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Biogeosciences Discussions

Interactive comment on "Short term variability of dissolved lipid classes during summer to autumn transition in the Ligurian sea (NW Mediterranean)" *by* M. Goutx et al.

M. Goutx et al.

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Final Author comments in response to the referree remarks on the manuscript entitled :

“ Short term variability of dissolved lipid classes during summer to autumn transition in the Ligurian sea (NW Mediterranean) by Goutx et al.

We greatly thank both reviewers for their carefull reviewing of the manuscript, and the detailed and constructive comments. All comments will be taken into consideration, which will certainly improve the quality and understanding of the study presented here.

Referee #2 (Anonymous) Note that Referee’s comments are underlined while

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author’s answers aren’t.

General comments

This work assesses the short time dynamics (variation in concentration and composition) of different dissolved lipid classes, in order to get out information on the role of physical and biological forcing on lipids concentration and distribution in the surface and mesopelagic layers of the Ligurian Sea, during a transition period from the summer oligotrophy to autumn. The paper provides the first data set on total lipid concentrations and lipid class composition in DOM from surface to the mesopelagic layer in the open Ligurian Sea. These data are novel and relevant, but the authors have to stress better their importance, taking also into account that lipids represents only a very low percentage of DOM. The data set is very large and the results support the interpretation and conclusion of the authors, but the text need some revisions. The structure could be improved and there are a number of sentences and concepts that should be clarified (see specific comments). The conclusion should better underline which is the contribution of this paper to advances in DOM characterization, in particular the authors should better evidence which information the distribution of the different lipid classes, observed in this study, can give on DOM origin and degradation. I recommend a careful check of the abbreviations, (they are many and there is often a little confusion) and of the units of measurement, for example the lipids concentration is expressed sometimes as μg C L-1, other times as μg L-1, both in the text and in the captions. I also recommend a general improvement of the English. Below I report a list of specific and technical observations.

AC. All comments from the referee will be considered. In particular, the writting of the conclusion will be strenghened in order to better show how studying lipid class biomarkers enabled to decipher the processes involved in the sources and transformations of DOM at short temporal scale during summer to autumn transition. In addition, the text will be polished by someone of english native language.

BGD

6, S583–S596, 2009

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Abstract

Pag. 29 lines 9-10. "Lipid class composition provided valuable information on the origin of DOM, and the changes that occurred during the period investigated." Which information lipid can give on the origin of DOM? What does the second part of this sentence mean?

AC. The sentence (line 9-10) seems a little bit confusing and will be deleted. Indeed, the answers to the question are given in the next sentence. Assuming that lipid biomarker patterns reflected (at least part of) DOM sources and degradation status....”.correlations between glycolipids and pigments ...suggested that picoeucaryotes were a major source of dissolved organic matter” and then ....lipid metabolites showed a greater degree of degradation of dissolved organic matter during this transition period.. The text will be rewritten and clarified.

Introduction Pag. 30 lines 2-3. The sentence "primary production by photosynthetic microorganisms is a major source of DOM" should be corrected. In the surface layer many are the mechanisms of DOM production, and the importance of one with respect to the other has not been assessed. This sentence should be rewritten, indicating that phytoplankton may be one of the most important source of DOM, or that primary production define the upper limit to DOM production because photosynthesis is the first step to transform inorganic carbon into organic material, but DOM can be produced at each step of the food web. AC.OK

Pag. 30 lines 5-6. "primarily composed of proteins, carbohydrates and lipids." Proteins, carbohydrates and lipids are the major constituent only of the fraction of DOM characterized at molecular level. This fraction represents about the 10% of DOM surface pool and a lower percentage of deep DOM (Benner, 2002). AC. The term primarily is misleading. The text will be changed according to both referee 1 and 2 remarks, and considering that protein, carbohydrates and lipids are the main component of freshly biosynthesized organic matter in the euphotic layer.

