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Interactive comment on "Cell-free extracellular enzymatic activity is linked to seasonal temperature changes in the Baltic Sea" *by* F. Baltar et al.

Anonymous Referee #2

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General comments: The authors attempt to address an interesting and important problem, with a time series of measurements of potential enzyme activities, including the fraction that is cell free. These measurements are a good start, but the data lack context, and the framework which the authors build does not adequately reflect current understanding of microbial community activities and dynamics.

Are other data in addition to T and chl a available? Salinity, DOC, circulation, other physical or chemical data for this site? A multitude of factors can affect potential enzyme activities, as well as the fraction of activity that is cell-free. Are cell counts available, or are there other studies done at the same location that provide a more in-depth background on the microbial ecology of the system? In particular, the Baltic has a

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very strong N-S salinity gradient that is linked with both compositional and functional changes in microbial communities (e.g. Dupont et al. (2014). "Functional tradeoffs underpin salinity-driven divergence in microbial community composition." PLOS one 9: 89549). It would be helpful to have some sense of microbial dynamics at this site; the T and chl a data are not really a sufficient context.

In its current form, this manuscript lacks sufficient significance and quality and presentation; it needs a lot of work.

Specific comments: Pg 2 lines 11, 12 Note that Arnosti (2011) is cited improperly ("This is why the activity of extracellular enzymatic activity (EEA) has been recognized as the rate-limiting step in organic matter degradation (Arnosti, 2011)." Arnosti (2011) does not state that EEA is the rate limiting step: that reference points out that EEA is the initial step in organic matter degradation, and discusses this idea at great length. These statements are not the same!

Lines 18, 19: earlier reports of cell-free activity, including Karner & Rassoulzadegan (1995), Obayashi & Suzuki (2008) and Keith & Arnosti (2001).

Note that one of the key points of the current ms - that enzyme activities may be decoupled from the producing organisms, and may contribute greatly to carbon cycling - is explicitly also discussed in Arnosti (2011), and summarized as one of the key points in that manuscript.

Lines 31 forward: Steen & Arnosti (2011) investigated cell-free enzyme lifetime in Arctic surface waters

Pg. 3: Line 7, delete 'the' before 'cell-free' Line 8, delete 'what are' before 'the factors' Line 8, this thought is incomplete. Do you mean 'little is known about the factors that control changes in the proportion of total EEA that is dissolved' ? Line 9, move 'is needed' to the end of the sentence Line 11: move the information about the specific enzyme activities assayed into another sentence following this one. Line 14: change

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'decipher what factors' to 'decipher the factors that control' Line 16, change to 'we hypothesized that there would be a strong link', or something of this sort- need to improve the phrasing.

Line 7 forward. This paragraph is not very clear – why will a long-term sampling strategy in particular help resolve the question of when and under what conditions cell-free EE is important? One could easily imagine that a detailed biochemical investigation of the nature and structure and dynamics of cell-free enzymes is what is needed (tho this of course is technically very challenging, and is a different question than the one examined here.) The whole paragraph needs more focus.

As it stands currently, this hypothesis is not very well phrased. At minimum, add another sentence or two: is this hypothesis based on rates of molecular motion, on rates of diffusion of enzymes and proteases, on an idea of the structural basis of enzymes that are produced under cold vs warm conditions, on kinetics of degradation/inactivation reactions?

Pg. 4 Were killed controls (autoclaved water) subtracted from the measured values?

Are other data (salinity, for example) available for this site? How variable are cell counts, or bacterial productivity, or DOM or POM or other biogeochemical parameters for this site?

Pg 5: The data presentation and discussion on pg 5 is not very satisfactory. The BGase and LAPase plots do appear to track one another to some extent, but having the APase activity on the same plot and scale makes something of a mess. Try plotting with a secondary Y axis for APase. More critically, it is difficult to figure out what connections there might be: as the text says, there are sometimes –but not always- coincidental peaks in these different activities, but this pattern is not very strong.

