Anna Makarewicz
Piotr Kowalczuk
Institute of Oceanology Polish Academy of Science
ul. Powstańców Warszawy 55
PL-81-712, Sopot, Poland

April 15, 2018

Prof. Dr. Oliver Zielinski,
Associate Editor Ocean Science
Head of Marine Sensor Systems
Vice Director Institute for Chemistry and Biology of the Marine Environment (ICBM)
University of Oldenburg, Germany
Cc/

Natascha Töpfer
Copernicus Publications
Editorial Support
Att. The response to review of manuscript by Makarewicz et al., 2017 submitted to Ocean Science and coded OS-2017-100 done by Reviewer#1, document reference OS-2017-100RC1

Dear Prof. Zielinski,

We thank the reviewers for their constructive comments. We have followed their guidance, and rewritten parts of the manuscript to place the work in better context. We have also gone through the text thoroughly to make any edits to the text to improve the flow and any grammatical errors we found that could be corrected. With this exercise we have also responded to all questions raised by Reviewer#1 and introduced necessary correction in revised manuscript.

The detailed comments to the Reviews are given below. After each of Reviewers single comments, our responses start with **Response:** (in bold)

Detailed Response to review by Reviewer #1:

General comments

The review submitted by Reviewer#1 concentrated mostly on methodological issues that we will explain in a point-by-point response below. The Reviewer#1 raised a concern about the main conclusion of our work that phytoplankton was the main source of the protein-like fraction of fluorescent dissolved organic matter, FDOM. We have come to this conclusion based on the simultaneous in situ measurements of protein-like FDOM fluorescence and chlorophyll *a* fluorescence performed with two different fluorometers integrated in one measurements system. The correlation coefficient R between those two variables was 0.804,

and the linear relationship between them explained 65% of variance (R^2 = 0.65). The regression analysis has been conducted on an extensive data set n = 24990 and results were very significant statistically ($p<0.0001$). The question put forward by Reviewer#1, that there was a very low determination coefficient values in the regression between protein-like FDOM fluorescence and chlorophyll *a* concentration concerned only analysis performed in case when chlorophyll *a* concentration was measured in discrete water samples. We admit that the relationship was weaker in this case, however it was statistically significant. This difference has been explained on Page 21 (lines 473 – 488) and can be attributed to time and space lag between instrumental measurements and water sample collection, reaching in some cases 1.5 hour and up to 3 nautical miles, respectively. This is caused by the "one instrument in water at a time" safety rule applied in almost all research vessels.

In addition, in both 2014 and 2015 we provided quantitative evidence of tight relationship between optical proxy for phytoplankton chlorophyll a concentration: the total absorption coefficient less due to water at 676 (atot-w(676)) and protein-like FDOM fluorescence (ICH3). The determination coefficient ranged between 0.423 and 0.860 depending on sampled water masses in a given year, see Figure S4 in supplement. We have also shown that there was a very tight coupling between total absorption coefficient less due to water at 676 nm, $a_{tot-w}(676)$ and chlorophyll *a* fluorescence intensity measured in different water masses in 2014 and 2015, Figure S3 in supplement. The determination coefficient between those variables ranged between 0.394 and 0.915 depending on sampled water masses in a given year. In addition to the quantitative analysis we have shown that there was a significant coupling in vertical distribution of protein-like FDOM fluorescence, ICH3 and $a_{tot-w}(676)$ and chlorophyll *a* fluorescence, see Figure 4, E, F, G, page 20.

