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 relate 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 (blue). 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.

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.

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.

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.

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.

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.

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.

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 abundant under more nutrient-depleted conditions?

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.

As requested we have revised the text, as follows: A latitudinal increase 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.
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.
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 - 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.

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.

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 microphytoplankton 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.

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.

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.

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.

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).

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

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.

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.

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.

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

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

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Abstract

The spatial distribution of heterotrophic prokaryotes was investigated during the Tokyo–Palau cruise in the western part of the North Pacific subtropical gyre (NPSG) along a north–south transect between 33.60 and 13.25° N. The cruise was conducted in three different hydrological areas identified as the Kuroshio region, the Subtropical gyre area and the Transition zone. Two eddies were crossed along the transect: one cold core cyclonic eddy and one warm core anticyclonic eddy and distributions of the heterotrophic prokaryotes were recorded. By using analytical flow cytometry and a nucleic acid staining protocol, heterotrophic prokaryotes were discriminated into three subgroups depending on their nucleic acid content (low, high and very high nucleic acid contents labeled LNA, HNA and VHNA, respectively). Statistical analyses performed on the dataset showed that LNA,

mainly associated with low temperature and low salinity, were dominant in all the hydrological regions. In contrast, HNA distribution seemed to be associated with temperature, salinity, Chl *a* and silicic acid. A latitudinal increase in the HNA/LNA ratio was observed along the north–south transect and was related to higher phosphate and nitrate concentrations. However, the opposite relationship observed for the VHNA/HNA ratio suggested that the link between nucleic acid content and oligotrophic conditions is not linear, underlying the complexity of the biodiversity in the VHNA, HNA and LNA subgroups. In the Kuroshio Current, it is suggested that the high concentration of heterotrophic prokaryotes observed at station 4 was linked to the path of the cold cyclonic eddy core. In contrast, it is thought that low concentrations of heterotrophic prokaryotes in the warm core of the anticyclonic gyre (Sta. 9) are related to the low nutrient concentrations measured in the seawater column. Our results showed that the high variability between the various heterotrophic prokaryote cluster abundances depend both on the mesoscale structures and the oligotrophic gradient.

1 Introduction

Marine heterotrophic prokaryotes play a key role in pelagic ecosystems both in terms of carbon sequestration and organic matter remineralisation. Their distribution is controlled by biotic (bottom-up control, top-down control by grazing, virus lyses) and abiotic variables (temperature, salinity, pressure, irradiance, nutrient concentrations). These possible limiting variables are shared with the autotrophic community and competition for resources inevitably occurs in order for each to survive in the same pelagic ecosystem. Competition between heterotrophic prokaryotes and phytoplankton for different forms of inorganic nitrogen and phosphorus has been clearly demonstrated both in laboratory experiments and in the open ocean (Currie and Kalff, 1984; Vadstein, 1998; Thingstad et al., 1998). Moreover, several studies have reported that dissolved organic compounds can be an alternative nutrient source for some nutrient-stressed phytoplankton (Duhamel et al., 2010; Girault et al., 2013a). The common utilization of the inorganic and/or organic matter, such as dissolved organic phosphorus, could lead to a tight coupling between the heterotrophic prokaryotes and photoautotrophs along an oligotrophic gradient. However, the relationship between heterotrophic prokaryote abundance and oligotrophic conditions is unclear, especially in terms of mesoscale structures such as eddies (Baltar et al., 2010; Lasternas et al., 2013). The differences within the same type of mesoscale circulation reported in the

literature highlights that the relationship between heterotrophic prokaryotes and photoautotrophs can be dependent on the identification of the different microorganisms making up the community (Girault et al., 2013b).

In this study, using analytical flow cytometry combined with fluorescent dyes we were able to identify three different subgroups among the bulk of heterotrophic prokaryotes: a group characterized by a very high nucleic acid content (VHNA), another by a high nucleic acid content (HNA), and finally a group with a low nucleic acid content (LNA). Previous studies have reported that the more active microorganisms seem to have the higher nucleic acid contents (Gasol et al., 1999; Lebaron et al., 2001). Complementary results have suggested that heterotrophic prokaryote activities are influenced by environmental parameters especially under oligotrophic conditions (Zubkov et al., 2001; Grégori et al., 2001, 2003a; Nishimura et al., 2005; Sherr et al., 2006; Bouvier et al., 2007). Using the basis of these previous reports, the oligotrophic conditions investigated in the western part of the NPSG during the Tokyo–Palau Cruise enabled us to examine the relationship between different groups of heterotrophic prokaryotes, as defined by different nucleic acid contents, and their environmental conditions.

Investigations into the heterotrophic prokaryote distribution in the western part of the NPSG are scarce and mostly restricted to the Kuroshio Current or the area near the Japan shelf during El-Niño events (Mitbavkar et al., 2009; Kataoka et al., 2009; Kobari et al., 2011). In contrast, the Tokyo Palau cruise was conducted during a strong LaNiña condition and over a large latitudinal gradient to include various seawater masses. In this work, we studied the extent to which abundance and distribution of various heterotrophic prokaryotic groups, defined by flow cytometry (VHNA, HNA, LNA) were influenced by phytoplankton distribution and environmental variables. The relationships between each heterotrophic prokaryote group and two different mesoscale eddies (one anticyclonic and one cyclonic) were also examined in order to identify any modification in organism distribution which could be related to the oligotrophic conditions found during the cruise.

2 Materials and methods

2.1 Study area and sample collection

This study was conducted from 17 January to 8 February 2011 on board RT/V Shinyo Maru during the Tokyo–Palau cruise. Samples were collected in the western part of the NPSG

between 33.60 and 13.25° N along the 141.5° E transect (Fig. 1). Twelve stations (Sta.) were sampled using 2.5 L Niskin bottles mounted on a rosette frame equipped with the Conductivity–Temperature–Depth (CTD) and in situ fluorometer system. Seawater was sampled without replicates at several depths between the surface and 200 m. Due to bad weather conditions, the seawater samples between station 1 and 4 were collected only at the surface (3 m) using a single Niskin Bottle. At these 4 stations, eXpendable Conductivity/Temperature/Depth profiling systems (XCTD) were used to measure temperature and salinity. The Brunt-Väisäla buoyancy frequency (N^2) was calculated using the exact thermodynamic expression reported by King et al. (2012) (equation 1).

$$N^2 = g^2\left(\frac{d\rho}{dp} - \frac{1}{c_s^2}\right) \qquad (1)$$

Where $\frac{d\rho}{dp}$ is the vertical gradient of *in-situ* density (ρ). The acceleration (g) due to the gravity was assumed to be constant during the Tokyo-Palau cruise (g=9.81) and the speed of sound (c_s) was calculated depending on the depth, salinity and temperature according to Del Grosso, (1974). The mixed layer depths were estimated as the depths at which the maximum stratification occurred (i.e., maximum of N^2 at each station). The irradiance was monitored at five stations (5, 7, 9, 11, 12) using a Profiling Reflectance radiometer (PRR 600 Biospherical Instrument®). The depth of the euphotic layer was estimated as the depth of 1 % of photosynthetically active radiation at noon.

2.2 Altimetry and large scale climatic conditions

The altimetry data (sea level anomaly) was produced by Ssalto/Duacs and distributed by Aviso, with support from CNES (http://www.aviso.oceanobs.com/duacs/). The sea level anomaly map centered on the 18 January 2011 was plotted using the Panoply software from NASA (http://www.giss.nasa.gov/tools/panoply/). This map was processed by compiling the data collected over a six weeks period before and after the chosen date (Fig. 1). The current sea maps provided by the bulletin of the Japanese coast guard were used to validate the satellite data and display the paths of both the cyclonic gyre and the Kuroshio Stream (http://www1.kaiho.mlit.go.jp/KANKYO/KAIYO/qboc/index_E.html).

2.3 Nutrient analyses

Nutrient samples were collected from Niskin bottles, immediately put into cleaned plastic tubes in the dark, plunged into liquid nitrogen and stored in the deep freeze (−60 ◦C) until analyses. The highly sensitive colorimetric method incorporating the AutoAnalyzer II (SEAL Analytical) and Liquid Waveguide Capillarity Cells (World precision Instruments), was used to determine nutrient concentrations (nitrate + nitrite, soluble reactive phosphorus and silicic acid) according to the methods listed in Hashihama et al. (2009) and Hashihama and Kanda (2010). Seawater collected at the surface of the western part of NPSG, which had been preserved for > 1 year, was used as nitrate + nitrite blank water. The blank water was analyzed using the chemiluminescent method described in Garside (1982). The detection limits for nitrate + nitrite, soluble reactive phosphorus and silicic acid were 3, 3 and 11 nM, respectively. Because soluble reactive phosphorus consists mainly of orthophosphate and nitrite was not substantially detectable, soluble reactive phosphorus and nitrate + nitrite are hereafter referred to as phosphate and nitrate.

The nutrient fluxes into the surface mixed layer were calculated using the equation $K\frac{dNut}{dz}$ where K is the local vertical diffusivity, *Nut* the concentration in nutrients (phosphates, nitrates or silicic acid) and $\frac{dNut}{dz}$ the vertical nutrient gradient. To compensate for irregular sampling depths among the stations, the nutrient profiles were linearly interpolated onto the 1 m grid. Then, vertical nutrient gradients were calculated between sequential depth bins (Painter et al., 2013). This method has the advantage to show the nutrient flux from a particular part of the water column. Due to the lack of an Acoustic Doppler Current Profiler (ADCP) on the ship, the local vertical diffusivity (K) was estimated using the literature (Table 1). Among the K values reported in the oligotrophic conditions, a vertical diffusion coefficient of 0.5 $cm^2.s^{-1}$ was chosen as a standard value (Table 1).

2.4 Chlorophyll *a* and flow cytometry analyses

The depth of the deep chlorophyll a maximum was determined from fluorescence profiles using the pre-calibrated in situ fluorometer. To measure chlorophyll a concentration, 250 cm^3 of seawater was filtrated through Whatman® nucleopore filters (porosity ~ 0.2 µm) using a low vacuum pressure (< 100 mm of Hg). Filters were then immersed into tubes containing N,N-dimethylformamide (DMF)- and stored in the dark at 4° C until analyses on shore.

Chlorophyll a was analysed using a Turner Designs fluorometer pre-calibrated with pure Chl a pigment (Suzuki and Ishimaru, 1990).