Moreover, the authors assert that BGase and LAPase show relative protein/polysaccharide degradation ratios. Note that more recent work has demonstrated

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that this perspective is likely limited. Multiple investigations of a range of peptidases substrates (see in particular the work of Obayashi & Suzuki Obayashi, Y. and S. Suzuki (2005). "Proteolytic enzymes in coastal surface seawater: Significant activity of endopeptidases and exopeptidases." Limnol. Oceanogr. 50: 722-726. Obayashi, Y. and S. Suzuki (2008). "Occurrence of exo- and endopeptidases in dissolved and particulate fractions of coastal seawater." Aq. Microb. Ecol. 50: 231-237. Bong, C. W., Y. Obayashi and S. Suzuki (2013). "Succession of protease activity in seawater and bacterial isolates during starvation in a mesocosm experiment." Aq. Microb. Ecol. 69: 33-46) which demonstrates that LAPase activity levels vary in a manner not indicative of other peptidase activities. See also the recent work of Steen et al. (2015; "Substrate specificity of aquatic extracellular peptidases assessed by competitive inhibition assays using synthetic substrates" AME 75: 271-281), who investigate the specificity of LAPase-hydrolyzing enzymes, and comment that "In some studies, Vmax for Leu-AMC hydrolysis is interpreted as a quantitative proxy of the total peptidolytic potential of a community (Kellogg et al. 2011). The results presented here show that approach to be invalid." Furthermore, the work of Arnosti (2011), which is cited in the ms, discusses the issue of the broad and variable spectrum of polysaccharide hydrolases present in the ocean; measurements of BGase do not present a complete or representative picture.

Note in addition that the peaks in BGase/LAPase ratios (which are not all that convincing..this plot shows a general increasing trend over the timecourse of the study, and the single large peak is driven by 2 measurements of BGase activity) do not in fact coincide with the chl a max, since the peak chl a in Fig 1B occurs in ca August and then in May, whereas Fig 2b shows peaks in June and August.

The comments on lines 19-22 on different phytoplankton blooms and enzyme activities is simply not very convincing: APase activity might be directly connected to phytoplankton, given the presence of APase in some phytoplankton, but the BGase and LAPase connection would be more distant, given that heterotrophic prokaryotes are the sources

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of these enzymes.

The discussion of all of these data in fact leave the reader with the impression that the authors don't quite know what to do with this long time series. More digging in the literature, discussion with other colleagues who have data from this site might help, but this is currently a very weak part of the manuscript.

Pg 6 The high fraction of dissolved activity is indeed an interesting observation. The authors need to discuss here some ideas about why this might be - certainly others have also at times high cell-free activities, but these activities are high for most of the annual cycles. Moreover, the changes in the cell-free activities for example between March and May, when the data spikes up and down with considerable frequency, would allow for some discussion of lifetimes (for activity to drop this much, some of the enzymes must either be removed, or they are advected away, and one is sampling a different patch of water; to evaluate this, more data on the study site and the dynamics of the water masses is needed.)

As others have doubtless pointed out, correlation is not causation: there is a statistical correlation between T and cell-free activity, but this is a long way from a coherent or plausible explanation. To what extent do cell counts, bacterial productivity, or microbial community composition change at this site on seasonal scales? What is the physical oceanography of this setting –what water masses are sampled? It is extremely likely that the nature and types of enzymes that hydrolyze leu-MCA, B-MUF, and alkaline phosphatase are likely quite different under different conditions of productivity, and with different seasons, all factors that are correlated with differences in microbial community composition and activity. See for example Arrieta & Herndl (2002; "Changes in β-glucosidase diversity during a coastal phytoplankton bloom." Limnol Oceanogr 47: 594−599). In any case, drawing parallels between community activities in the Arctic and the Baltic needs also to consider the differences between permanently cold and temperate environments, in terms of community composition and activities.

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With all of these issues that should be addressed, extending the discussion here to questions of global warming is much too far a reach.

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