Geographic distribution pattern of low and high nucleic acid content bacteria on a river-catchment scale

Jie Liu[A,B,1]*, Dan Ma*[A,1]*, Lili Ma*[A,C]*, Yuhao Song*[A]*, Guanghai Gao*[A]
and Yingying Wang[A,D]

[A]Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), Tianjin
  Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental
  Science and Engineering, Nankai University, Tianjin 300071, China.
[B]Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China.
[C]College of Chemistry and Chemical Engineering, Southwest Petroleum University,
  Chengdu 610500, China.
[D]Corresponding author. Email: wangyy@nankai.edu.cn

Abstract. Bacteria with low nucleic acid content (LNA) and high nucleic acid content (HNA) are widely distributed in aquatic environments. Most of the current understanding of these two subgroups is derived from studies in marine environments. In comparison, information on the spatial distribution of these two subgroups in freshwater environments is very limited. The present study analysed the biogeographical pattern of those two groups on a large-river scale (i.e. the Songhua River catchment, >1000 km). The results showed that the concentrations of LNA and HNA bacteria were distributed over a wide range from 5.45×10^4 to 4.43×10^6 cells mL^{-1}, and from 1.35×10^5 to 4.37×10^6 cells mL^{-1} respectively. The two groups have almost equal proportions in the Songhua River, with the average contribution of LNA bacteria reaching 47.0%. In comparison, the abundance of LNA bacteria in the mainstream was significantly higher than in the tributaries. The cytometric expressions (green fluorescence and side scatter) within LNA and HNA were strongly covaried, which implies that these two subgroups are intrinsically linked. Multivariate redundancy analysis indicated that both the abundance and cytometric characteristics of co-occurring LNA and HNA bacteria were regulated differently in the Songhua River. This suggests that LNA and HNA bacteria play different ecological roles in river ecosystems.

Additional keywords: biogeography, cytometric characteristics, flow cytometry (FCM), multivariate redundancy analysis.

Received 9 March 2016, accepted 15 November 2016, published online 2 February 2017

Introduction

Flow cytometry (FCM), which is based on fluorescent nucleic acid-staining technologies and is independent of microbial cultivability, has been rapidly applied in the field of aquatic microbiology (Wang *et al.* 2010; Koch *et al.* 2014). At least two major groups of bacteria, namely those with low and those with high nucleic acid content, can be distinctively characterised by FCM after staining with DNA-binding dyes (Bouvier *et al.* 2007; Wang *et al.* 2009). They were originally classified as 'Group I cells' and 'Group II cells' (Li *et al.* 1995), then as 'low nucleic acid-content (LNA) bacteria' and 'high nucleic acid-content (HNA) bacteria' (Lebaron *et al.* 2001). This classification was based on the different fluorescence intensity and side scatter signal of the cells as detected by FCM, where fluorescence intensity was regarded as an indicator of intracellular nucleic acid content (genome size and the number of genome copies) and side scatter as an index of cellular morphological characteristics (size, internal granulosity and cytoskeletal proteins; Bouvier *et al.* 2007). Although this classification is method-specific for FCM, it has been widely applied in different research fields (Bouvier *et al.* 2007; Van Wambeke *et al.* 2011; Huete-Stauffer and Morán 2012).

The question of whether LNA bacteria are active or inactive microbes has been controversial for a long time. HNA bacteria were commonly considered to be the active part of the microbial metabolic groups because of their high nucleic acid content and large bio-volumes, whereas LNA bacteria were regarded as inactive (Lebaron *et al.* 2001). When first observed in FCM, LNA bacteria were considered to be inactivated or dead cells and HNA bacteria were proposed to be an indicator of active bacteria (Lebaron *et al.* 2001). However, further studies have shown that LNA bacteria not only possess activities similar to

HNA bacteria (Wang *et al.* 2009), but also play an important role in various ecosystems (Gozdereliler *et al.* 2013). Multiple novel physiological strategies, for example, simplified metabolism, high affinity and binding protein-dependent uptake system and special cellular membrane constitution, enable LNA bacteria to respond quickly to various stresses, such as low substrate concentrations (Salcher *et al.* 2011), oxidising agents (Ramseier *et al.* 2011) and viral infection (Bouvier and Maurice 2011). LNA bacteria can even adopt a dormancy strategy to overcome unfavourable environmental conditions (Rösel and Grossart 2012). All of these observations indicate that the adaptability and competition of LNA bacteria should be higher than that of HNA bacteria, such that they become the predominant community in aquatic environments.

