Distribution Characteristics of Surface Planktonic Bacteria and Their Environmental Influencing Factors in Shenzhen Coastal Waters: Postprint

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Abstract

Surface water samples were collected in March, May, August, and October 2015 from the coastal waters of Shenzhen City (Pearl River Estuary, Shenzhen Bay, and Daya Bay). Flow cytometry was employed to determine the abundances of total bacterioplankton, high nucleic acid content bacteria subgroup (HNA), and low nucleic acid content bacteria subgroup (LNA), and to analyze their spatiotemporal distribution characteristics and elucidate the influence of environmental factors on the spatiotemporal distribution patterns of bacterioplankton. The results demonstrated that the average abundances of surface bacterioplankton in the Pearl River Estuary, Shenzhen Bay, and Daya Bay decreased sequentially, being 3.82$×10^{6}$ cells/mL, 7.67$×10^{6}$ cells/mL, and 3.38$×10^{6}$ cells/mL, respectively. In the Pearl River Estuary, bacterioplankton abundance increased from offshore to nearshore; in Shenzhen Bay, bacterioplankton abundance showed minor variation among stations within the bay; and in Daya Bay, spatial differences in bacterioplankton abundance were not significant ($P > 0.05$). Temporal differences in bacterioplankton abundance were primarily influenced by temperature, whereas spatial differences were mainly affected by nutrients and chlorophyll a. The spatiotemporal variability of HNA subgroup abundance was greater than that of the LNA subgroup; the HNA subgroup was significantly affected by temperature ($P < 0.01$), while the correlation between the LNA subgroup and temperature was not significant ($P > 0.05$). The effects of the environment on HNA and LNA subgroup abundances shared many similarities, yet the two subgroups exhibited different responses to certain environmental factors, suggesting that they may play partially overlapping but slightly different roles in coastal surface ecosystems.

Full Text

Distribution Characteristics of Bacterioplankton and Effects of Environmental Factors in the Coastal Surface Waters of Shenzhen

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Abstract: In marine ecosystems, the distribution pattern and abundance of bacterioplankton are directly related to environmental conditions. To study the effect of environmental factors on bacterial abundance, surface water samples were collected in March, May, August, and October 2015 from three coastal regions in Shenzhen, China: the Pearl River Estuary, Shenzhen Bay, and Daya Bay. The abundance of bacterioplankton, bacteria with high DNA content (HNA subgroup bacteria), and bacteria with low DNA content (LNA subgroup bacteria) in the coastal waters of Shenzhen were measured by flow cytometry to analyze their temporal and spatial distribution patterns and to explore their responses to environmental factors. The results showed that the average abundance of bacterioplankton in Shenzhen Bay (7.67×10^5 cells/mL), the Pearl River Estuary (3.82×10^5 cells/mL), and Daya Bay (3.38×10^5 cells/mL) exhibited a decreasing trend. The abundance of bacterioplankton in the Pearl River Estuary increased from offshore to nearshore sites; however, there were no significant differences in abundance among the sites in Shenzhen Bay ($p > 0.05$) and Daya Bay ($p > 0.05$). The temporal variation in bacterioplankton abundance in the three regions was mainly affected by temperature, whereas spatial variation was predominantly controlled by the concentration of chlorophyll a and nutrient matter, such as nitrogen and phosphorus. Additionally, the temporal and spatial variability of HNA subgroup bacterial abundance was greater than that of LNA subgroup bacteria ($p < 0.01$). The HNA subgroup bacteria were significantly affected by temperature ($p < 0.01$) whereas LNA subgroup bacteria were not ($p > 0.05$). The different responses of HNA and LNA subgroup bacteria to certain environmental factors imply that they play partially overlapping but slightly different roles in the coastal ecosystem.

