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Interactive comment on "Spring phytoplankton communities of the Labrador Sea (2005–2014): pigment signatures, photophysiology and elemental ratios" *by* Glaucia M. Fragoso et al.

Anonymous Referee #1

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General comments This work provides information on the phytoplankton groups found in the surface waters of the Labrador Sea. Pigment signatures determined with HPLC were analyzed with CHEMTAX to obtain the contribution of the various algal groups to the total chlorophyll a concentration. The authors also related the phytoplankton biogeographic distribution to the properties of the various water masses and the photophysiology of cells during the late spring /early summer over a 10 year period. The use of CHEMTAX for this data set is a novel application, however, a previous publication by Fragoso et al. in 2016 described the phytoplankton communities linked to the various hydrographical areas of the Labrador Sea at depths less than 50 meters using microscopy. Although both microscopy and CHEMTAX analytical methods are critical

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to any biogeographic examination of phytoplankton, I feel the two methods should have been combined into a single manuscript as they complement one another. Therefore, although I consider this work to be of value in its contribution to our understanding of the dynamics of the biogeochemical characteristics of the Labrador Sea, I feel its content fails to merit publication in present form.

Key problems that I feel need to be addressed include: 1) the absence of the initial CHEMTAX matrices and RMS errors 2) the organization of the methodology section; it is not well structured, it includes CHEMTAX results and lacks information (see specific comments) 3) the amount of information presented regarding taxonomy; species-specific information for the encountered groups of diatoms would have helped to understand differences on the photoprotective responses observed 4) of all the identified pigments (presented in Table 2) only the (DD+DT): chl a and DT:(DT+DD) are included for discussion on cell physiology. The authors should at least have included why they did not use the PPC:PSC, PPC:chl a or the pigment chlorophyllide a 6) the use of accepted and standardized abbreviations for the marker pigments and the phytoplankton groups in Tables 2 and 3 and throughout the text and finally 7) the correction of any incorrectly assigned references.

Specific comments

Introduction Line 54: change for Phaeocystis spp. colonies (> 100 μm). Line 83: update references. Line 84 what do you mean by "while the influence of phytoplankton composition on photophysiological patterns has not been investigated thoroughly?" please explain further.

Methods In general this section is not well structured and needs clarification and more detail.

Sampling and analysis are combined throughout this section and need to be presented with more organization. I recommend organizing this section into separate Study Area, Sampling and Biogeochemical Analyses sub-sections and limiting relevant data to rel-

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evant sub-sections.

Line 138: please include the number of stations sampled before fixed stations (was it 28 as in the previous work?). The number of depths sampled at each station should appear in the text as well.

Line 141: please write the specifications of the Seabird CTD system.

Lines 148-149: the description of how the total chl a was analyzed is presented before explaining how the collected samples for pigment analyses were filtered (probably on board?). Were samples for chl a fluorometric determination kept frozen at -20°C until analyses or at -80°C is a bit confusing. Was the extraction (90% acetone) performed by keeping the filters at -20°C for 24 h? or rather the filters were kept at -20°C until analysis (extraction for 24 h with 90% acetone)? Was acidification of the samples performed?

Line 151: I recommend changing this line to "samples for detailed pigment analysis were filtered onto 25 mm Whatman GF/F filters".

Lines 151-153 How much time passed between storage and analysis for the samples? Were the samples always analyzed in the same laboratory for every cruise over the 10-year period? Information on the maximum time of filtration is not provided and is very important for xanthophyll measurements. If too much passed while doing the filtration, the measurements of diatoxanthin are likely to be meaningless. This is also important for degradation pigment information, however the later data are not presented.

Line 153: were the nutrient samples kept frozen or refrigerated until analysis?

Pigment analysis Line 166: Was calibration done with external pigment standards obtained from DHI? Was the precision of the instrument tested? Is there a variation coefficient? Do you have limits of detection? Please at least provide the limits of detection and quantification and how were they estimated and if the pigments with concentrations below this limit were reported or not. All this information is relevant and missing.