Samples for heterotrophic prokaryotes were collected from the Niskin bottles and pre-filtered onto disposable 100 µm porosity nylon filters to prevent clogging of any in the flow cytometer. Seawater aliquots of 1.8 cm3 were fixed with 2 % (w/v final dilution) formaldehyde solution, quickly frozen in liquid nitrogen and stored in the deep freeze onboard (−60 °C) until analysis at the flow cytometry core facility PRECYM of the Mediterranean Institute of Oceanology (http://precym.mio.osupytheas.fr). In the PRECYM, samples were thawed at room temperature and stained using SYBR Green II (Molecular Probes®) methods detailed in Marie et al. (1999), Lebaron et al. (1998) and modified by Grégori et al. (2003b). The analyses were performed on a FACSCalibur flow cytometer (BD Biosciences®) equipped with an air-cooled argon laser (488 nm, 15 mW). For each particle (cell), five optical parameters were recorded: two light scatter signals, namely forward and right angle light scatters and three fluorescences corresponding to emissions in green (515–545 nm), orange (564–606 nm) and red (653–669 nm) wavelength ranges. Data were collected using the CellQuest software (BD Biosciences®) and the analysis and optical resolution of the various groups of heterotrophic prokaryotes were performed a posteriori using the SUMMIT v4.3 software (Beckman Coulter). For each sample, the runtime of the flow cytometer was 2 min and the flow rate set to 50 µL.min−1 (corresponding to the “Med” flow rate of the flow cytometer). Trucount™ calibration beads (Becton Dickinson Biosciences) were also added to the samples just prior to analysis as an internal standard to monitor the instrument stability and accurately determine the volume analyzed. Following the staining of the nucleic acid with SYBR Green II, heterotrophic prokaryotes, excited at 488 nm, were recorded and enumerated according to their right-angle light scatter intensity (SSC) which relates to the cell size and their green fluorescence intensity (515–545 nm) which relates to the nucleic acid content. As already widely described in the literature, several heterotrophic prokaryote groups can be optically resolved by flow cytometry depending on their average green fluorescence intensities related to their nucleic acid content : in this study, a group of cells with a lower green fluorescence corresponding to heterotrophic prokaryotes with a lower nucleic acid content (LNA), a group of cells displaying a higher green fluorescence corresponding to a higher nucleic acid content (HNA) and a last group of cells with the highest green fluorescence intensity corresponding to the highest nucleic acid content (VHNA) (Fig. 2). The overlap between the stained phytoplankton, in particular *Prochlorococcus* and *Synechococcus*, and the heterotrophic prokaryotes (in terms of green fluorescence and side scatter intensity)

was resolved by using red fluorescence (induced by the chlorophyll) to discriminate and identify the photoautotrophs (Sieracki et al., 1995). The heterotrophic prokaryote abundances were also expressed for each cluster (LNA, HNA and VHNA) in terms of carbon biomass using a conversion factor of 15 fg.C.cell^{-1} (Caron *et al.*, 1995).

Although this study focuses on the distribution of the heterotrophic prokaryotes, ultraphytoplankton was also investigated during the Tokyo-Palau project. Briefly, the ultraphytoplankton was sampled thanks to Niskin bottles and filtrated through a 100 µm mesh size. 4.5 cm^{-3} of subsamples preserved with 0.5 cm^{-3} of a 20 % formaldehyde solution (i.e., 2% final concentration) were put into 5 cm^{-3} Cryovials tubes. Similarly to the heterotrophic prokaryote samples, Cryovials tubes were rapidly frozen in liquid nitrogen and stored in a deep freezer (-60 °C) until analysis. Analyses were all performed in the same period than the heterotrophic prokaryotes and based on their light scatter and fluorescence emission properties. Ultraphytoplankton was discriminated in this study into five flow cytometry clusters (*Synechococcus*, *Prochlorococcus*, Picoeukaryotes, Nanoeukaryotes and Nanocyanobacteria-like) as described in Girault et al. (2013b).

2.5 Statistical analysis

To analyse the multivariate data set, principal component analyses (PCA) and redundancy analysis (RDA) were performed using the R software (vegan package) and the Biplot macro for Excel® (Lipkovich and Smith, 2002). PCA was performed in order to qualitatively identify the relationships between heterotrophic prokaryotes and the environmental variables (Pearson, 1901). Possible links between each heterotrophic prokaryote subgroup and their environmental variables were quantitatively examined using the RDA. For the RDA, the data set was log10 (x+1)-transformed to correct for the large differences in scale among the original variables. A Monte-Carlo test was used in order to test the significance of the RDA results. Partial RDAs were also carried out to evaluate the effects of each explanatory variable set on the heterotrophic prokaryote composition (Liu, 1997). The first RDA was performed on the whole data set by taking into account the heterotrophic prokaryotes as one single group. Additional partial RDAs were performed for each subgroup (LNA, HNA, and VHNA). The environmental variables in the additional partial RDAs were classified into three intercorrelated variable groups, namely: the depth-related parameters (phosphate, nitrate, depth), spatial-related parameters (temperature and salinity) and the phytoplankton-related parameters (Chl *a* and silicic acid). This decision was made considering the results of the PCA (environmental variables were separated into three

groups).

3 Results

3.1 Sampling sites and ultraphytoplankton distribution.

The cruise took place along a north–south transect in the western part of the NPSG (141.5° E) during a strong La-Niña climatic event. According to the temperature–salinity diagram presented in the study made by 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). The cruise crossed two main eddies identified in this study as a cold core cyclonic eddy (C), and a warm core anticyclonic eddy (A) (Fig. 1). Eddy C (31° N, 141° E) is located in the Kuroshio region and eddy A (20.5° N, 142° E) in the Transition zone. The distribution of the ultraphytoplankton assemblages observed during the Tokyo-Palau cruise was reported in detail in the study of Girault et al., (2013b). Briefly, ultraphytoplankton was characterized by an heterogeneous distribution of its phytoplankton groups associated with the complex distribution of the various seawater masses met during the cruise (including salinity front, subtropical countercurrent, eddies). Among the phytoplankton communities *Prochlorococcus* numerically dominated the ultraphytoplankton assemblages in the samples collected in the stratified oligotrophic areas such as the Subtropical gyre area and the Transition zone. Picoeukaryotes, Nanoeukaryotes and *Synechococcus* also constituted a significant part of the carbon biomass in the region depleted in phosphate and nitrate. The role of the cold core eddy C was reported at the surface where the highest concentration of Nanoeukaryotes in the surface sample was found in the very core of the cyclonic eddy (Sta. 3) and where, the *Synechococcus* outnumbered the *Prochlorococcus* abundance in the path of the cold core cyclonic eddy (Sta. 4). The Nanocynaobacteria-like group was reported to be controlled by the frontal system observed at station 9 rather than the concentration of inorganic nutrients.

3.2 Stratification of seawater masses and vertical nutrient fluxes

The Brunt-Väisäla buoyancy frequencies calculated from the CTD data set are characterized

by low N^2 values (<$2x10^{-4}$ s^{-2}) from the surface down to the 90 m depth (Fig. 3). Below this depth, the vertical distribution of N^2 was more irregular and reached at the maximum $1.09x10^{-3}$ s^{-2} at station 11 (90 m). Fig. 3 also shows that the depth of the N^2 maximum (thermocline depth) tended to be shallower in the southernmost part of the transect (Sta. 1, 185 m to Sta. 11, 90 m) underlying the strengthening of the upper thermocline when the heat flux at the surface is positive and wind mixing is low in the South part of the transect. Along the latitudinal transect, two particular values of the thermocline depths were found at station 3 (145 m) and at station 9 (140 m) corresponding to the cyclonic and anticyclonic eddies, respectively. Moreover, excepted at station 3 and station 9, the first increases of N^2 (> $2x10^{-4}$ s^{-2}) from the surface to the 200 m depth corresponded to the depth of the thermocline and indicated the lack of seasonal thermocline as already described in Sprintall and Roemmich (1999). The limit of the euphotic layer (defined by the depth with 1 % of the irradiance at the surface) was also plotted in Fig. 3. During the cruise, this limit varied from 84 m (Sta. 7) to 115 m (Sta. 12). Except station 11, the limit of the euphotic layer was located upper the thermocline. The average of the absolute difference between the euphotic layer and the thermocline depths was 34 ±11 m.

Figure 4 shows the vertical gradient of nutrients (phosphates, nitrates and silicic acid). The vertical phosphate profiles were characterized by a very low gradient (<1 $nM.m^{-1}$) in the upper 100 m from station 6 to station 12. Both positive and negative gradients were observed and no specific distribution between them was found. Under the depth of 100 m, higher phosphate gradients (> 3 $nM.m^{-1}$) were found and defined the phosphacline depths as displayed in Figure 6 of the study made by Girault et al. (2013b). Nitrates showed that vertical profiles closely corresponded to phosphates with negative or positive values lower than 5 $nM.m^{-1}$ and higher gradient below 100 m. The vertical distribution of the silicic acid gradient was more complex with moderate gradients ranging from 0.01 to 0.02 $\mu M.m^{-1}$ were observed in the upper 100 m depth at stations 5, 6, 7, and 12. Similarly to phosphates and nitrates, the highest gradients of silicic acide (0.04 $\mu M.m^{-1}$) were found below 100 m depth from station 6 to station 10. Taking into account all the panels of Figure 4, Station 8 showed a particular pattern between 100 m and 160 m depths where two superimposed high gradients were observed. The depths of these high gradients were found to be similar for phosphates and silicic acid (100-115 m and 130-155 m) but the vertical profile of nitrates gradient showed a slightly lower depth (130-140 m and 155-170 m).

By using a vertical diffusion coefficient of 0.5 $cm^2.s^{-1}$, the nutrient fluxes were calculated

from station 5 to station 11 (Table 2). Phosphate fluxes into the surface mixed layer were negative at stations 5 and 6 (-0.52 and -1.34 µmol.m^{-2}.d^{-1}, respectively) and positive from station 7 to station 11. The positive phosphate fluxes were maximum at station 7 (9.43 µmol.m^{-2}.d^{-1}) and decreased to reach 1.38 µmol.m^{-2}.d^{-1} at station 11. The percentages of diffuse flux per day relative to the standing stock in the mixed layer were particularly low and varied from -0.03 % (Sta. 6) to 0.76 % (Sta. 8). Nitrate fluxes into the mixed layer were positive and highly variable along the transect (~0 to 81.3 µmol.m^{-2}.d^{-1}). The percentage of daily diffuse supply relative to the pool reflects this result and varied from ~0 (Sta. 7 and 10) to 432 % (Sta. 8). The Silicic acid fluxes were globally higher than the phosphate and nitrate fluxes calculated in the mixed layer (up to 571.1 µmol.m^{-2}.d^{-1}; Sta. 9). The daily diffuse supply relative to the mixed layer pool was low and spread from 0.002 % (Sta. 5) to 0.48 % (Sta. 9).

