



1 Vertical stratification driven nutrient ratios to regulate phytoplankton 2 community structure in the oligotrophic western Pacific Ocean

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9
10 Abstract: Vertical stratification determined the variability of temperature and nutrient distribution
11 in upper seawater, thereby affecting the primary production of the ocean. Nutrients in the oligo-
12 trophic region vary in time and space, and thus phytoplankton vary in their vertical distribution.
13 However, the differences in the vertical distribution of phytoplankton have not been systematically
14 studied. This study investigated the spatial distribution pattern and diversity of phytoplankton
15 communities in the western Pacific Ocean (WPO) in the autumn of 2016, 2017 and 2018, as well as
16 the local hydrological and nutritional status. The Utermöhl method was used to analyze the relevant
17 ecological characteristics of phytoplankton in the surveyed sea area. In the three cruises investigated,
18 we show universal relationships between phytoplankton and (1) vertical stratification, (2) N:P ratio
19 (3) temperature and salinity. The potential influencing factors of physical and chemical parameters
20 on phytoplankton abundance were analyzed by structural equation model (SEM), determining the
21 vertical stratification index was the most important influence factor affecting phytoplankton
22 abundance and indirectly on phytoplankton abundance by dissolved inorganic nitrogen (DIN) and
23 Dissolved inorganic phosphorus (DIP). Vertical stratification determines the vertical distribution of
24 the phytoplankton community structure in the WPO. The areas with strong vertical stratification
25 (Group A and B) are more conducive to the growth of cyanobacteria, and the areas with weak
26 vertical stratification (Group C and D) are more conducive to the bloom of diatoms and
27 dinoflagellates.

28
29 Keywords: Vertical stratification; phytoplankton community; western Pacific Ocean; N:P ratio

30 31 1. Introduction

32 Phytoplankton provides more than half of global marine primary production, which can
33 actively maintain the stability of the entire ecosystem (Sun, 2011). Phytoplankton are the most
34 important ring of in the marine food chain (Qian et al., 2005), and changes in the phytoplankton
35 community structure may lead to changes in the entire food network. Wide-distributed in various
36 aquatic ecosystems, with small influence of human fishing activities, phytoplankton has become a
37 good indicator of marine environment and climate change (Tang et al., 2017), and it is of great
38 significance to study the phytoplankton community structure in marine ecology. The growth of
39 phytoplankton is closely related to the concentration of nutrients, while in the stable upper water the
40 heat chain controls the upward replenishment of the nutrients. Therefore, the effect of vertical
41 stratification on phytoplankton is particularly important.

42



1 WPO is not only the typical oligotrophic ocean among the global ocean, but also the highest
2 number of tropical storms and typhoons in the world. These Marine changes will not only affect the
3 local and marginal sea climate and ecological environment, but also have an important and far-
4 reaching impact on the whole tropical Pacific and even global climate change (Hu et al., 2015). The
5 annual average surface sea water temperature of the Western Pacific Warm Pool (WPWP) is not less
6 than 28 °C, and the evaporative heat (Hu, 2012) through heated seawater, radiant heat and latent
7 heat makes WPO generally 3-6 °C (Gordon et al., 1996) higher than the equatorial East Pacific water
8 temperature, with a profound impact on global climate change, especially in China and Southeast
9 Asia. In addition, the surface primary productivity is lower, which is a typical sea (Radenac, 2006)
10 with high temperature, low salinity and malnutrition. Because of typhoon, upwellings and various
11 kinds of physical mixing processes, vertical stratification of subtropical Pacific seawater (Emery et
12 al., 1982). Subsurface chlorophyll maximum (SCM) usually occurs near or at the bottom of the
13 euphotic layer of stable seawater during phytoplankton flowering (Yentsch, 1965). The distribution
14 of SCM is closely related to the depth and intensity of the thermocline, and the mixing of solar
15 radiation and wind-induced is the driver of regional consistency and latitudinal differences in the
16 thermocline. Therefore, it is characterized by SCM regions by latitude. The WPO in this
17 investigation belongs to the typical tropical tectonic sea (TTS) area. the TTS is representative of the
18 equatorial seawater structure. The ternary input through disturbance and mixing through the thermal
19 slope into the upper layer is maintaining the SCM (Herbland et al., 1979). The SCM in the tropical
20 WPO is 80 m (Dandonneau, 1979).

