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Biogeosciences Discussions

Interactive comment on "Ecology and biogeochemistry of contrasting trophic environments in the South East Pacific by carbon isotope ratios on lipid biomarkers" *by* I. Tolosa et al.

I. Tolosa et al.

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Responses to the Anonymous Referee #2

1) The conclusion section should be much more specific in what the study has achieved. More important are which factors might affect the natural variability in carbon isotope fractionation among algal taxa? Which are important and which not? How did they impact fractionation?
Conclusions have been modified as it has been reported in point 7) from Referee #1.

2). **One of my major concerns has to do with the calculation of ε_p. This calcula-**

tion has a number of uncertainties (e.g. are the compounds really that specific for these groups of organisms) but two major ones are obvious for this paper: the δ^{13}C of CO_2 was not determined but estimated and the estimated difference between the ^{13}C of the compound and that of the biomass. Concerning the first point it is a great pity that a study seemingly designed to look at the isotopic composition of biomarkers simply did not measure the 13C of DIC. A discussion on potential errors in the estimates of 13C CO_2 and thus ε_p would be very useful to constrain the impact of this omission.

Concerning the specificity of the used lipid biomarkers, we have included a more detailed description on their sources and specificity (L. 309-322):

"In particular, we focus on the long-chain unsaturated methylketone ($C_{37:2}$ alkenone) which is a marker for certain haptophyte algae (Conte et al., 1995, Volkman et al., 1995), the $C_{28}\Delta^{5,24(28)}$ sterol and HBI which are major components in many diatoms (Volkman and Hallegraeff, 1988, Volkman et al., 1994), the sterol dinosterol mainly derived from dinoflagellates (Robinson et al., 1994) and the n-C17 alkane derived from cyanobacteria and green algae (Han and Calvin, 1969, Winters et al., 1969). We note, however, that HBIs are not markers for all diatom species since they are mainly synthesized by centric (Rhizosolenia species) and pennate diatoms (Haslea, Navicula and Pleurosigma), whereas $C_{28}\Delta^{5,24(28)}$ sterol has also been found in some dinoflagellates and green algae (Volkman, 1986). Therefore, there might be an offset between the diatom sterols and the HBIs depending on the diatom species composition. In a similar way, all diatoms do not produce the $C_{28}\Delta^{5,24(28)}$ sterol, and dinosterol can also be present in certain diatoms (Volkman et al., 1986)."

According to the concerns with the calculation of ε_p, we have now discussed the variability on the calculation of ε_p which takes into account the potential variations of δ^{13}DIC as well as variations in the offset between the δ^{13}C of the bulk organic matter and that of the biomarker. In our original manuscript and following the work of other authors, e.g. Harada et al. (2003, GCA, 67, 1507), we used the reference value documented by Craig (1970) for surface water of the South Pacific, which was +2.2 ‰. Now, in the

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revised manuscript, we adopt a lower value (1.5‰) reported by Quay et al. (2003) who showed in an earlier paper that the δ^{13}C value of DIC in surface waters of the Pacific Ocean has decreased by about 0.4‰ between 1970 and 1990 (Quay et al., 1992). We modified the text (L 223-240) as:

"We adopt a constant value of δ^{13}bicarbonate for all sites and depths based on a) the low variability of δ^{13}DIC reported for the surface waters of the global ocean, including Pacific (1.55‰), Atlantic (1.56‰) an Indian Ocean (1.37‰) (Quay et al., 2003), b) the major contribution of bicarbonate in the total DIC pool (90% of the total) and c) the low variability of δ^{13}DIC in the upper water column (Kroopnick, 1985). Although meridional δ^{13}C variability is generally greater than zonal variability, surface δ^{13}C in the Pacific ocean varied only by 0.3‰ over the latitudes of the studied area. However, field data of δ^{13}C DIC in the Peru upwelling region ranged from -0.65 to 0.81‰ (Pancost et al., 1997, Bidigare et al., 1997) whereas in the other areas of the Pacific, it ranged from 1.20 to 1.85‰ (Bidigare et al., 1997). Based on the extreme values of δ^{13}C of DIC, ε_p might have a maximum variation of 2‰, whereas the typical variation in δ^{13}C of DIC of $\pm$0.5‰ results in a ε_p variation of $\pm$0.7‰. Therefore, the isotopic composition of DIC does not seem to have a major influence on the changing isotopic compositions of organic matter in the upper water column of the ocean, and the likely lower values of δ^{13}C DIC for the upwelling site would only accentuate the differences between the trophic environments, providing lower ε_p values for the upwelling sites".