Keywords: Shenzhen; coastal waters; flow cytometry; bacterioplankton; HNA subgroup bacteria; LNA subgroup bacteria

Introduction

Coastal waters connect terrestrial and marine environments and serve as important sites for global carbon cycling and storage. Compared to open oceans, coastal waters receive substantial terrestrial nutrient inputs and are more susceptible to human disturbance, often exhibiting higher primary and secondary productivity. Bacterioplankton, as the primary decomposers and producers in coastal ecosystems, play crucial roles in marine nutrient cycling and exert significant regulatory effects on marine ecological environments. Their abundance directly influences material transformation and energy flow within ecosystems. Bacterioplankton abundance is also affected by numerous environmental factors and serves as an important indicator of water quality, playing a significant role in studies of water pollution and eutrophication. As a key parameter in coastal ecosystems, investigating the spatiotemporal distribution characteristics of bacterioplankton and their relationship with environmental factors helps to understand marine ecological conditions and promotes comprehension of bacterioplankton ecological functions.

Flow cytometry (FCM) offers advantages including stability and reproducibility and has been widely applied in marine microbial research, including the determination of bacterioplankton abundance. Flow cytometers can also differentiate bacterioplankton stained with nucleic acid dyes into two subgroups: high nucleic acid content (HNA) and low nucleic acid content (LNA) subgroups, which may differ in growth rate and cellular activity. Some studies have indicated that the HNA subgroup exhibits higher growth rates than the LNA subgroup, while the LNA subgroup possesses stronger adaptability to certain stressful environments due to its special membrane structure and protein metabolism resistance mechanisms. To date, few studies have investigated the distribution patterns and ecological functions of these two subgroups in natural aquatic environments based on their nucleic acid content differences. Li et al. compared the distribution characteristics of HNA and LNA subgroups in the North Atlantic and eastern Mediterranean and analyzed their relationships with environmental factors, finding that HNA bacterial abundance showed significant correlation with chlorophyll content while LNA did not, indicating that the two subgroups contribute differently to marine nutrient cycling. Liu et al.' s research on the Haihe River supported that HNA and LNA subgroups also play different ecological roles in freshwater ecosystems, suggesting that these two bacterial groups may exhibit different distribution characteristics and ecological functions under different water conditions and environmental conditions. However, similar investigations have not been reported in China' s coastal waters.

Shenzhen borders the South China Sea, and its coastal waters are divided into eastern and western sections by the Kowloon Peninsula. The western waters include the Pearl River Estuary and Shenzhen Bay, while the eastern waters include Dapeng Bay and Daya Bay, providing an excellent environment for studying bacterioplankton distribution and function in coastal ecosystems. Water pollution in Shenzhen' s coastal waters, particularly in Shenzhen Bay and

the Pearl River Estuary, has remained uncontrolled and unresolved. Previous scholars have studied bacterioplankton abundance in Shenzhen Bay and Dapeng Bay, revealing poor water quality and severe eutrophication in both areas. Environmental factors show significant spatial variation among different coastal waters of Shenzhen. However, the overall spatiotemporal distribution patterns of bacterioplankton in Shenzhen' s coastal waters and their main environmental drivers remain unclear, and the distribution characteristics and ecological function differences of HNA and LNA subgroups in Shenzhen' s coastal waters have never been investigated.

This study used flow cytometry to determine the abundance of total bacterioplankton and HNA and LNA subgroups in Shenzhen' s coastal waters, explored their spatiotemporal distribution characteristics through variance analysis, and elucidated the relationships between bacterioplankton abundance and various environmental factors using Pearson correlation analysis. The results provide scientific basis for understanding the functional mechanisms and characteristics of bacterioplankton in coastal ecosystem nutrient cycling, and for rational development, utilization, and protection of marine resources.