3.3 Distribution of the heterotrophic prokaryotes

After staining with the SYBR green II fluorescent dye, three clusters of heterotrophic prokaryotes were characterized by their different green fluorescence mean intensities (Fig. 2). Figure 5 shows the abundance of each heterotrophic prokaryote cluster at the surface, along with latitude. In the surface samples of the Kuroshio region the average concentrations of LNA, HNA and VHNA were, 8.71x10^5±3.8x10^5, 3.27x10^5±1.4x10^5 and 2.64x10^5±1.2x10^5 cells.cm^{-3}, respectively. In the Subtropical area the average concentrations of LNA, HNA and VHNA were 6.01x10^5±1.2x10^5, 2.97x10^5±1.4x10^5 and 1.84x10^5±6.4x10^4 cells.cm^{-3}, respectively. In the Transition zone the average concentrations of LNA, HNA and VHNA were 5.18x10^5±1.8x10^5, 4.38x10^5±1.6x10^5 and 1.15x10^5±6.2x10^5 cells.cm^{-3}, respectively. Despite the high variability between the concentrations along the north-south transect, the distribution of the three heterotrophic prokaryote groups was characterized by a common maximum at station 4 and a minimum at station 9. At station 4 the concentrations of LNA, HNA and VHNA were 1.39x10^6, 5.03x10^5 and 4.35x10^{5}cells.cm^{-3}, respectively. In contrast, the concentrations of LNA, HNA and VHNA at station 9 were 2.07x10^5, 1.6x10^5 and 5.07x10^{4}cells.cm^{-3}, respectively. To a lesser extent, high concentrations of LNA (9.13x10^{5}cells.cm^{-3}) and HNA (3.62x10^{5}cells.cm^{-3}) were identified at the northernmost station of the Kuroshio region (at station 1).

The vertical distributions of heterotrophic prokaryotes were also investigated along the transect (Fig. 6). As for surface, the vertical distributions of all heterotrophic prokaryote groups are characterized by lower cell concentrations at station 9. In this very station both

LNA and HNA concentrations are significantly lower than at the other stations (Kruskal wallis test, n = 90, P value < 0.05). The LNA cluster is numerically dominant in 99 % of the samples. The VHNA concentrations are lower than the HNA in 75 % of the samples. The contributions of each heterotrophic prokaryote cluster in terms of carbon biomass integrated between the surface and the 200 m depth to the total heterotrophic prokaryote carbon biomass (sum of the biomasses of the three heterotrophic prokaryote clusters) is shown in Fig. 7. LNA numerically dominated the carbon biomass from surface to the depth of 200 m (Sta. 5 to Sta. 12). The latitudinal contribution of the LNA cluster to the total heterotrophic prokaryotes in terms of carbon biomass varied from 47 % (Sta. 9) to 63 % (Sta. 6). Contribution of the HNA cluster is characterized by a low percentage at stations 5 and 6 (22 % and 16 %, respectively) and a near constant contribution between station 7 and the southernmost station 12 (33 ± 2 %; n=6). The contribution of the VHNA cluster was nearly constant from station 5 to 9 (19 ± 2 %; n=5). Then, it reached the lower values in the Transition zone (14 % at Sta. 10, 5 % at Sta.11 and 12 % at Sta.12).

Figure 8a displays the ratios of HNA/LNA concentration depending on depth. In the Kuroshio region, ratios are low and varied from 0.29 (Sta. 2) to 0.44 (Sta. 3). In the Subtropical gyre area, the ratios varied from 0.16 (Sta. 5, 70 m) to 0.82 (Sta. 7, 10 m). The higher ratios (up to 1.03 at Sta. 10, 10 m) were observed in the surface layer of the Transition zone. In the Transition zone and the Subtropical gyre area the higher ratios measured were found between the surface and 100 m. Figure 8b shows the ratio of VHNA/HNA concentrations depending on depth. In the Kuroshio region the ratio varied from 0.53 (Sta. 3) to 1.46 (Sta. 2). In the Subtropical gyre area, the ratio varied from 0.10 (Sta. 7, 58 m) to 1.93 (Sta. 9, 175 m). In the Transition zone the ratio varied from 0.10 (Sta. 12, 70 m) to 1.47 (Sta. 12, 180 m). The average of the VHNA/HNA ratio (0.37 ± 0.35) in the Transition zone was the lowest of the three sampled regions (0.78 ± 0.44 in the Subtropical gyre; 0.88 ± 0.41 in the Kuroshio region).

3.4 Statistical analysis

Results of the Principal Component Analysis (PCA) and the Redundance Analysis (RDA) are shown in Figs. 9 and 10, respectively. The correlation circle of the PCA, displays the first two principal components (PC1 and PC2) which accounted for 32.44 and 27.67 % of the total inertia, respectively. The third and fourth principal components are not shown due to the low inertia exhibited (11 and 8 % of the total inertia, respectively) and the lack of any

clear ecological understanding. Silicic acid, Chl *a*, VHNA and LNA where differentiated from temperature and salinity by PC1, while PC2 mainly differentiated depth, nitrate, and phosphate (negative coordinates) from the HNA clusters (positive coordinate). Using hierarchical classification the sampling depths were separated into six different clusters (Table 3 and Fig. 9.) Cluster 1, black dot, characterized all the stations located in the Kuroshio region. Cluster 2 samples were collected at the edge of the Subtropical gyre but also contained the deepest sample collected at station 9 (200 m), which was in the anticyclone eddy in the transition zone. All samples in Cluster 3 were collected below a depth of 125 m where nitrate and phosphate concentrations were higher than for surface samples. This cluster was defined as the deep layer group. Cluster 4 samples were collected in the center of the subtropical gyre (stations 7 and 8) where heterotrophic prokaryote concentrations were at their maximum in the seawater column. Cluster 5 represented the samples collected in the anticyclonic eddy where a marked salinity has been reported (Girault et al., 2013b). Located in the Transition zone, at the southernmost stations the sixth and last cluster group was characterized by the highest salinity and temperature values. This last cluster (blue dots in the Fig. 9a) is distinguished from the deep layer group (Cluster 3, green dots) by the low nutrient concentrations measured in the upper layer.

A redundancy analysis (Fig. 10) was then performed to find out how the measured environmental factors influenced the distribution of heterotrophic prokaryote subgroups sampled during the cruise. The cumulative percentage of all canonical eigenvalues indicated that 69.1 % of the observed heterotrophic cluster variations were explained by environmental factors. The first two axes of the RDA explained 38 and 24 % of the total variance, respectively. Monte-Carlo tests for these two axes were significant (P value < 0.05, using 999 permutations) and suggested that environmental parameters might be important in explaining heterotrophic prokaryote distribution. The first axis is negatively correlated with salinity and positively correlated with the LNA cluster. The second axis is negatively correlated with temperature and the HNA cluster and positively correlated with the VHNA cluster. RDA suggested two main correlations between the LNA cluster and the phytoplankton-related variables (Chl a and silicic acid) and the HNA cluster with the depth-related variables (nutrients such as nitrate and phosphate and depth).

To confirm and quantify these possible correlations 4 partial RDAs were also performed: one partial RDA using all the heterotrophic prokaryotes at once and one additional partial RDA for each heterotrophic prokaryote subgroup (LNA, HNA and VHNA). Results of the partial RDA performed on all the heterotrophic prokaryotes showed that among the six

environmental variables measured during the cruise, salinity and temperature statistically contribute for 24 and 7.5 % of the variation of the heterotrophic prokaryotes, respectively. To a lesser extent, phosphate alone explained 3.5 % of the variability, whereas Chl a, nitrate, depth and silicic acid explained only 1.8, 1.7, 1.7 and 0.86 %, respectively. The partial RDAs performed either on LNA, or HNA, or VHNA indicated that environmental parameters can explain 60, 55 and 27 % of the total variance, respectively (Table 4). Partial RDA results showed that the spatial related parameters alone can explain up to 31 % of the variation in the heterotrophic prokaryote distribution. The depth-related parameters explained between 6 and 8 % of the variance and finally the phytoplankton-related group explained a maximum 4 % of the variance in the LNA heterotrophic prokaryotes. As far as the HNA cluster is concerned, the joint variation of the spatial- and phytoplankton-related parameters explained 22 % of the variance.

4 Discussion

4.1 Relationship between the heterotrophic prokaryotes and phytoplankton along the oligotrophic gradient

The spatial distribution of the heterotrophic prokaryote clusters defined by flow cytometry can be discriminated into three main areas that correspond to different seawater masses: (i) the Kuroshio region, where the highest heterotrophic prokaryote concentrations were measured, (ii) the Subtropical gyre and (iii) the Transition zone both characterized by a high variability in the heterotrophic prokaryote concentrations in the seawater column (Figs. 1, 5 and 6). Separation between the Subtropical gyre and the Transition zone was made using the salinity front observed south of station 8 (Girault et al., 2013b). The hierarchical classification performed on the first two axes of the PCA, statistically confirmed this latitudinal pattern and also provided additional information on the relationships between the environmental parameters and specific mesoscale structures encountered during the cruise. Discrimination of six different clusters highlighted the complex assemblages of the mesoscale structures in the three main areas as previously reported in the NPSG area (Aoki et al., 2002) (Fig. 9). For example, stations located in the Transition zone were statistically discriminated into two clusters (Clusters 5 and 6) due to the high salinity and temperature values in the anticyclonic eddy (station 9). In addition to the latitudinal variations, vertical distribution is also important and this is taken into consideration with cluster 3. This cluster

grouped the deep layer samples which were characterized by higher nutrient concentrations than found in the upper layer (negative coordinates on PCA2). An interesting result obtained from the PCA and RDA, is that PC1 characterized both the silicic acid and Chl a concentrations. This result suggests a possible link between the abundance of phytoplankton and silicic acid concentrations. Concerning the large phytoplankton, evidence of Si depletion (<11 nmol.L^{-1}) associated with bloom of diatoms was previously reported in the Kuroshio current and highlighted that under specific conditions such as an eddy, large phytoplankton can be controlled in part by the availability of the silicic acid in this area (Hashihama et al., 2014). However, the effect of silicic acid on phytoplankton over a larger scale is unexpected, as the lowest concentrations of phosphate and nitrate have been reported in the euphotic layer of the western part of the NPSG area, and Si : N : P stoichiometry measured during the Tokyo–Palau cruise identified nitrogen and/or phosphorus to be potential limiting factors (Hashihama et al., 2009, 2014; Girault et al., 2013b). The nature of the control and the role of microorganisms with a smaller size, including small diazotrophs, on the silicic acid uptake are still controversial. Based on the nutrient uptake values measured in the NPSG area, relationship between the very high efficient uptake of silicic acid and silicified organisms was reported in Krause et al. (2012). This high efficiency of silicic acid uptake may explain in part the correlation between these two variables as observed both in the PCA and RDA despite the low concentrations of large silicified organisms measured in this area (Girault et al., 2013a). On the other hand, measurements of the Si-bioaccumulation in some strains of *Synechococcus* were reported in Baines et al. (2012), suggesting that some organisms without a Si-skeleton may also be involved in this silicic acid-Chl *a* correlation. In the Tokyo–Palau cruise, high *Synechococcus* concentrations were measured in the Subtropical gyre and in the Kuroshio regions and may lead to an unidentified Si pool in these areas. Finally, the availability of a Si pool may be in part promoted by the regeneration mechanism initiated from the marine bacterial assemblages (Bidle and Azam, 1999). The association of silicic acid and Chl *a* was proposed in this study to quantify the extent to which environmental parameters can explain the variation in heterotrophic prokaryotes. This approach seemed to be ecologically sound for the Tokyo–Palau cruise, as demonstrated by the partial RDA (silicic acid and Chl a were grouped together).