3) **A more important concern is the estimated difference between the δ^{13}C content of the compound and the biomass. The authors have only two compounds for which this estimate is somewhat constrained in the literature, i.e. alkenones (though the estimates vary from 3.8 to 5.8‰ depending on each study; see Riebesell et al., 2000) and the C17 n-alkane (though this was determined for only 1 cultured mesophilic cyanobacteria). Interestingly, the authors take the 4.2‰ offset determined for the alkenones also as being the offset for the other eukaryotic compounds (p.4661; line 10). This is in contrast to a number of other studies which show that isotopic compositions of phytol and sterols vary widely**

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compared to biomass depending on species (-2 to +8‰; Schouten et al.,1998, Geochim. Cosmochim,. Acta ; Riebesell et al., 2000, GCA; summarized in Hayes J.M., 2001, Rev. Mineral. Geochem.) and also ignores the fact that phytol and sterols are isoprenoid compounds with different biosynthetic pathways than straight chain compounds. In addition, the authors do also not take into account that the isotopic difference between compound and biomass is not always constant (see eg Riebesell, 2000; discussion in Hayes, 2001). Hence, there is a great uncertainty associated with the calculation of ε_p and I wonder how valid this calculations really are in discriminating ε_p between the various phytoplankton taxa. At minimum the authors should acknowledge these uncertainties and discuss the potential range in errors in their ε_p calculations. Alternatively, and perhaps preferably, the authors should restrict themselves to calculating ε_p based on compounds which would make comparisons of ε_p relative to each other more difficult and potential differences in growth rates between algal taxa.

We agree with this comment and we have modified the text to include and discuss the variability on ε_p (L. 241-274):
"$\delta^{13}C_{pp}$ (primary photosynthate) for eukaryotic organisms was calculated by using a constant isotopic fractionation of 4.2‰ between photosynthetic lipids and algal biomass. This value has been provided by Popp et al. (1998a) for alkenones and has been used by other authors (Bidigare et al., 1997; Benthien et al., 2002, Harada et al., 2003, Benthien et al., 2005, Popp et al, 2006). Similarly, we adopted this value for the isoprenoid compounds, e.g. phytol and sterols, used in previous papers (Pancost et al., 1997, Pancost et al., 1999, Bidigare et al., 1999), thus allowing a comparison of ε_p calculated in the present study. However, the offset in $\delta^{13}C$ values for common lipids relative to the $\delta^{13}C$ value of biomass might vary considerably between microalgal species, biosynthetic pathways, the site of reactions in the cell (Shouten et al., 1998; Hayes 2001), and by variations in the relative amounts of the major biochemicals in the cell (i.e., proteins, carbohydrates, and lipids) which in addition have different $\delta^{13}C$

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values. Thus, lower isotopic offsets between lipids and total biomass are expected to occur in nutrient limited environments where higher cellular lipid contents relative to proteins and carbohydrates are found (Livne and Sukenik, 1992). This variability might accentuate the range of ε_p between the trophic environments, with higher ε_p values in low-nutrient waters compared to high-nutrient environments. Potential variations of ± 1 ‰ in the isotopic shift between the algal biomass and lipids might result in ε_p variations of ± 1 ‰.