BGD

6, S583–S596, 2009

Interactive Comment

Pag. 30 lines 6-9. "Understanding the dynamics of these biomolecules, their resistance to remineralization and their transformation to more complex and refractory substances is essential to predict the response of biogeochemical cycles to climatic changes." I suggest to correct this sentence underlining that these biomolecules can give information only about a very low percentage of DOM, as the authors report in the following sentence. AC. Although I agree that these molecules represent only a small percentage of total DOM, they are biosynthesized by living organisms and represent an important fraction in DOM, not in term of mass but as markers of some of the biogeochemical processes controling DOM distribution. Thus I would not underline that these biomolecules give information only about a very low percentage of DOM. I would rather stress the fact that understanding the dynamic of these biomolecules is important to identify the source, fate and transport of freshly biosynthesized DOM from the euphotic layer through the mesopelagic, under various environmental forcings, a unique ressources for numerous heterotrophic microorganisms in the oceans. This section will be rewritten. The relative importance of lipids in DOM will be reported.

Pag. 31 line 24. Correct the sentence:" The analytical TLC/FID technic on an "Iatroscan" apparatus..." AC. OK

Pag. 32 line 10. Why did you collect samples until 1000 m depth and not until the bottom? In this area, an increase in DOM concentration should be present close to the bottom, due to deep water formation; so it could be very interesting to have information on the occurrence of different lipids classes also in the deep waters, where DOM may be less degraded than in the intermediate layer. I think you should explain the choice to collect samples only until 1000 m. AC. We agree that sampling the deep water column could have been very informative and that it is an opened field of investigation . However, the objectives of the DYNAPROC2 mutidisciplinary cruise was focussed on the surface and mesopelagic processes, coupled to a high frequency sampling , which was not compatible with the study of the deep water column processes. According to the reviewer comments and also to the following comment (P32 lines 11-15), the

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6, S583–S596, 2009

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focuss of our interest in the surface and the mesopelagic layer will be clarified.

Pag. 32 lines 11-15. "Our overall objective...Mediterranean" I think you should clarify that your interest is mainly in the surface and mesopelagic layers. AC. OK see answer to the comment above

Material and methods Pag. 32 lines 20-21. What does "dynamics of the biological system" mean? To which parameters are you referring? AC. We referred to biological parameters including phyto-, microzoo-, macrozooplankton and bacteria biomasses and production rates during the period investigated.

Pag. 33 lines 1-3. "Additional 0-150 m surface profiles at C1", which kind of profiles were measured? CTD profiles? Or did you take samples for lipid analysis? Which was the frequency of this sampling? Did you perform the more frequent sampling only during C1 cycle? AC. The text will be clarified. Additional 0-150 m CTD/rosette sampling profiles were made during C1.

Pag. 34 lines 7-8. I suggest to indicate the sum of all lipid compound classes except hydrocarbons with the sigle: TLd-HC, as in the supplement table. AC. OK

Pag. 34 line 12. Why did you use two precombusted glass fiber filters? AC. This is the protocole to better prevent DOC contamination by particles.

Pag. 34 line 18. The concentration of the reference water for DOC analysis is in micromole per litre. In addition you should also indicate the batch you used, because the range of DOC concentration changes in the different batches.

AC. The text will be corrected. The concentration of the batch will be noticed correctly (in μM) and the bach reference will be added if available. An error in the reporting of the analytical precision will be changed.

“The Samples were filtered through 2 precombusted (24 h, 450°) glass fiber filters (Whatman GF/F, 25 mm), collected into precombusted glass tubes (closed with a screw cap and a Teflon liner), acidified with Orthophosphoric acid (H3PO4) and imme-

BGD

6, S583–S596, 2009

diately analysed on board on a Shimadzu TOC-V analyser. Dissolved organic carbon (DOC) was analyzed by high temperature catalytic oxidation (HTCO) (Sugimura and Suzuki, 1988; Cauwet, 1994, Cauwet 1999). Typical analytical precision is ś 0.1-0.5 (SD) or 0.2-1% (CV). Deep Sargasso Sea reference water (47 μmol L-1 C, ś0.5 SE, http://www. rsmas.miami.edu/groups/biogeochem/CRM.html) was injected every 10 12 samples to insure stable operating conditions”.