By using partial RDA analyses, the quantification of the effects of the environmental variables was in agreement with the PCA results. The hydrological parameters including temperature, salinity and to a lesser extent nutrients confirms the key role of the mesoscale

circulation. At the subgroup level LNA, HNA, and VHNA distributions appeared to be spatially different. This pattern is illustrated with the patchy distribution of the VHNA in comparison to the LNA and HNA distributions. With only 27 % of the variance explained, the distribution of VHNA is difficult to relate to the specific environmental parameters measured during the cruise, despite a non-negligible part of total variation explained by temperature and salinity (Table 4). The significant role of spatial-related variables is also observed in the LNA and HNA cluster distributions and matches well with the mesoscale circulation. “Pure” phytoplankton-related variables (Chl a, silicic acid) as a general control (bottom up) of the VHNA, HNA, and LNA distributions accounted only for a small fraction (1–4 %) of the explained variation. Indeed, in contrast to some recent investigations, this study suggests that Chl a and silicic acid variables are poorly correlated to the distribution of the various heterotrophic prokaryote subgroups (Sherr et al., 2006; Bouvier et al., 2007; Van Wambeke et al., 2011). However, when the phytoplankton-related variables were combined with spatial-related variables, the combination gave a negative loading for VHNA and LNA, while 22 % of the variance was calculated for the HNA. This may suggest that phytoplankton related variables are less important for VHNA and LNA than for HNA. This means that the variation in HNA is more likely to be spatial and phytoplankton dependent. The link between HNA and the spatial- and phytoplankton-related variables is not obvious in Fig. 9 because PCA cannot quantify the unique variation belonging to the specific variables. The partial RDA provided possible evidence and quantified that: (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.

4.2 Diffusive nutrient fluxes and their biological relevance

Relationships between the vertical distribution of nutrients and ultraphytoplankton were investigated in a previous article (Girault et al., 2013b). Results pointed out that both phosphate and nitrate concentrations were particularly low in the upper layer of the ocean and characterized an oligotrophic environment combined with a complex assemblage of seawater masses. Calculation of Brunt-Väisäla buoyancy frequencies confirmed the well stratified structure observed along the transect, especially at stations 10 and 11 where the higher values of N^2 were found. Contrasting to some other oligotrophic areas the depth receiving 1 % of the irradiance in surface (used to define the euphotic layer) was not coupled with the thermocline, suggesting that a part of the organic material could be

transported below the euphotic layer (by vertical migration of organisms for instance). In these low nutrient conditions, theoretical calculation of nutrient supplies from the mixed layer was investigated in order to estimate the influence of these nutrient supplies in the upper mixed layer (Tables 1 and 2). The results obtained should obviously be taken with caution, especially for nitrates due to the importance of diazotrophy as previously reported and to episodic dust deposition not negligible in the NPSG (Wilson, 2003; Kitajima et al., 2009; Maki et al., 2011). However, results of the phosphate and nitrogen diffuse fluxes were in agreement with the value reported in oligotrophic conditions (Gasol et al., 2009). Silicic acid diffuse fluxes were also in the range of values reported by Painter et al., (2014). Negative diffuse fluxes of phosphate observed at stations 5 and 6 resulted from the variation of phosphate concentration in the mixed layer and the depth of the phosphacline (found at ~200 m depths). The oscillation of positive and negative values in phosphate-depleted condition also pointed out the approximation linked to the limit of detection of the phosphate concentration (3 nM) in the oligotrophic upper layer. The comparison between the phosphate or silicic acid fluxes and the mixed layer integrated concentration of nutrients suggested that the daily diffuse fluxes were of minor importance to resupply nutrients to the surface Ocean. This result is coherent with the oligotrophic conditions observed during the cruise and emphasized the important role of the microbial loop to sustain the growth of organisms in the western part of the NPSG.

The particular high gradient in nutrients observed between 100 m and 155 m depth at station 8 matched very well with the possible presence of the subtropical counter current, STCC (Sta. 100 m to 130 m), as reported in Girault et al., (2013b). Despites no noticeable pattern of variation of buoyancy frequency at these depths, nutrient gradients clearly indicated two zones of high gradients separated by a layer of lower or null gradient. Interestingly, the depths with high nutrient gradients were similar for silicic acid and phosphates but higher nitrate gradients were located just beneath this layer. This result suggested that utilization of nitrate differed from phosphates and silicic acid in the vicinity of the STCC layer. In terms of nitrate daily flux related to the standing pool, an anomalous percentage of 432% was evidenced suggesting that diffusive role of nitrates linked to the STCC was particularly important. This anomalous percentage was the result of two mechanisms *i*) a nitrate-depletion in the mixed layer and *ii)* the depth of the nitracline corresponded to the depth of the thermocline. Association of nitracline with thermocline mathematically maximized the daily flux related to the standing pool and lead to this high percentage at station 8. Although, Figure 6 did not evidence particular distribution of

heterotrophic prokaryotes close to the STCC layer, integrated heterotrophic prokaryote abundance and carbon biomass of HNA in the Subtropical gyre area were maximum at station 8 (Fig. 7). This result is also observed with the ultraphytoplankton distribution where high concentrations were also found at this station (Girault et al., 2013b). Relationships between the STCC and microbial food web *via* the nutrient fluxes appeared to be an important mechanism to sustain the ecosystem in the Subtropical pacific gyre area. Although the statistical analyses were performed on the entire data set, RDA results tend to confirm and emphasize this relationship between the variance of HNA distribution, phytoplankton distribution and depth related parameters (Table 2).

4.3 The role of nutrients in the distribution of HNA and LNA

The partial RDA showed that the "pure" depth-related variables explained between 6 and 8 % of the total heterotrophic prokaryotes variance. These percentages are low but the sum of their joint effect (including the depth-related variables) can explain more than 26 % of the total variation in the LNA distribution. Differences in nutrient utilisation and requirements could also lead to different heterotrophic prokaryote distributions and a possible discrimination of certain organisms subject to the oligotrophic gradient. These variations can in part be observed thanks to the ratios of the abundances of the various clusters (Fig. 8). Figure 8 shows two opposite relationships between the nucleic acid content of the heterotrophic prokaryotes and the spatial distribution. Considering the VHNA/HNA ratio, a northward increase of the ratio was found in the upper layer (from the surface to 100 m) and suggested that these microorganisms with a very high nucleic acid content are outcompeted by the HNA prokaryotes in the most oligotrophic region (Transition zone). It should be noted that the relationship between the VHNA and HNA appeared to be rather centered on the boundary between the Transition zone and the Subtropical gyre areas, and not related to a continuous modification of the ratio along the gradient (average and standard deviation of VHNA/HNA ratio in the Kuroshio region are close to the ones in the Subtropical gyre area). In contrast, Figure 8a shows that HNA/LNA ratio increased from the northernmost station 1 to the southward station 12 in the upper layer (from the surface to 100 m), especially from station 6. In addition, a decrease in the HNA/LNA ratio was found below 100 m at station 7 in the Subtropical gyre area through to the Transition zone. In opposition to other cruises conducted in oligotrophic conditions, the Tokyo–Palau cruise demonstrated a latitudinal gradient in VHNA, HNA and LNA concentrations in the upper layer and from the surface to the deep layer (Van Wanbeke et al., 2011). Nutrient data

displayed in Fig. 6 (Girault et al., 2013b) showed that both phosphate and nitrate concentrations decreased between stations 6 and 7 but were measured in high concentrations under the thermocline. From the perspective of nutrients these results suggest that LNA is less abundant than HNA under low phosphate and nitrate conditions. This is in contrast with the hypothesis proposed for severely P-limited environments which suggests that inorganic phosphorus can exert more severe physiological constraints on the growth of HNA than LNA (Nishimura et al., 2005; Wang et al., 2007). However, it is important to note that both LNA and HNA clusters are likely to include different strains of microorganisms including species adapted to the warm, which have been shown to have lower minimal P cell quotas (Hall et al., 2008). The link between these warm-adapted species and the nucleic acid content is still unclear and depends on the type of environment studied. For example, the warm-adapted species of LNA were expected to have an advantage over cells with high nucleic acid content (HNA) in the warm resource limited environment of the Mediterranean Sea (Van Wanbeke et al., 2011). In contrast, the work of Andrade et al. (2007) found that LNA accounted for the high proportion of cells in cold and "nutrient rich" waters, whereas cells with higher HNA concentrations were prominent in the oligotrophic high temperature regions of the Southwest Atlantic Ocean. According to Andrade et al. (2007), the variation in the HNA/LNA ratio observed suggests that low nutrient conditions favour HNA cells over LNA cells. This result along with the statistical analyses performed in this study may suggest that HNA species are more warm-adapted than LNA in the Subtropical gyre and Transition zone. Decrease of the VHNA/HNA ratio also suggests that the numerically dominant species with high nucleic acid content (HNA) might be more warm-adapted than the cells with the highest nucleic acid content (VHNA). These contrasting results highlight the complex and non linear link between the cell nucleic acid contents and the various ecological meanings as reported in Bouvier et al., (2007) and Van Wanbeke et al., (2009).

