Biogeosciences
Discussions

Interactive comment on "Heterotrophic prokaryote distribution along a 2300 ㎞ transect in the North Pacific subtropical gyre during strong La Niña conditions: relationship between distribution and hydrological conditions" *by* M. Girault et al.

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Dear editors of the Biogeosciences and Reviewers, On behalf on my co-authors, I am pleased to submit the response to the reviewers of the manuscript entitled "Heterotrophic prokaryote distribution along a 2,300 km transect in the north Pacific subtropical gyre during strong La Niña conditions: relationship between distribution and hydrological conditions". We thank the editors and the anonymous reviewers for the very useful comments. They have helped to improve the quality of the new manuscript.

Anonymous Referee #1 -The authors present a useful set of hydrographic and bacterial abundance data obtained in a little-explored oceanic region, which are used to investigate possible connections between driving variables and microbial distributions. While the topic and the material is certainly of interest for the readers of Biogeosciences, the analysis has a number of limitations and should be significantly improved before publication. Of particular concern (as detailed below) is the way in which nutrient availability is assessed, lack of consideration of vertically integrated variables, use of bacterial abundance only and not bacterial biomass, lack of consideration of vertical mixing, and lack of focus on the underlying mechanisms that link hydrography with bacterial distributions. A large part of the manuscript deals with the potential role of nutrients in explaining the variability in bacterial group distribution, but I am not convinced that the authors have chosen the best approach to assess nutrient availability. Only nutrient concentrations, rather than nutrient supply rates, are considered. But in the surface layer of the tropical ocean, fast microbial consumption often result in a disconnect between nutrient concentration and nutrient supply rates. Changes in the latter may lead to changes in microbial abundance and diversity – without necessarily being reflected in changes of nutrient concentrations. This was shown by Gasol et al. (2009, Aq Microb Ecol 56:1-12): nutrient concentration in the upper mixed layer of the central tropical Atlantic is very low and relatively constant but diffusive fluxes change by more then 4 orders of magnitude, and had an impact on bacterial activity. The present manuscript requires a better characterization of nutrient supply if the relationship between potential nutrient limitation and bacterial group distribution is to be ascertained. One possibility is to compute vertical gradients in nutrient concentration and apply diffusivity values obtained from the literature (or use parametrizations based on measurements conducted during the cruise, e.g. vertical density gradients, wind speed, etc) to obtain estimates of vertical diffusive nutrient fluxes (see Gasol et al. 2009). Another approach, less accurate but also useful, would be to use the nutricline depth as a proxy for nutrient supply (Malone et al. 1993 Deep-Sea Res I 40:903-924; Cermeno et al. 2008 PNAS 105(51) 20344-20349). The analysis, based on a multivariate approach to re-

late different environmental and biological variables to bacterial abundance, suggests a rather 'static' view of the connection between environmental forcing and microbial distributions. No consideration is given to the role of vertical mixing and turbulence, but microbial populations are subject to vertical displacements, whose magnitude is likely to change significantly along the transect, and the authors coud explore this by calculating parameters such as the Brunt Vaaisala frequency.

***Answer: We thank Referee #1 for her/his comments on our manuscript. We did our best to address all the comments as described below, and in the new version of the manuscript. We globally share the same point of view about the important roles of both the vertical migration of organisms and the diffuse nutrient fluxes from the deep layer to sustain growth of organism in oligotrophic condition. At a large phytoplankton scale, several examples of microorganism able to mine nutrients in the deeper depth layer was reported in the NPSG and are in agreement with the hypothesis that mining nutrients can enhance the growth of large phytoplankton and associated living organisms (White et al., 2006). We also believe that mining scenario and role of nutrient fluxes can be in part outcompeted by episodic dust deposition event and of course the importance of diazotrophy in the upper layer of the NPSG (Wilson 2003; Kitajima et al. 2009; Calil et al., 2011). According to these reports in the literature, to the data set available (with a lack of organic nutrient concentrations) and the difficult task to measure in which extent the vertical migration, mining scenario, and nutrient fluxes control the pool of nutrients, our first version of the manuscript was written to represent the nutrient concentrations as an estimation rather than a dynamic stock that we could decompose in various measurable fluxes. However, we took into consideration the Referee's comments; and in this new version we have attended to decompose the role of nutrients in the upper layer by calculating the importance of the diffuse nutrient fluxes related to the integrate stock of inorganic nutrients (phosphate, nitrate and silicic acid) in the mixed layer. Our approach was based on the study of (Gasol et al., 2009) but the buoyancy frequency was calculated using the thermodynamic expression reported by King et al., (2012). This choice was motivated by the comments of King et al., (2012)