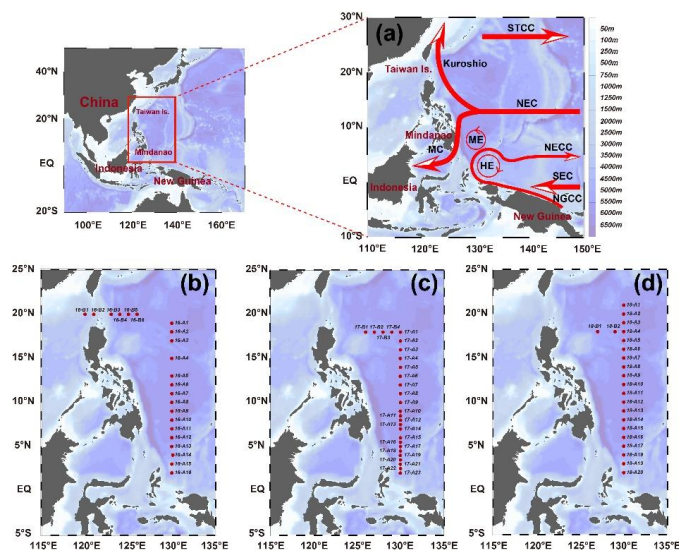
21
22 Most ocean waters in the global oceans are oligotrophic. With global warming and increased
23 stratification of seawater, these zones are expected to expand, leading to decreases in marine nutrient
24 fluxes and primary productivity (Capotondi et al., 2012; Gruber, 2011; Falkowski et al., 2007).
25 Eutrophic zones with intermittent or irregular nutrient pulses alter phytoplankton community
26 structure and are ideal for studying changes in phytoplankton community structure dynamics
27 (Siokou-Frangou et al., 2010; Lozier et al., 2011). Changes in seawater stratification and vertical
28 mixing may affect phytoplankton species composition, abundance, size structure, spatial
29 distribution, phenology, and productivity (Edwards et al., 2004; Behrenfeld et al., 2006; Daufresne
30 et al., 2009). This affects the function and biogeochemistry of marine ecosystems (Beaugrand et al.,
31 2009; Hoegh-Guldberg et al., 2010). Therefore, studying the ecological and physiological
32 mechanisms that control changes in phytoplankton community structure within vertical gradients is
33 essential to assess the response of marine systems to global climate change (Richardson et al., 2004).

34
35 At present, most studies on phytoplankton communities focus on the horizontal distribution at
36 the regional scale, while the vertical stratification of phytoplankton communities has been less
37 studied, and the factors affecting the vertical stratification of phytoplankton are still unclear. Since
38 WPO is a typical oligotrophic zone with severe vertical stratification and seawater stratification has
39 an important influence on the distribution of phytoplankton, it is necessary to study the vertical
40 stratification of phytoplankton in this region. Here we investigate how phytoplankton abundance
41 and community composition are related to vertical stratification along a latitudinal gradient in the
42 western Pacific during 2016-2018. Comparison between different geographical regions with
43 different vertical density distributions offers an unique opportunity to study how phytoplankton
44 dynamics change as stratification develops.



1
2 2. Materials and methods
3 2.1. Study area and sampling
4

5 This study relies on the shared voyage of the WPO (0-20 °N, 120-130 °E) commissioned by
6 the National Natural Science Foundation of China. Physical, biological, chemical and geological
7 surveys were carried out on the RV “*kexue*” from September to November in 2016, 2017, 2018. The
8 sampling stations in this study are shown in Figure 1. The sampling layers were 5, 25, 50, 75, 100,
9 150 and 200 m. Samples of phytoplankton from different water layers were placed in 1-L PE bottles,
10 fixed with formaldehyde solution (3%) and stored in a cool place. Samples of nutrient from different
11 layers were directly washed and packed in PE bottles soaked in hydrochloric acid solution with a
12 volume ratio of 1:5 (HCl: H₂O). The collected samples were frozen and stored at -20 °C for
13 laboratory nutrient analysis.



14
15 Figure 1. Stations in the WPO of three cruises. Stations in the 2016, 2017, 2018 cruise represented
16 in red, yellow and green triangles, respectively, (the same is relevant for the 2017 and 2018 cruises
17 represented in black dots)

18
19 2.2. Identification of Phytoplankton

20 In the laboratory, phytoplankton samples enumerated and identified by Utermöhl method under
21 an inverted microscope (Motic AE 2000). The identification of phytoplankton refers to Jin et al.
22 (1965), Isamu Y (1991) and Sun et al. (2002).

