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BGD 12, C6548–C6564, 2015

> Interactive Comment

Interactive comment on "Seagrass beds as ocean acidification refuges for mussels? High resolution measurements of pCO_2 and O_2 in a Zostera marina and Mytilus edulis mosaic habitat" by V. Saderne et al.

V. Saderne et al.

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We are very thankful to the two reviewers for offering generous recommendations on how to improve the manuscript and figures.

Referee 1 mostly suggests that, beside solid datasets, the article structure requires revision and clarification.

General comment is that the objectives of the manuscript are not clearly enounced and targeted, leading to organization and clarity issues



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We understand and agree and wish to thank the referee a lot for pointing this out so that we could make important improvements to the article. The article is torn between the primary aim of the study as enounced p11427-I15-20 and a discussion on inconsistencies encountered between the different variables of the carbonate chemistry measured. The latter became necessary due to the over-determination of the carbonate chemistry; measuring 3 variables, 1 continuously and 2 punctually. Referee 1 considers that the methodological part on comparing in situ carbon parameters are, despite its interest, not expected from the objectives and the primary aim of the study as mentioned before. We therefore propose to move the section from p11434 I4 to p11436 I19 to the appendix, This will sensibly shorten and clarify the article. Furthermore we will remove all paragraphs about upwelling and also mussel adaptation to hypoxia from discussion (p11439 I8-17). We will shorten the part about upwelling in introduction as well (p11426 I5-10). We will add a subsection in material and method describing Kiel bay hydrodynamic, as suggested.

The second associated comment is that the manuscript in its present state remains too descriptive with not enough quantitative aspects to explain observed O2 and carbonate parameter variations (statistical tests for significant variations or not between studied periods).

As suggested, we will produce test statistics comparing August and September carbonate chemistry dynamic in table 1 and 2. Accordingly we will produce one summary daily plot for O2 and carbonate system parameters with hourly means and SD for August and September. These daily plots will add valuable content to the manuscript as they close a gap between the numerical results presented in table 1 and the results presented through the time series data in figures 2, 3, 4, 7, 10.

SPECIFIC COMMENTS 1. Introduction P.11425, I.16-23: please in this paragraph emphasize the combined effect of ocean acidification and eutrophication over coastal systems as described in Cai et al. (2011).

BGD

12, C6548-C6564, 2015

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We will highlight the important eutrophication of the Baltic Sea and its recent history.

P.11425-26, I.24-10: I am wondering if this information about the effect of upwelling events (high pCO2/low O2) on the benthic metabolism is relevant as no such event occurred in the Kiel Bay in the present study contrarily to what was observed by Saderne et al. (2013) in the Eckernförde Bay?

We will shorten this part and move some of it to the future paragraph on Kiel bay hydrodynamic in material and methods.

P.11426, I.8-11: please specify here the location of the study carried out by Frieder et al. (2013).

We will include in the manuscript that the study by Frieder et al. (2013) was conducted with animals from San Diego area.

P.11426, I.15-21: please specify the different objectives of the study that come with the temporal description of O2 and carbon parameter variations. They will have to clearly appear in turn in result and discussion parts.

By shifting the methodological related parts from the discussion to the appendix, we will be able to strongly emphasize the objectives of the study. We aim to contribute to the debate on the potentialities of refuge against seawater corrosiveness given to calcifiers by vegetated habitats, in link with the ocean acidification thematic. - What are the fluctuations carbonate chemistry parameters undergo above a mussel patch within a seagrass meadow? - Regarding what is known in the literature about the effect CO32- and saturation states for CaCO3 isomorphs on mussel biomineralization, we quantify time windows of potentially favorable and disfavorable condition for mussel calcification. As secondary objective: O2 and spreading of hypoxic areas are strongly linked with ocean acidification, in coastal areas and particularly in the Baltic Sea. - What is the O2 dynamic at the site, and is there hypoxia occurring? We will clarify this in the future version of the abstract and introduction. Accordingly we will modify the

BGD

12, C6548-C6564, 2015

Interactive Comment



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title of the article.

