

## ***Interactive comment on “Distribution of ultraphytoplankton in the western part of the North Pacific subtropical gyre during a strong La Niña condition: relations with the hydrological conditions” by M. Girault et al.***

**Anonymous Referee #2**

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Overall quality/General comments

The authors present, the distribution patterns of ultraphytoplankton, heterotrophic prokaryotes and hydrographic variables in the subtropical western North Pacific during a period of La Niña. The majority of the manuscript comes across as being very descriptive, which is often a consequence of having a significant number of determinands at multiple stations. Although the manuscript focuses on the distribution of ultraphytoplankton, measurements of the microbial community and hydrographic variables are brought together using principle component analysis in an attempt to examine

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the relationships between these parameters. At present I am unconvinced about the relationships between microbial and hydrographic data (see concerns in Specific Comments below) but the manuscript might be publishable following revision, particularly of the section concerning relationships between parameters and clarification of other concerns and rewriting of the discussion relating to microbes/nutrient interactions. The discussion would also benefit from being shorter and focussing on fewer topics.

Specific Comments

A major part of the manuscript is based on an analysis of wide-ranging measurements using PCA and so the success of the manuscript hinges on figures 13 and 14. These were found to be somewhat unclear, crowded and therefore unconvincing. Whilst it is felt that there is definitely evidence that the microbial community DID change with latitude, the way in which it changed in relation to the hydrographic variables needs to be more convincingly articulated and presented to the reader.

Flow cytometry - The abundances of microbes measured by flow cytometry are often very low and a detection limit of 5 cells cm<sup>-3</sup> (P5712, L22-28) was stated. Therefore, the authors must state how they determined their detection limit and the degree of confidence they have in this detection limit. In addition to this, was there any replication in sample analysis? If not, what confidence do the authors have in the abundance estimates that they have generated and how can they evidence their confidence? Confidence in abundance estimates increases with the number of cells analysed. Typically, a count of 1000 ‘events’ results in an error in abundance estimation of approx. 5%, but this also depends on the volume of sample analysed. Therefore, the authors need to state what volume of sample was analysed for the different components of the microbial community, or at least provide information about the analysis time and flow rate, the latter of which should be possible as Truocount beads were used as an internal standard. These beads would also provide a means of testing sample analysis reproducibility, at least in well-mixed waters if there was no real sample analysis replication as there is often very little variation in cell numbers through the water column in mixed

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waters.

Separation of nano and picophytoplankton – The authors state that they used 2  $\mu\text{m}$  beads to separate the nano and picophytoplankton. I fail to see how this has been done. Size is measured relatively with a flow cytometer using forward scatter, primarily, however the light scattering properties of beads are very different to phytoplankton: the beads scatter far more light relative to phytoplankton of the same size and so would appear much larger in terms of their forward scatter signal. It should be possible to demonstrate where the 2  $\mu\text{m}$  beads were in relation to the phytoplankton as samples were run with 2  $\mu\text{m}$  beads. Fig 3a, shows beads in the plot. However, it is not known whether the beads are 2  $\mu\text{m}$  beads or the larger 4.2  $\mu\text{m}$  Trucount beads. I would have thought that both bead types would have been in the plot as that is what is reported in the methods (page 5707, lines 1-6).

Nanocyanobacteria – How were the authors able to discriminate nanocyanobacteria from cryptophytes? Molecular analyses? If there is no actual evidence of the identity of the high phycoerythrin-containing group, then the uncertainty needs to be stated. If it cannot be confirmed that the group in question is nanocyanobacteria then the section beginning on line 25 of page 5720 is brought into question and should be removed

Heterotrophic prokaryotes – Marie et al. (1999) did not use SYBR Green II. One assumes, therefore that Sybr Green I was used. Please confirm. This is important as Sybr Green II stains single stranded DNA and RNA, whereas Sybr Green I targets double stranded DNA.

Introduction – I feel I must disagree with the authors regarding their comments regarding difficulties using HPLC in oligotrophic waters. Yes, it is well known that pigment per cell of phytoplankton is low in surface waters of oligotrophic regions but by simply increasing the sample volume filtered it is still perfectly possible to measure pigment concentrations of even the smallest organisms such as Prochlorococcus.

Discussion – The authors should take a look at the literature pertaining to the Atlantic

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Ocean to gain a better understanding of the way in which microbes in oligotrophic waters are able to utilise apparently scarce nutrient resources.