23
24 2.3. Nutrient Analysis

25 The AA3 (SEAL, German) was used for the analysis and determination nutrient. Soluble
26 inorganic phosphorus (PO₄-P) was determined by the phosphomolybdenum blue method with the
27 limit of detection of 0.02 μmol L⁻¹; dissolved silicate (SiO₃-Si) was determined by the silicon
28 molybdenum blue method with the limit of detection of 0.02 μmol L⁻¹; nitrate (NO₃-N) was



1 determined by the cadmium column method with the limit of detection of $0.01 \mu\text{mol L}^{-1}$; nitrite
2 ($\text{NO}_2\text{-N}$) was determined by the naphthalene ethylenediamine method with the limit of detection of
3 $0.01 \mu\text{mol L}^{-1}$ (Dai et al., 2008). Ammonia ($\text{NH}_4\text{-N}$) was determined by the sodium salicylate
4 method with the limit of detection of $0.03 \mu\text{mol L}^{-1}$ (Guo et al., 2014; Pai et al., 2001). Nitrogen-to-
5 phosphorous (N:P) ratio was calculated by dividing nitrogen concentration ($\text{NO}_3^- + \text{NO}_2^-$) by
6 phosphate concentration.

7 8 2.4. Analysis and methods

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

$$11 \quad (S, T) = (S_{\text{ref}}, T_{\text{ref}} - \Delta T)$$

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

$$15 \quad \text{VSI} = \sum [\delta_\theta(m+1) - \delta_\theta(m)]$$

16 where δ_θ is the potential density anomaly, and m is the depth from 5 to 200 m.

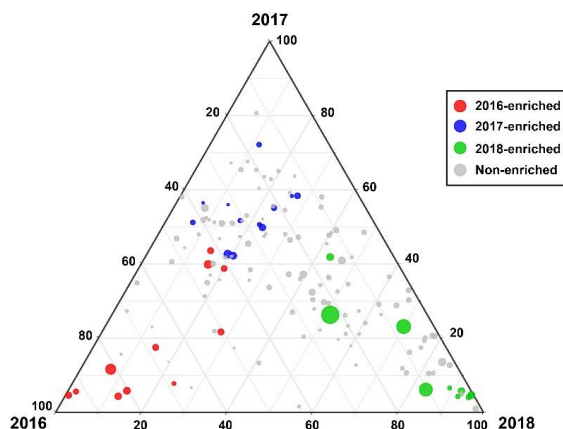
17
18 We clustered all species based on Bray-Curtis similarity distance for three years, and the results
19 showed four distinct regions using the Primer (version 6). Distance-based Redundancy analysis (db-
20 RDA) and Principal Co-ordinates Analysis (PCoA) were performed using the R package vegan
21 (version 2.5-7) (Jari Oksanen et al., 2020) to explain the relationship between the environmental
22 parameters (temperature, salinity, depth, VSI, DIN, DIP and Dissolved silicate (DSi)) and
23 phytoplankton community structure. The results were visualized using the R package ggplot2
24 (version 3.3.2). SEM was used to assess the relative direct and indirect impact of physical and
25 chemical parameters on phytoplankton abundance. The chi-square test (χ^2), comparative fit index
26 (CFI), and goodness fit index (GFI) were used to assess the model fit.

27 28 3. Results

29 3.1. Inter-annual variability of phytoplankton communities

30 Ternary diagram of the species distribution is presented in Figure 2. Unlike seasonal changes,
31 the interannual phytoplankton changes are relatively stable, and the sampling area and sampling
32 time from 2016 to 2018 are basically the same. Most phytoplankton species show a relatively even
33 distribution. Although some species have been recorded in different years. In 2016, 2017, and 2018,
34 a total of 305, 339, and 269 species were identified belonging to 76, 83, and 70 genera, mostly
35 dominated by Bacillariophyta, Pyrrophyta, Cyanophyta, and Chrysophyta. Because some points
36 overlap each other, more species are actually than shown in the graphical representation. However,
37 in the 2018 cruise, the abundance of *Trichodesmium* at certain sites was relatively high. In contrast,
38 other species did not show strong interannual changes.

39



1

2 Figure 2. Ternary Plot of species distribution in years. The red, blue and green dots respectively
3 denote the species enriched in the corresponding subregions, while the grey dots represent the
4 species with a relatively homogeneous distribution among subregions. The location of each dot was
5 determined by the contribution of the three regions to the relative abundance. The number of dots
6 indicates the species richness, and the size of a dot denotes the species' relative abundance.

