII reaction centers (RCII) are open. Estimate for chlorophyll a concentration.	Dimensionless
F_m	Maximum fluorescence, reached at n[th] flashlet of an FRRf single turnover measurement when all PSII reaction centers are closed.	Dimensionless
$1/\tau$	Rate of re-opening of a closed RCII	ms^{-1}
σ_{PSII}	Effective absorption cross section of PSII photochemistry	$nm^2\ PSII^{-1}$
Parameters calculated from parameters derived from fluorescence induction curve		
JV_{PII}	PSII charge separation rate per unit volume (see eq. [3])	μmol electrons $m^{-3}\ h^{-1}$
F_v/F_m	Quantum efficiency of PSII under dark conditions (see eq. [4])	Dimensionless
a_{LHII}	Absorption coefficient of PSII light harvesting (see eq. [5])	m^{-1}
[RCII]	Functional PSII reaction centers per volume (see eq. [6])	$nmol\ RCII\ m^{-3}$
Parameters derived from Fluorescence light curve (FLC)		
α_{PSII}	Initial slope of the FLC, an estimate of affinity for light	μmol electrons (μmol photons)$^{-1}$
E_k	Minimum saturating irradiance of fluorescence light curve	μmol photons $m^{-2}\ s^{-1}$
P_{max}	Maximum photosynthetic electron transport rate	μmol electrons $m^{-2}\ s^{-1}$
Parameters calculated from parameters derived from fluorescence light curve and irradiance		
Surface GPP	Surface Gross Primary Productivity (see eq. [3]) calculated based on the FLC-parameters and incoming irradiance.	$\mu g\ C\ L^{-1}\ h^{-1}$

K_a (m^{-1}) is an instrument specific factor necessary for obtaining absolutes rate of photosynthetic transport (see Oxborough et al. (2012) and Silsbe et al. (2015) for more information). The amount of reaction centres of PSII per cubic metre ([RCII]) was calculated as

$$[RCII]\ (in\ nmol\ m^{-3}) = K_a * \frac{F_0}{\sigma_{PSII}}$$

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and the approximate number of reaction centres per Chl *a* (note that the Chl *a* concentration is estimated based on minimum fluorescence value (F_0), and is therefore a mere estimation). If [RCII] is known the number of PSII units per mole Chl *a* (n_{PSII}) can be calculated:

$$n_{PSII} = \frac{[RCII]}{[Chl\ a]} \tag{7}$$

for more information on the calculation of [RCII] and a_{LHII} see Oxborough et al. (2012) and Silsbe et al. (2015).

Q_A reoxidation or rate of re-opening of a closed RCII was calculated as 1 divided by the time constant of re-opening of a closed RCII with an empty Q_B site (τ_{ES}) in ms^{-1}.

Standardized daily anomalies (Z-scores) were calculated for the photophysiological parameters as:

$$Z - score = \frac{x - daily\ mean(x_0 \ldots x_{24})}{Daily\ standard\ deviation(x_0 \ldots x_{24})} \tag{7}$$

Partial days were excluded because this could potentially offset the daily mean and standard deviation.

Gross Primary Productivity (GPP) was estimated ~~by integrating surface productivity over water depth. Volumetric P_{max} and α were derived~~ by fitting JV$_{PII}$ in µmol photons m^{-3} h^{-1} to equation 1 (the exponential model of Webb et al., 1974) to derive a volumetric P_{max} and ~~these parameters used to integrate productivity over depth where the light extinction~~α. GPP in ~~the water column~~µg C L^{-1} h^{-1} was ~~estimated as~~

$$E(z) = E_{surface} * e^{-K_d * z} \tag{9}$$

~~with E(z) being the irradiance at depth z. The value for~~ then calculated using equation 1 and incident surface irradiance. To avoid effects of changing incident surface irradiance (E$_{surface}$) ~~was held constant over~~on the ~~month~~spatial pattern and ~~calculated as the~~to be able to compare GPP between regions we used monthly average ~~light intensity over~~surface irradiances (E$_{surface}$) in our calculations of primary productivity. From 2010~~-to -~~2016 irradiance (400-700 nm) was measured at the roof of the NIOZ building in Yerseke using a LI-190 quantum PAR sensor~~. Hourly data were averaged~~ and hourly averages stored using a LI1000 datalogger. ~~The light extinction coefficient, K_d,~~E$_{surface}$ was then calculated ~~based on a vertical irradiance profile obtained with a QSP-200L Log Quantum Scalar Irradiance Sensor (Biospherical Instruments Inc., USA) which was conducted approximately 10 times per cruise (see Methods-Study Site and sampling). In order to interpolate between these profiles a correlation with the turbidity (in NTU, as measured by the Ferrybox) as predictor was determined based on linear regression: ln(K_d)=0.785*ln(Turbidity)-1.324 (n=71, R²=0.77, p<0.01). The calculated water column~~by averaging all irradiance data from the years 2010-2016 for the respective month. The primary productivity in electrons units was converted to carbon units by assuming 6 moles of electrons were required to fix one mole of carbon, based on a study in the adjacent Oosterschelde and Westerschelde estuaries (Kromkamp et al., in prep.).

2.3.2 CytoSense scanning flowcytometry

Single cell measurements of the phytoplankton community were ~~carried out~~conducted using a bench- -top scanning flowcytometer (Cytobuoy BV, the Netherlands) equipped with two lasers (488 nm and 552nm~~). Both lasers (~~; 60mW each~~)~~). Both laser beams were ~~continuously~~ca. 5 µm high and 300 µm wide and focussed on the same spot in the middle of the flow-through chamber ~~having a height of ca. 5 um and a width of 300 um.~~. The speed of the particles ~~is~~was ca. 2.2 m s⁻¹. ~~With this configuration Forward light Scatter (FWS) and Sideward light Scatter (SWS) of all particles were measured.~~ The system contained 3 fluorescence detector channels~~;~~ FLY separating fluoresced wavelengths of 550-600 nm (FLY; Phycoerythrin~~); FLO of~~), 600-650 nm (~~Chlorophyll b and~~FLO; Phycocyanin) and ~~FLR >~~above 650 nm ~~for~~(FLR; chlorophyll *a* ~~and c detection. Per cell the pulse shape recording and the parameters (FWS, SWS, FLR, FLO and FLY) plus their affiliates (length, total and maximum values) are recorded and saved.~~). Additionally, the Forward Light Scatter (FWS) and Sideward Light Scatter (SWS) of all particles were measured. The FCM was equipped with a double set of detectors (PMT's) for each of the three fluorescence channels to increase the dynamic range (Rutten, 2015). Per cell, the pulse shape of the parameters (FWS, SWS, FLR, FLO, and FLY) plus their affiliates (length, total and maximum values) were recorded and saved. The instrument was checked daily for drift using 3 µm Cyto-Cal™ 488 nm alignments beads (Thermo Fisher Scientific Inc., USA). Additionally, the FCM was equipped with an Image-in-flow camera to take pictures of the nano- and micro-phytoplankton~~, this~~. This allows for linking pulse shape recordings to microscopy results and thereby identification of represented phytoplankton groups -in respective- clusters.

Phytoplankton cells were clustered based on the pulse shape recording of the individually scanned phytoplankton. In this paper, we ~~mainly~~ discriminate the phytoplankton groups based on their size (pico, nano, and micro) and Orange/Red fluorescence ratio (hereafter O/R ratio; Table 2). The chosen cluster criteria were based on expert judgment (SeaDataNet, 2018) and corresponding to other studies (Sieburth et al., 1978; Vaulot et al., 2008). ~~; Easyclus~~The clustering was done using the software Easyclus 1.26~~,~~ (ThomasRuttenProjects, The

Table 2: The phytoplankton groups distinguished in the current study.

Name	Cluster criteria		Main corresponding taxonomic group(s)
	Length FWS	O/R-ratio	
Pico-Red	<4 µm*	<1	Pico-eukaryotes
Pico-Synecho	<4 µm*	>1	Synechococcus
Nano-Crypto	4-20 µm	>1	Cryptophycea
Nano-Red	4-20 µm	<1	Diatoms,Haptophytes, Dinoflagelates
Micro-Red	>20 µm	<1	Diatoms,Haptophytes, Dinoflagelates

*In june <6 µm

Netherlands~~). Size was calculated based on the length FWS. Length FWS was found to be a good estimate of the length of the particles because due~~) according to ~~the speed~~these criteria. Noise, air

bubbles and other potential outliers were removed. The acceleration of the particles in the sheath fluid ~~of~~positions the ~~FCM the organisms will flow~~cells along their long axis~~. We obtained a~~, which allows for size estimation based on the FWS pulse shape. A linear relation was found between Length FWS and measured length of diverse phytoplankton species~~, having an angle of inclination of almost 1 and R²=0.99. For organisms smaller than 5 µm there may be some deviation from this relationship due to~~ (Length FWS = 0.92*Measured length – 1.57; R^2=0.98; Rijkeboer, 2018). Size estimation is limited by the width of the laser beam (~~which is ~~5 µm) so estimations of cell sizes < 5 µm)~~. is not possible based on the FWS.~~

2.4 Data analysis

Outliers of the complete dataset were removed after visual inspection of pairplots made with the pairplot function of the ~~HihgstatLib~~HighstatLib.V4 script (Zuur et al., 2009~~)~~). For the FRRf data, quality control of the FLC fits was done based on the quality ratio of the induction curve fit per FLC light step and the ~~fitted F$_q$'/F$_m$'-E curves. A~~r^2 of the FLC fit. The quality ratio of the induction curve fit was calculated as the ratio of Fv or Fv' to the standard error (SE) of the linear regression of the saturation phase. FLC fits with an r^2 < 0.75, or with over 30% of the data points with a quality ratio below 6, were visually inspected and removed based on expert judgment. This led to the removal of 1% to 7% of the FLC fits per month. Unsatisfactory fits occurred when the auto-LED settings misadjusted the maximum irradiance or when fluorescence was too low to retrieve a reliable fluorescence signal. Especially at low biomass FLCs became noisy, therefore a minimum fluorescence signal was set for calculations of photosynthetic parameters~~, below~~. Below this blank corrected instrument-specific fluorescence signal F$_q$'/F$_m$' became noisy and often reached above the biologically unlikely limit of 0.65~~.~~ (Kolber and Falkowski, 1993). The datasets of the high-resolution measurements (FRRf, FCM, and Ferrybox) were linked using corresponding timestamps. When multiple measurements were performed within one FLC, the average was used.

~~Spatial clusters~~ To test whether environmental conditions (as present in the ~~Dutch North Sea~~ different months) had a significant effect on fluorescence as a predictor for Chl a concentration, an ANCOVA was performed with the month as a factorial predictor. To find regions with similar phytoplankton communities, data were ~~defined~~spectrally clustered using ~~R (version 3.4.1, R Core Team, 2017) with the additional~~the uHMM R package (Poisson-caillault and Ternynck, 2016~~)~~.) in the statistical software R (version 3.4.1, R Core Team, 2017). The package default settings normalize data before clustering~~,~~ and automatically find the number of clusters based on spectral classification and the geometry of the data. This new methodology is more robust than the classical hierarchical and k-means technics (Rousseeuw et al., ~~2013,~~ 2015). ~~Datapoints were then per cluster labelled and plotted on a map to visually identify regions. Cruises were analysed separately and not as one continuous time series as the time gaps between the sampling cruises were large. All photophysiological colinear predictors were removed (VIF>6; Zuur et al., 2009). The FCM phytoplankton groups based on total red fluorescence were included despite colinearity.~~

~~Separate stepwise multiple regressions were performed for all months combined by stepwise deletion of insignificant predictors. Predictors were~~Phytoplankton parameters were first tested for ~~colinearity~~collinearity and ~~all~~predictors with a variance inflation factor (VIF) over 6 were removed (Zuur et al., ~~2009. Interactions of the predictors were not included. Residuals were visually checked~~2009; see supplementary material for pairplots). This left for ~~normality by plotting a qqnorm plot of the residuals of the model and for homogeneity of the variances by plotting the residuals of the model against the fitted values and against each separate predictor.~~

the cluster analysis FCM-parameters Pico-red, Nano-red, Micro-red, and *Synechococcus* and the FRRf-parameters σ_{PSII}, F_v/F_m, a_{LHII}, $1/\tau$, E_k. Datapoints were then per cluster labeled and plotted on a map to visually identify regions. Principal Component Analyses (PCA) were performed to find which variables contributed most to the cluster results. The PCA's were based on correlation matrixes with scaled parameters to correct for unequal variances and was carried out with the prcomp() function in ~~-~~R (version 3.4.1, R Core Team, 2017). The PCA visualization was done using the supplemental R package factoextra (Kassambra and Mundt, 2017). Maps were made using QGIS v. 2.14.2 and other figures were made with ggplot2 in R (Wickham, 2009).