Previous studies of LNA and HNA bacteria have mainly focused on their distribution and dynamics in marine environments (Andrade *et al.* 2007; Huete-Stauffer and Morán 2012; Grob *et al.* 2013; García *et al.* 2014). It was reported that coastal seas had a lower proportion of LNA bacteria, such as on a transect from Delaware to the Sargasso Sea (35%), or the Grand Banks (22–42%) (Li *et al.* 1995; Li and Dickie 2001). In contrast, there was a higher proportion of LNA bacteria found in the coastal Canet lagoon (57%; Servais *et al.* 2003), eastern Mediterranean Sea (65%), North Atlantic (64%; Li *et al.* 1995) and the Gulf of Mexico (62%; Jochem *et al.* 2004). In comparison, only a few reports are available on LNA and HNA bacteria in freshwater environments (Ni *et al.* 2015; Read *et al.* 2015). For example, Joux and colleagues investigated the percentages of LNA and HNA bacteria and the effect of salinity and chlorophyll-*a* on those two groups along the Rhone River (France) (Joux *et al.* 2005). Wang and colleagues isolated three typical pure cultures of LNA that were closely affiliated to *Polynucleobacter* from freshwater environments in Switzerland (Wang *et al.* 2009). To the best of our knowledge, field data on the spatial distribution patterns of LNA and HNA fractions are very scarce in freshwater environments (Ni *et al.* 2015; Read *et al.* 2015), and there is no information concerning the changes in cytometric characteristics of LNA and HNA bacteria on a large river-catchment scale. In the current study, we profiled the relationship between the cytometric characteristics of LNA and HNA bacteria, illustrated the biogeographical patterns (i.e. main river *v.* tributaries and geographic distance) of their abundance on a river-catchment scale (over 1000 km), and evaluated the variation of the properties of these two subgroups by the multivariate ordination method.

Materials and methods

Sampling site

The Songhua River is located at a joint section of the temperate zone and the cold-temperature zone in north-eastern Asia (41°42 to ~51°38′N, 119°52 to ~132°31′E). It is the third-largest river in China, and drains 557 000 km² of land. The Songhua River derives from the Great Khingan and Baekdu Mountains and merges into the Amur River (the national boundary between China and Russia). Water samples from the river were taken over the period from June to August in 2010. Sampling sites were set every 40 km along the river and were orientated using a global positioning system (GPS; Fig. 1). In total, 98 water
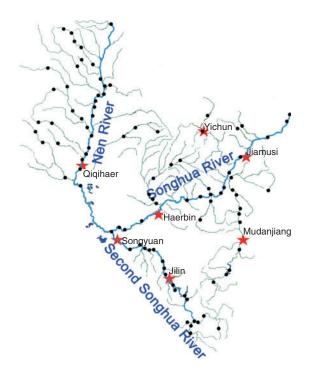

Fig. 1. Sampling sites in the Songhua River catchment. Black solid circles indicate the sampling sites and stars indicate the cities.

samples were collected. Samples were collected in clean and sterilised glass bottles and stored at 4°C during transportation and processed immediately within 24 h after sampling.

Flow-cytometric measurements

Flow-cytometric measurements were performed as described previously (Ma *et al.* 2013). Each water sample (1 mL) was stained with 10 μL mL^{-1} SYBR Green I (1 : 100 dilution in dimethyl sulfoxide as the working solution; Invitrogen, Eugene, OR, USA), and incubated in the dark for 15 min at room temperature before measurement. Flow cytometry was performed using a CyFlow Space instrument (Partec, Hamburg, Germany) equipped with a 50-mW argon ion laser, emitting at a fixed wavelength of 488 nm. Green fluorescence (FL1) was collected at 520 ± 20 nm. Cell population was gated on the two-parameter dot-plot of FL1 and side scatter (SSC). LNA and HNA bacteria were counted separately. The geometrical means of FL1 and SSC of the LNA and HNA subgroups were calculated. All samples were measured in triplicate. To avoid excessive cell aggregation, samples were diluted in cell-free water so that the concentration measured by FCM was always lower than 2×10^5 cells mL^{-1}. The detection limit was below 500 cells s^{-1}, with an average standard deviation of 5%.

Heterotrophic plate counts (HPCs)