7

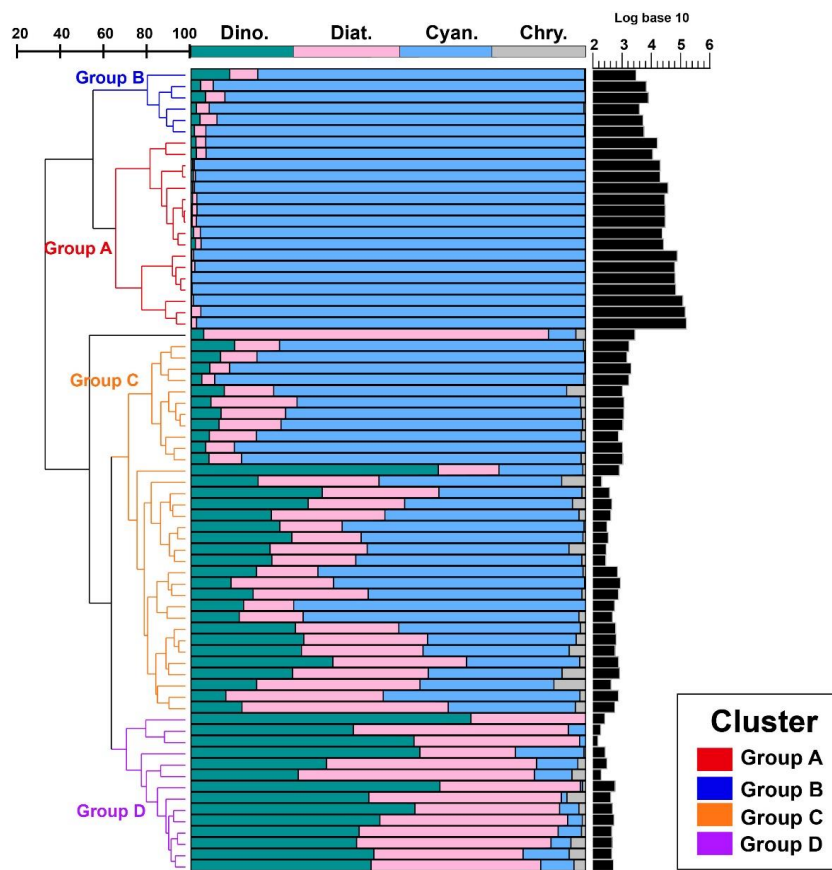
8 3.2. Phytoplankton community structure

9 Since there is little difference in inter-annual changes between species, we clustered all species
10 based on Bray-Curtis similarity distance for three years, and the results showed four distinct regions
11 (Figure 3). The cluster analysis divided the phytoplankton communities at the sampling sites for
12 three years into four groups. Cyanobacteria (>90%) were the dominant species in Group A and
13 Group B. The ratio of diatom to dinoflagellate in Group A (4.8) was higher than that in Group B
14 (1.4). Cyanobacteria were the dominant (66%) phytoplankton at stations of Group C, while diatoms
15 (18%) and dinoflagellates (14%) made up 32% of the population in this Group. Diatoms (43%) and
16 dinoflagellates (49%) dominated stations in Group D, accounting for about 92% of the total
17 phytoplankton. The proportion of chrysophyceae was low in all four groups (Table 1).

18

19 Principal Co-ordinates Analysis (PCoA) were performed to explain the relationship between
20 the environmental parameters (temperature, salinity, depth, VSI, DIN, DIP and DSi) and
21 phytoplankton community structure. PCoA analyzed the phytoplankton community structure of 4
22 groups (Figure 4). The horizontal and vertical axes explain 51.87% and 21.41% of the
23 phytoplankton community structure, respectively.

24



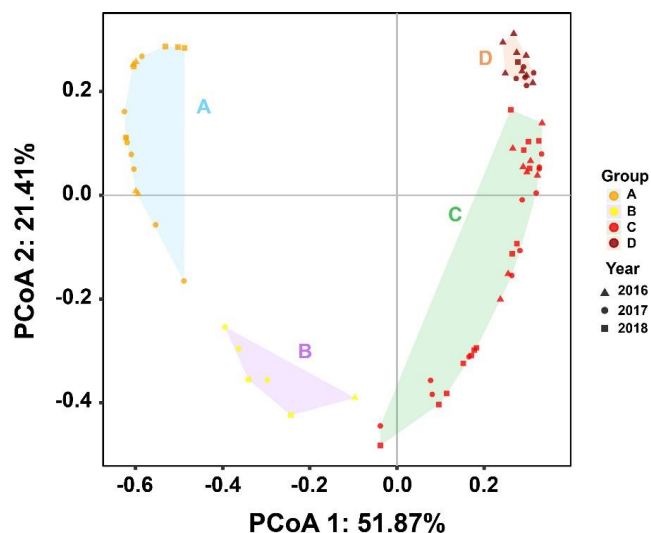
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2 Figure 3. Bray-Curtis similarity-based dendrogram showing averaged phytoplankton community
 3 composition and abundance for each station across the 3 cruises. For each station, community
 4 composition is indicated with bar plots, phytoplankton abundance is represented with black bars.
 5