3 Results

3.1 ~~Environmental~~Abiotic conditions

Environmental conditions in the Dutch North Sea ~~are~~were spatially heterogeneous and differed strongly ~~influenced by seasonal dynamics.~~between months. Sea surface temperature ~~increases~~increased from 9.5 ± 1.0 ºC in April to 19.0 ± 0.6 ºC in August (~~Table 3). Seasonal variations~~supplementary table S1). Differences in salinity ~~are~~between cruises were small with the highest monthly mean salinity ~~was measured~~ in April (34.1 ± 1.8~~), while spatial~~). Spatial variability ~~is high~~of salinity was higher with river influx decreasing the salinity ~~-~~down to 26 in the coastal zone. The monthly average of turbidity ~~does show seasonal variation and was clearly~~was higher in April (2.3 ± 3.0 NTU) in comparison to other months~~, which~~. This was also reflected in the K_d values, which were highest ~~K_d values~~in April (0.39 ± 0.28 m^{-1}~~)~~; supplementary table S1). It needs to be noted that monthly averages are not ~~completely~~fully comparable, because of differences in sampling route and stations (Fig. 3). ~~(Fig. 4).~~

Dissolved Inorganic Nitrogen (DIN; Nitrate+Nitrite+Ammonium) and silicate (Si) concentrations showed spatial variability and varied per cruise (supplementary table S2). Spatially, two trends were distinguishable: a coastal-offshore gradient and a longitudinal gradient. Per cruise the strength and position of these spatial gradients changed. The ~~average nutrient concentrations of all~~ coastal to offshore gradient moved shoreward from April to August and the southern stations ~~sampled during the different cruises is shown in Table 4. To see if nutrient~~were depleted earlier in the year in comparison to the more northerly stations. In April DIN and Si concentrations were on average higher and only potentially limiting ~~we used threshold concentration for DIN and (~~Si~~as 2 µmol L$^-$~~<1 ~~and PO$_4$$^{3-}$ as 0.2 µmol~~.8 µmol L^{-1} ~~(~~, DIN<2 µmol L^{-1}; Peeters and Peperzak et al~~, 1991, Philippart et al., 2007) although Ly et al. (2014) showed that for Wadden Sea phytoplankton phosphate can become limiting when values become lower than 0.13-0.16 µmol L^{-1}.~~

~~Nutrient concentrations show both high spatial and seasonal variability (supplementary Table 1). The general trend in all transects is an offshore moving gradient of decreasing nutrient concentrations. Offshore~~. (1990) and references therein) in the most Southerly part of the Dutch North Sea ~~(Walcheren transect) and at offshore~~ stations (>70 km offshore west of the Netherlands, >135 km North of the Netherlands~~) are DIN limited year-round, while regions closer to freshwater influx are DIN sufficient in all sampled months except August. This clearly reflects the input of the Rhine and its waters remained relatively close to the coast; no influence seems present at waters further than 70 km offshore. In April nutrient concentrations are on average higher and only potentially limiting in the most Southerly part of the Dutch North Sea (Walcheren transect) and further offshore (>70 km offshore west of the Netherlands, >135 km North of the Netherlands). In later months, nitrate and~~

~~silicate limitations gradually moves towards the coastal zone, with nutrient limitation at all sampled stations in August. Phosphate levels were generally quite).~~ In later months, DIN and Si limitations gradually moved towards the coastal zone. Stations closest to freshwater influx (Noordwijk 2 and 10) became DIN and Si-limited later in the year (supplementary table S2). The increased DIN concentration at the transect close to the Rhine outflow was absent seventy kilometers offshore (Noordwijk 70), suggesting that the Rhine water remained close to the coast. Phosphate concentrations were low and possibly limiting~~, with exception~~ throughout the Dutch North Sea (ortho-phosphate PO_4^{3-}<0.5 µmol L^{-1}; Peeters and Peperzak et al, 1990). With exceptions in April north of ~~the Wadden Islands up to 135~~Terschelling between 50 and 100 km offshore~~. Later in the year (~~ and in May at Noordwijk 2, a region with the high freshwater influx. In June and August~~)~~, phosphate concentrations recovered in the Southern part of the Dutch North Sea reaching up to 0.6 µM~~. Silicate, an essential element for diatoms, seemed to be present in limiting concentrations at all sampled times at the Walcheren stations, suggesting that the cruise in April was already beyond the peak of the diatom bloom. Nutrient ratios (DIN:DIP, DSi:DIP and DIN:DSi) suggest P-limitation, and for diatoms Si-limitation in April and May, and N-limitation in August, whereas in June values were closer (but below) to the Redfield Ratio. Apart from the seasonal trend of the nutrient limitation on the onshore-offshore gradient moving towards the coast, there also seems to be a south to north trend, with the southern Dutch North Sea being depleted earlier in the season in comparison to the more northerly area.~~ (supplementary table S2).
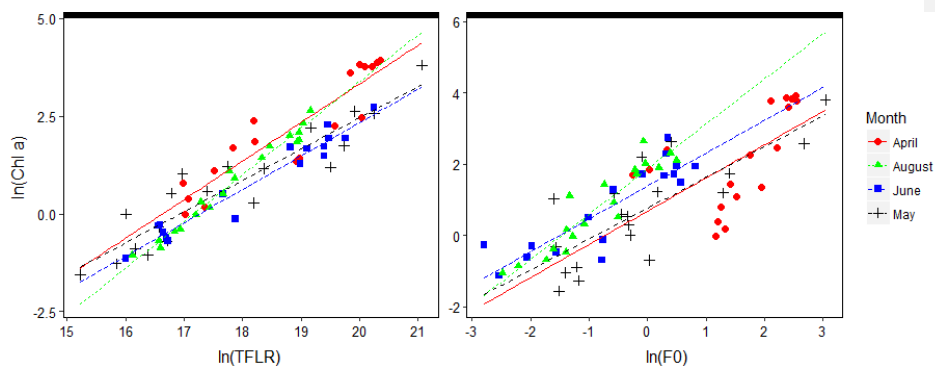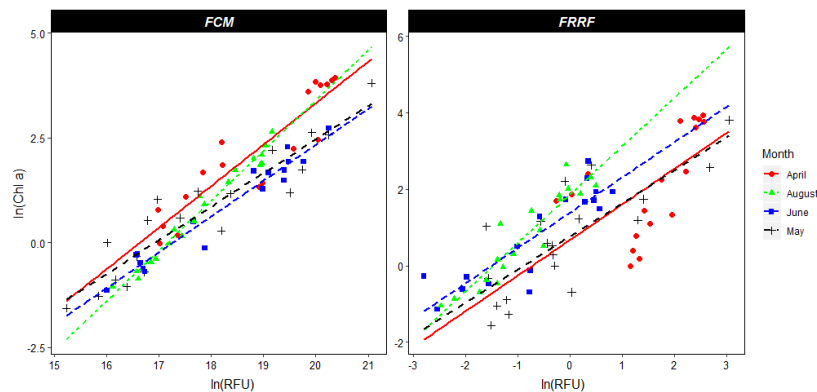
Figure 2: linear regression of the natural logarithms of Chl *a* concentration in µg L^{-1} as determined by HPLC (y-axis) and on the x-axis, ~~the natural logarithm of;~~ FCM-derived total red fluorescence (~~TFLR,~~in relative fluorensence units (RFU), left panel) and FRRf-derived minimum fluorescence (F$_0$ in RFU, right panel). Both FCM red fluorescence (p<0.01, adjusted R^2=0.90) and the FRRF F$_0$ (p<0.01, adjusted R2=0.66) are significant predictors for Chl *a* concentrations. The months (April, May, June and August) were a significant predictor of Chl *a* concentration for both the FRRf (p<0.05) and the FCM (p<0.01). The interaction between the x and y axis was only significant for the FCM data (p<0.05).

3.2 Phytoplankton ~~parameters~~

~~Information on total phytopankton~~ abundance ~~can be obtained from both FRRf and FCM (Fig. 2). The FCM provides data on abundance in~~and fluorescence

Before the ~~amount of cells per millilitre of seawater and gives an estimate of Chl *a* concentration based on the cumulative red fluorescence of all cells (hereafter TFLR). The FRRf also provides an estimate of Chl *a* based on the minimum fluorescence (F$_0$). Using cell count or fluorescence as predictor of phytoplankton presence yields contrasting results (Fig. 3), because of the wide range of phytoplankton cell sizes; microplankton have a substantially higher biomass, and thus fluorescence, per cell in comparison to picoplankton. So while the phytoplankton cell count is higher in June and August in comparison with April, the community in the former months consists of mainly pico-plankton which contribute little to total fluorescence resulting in a considerably lower fluorescence in June and August (Fig. 3). Fluorescence is therefore a better predictor of biomass or of Chl *a* concentration than cell counts, although Chl *a* concentration is a limited predictor of biomass because the Chl *a* concentration per cell is species-specific and is subject to phenotypic acclimation to abiotic conditions (Flynn, 1991, 2005; Geider et al., 1997).~~

~~Both the FRRf and FCM provide significant predictors of Chl a concentration (Fig. 2). When performing an~~ ANCOVA ~~with month as factorial predictor~~analysis, natural logarithm transformations were ~~necessary because~~required to correct for inhomogeneity of the ~~highly~~residuals and unequal variances between months. Both the FRRf F$_0$ (p<0.01, adjusted R^2=0.66) and FCM total red fluorescence (p<0.01, adjusted R^2=0.90) provided significant predictors of HPLC-derived Chl *a* concentration (Fig. 2). The ANCOVA with the FRRf-derived F$_0$ as Chl *a* predictor revealed that ~~Chl *a* concentrations significantly differed per month (p<0.01) but not~~ the slope~~, and that F$_0$ was a~~

significant predictor (p<0.01) of Chl *a* concentration (adjusted R^2=0.66). Yet, the FCM estimate of Chl *a* concentration (TFLR) was a better predictor (p<0.01) with an adjusted R^2 of 0.90. did not differ per month, but the intercept did (p<0.01). The ANCOVA with the FCM-derived TFLR as Chl *a* predictor resulted not only in a significant difference of the Chl *a* concentration per month (p<0.01) but also in a significantly different slope (p<0.05), suggesting that abiotic factors and phytoplankton community composition are other predictors that differ per month were influencing the amount of fluorescence per Chl *a* molecule (Fig. 2). In April and August the slope is steeper in comparison to May and June. An explanation could be the package effect (Dubinsky et al., 1986), where stacking of Chl *a* at low light intensities causes a shading effect within the cells and a steeper slope of Chl *a* concentration with *in vivo* fluorescence. Because there is a lack of agreement in photophysiological parameters between April and August, it is likely that the months do not have the same drivers for the steeper slope. In April the high σ_{PSII} coincides with high n_{PSII}, suggesting that although the chlorophyll self-shades it does not result in a lower absorption cross section because of the high amount of RCIIs in relation to Chl *a* molecules. In contrast, in August the phytoplankton community is nutrient limited and has a higher σ_{PSII}, in correspondence with results obtained by Kolber et al. (1988) who observed that nutrient limitation increases σ_{PSII}. Self-shading in this month is more likely a result of smaller cell size as indicated by the higher abundance of picoplankton (Table 3; Geider et al., 19862).

The FRRf yields other biomass related proxies next to the minimum fluorescence; the total absorption coefficient in the water (a_{LHII} in m^{-1}) based on the absorption of the photosynthetic pigments pigments associated with PSII and the amount of PSII reaction centres per volume ([RCII] in nmol RCII m^{-3}). Both are very strongly correlated to F_0. The ratio of RCII to a_{LHII} can vary by nature, affecting n_{PSII}. However, these three biomass related proxies show a perfect relationship to each other (Supplementary material). The minimum fluorescence measured with the FRRf (F_0) is related to the red fluorescence mearured with the FCM (TFLR; r=0.7). Interestingly,TFLR and F_0 are not correlated to total orange fluorescence, which indicates that the cyanobacterial picoplankton is not a fixed proportion of the total phytoplankton biomass (Supplementary material).

A pairplot analysis of the combined data of all cruises shows that some photosynthetic parameters are highly correlated (Supplementary material). The correlation of alpha and F_v/F_m, indicators for photosynthetic affinitiy and photosynthetic efficiency, are perfectly correlated (r=1). The parameters derived from the PE-curve also show high correlation, being dependent on the light acclimation state of the phytoplankton trends in the maximum electron transport rate (P_{max}) and the light saturation parameter (E_k) are similar. Surprisingly, alpha does not show any correlation with Ek, suggesting that the light affinity is not dependent on the level of irradiance where the PSII reaction centres become saturated, or that its value is also affected by nutrient limitation, obscuring a relationship with E_k. The effective absorption cross section per photosystem, σ_{PSII}, is very strongly negatively related to n_{PSII} (r=-0.9). This is to be expected; the larger n_{PSII}, the smaller the number of pigment molecules associated with it.