Culture studies of haptophytes have identified an isotopic shift ranging from 3.1 to 5.3 ‰ between primary photosynthate and alkenone biomarkers (Laws et al., 2001, Riebesell et al., 2000, Jasper and Hayes, 1990, and Popp et al., 1998). In contrast to alkenones, the isotopic offset between algal biomass and other eukaryotic lipid biomarkers is less constrained with reported offsets ranging from 2 to 8 ‰ for different cultures of phytoplankton taxa (Shoulten et al., 1998, Hayes, 2001). If we consider the upper and lower bound values of offsets found for phytol (-0.8 to 4.2 ‰) and diatom sterols (0.6 to 6.4 ‰) in marine diatom cultures, the extreme values of ε_p for phytol and diatom sterols differ by 5 and 6 ‰, respectively. One reported culture of dinoflagellate exhibited an isotopic fractionation between dinosterol and algal biomass of 4.5 ‰ (Shouten et al., 1998). For prokaryote organisms, δ^{13}Cpp was calculated from the n heptadecane supposing a constant isotopic fractionation of 8.4 ‰ reported by Sakata et al.,(1997)".

Overall, the carbon isotope fractionation for alkenones-producing haptophytes are little affected (± 1 ‰) by the variability of the offset between photosynthetic lipids and primary phtosynthate, whereas higher variability in the carbon isotope fractionation values for the diatom organisms might occur (up to 6 ‰). However, even if we take in account this variability, ε_p values for diatoms in the upwelling zone are always lower than those measured in the oligotrophic waters and therefore, the conclusions drawn from the previous results were not dependent on the data reduction.

4).**The isotopic composition of some biomarkers is already mentioned a few times in 3.1 and 3.2 while the real discussion takes place in 3.3. I would not**

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discuss the isotopes yet in 3.1 and 3.2.
We feel that some insights on the isotopic composition of some biomarkers in sections 3.1 and 3.2 are supporting the interpretation of these sections, and therefore, we consider that it is better to keep this insight as it was.

5) **They can still correlate ε_p to nutrients and CO_2 concentrations to investigate factors influencing isotopic fractionation. The discussion on the impact of nutrients and CO_2 concentrations on ε_p in 3.3. switches quite a lot and is not fully addressed. For example, on p. 4670, l. 9-16., the correlation for Si is given but not for the other nutrients as it is only stated that they are 8220;lower8221;. How much lower? Still significant? Perhaps the authors could give the results in a Pearson correlation matrix and indicate which correlations are reasonably significant (eg van Breugel et al., 2006, Am. J. Sci).**
We have carefully analysed the correlations between the ε_p and nutrients/CO_2. In fact, these correlations are driven by the gradient of the nutrients/CO_2 from oligotrophic to eutrophic sites. Yet all macronutrients have similar gradients and therefore we only discuss the correlation between nitrates and ε_p. In the revised manuscript, we have only included the ε_p values from the euphotic layer, considering that the eukaryotic lipids analyzed in samples from below the euphotic layer result from the synthesis, which took place within the euphotic layer and in the corresponding nutrient/CO_2 conditions. Correlations of ε_p in the euphotic layer vs nutrients were lower than those provided previously with samples from all depths. As it is shown in Figure 8, the high scatter of ε_p at lower nutrient concentrations and more consistently low ε_p values of the upwelling sites indicate general trends illustrated by the fitted curves. According to this, we have modified the text (L. 571-605) as:

"Plots of the carbon isotope fractionation of the different eukaryotic markers vs the three major nutrients in the euphotic layer showed similar trends. An example is given in Fig. 8 for the nitrate concentrations, showing a negative logarithmic curve for the diatom biomarker. ε_p values from nutrient-rich waters at eutrophic sites were

