Vertical stratification driven nutrient ratios to regulate phytoplankton community structure in the oligotrophic western Pacific Ocean

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Abstract: Vertical stratification determined the variability of temperature and nutrient distribution in upper seawater, thereby affecting the primary production of the ocean. Nutrients in the oligotrophic region vary in time and space, and thus phytoplankton vary in their vertical distribution. However, the differences in the vertical distribution of phytoplankton have not been systematically studied. This study investigated the spatial distribution pattern and diversity of phytoplankton communities in the western Pacific Ocean (WPO) in the autumn of 2016, 2017 and 2018, as well as the local hydrological and nutritional status. The Utermöhl method was used to analyze the relevant ecological characteristics of phytoplankton in the surveyed sea area. In the three cruises investigated, we show universal relationships between phytoplankton and (1) vertical stratification, (2) N:P ratio (3) temperature and salinity. The potential influencing factors of physical and chemical parameters on phytoplankton abundance were analyzed by structural equation model (SEM), determining the vertical stratification index was the most important influence factor affecting phytoplankton abundance and indirectly on phytoplankton abundance by dissolved inorganic nitrogen (DIN) and Dissolved inorganic phosphorus (DIP). Vertical stratification determines the vertical distribution of the phytoplankton community structure in the WPO. The areas with strong vertical stratification (Group A and B) are more conducive to the growth of cyanobacteria, and the areas with weak vertical stratification (Group C and D) are more conducive to the bloom of diatoms and dinoflagellates.

Keywords: Vertical stratification; phytoplankton community; western Pacific Ocean; N:P ratio

1. Introduction

Phytoplankton provides more than half of global marine primary production, which can actively maintain the stability of the entire ecosystem (Sun, 2011). Phytoplankton are the most important ring of in the marine food chain (Qian et al., 2005), and changes in the phytoplankton community structure may lead to changes in the entire food network. Wide-distributed in various aquatic ecosystems, with small influence of human fishing activities, phytoplankton has become a good indicator of marine environment and climate change (Tang et al., 2017), and it is of great significance to study the phytoplankton community structure in marine ecology. The growth of phytoplankton is closely related to the concentration of nutrients, while in the stable upper water the heat chain controls the upward replenishment of the nutrients. Therefore, the effect of vertical stratification on phytoplankton is particularly important.
WPO is not only the typical oligotrophic ocean among the global ocean, but also the highest number of tropical storms and typhoons in the world. These marine changes will not only affect the local and marginal sea climate and ecological environment, but also have an important and far-reaching impact on the whole tropical Pacific and even global climate change (Hu et al., 2015). The annual average surface sea water temperature of the Western Pacific Warm Pool (WPWP) is not less than 28 °C, and the evaporative heat (Hu, 2012) through heated seawater, radiant heat and latent heat makes WPO generally 3-6 °C (Gordon et al., 1996) higher than the equatorial East Pacific water temperature, with a profound impact on global climate change, especially in China and Southeast Asia. In addition, the surface primary productivity is lower, which is a typical sea (Radenac, 2006) with high temperature, low salinity and malnutrition. Because of typhoon, upwellings and various kinds of physical mixing processes, vertical stratification of subtropical Pacific seawater (Emery et al., 1982). Subsurface chlorophyll maximum (SCM) usually occurs near or at the bottom of the euphotic layer of stable seawater during phytoplankton flowering (Yentsch, 1965). The distribution of SCM is closely related to the depth and intensity of the thermocline, and the mixing of solar radiation and wind-induced is the driver of regional consistency and latitudinal differences in the thermocline. Therefore, it is characterized by SCM regions by latitude. The WPO in this investigation belongs to the typical tropical tectonic sea (TTS) area. the TTS is representative of the equatorial seawater structure. The ternary input through disturbance and mixing through the thermal slope into the upper layer is maintaining the SCM (Herbland et al., 1979). The SCM in the tropical WPO is 80 m (Dandonneau, 1979).

