

Referee N.J. Bale

#### General comments

This study examines the intact polar lipid (IPL) distribution in suspended particulate matter (SPM) from four stations in the oxygen minimum zone (OMZ) of the Eastern Tropical North Pacific (ETNP). It aims to link the IPL distribution of different water column zones with the microorganisms found there and to examine the ecophysical adaptations to the different zones of the OMZ. This is an extensive data IPL set and authors have theorized which groups of microorganisms are responsible for which IPLs. The strength, as I see it, in this study is the examination of how the IPL distribution changes across the different zones of the water column due to the changes in the bio-geochemical environment. Indeed, due to the generic nature of many of these IPLs it is only possible to put forward tentative assignments of their sources, whereas examining changes in the lipid composition with changing environmental parameters provides more solid information. Overall, I recommend this article for publication with the following edits and with suitable responses to my questions on the analytical methods.

We thank Dr. Bale for these constructive comments.

#### Specific comments

I have two specific comments.

My first specific comment relates to the extraction and analysis of these samples. The manuscript states that the samples were collected in 2007 and (presumably soon after) that they were extracted using Soxhlet-extraction with DCM:MeOH for 8 hours. From an IPL perspective this seems a “harsh” extraction method that has potential to destroy certain IPLs, resulting in a IPL distribution unrepresentative of that in nature. Could the authors comment on whether this would be their preferred method of extraction for IPLs or whether IPL analysis was not the original reason for the chosen extraction method? Indeed the first author has described utilizing the much gentler modified ‘Bligh and Dyer’ extraction method in other publications relating to IPLs. The authors also describe two different analytical methods used to quantify and to identify structures. Can you state whether analysis occurred soon after extraction in both cases? If not, how were the extracts stored and for how long? If the two analyses were carried out at different times, did extract storage introduce changes in the lipid distribution? I noted that the reference for the second LC-MS method applied (Wörmer et al., 2013), was published 6 years after the samples were collected. Based on the authors’ replies, suitable discussion of these issues should be included in the method section.

We have not performed a direct comparison of the Soxhlet extraction technique with the more common B&D ultrasonication technique using the same sample material, therefore we cannot comment on this with exclusive certainty. Nevertheless, we are not very worried about losing significant proportions of IPLs during Soxhlet extraction due to the following observations: (1) tests using microwave extraction showed optimal IPL yields at 70°C, indicating that IPLs may be more thermally stable than thought, (2) we detect presumably more labile compounds such as HPH-GDGTs in similar abundance using Soxhlet extraction as have been reported from other OMZ zones, indicating that these (presumably) more labile IPLs are not preferentially destroyed during Soxhlet extraction.

With respect to analyzing the samples at different time points: the first analysis using the quantitative data on the LC-ion trap-MS were performed in 2010 and 2011, while the QTOF samples were analyzed 4 years later in 2015. During this time the samples were stored in dry condition at -20°C. Again, we cannot state with absolute certainty that the IPL distribution has not been affected over time, however, based on the following lines of evidence, which were accumulated over more than a decade worth of experience in IPL analysis we are again not too worried about this: (1) we typically

analyze IPL standards every two months (together with our samples) that we store at -20°C over several years. So far these IPL standards have showed no selective degradation of compounds over time, thus indicating that relative IPL abundances will not be affected by storage at -20°C, (2) re-analysis of total lipid extracts that contained an internal standard within a 1.5-year timeframe gave similar absolute concentrations of different IPL species with different headgroups (this is unpublished data). This again indicates that there very likely are no significant selective changes in IPL abundance occurring over year-long storage of IPLs, at least none that would significantly affect the already existing uncertainties in IPL quantification.

Since also reviewer #3 expressed similar concerns, we added some explanatory sentences in the methods section with respect to these issues (see section 2.3).

My second specific comment relates to the length and depth of the discussion. This is a subject I find very interesting and yet I felt rather weighed down in information at certain points. I feel it would aid the reader if this was shortened and made more succinct.

We revised and shortened the discussion substantially from the originally 19 (Word) pages to 13 pages.

#### Technical corrections

Line 52 - change to “the subsequent”

Done.

Line 70 - change to “(ENTP), situated off the. . .”

Done.

Line 93 - change to “(IPL) are the main building blocks of cellular membrane and may”

Done.

Line 98 - change to “the North Sea”

Done.

Line 100 - you could also include in your reference list here the Western English Channel (White et al., 2015).