2. Material and methods 2.1. The site More characteristics of the studied site should appear in this part, i.e. previous studies carried out in this area, estimated seagrass and mussel covers (density, biomass, volume?) and hydrodynamic information (freshwater inputs, seawater exchange, distance from the shore, water residence time, currents, stratification?). A photo of this mosaic habitat if available could also be appreciated.

We will add a subsection in Material and Method describing Kiel bay hydrodynamics and describing better the mussel patch. An additional picture of the deployment site will be added and help to convey a better understanding of the site composition (see picture attached to this response). Referee 1's comments made us realize that we have not sufficiently explained one important aspect which is in return helpful to address some of the comments. We measured CO2 directly at the bottom and the sensors sat directly on a localized patch of mussels covering an area of 1-2 m² maximum. The patch is surrounded by seagrass. The manuscript discusses a case study of exemplary character. It is therefore localized and intended to contribute to the debate of vegetation refuge to OA stress. The results given cannot be transferred or extrapolated to the entire mosaic habitat, nor Kiel bay in general, since we only measure one part of the mosaic. That is also the reason why we did not engage into a comparison with other environments worldwide. We unfortunately cannot provide a quantitative, valid estimation of seagrass and mussel cover for the area.

2.2. In situ sensor suite A picture with captions of the system should be added. Was biofouling important in this area especially at this season? Please briefly specify advantages/disadvantages of the pCO2 measurement system used here (HydroC, Fietzek et al. 2014) compared to Equilibrator systems (Frankignoulle et al. 2001, Water Res., 35:1344-1347) or other available CO2 sensors in terms of precision, range and equilibration time.

BGD

12, C6548-C6564, 2015

Interactive Comment



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We do not have a quantitative measure of fouling. In 7 weeks, only a thin film of microalgae was found on the sensor. We will add additional information regarding the HydroC sensor and the conducted calibrations to the corresponding part in the Materials and Methods. Equilibrator-based flow-through systems for surface water pCO2 measurements are powerful instruments and prevailing setups to measure air sea fluxes of CO2 as they commonly also regularly determine atmospheric CO2 concentrations (e.g. Pierrot et al. 2009) The submersible and autonomously operating HydroC enables continuous pCO2 measurements directly at the point of interest while using the same proven measuring principle like the surface water setups (i.e. non-dispersive infrared absorption spectrometry). Since the flow through systems measure at the surface and are typically operated from a boat, while in the present study the submersible sensor was places directly on the mussel patch, these two technologies are not truly exchangeable. In contrast to the surface water flow-through systems, which are usually recalibrated every few hours (Pierrot et al. 2009) the HydroC can achieve pCO2 measurements at convincing quality through usage of a pre- and post-deployment calibration in combination with automatically conducted zero-measurements (see Fietzek et al. 2014). In Fietzek et al. 2014, several week long pCO2 data from different HydroC sensors was compared to equilibrator based flow-through pCO2 measurements. An overall mean deviation of -0.6 μ atm with an RMSE of 3.7 μ atm was found. The measuring range can be as high as 6000 μ atm depending on the calibration and response time can be as fast as approx. 1 min (t63) when operated with an underwater pump. The power demands of the HydroC either require an underwater power solutions or grid power through cable connection as achieved in this study.

2.3. Discrete sampling Please add the reproducibility (precision, uncertainty) obtained for DIC and nutrient analysis.

We will add those to material and methods. .

2.5. Community metabolism Calculations following Champenois and Borges (2012) should be detailed with written equations and also water column depth used to integrate

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12, C6548-C6564, 2015

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O2 fluxes.

We will detail the calculations with equations. The water column depth used was 2 m.

2.6. Calculation of the regional atmospheric pCO2 Please add the distance of the station from the study site and also pCO2 standard deviations (or ranges) obtained for August and September periods.