Most ocean waters in the global oceans are oligotrophic. With global warming and increased stratification of seawater, these zones are expected to expand, leading to decreases in marine nutrient fluxes and primary productivity (Capotondi et al., 2012; Gruber, 2011; Falkowski et al., 2007). Eutrophic zones with intermittent or irregular nutrient pulses alter phytoplankton community structure and are ideal for studying changes in phytoplankton community structure dynamics (Siokou-Frangou et al., 2010; Lozier et al., 2011). Changes in seawater stratification and vertical mixing may affect phytoplankton species composition, abundance, size structure, spatial distribution, phenology, and productivity (Edwards et al., 2004; Behrenfeld et al., 2006; Daufresne et al., 2009). This affects the function and biogeochemistry of marine ecosystems (Beaugrand et al., 2009; Hoegh-Guldberg et al., 2010). Therefore, studying the ecological and physiological mechanisms that control changes in phytoplankton community structure within vertical gradients is essential to assess the response of marine systems to global climate change (Richardson et al., 2004).

At present, most studies on phytoplankton communities focus on the horizontal distribution at the regional scale, while the vertical stratification of phytoplankton communities has been less studied, and the factors affecting the vertical stratification of phytoplankton are still unclear. Since WPO is a typical oligotrophic zone with severe vertical stratification and seawater stratification has an important influence on the distribution of phytoplankton, it is necessary to study the vertical stratification of phytoplankton in this region. Here we investigate how phytoplankton abundance and community composition are related to vertical stratification along a latitudinal gradient in the western Pacific during 2016-2018. Comparison between different geographical regions with different vertical density distributions offers an unique opportunity to study how phytoplankton dynamics change as stratification develops.
2. Materials and methods

2.1. Study area and sampling

This study relies on the shared voyage of the WPO (0-20 °N, 120-130 °E) commissioned by the National Natural Science Foundation of China. Physical, biological, chemical and geological surveys were carried out on the RV “kexue” from September to November in 2016, 2017, 2018. The sampling stations in this study are shown in Figure 1. The sampling layers were 5, 25, 50, 75, 100, 150 and 200 m. Samples of phytoplankton from different water layers were placed in 1-L PE bottles, fixed with formaldehyde solution (3%) and stored in a cool place. Samples of nutrient from different layers were directly washed and packed in PE bottles soaked in hydrochloric acid solution with a volume ratio of 1:5 (HCl: H₂O). The collected samples were frozen and stored at -20 °C for laboratory nutrient analysis.

Figure 1. Stations in the WPO of three cruises. Stations in the 2016, 2017, 2018 cruise represented in red, yellow and green triangles, respectively, (the same is relevant for the 2017 and 2018 cruises represented in black dots)

2.2. Identification of Phytoplankton

In the laboratory, phytoplankton samples enumerated and identified by Utermöhl method under an inverted microscope (Motic AE 2000). The identification of phytoplankton refers to Jin et al. (1965), Isamu Y (1991) and Sun et al. (2002).

2.3. Nutrient Analysis

The AA3 (SEAL, German) was used for the analysis and determination nutrient. Soluble inorganic phosphorus (PO₄-P) was determined by the phosphomolybdenum blue method with the limit of detection of 0.02 µmol L⁻¹; dissolved silicate (SiO₂-Si) was determined by the silicon molybdenum blue method with the limit of detection of 0.02 µmol L⁻¹; nitrate (NO₃-N) was...
determined by the cadmium column method with the limit of detection of 0.01 µmol L\(^{-1}\); nitrite (NO\(_2\)-N) was determined by the naphthalene ethylenediamine method with the limit of detection of 0.01 µmol L\(^{-1}\) (Dai et al., 2008). Ammonia (NH\(_4\)-N) was determined by the sodium salicylate method with the limit of detection of 0.03 µmol L\(^{-1}\) (Guo et al., 2014; Pai et al., 2001). Nitrogen-to-phosphorous (N:P) ratio was calculated by dividing nitrogen concentration (NO\(_3\)+NO\(_2\)) by phosphate concentration.