Findings presented above have been thoroughly discussed in section 4.4 (Pages 31-34) where we have reviewed recent studies providing evidence based on in situ and mesocosm studies that protein-like FDOM fraction has been controlled by phytoplankton dynamics. Recent studies by Chen et al., (2017) (Science of The Total Environment, v:599–600, pp: 355-363) and Reteletti-Brogi et al, 2018 (Science of The Total Environment, 627, 802-811) presented new data linking high abundance of protein-like fluorescence with autochthonous production by phytoplankton and ice algae in the water of Amerasian Basin of Arctic Ocean, sea ice and under ice water in Canadian Archipelago. However, this evidence has been based on limited samples size collected in single surveys. Our data set is unprecedented in volume acquired in repeated surveys in the same area over several consecutive years, and acquired with a custom-made fluorescence sensor (excitation/emission pairs to detect different CDOM pools) simultaneously (the in situ 3 channel WetStar FDOM fluorometer by WetLabs). Therefore we are confident that our conclusion are based on sound arguments.

At the end of his/her review Reviewer#1 has asked questions (Question 13), why the CDOM absorption is not well correlated with DOC concentrations? Well, the weak but statistically significant relationship between $a_{CDOM}(350)$ and DOC concentrations in the North Atlantic is not necessarily unexpected in waters of Atlantic origin. In principle, the $a_{CDOM}(\lambda)$ and DOC is not correlated on oceanic basin scales (Siegel et al., 2002, Nelson and Siegel 2013). The empirical relationships between the $a_{CDOM}(\lambda)$ and DOC reported in literature (e.g.

Massicotte at al., 2017 and references therein) were driven by concentration gradient in DOC and $a_{CDOM}(\lambda)$ found between terrestrial, freshwater, marine and coastal ocean environments. Most of estimated empirical relationships between DOC and $a_{CDOM}(\lambda)$ were derived for coastal regions where there was a local point source of terrestrial DOC (river outlet). Such relationships published for Arctic were usually established for coastal regions in the vicinity of North American and Siberian Rivers (Amon et al., 2012; Matsuoka et al., 2012; 2013; Gonçalves–Araujo et al., 2015; Pavlov et al., 2016; Mann et al., 2016). The environment-specific determination coefficient in relationships between the $a_{CDOM}(\lambda)$ and DOC estimated by Massicotte at al., (2017) was lowest for pelagic ocean and reached 0.44. Our results are in line with statements from Siegel et al., 2002 and Nelson and Siegel, 2013 that the $a_{CDOM}(\lambda)$ and DOC is not correlated in pelagic ocean, as our study region in the Atlantic water inflow regions has very limited input from land runoff and the salinity range we have covered is very limited and high (with occasional freshening due to sea-ice melt rather than terrestrial runoff).

We have tried the Fichot and Benner (2011, 2012) approach to link spectral slope $S_{275\text{-}295}$ with carbon specific $a^*_{CDOM}(\lambda)$, but results were unsatisfactory. Rather we have presented similar non-linear relationship between the $S_{300\text{-}600}$ with carbon specific $a^*_{CDOM}(\lambda)$, which worked fairly well, and was consistent with non-linear relationship between those parameters presented by Norman et al., (2011) in Antarctica. Again, the Fichot and Benner approach to link spectral slope $S_{275\text{-}295}$ with carbon specific $a^*_{CDOM}(\lambda)$ was derived for Gulf of Mexico, where there were contrasting concentrations and compositional CDOM properties exists between the coast near the Mississippi River mouth and central oligotrophic part of Gulf of Mexico (Carder et al., 1999). In our opinion we have presented a thorough discussion comparing our results with existing literature, (Section 4.3).

We have tried to explain most critical points raised by Reviewer#1 in this section. Detailed responses to all individual questions are given below.

Detailed responses:

Abstract 1) page 2 line 45 why you concluded that phytoplankton is the main source of protein-like fluorescence based on a r2=0.36?

Response: This question has been addressed in General Comments section of our response letter, see above. We have deleted part of this sentence from the abstract: "~~and between the protein like fluorescence intensity and chlorophyll *a* concentration in discrete water samples (R_2=0.36, p<0.0001, n=299),~~"

2) page 3, line 51. how did you arrive to this conclusion? of the Arctic Ocean (Arrigo et al., 2008), which could potentially contribute to increased production of autochthonous (marine) dissolved organic matter (DOM). what about the ice algae? they will disappear and they also contribute to CDOM.