Technical corrections – NOTE corrections in italics or explained 5701: Title, line 4 – change to, 'relationship with hydrological conditions' 5702 line 3 – a north-south transect (33.60N-13.250N) along the 141.50E meridian. . . Line 10 – explain warm pool Line 12 – However, - add comma Line 17 – Explain (35) Line 20 – the distribution Line 22 – remove (>100 m), add at depths >100 m after (STCC) 5703: line 13 – add comma after 200 m Line 14 – change hydrology to hydrography Line – 27 – change maximum to maxima 5704: Line 14 – comma after conditions Line 15 – s after cast Line 21 – with an in situ. . . 5705: line 13 – the MAGIC. . . Line 16 – n after show 5706: line 4 – filtered not filtrated Line 10 – remove s from otes Line 13 – using a FACSCalibur flow cytometer 5707: line 15 – separated from Synechococcus Line 25 – on the basis. . . 5708: line 14 – points below. . . Line 21 Hydrographic 5709: line 18 – remove the word 'the' before station 7 and station 8. 5710: line 1 – remove sides, replace with ends Line 4 – tions varied from. . . Line 5 – remove of and replace with in Line 4-6 – Sentence does not make sense Line 9 – remove spread and replace with varied Line 10 – comma after 8, remove s from phosphates Line 11 – Except for station 3, Line 12- at the surface Line 15 – at station 3 (remove the word the) Line 17 – replace among with for Line 18 – replace The with A Line 19 – remove discriminated the with was the, and silica (remove the) Line 21 – remove the word the before phosphorus Line 22 – consisting instead of consisted Line 24 – was instead of were Line – 26 Its Si:P, comma between 15 and observed Line 27 – at the surface, addy had instead of has Line 28 – the highest Si:P ratio of the cruise. 5711: line 2 - remove the s from fluorescences Line 16 – add a space after cells Line 21 – minimum abundance was found. Line 22 – replace from with between Line 23 – had the lowest concentration compared . . . Line 24 – remove communities and replace with populations, add The before distribution Line 25 – add the word the between at and surface, replace was with is, remove the word were, change ranging to ranged Line 26 – remove the word the Line 27 – remove the s from extremes 5712: line 4 – between stations Line 5 – remove group of the

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Line 18 – remove the s from picoeukaryotes Line 20 – replace is with was Line 24 – replace are with were Line 28 – replace under with below 5713: line 2 – move (MLD) to the other side of layers Line 3 – add the word the before MLD at the beginning of the sentence Line 4 – add the word the before MLD Line 12 – at a depth... Line 14 - remove has been Line 16 – remove on the first and replace with to, add the word a after for Line 17 – replace on with in, add an n after show Line 18 – replace both with the Line 24 – replace biomasses were with biomass was Line 25 – remove of, replace spread with varied Line 27 – was lower at ... 5714: line 1 – decreased Line 4 – replace is with was Line 5 – comma after transect, replace at with to Line 11 remove the Line 12 – replace are with were Line 13 – comma after however Line 20 erplace is with was Line 27 – hydrographic 5715: line 1 – replace is constituted with consists Line 2 – replace side with section Line 5 – add the afterin Line 20 – replace on with to Line 23 – move respectively to the end of the sentence Line 24 – differentiated 5716: line 2 – characterised Line 15 – add the after of Line 23 – remove the comma after 1994 and replace with a full stop Line 24 – replace at with to 5717: line 8 – comma after can, remove be and insert after part on the following line Line 18 – add the before STF Line 20 – comma after STCC, replace as for instance with including Line 24 – add the before STCC 5718: line 1 – add salinity unit after 0.05 Line 3 – comma after layer Line 5 – remove drastic Line 7 – replace is with was, twice Line 9 – add being before lower Line 13 – remove overall Line 16 – replace revealed with observed Line 23 – replace apparent with the Line 24 – add an s at the end of condition Line 27 – add the before surface 5719:line 1 – change maxima to maximum and move before was, removethe of after maxima Line 3 – add such after organisms, replace as well as with and Lines 2-4 – sentence does not make sense Line 4 - remove the word a Line 18 – remove overall Line 27 – move from the surface down to 200m depth and place it before prokaryotes on the following line, add an n after show 5720: lines 13-18 – 10nM phosphate is not limiting for Prochlorococcus or Synechococcus. Rethink what you want to say 5721: line 4 – add the after at Line 8 - remove also Line 10 – phosphate is not exhausted, merely at a very low concentration. It suits the numerically dominant

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autotrophs, namely Prochlorococcus and Synechococcus who are capable of turning over the pool of phosphorus at high rates and using phosphorus sources other than phosphate e.g. Hartmann, M., C. Grob, D.J. Scanlan, A.P. Martin, P.H. Burkill, and M.V. Zubkov. 2011. Comparison of phosphate uptake rates by the smallest plastidic and aplastidic protists in the North Atlantic subtropical gyre. *FEMS Microbiology Ecology* 78(2), 327-335. Line 20 - hydrographical Line 21 – relationship Line 26 – communities numerically dominated by... Line 27 – replace has with have 5722: line 2 – anticyclonic eddies lead to Line 3 – remove sea Line 28 – add the after at 5723: line 14 – add the after at Line 16 – sentence does not make sense Line 28 – remove the s at the end of despite 5724: line 12 – add the after at, Similarly Line 17 – replace is with was

The references and figure legends have not been checked by the reviewer

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Interactive comment on Biogeosciences Discuss., 10, 5701, 2013.

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