4.4 Distribution of the heterotrophic prokaryotes and eddies

During the Tokyo–Palau cruise the transect crossed a cold core cyclonic eddy near station 3 and a warm core cyclonic eddy at station 9. Cyclonic eddies usually enhance biological activities as reported by the measurements of carbon fixation, nutrient uptake, and oxygen production (Bidigare et al., 2003). At station 3, the pumping effect initiated by the cyclonic eddy was seen by recording the nutrient ratio and identifying the microphytoplankton taxonomy (Girault et al., 2013a, b). The high concentration of Chl a measured at station 3

and the numerical dominance of large phytoplankton agreed with the description of cold core cyclonic eddy event (Vaillencourt et al., 2003). A high concentration of heterotrophic prokaryotes was found at the edge of the cyclonic eddy (Sta. 4). The effect of the eddy on the similar concentrations of heterotrophic prokaryotes were measured at stations 2 and 3. In oligotrophic conditions, environmental factors controlling the distribution of the heterotrophic prokaryotes were compared in two extreme cases: the stations located under the influence of the eddy and the ones outside its influence (Baltar et al., 2010). However, few investigations target the distribution of heterotrophic prokaryotes along the spatial oligotrophic gradient (Thyssen et al., 2005) or take into account the age of eddy (Sweeney et al., 2003; Rii et al., 2007). With three stations only (stations 2, 3, 4), a snapshot of the eddy effects was presented and it remained difficult, not to say impossible, to describe the local effect of the eddy. However, by using satellite data and daily surface currents of the bulletin of the Japanese coast guard, it was possible to detect that the creation of the eddy structure was linked to the instability in the meander of the Kuroshio Current between 9 and 12 July 2010. This phenomenon has been commonly observed in this area (e.g. 17 April 2012; 14 May 2013) and its lifespan is usually about a month. In comparison, the sea surface current map suggested that the mesoscale structure observed during the cruise was older than six months. On the basis of the “closed” model proposed for an eddy, a six month old cyclonic eddy is associated with its decay phase, where intense blooms can be observed but which lack significant diatom abundance (Seki et al., 2001). During the cruise, the highest abundance of microphytoplankton was observed at station 3, suggesting that the classical biogeochemical properties normally associated with an eddy (i.e. single nutrient pulse, “closed system”) were not apparent in the cyclonic eddy encountered during the Tokyo–Palau cruise. This phenomenon has also been observed in other oligotrophic areas (Seki et al., 2001; Bidigare et al., 2003; Landry et al., 2008). According to Nencioli et al. (2008), the association of a horizontal translation gradient with multiple nutrient inputs might explain the variability in organisms between stations 2, 3, and 4. Indeed, the cold core of the cyclonic eddy moved to the north-west between December and the sampling time of the cruise. Station 4 therefore is the first station to be influenced by the eddy, the center of cyclonic eddy then moved towards station 3, but did not reach station 2 located in the vicinity of the Kuroshio Current. The path of the cold core cyclonic eddy could explain the possible decrease in the nutrient uptake from the bottom layer at station 4 and lead to an oligotrophic system dominated by regeneration processes. The high abundance of heterotrophic prokaryotes measured at the edge of the cyclonic eddy could be explained by

the high activity in the microbial loop. This difference in heterotrophic prokaryote abundance between the center and the edge of the eddy may be due to a more efficient vertical exchange of seawater masses which has been reported at the periphery of some eddies rather than at the center of them (Stapleton et al., 2002; Klein and Lapeyre, 2009). Similarly, the numerical dominance of Synechococcus, observed only once in the surface samples during the cruise, may explain the change in trophic conditions (Girault et al., 2013b).

The frontal structures observed between the Subtropical gyre and the Transition zone are usually defined by an accumulation zone for organic matter and an area of high heterotrophic prokaryote abundance is found (Arístegui and Montero, 2005; Baltar et al., 2009; Lasternas et al., 2013). However, the anticyclonic eddy at station 9 is characterized by the lowest concentrations of heterotrophic prokaryote clusters found during the cruise. The low concentrations of the dominant LNA cluster was also observable in terms of integrated carbon biomass, and highlighted the response of each cluster to the change of environmental conditions, such as the salinity front (Fig. 7), Among the environmental variables, low nutrient concentrations are expected to be one factor controlling the specific distribution of heterotrophic prokaryote clusters (Girault et al., 2013b). The partial RDA suggests that the spatial related variables are the most important, followed by the “pure” depth-related variables which explained between 6 and 8 % of the total variation in the heterotrophic prokaryote cluster abundances. The low difference in percentages between LNA, HNA, and VHNA clusters was in agreement with the constant numerically dominant group found between the surface and 160 m. This result suggested that the anticyclonic eddy did not enhance nor limit one particular heterotrophic prokaryote cluster in the upper layer (Fig. 6). However, below 160 m a high increase in VHNA and LNA abundance was measured compared to HNA. This result is uncommon in the meso- and bathypelagic zones of oligotrophic areas where the concentration of HNA and LNA heterotrophic prokaryotes decreased significantly with depth (Van Wambeke et al., 2011; Yamada et al., 2012). The increase in nutrient concentrations associated with the sloppy feeding mechanism initiated by the concentration of VHNA may partially lead to the high abundance of LNA observed at the bottom of the euphotic layer (Thyssen et al., 2005).

5 Conclusions

This study along a 2300 km transect in the North Pacific subtropical gyre area during a

strong La Niña condition showed that the heterotrophic prokaryote distribution is correlated with three different seawater masses identified as (i) the Kuroshio, (ii) the Subtropical gyre and (iii) the Transition zone. A latitudinal increase 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. The statistical analyses highlighted that the majority of the heterotrophic prokaryote distribution is explained by temperature and salinity. Nutrients and phytoplankton-related variables had different influences depending on the LNA, HNA and VHNA clusters. LNA distribution is mainly correlated with temperature and salinity while HNA distribution is mainly explained by an association of variables (temperature, salinity, Chl a and silicic acid). During the cruise, two eddies (one cyclonic and one anticyclonic) were crossed. The vertical distributions of LNA, HNA and VHNA were investigated. Based on the current surface map and the microorganism distribution, it is reasonable to form the hypothesis that the high concentration of heterotrophic prokaryotes observed at station 4 was linked to the path of the cold cyclonic eddy core. In contrast, in the warm core of the anticyclonic eddy, lower heterotrophic prokaryote concentrations are suggested to be linked to the low nutrient concentrations. All the results described in this study highlight the high variability of each heterotrophic prokaryote cluster defined by their nucleic acid content (LNA, HNA, and VHNA) with regard to the mesoscale structures and the oligotrophic gradient observed in situ within the area of the North Pacific subtropical gyre.

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Table 1. Literature estimates of vertical turbulent diffusivity rates obtained using different methods in the oligotrophic condition. *na* indicates information not mentioned.

Domain	Location	Depth (m)	Diffusivity ($cm^2.s^{-1}$)	Reference
North Pacific Subtropical Gyre	22°N-158°W	300-500	0.1-0.5	(Christian and Lewis, 1997)
	35–44°N, 150–170°W	*na*	0.2-0.4	(White and Berstein, 1981)
	10°N-40°N	0-1000	0.3	(Van Scoy and Kelley, 1996)
	22°N-158°W	*Euphotic*	1-2	(Emerson *et al.*, 1995)
Pacific Ocean	20°S-20°N	125	0.5	(Li *et al.*, 1984)
	20°N-60°N	100	1.8	(Li *et al.*, 1984)
Tropical North Pacific Ocean	5°N-10°N, 90°E	*na*	0.05-0.16	(King and Devol, 1979)
	10°N-15°N, 85°E	*na*	0.44-1.10	(King and Devol, 1979)
Subtropical North Atlantic	25°N, 28°W	300	0.12-0.17	(Ledwell *et al.*, 1998)
	28.5°N, 23°W	100-400	0.37	(Lewis *et al.*, 1986)
	31°N, 66°W	<100	0.35	(Ledwell *et al.*, 2008)

Table 2. Phosphate, nitrate and silicic acid diffusive fluxes into the surface mixed layer and the importance of supply term relative to the standing pool size.

Station	Latitude	Mixed layer depth (m)	Phosphate flux ($\mu mol.m^{-2}.d^{-1}$)	Daily diffusive supply relative to pool (%)	Nitrate flux ($\mu mol.m^{-2}.d^{-1}$)	Daily diffusive supply relative to pool (%)	Silicic acid flux ($\mu mol.m^{-2}.d^{-1}$)	Daily diffusive supply relative to pool (%)
5	28.98	141	-0.52	-0.01	3.63	0.01	2.25	0.002
6	27.16	136	-1.34	-0.03	12.88	0.04	54.09	0.03
7	24.83	109	9.43	0.69	0	0	142.21	0.21
8	22.83	101	7.78	0.76	81.3	432	351.36	0.39
9	20.78	140	6.48	0.68	13.91	5.7	571.1	0.48
10	19.98	95	1.38	0.16	0	0	0.006	0.01

Table 3. List of observations from stations 1 to 11 and their classification into six clusters according to the principal component analysis (PCA).

PCA Cluster	Observations	Latitude (°N)	Station	Depth (m)
1	1	33.6	1	0
1	2	33	2	0
1	3	31.6	3	0
1	4	31	4	0
2	5,6,7,8,9,10,11,12,13	28.6	5	0,40,60,70,78,80,100,120,140
2	15,16,17,18,19,20,21,22,23	27.1	6	0,25,60,75,80,90,100,115,125
2	32,33,34	24.5	7	75,90,101
2	40,41	22.5	8	110,125
2	55	20.5	9	200
3	14	28.6	5	160
3	24	27.1	6	150
3	42,43,44	22.5	8	135,150,165
3	54	20.5	9	160
3	61	19.6	10	125
4	25,26,27,28,29,30,31	24.5	7	0,10,25,40,58,59,60
4	35,36,37,38,39	22.5	8	0,25,50,75,95
5	45,46,47,48,49,50,51,52,53	20.78	9	0,25,50,75,100,120,130,140
5	59,60,62	19.6	10	75,100,150
6	56,57,58	19.6	10	0,25,50
6	63,64,65,66	17.2	11	0,30,45,60

Table 4. Partial redundancy analysis performed on each heterotrophic prokaryote cluster optically resolved by flow cytometry: low nucleic acid content (LNA), high nucleic acid content (HNA) and very high nucleic acid content (VHNA). According to the PCA results, Chl *a* and silicic acid are the phytoplankton-related variables. Temperature and salinity are the spatial-related variables. Nitrate, phosphate and depth are the depth-related variables. Negative values characterized the lack of any correlation between heterotrophic prokaryote clusters and the variables tested.