who evidenced that the widely used buoyancy frequency definition was incorrect and can lead to wrong instability diagnostic, especially in the Atlantic Ocean. The detailed buoyancy frequency equation and the estimation of the mixed layer depth were added in the materials and methods section (page 4, line 10-17). A figure of the vertical profile of N^2 in the mixed layer and the limit of the euphotic layer were also added into the manuscript (Fig. 3). Then, the vertical nutrient gradient profiles of phosphate, nitrate, and silicid acid were calculated in agreement with the study of Painter al. (2014) and Figure 4 was added (Page 5, lines 15-24). As we did not have ADCP data during the cruise, we used some vertical turbulent diffusivity rate values reported in the literature (Table 1) to select the most appropriate K in our study. Results and discussions about the role of diffusive nutrient fluxes were also developed in the new version of the manuscript. Except for nitrate, results pointed out that nutrients fluxes represent a very low percentage of integrative nutrient stock measured in the mixed layer, meaning that vertical diffuse nutrient fluxes are expected to be a minor nutrient supply phenomenon in the upper mixed layer. These results are in agreement with the study of Painter et al., (2014). The difference between the low (Phosphate and Silicic acid) and high percentages (nitrate) mainly results from the location of nutricline relatively to the mixed layer depth. For example, at Station 8, the depletion of nitrate in the upper layer and the nitracline depth which correspond to the mixed layer depth lead to an anomalous value of daily diffusive supply relative to the pool (432%).

References: White, A. E., Y. H. Spitz, and R. M. Letelier, Modeling carbohydrate ballasting by Trichodesmium spp. Mar. Ecol. Prog. Ser., 323, 35–45, 2006. Calil, P. H. R., S. C. Doney, K. Yumimoto, K. Eguchi, and T. Takemura , Episodic upwelling and dust deposition as bloom triggers in low-nutrient, low-chlorophyll regions, J. Geophys. Res.,116, C06030, doi:10.1029/2010JC006704, 2011.

-Given the sampling stations are far apart, and considering that the authors have conducted a relatively high-resolution sampling along the vertical, it could be useful to calculate vertically integrated abundance and or biomass for each group, and plot them

against environmental variables such as degre of stratificacion, mixing, nutricline depth, estimated vertical diffusive flux, etc. This approach is complementary to the multivariate analysis and well-suited to pursue a 'hypothesis-driven' analysis of the data. The vertical distribution of temperature and nutrient concentration should be shown, even though it has been presumably included in a previous article (Girault et al 2013b). However, these data are essential for the discussion of bacterial distribution and to make the present manuscript stand on its own.The authors use only abundance data but from a biogeochemical standpoint biomass can be more relevant. The flow cytometry data should allow calculation of cell biovolume and then an estimate of cellular biomass. Several studies that report on bacterial distribution over large spatial scales have used bacterial biomass as the key variable (e.g. articles by Zuvkov, Gasol, Moran and others). Microbial cell size is itself sensitive to both temperature and nutrient availability – therefore including cell size as a variable of study could provide additional insight.