~~Community composition is variable over the months with the biggest shift in community composition between May and June (Fig.~~ **3.3 Phytoplankton community composition**

In April the northern ~~2). In May mean F~v~/F~m~ is low (0.26 ± 0.09), suggesting physiological stress (Table 3). This coincides with a shift of nutrient sufficiency in the largest part of the sample region (with only potentially limiting conditions in the most southerly~~ part of the Dutch North Sea ~~and further offshore) in April to a larger region of nutrient limitation in May. In June the mean F~v~/F~m~ recovers and community composition changes. Both groups of picophytoplankton (Synechococcus and total) increase in relative abundance between May and June, while the nanophytoplankton shows a strong decrease (Fig. 3). Because the picophytoplankton fraction makes up for only a small part of the biomass, the microphytoplankton is the largest contributor to red fluorescence in June, although this group does not increase in relative abundance in comparison to May. After June the microplankton disappears, leaving 80% of the average community composition to picoplankton. The shift to smaller cell sizes at nutrient limiting conditions is not surprising because of the higher nutrient affinity of smaller cells.~~
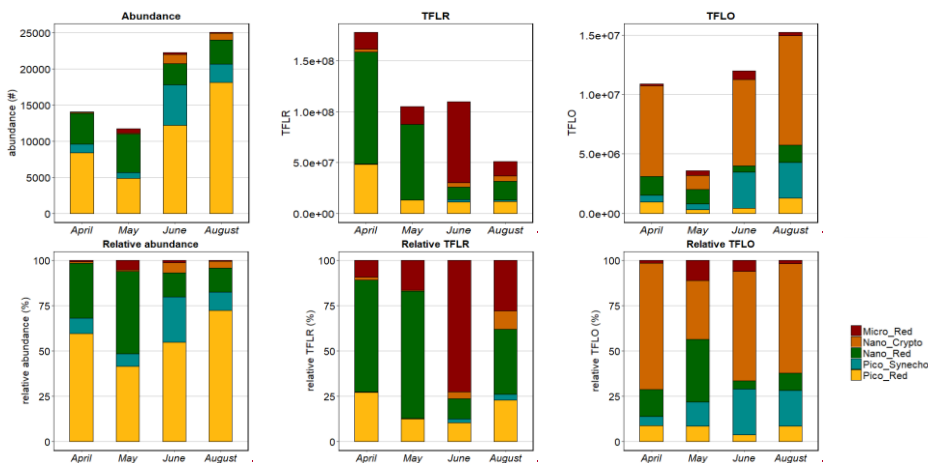
~~Figure 3: Phytoplankton abundance per phytoplankton group distinghuished with the flowcytometer, shown as average (relative) abundance per month (left), total red fluorescence(middle) and total orange fluorescence (TFLO; right).The upper graphs are absolute and lower graphs relative.~~

~~**3.3 Spatial distributions**~~

~~Both the biomass concentration and the phytoplankton community composition, expressed as percentage of the total cell numbers, showed a dynamic picture (Fig. 4). In all cases,~~

~~microphytoplankton < 10% of the total cell counts, although in terms of biomass they sometimes dominate (Fig.~~ was numerically dominated by ~~3).~~

~~In April, high biomass concentrations (using TFLR) are observed close to the Dutch Delta in the South of the Dutch EEZ and west to the island of Texel and Vlieland. Very low concentrations are found offshore, especially in the more central part towards the Doggersbank area. The north-western wedge of the Dutch North Sea was dominated by~~ picoplankton whereas the southern part and the north coastal area of the Dutch EEZ were numerically dominated by nanophytoplankton. ~~Orange fluorescent dominating species (like~~ The taxa with high phycoerythrin content (*Synechococcus* and Cryptophycea) ~~were~~ made up only a small proportion of the total phytoplankton community in April (generally less than 10~~%), however about 100km North~~ %) and were most abundant in the northern part of the ~~island of Vlieland there was a patch of water where they seem to dominate the phytoplankton~~ Dutch North Sea (Fig. ~~4e~~3e). Microphytoplankton represented always less than 3% of the total community. Highest microphytoplankton abundance ~~< 3%, and highest numbers were~~ was found close to the Dutch Delta and along the Noordwijk transect. ~~In April, the lowest F_v/F_m values (0.4-0.5) were found in the southern part of the Dutch EEZ. Highest values were found offshore on and towards the Terschelling transect, and at the coastal stations of the Noordwijk transect.~~

~~The situation in May is different from April (Fig. 4~~The spatial patterns of the phytoplankton community in May were smaller in comparison to April (Fig. 3, second column). ~~The higher biomass near the Texel and Delta area has mainly dissappeared, and is much more homogeously distributed while the community composition is very heterogeneous. The biomass concentration is about 50% lower than in April (Table 2, Fig. 3). Unfortunately, the RV *Zirfaea* did not sail to the NW edge of the Dutch coastal zone (Dogger Bank), but the higher dominance of Orange fluorescent dominated species on the Terschelling transect observed in April is still visible in May (Fig.~~ Picophytoplankton abundance was highest offshore~~4f). Along the Walcheren transect and at a section of the Terschelling transect (~60-135 km off the coast) the highest percentages of picophytoplankton were observed~~ (60-80%), whereas the highest percentage of nanophytoplankton was observed north of Terschelling 100 and in the coastal zone (Fig. 3f). Between May and June the community composition shifted and phytoplankton cell numbers increased. Both groups of picophytoplankton (*Synechococcus* and Pico-red) increase in relative abundance between May and June, while the nano-phytoplankton shows a strong decrease (Fig. 3). The highest abundance of pico-phytoplankton was observed offshore. The microphytoplankton was the largest contributor to red fluorescence in the coastal region, although this group does not increase in relative abundance in comparison to May (Fig. 3). ~~closer to the Frisian coast. Notice that this coast was visited twice in 2 days, and that abundance varied between those occasions (40-60% vs. 60-80%), yet the difference between visits was only 6 %; the first time around 64 % and the second time around 58 %. The two visits did fall into different diurnal time periods, the first time was at the end of the day (around 18:00h) while the second time was early morning (around 7:00h), but both of these time periods were in the flood tide. In May, F_v/F_m was in general much lower than in April (0.1-0.3) across most of the Dutch EEZ. Higher values were found in the southern coastal stations, a possible consequence of the outflow of the Scheldt River, and 70 km offshore Noordwijk. At both of these regions low values for F_v/F_m were found in April so possibly these phytoplankton~~ In August the pico-phytoplankton was dominating the phytoplankton communities ~~have already acclimated to low nutrient conditions. The range in σ_{PSII} was larger in May in comparison to April. A small area near the coast of Noordwijk showed low σ_{PSII} values. This might reflect Rhine River waters, but the effect was not noticable further north.~~
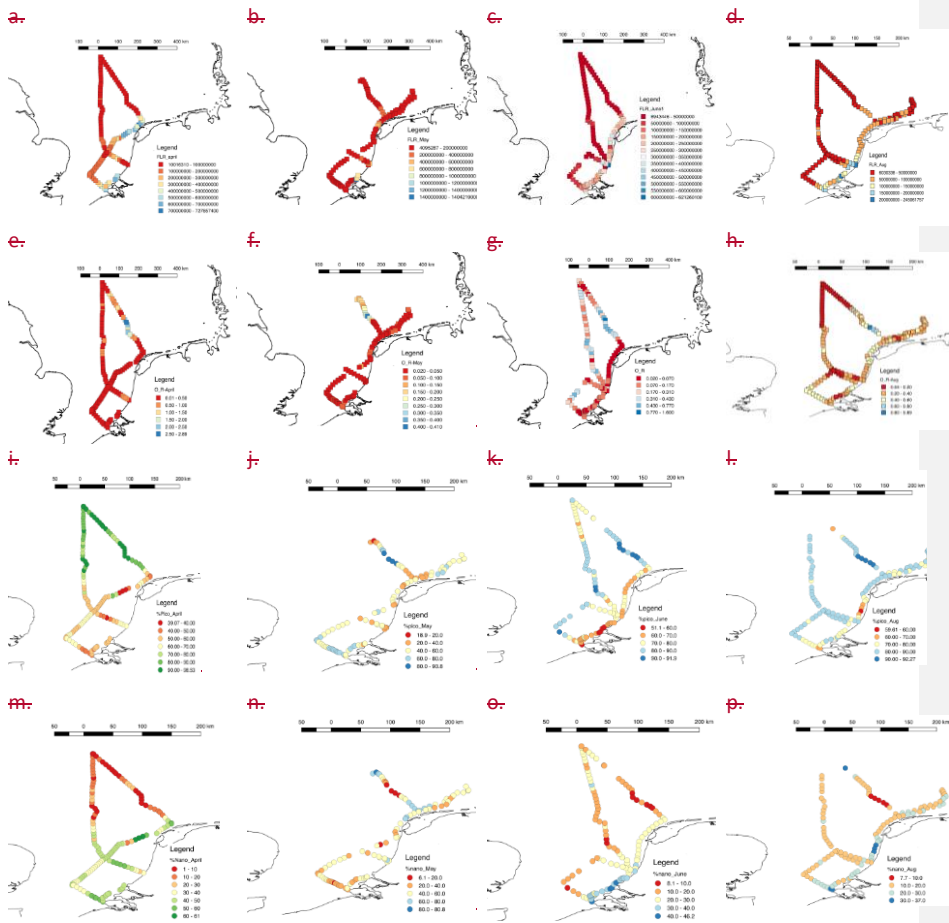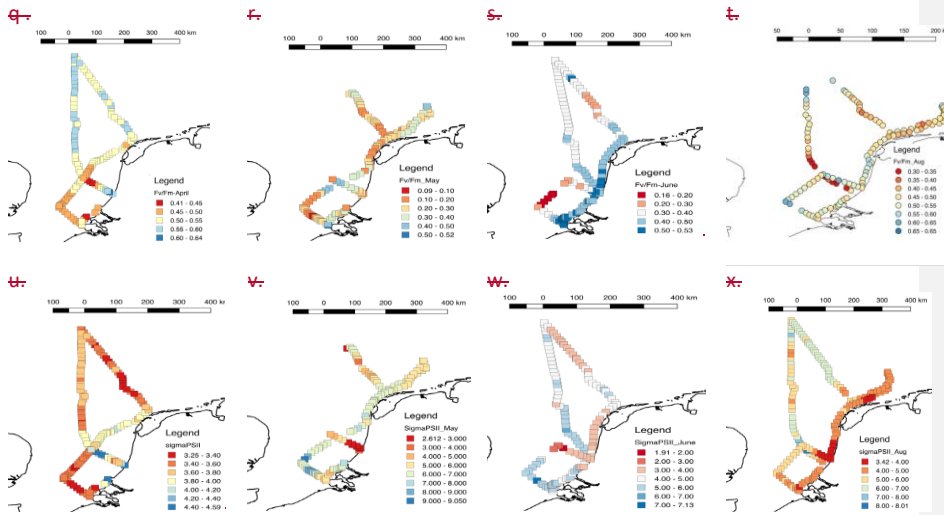
Figure 4: Phytoplankton biomass using FCM-derived total red fluorescence (first row; a-d), O/R ratio (second row, e-h), percentage pico (third row, i-l) and percentage nano (fourth row, m-p), F_v/F_m (fifth row, q-t) and σ_{PSII} (sixth row, u-x) in April 2017 (left panels), May 2017 (middle left panels), June 2017 (middle right panels) and August 2017 (right panels). Please note the different scaling, which was necessary to optimally visualize spatial heterogeneity.

June showed an increase in phytoplankton abundance, although the community changed toward less nanoplankton (Fig. 3). Highest biomass concentrations were found along the coast, along with highest nanophytoplankton proportional presence (Fig. 4, middle-right panels). Highest abundance of picophytoplankton was observed more offshore, although near the Dogger Bank there was a decrease in picophytoplankton abundance. The total O/R ratio showed an increased abundance of Orange fluorescent dominating species in most of the offshore waters between 50 and 150 km, and a decrease in relative abundance near the Doggersbank area (Fig. 4, middle-right panels). Microphytoplankton abundance was less than 8%, yet they represented the largest contributors to red fluorescence (Fig. 3). They show a somewhat patchy distribution along the coast and near the Dogger Bank. The F_v/F_m increased in comparison to May in the coastal region, but not in offshore regions in the Southern North Sea (Fig. 4s).

In August the increase in phytoplankton abundance observed in front of the Dutch Delta in June was still visible, but more northerly, the biomass seemed to decrease again (Fig. 4e). Unfortunately no data are available for July to check whether this community displaced northward over time. The picophytoplankton was present at highest abundance (> 80%)with an average contribution to total cell numbers of over 80% and only slightly lower values were observed (but still > 70%) along the southern Dutch coast, where the abundance of nanophytoplanktonnano-phytoplankton was higher. MicrophytoplanktonMicro-phytoplankton was hardly observed, only a small patch (2-3.5%) was observed near the coast of the province of North Holland. The effective absorption cross section was low in the coastal zone in comparison with offshore regions (Fig. 4x).although their high per cell red fluorescence, they contributed to up to half of the total red fluorescence in coastal regions.

