Multivariate analysis of phytoplankton community structure in Changli Gold Coast National Nature Reserve of Hebei Province in Spring, 2019

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Abstract. In order to research the characteristics of phytoplankton community structure in Changli Gold Coast National Nature Reserve, China, 14 survey stations were set up in this area in May 2019. The basic characteristics of phytoplankton community composition, cell abundance and community diversity were analyzed, and the Multi-Dimensional Scaling (MDS) was used to divide the structure of phytoplankton community. The results showed that a total of 30 species of phytoplankton were identified in this area, mainly diatoms, and the main ecological types were temperate inshore wide temperature and wide salt species. The average cell abundance of phytoplankton in May was 54.38×10⁴ cells/m³, and the main dominant species were Nitzschia sp., Skeletonema costatum and Noctiluca scientillans. The average value of Shannon-Wiener index was 2.26, The average value of evenness index was 0.75. The average of richness index was 0.73. The phytoplankton divided into 6 zones with significant differences by CLUSTER Analysis (P<0.001).

1. Introduction
Changli Gold Coast National Nature Reserve (hereinafter referred to as ‘Changli Nature Reserve’) is located in Changli County, Qinhuangdao City, northeast Hebei Province, China. The protected area is about 300 m² [1]. Due to the injection of rivers, the shallow sea area has low salinity, rich nutrition and appropriate water temperature, which has become an important habitat for the endemic species of amphioxus in the Bohai Sea [2]. At the same time, the region is also one of the first five national marine nature reserves approved by the State Council, mainly protecting the coastal natural landscape, the ecological environment and marine living resources.

Phytoplankton are highly diverse, microscopic, single-celled and rich organisms that form the basics of ecosystems of the marine food chain [3]. About half of the primary production is accomplished by phytoplankton [4]. It is also the material basis for the survival of marine life, maintaining the material cycle and energy flow of this ecosystem, it is also known as the ‘forest in the ocean’. The species diversity of phytoplankton and the dynamic change of community characteristics are important indexes to evaluate the nutrition level, pollution status, resource status, production potential and stability of marine ecosystem [5, 6]. The shift in species diversity of marine phytoplankton will change the primary productivity of the ocean, which in turn will affect the entire
Recent studies have also shown that changes in marine phytoplankton communities can reflect the change of environment. Understanding the driving factors for changes in phytoplankton species and structure remains an important goal of biological oceanography hereafter [8, 9]. Therefore, a comprehensive and in-depth study on the changes of phytoplankton community characteristics in Changli Gold Coast National Nature Reserve will have considerable application prospects and academic value for realizing the health assessment of the ecosystem, protecting the relative stability of the marine environment, maintaining the sufficient and stable fishery resources, and formulating environmental management strategies.

2. Materials and methods

2.1. Sample collection
Phytoplankton samples and seawater samples were collected at 14 stations in Changli Nature Reserve in May 2019, as shown in Figure 1. Collection, storage, transportation and pretreatment of phytoplankton in accordance with ‘The specification for Marine Monitoring’ (GB12763.6-2007) [10]. Sampling depth of sampling station is shown in Table 1. The samples of phytoplankton were collected by dragging from the bottom to the surface of the shallow water type III plankton network, and the samples were fixed and preserved with 5% formaldehyde. The samples of phytoplankton in the laboratory were placed in storage bottles and numbered. The samples were sent to the laboratory for concentration and treatment after 24 hours. Then the concentration counting method was used to count, and the 0.2ml was quickly taken from the phytoplankton counting frame with a sampling tube and covered with glass slides so that no bubbles were left. The samples were identified and the cells were counted under the inverted microscope (OLYMPUS CKX-53).

| Station | Depth (m) |
|---------|-----------|
| 1       | 6.0       |
| 2       | 9.5       |
| 3       | 10.0      |
| 4       | 11.5      |
| 5       | 14.5      |
| 6       | 11.5      |
| 7       | 12.5      |
| 8       | 14.0      |
| 9       | 12.0      |
| 10      | 13.7      |
| 11      | 12.5      |
| 12      | 13.0      |
| 13      | 16.0      |
| 14      | 16.0      |