2.4. Analysis and methods

A SBE911 CTD sensor and standard Sea-Bird Electronics methods were used to process recorded hydrological parameters. The depth of the mixed layer (ML) is calculated as

\[
(S, T) = (S_{\text{ref}}, T_{\text{ref}} - \Delta T)
\]

where \(S\) and \(T\) are the average salinity and temperature, respectively, and \(S_{\text{ref}}\) and \(T_{\text{ref}}\) are the temperature and salinity at 5 m, \(\Delta T\) is equal to 0.5 °C. We calculated the vertical stratification index (VSI) to indicate the degree of vertical stratification of the water column:

\[
\text{VSI} = \sum (\delta_\theta(m+1) - \delta_\theta(m))
\]

where \(\delta_\theta\) is the potential density anomaly, and \(m\) is the depth from 5 to 200 m.

We clustered all species based on Bray-Curtis similarity distance for three years, and the results showed four distinct regions using the Primer (version 6). Distance-based Redundancy analysis (db-RDA) and Principal Co-ordinates Analysis (PCoA) were performed using the R package vegan (version 2.5-7) (Jari Oksanen et al., 2020) to explain the relationship between the environmental parameters (temperature, salinity, depth, VSI, DIN, DIP and Dissolved silicate (DSi)) and phytoplankton community structure. The results were visualized using the R package ggplot2 (version 3.3.2). SEM was used to assess the relative direct and indirect impact of physical and chemical parameters on phytoplankton abundance. The chi-square test (\(\chi^2\)), comparative fit index (CFI), and goodness fit index (GFI) were used to assess the model fit.

3. Results

3.1. Inter-annual variability of phytoplankton communities

Ternary diagram of the species distribution is presented in Figure 2. Unlike seasonal changes, the interannual phytoplankton changes are relatively stable, and the sampling area and sampling time from 2016 to 2018 are basically the same. Most phytoplankton species show a relatively even distribution. Although some species have been recorded in different years. In 2016, 2017, and 2018, a total of 305, 339, and 269 species were identified belonging to 76, 83, and 70 genera, mostly dominated by Bacillariophyta, Pyrrhophyta, Cyanophyta, and Chrysophyta. Because some points overlap each other, more species are actually than shown in the graphical representation. However, in the 2018 cruise, the abundance of *Trichodesmium* at certain sites was relatively high. In contrast, other species did not show strong interannual changes.
Figure 2. Ternary Plot of species distribution in years. The red, blue and green dots respectively denote the species enriched in the corresponding subregions, while the grey dots represent the species with a relatively homogeneous distribution among subregions. The location of each dot was determined by the contribution of the three regions to the relative abundance. The number of dots indicates the species richness, and the size of a dot denotes the species’ relative abundance.

3.2. Phytoplankton community structure

Since there is little difference in inter-annual changes between species, we clustered all species based on Bray-Curtis similarity distance for three years, and the results showed four distinct regions (Figure 3). The cluster analysis divided the phytoplankton communities at the sampling sites for three years into four groups. Cyanobacteria (>90%) were the dominant species in Group A and Group B. The ratio of diatom to dinoflagellate in Group A (4.8) was higher than that in Group B (1.4). Cyanobacteria were the dominant (66%) phytoplankton at stations of Group C, while diatoms (18%) and dinoflagellates (14%) made up 32% of the population in this Group. Diatoms (43%) and dinoflagellates (49%) dominated stations in Group D, accounting for about 92% of the total phytoplankton. The proportion of chrysophyceae was low in all four groups (Table 1).

Principal Co-ordinates Analysis (PCoA) were performed to explain the relationship between the environmental parameters (temperature, salinity, depth, VSI, DIN, DIP and DSi) and phytoplankton community structure. PCoA analyzed the phytoplankton community structure of 4 groups (Figure 4). The horizontal and vertical axes explain 51.87% and 21.41% of the phytoplankton community structure, respectively.
Figure 3. Bray-Curtis similarity-based dendrogram showing averaged phytoplankton community composition and abundance for each station across the 3 cruises. For each station, community composition is indicated with bar plots, phytoplankton abundance is represented with black bars.

