

Dear Anonymous Referee #2

Tanks for your opinion here follow the answers of your questions:

1 - Measurements of primary production and respiration have always been the most accepted to assess net autotrophy and heterotrophy however, both measurements needs incubation procedures and the ultimate goal of our work is to assess ecological conditions in real time/on line.

We could use chlorophyll-a in vivo fluorescence to assess the photosynthetic activity of autotrophs. A possible way could use the red fluorescence profiles of each particle that is only provided by this cytometer and calculate the integral of these shapes as a measurement of chlorophyll biomass. There are some issues in this approach, since fluorescence data yield only relative photosynthetic electron transport rates; a direct conversion into absolute carbon fixation rates is not possible. It tends to overestimate primary production. The light scattering and the package effect of the cells influence the viability of the calculated fraction of absorbed PAR. The physiological regulation of electron partitioning under PAR saturation has to be studied in detail to understand **non-linear** relationship between fluorescence and gas exchange and the variability of O₂/CO₂ ratio have to be measured in situ. On the other hand, ¹⁴C incorporation rates can be influenced by “bottle effects”, especially near surface where photoinhibition and photorespiration can play a major role. Changes in physiological activity during bottle exposure have to be quantified in relation to the photosynthetic performance under natural conditions. We should have in mind that production always result in biomass increases and cell division (abundance).

In relation to respiration, considered as the major process of the biological oxidation of organic matter, it was achieved in laboratory experiments using flow cytometry and some fluorescent probes such as DiOC₆. It has shown to be sensitive to changes in the proton electrochemical potential difference, characterizing mitochondrial and plasmic membranes bearing the cell respiratory system in eukaryotic and prokaryotic cells. In mitochondria, the proton electrochemical potential is linked to the flux of oxygen uptake by a **linear** relationship. It enables a simple and direct conversion of fluorescence signals into respiration rates. However, the in situ respiration rate is generally low to be accessible by this available method.

2 – In relation to the present version of this study to ignores the presence of pigmented eukaryotes and flagellated heterotrophs (mixotrophs). It was my fault and will be recognized in the new version of this article. Sorry!

However, the assessment to these populations have been achieved by flow cytometry (in lab) using specific probes. For real time, the CytoBuoy system should be redesign in order to allow the use of beads and fluorescent probes on board. On the other hand, we have tested the image in flow module coupled to our instrument and many of these organisms have been detected and photographed (it will be another article). However, many of these are empty images that could be interpreted as noise. We are investigating it at this moment.

3 – Samples were collected only to make measurements of viruses, heterotrophic bacteria and nutrients. As nutrients are usually measured (weekly) by the Oceanographic lab of the Brazilian Navy, I present the same frequency. However, we have performed high frequency monitoring and as higher is the sampling frequency, closer to a chaotic behavior is the function monitored (abundances of phytoplankton).

The question is what will be the best sampling frequency representative of microbial behavior and interactions once they vary differently? In my opinion, it depends of the goal of the monitoring program.

4 – Dear referee, although unusual, it is possible. Not so good but the same can be viewed in some papers, like Seymour et al. MEPS, 288:1-8 for an example. Dilutions should be taken not only to minimize erroneous counts but we have also to avoid inhibition effects where the number of counted particles increases as higher is the dilution. The whole procedure of the method will be described as suggested by the referee #1.

5 – Sorry, another error. You are completely right, the symbol will be changed.

6 – All the abundance data were converted to milliliter and will be included in the corrected version.

7 – Date of sampling will also be indicated in the corrected version of Fig.5.