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1. Sampling

To investigate the distribution and variation patterns of total bacterioplankton and HNA and LNA subgroups in Shenzhen' s coastal surface waters, sampling stations were established in the Pearl River Estuary, Shenzhen Bay, and Daya Bay according to the geographical distribution of Shenzhen' s sea areas. Surface seawater was collected at each station using a water sampler. Salinity (Sal), dissolved oxygen concentration (DO), pH, and temperature (Temp) were measured on-site. For each water sample, 1.8 mL was transferred to sterile cryovials, fixed with glutaraldehyde at a final concentration of 1% (v/v), thoroughly mixed, rapidly frozen in liquid nitrogen, and stored in an ultra-low temperature freezer until bacterioplankton abundance determination. The remaining water samples were used for measurements of chlorophyll a (Chl-a), total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD).

[Figure 1: see original paper] Distribution of sampling sites in Shenzhen coastal waters

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2. Measurement of Environmental Parameters

Dissolved oxygen and temperature were measured on-site using a multiparameter water quality meter. The determination of total phosphorus and chemical oxygen demand followed the specifications in "Specifications for Oceanographic Survey - Marine Chemical Elements Investigation (GB/T 12763.4-2007)." Chlorophyll-a content was determined according to "Marine

Monitoring Code - Nearshore Pollution Ecological Survey and Biological Monitoring (GB 17378.7-2007)."

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3. Determination of Bacterioplankton Abundance

Flow cytometry was used to determine total bacterioplankton abundance and HNA and LNA subgroup abundances in water samples. Frozen samples were thawed and diluted 10-fold with 0.22 m membrane-filtered sterile buffer. A working solution of SYBR Green I (Invitrogen) fluorescent dye was prepared with particle-free 0.22 m filtered water. Approximately 10 L of appropriately concentrated 0.22 m fluorescent microsphere suspension was prepared as an internal reference for flow cytometric detection and counting. To 450 L of diluted sample, 12.5 L of SYBR Green I working solution was added, vortexed, and stained for 10 minutes at room temperature in the dark. After brief vortexing, the sample was analyzed by flow cytometry. This study used a FACSCalibur flow cytometer (BD Biosciences) equipped with an argon ion laser (excitation wavelength 488 nm, 15 mW).

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4. Data Analysis

Principal component analysis (PCA) of environmental parameters under different spatiotemporal conditions was performed using Canoco 5.0 software to clarify sea area environmental characteristics. Two-way ANOVA was conducted using IBM SPSS Statistics 19.0 to compare significant differences in bacterioplankton abundance (including total bacterioplankton, HNA, and LNA) across different times and spaces. Pearson correlation coefficients between bacterioplankton abundance (including total bacterioplankton, HNA, and LNA subgroups) and various environmental parameters were calculated to reveal environmental influences on bacterioplankton distribution and compare the functional characteristics of HNA and LNA subgroups in coastal nutrient cycling processes.

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1. Characteristics of Environmental Parameters in Shenzhen Coastal Surface Waters

During this investigation, seawater temperature ranged from 19.92 to 31.74°C, with an average of 25.80°C. The lowest monthly average temperature occurred in March (21.85°C), and the highest in August (28.50°C). Average salinity in the Pearl River Estuary, Shenzhen Bay, and Daya Bay was 13.36‰, 18.99‰, and 32.90‰, respectively. Chlorophyll-a (Chl-a) content in Shenzhen Bay and the Pearl River Estuary was significantly higher than in Daya Bay ($p < 0.01$).

The Chl-a content at the mangrove site (SZW07) was significantly higher than at other stations. Nitrate and ammonium concentrations in the Pearl River Estuary and Shenzhen Bay were significantly higher than in Daya Bay. Other environmental parameters showed no significant differences among the three sea areas.

Average values of environmental parameters in Shenzhen coastal waters (Pearl River Estuary, Shenzhen Bay, and Daya Bay)

Principal component analysis (PCA) was performed on environmental parameters from all stations across four months. The results revealed distinct environmental characteristics for the Pearl River Estuary, Shenzhen Bay, and Daya Bay. Sample points from Shenzhen Bay showed the most dispersed distribution across different months and stations, while those from Daya Bay were the most concentrated, indicating that Shenzhen Bay had the greatest spatiotemporal variation in environmental parameters, followed by the Pearl River Estuary, while Daya Bay showed the smallest variation. The Pearl River Estuary exhibited a particularly pronounced environmental gradient, with almost no spatial difference in October.