2.2. Data analysis

2.2.1. Analysis of species diversity. The data of species diversity was calculated by Primer 6.0. Shannon-Weaver diversity index can reflect the diversity of community species in the investigated sea area [11], and then evaluate it according to ‘Specification for offshore environmental monitoring ’ (HJ 442-2008) (Table 2) [12, 13]. Richness index is an index that represents the richness of species in a community. The species richness index is more dependent on the number of species. The more species, the larger the index, it can better reflect the change of the number of species. In this paper, Margalef formula is used to express [14]. The evenness index is calculated by Pielou formula [15]. The J value
range from 0 to 1, the distribution of interspecific individual number is uniform when J value is large. On the contrary, the distribution of interspecific individual number is not uniform when J value is small. The dominant species were selected with Dominance index $Y \geq 0.02$ [15].

The formula is as follows:

1. Shannon-Wiener index ($H'$)

   $$\sum_{i=1}^{S} p_i \log_2 p_i$$

2. Pielou index ($J$)

   $$J = \frac{\log H'}{\log S}$$

3. Margalef index ($D$)

   $$D = \frac{S - 1}{\ln N}$$

4. Dominance index ($Y$), $Y \geq 0.02$ was used to determine the dominant species.

   $$Y = \frac{n_i}{N} \times f_i$$

In the formula, $n_i$ is the total number of individuals in species $i$; $N$ is the number of individuals of all species; and $f_i$ is the frequency at which species $i$ appears at each sampling point. $S$ is the total number of species and $P_i$ is the proportion of species $i$ in the sample.

The Cluster analysis of phytoplankton community was carried out by Primer 6.0. First of all, the abundance of phytoplankton in each station was transformed by log(x+1), and the similarity Cluster analysis of Bray-Curtis was established. Then, the spatial distribution of phytoplankton community was analyzed by MDS. Finally, Analysis of similarities (ANOSIM) was used to test the significance of differences among phytoplankton communities in May. Station map were drawn by Mapinfo 12.0 and Surfer11.0, and other charts were drawn with Origin 8.0 and Excel.

### Table 2. ‘Specification for offshore environmental monitoring ’ (HJ 442-2008).

| $H'$ | $H' \geq 3.0$ | $2.0 \leq H' \leq 3.0$ | $1.0 \leq H' \leq 2.0$ | $H' \leq 1.0$ |
|------|---------------|------------------------|------------------------|-------------|
| Habitat quality grade | good | general | poor | very pool |

3. **Results and discussion**

#### 3.1. Species composition of phytoplankton

Figure 2 showed a total of 30 species of three different algal classes were identified, of which 22 species were Bacillariophyta, accounting for 88.87% of the total species abundance, 7 species were Pyrrophyta, accounting for 10.36% of the total species abundance, and 1 species of Xanthophyceae, accounting for 0.77% of the total species abundance.

As shown in Figure 3, the species were phytoplankton collected from each station in the investigated area range from 5 to 17 species, with an average of 9 species, of which 17 species are the largest station 1, and 5 species were the smallest station 8, station 12 and station 14.

Hao Luo et al. carried out ecological investigation in the area for 15 years in summer from 1999 to 2013. The results showed that the dominance was diatom [16]. Peng Ru-yan Zhang did also the same study in the spring, 2015. Her survey found that Pyrrophyta were the most species, mainly including *Noctiluca scintillans*, accounting for 89.50% [17]. Studies have found that diatoms prefer low temperature environments. The most suitable temperature was usually lower than 18°C [18], while the dinoflagellate community was positively correlated with temperature [19]. It was worth noting that *Chattonella marine* appeared in May 2019. The *Chattonella marine* Xanthophyceae, it is a harmful algal blooms and one of the most harmful red tide species in the world. In recent years, the algal red tide has occurred frequently in the coastal areas of Japan, Canada, Australia and China, resulting in the
death of a large number of fish, shrimp and shellfish, and has caused serious harm to mariculture and marine ecosystems in coastal waters [20]. Although the frequency and density of this algae appearing in the protected area is very small, it should be highly concerned and should not be ignored.