***Answer: These excellent comments were also taken into consideration. We have therefore calculated the latitudinal contribution (%) of each heterotrophic prokaryote group as defined by flow cytometry to the whole heterotrophic prokaryote biomass from the surface down to 200 m depth (Figure 7). The heterotrophic prokaryote abundances were converted in terms of carbon biomass using a conversion factor (15 fg.C.cell-1) as defined in the literature (Caron et al. 1995) (Materials and methods page 7 line 2-4).

-Most of the Discussion is focused on the relationship between environmental or 'potentially driving' variables and the abundance of the different bacterial groups but there is little consideration of the underlying mechanisms. For instance, when the authors write "i) the LNA distribution is mainly explained by temperature and salinity and ii) HNA distribution is mainly explained by an association of variables (temperature, salinity, Chl a and silicic acid) rather than a single environmental factor" they are essentially re-stating the results of the multivariate analysis. But the question is: How are temperature and salinity driving the distribution of LNA bacteria? Is there a physiologically or ecologically plausible mechanism that links directly salinity with LNA abundance? Or

maybe is it the case that salinity is just acting as a marker for other properties which are themselves driving the variability in LNA abundance? Beyond highlighting correlation between variables, the Discussion would benefit from a deeper consideration of the ultimate mechanisms that govern microbial distribution.

***Answer: Once again we thank Referee#1 for the constructive comments and suggestions. In the discussion part of the new manuscript, we have discussed further the results of the PCA and RDA statistical analyses. Obviously, to better address the results highlighted by the statistical analyses, some additional experiments would be required. Unfortunately, experiments necessary to address the physiological response of the microorganism to the change of salinity. (e.g. incubations in batch culture and monitoring abundance or physiological effect of salinity gradient on the heterotrophic prokaryote communities) were not performed onboard, and were out of scope of the Tokyo-Palau cruise. Therefore in our manuscript, the link between salinity, temperature and heterotrophic prokaryotes is based on hypothesis already described in the literature. To the best of our knowledge, our study is the first report of the heterotrophic prokaryote abundances in this western part of the NPSG. As a consequence, as the Referee correctly noticed for the part related to la Nina, the conclusion of our manuscript is speculative, and based on the results of experiments performed in various environmental conditions.

-Specific points -The last section of the Introduction is missing a set of specific hypothesis which are to be tested. Previously the section has discussed possible relationships between environmental variables such as degree of oligotrophy and relative abundance of different bacterial groups, but no specific prediction is made as to what was to be expected along the transect.

-Abstract, Line 12. The phrase 'associated with temperature and salinity' is not informative. It should be specified whether the association is with high/low temperature and/or salinity.

***Answer: We have corrected the sentence Page 2 lines 1-3 as follows: Statistical analyses performed on the data set showed that LNA, mainly associated with low temperature and low salinity, were dominant in all the hydrological regions.

-The different stations are grouped into different areas according to temperature and salinity (page 15801, line 8), but the detailed criteria used in the partition are not indicated.

***Answer: We have added more information on page 8 lines 6-11 as follows: According to the temperature–salinity diagram of the Tokyo –Palau cruise shown in the study of (Girault et al., 2013b), three main areas corresponding to the Kuroshio region (Sta. 1–4), the subtropical gyre (Sta. 5–8) and the Transition zone (Sta. 9–12) were discriminated (Fig. 1). The discrimination between the Kuroshio area and the Subtropical gyre seawater masses was confirmed by comparing the Tokyo-Palau data set and the studies of Sekine and Miyamoto (2002) and Kitajima et al., (2009).

-Mixed layer depth is calculated but seems not to be included in the multivariate analysis. Why is this? Vertical mixing can have a strong impact on important processes such as nutrient input and exposure to high irradiance, among others.