[Figure 2: see original paper] Principal component analysis (PCA) of environmental parameters in Shenzhen coastal waters (Pearl River Estuary, Shenzhen Bay, and Daya Bay)

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2. Spatiotemporal Distribution of Total Bacterioplankton Abundance in Shenzhen Coastal Waters

Bacterioplankton abundance in Shenzhen coastal surface waters ranged from 1.41×10^5 to 1.75×10^6 cells/mL. The highest value appeared in Shenzhen Bay, while the lowest occurred in Daya Bay. Average bacterioplankton abundance decreased sequentially in Shenzhen Bay (7.67×10^5 cells/mL), the Pearl River Estuary (3.82×10^5 cells/mL), and Daya Bay (3.38×10^5 cells/mL). August showed the highest average bacterioplankton abundance (5.80×10^5 cells/mL), while March showed the lowest (2.98×10^5 cells/mL).

[Figure 3: see original paper] Spatiotemporal variations of bacterioplankton abundance in Shenzhen coastal waters (Pearl River Estuary, Shenzhen Bay, and Daya Bay)

In the Pearl River Estuary, bacterioplankton abundance increased from the outer to inner bay in May, while other months showed no significant differences among stations. In Shenzhen Bay, there were no significant differences in bacterioplankton abundance among stations within the bay ($p > 0.05$). In May, bacterioplankton abundance at station ZJK03 was significantly higher than at ZJK01 ($p < 0.05$). In Shenzhen Bay, bacterioplankton abundance at station SZW04 was significantly higher than at SZW06 ($p < 0.05$). In Daya Bay, bacterioplankton abundance showed small variations across time and space. Bac-

terioplankton abundance in Shenzhen Bay was higher than in the Pearl River Estuary and Daya Bay at the same sampling time, except in March when it was higher than Daya Bay.

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3. Spatiotemporal Distribution of HNA and LNA Subgroup Abundances in Shenzhen Coastal Waters

Based on side scatter signals and fluorescence signals, bacterioplankton in samples from all sea areas showed clear clustering, with HNA and LNA bacterial subgroups distinctly separated, similar to classification phenomena reported in literature.

HNA subgroup abundance ranged from 2.20×10^4 to 8.31×10^5 cells/mL. The highest value appeared at station SZW06 in Shenzhen Bay, which was significantly higher than in the Pearl River Estuary and Daya Bay ($p < 0.01$). The lowest value occurred at station DYW11 in Daya Bay. In the Pearl River Estuary and Shenzhen Bay, HNA subgroup abundance was higher in May than in other months.

[Figure 5: see original paper] Flow cytometry plot of LNA and HNA bacteria

LNA subgroup abundance ranged from 3.74×10^4 to 1.13×10^6 cells/mL. The highest value appeared at station SZW07, and the lowest at station DYW12. The LNA subgroup proportion in total bacterioplankton ranged from 25.97% to 82.93%, with both minimum and maximum proportions appearing in Shenzhen Bay. The average proportions in the Pearl River Estuary, Shenzhen Bay, and Daya Bay were 56.58%, 58.71%, and 62.38%, respectively, showing small spatial variation.