Response: We assume that Reviewer refers to line 61 in the Introduction. In fact, this remark does not refer to our results, and the Reviewer#1 likely wanted this sentence to be clarified. Similar remarks have been noted by Reviewer #1 in question 3, therefore we have decided to rewrite this paragraph in the Introduction. We agree with Reviewer #1 that ice algae can be considered as a potential source of autochthonous CDOM/DOM, (e.g. Granskog et al., 2015; Reteletti-Brogi et al, 2018) and this has been addressed in General Comments as well (see above). Above we have given points supporting our conclusion that phytoplankton is a dominant source of CDOM in the Nordic Seas influenced by Atlantic Waters - we have used the term "study area" to specify this (line 51). We would like to underline that we have been conducting field surveys in the ice-free waters, see section 2.1., as our research vessel is not classified as icebreaker. We neither did present any data nor written any conclusions about the sea ice.

We have changed the corresponding paragraph as follow:

The rapid reduction of summer sea ice in the Arctic Ocean in the past decades has various repercussions on the structure and functioning of the Arctic marine system: forcing changes in physics, biogeochemistry and ecology of this complex oceanic system (Meier et al., 2014). One of the most significant consequence of observed rapid Arctic Ocean transition is an increase in the primary productivity of the Arctic Ocean (Arrigo et al., 2008), which could potentially contribute to increased production of autochthonous (marine) dissolved organic matter (DOM) in ice free and under ice waters. The sea ice is also a source of autochthonous CDOM/DOM, (e.g. Granskog et al., 2015; Anderson and Amon, 2015 Reteletti-Brogi et al, 2018). However DOC produced by sympagic algae has limited effect on overall organic carbon mass balance in the Arctic Ocean, as melting of one meter of sea ice would negligibly change DOC concentration in top 50 m of water column, assuming an averaged DOC content in the ice of 100 μMol C, (Anderson and Amon, 2015). Simultaneously, response of terrestrial ecosystems to temperature increase will accelerate permafrost thaw and increase the riverine discharge, resulting in more allochthonous (terrestrial) DOM being released into the Arctic Ocean (Amon, 2004; Stedmon et al., 2011; Anderson and Amon, 2015; Prowse et al., 2015, and references therein). Terrestrial DOM presents a considerable role in the carbon budget of the Arctic Ocean (Findlay et al., 2015; Stein and Macdonald, 2004), especially in coastal waters and continental shelf with large inflow of terrestrial DOM, which constitutes 80% of total organic carbon delivered by Arctic rivers (Stedmon et al., 2011).

Following references have been added to text:

Amon, R.M.W. 2004. The Role of Dissolved Organic Matter for the Organic Carbon Cycle. the Arctic Ocean, [in:] The organic carbon cycle in the Arctic Ocean, Stein, R., and Macdonald, R. W. (Eds.) Springer, Berlin, Heidelberg Chapter 4, 82-99.

Anderson and Amon, 2015. DOM in the Arctic Ocean. [in:] Biogeochemistry of Marine Dissolved Organic Matter, D. A. Hansell, D. A., and Carlson, C. A. (eds), 609–633.

Granskog, M. A., Nomura, D., Müller, S., Krell, A., Toyota, T., & Hattori, H. (2015). Evidence for significant protein-like dissolved organic matter accumulation in Sea of Okhotsk sea ice. *Annals of Glaciology*, *56*(69), 1–8. https://doi.org/10.3189/2015AoG69A002

Retelletti-Brogi, S., S-Y. Ha, K. Kim, M. Derrien, Y.K. Lee, and J. Hur, 2018. Optical and molecular characterization of dissolved organic matter (DOM) in the Arctic ice core and the

underlying seawater (Cambridge Bay, Canada): Implication for increased autochthonous DOM during ice melting. Science of the Total Environment 627, 802–811

3) page 3 line 65, which percentage to carbon budget? DOM presents a considerable role in the carbon budget of...