Pag. 34 lines 22-23. You indicate as biogeochemical parameters only CDOM and pigments, I think you should include also nutrients, DOC and oxygen. In addition, you use CDOM data in your paper, so I think you should report how you measured it, in the material and methods section. (Excitation emission matrix? Fluorescence emission spectra? Absorption spectra? Which wavelength did you use?) AC. Yes I agree with this remark (first sentence). The text will be corrected accordingly. About CDOM protocol, we do not report on the protocol of what we called the ancillary parameters (&2.4.) that are the topics of various companion papers in the special issue. However we will add one word for the CDOM analytical methodology (that is reported in Ghiglione et al. this issue)(see below).

Results Pag. 35 General hydrological conditions. To understand this paragraph we have to look at the figures reported by Andersen et al. 2008. The graphs in Andersen et al. 2008 paper, are limited to the layer 0-150 m, whereas your data arrive until 1000 m. In order to understand the relationship between physical forcing and lipids distribution, you should better describe hydrological condition observed below 150 m too. For example you could report potential temperature, salinity and dissolved oxygen vertical distribution from the surface to the bottom, in order to identify the different water masses occurring during the cruise in the study area. Did you observe the occurrence of the Levantine intermediate water? At which depth? Did you find lower lipids concentrations, or different lipids classes in the core of this old water? AC. We will consider new figures of temperature, salinity, oxygen, including the 150-1000 m water column and will look at the levantine intermediate waters composition.

BGD

6, S583–S596, 2009

Pag. 35 Lines 20-22. "We observed a strong water column stratification partially disrupted at the end of the cruise, low nutrients stocks and successive meteorological events." The last part of this sentence is not clear for me. What does the expression "successive meteorological events" mean? Where nutrients data are visible? They are not reported in the Andersen paper! AC. The text will be improved. Nutrients data are reported in Marty et al. 2008 DSR 55:1131

Pag. 36 lines 8-11. What is the meaning of the intrusion of low salinity water masses? Are they coming from the land? Clarify why you are interested in them. AC. The intrusion of low salinity water will be better described. “During the duration of the campaign, the observations were located beyond the front-Provençal Liguro and current Ligure. This assertion is established by the monitoring of T and S properties of the mixed layer and the dynamic height calculated for each CTD station (ten per day), by drift of the line of traps and a first display of ADCP currents. However, the appearance of desalinated water (S $<$38.2) indicates water intrusion of coastal origin, under the thermocline, in the range 50-150 m (Fig. phys), which cannot be explained by the existence of a Ligure current meander covering the Central Point. The Coastal watersorigin is determined by observations made on the radial side-large at the beginning of the campaign and two stations at the coast in mid and late season. The simplest explanation is based on the existence of baroclyne instability, exchanging of offshore and coastal waters over a small portion of the water column”.

Pag. 36 lines 20-21. "...but with hydrological and nutrient resources (N/P) similar to summer oligotrophic conditions (Andersen et al., this issue#(1)." Nutrients data are not reported neither in this paper nor in the Andersen et al. paper. AC. Nutrients data are reported in Marty et al. 2008 DSR 55:1131. The reference will be added.

Pag. 36 line 23. "TLd concentrations varied from 5.3 to 48.5 μg C L-1 (0.4 to 4 μM)". Why is the colour bar range (10-40 μg C L-1, figure 2) different from the range reported in the text? You should use the same word and/or abbreviation to indicate all lipid compound classes except hydrocarbons, in the paper. In the caption of

BGD

6, S583–S596, 2009

figure 2 you wrote "total biogenic lipids", in the caption of figure 3 "Total dissolved lipids (HC not included)", in the text "Total dissolved lipids TLd", this create a little confusion for the reader. AC. Same comment as for referee 1: Biogenic lipids are total lipids not including hydrocarbon. This will be explained in the revised version, we will also use the term LT-HC and LT homogeneously throughout the manuscript and in the figure caption.