The cultivation medium was composed of peptone (10 g L^{-1}), beef extract (3 g L^{-1}), NaCl (5 g L^{-1}) and agar (20 g L^{-1}), with a pH between 7.4 and 7.6. The medium was sterilised at 121°C for 20 min. A water sample of 100 μL was transferred to a plate with 15 mL of sterilised medium. The samples were incubated at 37°C for 48 h before the colonies were counted. All
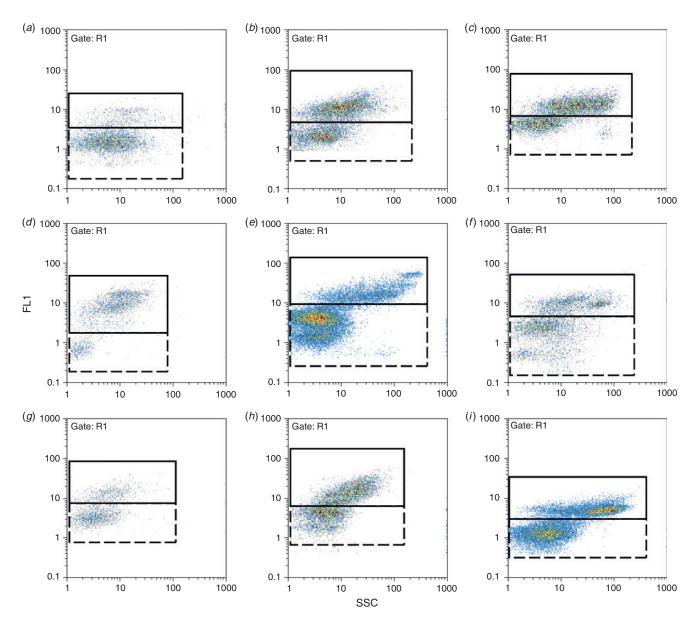
Fig. 2. Flow-cytogram example of bacteria with low nucleic acid content (LNA) and high nucleic acid content (HNA) in flow-cytometric plot. Dashed lines indicate LNA bacteria, and solid lines indicate HNA bacteria.

measurements were performed in triplicate, and three blanks were set as the control.

Water physicochemical properties

Water physicochemical properties (i.e. temperature, pH, conductivity, dissolved oxygen) were measured on site during the sampling process. Chlorophyll-*a* was determined using the traditional spectrophotometer method with extracting pigment by 90% acetone (Lorenzen 1967).

Statistical analysis

ANOVA analysis was used to assess the significance of differences in measured and calculated parameters. The ANOVA analysis was performed in R software (ver. 3.1.3, R Foundation for Statistical Computing, Vienna, Austria) using the package 'Vegan'. A multivariate redundancy analysis (RDA) was performed using Canoco software (Canoco for Windows ver. 4.5, Microcomputer Power, Ithaca, NY, USA) to illustrate the changes in the abundance and cytometric characteristics of LNA and HNA bacteria in response to geographic distance and environmental factors (Liu *et al.* 2015). The bacterial cytometric parameters and abundance data were centred and standardised before RDA, and the Monte Carlo test was used to examine the significance of the RDA analysis. At the same time, a generalised linear model (GLM) and generalised additive model (GAM) were applied in a stepwise manner to predict the abundance and flow cytometric characteristic (FL1 and SSC) of LNA and HNA bacteria in response to environmental ordination axes in RDA. The axes were constrained by environmental variables,
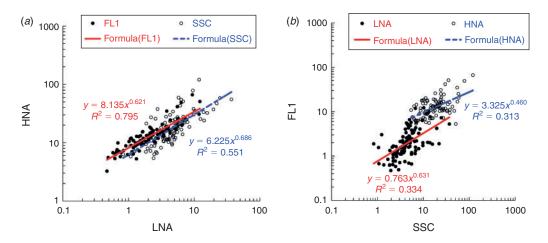
Fig. 3. (*a*) Relationship between high nucleic acid content (HNA) and low nucleic acid content (LNA) bacterial green fluorescence (FL1) and side scatter (SSC). (*b*) Relationship between green fluorescence and side scatter in HNA and LNA bacteria. Spearman correlation coefficient was calculated using regression analysis.

and the visualisation formula were constructed in terms of linear, quadratic or cubic degrees of GLM. *F*-statistics were employed to test the significance in GLM.

Results and discussion

The cytometric characteristics of LNA and HNA bacteria

The separation of aquatic microbes into LNA and HNA groups has been widely reported in marine environments. However, information on freshwater environments is very limited. In the current study, the distribution of LNA and HNA bacteria in a freshwater river catchment was evaluated on a large spatial scale (>1000 km). Flow cytometric analysis showed that the microbial communities were separated into at least two groups (LNA and HNA bacteria), which were observed in all water samples collected from the Songhua River. Neither group was associated with a fixed geometrical mean value of FL1 intensity or SSC in different samples (Fig. 2). Their cytometric characteristics changed greatly, both within a given ecosystem and along various environmental gradients, which revealed that the bimodal distribution was a general feature in bacterioplankton communities. The results showed that there was a positive and highly significant correlation between the cytometric characteristics (FL1 and SSC) of the LNA and HNA subgroups (FL1: Spearman's $r^2 = 0.795$, $P < 0.001$; SSC: Spearman's $r^2 = 0.551$, $P < 0.001$; Fig. 3a), whereas the FL1 and SSC were weakly correlated within each groups (LNA: Spearman's $r^2 = 0.334$, $P < 0.01$; HNA: Spearman's $r^2 = 0.313$, $P < 0.01$; Fig. 3b). In addition, because LNA and HNA were clearly distinguishable from cytometric parameters (FL1 and SSC), the derived variables $FL1_{HNA} : FL1_{LNA}$ and $SSC_{HNA} : SSC_{LNA}$ were used to represent the degree of the relative distances between LNA and HNA in FCM (Bouvier *et al.* 2007). The results showed that the relative distance of FL1 between these two subgroups was significantly higher than that of SSC (Fig. 4, ANOVA, $F = 51.0$, $P < 0.0001$). These results demonstrated that the cytometric parameters within LNA and HNA bacteria were not independent from each other and tended to covary within a given specific ecosystem, which supports the hypothesis that LNA and HNA
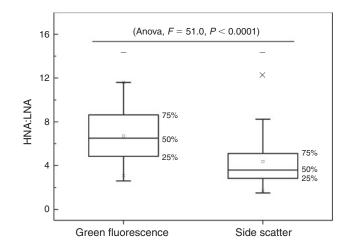