[Figure 6: see original paper] Spatiotemporal variations of HNA subgroup bacterial abundance in Shenzhen coastal waters (Pearl River Estuary, Shenzhen Bay, and Daya Bay)

[Figure 7: see original paper] Spatiotemporal variations of LNA subgroup bacterial abundance in Shenzhen coastal waters (Pearl River Estuary, Shenzhen Bay, and Daya Bay)

[Figure 8: see original paper] Percentage of HNA subgroup bacteria in bacterioplankton in Shenzhen coastal waters (Pearl River Estuary, Shenzhen Bay, and Daya Bay)

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4. Correlations Between Bacterioplankton Abundance and Environmental Parameters in Shenzhen Coastal Waters

Pearson correlation analysis results (Table 2) showed that total bacterioplankton abundance was extremely significantly positively correlated with TN, TP,

COD, NO_2^-, and Chl-a ($p < 0.01$), and significantly negatively correlated with salinity ($p < 0.05$). HNA subgroup abundance was extremely significantly positively correlated with temperature ($p < 0.01$), while LNA subgroup abundance showed no significant correlation with temperature ($p > 0.05$). The most notable difference between HNA and LNA subgroups was that HNA subgroup abundance showed significant positive correlation with temperature and Chl-a, while LNA subgroup abundance showed no significant correlation with temperature but significant negative correlation with salinity.

Pearson correlation coefficients of bacterioplankton, HNA subgroup, and LNA subgroup abundances with key environmental parameters in Shenzhen coastal waters (Pearl River Estuary, Shenzhen Bay, and Daya Bay)

Discussion

1. Environmental Effects on Bacterioplankton Abundance

Temperature is a crucial factor affecting bacterioplankton growth and the main cause of spatiotemporal differences in bacterioplankton abundance. Temperature indirectly affects bacterial metabolism by controlling intracellular enzyme activity. Within a certain range, bacterial growth rate is positively correlated with temperature. Temperature also indirectly affects bacterioplankton abundance and community composition by influencing the growth of primary producers such as filamentous green algae and their removal of nitrogen and phosphorus from water. This study showed that total bacterioplankton abundance was significantly positively correlated with temperature ($r = 0.352$, $p = 0.012$), and HNA subgroup abundance was extremely significantly positively correlated with temperature ($r = 0.511$, $p = 0$). These results indicate that bacterioplankton growth is more favorable in nutrient-rich areas.

Salinity, as an indicator characterizing ecological niches, indirectly affects bacterioplankton abundance and community composition in ecosystems by altering nutrient levels in water. This study showed a significant negative correlation between salinity and bacterioplankton abundance ($r = -0.369$, $p = 0.015$). Salinity concentrations increased sequentially in the Pearl River Estuary, Shenzhen Bay, and Daya Bay, while nutrient concentrations decreased significantly, and bacterioplankton abundance also decreased accordingly.

Chl-a serves as a good indicator of phytoplankton standing stock and can be used to study the relationship between bacterioplankton growth and reproduction and phytoplankton. Part of the dissolved organic matter in water is produced by phytoplankton photosynthesis, providing sufficient carbon sources for bacterioplankton secondary growth, while bacterioplankton metabolic products provide certain nutrients for phytoplankton. In Shenzhen' s coastal waters, bacterioplankton showed extremely significant positive correlation with Chl-a content ($r = 0.721$, $p = 0.006$), demonstrating the close relationship between

phytoplankton and bacteria. Chl-a content was significantly higher in Shenzhen Bay and the Pearl River Estuary than in Daya Bay, corresponding with higher bacterioplankton abundance in these areas.

Inorganic nutrients are important environmental factors in marine ecosystems, especially coastal waters, and represent the main factor causing spatial distribution patterns of bacterioplankton. Nutrient changes have extremely important impacts on biotic and abiotic components and their ecological functions in ecosystems. This study showed that bacterioplankton abundance was significantly positively correlated with DIN, NH_4^+, NO_2^-, and NO_3^- ($p < 0.01$). Due to terrestrial discharge from Shenzhen, nutrient concentrations in Shenzhen Bay and the Pearl River Estuary were significantly higher than in Daya Bay. Moreover, water circulation in these two bays is weak, making it difficult for nutrients to diffuse, resulting in higher concentrations inside the bays than outside. This leads to higher bacterioplankton abundance in Shenzhen Bay and the Pearl River Estuary compared to Daya Bay, consistent with previous research results.