		LNA	HNA	VHNA
Total explained variance		60%	55%	27%
Joint variation	Phytoplankton-related and spatial- and depth-related	6%	-1%	-1%
Partial joint variation	Spatial-related and phytoplankton-related	-1%	22%	-4%
	Spatial- and depth-related	9%	1%	5%
	Depth-related and phytoplankton-related	3%	1%	0%
Unique variation	Phytoplankton-related	4%	1%	1%
	Depth-related	8%	8%	6%
	Spatial-related	31%	23%	20%

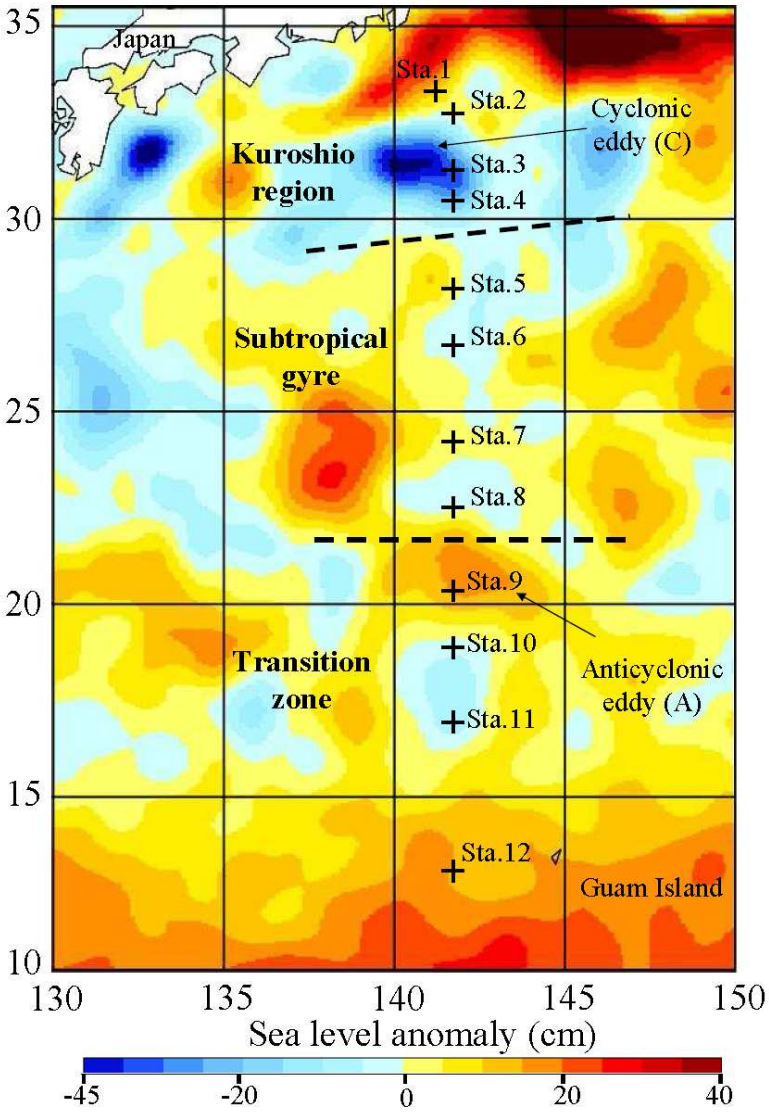

Figure 1: Map of the sea level anomaly (cm) in the west part of the North Pacific subtropical gyre. The sampling stations (black crosses) were separated depending on temperature and salinity into 3 areas: Kuroshio region (stations 1-4), Subtropical gyre (stations 5-8) and the Transition zone (stations 9-12).

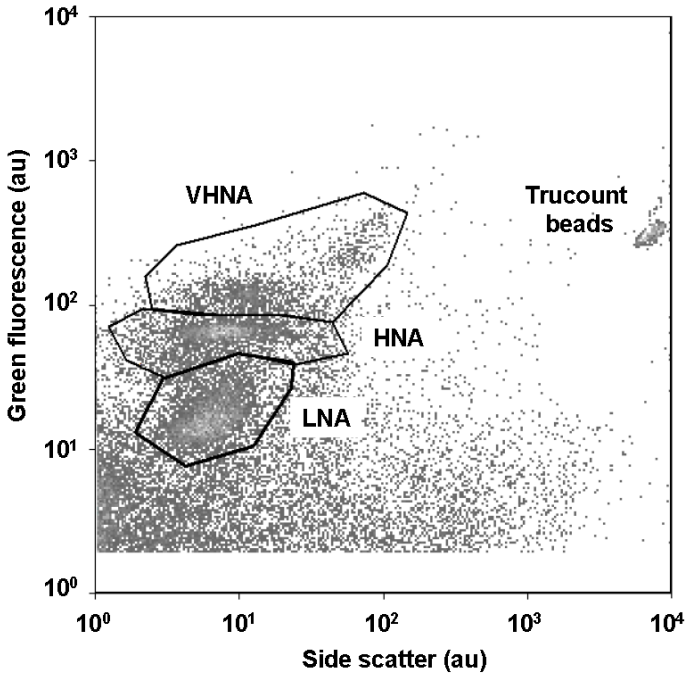

Figure 2: Example of the optical resolution obtained by the analytical flow cytometry of the heterotrophic prokaryote assemblages sampled during the Tokyo-Palau cruise at station 8 (25 m depth). Cytogram of green fluorescence intensity (SYBR Green II ®) versus side scatter intensity showed up three groups of heterotrophic prokaryotes : one defined by prokaryotes with a low nucleic acid content (LNA), one defined by prokaryotes with a high nucleic acid content (HNA) and one defined by those with a very high nucleic acid content (VHNA). Trucount calibration beads (Beckton Dickinson ®) were used both as an internal standard and to determine the volume analysed by the flow cytometer.

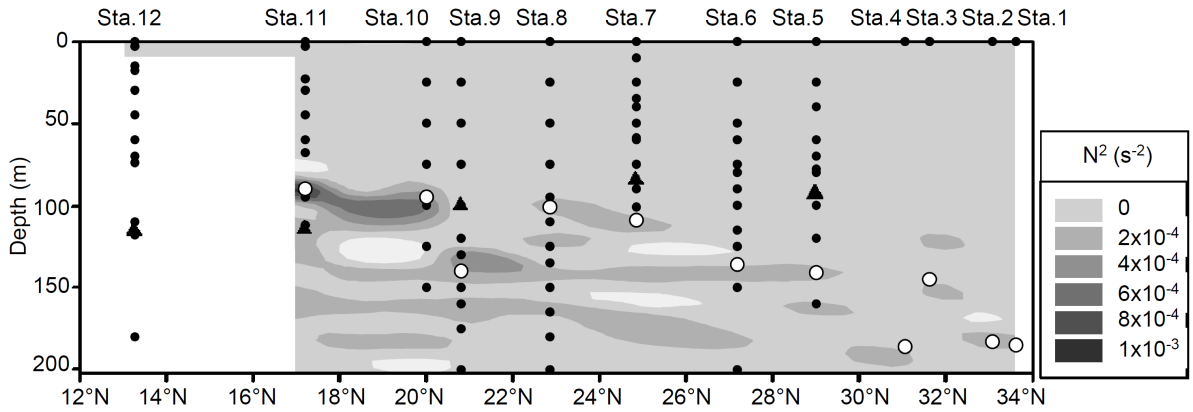

Figure 3: Vertical profiles of the Brunt-Väisäla buoyancy frequency (N^2) calculated from the temperature - salinity measurements. The white circles display the thermocline depth and the black triangles the depths of 1% of photosynthetically active radiation (limit of the euphotic zone).

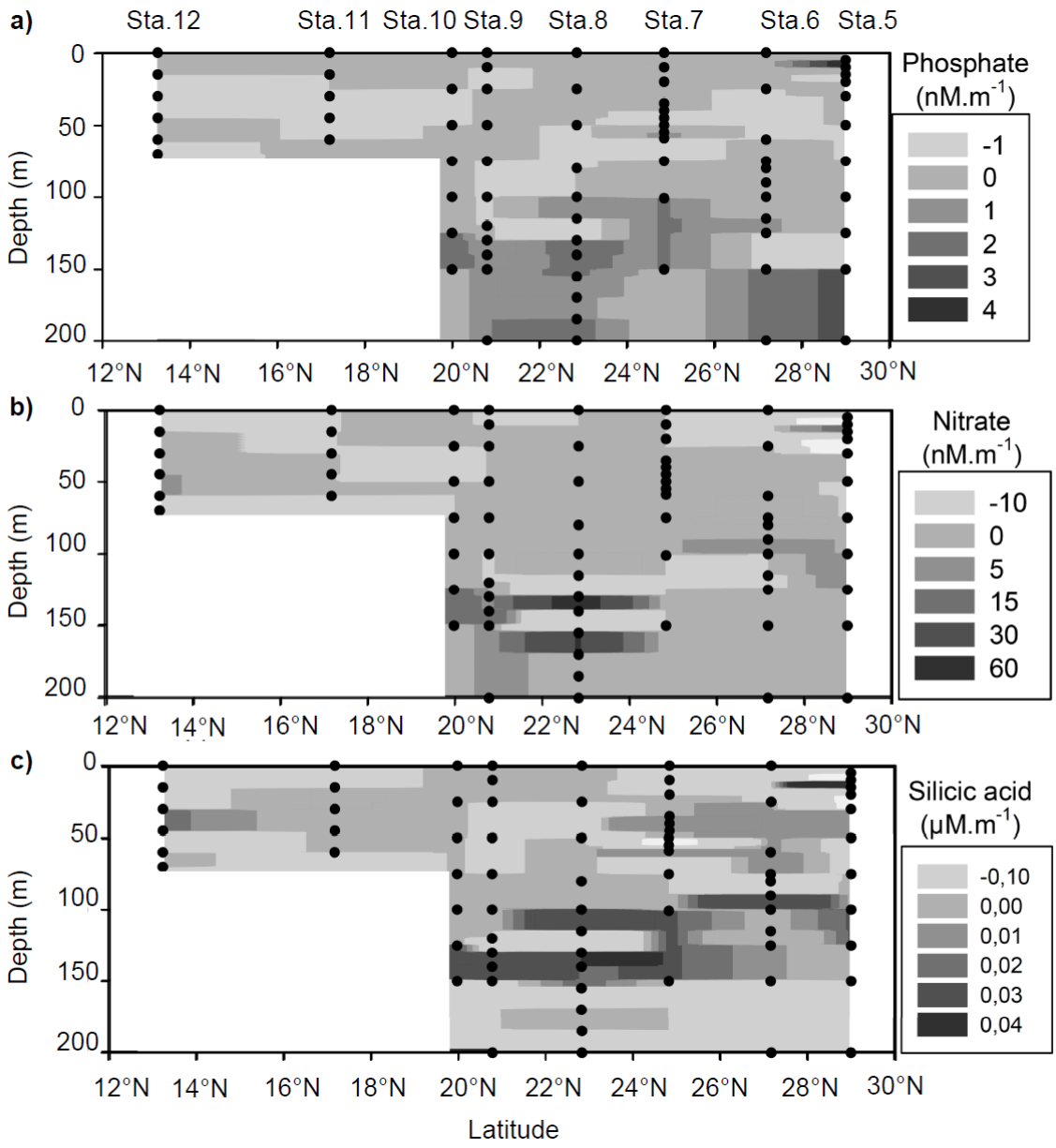

Figure 4: Vertical nutrient gradient (dNutrient/dz) of Phosphate (a), Nitrate (b) and Silicic acid (c), found between station 5 and station 12. The black dots display the sample depths and the names of the stations are indicated in the upper axes.

