

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

**Anonymous Referee #2**

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### General comments

The authors examined spatial (1 - 10 km scale) and temporal (week - seasonal scale) variations of the abundances of phytoplankton, heterotrophic prokaryotes and viruses in the Anjos Bay, Brazil. Simple correlation analyzes were conducted to examine relationships among biotic and abiotic (temperature and salinity) data. They propose to use the abundance ratios of autotrophic and heterotrophic plankton for the assessment of the “ecological status” of the bay. This manuscript has serious problems in science and presentations. Substantial revisions are required if it are to be published in a scientific journal.

Specific comments.

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1. I agree that the metabolic balance (the ratio of photosynthetic production, or P, and respiration, or R) can be a useful indicator of the “ecological status” of a given aquatic system. If a system is “net autotrophic ( $P > R$ )”, this would indicate that the food web of the system is supported by autochthonous (i.e., phytoplankton) production. Reversely, if a system is “net heterotrophic ( $P < R$ )”, one could argue that the food web of the system is subsidized by allochthonous sources of reduced carbon. This concept has been extensively developed in the literature of limnology (studies of lakes and rivers), although it is relevant to estuarine and marine systems as well. However, I disagree with the authors’ proposition that the “abundance ratio of heterotrophs and autotrophs”, as determined by the methods used in the present study, can be a useful indicator of the metabolic and ecological status of the Anjos Bay. Heterotrophic prokaryotes are more abundant than phytoplankton not only in the Anjos Bay but also in marine environments in general. However, this piece of information alone cannot be the evidence of the net heterotrophy of the sea. In short, investigators should determine primary production and respiration, not just abundances, in order to assess the relative magnitude of “heterotrophy” and “autotrophy”.

2. It is problematic that the present study fully ignores the presence of heterotrophic eukaryotes (flagellates and ciliates), which are often as abundant as autotrophic eukaryotes in coastal marine environments. The authors should also recognize that some of the “pigmented” eukaryotes are mixotrophs that display both the autotrophy and heterotrophy.

3. Although the authors used an “in situ” flow cytometry, the present study basically relied only on the data collected at the time of bottle sampling. I found no advantage of a high frequency monitoring.

4. I have concerns about the validity of the viral abundance data. In general, in marine surface waters, viruses are roughly 10 fold more abundant than prokaryotes. One should dilute viral samples before the injection of the sample to flow cytometer in order to minimize erroneous counts due to high event rates. This means that viruses and

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prokaryotes cannot be counted on a single run. In this regard, it is unusual to show both the viral and prokaryote plots on the same graph (Fig. 3).

5. The authors report that “V-1” viruses varied in the range of  $3.64E+03$  to  $1.92E+04$  whereas “V-2” viruses ranged from  $2.14E+03$  to  $4.13E+05$  (L8-10 P6250). Then, they state that “the total virus abundance varied from  $1.23E+05$  to  $3.62E+07$ ”. Assuming that the total is the sum of V-1 and V-2, why does the max value of the total exceed  $4.3E+05$  ( $1.92E+04 + 4.13E+05$ ) by two orders of magnitude?

6. This manuscript has serious deficiencies in terms of presentation. The most serious one is the lack of the explanation of units for the abundance of plankton and viruses in both the text and figures (only the exception is Fig 5, in which units, but not values, are indicated). For the descriptions of the statistical parameters, there are negative values for r square (e.g. L18 and L20 of P6250). They are obviously incorrect. I also have concerns about their results indicating that p values were always “= 0.05” (P6250): they should be read  $p < 0.05$ ?

7. Fig. 5: The authors should indicate the date of sampling.

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