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Biogeosciences Discussions

Interactive comment on "Inter- and intra-specimen variability masks reliable temperaturecontrol on shell Mg/Ca ratios in laboratory and field cultured*Mytilus edulis* and *Pecten maximus* (bivalvia)" *by* P. S. Freitas et al.

P. S. Freitas et al.

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We appreciate the thoughtful comment from both referees and hope we are able to properly address the issues raised. We have revised the manuscript carefully considering the advice and criticism offered by the referees. Below we explain the changes made in accordance to the comments by the referees.

C. Lazareth REFEREE (#1) COMMENTS Specific comments Major points "Oxygen stable isotopes: First of all, I will remove the whole part(s) concerning d18O because this is not the point here. These results do not support the problematic raised by the authors. In addition, there is only one sentence in the discussion talking about these

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results, L 1-2 p. 550; which, in my point of view, is irrelevant. Nothing in the abstract or conclusion concerning d18O so clearly must be removed unless more discussed. If the authors still want to keep these results, I would like to know which d18Oseawater they used when they talked about "d18Ocarbonate minus d18Oseawater". Do they used a mean d18Oseawater for all points or used mean d18Oseawater per growth period? As d18Oseawater was regularly measured, precise d18Oseawater can be used for each analytical point."

-Our original intention was to use the shell oxygen stable-isotope ratios to demonstrate that the new shell growth was laid down under oxygen-isotope ratio thermodynamic equilibrium. We acknowledge that we have not discussed these data in detail and in agreement with the referee have removed these data from the text. As a point of information: we used mean ?18Oseawater per growth period, the shortest temporal resolution record that relates to the shell samples.

"Shell ages: Are the specimens used not all juvenile? Something should be said about that as differences might be observed in shell chemistry before and after sexual maturity. Should be discussed somewhere."

-All specimens were from the same cohort and were less than one year of age at the time of the experiments, as stated in Sections 2.1 and 2.2. If significant differences occur in shell elemental composition in specimens before and after sexual maturity, this potentially could contribute to differences in Mg/Ca between the present study and previous studies (e.g. Lorrain et al., 2005 and Freitas et al., 2006) that used mature animals. However, whilst Mg/Ca data from laboratory- and field-cultured M. edulis specimens in the present study were similar, the latter animals may have reached sexual maturity during the present study. The relevant text in the manuscript has been changed (Section 4.1).

Environmental parameters: "L 11-13, p. 536 it is said that "animals of similar size were moved into separate aquaria each under different but constant temperatures and

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controlled food and light conditions". If temperature and food conditions are described, this is not the case for light. What do you mean by "constant light conditions"? Not discussed further. "

-Light was held constant during the experiments, i.e. there were no diurnal cycles. Tanks were covered with a mesh to shadow the animals and reduce light levels. The relevant text in the manuscript has been changed (Section 2.1).

"Concerning food, it is said that a constant quantity of food was supplied to the specimens but what about possible food supply via the natural seawater pumped into the aquariums? "

-Seawater is pumped from the Menai Strait into large holding tanks, where it resides in the dark for a few days; consequently, microalgae are unable to photosynthesise and the large majority of particles settle in these tanks during this period and do not reach the experimental aquaria. Nevertheless, a small amount of food, most likely as very small particles (biogenic or abiogenic in origin), may reach the aquaria. From practical experience this represents a very small contribution to the total food supply and the condition of any non-feed animals (i.e. without a supply of food other than any particles brought into the aquaria with the supply water) will degrade very rapidly. The relevant text in the manuscript has been changed (Section 2.1).

"The possible (or not?) influence of food intake, that is most probably different from animal to animal, on shell Mg incorporation is not discussed. That could be an additional hypothesis to explain the discrepancies observed (at the different levels). "

-Although the aquaria were vigorously mixed to maximize the homogeneity of food in the aquaria, it is likely that food intake varied from animal to animal. However, it is possible to assume that differences in food intake between animals most likely would be reflected in differences in growth rate and metabolic activity. We did not find any significant relationship between Mg/Ca ratios and either shell growth or respiration rates (the latter a measure of metabolic activity, unpublished data). The relevant text in the

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manuscript has been changed (Section 4.2).