Fig. 4. Relative distance between high nucleic acid content (HNA) and low nucleic acid content (LNA) bacterial cells in terms of green fluorescence and side scatter.

are possibly dynamic and intrinsically linked in aquatic microbial communities (Bouvier *et al.* 2007).

Geographic patterns in abundance of LNA and HNA subgroups

The concentrations of LNA and HNA bacteria ranged from 5.45×10^4 to 4.43×10^6 cells mL^{-1}, and 1.35×10^5 to 4.37×10^6 cells mL^{-1} respectively (Fig. 5a). The two groups are present in an almost equal proportion in the Songhua River catchment (average contribution of LNA cells reached 47%; Fig. 5b). The fact that LNA bacteria persist with high abundance within the river ecosystem suggests that LNA bacteria have a high viability in the microbial loop. It has been shown that heterotrophic nanoflagellates and other planktonic predators seem to prefer grazing on and selectively cropping the larger-sized and more active fraction of bacterioplankton (Corzo *et al.* 2005; Nishimura *et al.* 2005). Tadonleke and co-workers
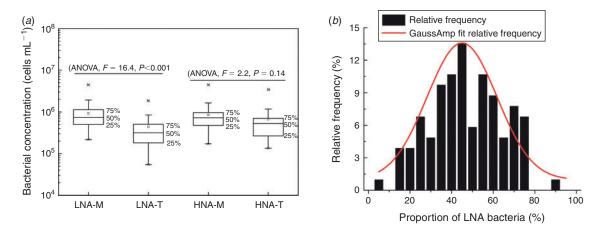
Fig. 5. Distribution pattern of the low nucleic acid content (LNA) and high nucleic acid content (HNA) bacteria in the Songhua River catchment. (*a*) Abundance boxplot, and (*b*) proportion frequency. LNA-M and HNA-M indicate the LNA and HNA bacterial concentration in mainstream, LNA-T and HNA-T indicate the LNA and HNA bacterial concentration in tributaries.

reported that *Cyclidium glaucoma* selectively grazed on HNA communities rather than on LNA (Tadonléké *et al.* 2005). Nishimura and colleagues also found that the grazing mortality of HNA bacteria was 2.8-fold higher than that of LNA bacteria in Lake Biwa (Nishimura *et al.* 2005). However, another study showed that LNA sometimes experienced higher grazing-loss rates than did HNA (Jochem *et al.* 2004). Differences in size-dependent grazing pressure and viral attack may enable LNA bacteria to dominate the community and play an important evolutionary and ecological role similar to that of the HNA in the microbial webs of the ecosystem (Morán *et al.* 2015).

Both LNA and HNA bacteria exhibited a clear geographical distribution pattern in the Songhua River. First, the concentration of LNA bacteria in the mainstream was significantly higher than that in the tributaries (Fig. 5*a*, ANOVA, $F = 16.4$, $P < 0.001$). In contrast, there was no significant difference between the concentrations of HNA bacteria in the mainstream and in the tributaries (Fig. 5, ANOVA, $F = 2.2$, $P = 0.14$). Second, the concentrations of both LNA and HNA bacteria decreased with an increasing altitude (LNA Spearman's $r = -0.311$, $P = 0.001$; HNA Spearman's $r = -0.197$, $P = 0.069$) and latitude (LNA Spearman's $r = -0.317$, $P = 0.001$; HNA Spearman's $r = -0.291$, $P = 0.003$), whereas the concentration of HNA bacteria was positively correlated with longitude (Fig. S1, available as Supplementary material for this paper).