**Figure 2.** Composition of phytoplankton phyla in Changli nature reserve, China.

**Figure 3.** Number of species every station in Changli nature reserve, China.

### 3.2. Abundance distribution of phytoplankton

It can be seen from Figure 4, the phytoplankton abundance distribution in each station of Changli nature reserve is very different in spring. The total abundance of phytoplankton collected from Changli Nature Reserve in spring 2019 was $534.51 \times 10^4$ cells/m$^3$, with an average abundance of $54.38 \times 10^4$ cells/m$^3$ for sampling site, the lowest abundance was at station 8, $1.06 \times 10^5$ cells/m$^3$, and the highest was at station 1, $418.83 \times 10^4$ cells/m$^3$. The ariatvion pattern of the phytoplankton cell abundance, in spring 2019, decreases off coast. The reason for the abundance of station 1 is that the density is about $218 \times 10^4$ cells/m$^3$, due to the reason of Skeletonema costatum.

**Figure 4.** Spatial variation of phytoplankton abundance in Changli nature reserve, China.
3.3. Composition of dominant species
The species and number of dominant species can reflect the change of diversity. When there are more dominant species of phytoplankton and there are no significant dominant species, it shows that the diversity of phytoplankton is higher. At this time, the structure of phytoplankton community is more complex and stable [21].

As shown in Table 3, there were three dominant species in spring 2019, which are Nitzschia sp., Skeletonema costatum and Noctiluca scientillans. The dominance of Nitzschia sp. was the highest, which was 0.15, and accounted for the largest proportion in station 1. The average number of cells of Skeletonema costatum was $16.46 \times 10^4$ cells/m$^3$, which appeared only at stations 1 and 2. The frequency of Noctiluca scientillans appeared the most, the abundance is the highest at station 9 and the lowest at station 14. Among them, Skeletonema costatum is a coastal diatom widely distributed along the coast of China. Although Skeletonema costatum is a species of red tide, it is not toxic [22, 23]. Skeletonema costatum is a typical, broad-salt coastal diatom that can survive in saltwater, brackish water and fresh water. It can grow when the water temperature is 0~37 °C and the salinity is 13-36, but the optimum range of colonization temperature and salt is 24–28 °C and 20–30 [24]. Secondly, nutrient elements are also the main factors affecting its growth. The reason for the high abundance of Skeletonema costatum at station 1 and 2 may be related to environmental factors. According to a new study recently published by Chinese and US researchers in the US Journal of Geophysical Communications, Noctiluca scintillans, a plankton known as ‘blue tears’, has been expanding along the coast of China in recent years. The number of outbreaks showed an increasing trend [25]. There are also a large number of reports of Noctiluca scientillans red tide in the four major sea areas of China. The red tide caused by Noctiluca scientillans occurs frequently in the southern and northern waters of China and in mariculture areas. Its outbreak frequency can reach about 50% of the total number of red tide [26-30]. According to the traditional view, Noctiluca scintillans itself is not toxic, and its main harm is that a large number of noctilucent algae seriously destroy the original marine ecological balance. It has a negative impact on the offshore aquaculture industry and coastal tourism industry, and then causes serious economic losses. However, some researchers have also revealed from the food chain level that after noctiluca preys on toxic finned algae and rhomboid algae, the toxic algae in their food bubbles will enter higher trophic organisms with the predation of Noctiluca scintillans [31]. Therefore, Noctiluca scintillans should be highly valued. According to previous studies, Skeletonema costatum and Noctiluca scientillans were common dominant species [16, 17], moreover, the density of plankton is $10^2$–$10^6$ cells/ml before the occurrence of red tide. This sea area is a national nature reserve, and there has never been red tide before. The habitat quality in this survey area is also good. Nevertheless, protecting Marine ecological environment should be done bit by bit.

| dominant species        | Average abundance $\times 10^4$ cells/m$^3$ | Occurrence frequency | Dominance degree |
|-------------------------|---------------------------------------------|----------------------|-----------------|
| Nitzschia sp.           | 11.16                                       | 0.50                 | 0.15            |
| Skeletonema costatum    | 16.46                                       | 0.14                 | 0.06            |
| Noctiluca scientillans  | 3.46                                        | 0.64                 | 0.06            |