***Answer: We agree with the Reviewer comment and we obviously think that the limits (depths) of the mixed layer and euphotic zone are important parameters. However, we believe that these very depths cannot be representative of the entire seawater column, and more especially of the distributions of the heterotrophic prokaryote communities in the samples collected at the surface. In this context, we did not take them into consideration in the multivariate analyses. These statistics were thus performed by using only the data set relative to the various variables measured at each sampling depth (nutrients, temperature, salinity, chlorophyll.a, etc.). Consequently, PCA and RDA were also calculated in agreement with this statement to explain the entire distribution of heterotrophic prokaryotes along the vertical profiles.

-Pages 15805-15806 There is a long discussion on the role of silicic acid which is

quite speculative. If no previous evidence is available to show that silica is limiting for phytoplankton in the region, the mechanistic linkage between silicic acid concentration and bacterial distribution is rather weak.

***Answer: We revised this part in order to emphasize the previous reports with our results (page 14, lines 4-20). The silicic acid part was firstly written to purpose an explanation of the PCA result. Indeed, the PCA showed that the silicic acid and the chlorophyll a vary along the first axis of the PCA. This result was unexpected for two main reasons: (i) the concentration of diatom is low, (ii) the phosphate or nitrate were more depleted in the upper layer. However, despites these two reasons, in the literature several evidences of the importance of silicic acid were also reported in the NPSG, (Baynes, 2012; Krause et al., 2012; Hashihama et al., 2014).

-Pages 15807-15808. The whole section on the role of climatic events such as El Nino/La Nina should be deleted as there is no data available to substantiate any claims on the topic.

***Answer: As suggested, we have deleted this section. However, although no comparison can be done with the existing data set, we actually believe that heterotrophic prokaryotes distribution can be significantly influenced by a large scale climatic variation such as the transition el Niño/la Niña. Our results on the ultraphytoplankton concentrations showed a significant difference between the samples collected during a El Niño or La Niña (Girault et al., 2013b). In the oligotrophic conditions such as the NPSG, the link between ultraphytoplankton and heterotrophic prokaryotes is probably important (due to the functioning of the microbial loop). In this context, we expected that this link could lead to a modification of the heterotrophic prokaryotes distribution depending on the environmental condition in this area. That is why we mentioned it in the first version of the manuscript.

-Page 15808, lines 26-28. If a high nucleic acid content is indicative of more active metabolism and faster growth, how do you explain that HNA bacteria are more abun-

dant under more nutrient-depleted conditions?

***Answer: Before answering this question, we must remind the Reviewer that the link between the nucleic acid content, cell activity, and growth are still unclear in the literature (Gasol et al., 1999; Bouvier et al., 2006). In agreement with literature and the comment of Referee 2 about the ecological role of nucleic acid content bacteria, we did not mention in this section that heterotrophic prokaryotes with HNA content correspond to cells with a more active metabolism. However, we observed that HNA are more abundant than LNA in the warm oligotrophic conditions and thus suggested that some heterotrophic prokaryote species among the HNA subgroup might be more warm-adapted than the LNA community in this warmer environmental condition. And the statistical analyses performed on these data confirm this observation. Numerous contradictions can be found in the literature about the explanation of the abundance of HNA and LNA subgroups depending on the environmental conditions, as reported in this section of the manuscript. All the studies cited in this manuscript provide very interesting conclusions. However, we believe that a study at the strain level (to address the biodiversity) would be very useful to link activity and nutrient concentration in the various environmental conditions met during the cruise. Following the original plan of the manuscript, we mention that an effort to better characterize the strains of HNA and LNA subgroups should therefore be taken into consideration in the future researches in the area of interest.

If, we discussed about the HNA and LNA at the subgroup level and put forward hypothesis that the HNA subgroup is only composed by one strain, we can then conclude that the low nutrient concentration observed in situ may result from the nutrient uptake competition between phytoplankton assemblages and active heterotrophic prokaryotes.

-Page 15812, line 7. Specify if the latitudinal increase in the HNA/LNA ratio is equatorward or northward.