Response: We agree with Reviewer#1 that this sentence wasn't clear. However, it is beyond the scope of this paper to place exact numbers on budget terms that have large margins of error even in the most up-to-date estimate of the carbon budget of the Arctic Ocean. We have changed it as follow:
Terrestrial DOM presents a considerable role in the carbon budget of the Arctic Ocean (Findlay et al., 2015; Stein and Macdonald, 2004), especially in coastal waters and continental shelf with large inflow of terrestrial DOM, which constitutes 80% of total organic carbon delivered by Arctic rivers (Stedmon et al., 2011).

4) line 71. please add Pegau reference to this list Hill, 2008; Granskog et al., 2007,

Response: Agree. The reference to:
Pegau,W. S. (2002), Inherent optical properties of the central Arctic surface waters, J.Geophys. Res., 107(C10), 8035, doi:10.1029/2000JC000382.
has been added to the revised manuscript text and reference list.

5) line 73 sorry this is not conclusive. UV can also produce radicals after interacting with CDOM resulting in more toxic and damaging effects! and preserves marine ecosystem from harmful ultraviolet radiation

Response: We agree with Reviewer suggestion. The sentence has been rewritten as follows:

Particularly in absence of sea ice, light absorbed by CDOM in visible part of the spectrum limits the light available for photosynthetic organisms (Arrigo and Brown, 1996), but also shields marine ecosystem from potentially harmful ultraviolet radiation strongly absorbing electromagnetic radiation in UVB and UVA (Erickson III et al., 2015). CDOM is also important substrate in photochemical reactions contributing to direct remineralization of organic carbon, production of bioavailable low molecular weight DOM but also formation of reactive oxygen species that could potentially be toxic to marine organisms (Mopper and Kieber, 2002, Kieber et al., 2003, Zepp, 2003).

Following references have been added to references list:

Arrigo K. and C. Brown, 1996. Impact of chromophoric dissolved organic matter on UV inhibition of primary productivity in the sea. Marine Ecology Progress Series, 140, 207-2016

Kieber, D.J., Peake, B.M., Scully, N.M., 2003. Reactive oxygen species in aquatic ecosystems. In: Helbling, E.W., Zagarese, H. (Eds.), UV Effects in Aquatic Organisms. Royal Society of Chemistry, Cambridge, pp. 251– 288.

Mopper, K., Kieber, D.J., 2002. Photochemistry and the cycling of carbon, sulfur, nitrogen and phosphorus. In: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, New York, pp. 455– 507.

Zepp, R.G., 2003. Solar ultraviolet radiation and aquatic biogeochemical cycles. In: Helbling, E.W., Zagarese, H. (Eds.), UV Effects in Aquatic Organisms and Ecosystems, vol. 1. The Royal Society of Chemistry, Cambridge UK, pp. 137– 184.

6)line 78, what fraction of DOM is CDOM? what fraction of CDOM is FCDOM?

Response: This is a good question, to which there is no consensus answer within the community working with DOM, and providing detailed answer to this question is beyond the scope of this paper. Stedmon and Nelson (2015) in their most recent book chapter presented only a qualitative schematic drawing of dissolved organic matter with subdivision for its chromophoric and fluorescent part with indication of elemental carbon, nitrogen, phosphorus, hydrogen and sulphur contribution. Unfortunately, no quantitative information is given (neither likely available). Similarly, the FDOM fraction has not been quantified as percent of CDOM or DOM (Stedmon and Nelson, 2015). Nelson and Siegel (2013) have defined CDOM as:

"Chromophoric dissolved organic matter (CDOM; also often referred to as gelbstoff or gilvin) is the fraction of DOM that interacts with solar radiation. CDOM compounds absorb light, and a fraction of them are also fluorescent. For the purposes of this review, we operationally define CDOM as material that passes through a submicron filter (usually 0.2–0.4 μm) and appreciably absorbs light in the solar radiation bands as found at the Earth's surface (e.g., UVB, UVA, and visible light; 280–700 nm). This definition practically excludes much of the DOM pool, which spectroscopically absorbs shortwave UV radiation but does not interact with light in the natural environment (Fichot & Benner 2011). We further operationally define the quantity of CDOM by its Naperian absorption coefficient at a reference wavelength. Quantification of CDOM in terms of mass or carbon content is not currently possible, so obviously optical characterization of any nature is relative to the exact composition of CDOM, which likely varies in both time and space."