"As natural seawater was supplied, what about possible Mg/Ca water content changes?If no important freshwater inputs are observed in the region, should just be said somewhere that Mg/Ca seawater content is constant. "

-Seawater salinity varied between 31.9 and 33.2 in the laboratory experiment and between 31.1 and 33.6 in the field experiment. Due to the conservative behaviour of seawater Mg and Ca concentrations Mg/Ca ratios are not expected to change with variable salinity, although the absolute concentrations of Mg and Ca can change with salinity. We did not observe any influence of salinity on shell Mg/Ca ratios. The relevant text in the manuscript has been changed (Section 4.2).

"Nothing on possible water pH changes and potential influence on shell Mg/Ca ratios."

-We measured pH (unpublished data) during both the laboratory and field experiments, but this seawater parameter did not influence shell Mg/Ca ratios. pH varied between 7.83 and 8.11 in the laboratory experiment and between 7.87 and 8.36 in the field experiment. The relevant text in the manuscript has been changed (Section 4.2).

Analytical points: "I find the analytical methodology description hard to follow or even not clear at all (p.542). "

-We have improved the clarity of the text in this section.

"L 3: You talk about "synthetic standard solution in the range of 0t25 mmol/mol for Mg/Ca". Apart of the "t" in "0t25", which is probably a comma in fact, you are not giving a "range" but a single value and I wonder why you are giving a ratio (Mg/Ca) rather than a Mg concentration, as you do for Ca. "

-The t is a formatting error; the text should read "0-25 mmol/mol for Mg/Ca", which is the range of Mg/Ca ratios used for the calibration. Mg/Ca ratio calibration was completed according to the intensity-ratio method described by deVilliers et al. (2002), an approach based on molar ratios rather than concentrations and which results in more

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precise molar ratios. Mg concentrations can be easily determined from the Mg/Ca molar ratio and Ca concentrations.

"What are the "N" you are giving into parenthesis (and this question is valuable all along the paper)? Do you mean you analyse 304 std solutions (so, much more than the number of your shell sample)? "

-N is the number of samples, defined implicitly in Section 2.3.2.

"L 5: "The smallest milled powder samples were analysed at 30μg/ml"; 30μg/ml of what? Of Ca? So less than your less concentrated std solution? "

-By N=304 we mean that 304 samples were analysed at a Ca concentration of 60 μg/ml, 161 at a Ca concentration of 50 μg/ml and 102 at a Ca concentration of 30 μg/ml. Calibration was done at Ca concentrations of 60, 50, and 30 μg/ml. We accept that the manuscript text is not very clear on this subject and have removed ambiguity.

"L 8: The intermediate calibration standard is at "16 mmol/mol" of Mg/Ca (not specified). Either you specify at the beginning of the paragraph that all data in mmol/mol will stand for Mg/Ca ratios or you must specify each time. "

-The manuscript text has been changed.

"L 9: "and data then were corrected accordingly" Which means? What percentage of derive was set as the maximum authorized?"

-Following ICP-AES analyses, we checked and corrected for signal intensity drift. We assumed a linear change in the signal intensity, derived from individual element emission lines, between two consecutive intermediate standard solutions (each pair of these solutions bracketing a number of sample unknowns). Sample data were corrected accordingly, i.e. by the fraction of total change that corresponded to their sequence position between their two bracketing intermediate standard solutions. In addition, we included additional standard solutions and CRM's as unknowns within the sample sequence to check the validity of the drift correction procedure. Table 2 confirms the

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precision and accuracy of the Mg/Ca ratios obtained in this study.

"Analytical precision (expressed as relative standard deviation or RSD) was 0.5% for the laboratory cultured specimens (N=86) and 1.3% for the field cultured specimens (N=29)." How was this analytical precision calculated? Are these RSD the ones from different replicates of each sample analyses (e.g. mean of 3 replicates per unknown solution)? Why is this analytical precision different between laboratory and field specimen?"