***Answer: As requested we have revised the text, as follows: A latitudinal increase

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in the HNA/LNA ratio was found along the equatorward oligotrophic gradient and suggested different relationships between the various heterotrophic clusters and the environmental variables measured in situ during the cruise. (Page 20 line 13-15)

-The contouring in Fig. 4 gives too much weight to the horizontal axis, resulting in features which are not really supported by the data. Considering the long distance between stations, the contouring should emphasize the vertical variability without presenting horizontal features which are not based on actual measurements but are just extrapolations from the contouring software.

***Answer: The software used in the study linearly interpolated the mesh grid, depending on the depth and latitude. The choice of a 3-Dimentional mesh grid was motivated to better represent the variability intra- and inter- stations. To improve the Figure, we have modified the solid line into dash line according to the Reviewer's suggestion.

-Section 4.3 of Discussion is quite long and speculative. Considering the (inevitably for a long transect such as this one) poor horizontal resolution of the survey, not much can be said confidently about the role of mesoscale features on microbial distributions. This section should be shortened, and the related conclusions toned-down and perhaps omitted from the Abstract.

***Answer: We have shortened the Discussion section to focus on the main results. And to improve the quality of the section we also have added some sentences according to the suggestions of the 2nd Referee.

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-Anonymous Referee #2 -Received and published: 18 December 2014 -Review of manuscript "Heterotrophic prokaryote distribution along a 2300km transect in the North Pacific subtropical gyre during strong La Niña conditions: relationship between distribution and hydrological conditions" by M. Girault et al. The authors explored the spatial distribution of heterotrophic prokaryotes along a northsouth latitudinal transect (33_N

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- 12_N) crossing three different hydrographic areas (Kuroshio region, Subtropical gyre and transition zone). The biotic and abiotic parameters collected were used to investigate the relationships between the environmental parameters and the three prokaryotic populations (VHNA, HNA and LNA) distinguished by flow cytometry and nucleic acid staining according to their green fluorescence versus side scatter signature. Furthermore, the authors analyzed the results obtained using principal component (PCA) and redundancy analysis (RDA) in order to statistically identify the main parameters controlling the prokaryotic distribution. Finally, the authors showed a significant correlation between the hydrographic conditions and the prokaryotic communities distinguished by flow cytometry.

***Answer: We thank you very much for the corrections and your numerous suggestions. We took them into consideration to improve the manuscript. You'll find our answers in blue following each of your comment below.

-Major Comments -The manuscript presents a very interesting dataset in a poorly study area, however the data analysis needs to be substantially improved before publication. The statistical analyses presented do not allow to answer the main scientific question of the manuscript, i.e. "Which are the main controlling factors for the three prokaryotic populations along a north-south latitudinal transect characterized by different hydrographic conditions?" Furthermore, the discussion is often very descriptive and speculative, hence I strongly suggest the authors to refocus the manuscript pointing out the main findings according to the new results obtained. Finally, I find La Niña section not relevant for the manuscript, as there is no data available to prove any effect of La Niña on the distribution of the prokaryotic community.

***Answer: As mentioned by Referee #2, the study took place in a poorly studied area. In addition, the NPSG is a complex area where several seawater masses and mesoscale circulations are mixed together. In these complex environmental conditions and due to the lack of data, the identification of the main controlling factors is obviously difficult and depends on the scale of the study. For example microphyto-