Furthermore, the results also suggested that the distribution pattern of bacterial abundance tends to be temperature related (LNA: Spearman's $r = 0.372$, $P = 0.001$; HNA: Spearman's $r = 0.271$, $P = 0.006$). It was reported that warm-adapted bacteria could have lower minimal P cell quotas than do cold-adapted bacteria (Hall *et al.* 2008), which would confer an advantage to cells with low nucleic acid content (because nucleic acids are an important intracellular source of P) in warm-temperature and resource-limited environments (Van Wambeke *et al.* 2011). The change of temperature, coupled with regional-scale latitudinal gradients or sometimes local weather contingencies, will affect microbial activity, metabolism and growth rate, and hence the abundance of LNA and HNA. This result is consistent with that of a previous study in which
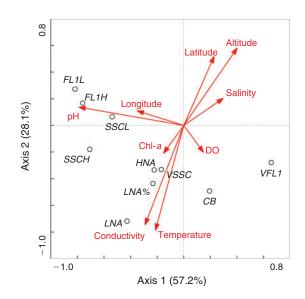

Fig. 6. Redundancy analysis of low nucleic acid content (LNA) and high nucleic acid content (HNA) bacteria with variable factors in the Songhua River. FL1, green fluorescence; FL1L and FL1H, FL1 of LNA and HNA bacteria; SSC, side scatter; SSCL and SSCH, SSC of LNA and HNA bacteria; VFL1, FL1 ratio of HNA to LNA; VSSC, SSC ratio of HNA to LNA; LNA and HNA, the LNA and HNA bacterial concentration; LNA%, the percentage of LNA in total bacterial concentration; CB, concentration of cultivable bacteria.

Huete-Stauffer and Morán found that the net growth rates of LNA bacteria were strongly correlated with temperature in the southern Bay of Biscay coastal waters (Huete-Stauffer and Morán 2012). Additionally, the concentration of LNA bacteria also displayed an altitudinal dependence in the present study. The elevation of altitude would cause a temperature decrease, changes in hydrologic flow and stability, and the river biotope, which would lead to variation of organic substances and inorganic nutrients, thereby influencing the biotic activities in the river. It should be noted that the altitude range (less than 1000 m) in the present study was limited. Therefore, further
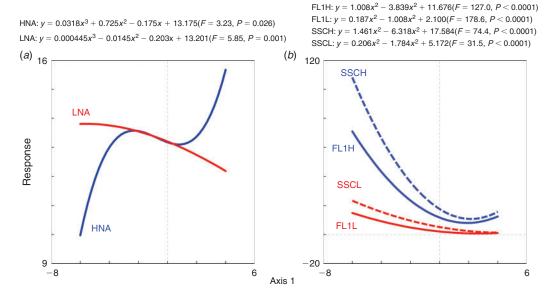
HNA: $y = 0.0318x^3 + 0.725x^2 - 0.175x + 13.175 (F = 3.23, P = 0.026)$
LNA: $y = 0.000445x^3 - 0.0145x^2 - 0.203x + 13.201 (F = 5.85, P = 0.001)$

FL1H: $y = 1.008x^2 - 3.839x^2 + 11.676 (F = 127.0, P < 0.0001)$
FL1L: $y = 0.187x^2 - 1.008x^2 + 2.100 (F = 178.6, P < 0.0001)$
SSCH: $y = 1.461x^2 - 6.318x^2 + 17.584 (F = 74.4, P < 0.0001)$
SSCL: $y = 0.206x^2 - 1.784x^2 + 5.172 (F = 31.5, P < 0.0001)$

Fig. 7. The differentiation in the changes of (*a*) abundance and (*b*) cytometric characteristics of low nucleic acid content (LNA) and high nucleic acid content (HNA) bacteria, in response to driving factors, which are represented by Axis 1. Bacterial concentration was converted by natural logarithm in (*a*). General linear modelling (GLM) was applied in a stepwise manner to predict the abundance and flow-cytometric characteristics (green fluorescence (FL1) and side scatter (SSC)) of LNA and HNA bacteria in response to environmental ordination axes in redundancy analysis. The visualisation formula were constructed in terms of linear, quadratic or cubic degrees of GLM. *F*-statistics were employed to test the significance in GLM.

studies covering larger ranges are needed for a better understanding of the altitudinal effect on the distribution of LNA and HNA bacteria.

Factors influencing the variation in LNA and HNA

Redundancy analysis (RDA) was used to explain the variance of LNA and HNA bacteria. A Monte Carlo test showed that the cytometric characteristics and the abundance of two subgroups were significantly correlated with the extrinsic factors ($F = 13.3$, $P = 0.002$). Axis 1 and Axis 2 explained 57.2 and 28.1% of the variation of bacterial properties respectively, and the RDA showed that pH was the main factor driving the variation of LNA and HNA (Fig. 6). Because it accounts for the major variance of the subgroup properties, Axis 1 was selected to represent the explanatory variables, and used to compare the differentiation between LNA and HNA and changes in cytometric characteristics to drive factors in GLM. The results showed that the response of the abundance of LNA and HNA bacteria to changes in environmental variables differ greatly in the Songhua River (Fig. 7*a*). Both the variability of SSC and FL1 in HNA were more sensitive to the explanatory axis than those in LNA (Fig. 7*b*). The study presented here showed that, although cytometric characteristics (FL1 and SSC) of LNA and HNA bacteria tend to covary within an ecosystem, the abundance of LNA and HNA bacteria changes greatly and differently along environmental gradients, as well as the green fluorescence (FL1) and side scatter (SSC), which implies that LNA and HNA may be regulated differently.