-Analytical precisions were obtained from synthetic std solutions run as unknowns. These RSD values are not the ones derived from replicate sample analyses, which we have termed sample precision. The analytical precisions are different between the laboratory and field experiments simply because these samples were analysed during different sessions on the ICP-AES. The manuscript text has been changed to show this difference.

"In the laboratory culturing experiments, sufficient material was not available from any one growth interval to enable replicate analyses for an assessment of true sample precision; in the field experiment, however, sample precision was better than 6.2% RSD for replicate measurements (N=3) of the same milled powder samples obtained from five M. edulis specimens." Can be cut into two sentences and not clear enough. If I understand well (still, we must guess), you managed to have one large powder sample that allowed you 3 replicate measurements but 3 replicate measurements of the same solution or do you split you powder into 3 (which is probably what you have done)? And you obtained such large sample for 5 shells so you have 5 measures of the true sample precision? That's it? If this is 3 replicate measurements of the same solution (I don't think it is), 6.2% is quite a "bad" value. If this is analyses of split milled samples, this value is understandable. "

-The referee is correct in her interpretation of the meaning of the sample precision values. For five of the field experiment M. edulis shells it was possible to obtain large

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powder samples that subsequently were each split into three powder sub-samples that were analyzed as independent solutions. The clarity of the relevant manuscript text has been improved.

"As this part is already confused, I would remove the results concerning the ongoing international calibration study (even if I can understand that the authors wish to get those data published). In addition, these results are of course not discussed. That will also remove a table. For me, these results are unnecessary here. "

-We disagree with the referee's suggestion. Inclusion of the CRM data illustrate the accuracy of the Mg/Ca molar ratios measured in this study and permit comparisons with previous and future studies; such comparisons are not possible without such an approach. Furthermore, in view of the variability of Mg/Ca ratios evident in the present study, it is fundamentally important to justify the accuracy (and precision) of our Mg/Ca measurements. Also, these results contribute to the ongoing evaluation of CRM's between different research groups.

"You referred to Freitas et al. 2005, 2006 for accuracy but that would be nice to specify here that accuracy."

-The manuscript text has been changed.

"In fact, a kind of summary of the analytical precision and accuracy (table or even just a final sentence?) would be nice also. "

-Such a summary can easily be evaluated using Table 2.

"In Figure 6 and 7, you talk about twice the analytical precision which corresponds to 0.1 mmol/mol for Mg/Ca, why not give that in the text?"

-In the manuscript text we stated the analytical precision as a percentage. In (old) Figures 6 and 7, for clarity and necessity, we have presented the precision for the intermediate std solution as ś one standard deviation.

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"I would find a kind of recapitulative table, with mean Mg/Ca results from that study and those from previous work, really helpful for the reader, particularly to follow up the discussion. That would also allow the authors to reduce the text somehow. "

-The referee has not been explicit in her suggestion and it not clear to us what data would be included in such table. Consequently, we feel unable to address this comment further.

"Same thing could be done for regressions (quite heavy in the text)."

-A new table presents a summary of the regressions of Mg/Ca with temperature.

Other points (in the order of appearance in the text) "L 20-21, p. 533: The references cited are all about foraminifera and corals. Is it not possible to add a reference on bivalves? "

-Citations to Weidman et al. (1994) and Schone et al. (2004) are now included.

"L 24, p. 538: "each shell was identified by a mark hand drilled on its surface" Didn't this handling involve stress in the animals? Is there something identifiable at the shell surface, for shell secreted after this drilled mark? "

-We have assumed any such stress to have been small and restricted to the marking event that took place prior to the experimental period. The drill mark was superficial and did not disturb subsequent growth. Natural features and marks present on the shell outer surface potentially could have been used to identify individual animals, but the risk of misidentification was considerably higher. Drill marks allowed us to identify individual animals unequivocally during the experiment.

"L 18-19, p. 539: I do not understand what you mean by saying that: "by assuming shell growth rate to be constant during each growth interval" here. "

-We have assumed shell growth to be continuous between two consecutive measurements of shell size, i.e. during one growth interval, and that it occurs at a constant

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rate. Therefore, we have assumed the incremental change in size with time during one growth interval to have been linear. The clarity of the relevant manuscript text has been improved.