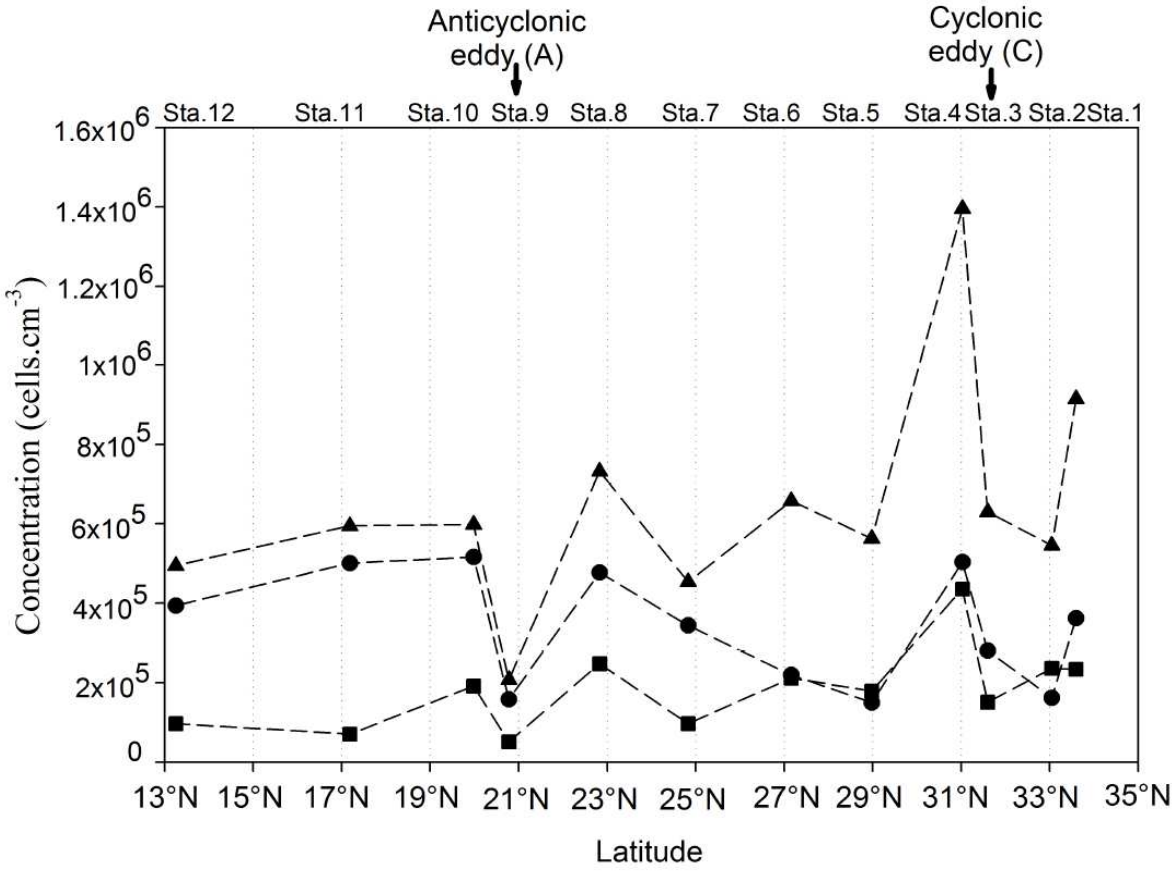

Figure 5: Latitudinal distribution of the heterotrophic prokaryote abundances at the surface along the 141.5°E meridian. (▲) is LNA heterotrophic prokaryotes, (●) the HNA heterotrophic prokaryotes and (■) the VHNA heterotrophic prokaryotes. Sampling stations are indicated on the upper scale axis.

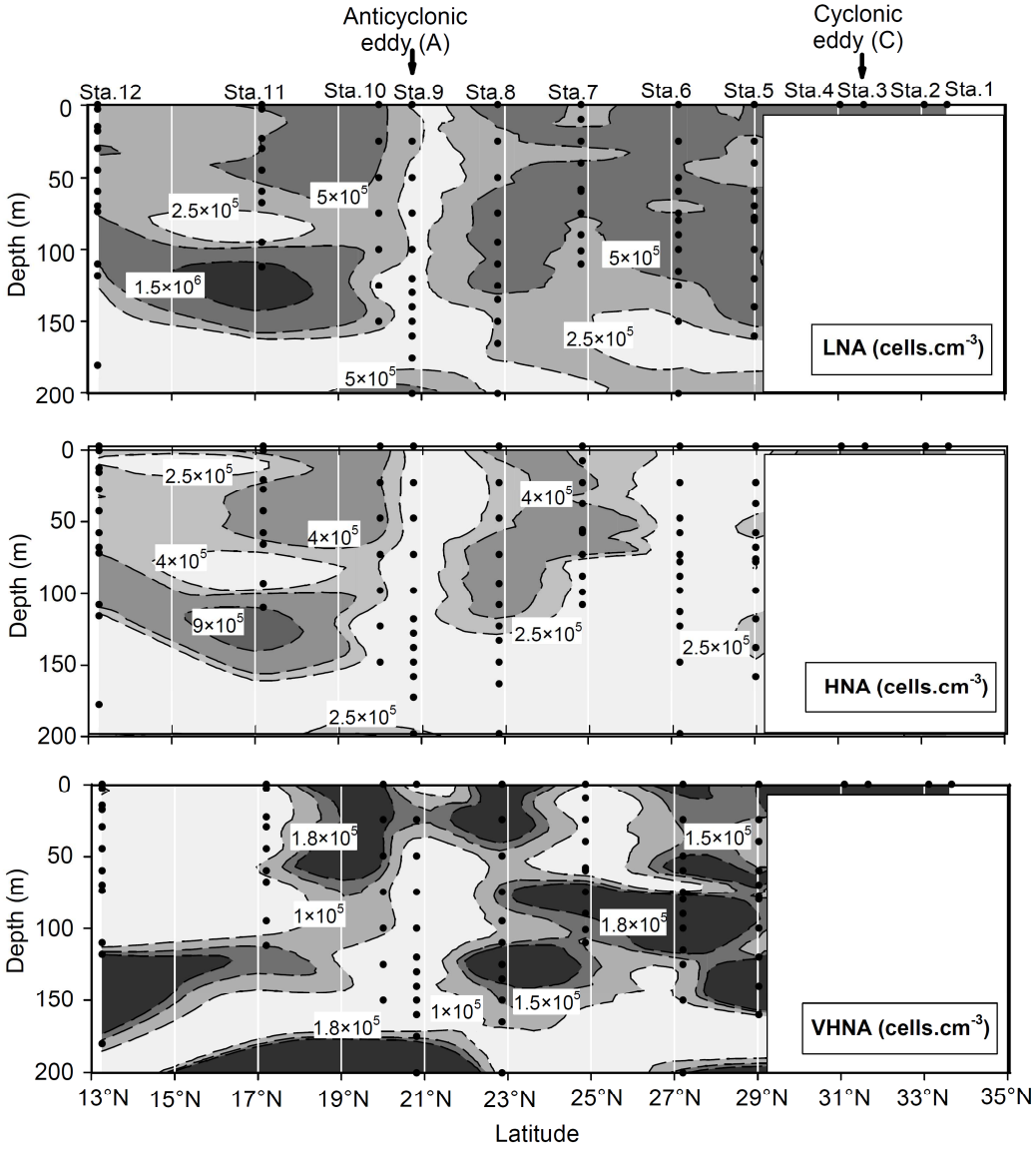

Figure 6: Vertical concentration (cells.cm^{-3}) of LNA, HNA, and VHNA heterotrophic prokaryotes interpolated along the transect during the Tokyo-Palau Cruise. The black dots are the depths sampled.

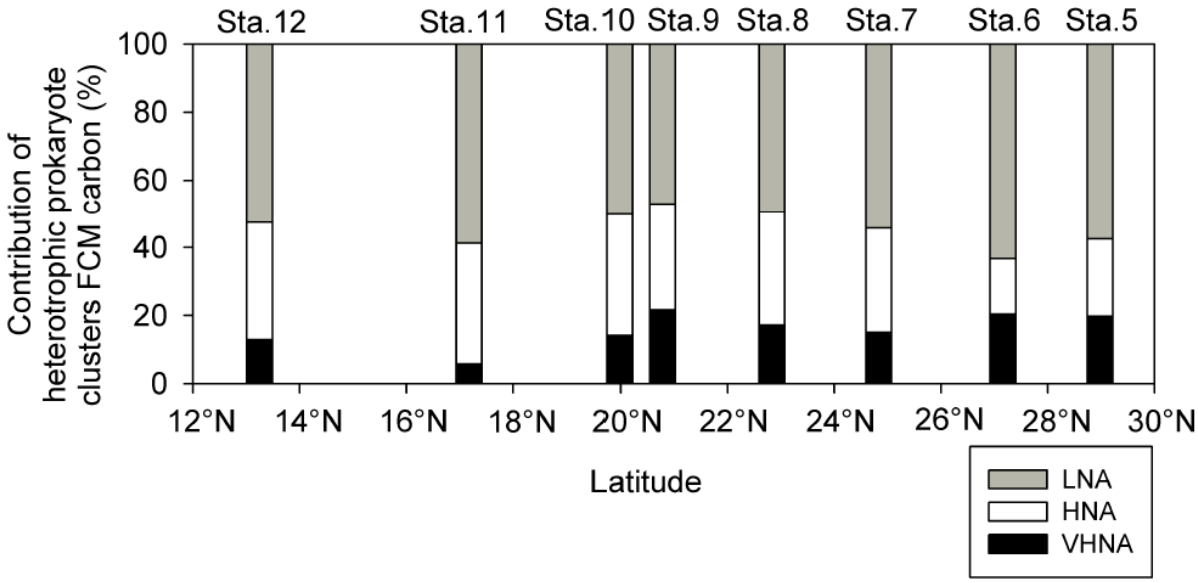

Figure 7: Latitudinal contributions (%) of each heterotrophic prokaryote cluster (LNA, HNA, VHNA) as defined by flow cytometry (FCM) to the whole heterotrophic prokaryote biomass integrated between surface and 200m depth.