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much lower compared to those in nitrate limited conditions of the Gyre. However, in oligotrophic waters, the high scatter of ε_p indicates that other factors than the major nutrients are probably affecting the carbon isotope fractionation. This is illustrated by the small effect of nitrate concentrations on the carbon isotope fractionation of the haptophytes (alkenones).
The carbon isotope fractionation of eukaryotic markers showed also a negative trend with $[CO_2]_{aq}$. (Fig. 9). These relationships deviate from the previously reported general oceanic trend (Rau et al., 2001) and culture studies (Burkhardt et al., 1999a) where carbon isotope fractionation increases ($\delta^{13}C$ decrease) when $[CO_2]_{aq}$ increases. However, this apparent deviation has already been observed in Peruvian upwelling waters where it was suggested that a diatom carbon concentrating mechanism (CCM) was likely the cause of the lower ε_p of diatoms in these waters with high $[CO_2]_{aq}$. In the present study, we also observed a small effect of $[CO_2]_{aq}$ on isotope fractionation of alkenones, which agrees with other studies which privileged potential changes of ε_p due to growth rate and carbon uptake mechanisms in E. huxleyi (Benthien et al., 2007; Bidigare et al., 1997). A similar trend was found between $[CO_2]$ and the ε_p of n C17 (data not shown) which is consistent with a previous work (Popp et al., 1998b) who found for Synechococcus that ε_p is independent of the concentration of dissolved CO_2, likely because its cell geometry guarantees a large CO_2 supply."

6) **Even more importantly for p.4673 l6-12 where they discuss correlations but do not give any values about the degree of correlation. Significant? Is it the same correlation as Bidigare et al, 1997 and Benthien et al.? This would give much more value to this discussion.**
As it has been explained above, these correlations are dependent on the gradient of nutrients/CO_2 along the different trophic sites, and since the surface-water concentrations of dissolved CO_2 and nutrients are positively correlated in the oceanic waters, we decided not to discuss the significance of the different correlations. Moreover, these correlations are based on b-values, which are distributed into two well defined

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clusters, corresponding to the two contrasting environments, the oligotrophic and the upwelling sites. Data with intermediate values to corroborate the observed trend is lacking. According to that we have modified the text (L 694-711) as:
"The b values for the alkenone synthesizer phytoplankton were well distinguishable between the two contrasting environments: low at the oligotrophic sites and a high value in the upwelling zone. Due to the natural correlation between concentrations of dissolved CO_2 and nutrients, b values obviously co-varied with the concentrations of silicate, nitrate and phosphate. However, at the very low phosphate levels (<0.4μM) of the oligotrophic sites, b values showed relatively high variation (75-160$‰\mu$mol kg^{-1}) and compared very well with the corresponding values reported by Bidigare et al.1997, but also with those from other oligotrophic areas (Laws e al., 2001, Benthien et al., 2002). This confirms the interpretation given by these authors that growth rates may be controlled by some trace micronutrient (e.g. Zn) (Bidigare et al., 1997; Shaked et al., 2006), and/or that adaptation of the phytoplankton physiology to the low nutrient waters might result in higher variability in the efficiency of the different carbon uptake mechanisms".

7) **One factor which is not discussed is light limitation which, in cultures, has shown to give different fractionation patterns than with nutrient-limited cultures. This discrepancy has to some extent been resolved by Cessar et al. (2006, Geochim. Cosmochim. Acta) and some discussion on this with respect to this data would be useful.**
We agree that light might affect the carbon specific growth rate of microalgae, especially under nutrient-replete conditions and light limitation. For the issue of light energy effects on ε_p, we have included the photosynthetically active radiation (PAR) parameter at each site in Table 2. According to this point we have included the following paragraph in section 3.3. (L. 615-626):
"Light is another factor which may decrease the carbon isotope fractionation under low-light saturation levels since it has opposite effects on the ε_p compared to nutrient-

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limited conditions (Rost et al., 2002, Cassar et al, 2006). However, despite light limiting conditions at 40 m (Table 2), phytoplankton sampled at these depths are not necessarily light limited. In hydrodynamically active zones like the upwelling, it can reasonable be admitted that phytoplankton cells produce under light conditions averaged over the mixed layer and not encountered at the depths they were sampled. Hence, it can be excluded that irradiance affected the isotopic fractionation of the different phytoplankton taxa in the upper mixed layer of the upwelling area ".