Pag. 36 line 25. "The highest values were found in the 0-150 m surface layer (Fig. 2). The concentration gradient with depth was more pronounced during Leg 1, with values $<$18 μg C L-1 below 100 m, than during Leg 2 when concentrations below the surface layer were up to 40 μg L-1 (Fig. 2)." I suggest to rewrite this sentence, in fact the highest TLd-HC values are visible in fig. 2 in the upper 50 m of the water column (with the exception of the data collected the 19/09 and the 4/10). The depth at which you found TLd-HC concentrations up to 40 μg C L-1 during Leg 2 should be indicated. A C is missing after 40 μg. AC. OK

Pag. 37 lines 3-5. What it is visible in figure 3 is not in agreement with the sentence "The day/night pattern of TLd profiles was not significant throughout the period investigated although lower concentrations at night than during the day were observed during Leg 2 (Fig. 3)". The observation that lower concentrations were observed at night than during the day is not true for all the depths. In the Leg 2 below 500 m values are higher during the night than during the day. In addition, I suggest to report a zoom of the first 150 m of these graphs, in order to better investigate the day/night pattern at surface in which I guess a trend should be visible. In the figure 3 you should indicate Leg 1 and Leg 2. AC. OK. The sentence will be rewitten in order to better fit with the figure.

Pag. 37 lines 8-10. "almost homogeneous values down to a minimum concentration at 1000 m depth (39.8´s0.7 μM) and no apparent difference between days and 10 night (Fig. 4)". Control this sentence, the verb is missing! AC. OK

Pag. 37 lines 14-15. "The only significant re-increase of DOC (+20 μg L-1) at

BGD

6, S583–S596, 2009

depth (700 m) was noticeable later at the end of Leg 2 (12 October)". 20 μg C L-1 correspond to 1.67 μM, this value is lower than analytical precision of DOC measurement (2 μM), I don’t think this is a significant increase of DOC.

AC. We agree that the increase of 20μg C L-1 is small so we changed a little bit the sentence in the text, replacing "The only significant re-increase of DOC" by "The only small re-increase of DOC".

However, this increase is real. There was effectively an error in the material and method section. First the analytical precision of measurements was not 2 μM but the real sentence was "the precision of this method is better than 2 % (Cauwet 1999)". In fact, this precision has been even improved since 1994 as the analyses now are done on the last generation of TOC analyzer, more accurate (the TOC-V instead of the TOC-5000 in 1994). Finally, we would rather like to talk in term of SD or/and CV% for the analytical precision of the analyses to avoid any confusion. Another thing is that analyses were performed on board immediately after sampling and the samples were not stored.

So the paragraph 2.3. will be changed to: Samples were filtered through 2 precombusted (24 h, 450°C) glass fiber filters (Whatman GF/F, 25 mm), collected into precombusted glass tubes (closed with a screw cap and a Teflon liner), acidified with Orthophosphoric acid (H3PO4) and immediately analysed on board on a Shimadzu TOC-V analyser. Dissolved organic carbon (DOC) was analyzed by high temperature catalytic oxidation (HTCO) (Sugimura and Suzuki, 1988; Cauwet, 1994, Cauwet 1999). Typical analytical precision is ś 0.1-0.5 (SD) or 0.2-1% (CV). Deep Sargasso Sea reference water (47 mol L-1 C, ś0.5 SE, http://www. rsmas.miami.edu/groups/biogeochem/CRM.html) was injected every 10–12 samples to insure stable operating conditions.

Ref Cauwet G. 1994. HTCO method for dissolved organic carbon analysis in seawater : influence of catalyst on blank estimation. Mar. Chem., 47 (1) : 55-64.