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

Species	Group A	Group B	Group C	Group D
Diatoms	1.07	4.09	18.32	43.23
Dinoflagellates	0.22	3.00	14.06	48.91
Cyanobacteria	98.69	92.66	66.18	5.82
Chrysophyceae	0.01	0.24	1.45	2.04

8



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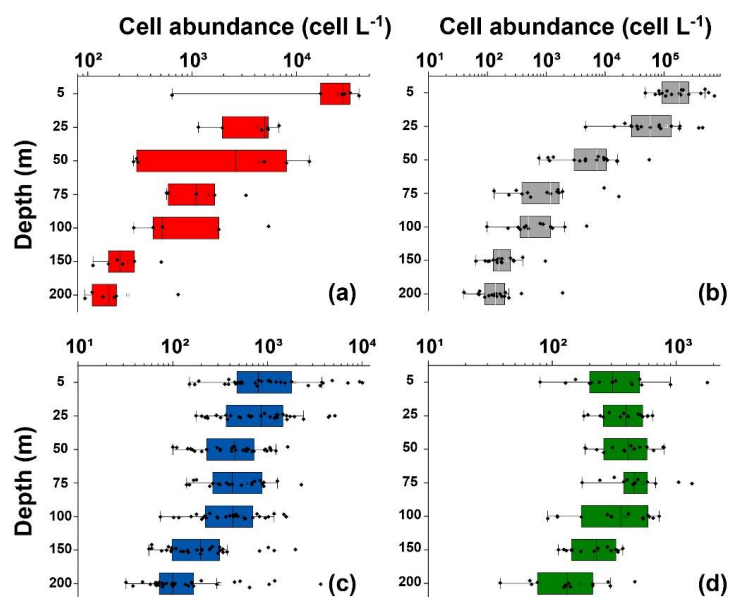
2 Figure 4. Principal Co-ordinates Analysis for groups. Triangles, circles and squares represent the
3 2016, 2017 and 2018 stations respectively. Different colors represent different groups.

4

5 3.3. Vertical distribution of phytoplankton cell abundance

6 The vertical distribution of phytoplankton can be represented by the Box-whisker diagram
7 (Figure 5). The maximum cell abundance of phytoplankton appears on the surface. As the water
8 depth increases vertically, the cell abundance of the four groups of phytoplankton gradually
9 decreases. At depths ranging from 5 m to 75 m, phytoplankton cell abundance was highest in Group
10 A, followed by higher cell abundance in Group B. At 5 m depth, phytoplankton cell abundance
11 reached a maximum and the difference between the four groups was the greatest. The average cell
12 abundance in Group A reached 234.97×10^3 cells L^{-1} , while the average abundance in Group B was
13 24.41×10^3 cells L^{-1} , Group C was 1.94×10^3 cells L^{-1} , and Group D only has 0.44×10^3 cells L^{-1} . It
14 can be seen that the difference in cell abundance between the four groups is obvious. Starting from
15 100 m, the difference in phytoplankton cell abundance between the four groups decreased. Up to
16 200 m, phytoplankton abundance was lower and differences in biomass among all groups were not
17 significant.

18

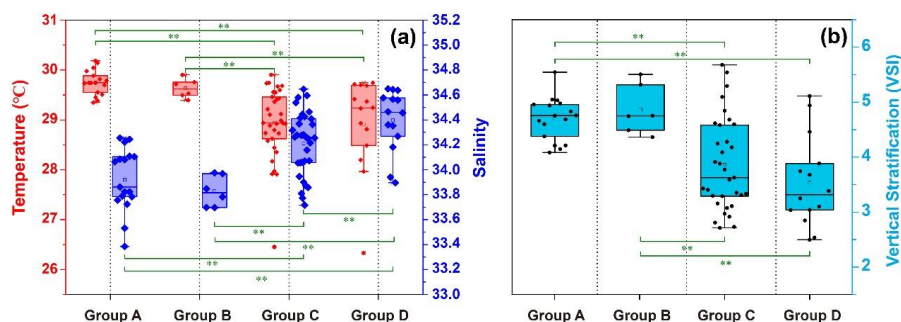


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2 Figure 5. Vertical distribution of phytoplankton cell abundance.