The total microbial abundance is commonly regarded as a variable to track the productivity index of the ecosystem, such as

chlorophyll-*a* stocks or bacterial production. In the present study, the concentration of LNA bacteria was positively correlated with the chlorophyll-*a* concentration, which is similar to the results of previous reports (Bouvier *et al.* 2007; Van Wambeke *et al.* 2011). Our results further showed that the correlation of chlorophyll-*a* with LNA bacteria was more significant than that with HNA (LNA: Spearman's $r = 0.222$, $P = 0.03$; HNA: Spearman's $r = 0.076$, $P = 0.47$), which suggests that LNA bacteria would be more responsive than HNA bacteria to high phytoplankton stocks in freshwater environments. In contrast, a different correlation between LNA bacteria and chlorophyll-*a* was observed in marine environments. For instance, Huete-Stauffer and Morán found a strong negative correlation between the net growth rates of LNA bacteria and chlorophyll-*a* in temperate coastal waters (Huete-Stauffer and Morán 2012). Corzo and co-workers reported that both FL1 and SSC of HNA were positively correlated with chlorophyll-*a*, but no similar correlation was observed for the LNA fraction in Antarctic waters in the Austral summer (Corzo *et al.* 2005). These might be the results of different compositions and ecological roles of LNA and HNA in aquatic environments. In addition to the physicochemical properties studied here, dissolved organic matter may also play an important role in regulating the distribution of LNA and HNA bacteria. The strong correlation between community dynamics, LNA : HNA ratio and dissolved organic matter quality and quantity has been observed in marine ecosystems (Belzile *et al.* 2008; Kaartokallio *et al.* 2016). Hence, further study on the correlation between dissolved organic matter and distribution of LNA and HNA bacteria in freshwater ecosystems is needed.

The present study focused on the geographic distribution of LNA and HNA bacteria. One major aspect that was not covered here is the temporal shifts in LNA and HNA bacteria. It has been demonstrated that seasonal changes have important influence over microbial community dynamics in marine environments (Belzile *et al.* 2008; Gilbert *et al.* 2012). Our previous study also showed that the temporal changes had stronger effects on the patterns of LNA and HNA than that of nutrient conditions in an exorheic river (Liu *et al.* 2016). The sampling period (i.e. June–August) in the present study was chosen because of the relative stability of the whole river catchment during this time. However, rivers are highly dynamic systems and information of the bacterial changes over time would offer important insights into the distribution of LNA and HNA bacteria in river catchments. Finally, the river catchment studied in the present study is still spatially limited; data from various rivers and streams would lead to a better understanding of the geographic distribution and dynamics of LNA and HNA bacteria in freshwater environments.

Conclusions

In summary, our study investigated the geographical distribution of LNA and HNA bacteria over a large river-catchment scale. The existence of LNA and HNA counterparts varied widely over the whole river catchment, and the LNA bacterial abundance was significantly higher in the mainstream than in the tributaries. The strong covariation of cytometric expressions between LNA and HNA bacteria indicated that these two subgroups are intrinsically linked components in aquatic bacterial communities. The multivariate analysis further revealed that the abundance and cytometric characteristics of these two co-occurring subgroups are regulated differently, which implies that LNA and HNA bacteria play different ecological roles in river ecosystems.

Acknowledgements

References

Andrade, L., Gonzalez, A. M., Rezende, C. E., Suzuki, M., Valentin, J. L., and Paranhos, R. (2007). Distribution of HNA and LNA bacterial groups in the southwest Atlantic Ocean. *Brazilian Journal of Microbiology* **38**, 330–336. doi:10.1590/S1517-83822007000200028

Belzile, C., Brugel, S., Nozais, C., Gratton, Y., and Demers, S. (2008). Variations of the abundance and nucleic acid content of heterotrophic bacteria in Beauford Shelf waters during winter and spring. *Journal of Marine Systems* **74**, 946–956. doi:10.1016/J.JMARSYS.2007.12.010

Bouvier, T., and Maurice, C. F. (2011). A single-cell analysis of virioplankton adsorption, infection, and intracellular abundance in different bacterioplankton physiologic categories. *Microbial Ecology* **62**, 669–678. doi:10.1007/S00248-011-9862-3