We have also discussed the effect of light on the distribution of δ^{13}C of alkenones in the gyre (Fig. 6, L. 508-516) as follows:

"Figure 6 illustrates the carbon isotope composition of the diunsaturated alkenone together with the total concentrations of $C_{37:2}$ alkenones. More enriched $\delta^{13}C$ values were obtained for alkenones measured at the depth of the chlorophyll maximum, whereas the higher concentrations of alkenones found at 125 m depth were associated to lower $\delta^{13}C$ values. Change in irradiance could partially explain the abrupt change of the carbon isotope composition of the alkenones, since lower photon flux density leads to a lower ^{13}C discrimination increasing the $\delta^{13}C$ values (Rost et al. 2002, Thompson and Calvert, 1995). As it is shown in Table 2, the % PAR values at depths higher than 125 m were lower than 1%, which would mean that haptophytes were light-limited at these high depths. Consequently, the more enriched $\delta^{13}C$ values of the alkenone markers at the depth of the chlorophyll maximumm might infer that haptophytes are under limited light conditions."

8) **Regarding the discussion on the origin of biomarker lipids there is often reference to data published in separate accompanying papers. Presumably these will become available for inspection once they are published but for the reader it would be quite helpful if these data were presented in the figures in some form or another. Eg. P. 4665, l. 12.**

The diagnostic pigments as well as other references used in the discussion that are

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referred to in the text have been recently published in the same special issue of the BIOSOPE project (Grob et al., 2007, Gomez et al., 2007, Ras et al., 2008, Van Wambeke et al., 2008). However, we have shown the 19'HF profile because it provided a particular insight for the discussion of our alkenone-producing haptophytes distribution. We decided to show quantitative data used to underpin our findings and to reference data for a more used qualitative discussion.

9) **Title: A bit of an odd title and awkwardly phrased. I presume you are not looking at the ecology of an environment but of phytoplankton? Furthermore, I prefer the phrasing 8220; stable carbon isotopic compounds of lipid biomarkers8221; p. 4654, l. 2: remove different p. 4656. L. 14-16. I do not think your study evaluates sources of organic matter but rather sources of biomarker lipids as outlined later. I suggest to rephrase the last paragraph.**
According to these suggestions, a) we have modified the title to: *"Distribution of lipid biomarkers and carbon isotope fractionation in contrasting trophic environments of the South East Pacific",* and b) we changed the concerned phrasings and rephrased the requested paragraph within the text: *"Our field study uses molecular and stable carbon isotopic ratios of specific lipid biomarkers to evaluate their organic sources and to explore variations in the biogeochemistry of the particulate organic matter in the different hydrodynamic and trophic environments from the South East Pacific".*

10) **p. 4660. L. 14. Note that TMS is actually not a very good derivatization agent for isotopic analysis though likely in this study it will not have a great impact (Shinebarger et al., 2002, Anal. Chem. p. 6244).**
It has been reported that TMS derivatives are not well suited for combustion interfaces as the formed SiO_2 can deteriorate the oxidation reactor, by sequestering C in the combustion tube during the oxidation process of compounds. As a result, some likely alteration on the measured ^{13}C might occur. However, this drawback should be minor with a regular oxidation of the oxidation reactor and in the case of sterols, where the

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great number of carbons (at least 27) minimizes the impact. Moreover, a great number of authors are using this derivatization agent for GC/C/IRMS because of its advantage of not having kinetic isotope effects (Rieley, 1991, The Analyst, 119, 915 - 919; Jones et al., 1991 Biol. Mass Spectrom., 20, 641-646, 1991.; Pancost et al., 1999; Pancost et al., 1997; Riebesell et al., 2000; Schouten et al., 1998).