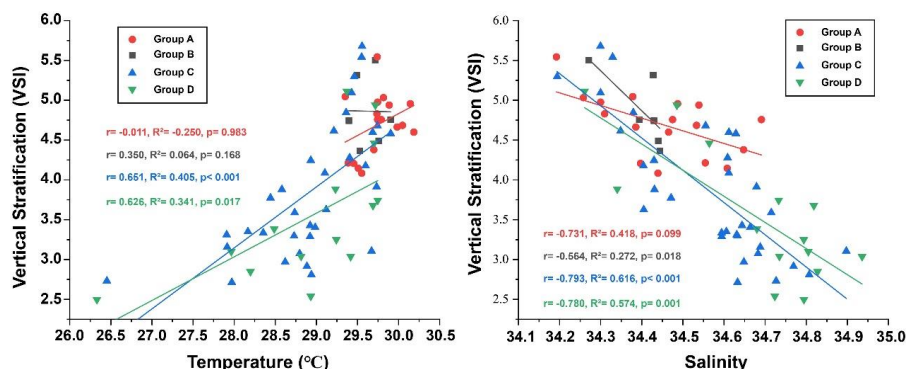
3
4 3.4. Temperature, salinity and vertical stratification index

5 Temperature, salinity and the vertical stratification index (VSI) of these four groups are shown
6 in Figure 6. Group A (average 29.8 °C) and B (average 29.6 °C) has high temperature, but the salinity
7 of Group A (average 33.9) and B (average 33.8) was low. The temperature of Group C (average
8 28.9 °C) and D (average 28.9 °C) was low, but the salinity of Group C (average 34.2) and D (average
9 34.4) was high (Figure 6a). From the characteristics of salinity and temperature, the strong spatial
10 variability of T-S is obvious. We also calculated the vertical stratification index of the four groups
11 (Figure 6b). Compared with Group C (average 3.86) and D (average 3.54), the values of VSI in
12 Groups A (average 4.69) and B (average 4.86) were markedly higher, and Group A has the highest
13 VSI. There are obvious differences between the four groups, that is, the first two groups performed
14 high stratification index.

15
16 The vertical stratification index is linearly fitted to temperature (Figure 7a) and salinity (Figure
17 7b). The fitting results show that the temperature is positively correlated with the vertical
18 stratification index. The VSI of all groups was negatively correlated with salinity. It can be seen that
19 the change of temperature and salinity are more pronounced in the vertical direction. In Group A
20 and B with high stratification index, the changes in temperature and salinity within the group were
21 moderate. However, in Group C and D with a small stratification index, the temperature and salinity
22 changed greatly within the group.



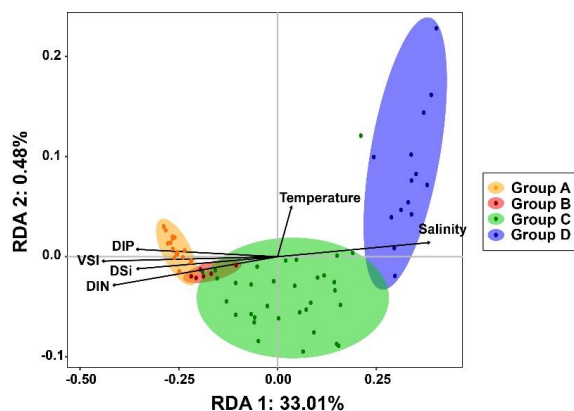
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 2 Figure 6. Temperature and salinity (a), vertical stratification index (b) of the four groups. ** indicate
 3 significance levels at $p < 0.05$.
 4



5
 6 Figure 7. Linear fitting of the vertical stratification index to temperature (a) and salinity (b) of the
 7 four groups.
 8

9 3.5. Relationships between phytoplankton and environmental factors

10 The structure of the overall phytoplankton community from four groups was explored through
 11 redundancy analysis (Figure 8). It can be seen that the abundance of phytoplankton is related to VSI,
 12 nutrients, temperature and salinity. There were significant differences in phytoplankton community
 13 structure across groups, in particular Group A and Group B communities clearly differed from
 14 Group C and Group D communities. Cyanophyta were significantly more abundant at Group A, B
 15 than Group C, D ($p < 0.001$). RDA model showed that the phytoplankton community in Group A
 16 and Group B had higher DIP, VSI, DSI and DIN concentrations than the latter two groups. The
 17 abundance of diatoms and dinoflagellates in Group D was higher, and they performed more sensitive
 18 to salinity changes.
 19



1

2 Figure 8. Redundancy analysis of the phytoplankton community. Ellipticals of different colors
 3 represent different groups. The RDA model interprets the total variance by 33.63%.