plankton was reported to be potentially limited in phosphate in the western part of the NPSG (Hashihama et al., 2009; Kitajima et al., 2009). However, when we decomposed microphytoplankton into diatoms and dinoflagellates, utilisation of phosphate varied significantly and lead to some contrasting conclusions (Girault et al., 2013a). At the ultraphytoplankton and heterotrophic prokaryotes level we obviously believe that the environmental factors influence their distribution as observed for the microphytoplankton assemblages. PCA and RDA results highlighted from all the data collected two main features: i) LNA cluster distribution is explained by temperature and salinity, ii) HNA cluster is mainly explained by an association of variables (temperature, salinity, Chl.a and silicic acid). This second result emphasizes the complex identification of a single limiting factor and also pointed out the link between the phytoplankton parameters (Chl.a) and one subgroup: the HNA. At the subgroup level, the HNA subgroup could appear to some extent more active in the seawater column as its variances can be explained by the highly dynamic variation of phytoplankton. However, as mentioned in the response to Referee #1 and according to the numerous contrasting results highlighted in the study of Bouvier et al. (2006), it would be more correct to indicate that the numerically dominant species in the HNA subgroup are more related with the autotrophic clusters than the numerically dominant species in the LNA subgroup. The nature of this link seems also particular because the HNA cluster is more abundant in oligotrophic conditions where ultraphytoplankton concentrations (excepted nanocyanobacteria) were low.

-The authors statistically analyzed the "phytoplankton-related variables (Chl.a and silicic acid)", however; they never included the pico-phytoplankton (Prochlorococcus, Synechococcus and pico-eukaryotes) counts obtained by flow cytometry in the analyses. Thus, they did not use this data in the manuscript, although they mention to have it. I suggest the author to include this data in the next manuscript version.

***Answer: We took into consideration your request. In the first version of the manuscript, we did not detail the results because discussion would have been too long

and the main results were already published (Girault et al., 2013b). In this new version we decided to help the reader to summarize the most relevant information relative to the distribution of ultraphytoplankton (page 7 lines 5-15; page 8 lines 14-29).

-In the manuscript the authors discussed the role of nutrients in the distribution of HNA and LNA populations. What about the VHNA population? Please include the VHNA population in the discussion. Instead of using the HNA/LNA ratio in your analyses you could use the relative contribution of the three prokaryotic populations to the bulk prokaryotic community.

***Answer: We have revised the manuscript in order to take into consideration the VHNA cluster in the discussion (page 11 lines 20-26; page 17 lines 16-28; page 18 lines 21-26). We also added Figure (8b) in order to display the vertical distribution of the VHNA/HNA ratio. We have selected these two figures only because no significant relationship was found between each other subgroups.

-Minor Comments -Page 15801, line 16-23. This sentence can be moved to the methods section.

***Answer: We moved the sentence (Page 6 lines 25-32)

-Pages 15801-15802. Please add the standard deviation to the average concentration of LNA, HNA, VHNA populations.

***Answer: We have added the standard deviation to the average of the LNA, HNA and VHNA concentrations. (Page 10 lines 18-23)

-Page 15802. Please consider using in this section the relative contribution of the three prokaryotic populations instead of the HNA/LNA ratio (figure 5).

***Answer: In the new version of the manuscript we have considered the three subgroups (Page 11 lines 20-26; Page 17 lines 16-28; Page 18 lines 21-26).

-Page 15805-15806. The paragraph has to be revised in a more concise way, the

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discussion on the role of silicic acid is too long and speculative.

***Answer: We have modified this paragraph and improved the connection between the various arguments presented. (Page 14 lines 4-20)

-Page 15806. Here for the first time the authors discussed about Synechococcus abundance in the Subtropical Gyre and in the Kuroshio regions, however this data is not presented at all in the results section. Please add more information about the picophytoplankton counts along the transect.

***Answer: We have added information about the ultraphytoplankton distribution in the Material and Method section (Page 7 line 3-13) and in the Results as well (Page 8 lines 14-29).

-Pages 15807-15808. As I mentioned before I find La Niña section not relevant for the manuscript.

