Reply to reviewers comments on "Spring-time zooplankton size structure over the continental shelf of the Bay of Biscay" by P. Vandromme et al.

Dear Drs. Bachillier and Basedow,

We are greatly thankful for the reviews, corrections and comments you made on our manuscript. One of the main concerns was about the general structure and language of the manuscript. We integrated the extensive corrections made by Dr. Bachillier, which greatly improved the manuscript. We removed all repetitions and useless parts that were already described in other works; we also made the text clearer by moving some part from discussion and results to materials and methods as highlighted by reviewers. The text in M&M and Results sections were reduced by about 35%. All together, the text was reduced by about 30%. We removed also figs. 2 and 9 from the manuscript and simplified figs. 3, 4, 8, 10 and 13 to make them more readable and focus on the essential information. We greatly improved the grammar and style of the manuscript by making it proof read by a native English speaker.
We improved the discussion by adding more references, simplifying the message on methods by removing parts already mentioned in the M&M or Results sections and focusing more on ecological features.

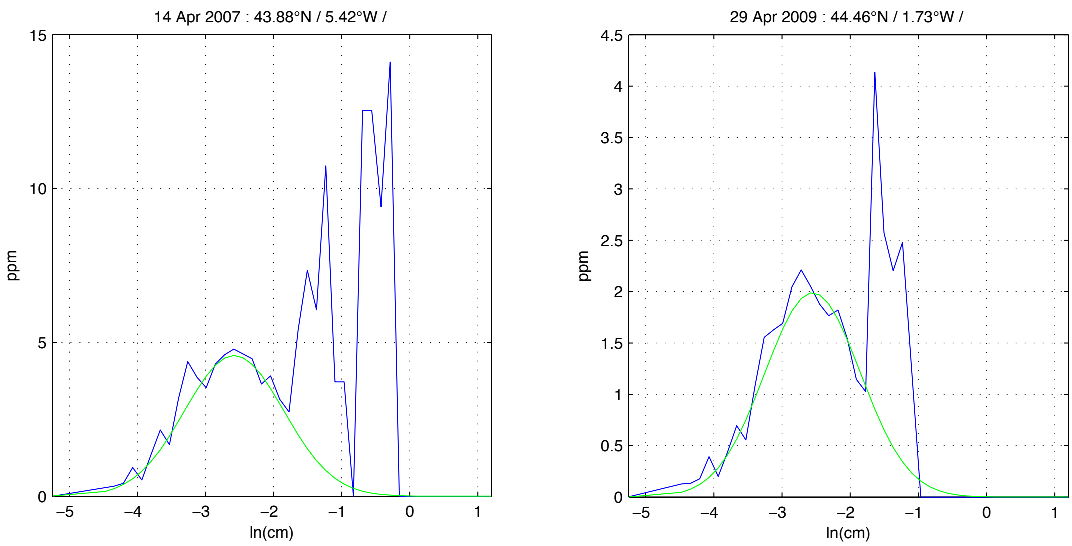
Methodological considerations:

Net clogging:

The net clogging was of great importance in previous work, such as Nogueira et al. (2004), which used a mesh of 20µm. In this work they used a flowmeter allowing an estimation of the clogging, which we didn't had here. In our work we used a typical WP2 net with a mesh size of 200µm, a diameter of 57cm at the mouth, a cylindrical section of 95cm and a conical section of 166cm. This make a ratio filtering area / mouth area (adjusted to porosity) of about 6:1 (Hernroth 1987, Keen 2013). This ratio corresponds to the minimum proposed by Ohman (2013) for efficient sampling, yet, a ratio of 10:1 to prevent clogging was preconized for eutrophic environment (Ohman 2013). However the clogging depends greatly on the distance towed (by proxy the total volume sampled). In our case the maximum depth was of 100m, making, with a sampling area of $0.25m^2$, $25m^3$ sampled. Smith et al. (1968) proposed an equation for the minimum acceptable filtering / mouth area ratio (OAR, adjusted to net porosity) in oligo- and eutrophic waters according to the total volume sampled. In our case the minimum OAR should be of 3.9:1, which is below our of 6:1. Considering that in most of the case where eutrophic water were encountered the bottom depth was lower than 100m this decrease again the minimum acceptable OAR and make us confortable in avoiding any clogging. Moreover, according to other tests (Unesco, 1968), the WP2 200µm net can be towed in eutrophic water during at least 4min before the efficiency drops below 85% because of the clogging. With a vertical towing speed of 0.5m/s, this makes a distance of 120m, longer than the maximum of our tows. Although we didn't used a flowmeter to directly measure the filtering efficiency we feel quite confident on the quasi-absence of clogging regarding the mesh size of 200μm and the limited volume sampled.