4

5 Table 2. Average (\pm standard deviations) values for nutrients ($\mu\text{mol/L}$), temperature ($^{\circ}\text{C}$), salinity for
 6 each phytoplankton community group identified by the cluster analysis in the WPO.

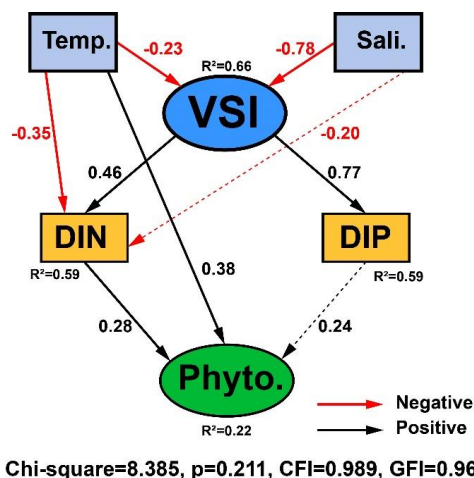
	Group A	Group B	Group C	Group D
Temperature	25.30 \pm 1.06	24.45 \pm 1.85	24.92 \pm 1.32	25.41 \pm 1.23
Salinity	34.45 \pm 0.14	34.40 \pm 0.07	34.56 \pm 0.16	34.68 \pm 0.20
DIP	0.28 \pm 0.07	0.18 \pm 0.13	0.16 \pm 0.13	0.13 \pm 0.10
DIN	4.49 \pm 1.76	5.43 \pm 2.71	2.62 \pm 1.89	1.80 \pm 1.08
DSi	2.93 \pm 1.05	4.13 \pm 2.15	1.90 \pm 1.47	1.44 \pm 0.95

7

8 3.6. Direct vs. indirect effects of environmental parameter on phytoplankton abundance

9

10 The causal relationships between measured phytoplankton abundance and relevant physical
 11 and chemical parameters were examined using SEM, using interactions between temperature,
 12 salinity, VSI, DIN and DIP (Figure 9), as theoretical and experimental data indicated the importance
 13 of these variables. The model results showed that temperature, DIP and DIN had a direct effect on
 14 phytoplankton abundance, with temperature having the largest direct effect on phytoplankton
 15 abundance (0.38), followed by DIN (0.28) and DIP (0.24). Temperature, salinity and VSI had
 16 indirect effects on phytoplankton abundance, with temperature and salinity having negative indirect
 17 effects on phytoplankton abundance (-0.17 and -0.30) and VSI having positive indirect effects (0.31)
 18 (Figure 9). From the results of the total effect, only salinity had a negative effect on phytoplankton
 19 abundance (-0.30), while both temperature and VSI had positive effects on phytoplankton
 20 abundance (0.20 and 0.312), with VSI having the largest total effect (Fig. 9). Although the direct
 21 effect of temperature on phytoplankton abundance was significant, it was partially offset by the
 22 indirect negative effect, while VSI had no direct effect on phytoplankton abundance, but its larger
 23 indirect effect resulted in its total effect still being the largest. Both DIN and DIP had positive effects
 24 on phytoplankton abundance, but the effect of DIN was greater, but since the vertical distribution
 25 of DIN and DIP had stronger variability, more specific analyses of DIN and DIP will be conducted
 later.

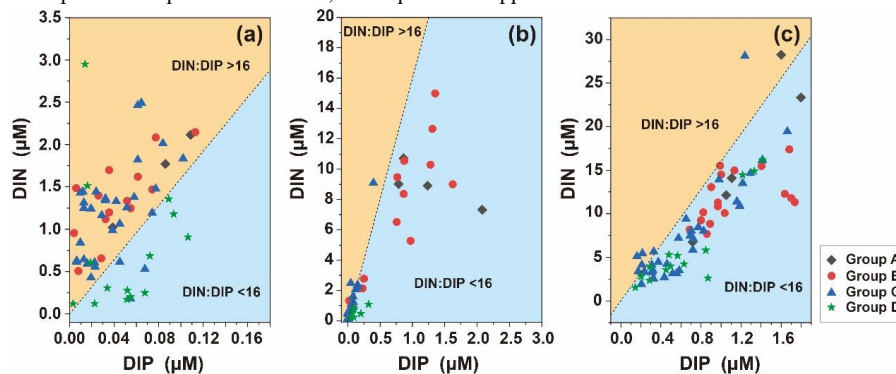


1

2 Figure 9. Structural Equation Model (SEM) analysis examining the effects of temperature, salinity,
 3 VSI, DIN and DIP on phytoplankton abundance. Solid black and red lines indicate significant
 4 positive and negative effects at $p < 0.05$, black and red dashed lines indicated insignificant effects.
 5 R^2 values associated with response variables indicate the proportion of variation explained by
 6 relationships with other variables. Values associated with arrows represent standardized path
 7 coefficients.