3.4 ~~Spatial clustering~~Photophysiology

In April, the photophysiology of the phytoplankton communities in the Dutch North Sea showed low variability. The F_v/F_m values stayed above 0.5 in northern regions and above 0.4 in southern regions (Fig. 4a). The σ_{PSII} stayed in a narrow range between 2.5-4 nm^2 PSII^{-1} (Fig. 4e). The E_k in April showed more variability in comparison to the F_v/F_m and σ_{PSII}. In the coastal zone, the E_k was lower off the coast from Walcheren and higher off the coast from Noordwijk. In offshore regions, no clear spatial patterns were present (Fig. 4i). In May photophysiological parameters of the phytoplankton communities in the Dutch North Sea were strongly heterogeneous with only smaller scale spatial patterns (Fig. 4b,f,j). F_v/F_m was in general lower in May (0.1-0.5) than in April (>0.4) across most of the Dutch EEZ (Fig. 4b). In May the σ_{PSII} was high (average 5.9 nm^2 PSII^{-1}) across the Dutch North Sea, except near the coast of Noordwijk (Fig. 4f). ~~Spatial clustering (uHMM) of photophysiological characteristics and phytoplankton community composition was performed to get an overview of spatial heterogeneity and the variability over the season (Fig. 5). Colinear variables were removed based on the variable inflation factors (VIF>6), which resulted in removal of the photophysiological parameters P~~$_{max}$~~, F~~$_v$~~/F~~$_m$~~, a~~$_{LHII}$~~, n~~$_{PSII}$~~, and the FCM-parameter of the total red fluorescence. The five defined phytoplankton groups (Table 2) had a higher VIF than 6 (maximum 9.6), but were retained for the sake of completeness. The remaining variables were the five FCM-defined phytoplankton groups (Table 2), the total O/R ratio and five photophysiological parameters (1/τ, [RCII], σ~~$_{PSII}$~~, α and E~~$_k$~~). PCA analysis was performed to get an overview of the variables that explained most of the variation of the identified spatial clusters and to understand main drivers per region. The first two components of the PCA analyses explained 49.2% (June) to 59.7% (August) of the variance.~~

~~UHMM analysis resulted in identification of two to four spatial clusters, which were not uniform over the months. In April the most distinct phytoplankton community is found in the Northern part of the Dutch North Sea (Fig. 5). The biomass concentration in this region is low and the phytoplankton community characterized by a high O/R ratio and high photosynthetic affinity (α). In April the whole coastal region is identified as the same spatial cluster, with high biomass concentration and a variable phytoplankton community. A small region offshore (~ 70 km) in the Southern North Sea has a very uniform phytoplankton community, which seems mainly consisiting of microphytoplankton with a low light saturation level (E~~$_k$~~) and low effective absorption cross section (σ~~$_{PSII}$~~). In May and June, the phytoplankton community in the Dutch North Sea seems quite uniform with only two distinct spatial clusters. In June these clusters are clearly divided in a coastal and an offshore zone, while in May the clusters are spatially less well defined. The uniform phytoplankton community found in April in a small region offshore (~ 70 km) in the Southern North Sea remains present but in May a similar community is found in the eastern coastal region. Unfortunately, this region was not sampled in April. This spatial cluster is still very uniform, but not with distinct drivers in comparison with the other spatial cluster. It seems mainly typified by a high effective absorption cross section and low biomass and low O/R ratio. In June a distinct separtion between coastal and offshore phytoplankton communities is present. The offshore phytoplankton community is consisting of a diverse phytoplankton community while the coastal phytoplankton community is consisting of mainly micro phytoplankton with low light saturation level and high photosynthetic affinity (α).~~

~~August was the most heterogeneous month with four different phytoplankton communities (Fig. 5). The first phytoplankton community, which covers the most Northern most part of the Dutch North~~

Figure 5: Maps separated per month of spatial clusters as defined by uHMM clustering (left) and a bi-plot of the PCA of the data (right) with as variables the FCM-based parameters O/R ratio and the total red fluorescence of the five described phytoplankton groups (Table 2) and non-colinear FRRf-parameters on photophysiology (1/τ, [RCII], σ$_{PSII}$, α, E$_k$). In the bi-plot of the PCA colors represent assigned spatial clusters with confidence elipses (confidence 95%). Overlapping conficence elipses suggest a high similarity between groups while the size of the elipse is a measure of variability within the group.

In April the uHMM did not visualize the phytoplankton community with distinct O/R ratio north of Terschelling (Fig. 4e). Manual increase of the number of states in the spectral classification to four did not result in a different spatial cluster at the aforementioned location (Fig. 6), but instead split up the coastal spatial cluster with a distinct community off the coast of Noordwijk and a small patch to the north-west. Forcing another spatial cluster did result in a visualisation of the community with high O/R ratio north of Terschelling (Fig. 6), but cluster this region with a more northern part of the transect and the coastal region of Noordwijk These regions do also show a higher O/R ratio in the spatial map (Fig. 4e).

PCA - low resolution data

In the same region, the E_k was high (> 450 µmol photons m^{-2} s^{-1}), but this concurrent signal (high E_k, low σ_PSII) did not occur in other regions of the Dutch North Sea. The E_k across the Dutch North Sea in May was heterogeneous without large-scale spatial patterns. In June the spatial patterns in the photophysiology of the phytoplankton in the Dutch North Sea were less heterogeneous and larger mesoscale spatial patterns could be

identified. The F_v/F_m values recovered in comparison to May to above 0.4 in the coastal zone, but not in offshore regions in the Southern North Sea. The F_v/F_m of the southern offshore
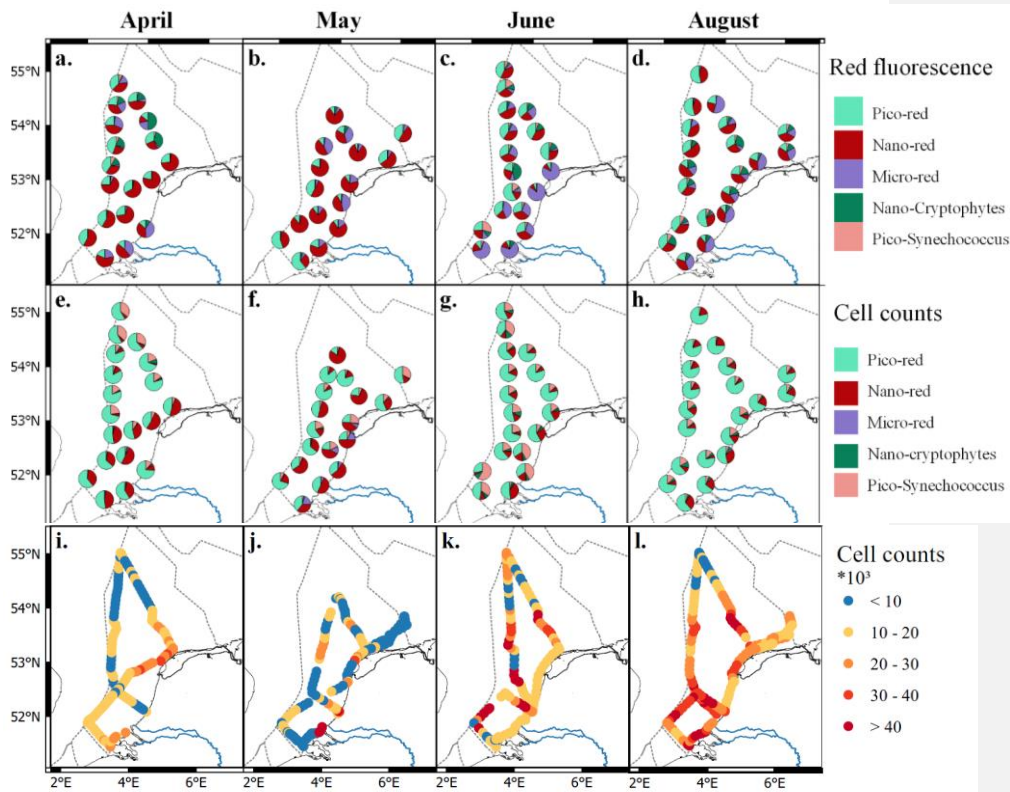
Figure 3: Relative phytoplankton community composition using FCM-derived total red fluorescence (first row; a-d) and cell numbers (second row, e-h) in April, May, June and August (from left to right). The groups are clustered according to table 2.

phytoplankton, between Walcheren 70 and Noordwijk 70 (Fig. 1), remained low (<0.2; Fig. 4c). The σ_{PSII} was lower in comparison to May across the Dutch North Sea, apart from the southern offshore region (Fig. 4g). In a small region around Noordwijk 70, the phytoplankton community had a particularly low σ_{PSII} (<2.5 nm^2 PSII^{-1}) which did not present itself in anomalies in the other photophysiological parameters. The E_k in June was low in the Northern coastal zone and higher in offshore regions (Fig. 4k). In August the F_v/F_m recovered across the Dutch North Sea (Fig. 4d). The σ_{PSII} was high in the northern offshore region, and comparable to June in the rest of the Dutch North Sea (Fig. 4h). In August the regions of the Noordwijk coast and the of the Wadden Island coast were sampled twice, on two different times of the day. This repeated measurement resulted in a higher E_k, suggesting diurnal variability. To further investigate daily patterns standardized daily anomalies (z-scores) were calculated. These show a clear diurnal trend in photosynthetic activity (Fig. 5). The F_v/F_m was lowest during the middle of the day, while Ek, $\sigma_{PSII,}$ and 1/τ peaked during the middle of the

day. As E_k was strongly correlated to P_{max} (Fig. S2); a clear diurnal pattern was also present in the photosynthetic electron transport rate.
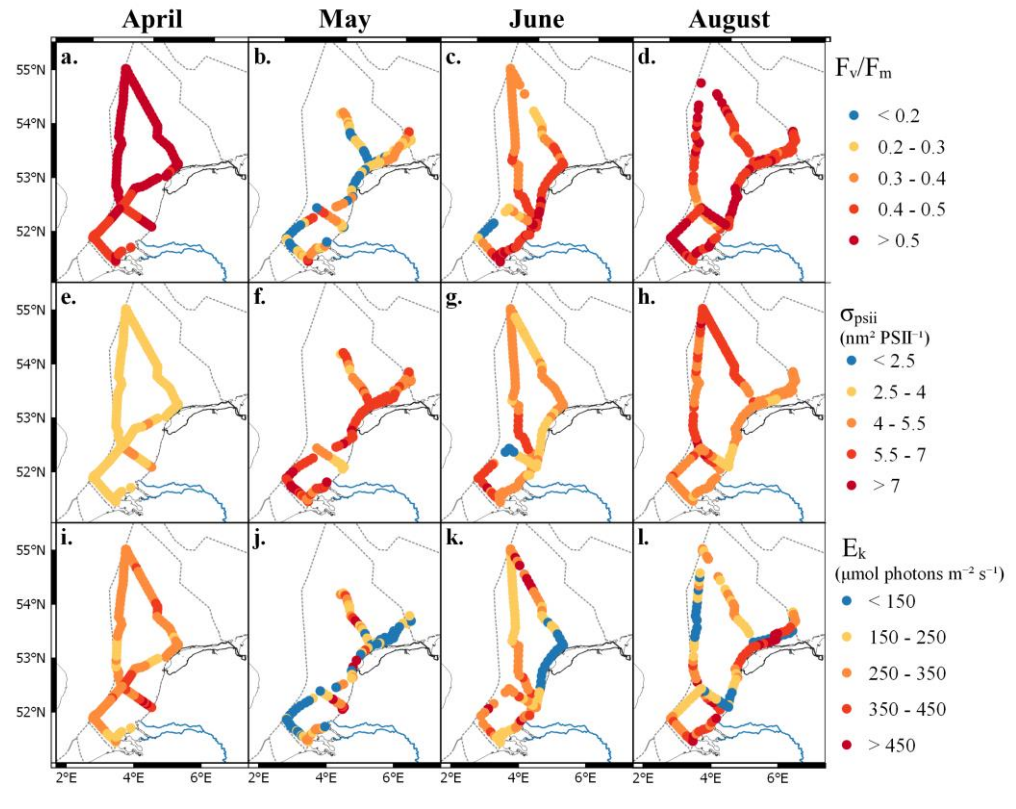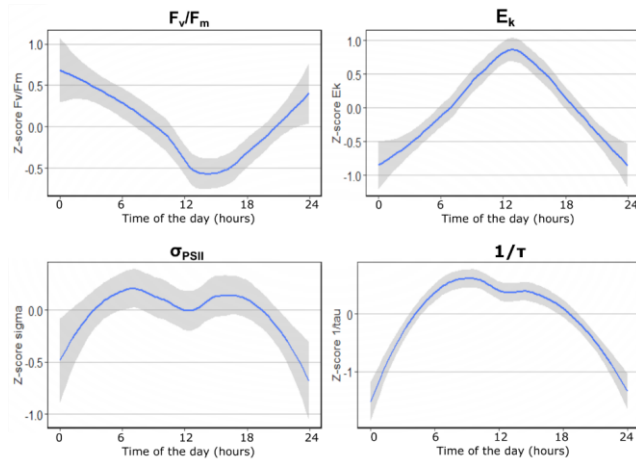
Figure 5: Standardized daily anomalies (z-scores) of F_v/F_m, E_k, σ_{PSII} and $1/\tau$ showing the diurnal trends in photophysiological data. On the x-axis the time of the day and on the y-axis the z-score.

3.5 Gross primary productivity

Gross primary productivity ranged from 0.35 µg C L^{-1} h^{-1} in June to 602 µg C L^{-1} h^{-1} in the coastal zone in May (Fig. 6). The average GPP was highest in April and lowest in August. Monthly averages ranged from 116 ± 59 µg C L^{-1} h^{-1} in April and 8.7 ± 8.3 µg C L^{-1} h^{-1} in August, although these averages are not completely comparable due to different ship routes per month (Fig. ~~4)~~.