Bouvier, T., del Giorgio, P. A., and Gasol, J. M. (2007). A comparative study of the cytometric characteristics of high and low nucleic-acid bacterioplankton cells from different aquatic ecosystems. *Environmental Microbiology* **9**, 2050–2066. doi:10.1111/J.1462-2920.2007.01321.X

Corzo, A., Rodriguez-Galvez, S., Lubian, L., Sobrino, C., Sangra, P., and Martinez, A. (2005). Antarctic marine bacterioplankton subpopulations discriminated by their apparent content of nucleic acids differ in their response to ecological factors. *Polar Biology* **29**, 27–39. doi:10.1007/S00300-005-0032-2

García, F. C., Lopez-Urrutia, A., and Morán, X. A. G. (2014). Automated clustering of heterotrophic bacterioplankton in flow cytometry data. *Aquatic Microbial Ecology* **72**, 175–185. doi:10.3354/AME01691

Gilbert, J. A., Steele, J. A., Caporaso, J. G., Steinbrück, L., Reeder, J., Temperton, B., Huse, S., McHardy, A. C., Knight, R., Joint, I., Somerfield, P., Fuhrman, J. A., and Field, D. (2012). Defining seasonal marine microbial community dynamics. *The ISME Journal* **6**, 298–308. doi:10.1038/ISMEJ.2011.107

Gozdereliler, E., Boon, N., Aamand, J., De Roy, K., Granitsiotis, M. S., Albrechtsen, H.-J., and Sorensen, S. R. (2013). Comparing metabolic functionalities, community structures, and dynamics of herbicide-degrading communities cultivated with different substrate concentrations. *Applied and Environmental Microbiology* **79**, 367–375. doi:10.1128/AEM.02536-12

Grob, C., Ostrowski, M., Holland, R. J., Heldal, M., Norland, S., Erichsen, E. S., Blindauer, C., Martin, A. P., Zubkov, M. V., and Scanlan, D. J. (2013). Elemental composition of natural populations of key microbial groups in Atlantic waters. *Environmental Microbiology* **15**, 3054–3064.

Hall, E. K., Neuhauser, C., and Cotner, J. B. (2008). Toward a mechanistic understanding of how natural bacterial communities respond to changes in temperature in aquatic ecosystems. *The ISME Journal* **2**, 471–481. doi:10.1038/ISMEJ.2008.9

Huete-Stauffer, T. M., and Morán, X. A. G. (2012). Dynamics of heterotrophic bacteria in temperate coastal waters: similar net growth but different controls in low and high nucleic acid cells. *Aquatic Microbial Ecology* **67**, 211–223. doi:10.3354/AME01590

Jochem, F. J., Lavrentyev, P. J., and First, M. R. (2004). Growth and grazing rates of bacteria groups with different apparent DNA content in the Gulf of Mexico. *Marine Biology* **145**, 1213–1225. doi:10.1007/S00227-004-1406-7

Joux, F., Servais, P., Naudin, J. J., Lebaron, P., Oriol, L., and Courties, C. (2005). Distribution of picophytoplankton and bacterioplankton along a river plume gradient in the Mediterranean Sea. *Life and Environment* **55**, 197–208.

Kaartokallio, H., Asmala, E., Autio, R., and Thomas, D. N. (2016). Bacterial production, abundance and cell properties in boreal estuaries: relation to dissolved organic matter quantity and quality. *Aquatic Sciences* **78**, 525–540. doi:10.1007/S00027-015-0449-9

Koch, C., Harms, H., and Muller, S. (2014). Dynamics in the microbial cytome: single cell analytics in natural systems. *Current Opinion in Biotechnology* **27**, 134–141. doi:10.1016/J.COPBIO.2014.01.011

Lebaron, P., Servais, P., Agogué, H., Courties, C., and Joux, F. (2001). Does the high nucleic acid content of individual bacterial cells allow us to discriminate between active cells and inactive cells in aquatic systems? *Applied and Environmental Microbiology* **67**, 1775–1782. doi:10.1128/AEM.67.4.1775-1782.2001

Li, W. K. W., and Dickie, P. M. (2001). Monitoring phytoplankton, bacterioplankton, and virioplankton in a coastal inlet (Bedford Basin) by flow cytometry. *Cytometry* **44**, 236–246. doi:10.1002/1097-0320(20010701)44:3<236::AID-CYTO1116>3.0.CO;2-5

Li, W., Jellett, J., and Dickie, P. (1995). DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. *Limnology and Oceanography* **40**, 1485–1495. doi:10.4319/LO.1995.40.8.1485

Liu, J., Wang, J., Gao, G., Bartlam, M. G., and Wang, Y. (2015). Distribution and diversity of fungi in freshwater sediments on a river catchment scale. *Frontiers in Microbiology* **6**, 329. doi:10.3389/FMICB.2015.00329

