Interactive comment on "High resolution *in situ* measurements of phytoplankton photosynthesis and abundance in the Dutch North Sea" *by* Hedy M. Aardema et al.

Anonymous Referee #1

Received and published: 25 June 2018

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).

General comments: - 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 emphasising more the

estimates of PP throughout the ms. - 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. - 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. - 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. - Part of the text requires rewording – see technical section below for details.

Specific comments - 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. - 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? - 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 consistemcy. 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). - 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 - 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. - Conclusions – I would suggest to highlight the importance of this study for monitoring programme. Also, a bit more considerations on combining low and high-resolution measurements would be useful. 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.

Technical corrections Page 1: 23-26 – rewording is needed Page 1 30 -keywords, consider adding primary productivity Page 2: 10-12 – this sentence would fit better at the start of the paragraph. It also requires references Page 3: 5 – 'a sum': consider replacing with 'a combination' Page 3: 23 - 'pigment ratio' slightly incorrect as the ratio considered is of fluorescence 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 Page 4: 3-5 – not clear, needs rewording Page 5: 1- would be useful to have the exact dates of the surveys. 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?) 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). Page 6: 16 & 18 – acronyms (e.g. NPQ and F0') should be explained when used the first time Page 8: 12-13 – formula 8 is missing Page 8: 17 – need rewording Page 8: 20-21 – it is not clear how surface irradiance was calculated; please reword

this section 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, Page 10: 2 – outliers – specify which analysis you are referring to (e.g. outliers from the fRRF?) Page 10: 5 – provide a reference for the value of 0.65 Page 10: 12 – please specify which are the photophysiological variables considered Page 10: 13 – acronyms (VIF) should be defined here Page 11: 20 – 'nitrate': should this be 'DIN'? 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 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. Figure 2 provide equations of linear regressions with R2 and significance Page 14: 27 – 'suggesting physiological stress', please provide reference Page 16: 9 – it is not clear to which phytoplankton group the % are referring to. Page 16: 14 – please specify which are 'these regions' 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. 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). 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. 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 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. 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. 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). 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. Page 23: 6-7 – this paragraph is not clear particularly what is meant with 'opposite' 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 Page 24: 15 – please indicate how much of the variability in PP is explained by the stepwise regression (e.g. R2?). Page 25: 4 – reword please. Page 26: 2-5 – require rewording particularly the need to clarify and be more specific on the work done in this study. 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. 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). Page 26: 24-25 – please reword Page 27: 8-9 – repetition of method; should be deleted. Page 29: Figure 10 legend, possibly just my issue, I don't see the similarity between the two figures. Page 30: 13 – 'low resolution'... should this be 'high-resolution'?

———————————————————