“The combined effects of seasonal community succession and adaptive algal physiology on lipid profiles of coastal phytoplankton in the Western English Channel. D.A.White , C.E.Widdicombe, P.J. Somerfield, R.L. Airs, G.A. Tarran, J.L. Maud, A. Atkinson. Marine Chemistry 177 (2015) 638–652).”

Done.

Line 129 - Remove ‘here’ to read “Notably, replacing. . .”

Done.

Line 141 - change to “extension of that of Xie”

Done.

Line 142 - change to “at two stations described here (station 1 and 8)”

Done.

Line 159 - Should this be “VERTEX I and II” ?

Yes, this has been corrected.

Line 160 The Martin et al. (1987) reference is missing from your reference list.

Is now added.

Line 177 - should define GFF at first use.

Done.

Line 223 - insert “response could not be corrected for”

Done.

Line 350 - The term amino lipid and betaine lipid seem to be used interchangeably throughout the manuscript. Could this be defined at one point?

This should not be the case, because aminolipids refer to the sum of betaine lipids, ornithine lipids and the unknown ALI and ALII (see also legend in Fig. 4) and betaine lipids are just betaine lipids, i.e. in our case DGTS and OH-DGTS. Since ornithine lipids and the unknown AL are often negligibly low in abundance the bulk of aminolipids are indeed dominated by betaine lipids. We checked the manuscript to make sure this distinction is clear and that these terms were not used interchangeably.

Lines 434-439 - This introductory sentence is too long to read and needs to be broken up or shortened.

Done.

Line 436 - replenishment that produces

Done.

Line 454 - “Podlaska et al. (2012)”

Done.

Line 482 - “by Xie et al. (2014)”. I have noticed this citation format error in more places. Please change throughout.

Done.

Line 489 - “coinciding with high Chl-a concentrations, reflecting”

Done.

Line 489 - What do you mean eukaryotic rather than microbial? Eukaryotes can be microbes and microbes can be eukaryotic. Do you mean eukaryotic rather than prokaryotic? But also in your results section, 3.1, you state that *Prochlorococcus* (not eukaryotes) were an important component of the photoautotrophic community. Hence I think the correct thing would be to say “photoautotrophic rather than heterotrophic”. Is this not what you wanted to say here? Check you have this correct throughout the manuscript.

We agree that term prokaryote should be used here, rather than microbe. We checked for consistency throughout the manuscript.

Line 497 - pluralize “IPLs”

Done.

Lines 540 - 551 - This section is confusing because you contradict each statement. You state that Eukaryotic phytoplankton and cyanos are assumed to be a major source of PG-DAG. Yet you then state that heterotrophic bacteria can also be a dominant source. Maybe using conjoining words “however” and “although” would make this section flow nicely.

We revised this section for clarity.

Line 547 - change to “we therefore suggest that”

Done.

Line 547 - remove heterotrophic bacteria. Cyanobacteria are not heterotrophic bacteria.

Done.

Line 576 - insert “abundance of less”

Done.

Line 586 - change to “fatty acid” or “acyl” rather than “fatty acyl”

Done.

Line 601 - change to “are <20”

Done.

Line 637-638 - remove tab within word “thaumarchaeota”

Done.

Line 648 - insert commas “zone, and that. . .1993), became”

Done.

Line 667 - replace “microbial” with “bacterial”

Done.

Line 679 - remove “shallower” as it is redundant.

Done.

Lines 694 - 670 - Please make this long sentence shorter or break it into two. You repeat the same words “exported, fossil and signal” twice.

This entire section has been revised and shortened.

Line 767 - insert “waters of the phosphorus-limited”

This entire section has been revised and shortened.

Line 768 - insert “to the phosphorus-replete”

This entire section has been revised and shortened.

Line 770 - insert “observation, the relative abundance”

This entire section has been revised and shortened.

Line 808 - change to “a myriad of bacterial sources”

This entire section has been revised and shortened.

Table 1 - Make the columns wider so that the cell contents all lie on one line. Table 1 caption - should this read “where  $p < 0.05$ ”

The table has been revised accordingly.

Figures 1,2,3 and 5 - Can you indicate the four water column zones on these figures. Perhaps with lines that join the specific depths at which the regions are defined (as was done in figures 4 and 6).

Figures 1, 2, 3 and 5 have been completely revised according to reviewer #3 request and the water column zones are now indicated.

Figure 2 - unnecessary brackets around nitrite in panel b

Figures 2 has been completely revised