~~Figure 7 shows the spatial heterogeneity of gross primary productivity per month. April corresponds to the month with the highest biomass concentration and higher nutrient concentration in comparison to the other months, resulting in higher GPP. In the coastal zone nutrients concentrations and turbidity are higher due to river water influx, but GPP in this region is lower. In April, phytoplankton populations were not nutrient limited in most areas which makes light availability a better predictor for primary productivity. In April offshore water column productivity shows quite some spatial variability and it is higher in comparison to the coastal zone, likely due to a lower light attenuation in the offshore water column, while in the other months the opposite appears, confirming this hypothesis. GPP is highest offshore (> 800 mg C m⁻²~~6). In April, spatial heterogeneity in GPP was low. Highest rates in April were measured offshore (> 250 µg C L^{-1} h^{-1}) ~~along the 70 km line to west of Den Helder~~ and ~~high near~~in the coastal regions close to the Wadden ~~Sea.~~Islands (Terschelling 10 in Fig. 1). In May, the GPP was heterogeneous without a clear spatial ~~variability is limited with some very local high GPP values (> 600 mg C m⁻² h⁻¹), but most values show~~pattern. Most production rates stayed below ~~213 mg C m⁻²~~30 µg C L^{-1} h^{-1}, with local GPP peak rates over 600 µg C L^{-1} h^{-1}.in the southern coastal zone. In June ~~the Dutch North Sea showed slightly more spatial variability, but~~, the GPP was on average lower than in May. ~~and showed more large-scale spatial patterning.~~ Highest values in June were observed (~~300-400 mg~~30-40 µg C ~~m⁻²~~L^{-1} h^{-1}) northwest of Noordwijk~~, where in April the values were low. In August, a similar spatial distribution with low GPP is visible as in June~~. In August, GPP was low throughout the Dutch North Sea with the

majority of ~~values~~productivity rates staying below ~~100 mg~~10 µg C $m^{-2}$$L^{-1}$ h^{-1}. ~~Yet,~~In the ~~GPP~~southern coastal zone, slightly higher rates ~~on the Terschelling transect~~ were ~~about twice as high as in June.~~
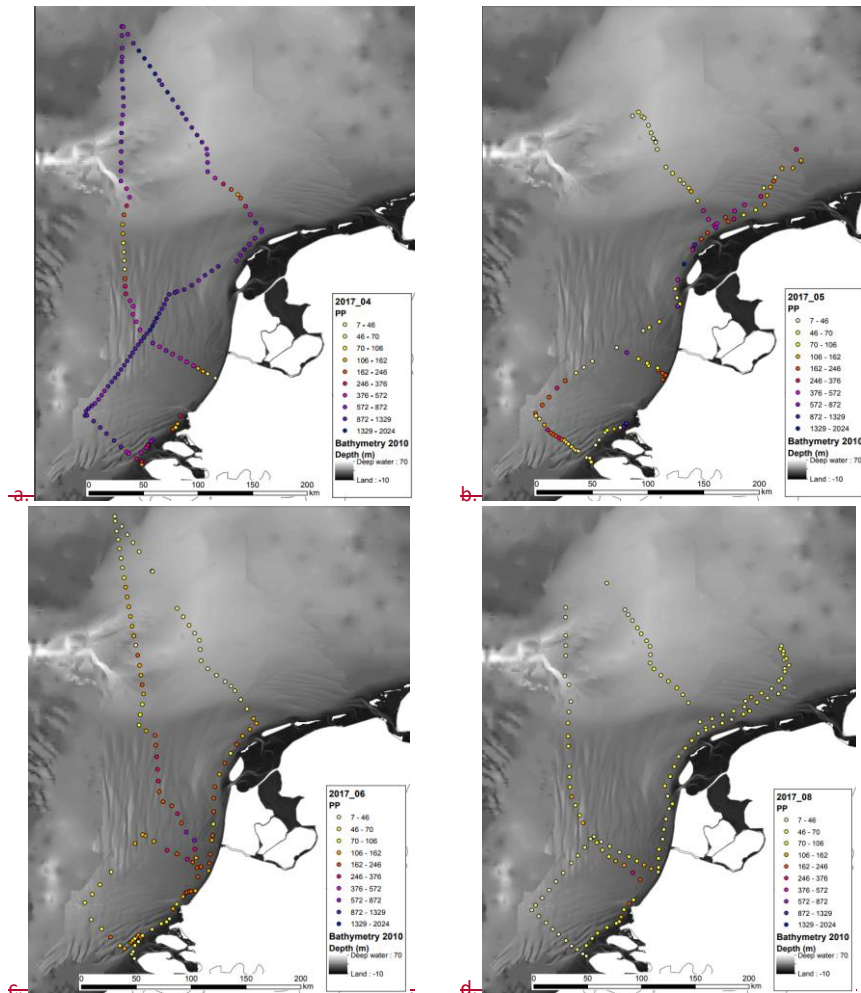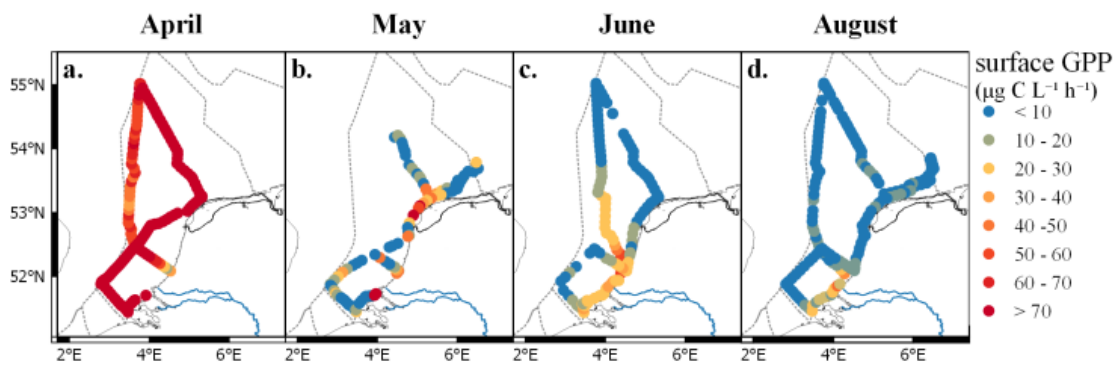
~~Figure 7: Water column integrated gross primary productivity (mg~~found, reaching up to 50 µg C $m^{-2}$$L^{-1}$ h^{-1}~~) per month (a, April, b. May, c. June, d. August).~~

Figure 6: Gross primary productivity of the surface (a-d; in µg C L^{-1} h^1) per month (from left to right: April, May, June and August). Colors represent rates, where blue is low and red is high (see legend).

3.5 Spatial clustering

Strong collinearity between measured parameters was present. For spatial clustering these were removed based on the variable inflation factor (VIF>6; see supplementary material for pairplots), which resulted in the removal of the photophysiological parameters P_{max}, $α$, a_{LHII}, n_{PSII}, the FCM-parameter of the total red fluorescence and the GPP. From the five defined phytoplankton groups (Table 2), the nano-crypto group was not used in the clustering because of collinearity (VIF>6). The remaining variables were the abundance of the remaining four FCM-defined phytoplankton groups (Pico-Red, Pico-Synecho, Nano-Red, and Micro-Red), the total O/R ratio and five photophysiological parameters (F_v/F_m, $σ_{PSII}$, $1/τ$, [RCII], and E_k). For an overview of the collinearity between variables, see the pairplots in the supplementary material.

Spectral cluster analysis resulted in the identification of two to four clusters in each cruise. Most of these clusters were spatially separated and therefore, can be considered as regions with distinct phytoplankton communities (Fig. 7). In April, the clustering resulted in three clusters with a clear spatial pattern. In the PCA, the variables that contributed most to the first principal component were all biomass related; [RCII] and a_{LHII}, related to the photosynthetic capacity per reaction center and per volume, and the abundance of the Nano-red group. The second principal component has photosynthetic parameters as two main contributors
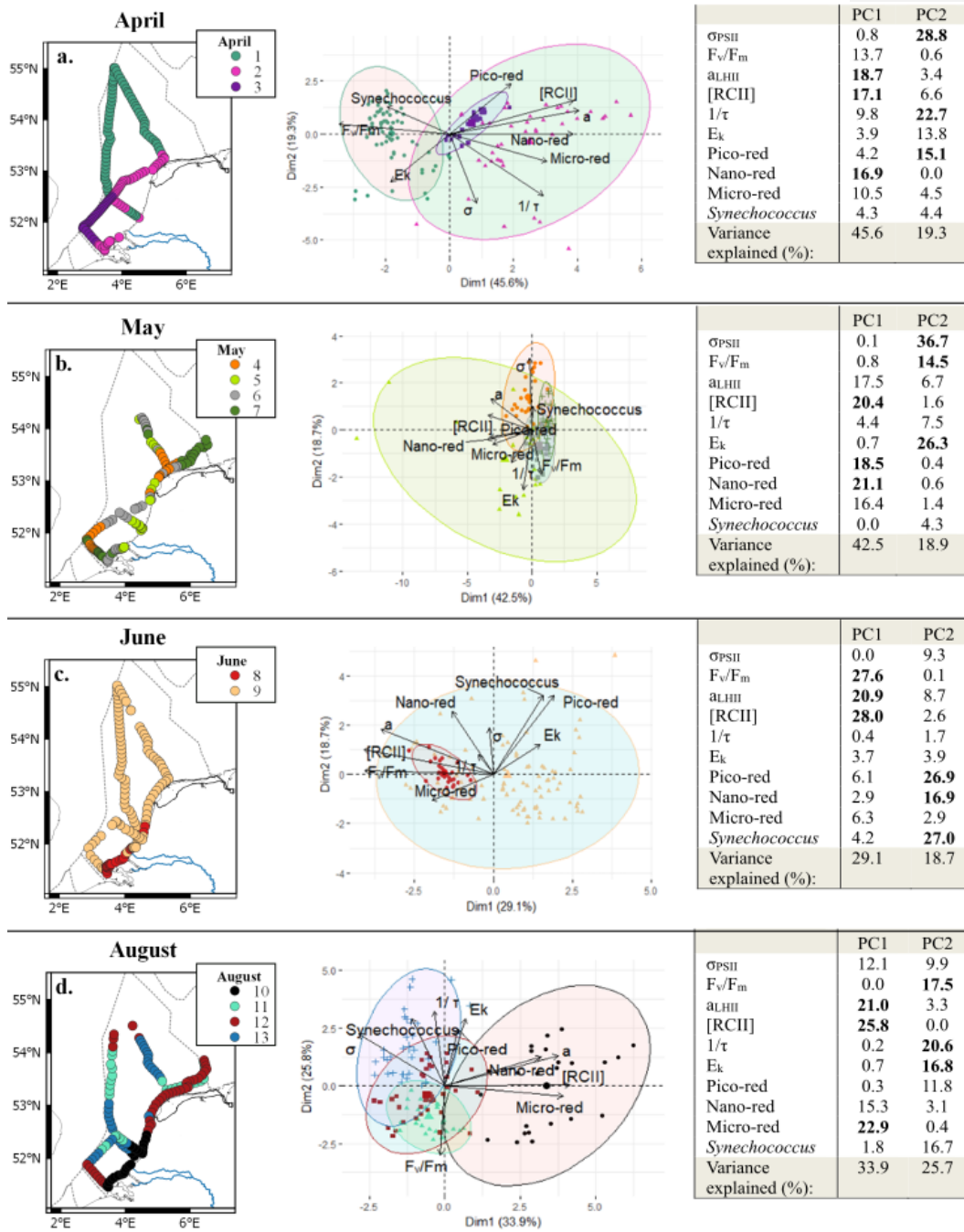
Figure 7 tables:

April

	PC1	PC2
σ_{PSII}	0.8	**28.8**
F_v/F_m	13.7	0.6
a_{LHII}	**18.7**	3.4
[RCII]	**17.1**	6.6
$1/\tau$	9.8	**22.7**
E_k	3.9	13.8
Pico-red	4.2	**15.1**
Nano-red	**16.9**	0.0
Micro-red	10.5	4.5
Synechococcus	4.3	4.4
Variance explained (%):	45.6	19.3

May

	PC1	PC2
σ_{PSII}	0.1	**36.7**
F_v/F_m	0.8	**14.5**
a_{LHII}	17.5	6.7
[RCII]	**20.4**	1.6
$1/\tau$	4.4	7.5
E_k	0.7	**26.3**
Pico-red	**18.5**	0.4
Nano-red	**21.1**	0.6
Micro-red	16.4	1.4
Synechococcus	0.0	4.3
Variance explained (%):	42.5	18.9

June

	PC1	PC2
σ_{PSII}	0.0	9.3
F_v/F_m	**27.6**	0.1
a_{LHII}	**20.9**	8.7
[RCII]	**28.0**	2.6
$1/\tau$	0.4	1.7
E_k	3.7	3.9
Pico-red	6.1	**26.9**
Nano-red	2.9	**16.9**
Micro-red	6.3	2.9
Synechococcus	4.2	**27.0**
Variance explained (%):	29.1	18.7

August

	PC1	PC2
σ_{PSII}	12.1	9.9
F_v/F_m	0.0	**17.5**
a_{LHII}	**21.0**	3.3
[RCII]	**25.8**	0.0
$1/\tau$	0.2	**20.6**
E_k	0.7	**16.8**
Pico-red	0.3	11.8
Nano-red	15.3	3.1
Micro-red	**22.9**	0.4
Synechococcus	1.8	16.7
Variance explained (%):	33.9	25.7

Figure 7: Overview of the spectral cluster analysis based on the non-collinear phytoplankton parameters (FCM: Pico-red, Nano-red, Micro-red, *Synechococcus*. FRRf: σ_{PSII}, F_v/F_m, a_{LHII}, $1/\tau$, E_k) separated per month (top to bottom: April, May,

June and August). With on the left clusters visualized on maps and in the middle the bi-plots of the PCA of the data with confidence ellipses per cluster (confidence 95%). In all graphics clusters are visualized by different colors as shown in the legend inset. Of the confidence ellipses the border lines (and not the fill) correspond to the clusters. In the bi-plot overlapping confidence ellipses suggest a high similarity between groups while the size of the ellipse is a measure of variability within the group. On the right the table of the PCA analysis with contribution in % of the different variables, in bold the three variables that contribute most to the principal component.

(σ_{PSII} and $1/\tau$; 51.5%). Cluster one covered most of the Northern part of the Dutch North Sea, and a small part of the Noordwijk transect to the coast. The bi-plot of the PCA showed that the first cluster was negatively correlated to the main contributors of PC1 ([RCII] and a_{LHII}; Fig. 7), so this region consisted of a phytoplankton community with lower photosynthetic capacity per liter. The coastal region was separated into two clusters, 2 and 3, with overlapping confidences ellipses (Fig. 7).