Table 1. The percentages (%) of diatoms, dinoflagellates, cyanobacteria and chrysophyceae in the four Groups respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>1.07</td>
<td>4.09</td>
<td>18.32</td>
<td>43.23</td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td>0.22</td>
<td>3.00</td>
<td>14.06</td>
<td>48.91</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>98.69</td>
<td>92.66</td>
<td>66.18</td>
<td>5.82</td>
</tr>
<tr>
<td>Chrysophyceae</td>
<td>0.01</td>
<td>0.24</td>
<td>1.45</td>
<td>2.04</td>
</tr>
</tbody>
</table>
3.3. Vertical distribution of phytoplankton cell abundance

The vertical distribution of phytoplankton can be represented by the Box-whisker diagram (Figure 5). The maximum cell abundance of phytoplankton appears on the surface. As the water depth increases vertically, the cell abundance of the four groups of phytoplankton gradually decreases. At depths ranging from 5 m to 75 m, phytoplankton cell abundance was highest in Group A, followed by higher cell abundance in Group B. At 5 m depth, phytoplankton cell abundance reached a maximum and the difference between the four groups was the greatest. The average cell abundance in Group A reached $2.3497 \times 10^3$ cells L$^{-1}$, while the average abundance in Group B was $2.41 \times 10^3$ cells L$^{-1}$, Group C was $1.94 \times 10^3$ cells L$^{-1}$, and Group D only has $0.44 \times 10^3$ cells L$^{-1}$. It can be seen that the difference in cell abundance between the four groups is obvious. Starting from 100 m, the difference in phytoplankton cell abundance between the four groups decreased. Up to 200 m, phytoplankton abundance was lower and differences in biomass among all groups were not significant.
3.4. Temperature, salinity and vertical stratification index

Temperature, salinity and the vertical stratification index (VSI) of these four groups are shown in Figure 6. Group A (average 29.8 °C) and B (average 29.6 °C) has high temperature, but the salinity of Group A (average 33.9) and B (average 33.8) was low. The temperature of Group C (average 28.9 °C) and D (average 28.9 °C) was low, but the salinity of Group C (average 34.2) and D (average 34.4) was high (Figure 6a). From the characteristics of salinity and temperature, the strong spatial variability of T-S is obvious. We also calculated the vertical stratification index of the four groups (Figure 6b). Compared with Group C (average 3.86) and D (average 3.54), the values of VSI in Groups A (average 4.69) and B (average 4.86) were markedly higher, and Group A has the highest VSI. There are obvious differences between the four groups, that is, the first two groups performed high stratification index.

The vertical stratification index is linearly fitted to temperature (Figure 7a) and salinity (Figure 7b). The fitting results show that the temperature is positively correlated with the vertical stratification index. The VSI of all groups was negatively correlated with salinity. It can be seen that the change of temperature and salinity are more pronounced in the vertical direction. In Group A and B with high stratification index, the changes in temperature and salinity within the group were moderate. However, in Group C and D with a small stratification index, the temperature and salinity changed greatly within the group.
3.5. Relationships between phytoplankton and environmental factors

The structure of the overall phytoplankton community from four groups was explored through redundancy analysis (Figure 8). It can be seen that the abundance of phytoplankton is related to VSI, nutrients, temperature and salinity. There were significant differences in phytoplankton community structure across groups, in particular Group A and Group B communities clearly differed from Group C and Group D communities. Cyanophyta were significantly more abundant at Group A, B than Group C, D (p < 0.001). RDA model showed that the phytoplankton community in Group A and Group B had higher DIP, VSI, DSi and DIN concentrations than the latter two groups. The abundance of diatoms and dinoflagellates in Group D was higher, and they performed more sensitive to salinity changes.
Figure 8. Redundancy analysis of the phytoplankton community. Ellipticals of different colors represent different groups. The RDA model interprets the total variance by 33.63%.

Table 2. Average (±standard deviations) values for nutrients (μmol/L), temperature (°C), salinity for each phytoplankton community group identified by the cluster analysis in the WPO.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>25.30±1.06</td>
<td>24.45±1.85</td>
<td>24.92±1.32</td>
<td>25.41±1.23</td>
</tr>
<tr>
<td>Salinity</td>
<td>34.45±0.14</td>
<td>34.40±0.07</td>
<td>34.56±0.16</td>
<td>34.68±0.20</td>
</tr>
<tr>
<td>DIP</td>
<td>0.28±0.07</td>
<td>0.18±0.13</td>
<td>0.16±0.13</td>
<td>0.13±0.10</td>
</tr>
<tr>
<td>DIN</td>
<td>4.49±1.76</td>
<td>5.43±2.71</td>
<td>2.62±1.89</td>
<td>1.80±1.08</td>
</tr>
<tr>
<td>DSi</td>
<td>2.93±1.05</td>
<td>4.13±2.15</td>
<td>1.90±1.47</td>
<td>1.44±0.95</td>
</tr>
</tbody>
</table>