There is a distance of approximately 135 km as the crow flies between the atmospheric measuring station and the deployment site. Since we obtained our pCO2 measurements at the bottom and we do not have any nearby measurements of atmospheric pCO2, we only considered monthly means for our qualitative analysis. We will add the requested information to the text by writing: "Dry atmospheric half-hourly measured CO2 mole fractions from Umweltbundesamt, Station Westerland, location: 8.308208 °E and 54.924967 °N, were averaged for the months August (08–31 August 2013) and September (01–27 September 2013); 391 \pm 7 and 395 \pm 14 ppm (mean \pm standard deviation) respectively. Thereof pCO2 in wet air (100% relative humidity) of 385 and 388 μ atm for August and September respectively were derived at local measurement conditions; i.e. using an averaged sea surface temperature (18.4 \pm 0.6 and 16.1 \pm 1.0 °C) and ambient pressure readings (1019.6 \pm 4.2 and 1015.7 \pm 7.5 mbar; both parameters from GEOMAR meteorological station) as well as the salinity measured in this study (18.4 \pm 0.6 and 16.1 \pm 1.0)."

3. Results This part along with tables and figures need to be reorganized and modified to clearly open on the discussion. I propose to authors to describe studied parameters for both periods with (i) environmental parameters (water temperature, salinity that is not described in the manuscript, irradiance, wind direction/speed, nutrients: : :) illustrated by a new table and figure and (ii) O2/carbonate parameters with one table and figures. Tables and Figures as they appeared in the submitted manuscript are not specified neither in the right order and could be merged. For instance Tables 1 and 2 could be associated whereas Table 3 is not necessary. Statistical tests should be

12, C6548–C6564, 2015

Interactive Comment



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added in the result part to characterize each period according to studied parameters (significant differences etc.). Figure 5 and associated paragraph could be displaced in the discussion part opening on community metabolism over coastal ecosystems (see previous/next comments).

We regret that some of the disorder relates to the mistake that tables and table captions for 2 and 3 were unfortunately swapped. We will merge table 1 and 2 (with caption from table 3) in a revised manuscript version. We will also add daily plots for O2 and carbonate system parameters with hourly means and SD for August and September and statistical tables of test statistics comparing August and September carbonate chemistry dynamic. We will further merge Fig 6 and table 3 (with caption from table 2) as these two are directly related. We think table 3 (with caption from table 2) is essential since salinity to alkalinity regressions are the base for the creation of DIC / pH / CO32and omega time series. We will merge figure 2, 3 and 4 to enhance clarity. Fig 7 will be merged with 10 since they both show parameters derived from the calculated TA series and measured pCO2. We will further discuss the obtained temperature and salinity time series in the manuscript, which has not happened in the present document so far and add corresponding statistical values to table 1 as well. Since it is out of the intents of the article, we do not wish to present data on wind and irradiance although these, obtained from a local weather station, have been used to calculate NPP, GPP and CR. Community metabolism was only calculated to explain the dynamic of the carbonate chemistry we measured at the site over the study period. They do not represent a key result of the article. Therefore, we will displace Fig. 5 and associated paragraph to the discussion as suggested.

4. Discussion The discussion part, as already explained needs to be reorganized, clearer and more consistent based on O2/carbonate parameter process and control explanations with quantitative aspects. - The discussion starts with methodological considerations comparing in situ carbon parameters measurements and calculations (p.11434, I.4-p.11436, I.19) before continuing with pCO2/O2 controls/mechanisms

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12, C6548-C6564, 2015

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(p.11436, I.20-p.11438, I.16), mussel/ seagrass interactions (p.11438, I.17-p.11439, I.7) and ending by mussel adaptation to OA (p.11439, I.8-29). Despite its interest, this long methodological part is not really expected since it is not clearly enounced in the objectives. It then needs to be better integrated in the manuscript structure.

We agree and since referee 1 suggests that the methodological considerations weaken the clarity of the manuscript in the present form, we will move it to the annex. Along with a general restructuring and enhanced focusing of the text, this will therefore greatly clarify the manuscript.