A stepwise multiple linear regression (n=61) for all months combined revealed that significant interactions included photophysiology (α), phytoplankton biomass (T) and abiotic predictors (Turbidity, DIN, time of the day and temperature). A log transformation of the GPP was necessary to correct for the heteroscedasticity of the data. Colinear predictors were removed before analysis (VIF>6), which included F_v/F_m (collinear with alpha), E_k (collinear with P_{max}), n_{PSII} (collinear with σ_{PSII}), [RCII] and a_{LHII} (colinear with total red fluorescence). Statistically non-significant predictors included the percentage of micro or picoplankton, maximum rate of photosynthesis, silicate and phosphate concentration, effective absorption cross section, salinity, the mean O/R ratio, and spatial predictors (latitude, longitude). Significant abiotic predictors included the quadratic of the time of the day (hours), turbidity, temperature and DIN (Table 4). Surprisingly, temperature and DIN concentrations are negatively correlated with the GPP, which could be a biased effect of not separating different months.

As shown above, variability in the data is related to the month (i.e. seasonal patterns) of sampling and the area sampled (i.e. to different abiotic and biotic factors). However, physiological activity can also be influenced by diurnal patterns, and we therefor investigated if our data show might be influenced by influenced diurnal influenced patterns in photosynthetic activity. This was done by calculating the z scores per day and plot these as a function of the diurnal time. The results show clear diurnal trends in photosynthetic activity (Fig. 9). P_{max}, E_k, σ_{PSII} and $1/\tau$ are all higher during the day than at night, while F_v/F_m and α are lowest in the early afternoon (Fig. 9).
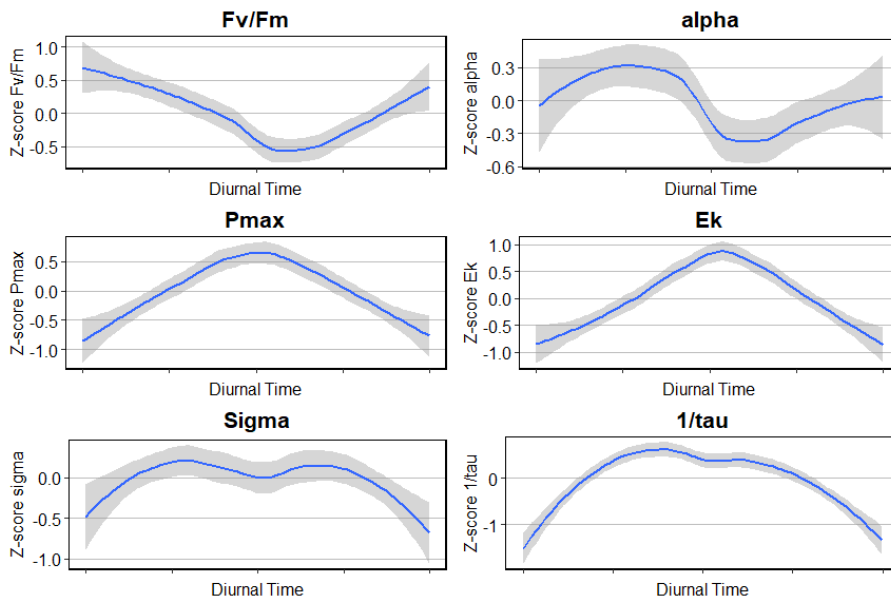
The confidence interval of cluster 2 is larger than cluster 3, suggesting that the phytoplankton community in cluster 2 was more heterogeneous. Both clusters are positively correlated to the main contributors to PC1 ([RCII] and a_{LHII}), meaning these clusters consists of a community with higher photosynthetic capacity per volume. In May, the cluster analysis resulted in four different clusters, but without a well-defined spatial pattern. The PCA biplots showed that the confidence interval of cluster 5 overlaps most of the other clusters, indicating that this cluster has weak support. E_k was negatively correlated with cluster 4 and σ_{PSII}, suggesting that cluster 4 contained low light acclimated algae. In contrast, in June only two clusters with a distinct separation between coastal and offshore phytoplankton communities were found. The PCA showed that the offshore phytoplankton community was consisting of a diverse phytoplankton community while the coastal phytoplankton community with high F_v/F_m, a_{LHII} and [RCII]. In August not all clusters were spatially separated (Fig. 7). Different clusters were appointed to the same region visited within a two-day time span twice; in the north-eastern coastal region and at the transect of Noordwijk. Both times, cluster 11 is one of the overlapping spatial clusters. Cluster 11 corresponds to only night-time sampling periods and was defined by low E_k and low $1/\tau$, indicative of a low light acclimated phytoplankton community. This suggests that cluster 11 was a temporal cluster instead of a spatial cluster. To test this we repeated the analysis for the month of August but only including the measurements performed within an 8-hour timeframe around noon (12:00±4h; see supplementary material Fig. S4). In this timeframe, the southern coastal zone was distinct from the rest of the Dutch North Sea and corresponded to cluster 10 in the analysis of the complete dataset (Fig. 7d), so this cluster was defined by spatial variability. Cluster 12 and 13 were grouped together in the 12±4h timeframe as cluster 1. Cluster 11 was not

recognized as a cluster within the 12±4h timeframe, so seemed indeed controlled by temporal rather than spatial variability.

4 Discussion

This The objective of this study examineswas to evaluate the use of added value of FRR fluorometry and flowcytometry for monitoring purposes. During four cruises spread over 5 months, a wide variety of environmental conditions and phytoplankton community states were sampled. Here, this data is used to evaluate the potential of this approach to be developed as a novel method to improve existing monitoring (OSPAR, MSFD).

Biomass is an important parameter to understand the role of phytoplankton in the ecosystem and biogeochemical cycles. Its direct measurement using high-resolution methods is challenging. Chlorophyll *a* concentration is often used as an estimate for biomass, although the Carbon:Chl *a* ratio is dependent on abiotic conditions and species-specific phenotypic plasticity and chlorophyll *a* is therefore not directly related to biomass (Flynn, 1991, 2005; Geider et al., 1997; Alvarez-Fernandez and Riegman, 2014; Halsey and Jones, 2015). In this study, chlorophyll concentrations are estimated by red fluorescence, which results in a good fit for both the FRRf (adjusted R^2=0.66) and the FCM (adjusted R^2=0.90). The impact of abiotic conditions on fluorescence as a predictor for chlorophyll *a* content was tested by comparing the relationship in the different months. Only the flowcytometer data were significantly affected by environmental conditions. The different environmental conditions per month did not affect the regression line of the FRRF data. Since the two instruments differ in optics as well as measurement set-up (measurements to supplement low-resolution monitoring. . Multiple per cell vs bulk), differences are not surprising. The different measurement set-up, with the flowcytometer measuring the fluorescence per particle, while the FRRf does a measurement of the bulk sample, might blur the effect of environmental conditions. In a bulk measurement, other particles in solution scatter the excitation and emission photons, plus the emitted fluorescence of the phytoplankton is subject to reabsorption, especially at higher biomass densities. Yet, the difference most affected by environmental conditions is the fluorescent state of the photosystems. The strong laser of the flowcytometer can only measure the maximum fluorescence (F_m), which is a parameter more prone to quenching than the minimum fluorescence measured by the FRRf. The lower sensitivity to environmental conditions implies that the FRRf is better suited to estimate chlorophyll *a* concentration in comparison to the FCM. Other studies that estimate chlorophyll *a* concentrations with FCM and fluorimeter also find better fits using the bulk measurements by a fluorimeter in comparison to the per cell flowcytometric measurements (Thyssen et al., 2015; Marrec et al., 2018). An alternative to the controversial use of chlorophyll *a* as an estimate for biomass is the derivation of biomass or biovolume from cell counts. This requires assumptions on cell size, cell shape and carbon content per biovolume (Tarran et al., 2006). Another alternative is to derive biovolume from scattering properties of the cell using a pulse shape recording flowcytometer as used in this study. This relationship appears to be taxon-specific (Rijkeboer, pers. comm.) and needs to be further explored by comparison of calculated biovolume

(based on the Image in Flow pictures) and the flowcytometric properties of the cell. The FRRf offers the possibility to circumvent the use of phytoplankton biomass as a necessary parameter to estimate primary productivity altogether by estimating the amount of Photosystem II Reaction Centers or total absorption by the PSII concentration (i.e. a_{LHII}; Oxborough et al., 2012). As long as there is no uncontroversial method to derive phytoplankton biomass, calculation of multiple parameters ~~with high~~ and critical evaluation remains necessary.

The FCM was able to visualize the spatial ~~resolution provide an overview of current conditions. Spatial clustering may serve as an example of how to use multiple high-resolution parameters in a monitoring program. Lastly, the water column integrated gross primary productivity of~~ variability of the phytoplankton community in the Dutch North Sea. The typical spring bloom was partly captured during the cruise of April, with high total fluorescence and high relative abundance of micro- and nanophytoplankton in comparison to other months. In contrast, in August the community was dominated by picophytoplankton with only sporadic observations of microphytoplankton. In addition, spatial variability in size distribution was clearly visible as a stronger presence of microphytoplankton in coastal regions than offshore. Microphytoplankton are a better food source for higher trophic levels than picophytoplankton. Picophytoplankton is part of the microbial food web, with less trophic efficiency and low contribution to carbon export (Azam et al., 1983; Finkel et al., 2010). The shift from nanophytoplankton-dominated communities in April to picophytoplankton-dominated communities in August therefore implicates that over the year the tropic efficiency and carbon export decrease. These spatial and temporal changes are a yearly phenomenon, influenced by the strong seasonal dynamics in the Dutch North Sea that affects the spatial distribution and community composition of the phytoplankton community (Baretta-Bekker et al., 2009; Brandsma et al., 2011). ~~is estimated and its main forcing factors are identified.~~ It is important to monitor interannual variability over the years to monitor changes in biogeochemical cycles and the carrying capacity of the ecosystem. To increase the informational value of the flowcytometry data beyond size, the FCM clusters would need to reflect taxonomic or functionally relevant groups. Interesting groups include calcifiers, silicifiers, DMS producers (such as *Phaeocystis*) or nitrogen fixers (le Quéré et al., 2005). The lack of identification of distinct clusters makes this so far impossible, although some species are recognizable as *Phaeocystis* sp. (Rijkeboer, unpublished). Marrec et al. (2018) manually separated up to 10 phytoplankton groups from the data of the Cytosense flowcytometer. Yet, most of these groups comprise many taxonomic genera and, apart from the size or pigment composition, hindering further interpretation of their role in the ecosystem or biogeochemical cycles. However, the distinction between different pigment groups can provide useful information on food web functioning. Chlorophyll-*c* containing algae (Chromista) contain long-chained essential fatty acids like docosahexaenoic acid (DHA) and eicosapenthic acid (EPA) which are lacking in green algae (some Prasinophycaea excepted) or cyanobacteria (Dijkman and Kromkamp, 2006). Thus, information about food quality can be obtained from FCM. For detection of nuisance phytoplankton, distinct clusters are lacking. Yet, toxicity in phytoplankton can differ even between strains within one species, so finding a distinct cluster by flowcytometry is challenging (Tillman and Rick, 2003). However, the identification of 'suspicious' clusters with potential toxic species could already be helpful. These suspicious clusters can flag sampling points to be further inspected by a specialist using microscopy.

Monitoring of the photophysiology of the phytoplankton by FRR fluorometry can supplement the flowcytometry measurements. For instance, the hypothesized spring bloom detected by the

flowcytometer in April is confirmed by photophysiological parameters; photophysiology was uniform and primary productivity high. Between April and May, the efficiency of PSII (F_v/F_m; Fig. 4) decreased throughout the Dutch North Sea. A decreasing F_v/F_m is generally associated with limiting nutrient conditions or other abiotic, but can also reflect a change in community composition stressors (Suggett et al., 2009b; Kolber et al. 1988; Kolber and Falkowski, 1993; Beardall et al. 2001; Ly et al. 2014). Photophysiological parameters vary per taxonomic group; smaller taxa typically have lower F_v/F_m values and higher σ_{PSII} values (Kolber et al., 1988; Suggett et al., 2009b). No major shift in community composition was identified by flowcytometry between April and May. This suggests that an abiotic stressor, such as the nutrient limiting conditions in a large part of the Dutch North Sea, instead of the community composition was driving the decrease in efficiency of PSII. In contrast, the recovery of the F_v/F_m between May and June did coincide with a shift in community composition. In May the phytoplankton communities were mostly nanophytoplankton-dominated, while in June the communities were dominated by picophytoplankton (offshore) and microphytoplankton (coastal). So, although recovery of the F_v/F_m can also occur as an adaptation of the phytoplankton to nutrient limiting conditions (Kruskopf and Flynn, 2005), it seems that the shift in community composition was the major driver for the recovery of the F_v/F_m between May and June. These findings are a good example of how concurrent measurements by flowcytometry and Fast Repetition Rate fluorometry can supplementary improve ecosystem understanding. When including photophysiology (or photophysiology based GPP estimates) in a monitoring program, it is critical to consider methodological constraints (Hughes et al., 2018). For instance at low phytoplankton abundance, the fluorescence signal becomes too noisy for calculation of parameters. Moreover, blank correction is essential for retrieving accurate FRRf data (Cullen and Davis, 2003). FRRf measurements are affected by the interference of colored dissolved matter which can lead to under or overestimation of some parameters (like F_v/F_m; Cullen and Davis, 2003). The blank correction is a manual measurement and should be done regularly and at least when abiotic conditions change (Hughes et al., 2018). For monitoring purposes, it is important to take into account diurnal variability. Diurnal trends make extrapolation to daily rates challenging. Most photophysiological parameters we measured showed diurnal trends (Fig. 5). The diurnal trend is dictated by the phytoplankton cell cycle, a circadian oscillator and photophysiological response to varying irradiance (Suzuki and Johnson, 2001; Cohen and Golden, 2015; Schuback et al., 2016). Phytoplankton use photophysiological plasticity to minimize photodamage and optimize growth under fluctuating irradiance (Schuback et al., 2016; Behrenfeld et al., 2002). The electron requirement for carbon fixation is also subject to diurnal variation (Schuback et al., 2016; Lawrenz et al., 2013; Raateoja, 2004). To interpret spatial variability separately from temporal variability and to provide a more reliable estimate of gross primary productivity, Schuback et al. (2016) suggest a correction with normalized Stern-Volmer quenching (NPQ_{NSV}). This approach needs further research, for example by using a Lagrangian approach where the photosynthetic activity of the same population is followed during the day. Until a reliable correction method has been established, a monitoring program including photophysiology should account for diurnal variability, for instance by using only measurements collected in a certain timeframe or from buoys. Despite the limitations of GPP estimates by variable fluorescence, our results clearly show large spatial variability in gross primary production that is not explained by diurnal variability. This spatial heterogeneity is not fully captured by sampling at the standard low-resolution monitoring stations, showing the added value of our approach.