3.6. Direct vs. indirect effects of environmental parameter on phytoplankton abundance

The causal relationships between measured phytoplankton abundance and relevant physical and chemical parameters were examined using SEM, using interactions between temperature, salinity, VSI, DIN and DIP (Figure 9), as theoretical and experimental data indicated the importance of these variables. The model results showed that temperature, DIP and DIN had a direct effect on phytoplankton abundance, with temperature having the largest direct effect on phytoplankton abundance (0.38), followed by DIN (0.28) and DIP (0.24). Temperature, salinity and VSI had indirect effects on phytoplankton abundance, with temperature and salinity having negative indirect effects on phytoplankton abundance (-0.17 and -0.30) and VSI having positive indirect effects (0.31) (Figure 9). From the results of the total effect, only salinity had a negative effect on phytoplankton abundance (-0.30), while both temperature and VSI had positive effects on phytoplankton abundance (0.20 and 0.312), with VSI having the largest total effect (Fig. 9). Although the direct effect of temperature on phytoplankton abundance was significant, it was partially offset by the indirect negative effect, while VSI had no direct effect on phytoplankton abundance, but its larger indirect effect resulted in its total effect still being the largest. Both DIN and DIP had positive effects on phytoplankton abundance, but the effect of DIN was greater, but since the vertical distribution of DIN and DIP had stronger variability, more specific analyses of DIN and DIP will be conducted later.
Figure 9. Structural Equation Model (SEM) analysis examining the effects of temperature, salinity, VSI, DIN and DIP on phytoplankton abundance. Solid black and red lines indicate significant positive and negative effects at p < 0.05, black and red dashed lines indicated insignificant effects. R² values associated with response variables indicate the proportion of variation explained by relationships with other variables. Values associated with arrows represent standardized path coefficients.

We analyzed the N:P ratios of the surface layer, SCM and 200 m. The N:P ratio in the surface layer (N:P>16) indicates phosphorus limitation, which is consistent with the SEM analysis (Figure 10). the trophic structure of the SCM layer changes, (N:P<16) indicates nitrogen limitation, and the depth continues to increase to the bottom of the poleward layer and stabilizes around (N:P=16), indicating that at the bottom of the poleward layer, as phytoplankton abundance decreased and interspecific competition decreases, the trophic ratio approaches the Redfield ratio.

Figure 10. Distribution of phytoplankton community in DIN and DIP. (a): 5 m, (b): SCM, (b): 200 m. The dashed line indicates Redfield ratio N:P= 16:1.

4. Discussion
4.1. Comparison with historical data
The Kuroshio and the WPWP are the key regions of air sea interaction in the WPO (Zhang et al., 1999). The Kuroshio has the characteristics of large flow, high speed, high temperature and high salinity. It was a strong western boundary warm current in the Pacific Ocean. Previous surveys have less about the phytoplankton community structure in this study area (Table 3). Previously, samples were collected by net capture, while the samples collected by trawling would reduce the phytoplankton abundance of small volume, thus underestimate the phytoplankton abundance in the investigated ocean. In this study, phytoplankton samples were collected from water samples, which better reflected the phytoplankton community structure and cell abundance. Sun et al. (1997) only studied diatoms, and Liu et al. (1997) only studied dinoflagellates. Chen et al. (2018a) and others proposed that the abundance of *Trichodesmium* was higher in the Kuroshio area due to the deeper nitrate thermocline and the lower nitrate concentration in the surface layer. It can be seen that the density of *Trichodesmium* in Kuroshio region was very high, which was consistent with the previous study. Previous studies mostly focused on the vertical trawl and the horizontal distribution of phytoplankton in the entire water column, while the effect of vertical stratification on phytoplankton has been ignored.