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Table 2: In this table and throughout the manuscript the authors should follow the abbreviations for phytoplankton pigments and pigment formulae suggested in the Scientific Council for Oceanic Research (SCOR), Jeffrey et al. 1997 or in Higgins et al. 2011 In: Roy S, Llewellyn CA, Egeland ES, Johnsen G (eds) Phyto- plankton pigments: characterization, chemotaxonomy and applications in oceanography. Cambridge University Press, Cambridge, p 257−301. Please, don't use capital letters or other abbreviations that are not standardized!

This table should summarize the distribution of major taxonomically significant pigments found in the various algal groups during the study. This is poorly done in its current form. The authors should avoid ambiguity. For example when referring to 19'-hexanoyloxyfucocanthin (Hex-fuco), it should be mentioned that is a major pigment in haptophytes and dinoflagellates (Type-2, lacking peridinin), instead of "some dinoflagellates" or "various". This information –if provided here-would improve significantly the reading of the few next sections dealing with the marker pigments used for the CHEMTAX analysis. Only if the authors are more specific, the use of the references Jeffrey et al. 1997 or Higgins et al. 2011 make sense. Please delete the reference column of this table unless is useful (not the case in its present form).

Chlorophyll c1 + c2 should stay as Chlorophyll c1 + c2. Please avoid the use of CHLC12.

Zeaxanthin is a minor pigment present in various groups as cyanobacteria, however this group is supposed to be practically absent in polar waters. Although Blais et al. 2012 showed that cyanobacteria may be underestimated in polar regions (Beaufort Sea & Baffin Bay). Did the authors find presence of cyanobacteria using epifluorescence microscopy? Also did the authors perform any correlation analyses between prasinoxanthin and zeaxanthin to prove that the zeaxanthin encountered did or did not correspond to a group of prasinophytes-containing zeaxanthin? Please provide this information.

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Pigment interpretation There are major problems with this section. The title itself is more like the title of a results section. Actually the authors use the title “CHEMTAX interpretation” as a section included in the results. I suggest the authors change the title of the pigment interpretation section to HPLC pigment data or Clustering of HPLC data for CHEMTAX or CHEMTAX analysis or something similar-

This section is not well structured and difficult to follow partially because the authors explain the use of the selected initial pigment ratios while presenting the output matrices after the CHEMTAX analyses (Table 3). This is confusing for the reader. The initial ratio matrices used to seed CHEMTAX are not presented or explained with detail. Instead ambiguous information is presented e.g. “diatoms were identified as containing high fucoxanthin to chl a ratios”

Line 171: change it for Mackey et al., 1996, version 1.95. The following paragraph is not straightforward. The information on how CHEMTAX works in general and how version 1.95 works lacks clarity. This later version is a significant improvement on CHEMTAX application since the software sets up the multiple (60) initial pigment ratio matrices to obtain the more stable final values (as was recommended for example by Latasa 2007) and was actually used and described by Wright et al. 2009 and other authors before Coupel et al. 2015! Please add the references.

Line 179 to the end of the paragraph: please use the standardized abbreviations and you should at least explain why you decided to choose these particular marker pigments for the CHEMTAX analysis. Your microscopy results from the previous work should help here in a more detailed way.

Line 183: Again, please refer to Mackey et al. 1996 before more recent studies.

Line 191 to 197: Is figure 2 referring to the mean relative concentration of the main marker pigments to total accessory pigments (wt:wt) encountered or to chl a or total chl a or is based on the pigments absolute values? Unclear. It would have been helpful to include in this figure the biogeographical region linked with each cluster (as in figure

3).

Line 198: you already explained this earlier (lines 173-74). I think this is not very well explained and this may be the reason why you mentioned it again here. Line 199-200: “To satisfy this requirement, initial pigment ratios were carefully selected and applied to each cluster”. This should actually be mentioned earlier in this section when you explain and justify why you use the selected pigment markers that best describe the phytoplankton community of your study area.

