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Biogeosciences Discussions

Interactive comment on "Assessing the ecological status of plankton in Anjos Bay: a flow cytometry approach" *by* G. C. Pereira et al.

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Dear Author,

This work deals with a great amount of data, some of them collected with rather new technologies such as automated and in situ flow cytometry. Since I have worked a lot with this instrument and analysed large amounts of samples, I think to be in a good position to provide helpful comments.

P6247 l19: flow cytometry does not analyse total suspended particles but particles present in the analysed volume passing through the flow cell and whose scatter signal is large enough to be recorded. P6247 l22: can you estimate the lower size level of analysed particles? P6248 l3: I do not understand your flow rate definition, 2 mm.s-1

? it should be more understandable if you express it in terms of mm3.s-1 ? What was the trigger level ? P6248 l4-5-6: It would have been great, I would say fundamental, to analyse a sample of suspended beads with known size and fluorescence, and it could have been done at this time, since you were moving the buoy. P6248 l16: In my experience, it is possible to have an estimation of stained cyanobacteria by plotting orange fluorescence vs green fluorescence on conventional bench top flow cytometers. Since it seems you do not have the lower level of detection of the Cytobuoy flow cytometer, how can you be sure you detect all cyanobacteria with it? P6249 l3 or Figure 2: Can you provide a figure that makes evidence of the lower detection level of the instrument? It corresponds to the background noise and the inert particles passing through the laser beam. It is crucial to evidence it on each flow cytometer, in order to estimate the detection limit and be convinced that what you see as fluorescing particles corresponds to phytoplankton. P6249 l4: As I explained previously, beads should have been measured, and why not before and after the mooring of the cytometer, or by connecting a tube to a sample with beads. This is a necessary control of the optical system stability over such a long analysis period. With less precision but that could be related to a control, is the stability of the different recorded parameters for a defined cluster along the analysed period. Small shifts should be linked to cell division, for exemple; while large shifts could be linked to instrumental errors. P6249 l4: You should say “Figure 2a presents a distribution of the analysed suspended particles based on their lengths and forward scatter signals” and not “all suspended particles”.

P6249 l4: You use the word Microalgea. Do you mean that you only collect signals of cells > 20 μm ? What about nanoplankton and picoplankton ?

P6249 l10: what is the unit of the abundance values?

P6249 l13: Flow cytometry does not account for total suspended particles. If the trigger level is at the lower level, then you also record huge amounts of instrument background noise that is also counted as particles. Furthermore, what is the definition of suspended particles, does it fit with the lower detection level of the flow cytometer?

BGD

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In order to have an estimation of what is found in your samples; could you also provide the cell-size estimation on the basis of the instrument outputs: TOF and FWS length? There will be an overestimation for cells under 5 μm. In order to define their size, you could analyse beads of 1, 3 and 6 μm and define the FWS length/beads size relation. The size estimations should be accurate with cells around 10 μm. You should check it with 10 μm beads.

Best Regards

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Interactive comment on Biogeosciences Discuss., 7, 6243, 2010.