Table 3. Historical data of the phytoplankton community in the WPO.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sampling areas</th>
<th>Layer /m</th>
<th>Number of species</th>
<th>Sampling types</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018.10</td>
<td>2°-20°N, 120°-130°E</td>
<td>0-200</td>
<td>305</td>
<td>Water samples</td>
<td>This study</td>
</tr>
<tr>
<td>2017.10</td>
<td>2°-18°N, 126°-130°E</td>
<td>0-200</td>
<td>339</td>
<td>Water samples</td>
<td>This study</td>
</tr>
<tr>
<td>2017.08</td>
<td>10.3°-10.9°N, 139.8°-140.4°E</td>
<td>0-200</td>
<td>147</td>
<td>Water samples</td>
<td>Dai et al., 2020</td>
</tr>
<tr>
<td>2017.08</td>
<td>21°-42°N, 118°-156°E</td>
<td>0-200</td>
<td>235</td>
<td>Water and net samples</td>
<td>Lin et al., 2020</td>
</tr>
<tr>
<td>2017.05</td>
<td>21°-42°N, 118°-156°E</td>
<td>0-200</td>
<td>248</td>
<td>Water and net samples</td>
<td>Lin et al., 2020</td>
</tr>
<tr>
<td>2016.09</td>
<td>2°-21°N, 127°-130°E</td>
<td>0-200</td>
<td>269</td>
<td>Water samples</td>
<td>This study</td>
</tr>
<tr>
<td>2016.09</td>
<td>0°-20°N, 120°-130°E</td>
<td>0-200</td>
<td>243</td>
<td>Net samples</td>
<td>Chen et al., 2018b</td>
</tr>
<tr>
<td>2014.08</td>
<td>0°-21.5°N, 121°-135.5°E</td>
<td>0-300</td>
<td>199</td>
<td>Net samples</td>
<td>Chen et al., 2018a</td>
</tr>
<tr>
<td>1997.07</td>
<td>23°30'-29°30'N, 122°30'-130°30'E</td>
<td>0-200</td>
<td>227</td>
<td>Net samples</td>
<td>Sun et al., 1997</td>
</tr>
<tr>
<td>1997.07</td>
<td>23°30'-29°30'N, 122°30'-130°30'E</td>
<td>0-200</td>
<td>251</td>
<td>Net samples</td>
<td>Liu et al., 1997</td>
</tr>
</tbody>
</table>

4.2. Relationship between N:P ratio and vertical distribution of phytoplankton

Research on the factors that control the structure of the phytoplankton community has been carried out for decades, but the hypothesis of nutrient concentration limit and ratio has not been fully explained in terms of affecting the structure of the phytoplankton community (Gao et al., 2019). In the four groups we studied, surface seawater N:P>16 indicated that phosphorus was limited in surface seawater, which was associated with a high abundance of *Trichodesmium* (Figure 10). The relationship between *Trichodesmium* and nitrogen fixation has been demonstrated many years ago (Grosskopf et al., 2012; Luo et al., 2012; Zehr, 2011). The presence of slight nitrogen limitation in surface seawater in Group D was consistent with the low abundance of *Trichodesmium*, which was consistent with studies on the abundance of *Trichodesmium* in the region (Chen et al., 2019; Sohm et al., 2011). As the most oligotrophic ocean around the world (Hansell et al., 2000), nutrients have become an important factor that determined the distribution of phytoplankton in the WPO. Where nutrition was limited, diatoms and dinoflagellates were more susceptible, especially under...
phosphorus limitation (Egge, 1998), which corresponds to the high abundance of Group D diatoms and dinoflagellates. In this study, the vertical pattern of N:P ratios indicated differences in nutrient composition across the vertical gradient. The N:P ratio of the surface layer (N:P>16) indicated phosphorus limitation, the structure of nutrient in the SCM layer changes, and (N:P<16) indicated nitro-gen limitation, the depth continues to increase to the bottom of the euphotic layer and was stable near (N:P=16), indicated that in the bottom of the euphotic layer, with phytoplankton abundance decreased, interspecific competition reduced, and the nutrient ratio near the Redfield ratio. The differences in nutrients should take into account the vertical distribution pattern of phytoplankton abundance. Diatoms had higher phosphorus requirements than other phytoplankton groups, which may be reflected by the lower N:P ratio in diatoms than in other groups (Hillebrand et al., 2013).