Liu, J., Hao, Z., Ma, L., Ji, Y., Bartlam, M. G., and Wang, Y. (2016). Spatio-temporal variations of high and low nucleic acid content bacteria in an

exorheic river. *PLoS One* **11**, e0153678. doi:10.1371/JOURNAL.
PONE.0153678

Lorenzen, C. J. (1967). Determination of chlorophyll and pheopigments:
spectrophotometric equations. *Limnology and Oceanography* **12**,
343–346. doi:10.4319/LO.1967.12.2.0343

Ma, L. L., Mao, G. N., Liu, J., Yu, H., Gao, G. H., and Wang, Y. Y. (2013).
Rapid quantification of bacteria and viruses in influent, settled water,
activated sludge and effluent from a wastewater treatment plant using
flow cytometry. *Water Science and Technology* **68**, 1763–1769.
doi:10.2166/WST.2013.426

Morán, X. A. G., Alonso-Sáez, L., Nogueira, E., Ducklow, H. W.,
González, N., López-Urrutia, A., Díaz-Pérez, L., Calvo-Díaz, A.,
Arandia-Gorostidi, N., and Huete-Stauffer, T. M. (2015). More, smaller
bacteria in response to ocean's warming? *Proceedings of the Royal
Society of London – B. Biological Sciences* **282**(1810), 369–374.
doi:10.1098/RSPB.2015.0371

Ni, Z. X., Huang, X. P., and Zhang, X. (2015). Picoplankton and virio-
plankton abundance and community structure in Pearl River Estuary and
Daya Bay, South China. *Journal of Environmental Sciences* **32**,
146–154. doi:10.1016/J.JES.2014.12.019

Nishimura, Y., Kim, C., and Nagata, T. (2005). Vertical and seasonal
variations of bacterioplankton subgroups with different nucleic acid
contents: possible regulation by phosphorus. *Applied and Environmental
Microbiology* **71**, 5828–5836. doi:10.1128/AEM.71.10.5828-5836.
2005

Ramseier, M. K., von Gunten, U., Freihofer, P., and Hammes, F. (2011).
Kinetics of membrane damage to high (HNA) and low (LNA) nucleic
acid bacterial clusters in drinking water by ozone, chlorine, chlorine
dioxide, monochloramine, ferrate(VI), and permanganate. *Water
Research* **45**, 1490–1500. doi:10.1016/J.WATRES.2010.11.016

Read, D. S., Gweon, H. S., Bowes, M. J., Newbold, L. K., Field, D., Bailey,
M. J., and Griffiths, R. I. (2015). Catchment-scale biogeography of
riverine bacterioplankton. *The ISME Journal* **9**, 516–526. doi:10.1038/
ISMEJ.2014.166

Rösel, S., and Grossart, H.-P. (2012). Contrasting dynamics in activity and
community composition of free-living and particle-associated bacteria in
spring. *Aquatic Microbial Ecology* **66**, 169–181. doi:10.3354/AME01568

Salcher, M. M., Pernthaler, J., and Posch, T. (2011). Seasonal bloom
dynamics and ecophysiology of the freshwater sister clade of SAR11
bacteria 'that rule the waves' (LD12). *The ISME Journal* **5**, 1242–1252.
doi:10.1038/ISMEJ.2011.8

Servais, P., Casamayor, E. O., Courties, C., Catala, P., Parthuisot, N., and
Lebaron, P. (2003). Activity and diversity of bacterial cells with high and
low nucleic acid content. *Aquatic Microbial Ecology* **33**, 41–51.
doi:10.3354/AME033041

Tadonléké, R. D., Planas, D., and Lucotte, A. (2005). Microbial food webs in
boreal humic lakes and reservoirs: ciliates as a major factor related to the
dynamics of the most active bacteria. *Microbial Ecology* **49**, 325–341.
doi:10.1007/S00248-004-0232-2

Van Wambeke, F., Catala, P., Pujo-Pay, M., and Lebaron, P. (2011). Vertical
and longitudinal gradients in HNA–LNA cell abundances and cyto-
metric characteristics in the Mediterranean Sea. *Biogeosciences* **8**,
1853–1863. doi:10.5194/BG-8-1853-2011

Wang, Y. Y., Hammes, F., Boon, N., Chami, M., and Egli, T. (2009).
Isolation and characterization of low nucleic acid (LNA)-content bacteria.
The ISME Journal **3**, 889–902. doi:10.1038/ISMEJ.2009.46

Wang, Y. Y., Hammes, F., De Roy, K., Verstraete, W., and Boon, N. (2010).
Past, present and future applications of flow cytometry in aquatic
microbiology. *Trends in Biotechnology* **28**, 416–424. doi:10.1016/J.TIB
TECH.2010.04.006