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Heterotrophic prokaryote distribution along a 2300 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
⁵ 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 temperature and salinity, were dominant in all the hydrological regions. In contrast, HNA distribution seemed to be associated with temperature, salinity, ChI *a* and silicic acid. A latitudinal increase in the
- ¹⁵ HNA/LNA ratio was observed along the north–south transect and was related to higher phosphate and nitrate concentrations. 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
- ²⁰ 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 La-Niña 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 informed a bundance and bundance. The multi-active distribution of the strength and bundance.
- ¹⁰ influenced by phytoplankton distribution and environmental variables. The relationship 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.

15 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 mixed layer depths were



estimated according to the $\Delta \sigma_{\theta} = 0.15 \text{ kgm}^{-3}$ (relative to z = 10 m; Thomson and Fine, 2003). 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.

5 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).

15 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.

2.4 Chlorophyll *a* and flow cytometry analyses

⁵ 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 cm³ 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). TrucountTM 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 (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).

2.5 Statistical analysis

- ¹⁵ 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 $\log_{10} (x+1)$ -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).

5 3 Results

3.1 Sampling sites

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. By using the temperature–salinity diagram, 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) (Girault et al., 2013b). 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.

3.2 Distribution of the heterotrophic prokaryotes

After staining with the SYBR green II fluorescent dye, three clusters of heterotrophic prokaryotes were identified by different green fluorescence mean intensities: 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). Figure 3 shows the abundance of each heterotrophic prokaryote cluster at the surface with latitude. In the surface samples of the Kuroshio region the average concentrations of LNA, HNA and VHNA were 8.71 × 10⁵, 3.27 × 10⁵ and 2.64 × 10⁵ cells cm⁻³, respectively. In the Subtropical area



the average concentrations of LNA, HNA and VHNA were 6.01 × 10⁵, 2.97 × 10⁵ and 1.84 × 10⁵ cells cm⁻³, respectively. In the Transition zone the average concentrations of LNA, HNA and VHNA were 5.18 × 10⁵, 4.38 × 10⁵ and 1.15 × 10⁵ 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 the LNA, HNA and VHNA were 1.39 × 10⁶, 5.03 × 10⁵ and 4.35 × 10⁵ cells cm⁻³, respectively. In contrast, the concentrations of LNA, HNA and VHNA at station 9 were 2.07 × 10⁵, 1.6 × 10⁵ and 5.07 × 10⁴ cells cm⁻³, respectively. To a lesser extent, high concentrations of LNA (9.13 × 10⁵ cells cm⁻³) and HNA (3.62 × 10⁵ cells cm⁻³) were identified at the northernmost station of the Kuroshio region (at station 1).

The vertical distributions for the concentration of heterotrophic prokaryotes were also investigated along the transect (Fig. 4). As at the surface, the vertical distributions are characterized by lower cell concentrations for all heterotrophic prokaryote groups at station 9. In this station both the 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 flow cytometer samples. The VHNA concentrations are lower than the HNA in 75% of the flow cytometer samples.

Figure 5 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.

Discussion Paper **BGD** 11, 15793-15826, 2014 **Heterotrophic** prokarvote distribution in the **Discussion** Paper **NPSG** M. Girault et al. **Title Page** Introduction Abstract Discussion Paper Conclusions References **Figures** Close Back **Discussion** Paper Full Screen / Esc **Printer-friendly Version** Interactive Discussion

3.3 Statistical analysis

Results of the Principal Component Analysis (PCA) and the Redundance Analysis (RDA) are shown in Figs. 6 and 7, 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 1 and Fig. 6.) 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. 6a) 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. 7) 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
⁵ 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 ChI *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

- 20 HNA, or VHNA indicated that environmental parameters can explain 60, 55 and 27 % of the total variance, respectively (Table 2). 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 %
- ²⁵ 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, 3 and 4). 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
- ¹⁵ 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. 6). 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. Although low concentrations of silicic acid have been reported
- in cyclonic mesoscale eddies, 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 (Hashihama et al., 2009, 2014). The Si: N : P stoichiometry measured during the Tokyo–Palau cruise



identified nitrogen and/or phosphorus to be potential limiting factors (Girault et al., 2013b) as found by Hashihama et al. (2009). Nutrient uptake rates were also taken into account in order to describe the nutrient conditions found during the cruise. According to the nutrient uptake values measured in the NPSG area, there is a relationship

- ⁵ between the very high efficient uptake of silicic acid and silicified organisms as reported in Krause et al. (2012). This synergy was suggested to maximise biomass without leading to a secondary growth limitation. According to Krause et al. (2012), the high efficiency of silicic acid uptake may explain in part the correlation between these two variables observed both in the PCA and RDA despite the low concentration of
- ¹⁰ 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* as reported in Baines et al. (2012), suggested 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. 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
- 20 ecologically sound for the Tokyo–Palau cruise, as demonstrated by the partial (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 ²⁵ 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 2). 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 phytoplanktonrelated 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. 6 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.