"L 11, p. 540: "depth and width of milling were controlled carefully": be more specific please. "

-The depth of milling was controlled, as best as possible for hand-milling, by milling under a binocular microscope. It was possible to make an estimate of the depth of milling relative to the diameter of the drill bit which was 0.6 mm. Width of milling across individual shells was kept as similar as possible and powder samples were collected from shell sections with the smallest curvature. In addition, we sectioned several shells along the main growth axis and cut through the milled sections; a scanning electron microscope then was used to accurately assess the depth of milling. Nevertheless, we have toned down this text in the manuscript.

"L 23, p. 540: "for measurement purposes": It is said L. 17-19, p. 537 that "Each time the M. edulis specimens were removed from the aquaria they were exposed to the air for 5 to 6 h" Five to six hours for "measurement purposes"?"

-M. edulis were intentionally exposed to the air for 5 to 6 hours to develop growth marks in their shells that then could be used as a fixed time marks during the experimental period. This duration of exposure resulted in the deposition of a visible growth mark, and in that sense was for shell growth measurement purposes.

"L 3-7, p. 541 (last sentence before § 2.3.2.): So you mean that the disturbance marks where not sampled? Specify if this was intentional (I guess so) and why. "

-We intentionally avoided sampling of powders from shell deposited during periods of air exposure, in order to minimize sampling of regions of the shell that might have had Mg/Ca ratios influenced by the disturbance mark, i.e. due to the findings of Lorens and Bender (1980) that "transition zone calcite" can exhibit more variable Mg/Ca ratios. The

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clarity of the relevant manuscript text has been improved.

"L 14-15, p. 541: "On such occasion..."; not clear to me. "

-This text has been changed to read: "Whenever the amount of shell growth permitted more than one sample was collected from a single growth interval (2=N=4)."

"Title 2.4, p. 541: I would change it by "Mg/Ca analyses" (or at least change "elemental ratio" by Mg/Ca). "

-The referee's suggestion has been accepted and a change made to the manuscript text.

"L 8, p. 545: Why not give a mean (all analyses species by species for each given temperature) ´s 1 sigma;? That will be clearer. "

-The referee's suggestion has been accepted. The manuscript text now reads: "Variability of shell Mg/Ca ratios at each temperature is very large for both species (Figs. 6a and 7). Mean Mg/Ca ratios (ś one sigma) at each temperature were for M. edulis 4.70 ś0.83 mmo/mol at 10.76 ś0.41°C, 4.03 ś0.86 mmo/mol at 11.96 ś0.12°C, 5.25 ś0.74 mmo/mol at 15.61 ś0.12°C (experiment one), 6.32 ś1.33 mmo/mol at 15.54 ś0.25°C (experiment two), 5.72 ś0.65 mmo/mol at 18.39 ś0.05°C, 7.73 ś1.03 mmo/mol at 20.23 ś0.22°C; and for P. maximus 15.88 ś4.02 mmo/mol at 10.76 ś0.41 °C, 17.11 ś3.62 mmo/mol at 15.54 ś0.12°C, 21.00 ś3.72 mmo/mol at 20.23 ś0.22°C."

"L 16-18, p. 545: I do not find the statistic results for significant differences for Mg/Ca ratios between experiments two and experiment one. And are Mg/Ca ratios between experiments one and two really significantly different for around 12 and 15_C? No so evident on Fig. 6a, particularly for 12_C for which Mg/Ca ratios seem similar for both experiments. And not discussed."

-Statistical data for such a difference are shown later in the manuscript text, starting at line 14, p. 546. The first paragraph of Section 3.2 was solely to present the range of Mg/Ca ratios and a summary of the main results, which then are developed further in

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the remainder of this section. Comparisons were made for the regressions of the two experiments and the only temperature present on both experiments was 15°C.

"L 7-8, p. 546: This sentence is a repetition of the L18, p. 545. "

-The sentence has been deleted.