Since authors observed large differences between measured and calculated pCO2 especially for high values, have they tried other model, calculations for comparisons, i.e. the CO2 system calculations from Lewis and Wallace (1998)?

The choice of a set of dissociation constants for carbonate system calculation is indeed of relevance. We used the set of constants from Millero et al. 2006 which is suitable for estuarine and marine waters and which was used by Kulinski et al. 2014 on their seminal paper on Baltic sea carbonate chemisty. In the article we erroneously wrote Millero 2010, which will be corrected within a revised version of the manuscript. Other sets of constants (instead of Millero et al. 2006) that could have been used are: -Millero 2010 - Mojica Prieto et al. 2002 - Mehrbach et al 1973 in the form of the re-fit by Dickson and Millero, 1987 The figure 2 attached to this answer shows the difference in pCO2 calculated from the discrete DIC and TA samples from our study in the form pCO2, Millero et al. 2006 minus the pCO2 as calculated by using the set of constants indicated within the plot legend. The Δ pCO2 values are plotted as a function of pCO2, Millero et al. 2006 for better visualization. The relative deviation compared to Millero et al. 2006 values used in our article is maximum when compared with results using Mojica Prieto 2002 constants with approx. 4.1%. Qualitatively the choice of constants does not change the carbonate system discussion of the manuscript; still it adds more content and we therefore plan to include little additional information in this within the carbonate system calculation part in the appendix.

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12, C6548-C6564, 2015

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I agree about the effects of organic acids contribution to TA and uncertainties associated to pCO2 calculations (see for instance Abril et al. 2015, Biogeosciences,12:67-78). Thus I am again wondering about the contribution of freshwater inputs to the Kiel Bay and its influence on carbon parameter variations, i.e. pCO2 or TA with the decrease observed the 03 September 2013 (Fig. 6.) associated to the salinity decrease (Fig. 4). Similarly, authors partly attributed measured and calculated pCO2 discrepancies to small-scale gradients (p.11436, I.6) but nothing is explained about the hydrodynamic or spatial heterogeneity of the studied site neither supported by references to address this assumption.

The only (noticeable) source of freshwater to Kiel bay is a modest river on the other side of the bay, the Schwentine, at a distance of approx. 2 km south-east. We ignore the influence of it on our site. We will add this to the subsection in Material and method describing Kiel bay hydrodynamics. We do not have an explanation for the salinity drop of the week around September 3rd. No particular rainfall appeared that day and the wind speed and direction was not particularly different from other days/weeks. We will precise that aspect in the article.

Could authors explain TA versus Salinity regression R2 differences between August and September in Fig. 6?

We do not have an explanation for this phenomenon, which we do not attach major value to. The approach of deriving calculated total alkalinity values as a function of salinity is justified through the nature of the sum parameters total alkalinity and salinity. As soon as composition changes occur for either one or the other parameter the quality of correlation will be affected as seen clearly for the major difference between the August and September regression.

P.11436-11437, I.27-11: this part on O2/pCO2 mechanisms/controls/variations need to be developed with for instance daily plots, correlations: Authors explained pCO2 variations with in situ biological activities of seagrasses and mussels without quantitative

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12, C6548-C6564, 2015

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considerations.

As suggested, we will produce test statistics comparing August and September carbonate chemistry dynamic in table 1 and 2. Accordingly we will produce summary daily plots for O2 and carbonate system parameters with hourly means and SD for August and September, which strongly contribute to the value and clarity of the conducted analysis. We are reluctant to produce correlation with test statistics (R², F-stat, p value) between time series since it is in violation of the hypothesis underlying this kind of tests (linearity, independence of observations, homoscedasticity, and normality). All observation have to be independent, what is of course not the case in a time series, observation at t depend of the observation at t-1, t-2, t-3 (serial autocorrelation). To our knowledge, there are methods in time series analysis to circumvent these problems (autoregressive models) but these are very advance statistics, far beyond our knowledge and they are not always applicable. See: Shumway, R. H., & Stoffer, D. S. (2013). Time series analysis and its applications. Springer Science & Business Media. for further explanations.

