

Interactive comment on “A quest for the biological sources of the ubiquitous long chain alkyl diols in the marine realm” by Sergio Balzano et al.

Sergio Balzano et al.

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Dear Reviewer 2

Thanks for evaluating our manuscript. You can find the responses to your comments

Sergio Balzano

Anonymous Referee #2 Received and published: 5 July 2018 Balzano et al. describe the attempt to attribute biological sources to long chain diols (LCDs), which are compounds with a great proxy potential and widespread both in the marine water column and sediments. The study relies on the analysis of suspended particulate matter in order to compare the distribution of concentration of LCDs to environmental parameters and abundance of potential LCD-producers. Unfortunately, this approach is not

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successful, and little information regarding LCD production can be gained. Instead, the authors provide an interesting discussion on the suitability of the combined biomarker-genetics approach. Overall, even if the results do not allow to narrow down the biological sources of LCDs, investigation is sound, and the paper is well written. A few comments are shown below.

Balzano et al.: We thank Ref. 2 for their positive comments on our manuscript. Please find below detailed answers:

Referee #2. -Abstract: In the abstract, the authors claim that “the contributions from two taxonomic classes to which known producers are affiliated. . . followed a similar trend to that of the concentrations of C30 and C32 diols”. This statement seems to suggest a source relationship. However, in the manuscript the authors inform that correlation is low (l. 531) and that it might be that “co-occurrences are simply driven by other environmental conditions leading to similar spatial distributions” (l.533). In my opinion both statements are not consistent and the abstract should be rephrased in order to clearly state that no informative correlation between LCD and putative LCD-producers could be established.

Balzano et al.. The reviewer is correct that in the abstract we suggest a relationship but in the manuscript we nuance this. We will rephrase the abstract to say that we did find a correlation between two OTUs from Chyrsophyceae with the major diol but that this correlation is weak and might be indirect.

Referee #2. Abstract: In the manuscript, three scenarios are discussed that explain why the correlation between LCD and potential LCD-producers is so weak: contribution of fossil LCDs, undersampling of potential LCD-producers because of their low number of rRNA gene copies per cell or LCD being produced by other species. However, in the abstract only the first hypothesis is mentioned. In my opinion, presenting all three scenarios would strengthen the manuscript.

Balzano et al.. We agree that all three scenarios should be mentioned in the abstract

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and will include this in the revised version.

Referee #2. -Discussion (from I.387 on): It is argued that the C28 1,13-diol can't be correctly interpreted because of its low abundance. However, C28 1,14-diol doesn't seem to be more abundant and is discussed with a lot of detail, and concerns regarding its abundance are not expressed.

Balzano et al. The C28 1,13 diol was only detected in 19 out of 71 samples, whereas the C28 1,14 diol was found, although often in low amounts, in all samples. We compared the distribution of the C28 1,14 diol with that of C30 1,14 diol, since both compounds can be biosynthesised by *Proboscia* spp. (Sinninghe Damsté et al., 2003). In contrast it has been suggested that the C28 1,13 diol can derive from the same organisms producing the C30 1,15 diol (Rampen et al., 2014). We could not compare the distribution of these two compounds because while the former was present below detection levels in 27 % of the samples, the latter was found in all the samples. We will clarify that the difficulties in the interpretation of the C28 1,13 diol are due not only to its low abundance but also to the fact that it was not detected in some of our samples.

Referee #2. -Discussion (from I.563 on): Regarding the possibility of fossil LCD contributing to the signal I have a few comments/suggestions. (1) Is there any information available on the residence time of SPM in a system like the one studied? Is the claim that LCD may accumulate as SPM for years (I. 581) consistent with such residence times? (2) Bale et al. (2018) employed very similar (or actually the same?) samples from the same location to study biological sources of cyanobacterial lipids and were quite successful. However, these lipids have also been shown to persist over longer time scales (e.g. Bauersachs et al. 2010). This should be mentioned and the difference to LCD discussed (3) I would appreciate some hypothesis on LCD production, even if they are fossil to some degree. Do the authors expect seasonal production and therefore absence of producers during sampling? Export from land/freshwater systems? Production by a small population and massive accumulation? Which are the sources fueling this hypothetical fossil pool of LCDs?

Balzano et al. 1) Unfortunately, we could not find any study reporting the residence time of SPM in the Amazon Shelf or in the area or the tropical north Atlantic Ocean studied here. Most studies focus on the turnover of dissolved organic matter rather than particulate organic matter. 2) Yes, Bale et al. (2018) analysed samples from the same (HCC) oceanographic cruise. The authors analysed intact polar lipids, which are more suitable indicators of “fresh” organic matter. 3) Most producers of the LCDs measured here were indeed unlikely to be present in seawater during sampling. Whether LCD-producers occurred during another period of the year, or in other locations, is unfortunately unknown, also as we do not know who the main producers are. Export from freshwater is unlikely because only 6 stations (7-13) were slightly affected by Amazon River as shown in the salinity profile (Fig. 1). In general, the Amazon River input is low for the period of the year in which sampling took place (Molleri et al., 2010).

Minor comments: Referee #2 -I.117 (also legend for figure 1), what does HCC stand for?

Balzano et al. That is the cruise name, it stands for HeteroCystous Cyanobacteria, which was the original focus of that cruise.

Referee #2 -when expressing ratios of, for example, solvents (e.g. I.159 “HCl: MeOH (1:1)”) empty spaces before and/or after the colon are not employed consistently. Please check throughout the text. Balzano et al.: Ok

Referee #2 -I. 184: as far as I know, it is recommended to write “m/z” (mass to charge ratio) in Italics. Balzano et al.: Ok

Referee #2 -I. 296. Please use either “Station” or “Stn.” consistently. Balzano et al.: Ok

Referee #2 -Figure 1: do de dots represent sampling depths? Please explain in legend. Balzano et al. :yes they do, we will clarify that in the legend.

Referee #2 -Figure 1: Bale et al. (2018) used chl-a obtained by fluorescence instead

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of the extraction-based approach used here, and those data seem to have a better coverage/ resolution. Could you please explain why you are not using them?

Balzano et al.: We preferred using Chl-a data based on methanol extraction and HPLC analyses as we could report these data as pg L⁻¹.

Referee #2: Should Figure 4 maybe also be in colour (like Fig. 1 and 2)?

Balzano et al.: Figs. 1-2 report different parameters which vary along a continuum (temperature, salinity, concentrations) whereas Fig. 4 is reporting the number of reads associated with specific taxa. We did not detect these reads in many samples, which appear in white and we do not see a specific need for using several colours.

Referee #2: Consider adding P-values to figure 5.

Balzano et al.: Ok, we will add this.

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