Line 204: The authors should justify why they have used the “high light” field ratios from Higgins et al. 2011. Moreover, considering the importance on the photo-physiological results obtained in this study why is there not more information beside the irradiance of the experimental incubations? Was the PAR incident irradiance measured at the sampling sites?

Line 205: “Prasinophytes were separated into type 1 (containing prasinoxanthin) and type 2 (lacking prasinoxanthin)”. Both genera were observed in light microscope counts (Fragoso et al. 2016)” What do you mean? Fragoso et al. 2016 enumerated pico-phytoplankton (M. pusilla < 2 um)?

Line 209: Did the authors detect by HPLC the unknown carotenoid that characterizes the unique pigment signature of M. pusilla? Did they detect the pigment micromonal in their samples? or micromonol?

Line 211: “In addition to prasinophytes –type 2 (type 2A in Higgins et al. 2011- I assume), zea is also the major accessory pigment of cyanobacteria etc.. unclear paragraph.

Line 215: ”Prasinophytes (type-1, Higgins et al. 2011) indeed contain chl b so do chlorophytes and they can be distinguished by their relative ratios of lutein to chl b (Higgins et al. 2011). Was lutein detected with the HPLC analyses? Again correlations would have helped here.

Line 218: I suggest the authors change Dino-2 class for Dino2 (dinoflagellates type-2). Avoid the use of class, use what is suggested by Higgins et al. 2011. As mentioned before, this could have been nicely done in Table 2.

Line 220: Why did the authors use the term Cryptophycea instead of cryptophytes?

Line 256: Please refer to algal groups or phytoplankton groups based on pigment composition instead of “class”.

Results Line 294-296: Where is cluster C1 mentioned in this section to explain Figure 4?

Line 380: Why do you present saturation irradiances here as Wm-2 when in the methodology (line 237) you mentioned the 30 different irradiance levels is expressed as μmol quanta m-2s-1. Please use same units everywhere.

Line 382: What was the % contribution of DD, DT and β,β-carotene to the total PPC for clusters C3b and C2?

Line 381: DD+DT/Chl a; clusters C3b and C2 have also the lowest chl a concentration. However the level of deepoxidation is higher for these two cluster. How do your DDDT/chla and PPC/PSC ratios compare with other studies for the Arctic during spring/summer transition? Actually you don’t present PPC/PSC, why?

Legend of figure 3: would be better if each variable and parameter is related to the corresponding panel.

Discussion Very little information is discussed about spatial and temporal incident PAR irradiance variation.

Line 405: Chlorophytes have also been associated with land-fast ice in the Arctic (e.g. Palmer et al. 2011).

Lines 524-529: I think this is a very interesting result and a interesting point for discussion. Here is where species identification for the diatom groups of Arctic and Atlantic

waters would have been helpful. How do these results compare to other Arctic studies?

Lines 540 to 550: This paragraph deserves a better explanation with at least details on the microscopic most abundant genera for diatoms.

Lines 564 to 575: is more a repeated line of the introduction.

Lines 564 to the end: The resulting ratios of the final CHEMTAX analysis should have been discussed here, at least accordance/discrepancies with past studies in the polar environment. The interesting comparison among the carbon biomass- estimated from CHEMTAX and the estimated by microscopic observations- should have been better structured and compared with other studies.

Lines 987 to 993: please relate each variable to the corresponding panel.

References need further formatting review.

Latasa M (2007) Improving estimations of phytoplankton class abundances using CHEMTAX. Mar Ecol Prog Ser 329:13−21

Wright SW, Ishikawa A, Marchant HJ, Davidson AT, van den Enden RL, Nash G (2009) Composition and significance of picophytoplankton in Antarctic waters. Polar Biol 32:797−808

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