However, in Shenzhen Bay, although nutrient concentrations decreased from inside to outside the bay, bacterioplankton abundance at station SZW05 was the highest, without showing a similar decreasing pattern among stations within the bay. According to existing research, when nutrient concentrations exceed certain values, severe eutrophication leads to massive proliferation of plankton, causing dissolved oxygen content to decrease and the entire ecological environment to change, thereby seriously affecting bacterioplankton growth and reproduction. It is reasonable to believe that in Shenzhen Bay, excessively high nutrient concentrations caused phytoplankton biomass to increase sharply, leading to rapid decreases in dissolved oxygen content while introducing large amounts of nitrogen and phosphorus into the water, ultimately resulting in reduced bacterioplankton abundance.

2. Environmental Effects on HNA and LNA Subgroup Abundances

Two-way ANOVA results showed that both temporal and spatial differences in HNA subgroup abundance were extremely significant ($F = 3.249$, $p < 0.0001$), with temporal and spatial differences contributing equally to the spatiotemporal distribution pattern of HNA subgroup abundance. In contrast, LNA subgroup abundance showed no significant temporal variation ($F = 1.504$, $p = 0.219$) but extremely significant spatial variation ($p < 0.01$). This study indicates that in Shenzhen' s coastal waters, temperature is the main factor causing temporal differences in HNA subgroup abundance, while nutrients and Chl-a are the main factors causing spatial differences. Similar conclusions have been reported in other sea areas.

The greatest difference between HNA and LNA subgroups in their correlation with environmental factors is that HNA subgroup abundance showed extremely significant positive correlation with temperature ($r = 0.416$, $p < 0.01$), while

LNA subgroup abundance showed no significant correlation with temperature ($r = 0.182$, $p = 0.243$). Temperature indirectly affects bacterial growth and reproduction by controlling intracellular enzyme activity, but the enzymes related to cellular metabolism in the two bacterial subgroups have different sensitivities to temperature. When temperature reaches a certain level, the effect of temperature on bacterioplankton decreases. Shiah and Ducklow' s research on Chesapeake Bay showed that when water temperature exceeded 10°C, bacterial metabolism was no longer significantly affected by temperature. In this study, the water temperature range was 19.92–31.74°C. Within this range, as temperature increased, enzyme activity continued to increase, and HNA subgroup cellular enzyme activity approached its maximum. The correlation coefficient between total bacterioplankton abundance and temperature was 0.352, while that between HNA subgroup and temperature was 0.416, indirectly indicating that HNA subgroup is the dominant group in total bacterioplankton.

Previous studies have shown that HNA subgroups tend to grow in nutrient-rich environments. This study' s results show that, except at individual stations, HNA subgroup abundance was higher than LNA subgroup abundance in most nutrient-rich areas, accounting for an average of 60.34% of total bacterioplankton. However, Liu et al.' s research on the Haihe River showed that both subgroups' temporal differences were controlled by temperature, with large temperature variation ranges allowing the correlation between LNA subgroup and temperature to become apparent. The study location was at 38°N, with maximum temperatures above 30°C and minimum temperatures below 10°C, which differs from this study' s results.

Conclusion

Bacterioplankton abundance in Shenzhen' s coastal waters showed significant temporal and spatial differences, with temporal differences mainly affected by temperature and spatial differences predominantly controlled by nutrients and Chl-a. HNA subgroup temporal distribution patterns were controlled by temperature, while spatial distribution patterns were controlled by nutrients and Chl-a. LNA subgroup temporal differences were not significant, but spatial differences were significant and mainly controlled by nutrients and Chl-a. The effects of environmental factors on HNA and LNA subgroup abundances share some similarities, but their different responses to certain environmental factors imply that they play partially overlapping but slightly different ecological roles in coastal ecosystems.

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Note: Figure translations are in progress. See original paper for figures.