It has been previously suggested that the effects of large scale climatic events such as El Niño-La Niña on inter-annual heterotrophic prokaryote abundances are important in the NPSG area (Campbell et al., 1997). However, since this pioneer study, where both the microbial loop and climatic structure were taken into consideration in the oligotrophic region of the Pacific Ocean (Brown et al., 2003) studies (and data sets),

on heterotrophic prokaryote distribution remain scarce. The area investigated during the Tokyo Palau cruise is particularly lacking in data and to the best of our knowledge, this is the first study addressing the concentration and distribution of heterotrophic prokaryotes. Obviously, with this lack of data, descriptions, comparison between data sets and discussions related to the potential influence of La Niña conditions on



the heterotrophic prokaryote distribution are particularly complex to do. However, according to the significant differences reported in the ultraphytoplankton assemblages and the hydrological parameters, it is reasonable to expect that within the microbial loop, heterotrophic prokaryote distribution should also be influenced by such large

⁵ scale climatic events (Girault et al., 2013b). In this context, the characterisation of the climate forcing on the microbial loop should also be investigated in periods outside of La Niña in order to confirm this hypothesis and evaluate the part of the El Niño Southern Oscillations in the heterotrophic prokaryote distribution, variance and impact on the ecosystem.

10 4.2 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. Figure 5 shows that the HNA/LNA ratio increased from stations 1–12 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 contrast to other cruises conducted in oligotrophic

- through to the Transition zone. In contrast to other cruises conducted in oligotrophic conditions, the Tokyo–Palau cruise demonstrated a latitudinal gradient in both 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
- ²⁵ 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.

4.3 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 heterotrophic prokaryotes appeared to be more significant in the south part of the eddy,



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 restrict the probability of the probab
- 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

- ¹⁵ Is characterized by the lowest concentrations of heterotrophic prokaryote clusters found during the cruise. 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 spatialrelated 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. 4). 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 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 15 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 20 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

²⁵ observed in situ within the area of the North Pacific subtropical gyre.

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BGD 11, 15793-15826, 2014 **Heterotrophic** prokarvote distribution in the NPSG M. Girault et al. Title Page Introduction Abstract Conclusions References Tables **Figures** Close Back **Discussion** Paper Full Screen / Esc **Printer-friendly Version** Interactive Discussion

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PCA Observations Depth (m) Latitude Station Cluster (° N) 1 33.6 1 0 1 2 33 2 0 1 3 3 1 31.6 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 22.5 110, 125 8 2 55 20.5 9 200 3 5 14 28.6 160 3 24 27.1 6 150 3 42, 43, 44 22.5 8 135, 150, 165 3 54 20.5 9 160 3 61 10 125 19.6 4 25, 26, 27, 28, 29, 30, 31 24.5 7 0, 10, 25, 40, 58, 59, 60 35, 36, 37, 38, 39 22.5 8 4 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 75, 100, 150 10 6 10 56, 57, 58 19.6 0, 25, 50 6 63, 64, 65, 66 17.2 11 0, 30, 45, 60

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



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Table 2. 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	3%	1%	0%
	phytoplankton-related			
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).

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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 $II^{(0)}$) vs. 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. Latitudinal distribution of the heterotrophic prokaryote abundances at the surface along the 141.5° E meridian. (\blacktriangle) is LNA heterotrophic prokaryotes, (\bullet) the HNA heterotrophic prokaryotes and (\blacksquare) the VHNA heterotrophic prokaryotes. Sampling stations are indicated on the upper scale axis.











Figure 5. Ratios of the abundance of HNA heterotrophic prokaryotes to the abundance of LNA heterotrophic prokaryotes with depth. 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, 9, respectively. The black squares, circles and triangles are stations 10, 11 and 12, respectively.





Figure 6. 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 7. 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.