***Answer: According to Referee #1 and to your comment, we have decided to delete the La Niña part in the new version of the manuscript because indeed we do not have any direct proof or any additional data set from the literature to compare both situations. However, we sincerely believe that the heterotrophic prokaryote distribution may vary depending on such a large scale climatic event. As ultraphytoplankton abundances were reported to be highly different during la Niña and el Niño (Girault et al., 2013b) and variance of some heterotrophic prokaryotes (HNA group) was explained by the phytoplankton cluster (this study), we can reasonably consider that one part of the variance of the heterotrophic prokaryotes could be explained by the large scale climatic event such as the transition el Niño/la Niña. That is why we mentioned it into the first version of the manuscript.

-Pages 15809. So far it is not really clear what is the ecological role of the prokaryotic populations distinguished with the flow cytometer (HNA versus LNA) (Bouvier et al. 2007 EM). Please comment on that in the manuscript.

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***Answer: We agree with your interesting comment and have addressed this point in the Discussion section (Page 18, lines 21-26).

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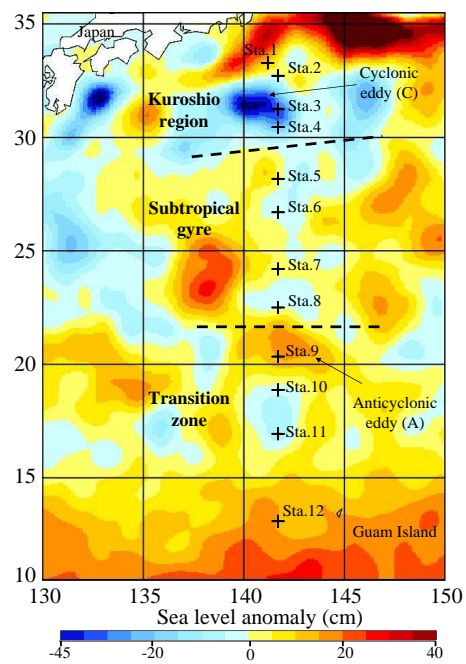
Fig. 1.

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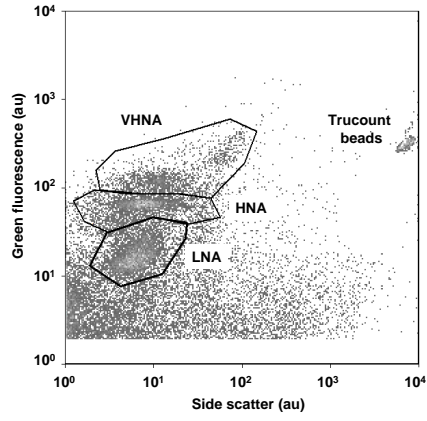
Fig. 2.

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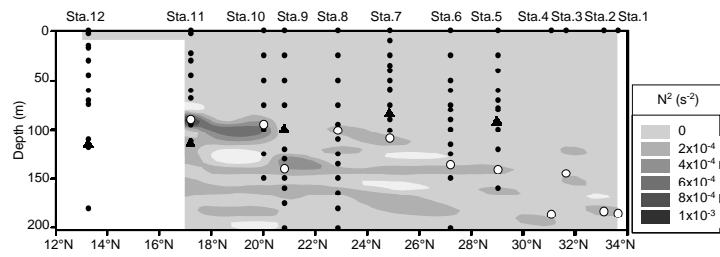
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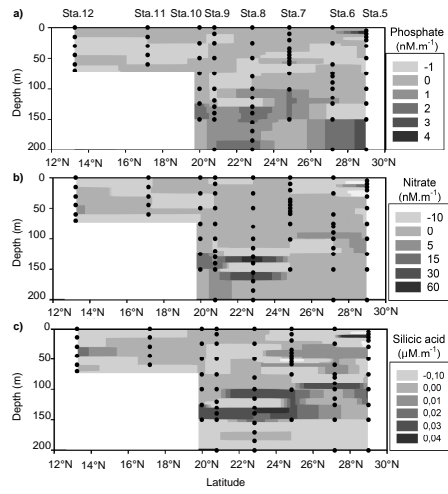
Fig. 4.