8

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



15

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

18

19 4. Discussion

20 4.1. Comparison with historical data



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

16

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

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

18

19 4.2. Relationship between N:P ratio and vertical distribution of phytoplankton

20 Research on the factors that control the structure of the phytoplankton community has been
21 carried out for decades, but the hypothesis of nutrient concentration limit and ratio has not been
22 fully explained in terms of affecting the structure of the phytoplankton community (Gao et al., 2019).
23 In the four groups we studied, surface seawater N:P>16 indicated that phosphorus was limited in
24 surface seawater, which was associated with a high abundance of *Trichodesmium* (Figure 10). The
25 relationship between *Trichodesmium* and nitrogen fixation has been demonstrated many years ago
26 (Grosskopf et al.,2012; Luo et al., 2012; Zehr, 2011). The presence of slight nitrogen limitation in
27 surface seawater in Group D was consistent with the low abundance of *Trichodesmium*, which was
28 consistent with studies on the abundance of *Trichodesmium* in the region (Chen et al., 2019; Sohm
29 et al., 2011). As the most oligotrophic ocean around the world (Hansell et al., 2000), nutrients have
30 become an important factor that determined the distribution of phytoplankton in the WPO. Where
31 nutrition was limited, diatoms and dinoflagellates were more susceptible, especially under



1 phosphorus limitation (Egge, 1998), which corresponds to the high abundance of Group D diatoms
2 and dinoflagellates. In this study, the vertical pattern of N:P ratios indicated differences in nutrient
3 composition across the vertical gradient. The N:P ratio of the surface layer (N:P>16) indicated
4 phosphorus limitation, the structure of nutrient in the SCM layer changes, and (N:P<16) indicated
5 nitro-gen limitation, the depth continues to increase to the bottom of the euphotic layer and was
6 stable near (N:P=16), indicated that in the bottom of the euphotic layer, with phytoplankton
7 abundance decreased, interspecific competition reduced, and the nutrient ratio near the Redfield
8 ratio. The differences in nutrients should take into account the vertical distribution pattern of
9 phytoplankton abundance. Diatoms had higher phosphorus requirements than other phytoplankton
10 groups, which may be reflected by the lower N:P ratio in diatoms than in other groups (Hillebrand
11 et al., 2013).

12

13 4.3. Vertical stratification determined the vertical distribution of phytoplankton

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

27

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



1 D regions leads to the homogeneity of temperature, salinity, density and nutrients in the upper part
2 of 200 m in the vertical direction. The frequency and abundance of dinoflagellates in Group C and
3 D are higher, which is consistent with the environment where they are more inclined to vertical
4 stratification and weaker (Perez et al., 2006). The vertical distribution of zooplankton has shown
5 that, vertical stratification can hinder the migration of small zooplankton populations, and indicate
6 different grazing pressures (Long et al., 2021; Mitra et al., 2005). Further research can consider the
7 difference in predation pressure of different zooplankton predators on the composition of the
8 phytoplankton community in different regions. Phytoplankton stratification may cause thin-layer
9 algae blooms and other phenomena, which will not be discussed in this article, and the influence of
10 phytoplankton stratification can be further studied in the future.

11

12 5. Conclusions

13 This study investigated the phytoplankton community structure in the WPO in the autumn of
14 2016, 2017, and 2018. The interannual changes of phytoplankton were not significant, and they
15 were mainly composed of cyanobacteria, diatoms and dinoflagellates. WPO as a typical oligotrophic
16 ocean, and due to the thermocline layer resulting in weak water exchange capacity, the upper layer
17 of seawater stratification is serious. Our results show that phytoplankton exhibited strong variability
18 in vertical distribution. The potential influencing factors of physical and chemical parameters on
19 phytoplankton abundance were analyzed by SEM, determining the vertical stratification index was
20 the most important influence factor affecting phytoplankton abundance and indirectly on
21 phytoplankton abundance by DIN and DIP. The areas with strong vertical stratification (Group A
22 and B) were more conducive to the growth of cyanobacteria, and the areas with weak vertical
23 stratification (Group C and D) were more conducive to the bloom of diatoms and dinoflagellates.

24

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