Cauwet G., 1999. Determination of dissolved organic carbon (DOC) and nitrogen

BGD

6, S583–S596, 2009

(DON) by high temperature combustion. In: K. Grashoff, K. Kremling and M. Ehrhard (Eds), Methods of seawater analysis, 3 rd edition. Wiley-VCH, Weinheim. pp. 407-420.

Sugimura Y, Suzuki Y., 1988. A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Mar Chem 24:105-131.

Pag. 37 line 23-24. "Daily variations of TLd contribution to DOC were not significant." Where these data are visible? AC. Data were calculated from the supplement table. As they were not significant, they are not presented in the manuscript . The sentence :”Data not shown will be added line 24.

Pag.38 lines 1. Why did you define in the text "chloroplast lipids" and you call them phytodetritus in the caption of fig.6? I think that it is less confusing if you use the same word. AC. OK

Pag. 38 lines 4-6. "Except hydrocarbons, the glycolipids (monogalactosyldiglycerides) were the major lipid class, followed by pigments, monoglycerides and non nitrogen containing phosholipids (phosphoglycerides)." Looking at table 2, it seems that the major lipid class after glycolipids (25.09%) and pigments (13.58%) are the free aliphatic alcohols (12.34%), the monoglycerides (11.84 %) and free fatty acids (11.38%), then the phospholipids (3.71%). Why don’t take into account free fatty acids and free aliphatic alcohols? AC. OK There might be an error in the sentence. Free fatty acids and free aliphatic alcohols are described later in the text line 14-15-16, in the metabolites pool. Monoglycerides are reported twice line 5 and line 15. The text will be corrected.

Pag. 38 lines 18 and 20. I don’t find PL between the abbreviation in table 2. What does it mean? Does it indicate phospholipids? AC. Yes, it is phospholipids, abbreviation table will be checked.

Pag. 38 line 27. "During Leg 1, TG integrated values were higher in the 0-150 m

BGD

6, S583–S596, 2009

surface waters than in the 150-1000 m mesopelagic layer, whereas an opposite pattern prevailed during Leg 2 that is TG integrated values in the mesopelagic layer were much higher than TG integrated values in surface layer (Fig. 8, upper panel). During Leg 2, night concentrations were higher than day concentrations in the mesopelagic layer." This sentence should be rewritten more carefully, in fact TG night integrated values were always lower at surface (0-150 m) than in mesopelagic layer, if we exclude integrated night value observed at 278 day. An opposite trend was observed for the integrated day values, they were always higher at surface than in the mesopelagic layer. AC. OK

Pag. 39 lines 6-8. "Finally, the hydrocarbon pool varied in the range 0.9-21.9 μg C L-1 amounting 6.3´s1.8%, on average, of the total lipids (Table 2). An increase of concentrations at 400-800m depth occurred in 6-8 October (Fig. 7, lower panel)." I suggest to shift this sentence at page 38 line 22. Otherwise you should move figure 7b after figure 8. AC. OK

Pag. 39 line 11. As I wrote above, you didn’t define CDOM. You have to explain how you determined it in the material and methods section. AC. The paper focuses on lipid material in DOC. All “ancillary parameters” have been the topic of other papers and/or are included in the Ghiglione et al. (this issue) publication. In this paper, as for all other parameters the protocole for CDOM analysis is given : Colored dissolved organic matter (CDOM) substances were extracted from 500 ml sea water samples by adsorption on C18 microcolumns. CDOM was eluated from the microcolumn by methanol and quantified by light-absorbance at 412nm. We will make reference to this paper (Ghiglione et al. this issue) rather than describing again the protocole for CDOM analysis. However,a little more informations about CDOM will be given.

Pag. 41 line 3. Probably figure 9 and 10 are reversed. You should refer to figure 10, not 9! AC. OK

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6, S583–S596, 2009

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Pag. 41 lines 9-10. "...or exported to depth by other means than the mixing of water masses." Which mechanisms are you thinking about? AC. We think about DOM release from POM during particle sinking, and zooplankton migration.