4.3. Vertical stratification determined the vertical distribution of phytoplankton

With global climate change, the marine oligotrophic regions continue to expand, and seawater stratification is intensified, which is the main problem facing marine phytoplankton community structure. WPO is a typical oligotrophic area with severe stratification. We found that the interannual variation of phytoplankton in stable oligotrophy is not significant, and the intensity of vertical stratification can adjust different environmental resource constraints (nutrients, temperature, salinity), thus forming 4 contrast environments with varying degrees of limit the community structure of phytoplankton. Comparative analysis of the phytoplankton community composition of the four groups shows that the phytoplankton is mainly strongly affected by the vertical stratification, which corresponds to the previous research (Bouman et al., 2011; Hidalgo et al., 2014; Mojica et al., 2015). Vertical stratification limits the replenishment of nutrients in the deep layer, and aggravates the formation of the thermocline, which affects the N:P ratio, thereby restricting the vertical migration of phytoplankton, or affecting the physiology of heat-driven phytoplankton growth and mortality variety (Alex et al., 2020).

Previous studies on models and field experiments have shown that the species composition of phytoplankton communities is significantly affected by vertical turbulent mixing changes (Huisman et al., 2004). There is a strong coupling between the nutrient supply rate and the photosynthetic performance of phytoplankton (Bouman et al., 2006), and the phytoplankton biomass and primary production in eutrophic areas are high (Richardson et al., 2019). The vertical stratification directly limits the supply of nutrients. The vertical stratification index reflects the potential causes of vertical stratification on various physical and chemical processes (such as regulating the utilization of light and nutrients in the ocean), which in turn affects phytoplankton dynamics. According to our results, from the equator to the north, as the latitude increases, the VSI decreases, and the phytoplankton community structure changes from cyanobacteria to diatoms. Phytoplankton abundance was significantly different in the water layer above SCM, and the water layer below SCM tends to be stable, and the surface phytoplankton abundance was usually greater than that of the SCM layer, which was related to the surface layer high abundance Trichodesmium. From our results, the highly stratified region was more suitable for the growth of Trichodesmium, while the region with low vertical stratification seems to be more conducive to the survival of diatoms and dinoflagellates.

Due to their poor activity and high potential growth rate, diatoms can reproduce rapidly in the circulation and the water with high nutrient content. The weak vertical stratification of Group C and
D regions leads to the homogeneity of temperature, salinity, density and nutrients in the upper part of 200 m in the vertical direction. The frequency and abundance of dinoflagellates in Group C and D are higher, which is consistent with the environment where they are more inclined to vertical stratification and weaker (Perez et al., 2006). The vertical distribution of zooplankton has shown that, vertical stratification can hinder the migration of small zooplankton populations, and indicate different grazing pressures (Long et al., 2021; Mitra et al., 2005). Further research can consider the difference in predation pressure of different zooplankton predators on the composition of the phytoplankton community in different regions. Phytoplankton stratification may cause thin-layer algae blooms and other phenomena, which will not be discussed in this article, and the influence of phytoplankton stratification can be further studied in the future.

5. Conclusions
This study investigated the phytoplankton community structure in the WPO in the autumn of 2016, 2017, and 2018. The interannual changes of phytoplankton were not significant, and they were mainly composed of cyanobacteria, diatoms and dinoflagellates. WPO as a typical oligotrophic ocean, and due to the thermocline layer resulting in weak water exchange capacity, the upper layer of seawater stratification is serious. Our results show that phytoplankton exhibited strong variability in vertical distribution. The potential influencing factors of physical and chemical parameters on phytoplankton abundance were analyzed by SEM, determining the vertical stratification index was the most important influence factor affecting phytoplankton abundance and indirectly on phytoplankton abundance by DIN and DIP. The areas with strong vertical stratification (Group A and B) were more conducive to the growth of cyanobacteria, and the areas with weak vertical stratification (Group C and D) were more conducive to the bloom of diatoms and dinoflagellates.

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References


Beaugrand, G.: Decadal changes in climate and ecosystems in the North Atlantic Ocean and adjacent


Grosskopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M., Lavik, G., Schmitz, R.