Our definition, given in the Introduction comprise in a shorter form of definition given by Nelson and Siegel (2013). The research community has also consistently used optical properties to characterize CDOM in oceanic environments, following concepts developed by Jerlov (1968) over 50 years ago.

7) page 4 line 100, upstream you meant? changes associated with CDOM in the areas downstream of the Atlantic Water inflow region

Response: We agree with Reviewer#1. North Atlantic south of Nordic Seas, are upstream in the North Atlantic Current. Changed accordingly.

8) page 8 line 209, S between 300 and 600 nm line 218 why additional acdom375 and acdom443 when actually the range is 300 and 600 nm? line 224 why to use micron^{-1} use nm^{-1}

Response: We have calculated spectral slope coefficient in the spectral range 300 – 600 nm. We have included additional CDOM absorption coefficient values at 375 and 443 nm, $a_{CDOM}(375)$ and $a_{CDOM}(443)$, to enable direct comparison of results with presented in other relevant studies e.g. Stedmon and Markager, 2001, Matsuoka et al., 2011 2012, 2013, 2017; Granskog et al., 2012; Hancke et al., 2014; Gonçalves–Araujo et al., 2015; Pavlov et al., 2015. The values of the slope coefficient have been scaled by multiplying by 1000, and given

in units μm-1 for better visualization in tables and figures, which is consistent with Stedmon and Markager, 2001, 2003, Kowalczuk et al., 2006, Stedmon and Nelson, 2015 among others.

9) page 9 equation 3, why the use of spectrophometry for chl? this is an old technique that has a larger error and is less sensitive than fluorometry or HPLC. What is the error of this emthod? Did you compare this method with HPLC or fluorometry nonacidification technique?

Response: We agree with Reviewer#1 that the HPLC method is most accurate way for estimation of chlorophyll *a* concentration, however due to its high cost and time-consuming analysis, we have chosen the spectrophotometric method, because it is simple, fast, low-cost, and not dependent on external standards. The spectrophotometric method was the most convenient way for processing large number of collected samples. We worked in mesotrophic and eutrophic waters, where the chlorophyll *a* concentration varied between 0.1 to 15 mg m^{-3}. The spectrophotometric method for determination of chlorophyll *a* concentration originally developed by Lorentzen (1967) has been recommended for use in mesotrophic and eutrophic waters by "Guidelines for the Baltic monitoring program". Baltic Sea Environment Proceedings, 27D, Helsinki Commission, Publication BSEP27D, Helsinki, 1988. We agree that fluorometric method of chlorophyll *a* concentration is more sensitive, however it is also heavily dependent on the fluorescence quantum yield, that is different for various phytoplankton groups and the fluorometer must be routinely calibrated against the external standards. Recently, there has been observed a rapid and dramatic change in phytoplankton phenology in Nordic Seas, where dominant diatoms have been replaced by coccolithophores advancing northward with warm Atlantic Water (Oziel et al., 2017). So, in our opinion the fluorometric method of chlorophyll *a* concentration measurements could be biased by variable quantum yields. The spectrophotometric method based on extracted pigments absorption measurements is also much closer to measured optical Chl a proxies e.g. $a_{tot-w}(676)$.

The comparison between spectrophotometric method and HPLC method of chlorophyll *a* concentration measurements used in our lab has been previously presented by Darecki and Stramski, 2004, who found very good agreement between those two methods.