Authors could quantify this effect as well as the temperature effect using Takahashi et al. (2002) equations. Dai et al. (2009) were able to distinguish relative contributions from photosynthesis/respiration, calcification/dissolution and temperature to pCO2 variations over a coral reef ecosystem at Xisha Islands (see Fig. 11). It could be interesting to have a similar approach for the present study.

In our study we attribute all variations to photosynthesis and respiration. Day night cycles of pCO2 are produced by photosynthesis and respiration by seagrass. Respiration by heterotrophs concurs to the general elevation of the mean pCO2 and, to an amplification of CO2 variations because of shift of the DIC speciation toward dissolved CO2 as explained p 11437. The question of the influence of calcification / dissolution is very relevant. Although we do not evocate it in the manuscript, we searched for a potential effect of calcification by the mussels on carbonate chemistry. It is known that in coral reefs, day-night patterns of calcification exist influencing the water column chemistry

12, C6548–C6564, 2015

Interactive Comment



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as shown by Drupp 2011. Every \sim 5 days we took samples of alkalinity at sunset and noon in the aim to identify daily calcification patterns by the mussels. After removing the influence of salinity, we did not find any significant pattern (systematic anomaly between sunrises and noons) and so we concluded that calcification / dissolution was not to be considered at our exemplary site. Net variations of TA between sunset and noon (after subtracting the part of TA variation caused by salinity variation) are -2.8 \pm 22.3 for the overall measurement period (mean \pm SD, n= 10 days). Presumably there was not enough mussel biomass to see calcification effect on the water column at the given conditions, or the mussels have no clear temporal pattern of calcification, or they did not calcified significantly over the course of the deployment. Thus, we did not want to speculate on this and simply ignored that aspect in the article to do not add more complexity. Regarding temperature, we observed daily of 18.7 \pm 0.5 for August and 16.3 \pm 1 for September. The daily amplitude of variations (mean \pm SD) was 1.08 \pm 0.3 for August and 0.8 \pm 0.3 for September. According to Takahashi's equation the temporal effect on CO2 dissolution is approx. 4% per 1°C temperature change. This effect is small compared to the mean daily pCO2 amplitudes of 880 and 737 μ atm for August and September respectively, which resembles daily changes of 140% and 114% of related to the mean pCO2 values of given months. Therefore, we neglected this aspect in our analysis, since the observed small variations in temperature do not have strong enough impact on the observed pCO2 dynamics.

What about the importance of non-autochthonous processes on O2/pCO2 variations? Could freshwater inputs or water mass advection also explain high pCO2 values observed in the studied site?

The only noticeable fresh water input is the river Schwentine, we hardly think it has any effect on our site although we cannot prove it. We could not determine the influence of water mass advection in our study, however, the canopy of the seagrass meadow surrounding our 1-2 m² patch surely influences the local currents. Moreover, water inlets of the sensors are at ~20 cm of the ground and water velocity is decreasing to

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12, C6548-C6564, 2015

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0 at the contact of the substratum. See the review of Hurd et al., 2000). Processes of benthic boundary layer surely acted full strength and greatly influenced the data for CO2 and O2 we obtained (and the mismatches between discrete samples and continuous series we found). However we do not have three-dimensional measures of fluxes that only an eddi-covariance system could have achieved.

P.11437, I.12-15: the described mechanism is not clear, what do authors mean with "shift" and "amplifying"