Pag. 41 line 12. What does "photosintetically-produced DOM" mean? Does it mean DOM produced by phytoplankton during photosynthesis? AC. yes

Pag. 41 line 19. I didn’t find Morris et al., 1983 in the references AC.OK

Pag. 41 lines 19-22. "In these studies, only intracellular TG concentrations were related to Chl-a, which supported the conclusion by the authors that nitrogen availability was the major factor driving lipid content in particles at the time of phytoplankton blooms;" I don’t understand this sentence, why the observation that intracellular TG concentrations were related to Chl-a indicates that nitrogen availability was the major factor driving lipid content in the particles, at the time of phytoplankton bloom? AC. This sentence is too heavy and far from the focus of the paper. We will delete.

Pag. 42 lines 11-21. Where are these relationships? AC. All correlations between parameters have been done. Reference to Table 3 will be added. Table 3 will be checked.

Pag. 43 lines 6-7. Where are these relationships? AC. Reference to Table 3 will be added.Table 3 will be checked

Pag. 44 line 13. What does "microbiological production" mean? Bacterial lipid consumption or lipids release by microorganisms? AC. Microbiological production includes both primary production and bacterial production

Pag. 44 line 27. I suggest to report the abbreviation HC in this line after hydrocarbons, it is simpler to understand the following sentences for the reader. AC. OK

Pag. 45 line 11. I suggest to report the abbreviation PL in this line after phospholipid, it is simpler for the reader to understand the following sentences AC.OK

BGD

6, S583–S596, 2009

Interactive Comment

Pag. 45 11-13 Where is it visible this significant re-increase of DOC at depth? AC. In the Supplement table (The reference to the table will be added in the text of the revised version).

Pag. 45 line 27 What is BB? AC. BB is bacterial biomass, The full words will be included.

Pag. 46 lines 6-10. "It also supports the conclusion of Bendtsen et al. (2002) about the Greenland Sea, that deep sea labile DOC may only be explained by a DOC released from the sinking flux of POC. Dissolution of POM would led to formation of colloids known to preferentially shelter phospholipids (Liu et al., 1998)." Are POC concentrations observed at this depth enough to explain this hypothesis? AC. A sentence about POC concentrations will be added.

Pag. 46 line 12. Why did you use a low salinity index, instead of salinity? Which is the advantage of this index? AC. The salinity index is comprised between 0 and 1. It enables to characterize a water layer in term of salinity anomaly.

Pag. 46 line 19. You should refer to figure 9 not 10 AC. OK Pag. 47 line 7. Define PAH AC. OK Pag. 47 line. Define HC AC. OK

Pag. 47 lines 9-10. Do you have any idea to explain this surprising finding? AC. Difficult to explain without any hydrocarbon molecular analyses. May be the atmospheric depositions are the major vehicle for introducing hydrocarbons into the NW Mediterranean and are more important in offshore waters.

AC. We will correct all tables and figures according to the referee's remarks :

Table 1. You indicate the layer 0-1000 m as water column, whereas the depth of the station is higher than 2000 m (fig. 1) Table 2. You indicate the layer 0-1000 m as water column in this table too. The concentrations are in μg C L-1? Table 3 It is not readable, use a bigger character!

Figure Figure 3. The X scale of the first graph should be the same of the other ones.

BGD

6, S583–S596, 2009

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What does the dotted line indicate? Figure 4. Should the symbols be different for each cast? Figure 6. What indicate the number above each graph? How can I discriminate night and day data? Are the night data indicate with a grey square? Put this information in the caption. Figure 8 Substitute "Jours Julien" with "Julian days". Indicate the first and second leg in the figure, or in the caption Figure 9 and 10 are inverted Figure 10. What does LT-HC mean? Use the same abbreviation reported in the text!

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