Phytoplankton biomass does not necessarily reflect primary productivity, as high grazing pressure can keep biomass low while production is high. This is clearly visualized by the lack of resemblance between patterns in cell numbers (Fig. 3 a-d) and gross primary productivity (Fig. 6). Gross primary

productivity estimates by FRR fluorometry are based on measurements of the first step of photosynthesis; the efficiency at which photons are captured and electrons transferred. However, to interpret gross primary productivity in an ecological or biogeochemical meaningful way, the FRR units of electrons per unit time need to be converted to carbon units. In general, gross photosynthesis correlates well with photosynthetic oxygen evolution (Suggett et al., 2003), and multiple studies have shown a good correlation between 14C-derived estimates of primary productivity and FRRf-derived estimates using a constant conversion factor (Melrose et al., 2006; Kromkamp et al., 2008). ~~Environmental conditions in the Dutch North Sea are spatially heterogeneous and strongly influenced by seasonal dynamics. The timing of the phytoplankton bloom period corresponds well to the study of Baretta-Bekker et al. (2009) on phytoplankton dynamics in the Dutch North Sea from 1991 to 2005. In April we covered a phytoplankton bloom period, typified by high biomass concentrations, high quantum efficiencies and electron transport rates of PSII. The May cruise covered the collapse of the phytoplankton bloom period, as shown by the lower average quantum efficiency of PSII and high variability in biomass concentrations (Table 3). The cruises in June and August covered a low Chl *a* period and a second late summer bloom period was not detected. Although a second bloom period is known to occur in some regions of the Dutch North Sea, an onset later than August is not unusual (Baretta-Bekker~~However, in reality, this parameter is not a constant, as along the pathway from electron to carbon atom electrons are consumed by other cell processes (Flameling and Kromkamp, 1998; Halsey and Jones, 2015; Schuback et al., 2016). As the cell processes from photon absorbance to carbon assimilation are known to vary with abiotic conditions, we expect that identification of biogeographic regions can aid in predicting regional $\Phi_{e,C}$ (Lawrenz et al., 2013). Calibration with other methods, such as concurrent C14 of C13 incubations, could help to better understand the processes from electron excitation to carbon fixation. However, these methods introduce other uncertainties; they measure a productivity in between net and gross primary productivity, depending on the incubation time and growth rate of the phytoplankton (Halsey and Jones, 2015). For now, a reliable GPP estimate in carbon units from FRR fluorometry requires more research and estimates provide relative rather than qualitative values. Despite its limitations, the ability to study life phytoplankton rates without long-term incubation effects, makes the method promising. Additionally, the high sampling resolution allows for identification of extra sampling points based on real-time projections, opening up early warning methodologies. For example, in the April cruise both Noordwijk 70 and Terschelling 235 km show high gross primary productivity, but in between both high and low productivity rates occur which are not detected with the current sampling program (Fig. 6). Extrapolation of surface measurements to water column estimates is required to assess the carrying capacity of the ecosystem and the contributions to biogeochemical cycles. Surface water measurements are only a good reflection of the water column when mixed layer depth is deeper than the euphotic zone. Stratification or mixed layer depth shallower than the euphotic zone can result in subsurface chlorophyll maximum layers and significantly different phytoplankton community (Latasa et al., 2017). Only frequent CTD casts equipped with PAR sensor can determine the vertical heterogeneity, mixed layer depth and the light extinction in the water column.

High-resolution methods such as the FRRf and the flowcytometer result in a multitude of parameters. Cluster methods can be helpful in bringing together these parameters for interpretation. The spectral clustering ~~et al., 2008). Generally, pico-autotrophs contributed considerably to cell numbers but covered only a small fraction of the total biomass (Fig. 3). As nutrient limitation progressed from April to August, the relative abundance of picoplankton reached over 80%, which corresponded to less than 30% of the relative fluorescence. In June and August the~~

molar nutrient N:P ratios were generally below the Redfield ratio and concentrations were in the limiting range, suggesting that phytoplankton populations were N-limited in a large part of the Dutch North Sea. This impacts the community composition: generally it is assumed that nutrient limitation favours small cell size, because of the higher surface to volume ratio of smaller cells, and that fluctuating nutrient concentrations favour larger cells due to their greater maximum uptake rate and storage capacity (Stolte and Riegman, 1995; Giannini and Ciotti, 2016; Philippart et al., 2000), and the shift towards smaller species observed by us using FCM is thus in accordance with this theory. The change in community composition over the season has implications for the whole ecosystem, because microphytoplankton is a better food source for higher trophic levels than picophytoplankton, which is more involved in the microbial food web, with less trophic efficiency and low contribution to carbon export (Quere et al., 2005). Nutrient limitation does not only affect community cell size but also low values of F_v/F_m are often related to nutrient limitation (Kolber et al. 1988, Kolber and Falkowski 1993, Beardall et al. 2001, Ly et al. 2014), although this is not always the case, and it seems likely that after acclimation to limiting nutrient conditions F_v/F_m can recover again as was seen in the current study in June (see also Kruskopf and Flynn, 2006). The σ_{PSII} was negatively associated with DIN and turbidity in the PCA on the low resolution data (Fig. 8), and although this value is assumed to vary per taxonomic group, it is not associated with any flowcytometer group (Kolber et al., 1988; Suggett et al., 2009), hence most of the variability seems to be driven by light and nutrient conditions. The values for the effective absorption cross section are slightly lower but in similar range to other studies (Suggett et al., 2009).

The gross primary productivity as found in the current study was both spatially and temporally variable. Average surface productivity of 44 ± 64 µg C L^{-1} h^{-1} and peak primary productivity in April and lower values the rest of the year is in agreement with earlier studies in the North Sea coastal zone (Brandsma et al., 2011). To interpret water column integrated primary productivity in an ecological or biogeochemical meaningful way, the FRR units of electrons per unit time were converted to carbon units. Gross photosynthesis correlates well with photosynthetic oxygen evolution (Suggett et al., 2003), and multiple studies have shown good correlation between ^{14}C-derived estimates of primary productivity and FRRf derived estimates (Melrose et al., 2006; Kromkamp et al., 2005, 2008). In this study the estimate of 6 moles electrons per mole carbon atom was used, based on a study in the same biogeographic region by Kromkamp et al. (in prep.). This a simplified assumption because the conversion from electron transport rate to gross primary productivity is complicated and depends on the consumption of electrons by other cell processes (Flameling and Kromkamp, 1998; Schuback et al., 2016). The conversion factor from electron flux to carbon fixation depends on biogeographic region and taxonomy, but is also subject to diurnal variation (Schuback et al., 2016; Lawrenz et al., 2012; Raateoja, 2004). As we passed several "biogeochemical" provinces, indicated by the cluster analysis, it is difficult to separate diurnal variability from variability introduced by phytoplankton in different biogeochemical areas. However, as we observed this diurnal variability also in the same clusters on a number of occasions, it is clear that diurnal variability is inherent in our analysis. For future studies it is advised to include Langragian based approach where the same phytoplankton community can be followed during a complete light-dark cycle. The diurnal trend in coupling of electron flux and carbon fixation is dictated by cell cycle, a circadian oscillator and irradiance, and photophysiological plasticity minimizes photodamage and optimizes growth under fluctuating light and nutrient concentrations (Claquin et al., 2014; Cohen and Golden, 2015; Schuback et al., 2016). To interpret spatial variability separately from temporal variability and to provide a reliable estimate of gross primary productivity, Schuback et al. (2016) suggest a correction with NPQ$_{NSV}$, which needs further research in order to get

a more reliable GPP value. The presence of non-photochemical quenching (NPQ) makes the interpretation of most photophysiological parameters complicated in our study because the lack of dark acclimation time decreases the comparability between samples. Most photophysiological parameters we measured showed diurnal trends, although, but as said, this is likely not only due to NPQ but also to phytoplankton cell cycle (Claquin et al., 2014; Schuback et al., 2016) and rhythms driven by a circadian oscillator (Cohen and Golden, 2015). But although the presence of NPQ compromises the use of fluorescence as estimate for chlorophyll concentrations, the good relationship between the HPLC derived Chl a concentration and fluorescence of the FRRf and FCM suggest that most of the NPQ is dissipated during the time in the tubing and low light acclimation. The clear diurnal trends we observed are in agreement with previous studies and is usually explained by photophysiological plasticity to minimize photodamage (Schuback et al., 2016; Behrenfeld et al., 2002). This makes interpreting spatial patterns difficult as temporal and spatial patterns occur simultaneously, yet, spatial patterns were generally more prominent than the diurnal oscillations.

Primary productivity is an important but difficult to estimate parameter. Its importance is evident, being at the base of the marine food web. In recent decades primary productivity in the North Sea seem to decline, with implications for the ecosystem structure and fisheries productivity. Capuzzo et al. (2017) and Cloern et al. (2014) see a global declining trend in primary production measurements. This is worrying as marine ecosystems face many changes and possible threats caused by global warming and increased use of marine resources by man. Remote sensing methods and models are used to estimate primary productivity, but despite improvements in satellite capabilities and ocean colour analyses, the current global annual NPP estimates are uncertain (Silsbe et al., 2016). One of the reasons is that for satellite estimates or modelling purposes variation in phytoplankton community composition or physiology are usually not included. Primary productivity is then estimated solely based on abiotic factors in combination with Chl *a* estimates (Cole and Cloern, 1987; Behrenfeld and Falkowski, 1997; Westberry et al., 2008; Westberry and Behrenfeld, 2013), although some models include P^B_{max} as parameters, which is parameterized from temperature only. Yet, Chl *a* and abiotic conditions alone are limited predictors of biological processes, because the Carbon:Chl *a* ratio is not only dependent on abiotic conditions but also to species-specific phenotypic plasticity needed to acclimate to those abiotic conditions (Flynn, 1991, 2005; Geider et al., 1997; Alvarez-Fernandez and Riegman, 2014) and Chl *a* is still difficult to measure in turbid case-2 waters. Therefore, *in vivo* measurements are required to calibrate remote sensing based models while *in vivo* high resolution methods require remote sensing methods to extrapolate over a wider spatial and temporal scale and we suggest that automated production measurements based on FRRf methodology can fulfil this role.