"L 7-8, p. 549: "i.e. between milled samples...shell level)." You want to say for the monitored laboratory experiment at a constant water temperature here? Because otherwise Mg/Ca changes could be related to water temperature changes (in the field experiment). "

-The referee is correct that we meant for the laboratory culture experiment only and the manuscript text has been changed.

"L 26-27, p. 549: What are the N here (because it refers to the Freitas et al., 2006 work)? Where can we find in the Freitas et al., 2006 study the "Mg/Ca ratios varied by up to 4.06 and 5.61 mmol/mol"? On their Fig. 8? "

-N is the number of samples. The Mg/Ca data can be found in the supplementary data table in Freitas et al. (2006).

"L 29, p. 549: Mg/Ca ranges for field-cultured M. edulis shells (this study) are given (start of § 3.3) but intra-individual variability are not given as numbers, that complicates the comparison with previous data given in the former § p. 549. Also, former studies results are given as "Mg/Ca ratios differences". I found these ratios for the present study for the laboratory shells only. "

-Data for Mg/Ca ratios, temperature, shell growth rate and salinity from the laboratory and field experiments are presented as supplementary material. "P 550: Grey points on Figure 8 are not discussed (and are not specified in the figure caption also). "

-In the legend of Figure 8b the grey dots are identified as data from field-cultured M. edulis. The figure legend has been increased in size to improve clarity.

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"L 15-16, p. 553: "with only a weak correlation for P. maximus (r2=0.21, p<0.001)." A word could be added on the relationship observed between P. maximus Mg/Ca ratios and salinity by Freitas et al., 2006. "

-The manuscript text now reads "The strength of this correlation between shell Mg/Ca ratios and salinity is, however, of comparable magnitude to that observed between temperature and shell Mg/Ca ratios (r2=0.21, p<0.001) and slightly lower than previously observed for shell Mg/Ca ratios and salinity (r2 = 0.36, p<0.001) in P. maximus (Freitas et al., 2006)."

"L 10-22 p. 554: Background on small scale Mg heterogeneities in M. edulis shell is given here. But the points reported in that paragraph are not discussed in relation with the present study. So, either the authors add some discussion or that paragraph can (must?) be omitted. "

-Text has been added to the discussion to make this text more related to the present study.

Technical corrections "L 19, p. 534: in the parenthesis citing "Klein et al., 1997; Immenhauser et al., 2005"; I would add an "e.g." at the beginning, because there are other. " -The referee's suggestion has been accepted.

"L 24, p. 534: is "M. edulis. edulis species" correct? (Twice "edulis")" -Second edulis has been deleted.

"L 13, p. 541: I would add a comma after "permitted"." -The referee's suggestion has been accepted.

"Title 3.2; p. 545: change "calcite mg/ca" by "calcite Mg/Ca"" -The title cases have been corrected.

"Equation (2), p. 546: parenthesis missing. "N" still undefined. " -The referee's suggestion has been accepted.

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"L 15, p. 547: Shouldn't the authors refer to the figure 6b here rather than on figure 6 in entirety? " -The referee's suggestion has been accepted.

"L 11, p. 552: "This consideration thus prohibits", why "thus" here? " -The word has been removed.

Figures: "Figure 1: Can you increase the police points for the names? " "Figure 2: Same thing. T1; T2, T3, M really smalls. " -The referee's suggestions have been accepted.

"Figure 4b: Two shells from the short-deployment experiment were analyzed. Should be indicated in the figure caption (only one curve). " -The figure caption has been amended.

"Figure 6b: Why not reduce the Mg/Ca scale (from 0 to 10 mmol/mol), because we can Not see anything like that! I do not understand "Each point represent...individual growthinterval"." -The referee's suggestion has been accepted.

"Figure 7: On the graphs, "´s 1 sigma" unreadable. And, is it not 2 sigma? (in the caption,you said "twice the analytical error"). The ´s 0.1 mmol/mol analytical error is not clearly stated in the text. " -The error plotted is ś 1 sigma; the size on the figure has been increased.