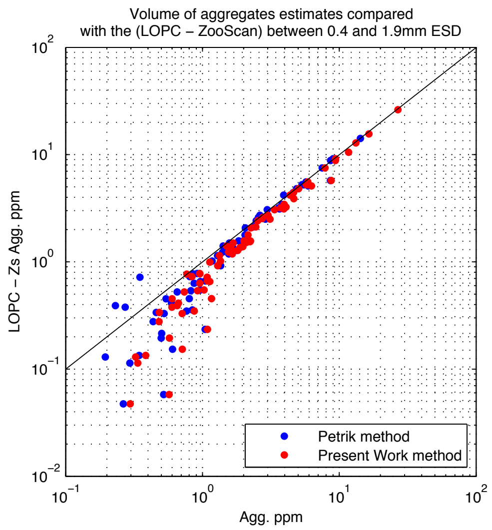
Petrik et al. LOPC corrections:

The work of Petrik et al. proposes a different methodology to extract the non-living particles from the LOPC. In their case they were interested in removing the zooplankton (considered as a noise) to study the non-living particles. We were more focused on the zooplankton, yet, the methodologies, although different in the philosophy, could be compared. Petrik et al. considered the volume spectrum of non-living particles to be a log-normal distribution with a mean always lower than 0.8mm ESD. Therefore, they fitted such a distribution (using the matlab function fminsearch) to the volume spectra of the LOPC. In our case we have the zooplankton data for some station and so we have a mean to evaluate the correction made on the LOPC and to link it to environmental data. At first we didn't considered making a detailed comparison with the work of Petrik et al. because of the strong differences in the methodology used. Yet, as highlighted by the reviewers, such a comparison could be of interest. We thus re-programmed the method of Petrik et al., of which an example of fit could be seen below:

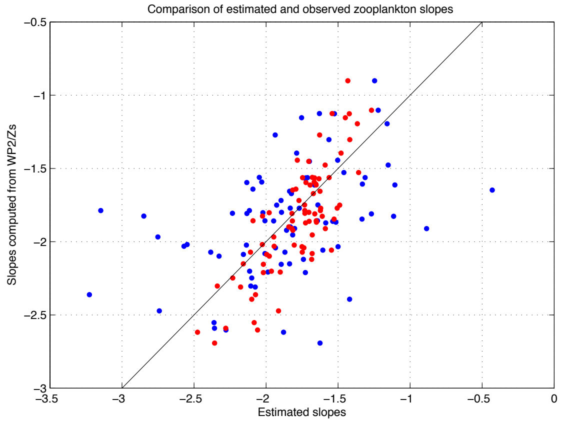


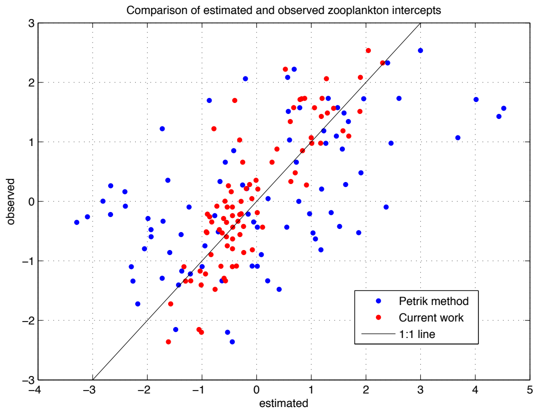
It has to be mentioned that we had some trouble making the algorithm work. In their formula of the cost function, the value f1 is 1 if the fit is higher than the LOPC and 0 otherwise. Yet, this makes the cost function to be 0 when the fit is below the LOPC for every point but not just below. It resulted for us to a completely flat curve being selected every time as the best fit. We had to change the f1 value to 5 when it was 1 before and to 1 when it was 0 before. We don't know if it was a small mistake in the initial paper by Petrik et al., or in the script we have rewritten, but it was, for us, the only way to make the algorithm work. We then used the notation and conversion of Petrik et al. to extract the total volume of aggregates. We calculated also the total volume of the difference

(LOPC – WP2/Zs), which we had considered being the best estimate of the aggregates, and the volume of aggregates reconstructed from the calculated slope and intercept of the present work. The results could be seen on the figure below. No big differences appear between the two methods which are both significantly correlated with the volume of aggregates estimated as the differences between LOPC and WP2/Zs. Larger errors seem to occur when the volume of aggregates is small which can be explained by the noise generated by the proximity between LOPC counts and WP2/Zs ones when subtracting each other. This figure is different from the ones shown in the manuscript since we used here the notation of Petrik et al. and we show the total volume of aggregates. In our work we focused on the zooplankton slopes and intercept. Here the total volume is a different measure that could be very sensitive to large objects. As seen on the examples of size spectra of LOPC and zooplankton shown in the manuscript (in log scale), the zooplankton generally represents only a small part of the total volume of particles.



On the other side, we tried to use the method of Petrik et al. to estimate the characteristics of the zooplankton community, as in our work. We see on the figures below that slopes values are of the same order but the correlation is lower with the method of Petrik et al. (blue dots) than with the method proposed here (red dots). For the intercepts, there is again the same order but a lower correlation with the Petrik method. These results appear realistic in the view of our work. The Petrik method take into account only the LOPC data and looking at the results of the stepwise RDA performed, the LOPC characteristics explained about ~45% of the total variance, while with additional environmental information it is increased to ~75% explaining the differences in the correlation seen in the figures below (which are comparable to fig. 5AB of our manuscript).





These results tend to validate on one hand the method followed by Petrik et al. that the distribution of aggregates could be estimated directly from the LOPC by a log-normal fit on the volume spectra, the residuals being the zooplankton. The distribution of the zooplankton estimated that way is comparable to the one observed by direct measurements. And on the other hand these results validate also the method followed in the present work. As shown here, the part of the LOPC that is composed of living, and non-living, particles is not fully explained by the shape of the total particles (LOPC). Our work, using direct zooplankton observations, which were not available for Petrik et al., allows going into more details. If the global aim of the study is to extract the non-living particles total volume, then the method of Petrik et al. appears to be valid. However, for a more detail description of the zooplankton size distribution it appears necessary to perform a correction of the LOPC based on a wider range of environmental variables. We propose to add this comparison into more detailed in the revised manuscript as it strongly support the significativity of both methods.

<u>Taxonomic information:</u>
It is true that the mesh used undersamples some groups. Yet, for correction of the size spectra we took only sizes larger than ~400µm ESD, which is, according to literature, the size at which a net with a mesh of 200µm is quantitative. The problem of undersampling may be of importance for the stepwise RDA performed on taxonomic group. Some groups include species of various sizes and if there is a geographic gradient, such as coast/offshore, in the mean size of the group this could induce a virtual gradient in the abundance of the group, e.g., the undersampling being stronger at the coast. Such groups could then appear to explain more variance than they actually do. However, the stepwise RDA performed on taxonomic groups is only informative in the manuscript and is not used to perform the corrections.