11)**p. 4660, l. 20; Besides abundance, the isotopic data of sterols are notoriously difficult to obtain due to co-elutions of other sterols. Perhaps it would be useful to provide some comments on this or a chromatogram in the supplementary material showing how the sterols are separated.**
We agree that isotopic data compounds that co-elute are not accurate and it will depend on the abundances of both compounds. We have now specified within the text and illustrated in Annex A, that the major compound 24-methylcholesta-5,24(28)-dien-3ß-ol ($C_{28}\Delta^{5,24(28)}$) stenol was integrated together with their minor stanol pair compound (24-methyl-5α-cholest-24(28)-en-3ß-ol, $C_{28}\Delta^{24(28)}$) to yield a single $\delta^{13}C$ value for both compounds because of incomplete chromatographic separation. The other target sterol compound, dinosterol ($C_{30}\Delta^{22}$), was well separated chromatographically from other compounds.

12)**p. 4663, l. 9: C25 HBI are excellent biomarkers for diatoms (Volkman et al., 1994; Org. Geochem.; publications by the Plymouth group) and I wonder why the authors did not report their isotopic compositions and estimated ε_p. This would make a nice comparison with the 8220;diatomsterol8221; data.**
Unfortunately, we could not provide the isotopic composition of the C25 HBI from the different sites because of their low peak abundance in the oligotrophic areas which prevents reliable and accurate $\delta^{13}C$ data measurements.

13) **p. 4664, l. 6. Linear alcohols p. 4664, l. 10: I am unfamiliar with the idea that monounsaturated C20 and C22 fatty acids are markers for herbivorous zooplankton. Are there more examples of this besides the Lee et al. reference?**

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More references providing examples on the C20:1 and C22:1 fatty acids as markers of herbivorous mesozooplankton are:
Graeve, M., Hagen, W. and Kattner, G.: Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods, Deep Sea Research Part I: Oceanographic Research Papers, 41, 915, 1994.
Dalsgaard, J., John, M.S., Kattner, G., Müller-Navarra, D. and Hagen, W.: Fatty acid trophic markers in the pelagic marine environment, Advances in marine biology, 46, 225-340, 2003.

14) **p. 4665, l. 15. The same, I am unfamiliar with this ratio, please provide a reference. Cholesterol is also abundantly present in algae (eg Volkman et al., 1986; Org. Geochem.).**
Although cholesterol is present in many classes of algae, it is considered a typical marker molecule for zooplankton derived organic matter supply because its concentration becomes enriched after passing the organisms in relation to the algal diet (Harvey et al., 1987). Therefore, the relative abundance of cholesterol over phytosterols has been used as a relative indicator of the presence of zooplankton over phytoplankton (Muhleback and Weber, 1998; Tolosa et al., 2003, Tolosa et al., 2005). This statement has been introduced in the revised version.

15)**p. 4666, l. 22-24. Interesting conclusion. Does this mean that the isotope values of lipids perhaps also represent a living and fossil component and thus not always match in situ conditions?**
Since a lipid molecular peak might include both the living and detrital component, the isotope values will integrate the mixed components, representing the in situ conditions of the environment. For instance, in the euphotic zone, it will mainly represent the living component whereas in the deeper zone of the water column, it will represent more the fossil component, depending on the extent of degradation of the compounds within the water column which varies from compound to compound.

16)**p. 4668, l. 21: As far as I know the UK37 is not a growth index.**

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We agree with the reviewer and we have now corrected the mistake.

17)**p. 4667, l. 19: I do not think you want to distinguish between different CO_2 fixation pathways as you are only looking at compounds produced via Rubisco. I presume you mean CO_2 indirectly via bicarbonate or via diffusion.**
We assumed that the reviewer is referring to page 4669, and not 4667. Anyway, at the beginning of section 3.3, we introduced the carbon isotope fractionation produced by all different pathways in a general way, and not only that produced via Rubisco. Therefore, we consider worth leaving here the sentence as it was.

18) **Tables 4-5. A large number values are reported in too many significant numbers here (e.g. 15.76 ng l^{-1} for concentration or 1.6 d^{-1} for growth rate). Please decrease this to a sensible number.**
We have now decreased the significant numbers on Tables 4-5.

Interactive comment on Biogeosciences Discuss., 4, 4653, 2007.