10) page 9 equation 4, I disagree. You cannot mix apples with bananas. DOC is not DOM unless you estimate DOM based on DOC with a curve or factor., same for equation 5

Response: We disagree. Specific DOC absorption (a* or SUVA) is a standard way adopted to examine CDOM properties in many studies measuring CDOM and DOC, and can provide insights about the quality of CDOM relative to DOC (Weishaar et al., 2003; Stedmon and Nelson , 2015; Massicotte et al., 2017). Principle goal of our study was to characterize the CDOM and FDOM optical properties and to identify their primary sources. As we mentioned in the answer to question 6, contributions of CDOM and FDOM to DOM (or DOC are generally unknown. Both CDOM and FDOM are characterized in aquatic environment through optical properties, Siegel and Nelson (2013). Optical properties normalized to DOC concentration are so called specific (in our case specific to carbon) optical properties and express the absorption crossection of the unit of mass of the substance. This specific optical crossection could be used as a measure of converting optical biogeochemical proxy into a concentration of substance (in this case carbon). Some of the specific optical properties have also biogeochemical meaning because they are related to diagenetic state of the substance, its chemical composition and molecular weight. Therefore, these variables were included in our manuscript. Stedmon and Nelson (2015) as well as Massicotte et al. (2017) advocated for

a use of those variables and ancillary parameters useful to characterize CDOM/FDOM and helpful in source identification. Therefore, we included those variables in our manuscript as they are relevant for the CDOM community, and would like to keep them.

11) line 305, how did you calculate the offset of wetstar-3 fluorescence measurements? reference with respect to nanopure? constant temperature?

Response: We have estimated a time drift of the fluorometer response, which was calculated by difference in raw counts values measured within similar pressure, salinity and temperature ranges (at ca. 200 m depth, temperature, 6.5 deg C. salinity >34.9) in the core of Atlantic Water, which we assume has not changed between years. The average difference in measured raw counts values in each channel in 2015 relative to 2014 was attributed to a drift of the optical detector, causing a deterioration of sensitivity and increase of raw counts. We calculated the average difference, and this was subtracted from all recorded raw counts in each channel measured during 2015 survey.

12) S slope without units?

Response: We could not identify the manuscript line where spectral slope unit was missing.

13) figure 3 is hard to interpret due to the vertical variability of properties

Response: We have foreseen this problem and Figure 3a presented a vertical distribution of sea water properties, giving a color scale of depth as third variable. An example of vertical distributions of ICH1 is shown in Figure 4.

14) many questions but fewer explanations or explanation attempts. beyond sampling aliasing, Why not acdom350 not well related to DOC? why not links between s275-295 and DOC? what is the linkage between particulate iron and absorption slopes?

Response: We have responded to this question in more detail in the General Response section (See above). In brief, we sampled a pelagic environment with narrow salinity range, and we believe that this in part explains why we cannot use CDOM as predictor of DOC. The area of study is not substantially influenced by riverine sources., therefore the we did expect that CDOM optical properties will predicts DOC in this environment with high accuracy.

At this point we can also address the last question concerning the iron. First of all, we have been analyzing properties of dissolved substances not particulate, therefore particulate iron did not affect our CDOM absorption measurements. At this point we could expresses how much useful was inclusion of SUVA254 variable in our analysis. Stedmon and Nelson (2015) in their book chapter has stated the variability of SUVA254 is between 0.5 – 5 m^2 $g^{-1}C$ in oceanic environment, and values over the 5 m^2 $g^{-1}C$ indicated the possible interference of dissolved iron on optical properties of CDOM. In this study SUVA254 was in the range of 0.56 – 2.54 m^2 $g^{-1}C$, with average value of ca. 1.7 m^2 $g^{-1}C$, which is a typical value for pelagic ocean (Massicotte et al., 2017). Based on this we can conclude that iron in dissolved and particulate phase had a negligible effect on CDOM/FDOM optical properties in our study area.