Depth integration of high-resolution measurements is a complicated estimate, depending on light penetration through the water column and assuming vertical homogeneity. For most part of the year, the assumption that the mixed layer depth (MLD) reaches below the euphotic zone and causes vertical homogeneity in photoacclimation and community composition, is a safe assumption for the Dutch North Sea, yet short-lived thermal stratification is a regional phenomenon in summer (Van Leeuwen et al., 2015). This short-lived thermal stratification can result in subsurface chlorophyll maximum layers, which, when MLD is shallower than the euphotic zone, will result in a phytoplankton community with distinctly different photophysiological characteristics. Additionally, to calculate water column productivity, an assumption on light penetration through the water column is needed. In this study, light extinction was actively measured approximately ten times per

~~cruise and based on the correlation with turbidity these figures are spatially interpolated using linear regression. Although the light attenuation in the water column is strongly influenced by turbidity, the situation is more complex involving not only underwater processes (absorption and scattering) but also surface processes like reflection and refraction (Brown et al., 1984). Additionally, turbidity is measured in the near-infrared (880 nm), but different substances in the water have characteristically shaped light absorption spectra and photosynthetic active radiation spans a wide range of wavelengths (400-700 nm), this nonlinearity can make the light attenuation coefficient based on turbidity a rough estimate (Kirk, 1994), but as we observed a good correlation between turbidity and K_d (r^2=0.77), we assume our K_d estimates are reliable.~~

~~The use of automated cluster analyses to interpret spatial heterogeneity is a necessity when dealing with the high amount of data collected by high-resolution methods. The unsupervised Hidden Markov Model (uHMM)~~ method used in this study was originally designed to detect phytoplankton blooms and understanding the involved dynamics~~, but here used to identify different phytoplankton communities at the regional scale~~ (Rousseeuw et al., 2015~~). In general, we see in all months a~~; Lefebvre and Poisson-Caillault, 2019). This spectral cluster analysis on parameters from the FRRf and the flowcytometer allowed for the identification of distinct phytoplankton communities or biogeographic regions that differed per cruise. A clear distinction between phytoplankton communities of the coastal zone and ~~off-coast regions. A further separation between the Dutch south coast and the coast off~~offshore regions could be made in all months, except May. In two cruises, in April and June, it was indeed possible to identify regions with distinct phytoplankton communities. During the ~~northern Wadden Islands can usually be made. A separate off-coast area seems to be~~cruise in May, the ~~southernmost study area, i.e. the northern corner of the Walcheren transect. August is the most~~clustering did not result in clear mesoscale patterns but was heterogeneous ~~month, while both biomass and nutrient concentrations are low, suggesting that niche differentiation is more strongly present than in other months. Broadly, August conditions correspond to the hydrographical regions formerly identified in the~~over the whole Dutch North Sea ~~(Fig. 10; Van Leeuwen et al., 2015; Capuzzo et al., 2015). However~~. Unfortunately, the model was not able to ~~automatically~~ visualize all spatial heterogeneity.
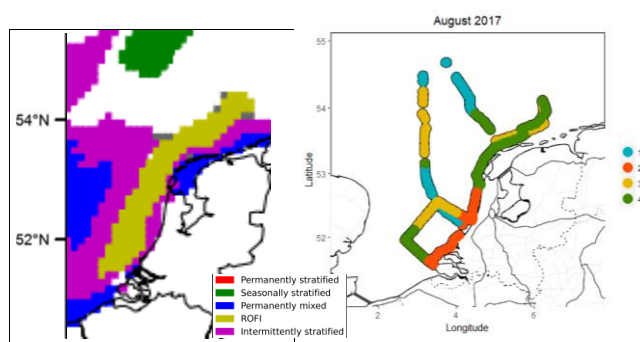
~~Figure 10: hydrographical regions as defined by Van Leeuwen (2015; left) and spatial clusters by uHMM clustering in August (right).~~

~~In April a distinct phytoplankton community was present~~For instance, in April off the coast from Terschelling~~, only resulting in~~ a distinct community with a high abundance of phycoerythrin-containing taxa did not result in a separate cluster ~~when manually increasing the number of spatial clusters from three to five~~. Additionally, temporal variation (i.e. day-night differences) was interfering with the spatial clustering in August. ~~Although~~So, although such models are useful for visualization and following changes in spatial heterogeneity, input and output need to be critically evaluated before implementation~~.~~ in monitoring programs. To test whether the differences between months result from seasonal variation or other factors, results over multiple years and additional seasonal cruises need to be made to better characterize the heterogeneity of the phytoplankton community structure.

~~Currently, biological monitoring of phytoplankton in the Dutch North Sea is dictated by the requirements set by OSPAR and the EU Marine Strategy Directive and limited to HPLC analysis of Chl *a* concentration, microscopy counts of *Phaeocystis* cells, and at a few stations, coccolithophores or toxic dinoflagellates. Unfortunately, sampling points were reduced from almost 70 in 1984 to less than 20 today, while strong seasonal patterns, high riverine input, and tidal forces make the Dutch North Sea a region with high spatiotemporal variability. At the same time, the Dutch North Sea is an area under high anthropogenic pressure, which has led to substantial biogeochemical changes over the past decades (Burson et al., 2016; Capuzzo et al., 2015 and 2017). These abiotic changes affect biology, with potential large implications for ecosystem function and services (Prins et al., 2012; Capuzzo et al., 2017; Burson et al., 2016).~~ The purpose of a phytoplankton monitoring program is to monitor the presence of functional types of phytoplankton, including the harmful taxa, the carrying capacity of the ecosystem and changes in biogeochemical cycling. The objective of this study was to evaluate the use of FRR fluorometry and flowcytometry for such monitoring purposes. The four conducted cruises spread over 5 months offered a wide variety of environmental conditions and phytoplankton community states, which the utilized methods were able to visualize. Inclusion of high-resolution methods in monitoring programs allows for analysis of finer scale events. Furthermore, it allows for analysis of living phytoplankton and is thereby able to measure rates and avoid effects of preservation and storage of samples. Another advantage is that high-resolution methods allow for easier comparison between countries, once common protocols are established. Nevertheless, low-resolution methods remain a necessity for more detailed taxonomic analysis, extrapolation over the entire water column, to calibrate and to correct for blanks. Data analysis is a challenge when implementing high-resolution methods, where cluster methods could simplify and standardize analysis. The cluster analysis of flowcytometric data has potential for improvement to increase the informative value of the method. Especially identification of phytoplankton clusters with a functional quality, such as nitrogen fixers, calcifiers, DMS-producers or clusters with high food quality, would be helpful for interpretation of ecosystem dynamics and biogeochemical fluxes. Regarding the FRRf, the main challenge is converting the electron transport rate to gross primary productivity in carbon units. Further research in these topics would benefit the implementation of these methods into monitoring protocols. Furthermore, it is important to account for diurnal patterns in monitoring set-up to be able to distinguish between diurnal and spatial variability. Possibly the diurnal variability could be modeled, but more studies with a Langragian based approach are needed for a better understanding of the impact of diurnal variability in the data. The combination of high-resolution in situ methods with remote sensing has the potential to further increase the spatial and temporal scale. Estimating biological parameters using remote sensing is challenging, especially in turbid, case-2 waters (Gohin et al., 2005; van der Woerd et al., 2008). Therefore, *in vivo* measurements are required to calibrate remote sensing based models and we

suggest that automated flowcytometry and production measurements based on FRRf methodology can fulfill this role. Overall, our proposed high-resolution measurement set-up has the potential to improve phytoplankton monitoring by supplementing existing low-resolution monitoring programs.

Data availability

Author contribution

HMA, MR, JCK and AV designed the measurement set-up. HMA, MR and JCK collected the data. HMA, MR, AL and JCK contributed to data processing. HMA and JCK prepared the figures and tables. HMA, MR, JCK, AL and AV prepared the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Systematic and sufficient monitoring of these changes is of crucial importance to recognize threats, and, once identified as such, develop mitigation actions. The current low-resolution monitoring program is clearly not able to cover the entire biological variability. For instance in April, both Noordwijk 70 and Terschelling 235 km show high gross primary productivity, suggesting that production of the entire area between these points is similar, but both high and low productivity rates occur (Fig. 7). Although extra sampling points in clearly deviating areas would be very useful, because only low-resolution offer the level of detail which is required to identify toxic, keystone or invasive species, adding high-resolution methods to the current monitoring program will already allow for obtaining sensible information between sampling points. A smart monitoring system should use high-resolution methods as it delivers information which is difficult to obtain otherwise, can be used to calibrate and validate remote sensing model and can also be used to identify extra sampling points, possibly even based on real-time projections, opening up early warning methodologies.

5 Conclusions

The combination of FRR fluorometry and flowcytometry offers an elaborate view of the phytoplankton community. Accounting for diurnal patterns and identification of FCM clusters for functional types such as nitrogen fixers, calcifiers or DMS-producers are steps needed to increase the value for interpretation ecosystem dynamics and biogeochemical fluxes.

Data interpretation may be supported by automated cluster analyses, such as the uHMM used in the current study, to interpret spatial heterogeneity and to deal with the high amount of data collected by high-resolution methods. However, our model needs to be improved to capture more of the spatial heterogeneity present in ecology of the Dutch North Sea. Overall, the addition of high-resolution monitoring is a very useful supplement to current monitoring to improve.

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Table 1: The derived photosynthetic parameters used in this study (see Oxborough et al. (2012) and Silsbe et al. (2015) for more information).

	Description	unit
C	Fraction of RCIIs in the open state	Dimensionless
$F^{(t)}$	Fluorescence at zeroth flashlet of an ST measurement when $C > 0$ (under ambient light)	Dimensionless
$F_m^{(t)}$	Fluorescence when $C = 1$ (under ambient light)	Dimensionless
$F_q^{(t)}$ or F_v	$\Delta F^{(t)}$, variable fluorescence	Dimensionless
$F_q^{(t)}/F_m^{(t)}$	Fluorescence parameter providing an estimate of PSII efficiency under ambient light (under ambient light)	Dimensionless
F_v/F_m	Quantum efficiency of PSII	Dimensionless
σ_{PSII}	Absorption cross section of PSII photochemistry	nm^2 PSII^{-1}
[RCII]	Concentration of functional RCII	nmol RCII m^{-3}
a_{LHII}	Absorption coefficient of PSII light harvesting	m^{-1}
α	Light utilisation efficiency	μmol electrons (μmol photons)$^{-1}$
E_k	Minimum saturating irradiance of fluorescence light curve	μmol photons m^{-2} s^{-1}
P_{max}	Maximum photosynthetic electron transport rate	μmol electrons m^{-2} s^{-1}
JV_{PII}	PSII flux per unit volume	mol electrons (PSII m^{-3}) d^{-1}
GPP	Gross Primary Productivity	mg C m^{-2} h^{-1}
n_{PSII}	Number of [RCII] per mole Chl a	mol RCII mol^{-1} chla
$1/\tau$	Rate of re-opening of a closed RCII with an empty Q_B site	ms^{-1}
K_a	Instrument type-specific constant allowing for direct calculation of [RCII] and JV_{PII} from FRR data	m^{-1}

Table 2: The phytoplankton groups distinguished in the current study.

	Length FWS	Main corresponding taxonomic group
Pico-Red	<4 µm*	Pico-eukaryotes
Pico-Synecho	<4 µm*	e.g. Synechococcus
Nano-Crypto	4-20 µm	Cryptophycea
Nano-Red	4-20 µm	Diatoms, Haptophytes
Micro-Red	>20 µm	Diatoms, Haptophytes

*In june <6 µm

	April	May	June	August
Abiotics				
Salinity (‰)	34.1 ± 1.8	33.5 ± 2.3	33.6 ± 1.8	34.0 ± 1.3
SST (°C)	9.5 ± 1.0	12.1 ± 1.1	15.5 ± 1.8	19.0 ± 0.6
Turbidity (NTU)	2.3 ± 3.0	1.1 ± 0.8	1.3 ± 1.3	1.2 ± 0.7
PO_4 (µM)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
Si (µM)	2.1 ± 2.2	1.6 ± 1.1	1 ± 0.6	1.3 ± 1.1
NH_4 (µM)	0.7 ± 0.7	1.3 ± 0.9	1.5 ± 1.2	0.4 ± 0.4
NO_3 (µM)	9.3 ± 11.4	3.5 ± 5.6	0 ± 0	0.2
DIN:DIP	43.8 ± 53.2	25.5 ± 36.7	9.0 ± 6.9	2.4 ± 1.9
DSi:DIP	7.9 ± 9.3	9.2 ± 8.5	4.0 ± 2.5	5.3 ± 3.7
K_d (m^{-1})	0.39 ± 0.28	0.33 ± 0.12	0.30 ± 0.20	0.25 ± 0.14
Biotics				
Chlorophyll a (µg L^{-1})	18.32 ± 19.71	5.67 ± 10.39	4.08 ± 4.11	3.98 ± 3.91
F_v/F_m	0.52 ± 0.04	0.26 ± 0.09	0.40 ± 0.09	0.48 ± 0.07
σ_{PSII} (nm^2 $PSII^{-1}$)	3.66 ± 0.27	5.92 ± 1.35	4.59 ± 0.88	5.26 ± 1.07
[RCII] (*10^{-9} nmol RCII m^{-3})	31.3 ± 17.1	6.94 ± 10.5	4.13 ± 2.78	2.21 ± 1.84
n_{PSII} (*10^{-4} RCII (Chl a)$^{-1}$)	8.02 ± 0.55	8.94 ± 6.05	6.65 ± 1.68	5.95 ± 1.15
$1/\tau$ (ms^{-1})	0.24 ± 0.06	0.52 ± 0.10	0.49 ± 0.07	0.62 ± 0.12
α	0.53 ± 0.03	0.25 ± 0.09	0.39 ± 0.08	0.48 ± 0.08
E_k	300 ± 52.5	223 ± 147	253 ± 124	277 ± 137
P_{max}	158 ± 30	56.5 ± 42.4	97.5 ± 47.7	130 ± 60.4
GPP water column (mg C m^{-2} h^{-1})	781 ± 409	207 ± 277	136 ± 101	68.4 ± 39.1
GPP surface (µg C L^{-1} h^{-1})	115.7 ± 58	27.5 ± 72	16.5 ± 13	8.7 ± 8.3
O:R ratio	0.31 ± 0.51	0.06 ± 0.07	0.19 ± 0.20	0.28 ± 0.16
Relative abundance microplankton (%)	1 ± 1	4 ± 4	2 ± 2	1 ± 1
Relative abundance Nanoplankton (%)	32 ± 17	44 ± 16	23 ± 8	18 ± 6
Relative abundance Picoplankton (%)	68 ± 17	52 ± 52	75 ± 9	82 ± 6

Table 4: Coefficients of the stepwise multiple linear regression (n=61) for ln(GPP) with p<0.05 and VIF<6

	coefficients
Intercept	5.613
alpha	2.916
Turbidity	$-9.929*10^{-2}$
DIN	$-3.567*10^{-2}$
Temperature	$-1.887*10^{-2}$
Total red fluorescence (biomass)	$2.833*10^{-9}$
Hours	$4.141*10^{-2}$