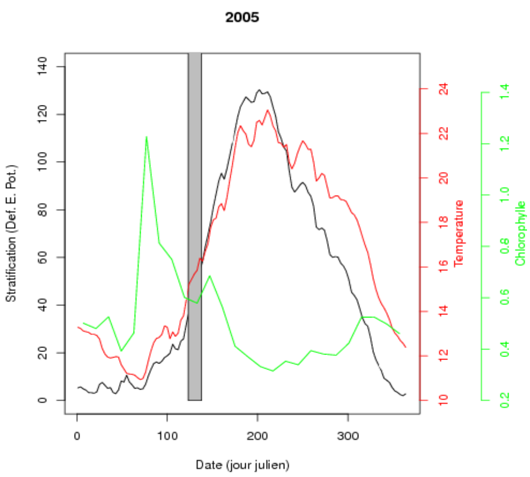
Ecological discussion

Dr Basedow makes the proposition of discussing the size spectra and their variability in light of temporal variability (pre-bloom, bloom, post-bloom), in relation to hydrography, and in connection to other studies.

This is true that we have focused our description in the discussion on the strong spatial patterns of the zooplankton community, based on biomass and slope results as climatologies, not showing explicitly the interannual variability, except in the presentation of the clustering results. This was for several reasons :

- first is the fact that we only have an annual snapshot from the surveys, that however lasts 1 month (2 months when French and Spanish surveys are combined) from the south to the north of the studied area, during a fast evolving season with fast changing environment. So comparing years based on these snapshots does not really make any sense, except looking on a more local scale (which was not the first idea) and on timing regarding local blooms and hydrography.
- However, and this is a second reason, even if the environment is evolving fast (increase of SST and stratification, bloom occurrence), spatial patterns for size spectra are quite stable among years, with cross-shore gradient over the French shelf, difference between northern and southern part of the French shelf, and between French and Iberian shelf. This gives support for a focus on the spatial description. One reason can be that blooms have already occurred everywhere in the Bay (from March to April) when the sampling is performed (from mid-April to end of May), even if secondary blooms may occur. Comparing winter to May to July would certainly give a different perspective. Discussion will be completed in this sense.
- Third reason is that getting information on the temporal evolution is not straightforward, and in any case not possible from the survey data. A solution is to satellite data, even if only providing surface data while our zooplankton information is integrated over the water column.

Nonetheless, we investigated the potential for improving the ms. in the direction of interpreting the interannual variability. For each of the sampled year, we then looked at the timing of the sampling with respect to the chlorophyll evolution, as well as temperature and stratification, as illustrated for 1 year in the figure below.



Timing of the survey in the southeastern Bay of Biscay (Julian day) with evolution of chlorophyll (ocean color data), surface temperature and stratification from an hydrodynamic model)

These data are actually being compiled and analyzed in our laboratory by other colleagues and will be the subject of future publications. Adding such data is possible but will however strongly lengthens the revised manuscript. It is nonetheless a possibility, if the reviewer and the editor find it necessary, to discuss the timing of each survey, by sub-areas, according to local blooms, interannual variability in temperature and stratification, with regard to the representativeness of each cluster, per year, in these sub-areas.

Other revisions were made in the discussion section according to the comment of the reviewers, mainly by adding more references and more detailed discussion on the ecological points as well as a more synthetic message. We added some new recent references for discussing the trophic link with small pelagic fishes and the relation to hydrological features into more detail and also to extend the results that relate size-spectra features to temporal environmental conditions in other area (e.g., the recent work of Marcolin et al. 2013) to increase the relevance of our results to a broaden community.

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Smith, P.E., R.C. Counts, and R.I. Cutter. (1968). Changes in filtering efficiency of plankton nets due to clogging under tow. *J. Cons. int. Explor. Mer* 32(2):232-248.

Marcolin, C. D. R., Schultes, S., Jackson, G. a., & Lopes, R. M. (2013). Plankton and seston size spectra estimated by the LOPC and ZooScan in the Abrolhos Bank ecosystem (SE Atlantic). *Continental Shelf Research*, *70*, 74–87. doi:10.1016/j.csr.2013.09.022