By shift we refer to the fractionation of DIC, as generally illustrated by a Bjerrum plot. We refer to a shift towards the lower pH conditions (which is typically the "left hand side of the plot"): more dissolved CO2, less CO32-. We will clarify this, especially since we used twice the word "shift" within few lines to talk about different processes. By "amplifying" we mean wider amplitude of variation. The mechanism is described I. 2 to 11. These are properties of the carbonate system. If you consider two DIC series, with similar amplitude of variations but different absolute values, then the series with the highest absolute DIC value will have the wider amplitude of pCO2. For example consider two DIC variations at TA 1900 μ mol kg-1, salinity 15 and temperature 16°C (calculations carried out by using R package Seacarb): 1> DIC varies between 1800 μ mol kg-1 and 1900 μ mol kg-1 (100 μ mol kg-1 variation) => pCO2 varies from 375 μ atm and 1095 μ atm resembling a variation of 720 μ atm. 2> DIC varies between 1900 μ mol kg-1 and 2000 μ mol kg-1 (still 100 μ mol kg-1 variation but 100 μ mol kg-1 larger DIC values). => pCO2 varies from 1095 μ atm to 2839 μ atm resembling a variation of 1844 μ atm. The DIC amplitude of variation is in both case 100 μ mol kg-1 but there is a 256 % increase of the pCO2 amplitude of variation. A precise explanation of this relationship is beyond the scope of the article.

P. 11438, I.3-6: O2 decrease and pCO2 increase between the two periods need to be support by statistical tests

We will produce test statistics comparing August and September O2 and carbonate

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12, C6548-C6564, 2015

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chemistry dynamics.

P. 11438, I.11-16: the comparison with upwelling effect on pCO2 variations is repetitive in the submitted manuscript whereas no such event occurred during the study. Even if this latter can be cited, Fig. 9 is not justified. In the same way, p.11439, I.8-15, the paragraph about blue mussel adaptation to hypoxia is off-topic in the discussion.

We will remove all paragraphs about upwelling and also mussel adaptation to hypoxia from discussion.

P.11438, I.27: could author estimate mussel/seagrass density in the present study?

Unfortunately, we do not have such estimates.

I suggest to authors to replace these discussion parts by one dealing with community metabolism estimations over coastal systems with comparisons with similar/contrasted ecosystems and methods (Champenois and Borges 2012; Rheuban et al. 2014, L&O, 59:1376-1387; Reidenbach et al. 2013, L&O: Fluids and Environment, 3:225-239; Martin et al. 2005, Aquatic Botany, 83:161-174;). To go further in this way, would it be possible to estimate CO2 fluxes with the atmosphere choosing a good parameterizetion of the gas transfer velocity (K600) and supposing/proving a good mixing of the water column, i.e. similar benthic and sub-surface pCO2?

We understand the suggestion of the referee, however we think that this is beyond what our data can do. As answered above, our study is a case study extremely localized, to intervene in the debate of vegetation as refuge for calcification. This is a case study, The results cannot be extrapolated to Kiel bay, since we measured on one mussel patch within a wider and more diverse habitat. That is the reason why we did not discussed the comparison with other ecosystems, or calculated fluxes of CO2 with atmosphere, in the aim of evaluating the contribution to the atmosphere of this kind of habitat. Accordingly, the community metabolism we calculated is not a major result of our paper but there only to bring some elements of explanation in discussion to the BGD

12, C6548-C6564, 2015

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observed carbonate chemistry dynamic. We will really put more emphases on this aspect of our study on the next manuscript.

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12, C6548–C6564, 2015

Interactive Comment

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pCO2 measuring systems and data-reduction routines, Deep. Res. Part II Top. Stud. Oceanogr., 56(8-10), 512–522, doi:10.1016/j.dsr2.2008.12.005, 2009.

Fig 1. Photo of the sensor suite at the sampling site.

Fig. 2: This plot shows the difference in pCO2 (Δ pCO2) calculated from the discrete DIC and TA samples from our study in the form pCO2, Millero et al. 2006 minus the pCO2 as calculated by using the set of constants indicated within the plot legend. The Δ pCO2 values are plotted as a function of pCO2, Millero et al. 2006 for better visualization. The relative deviation compared to Millero et al. 2006 values used in our article is maximum when compared with results using Mojica Prieto 2002 constants with approx. 4.1%.

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12, C6548–C6564, 2015

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Fig. 1. Figure 1

BGD

12, C6548–C6564, 2015

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12, C6548-C6564, 2015



Fig. 2. Figure 2

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