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).**

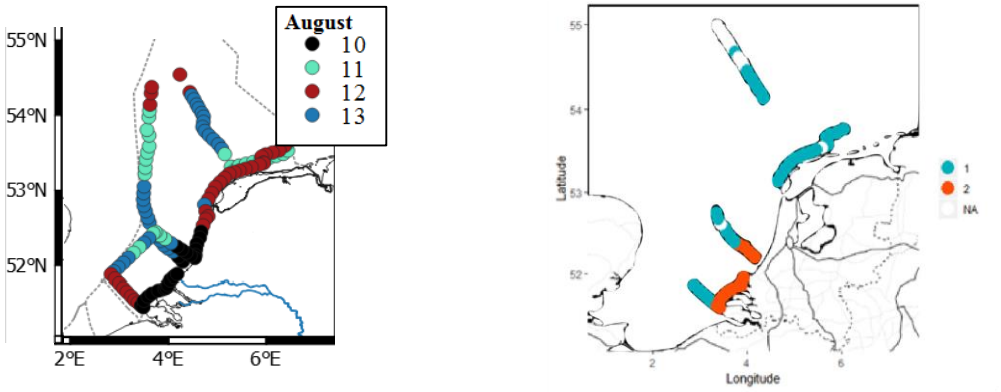

Fig. R1: : Maps of clusters as defined by spectral clustering of the whole dataset (left) and only the measurements at 8h around noon (8:00h to 16:00h). Based on the FCM-based five described phytoplankton groups (Table 2) and non-collinear FRRf-parameters on photophysiology (F_v/F_m, $1/\tau$, [RCII],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