"Figure 8b: No reference for the grey points (M. edulis; Menai Strait). What is the dashed line? As individual points of the Vander Putten et al., 2000 study are not shown, why not display their results as a light shaded area? Legend of the points on the figure (8a and 8b) quite small. " -The grey points are the data presented in this study. The size of the points in the legend has been increased. The dashed line shows the relationship between Mg/Ca and seawater temperature from Vander Putten et al. (2000); In the absence of individual data points we used this relationship to present the variation of Mg/Ca with temperature presented by Vander Putten et al. (2000).

References "L 27, p. 556: change "Debeney" by "Debenay" L 17, p. 557: "Elferfield et

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al. 2002"; cited in the text as "Elderfield et al. 2001" (L 10, p.534) L 21, p. 557: "Erez et al., 2003"; cited in the text as "Erez et al., 2005" (L 14, p. 534) L 1, p. 558: "Gardner et al., 1992"; change page number from "219-213" by "219- 243" L 12, p. 559: change "Papanicolau" by "Papanicolaou" L 9, p. 560: change "Oxyegn" by "Oxygen" (twice)" -All of these changes have been made to the manuscript text.

Anonymous REFEREE (#2) COMMENTS "I have only a few minor comments: - In the 2.1 Subsection, nothing is said about mortality rates of specimens. " - Mortality rates during the experimental period were zero for both Mytilus edulis and Pecten maximus.

" P. 539, last line: as described in section something is missing. " -The text has been removed

" P. 540, lines 8-9: why at 200 μm? how can the authors determine this depth under the binocular? " -It was possible to estimate the depth of milling relative to the diameter of the drill bit, which was of a 0.6 mm diameter. In addition, we sectioned several shells along the main growth axis, cutting through the milled sections, and used a scanning electron microscope to accurately record the depth of milling. Nevertheless, we have toned down this text in the manuscript.

" P. 540, line 25: has instead of have? " -The manuscript text has been changed.

" P. 542, line 4:0t25mmol, is this right? " -The manuscript text has been changed to read "...0-25 mmol/mol...".

" Regarding preparation methods, a photo showing the growth ruptures would help. Have the authors taken into account that the growth margins of both species are different,i.e., acute wedge-like in Mytilus and abrupt in Pecten? " -We do not believe that a photo of growth ruptures adds anything substantial to the manuscript, since the milling of carbonate powders avoided these regions of the shells. We are aware of the differences in the growth margins of the two contrasting species; however, such differences are not significant in this study since the powder samples were obtained by milling the

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outer surfaces of the new growth region in the shells.

" P. 544: there is no comment to figure 4b. " -A comment on Fig. 4b appears on P547, line 18 in Section 3.3, which describes the variation of Mg/Ca ratios from the field-culturing experiment.

" P. 547: line 10: in lower case (records). -The manuscript text has been corrected.

" P. 547: line 15, Fig. 4b is referred, but not commented on earlier. " -Only in Section 3.3 do we present Mg/Ca data from the field-culturing experiment, as such it does not make sense to comment on Fig 4b any earlier in the manuscript text.

" P. 553, paragraph beginning in line 3: when speaking of salinity, which is exactly meant, Mg/Ca? or salt concentration? May be the papers of Stanley & Hardie, 1998, Palaeo-3, 144, 3-19, and Stanley et al, 2002, PNAS 99, 15323-15326, should be cited in this context. " -We refer to salinity in terms of salt concentration, not Mg/Ca ratios. Mg/Ca ratios should not change with variations in salinity, although the absolute concentrations of Mg and Ca in seawater could change. We do not believe that the papers cited by the referee are pertinent to this study.

" P. 555 lines 18-19: where are the data backing this conclusion provided? " -These data are discussed in Section 4.2.

" P. 557: Elderfield 2002 appears as 2001 in p. 534. - Figure 5: Wanamaker appears incorrectly as Wannamaker in the figure and in its caption. " -The Elderfield reference on P534 was incorrect and has been changed to 2002. Figure 5 has now been deleted from the manuscript.

" Figure 8a is exactly the same as 6a. One of them should be removed. " -The separation of data into Figures 8a and 8b was done for clarity and we think it justifies the use of figure 8a.

Interactive comment on Biogeosciences Discuss., 5, 531, 2008.