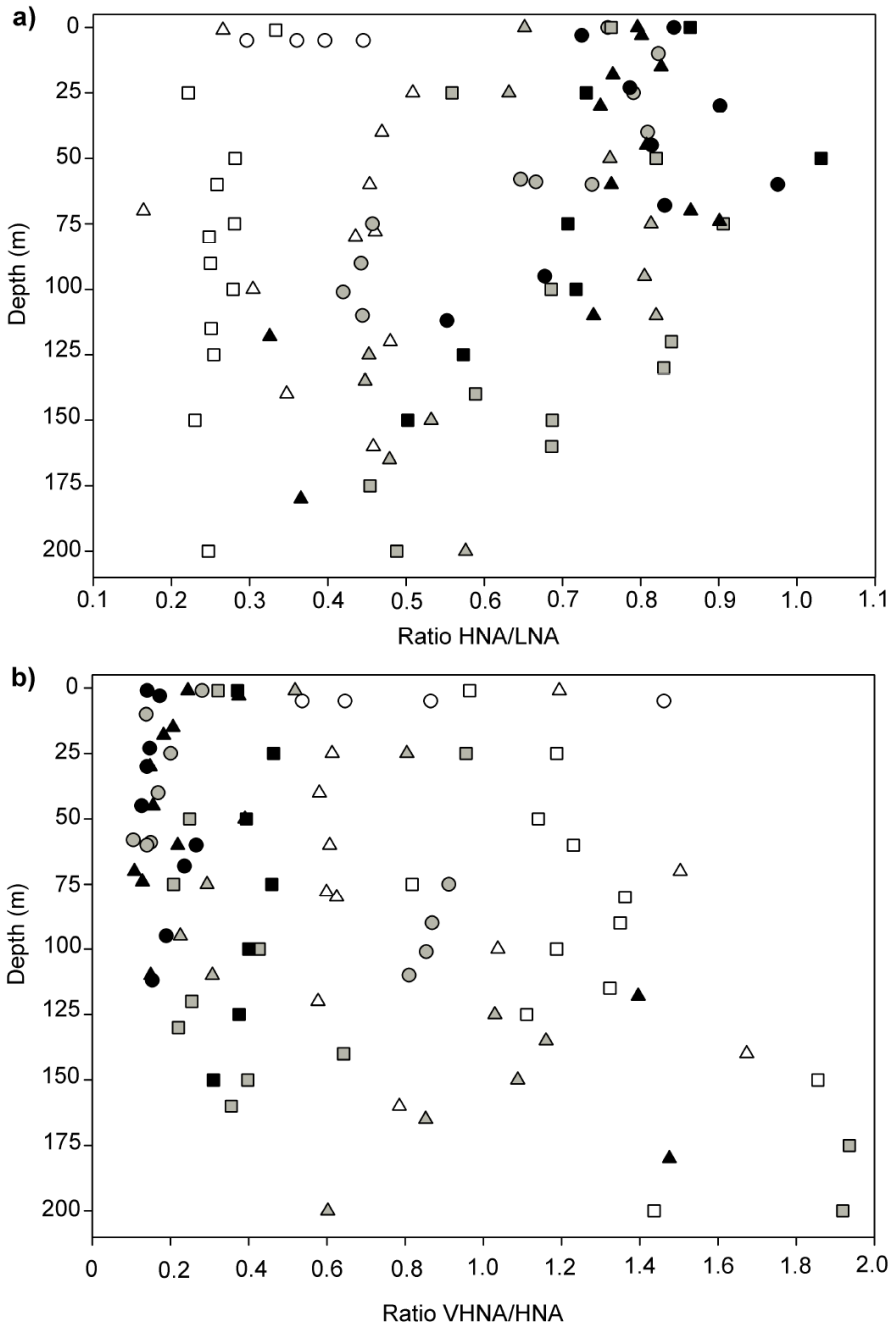

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Figure 8: Ratios of the abundances between the heterotrophic prokaryote clusters according to depth. a) shows the ratio of the abundances of HNA/LNA clusters while b) shows the ratio of abundances of VHNA/HNA clusters. The white circles are stations 1, 2, 3 and 4. The white triangles and the squares are stations 5 and 6, respectively. The grey circles, triangles, and squares characterize stations 7, 8 and 9, respectively. The black squares, circles and triangles are stations 10, 11 and 12, respectively.

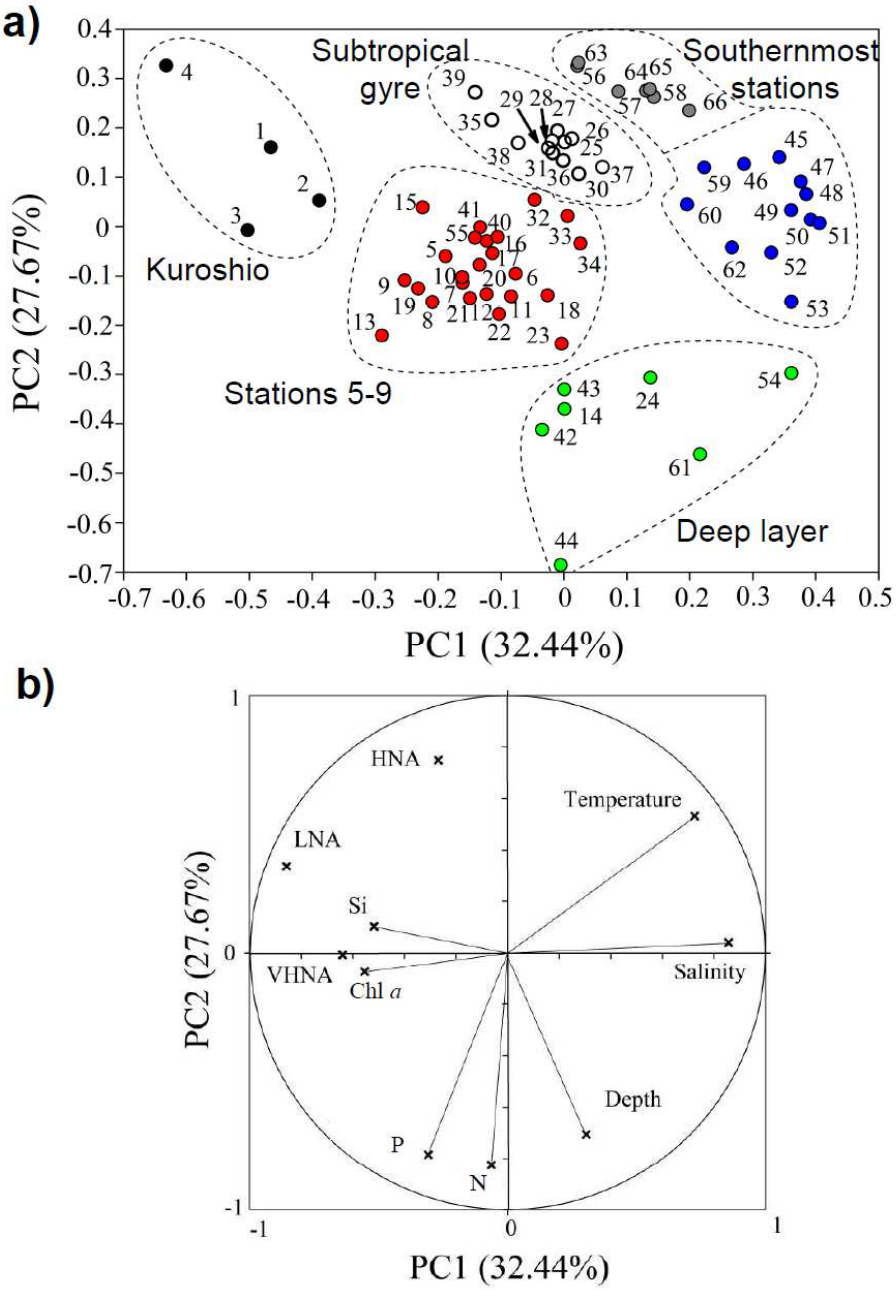

Figure 9: Hierarchical clustering illustrated for the first two principal components of the principal component analysis performed with the data collected from stations 1 to 11 (a). According to the classification (Table 1) the sampling depths (numbers) were discriminated into 6 clusters: one characterizes the Kuroshio region (Cluster 1, black), another incorporates stations 5 to 9 (Cluster 2, red), a third one the deep layer (Cluster 3, green) and the last three clusters characterize the subtropical gyre (Cluster 4, white) and the southernmost stations (5, blue and 6, dark grey). The circle (b) shows the first two dimensions of the principal component analysis. The environmental variables taken into consideration are temperature, salinity, depth, nitrate (N), phosphate (P), silicic acid (Si), and chlorophyll *a* (Chl *a*).

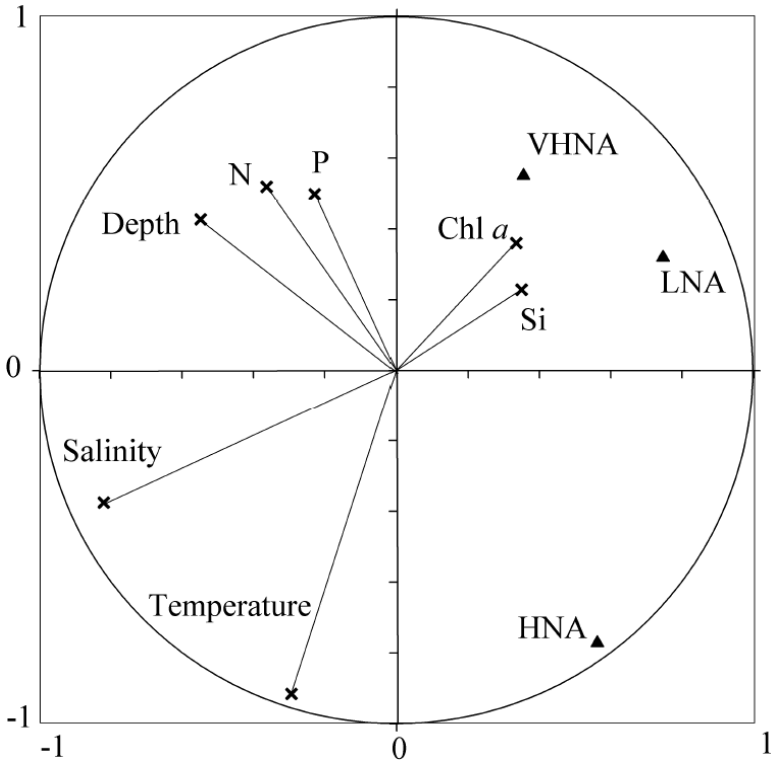

Figure 10: Correlation plot of the redundancy analysis (RDA) on the relationships between the environmental variables and the three subgroups of heterotrophic prokaryotes observed during the cruise (LNA, HNA, VHNA). Chl *a*, N, P, and Si stand for chlorophyll *a*, nitrate, phosphate, and silicic acid, respectively.