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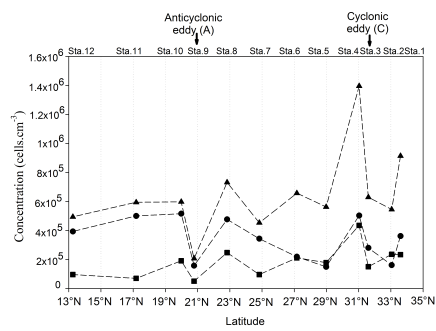
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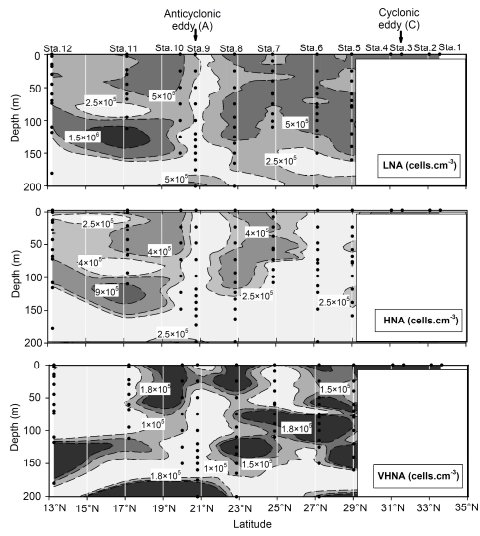
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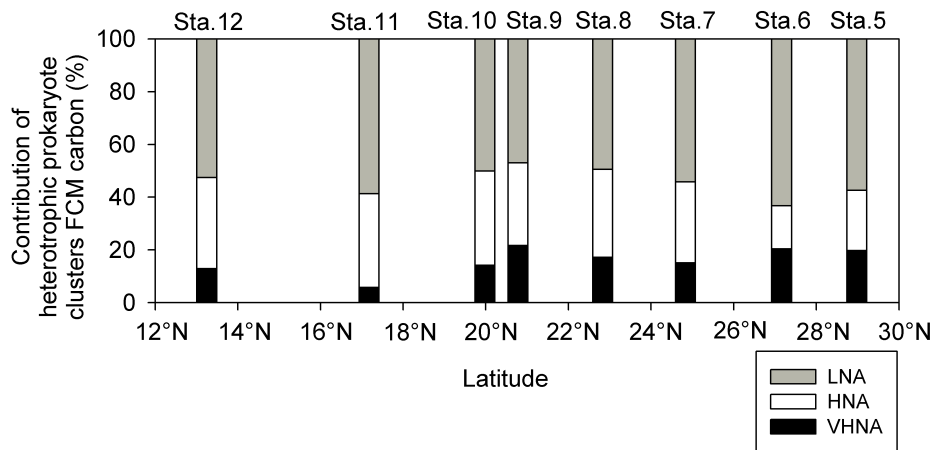
Fig. 7.
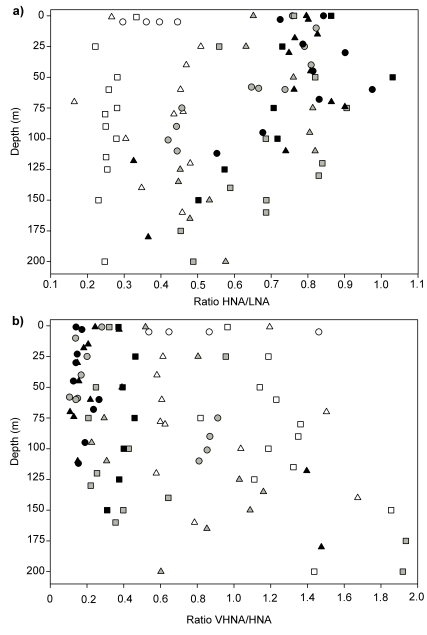
Fig. 8.

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Fig. 9.

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Fig. 10.

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