3.4. Species diversity of phytoplankton
Biodiversity index, included species richness, diversity and evenness, the comprehensive analysis of biodiversity index can reasonably evaluate the diversity of marine plankton, which is closely related to community stability and had been widely used [32]. It is also a potentially useful tool for assessing water quality, and in general, the higher the index value, the better the water quality [33]. In most of the diversity indices, the more species that make up the community, the larger the diversity index value and the less reproducible. The stability of the community with large diversity index is also large.
In spring 2019, Figure 5 showed the average Shannon-Wiener index \((H')\) was 2.26, the fluctuation range of the stations was from 1.34 to 2.92, the minimum value was at station 8, and the maximum value was at station 2. The diversity index of the southern sea area was higher than that in the northern sea area. The reason for the lower index of the station 8 is that the number of species is low. According to the biodiversity evaluation index in ‘Specification for offshore environmental monitoring’ (HJ 442-2008), the habitat quality was general. The average evenness index \((J)\) was 0.75, the community was uniform, the value fluctuated from 0.39 to 0.98, the minimum value still appeared at station 9, and the maximum value appeared at station 14, and the change characteristics were similar to that of Shannon-Wiener index. The reason for the small number of stations is because there are fewer species, and some dominant species have abundance, which limits the growth of other algae and causes uneven distribution between species. The richness index \((D)\) has also obvious spatial difference, the average was 0.73, the range of variation was 0.41 ~ 1.42, the minimum value was at station 12, and the maximum value was at station 2. The richness index of the west inshore sea area was higher than that in the east.

![Figure 5](image_url)

**Figure 5.** The spatial variation trend of index in Changli nature reserve, China.

### 3.5. CLUSTER Analysis

Based on the data of species and abundance of phytoplankton every station, the spatial distribution characteristics of phytoplankton community structure in Changli Gold Coast National Nature Reserve were discussed by CLUSTER Analysis and MDS analysis. The results of cluster analysis showed that the similarity of community structure every station in the survey area was low (Figure 6). In spring, according to the similarity coefficient of 20% of Bray-Curtis similarity coefficient, the community structure was divided into 6 groups, and the phytoplankton in the reserve could be divided into 6 groups. The single factor similarity analysis showed that there were obvious spatial differences in community composition. The single factor similarity analysis of (ANOSIM) showed that there were significant spatial differences in community composition \([34, 35, 36]\). The pressure coefficient of two-dimensional MDS was 0.15, less than 0.2, indicating that the MDS diagram can correctly explain the similar relationship between stations \([37]\). Among them, group 1 includes station 1 in inshore waters, *Nitzschia* sp. and *Skeletonema costatum* were dominant. The number and abundance of species were the largest in all stations. Group 2 included station 8 with less species, dinoflagellates were dominant. Group 3 included stations 6 and 12, less species and no significant difference in abundance of all species. Group 4 included station 3, 4 and 5, *Ditylum brightwellii* was dominant, belonging to temperate offshore plankton. Group 5 included station 10, 13 and 14, *Noctiluca scintillans* were dominant. Group 6 included station 2, 7, 9 and 11, *Noctiluca scintillans* and *Skeletonema costatum* and *Noctiluca scintillans* were dominant.
Figure 6. CLUSTER analysis and MDS analysis of phytoplankton samples from different stations.

4. Conclusions
The main results were as follows:
(1) A total of 3 phylum and 30 species of phytoplankton were identified, of which diatoms were the most, followed by dinoflagellates.
(2) The abundance of phytoplankton was $1.06 \times 10^4$ cells/m$^3$ – $418.83 \times 10^4$ cells/m$^3$, and the average abundance was $54.38 \times 10^4$ cells/m$^3$.
(3) There were three dominant species, which were *Nitzschia* sp., *Skeletonema costatum* and *Noctiluca scintillans*.
(4) The range of Shannon-Wiener Index was 1.34–2.92, the value of Pielou index index was 0.39–0.98, and the richness index was 0.41–1.42.
(5) The results of CLUSTER Analysis and MDS analysis showed that the community structure was divided into 6 groups, and the phytoplankton in the reserve could be divided into 6 groups. The single factor similarity analysis showed that there were obvious spatial differences in community composition. The results of single factor similarity analysis showed that there were obvious spatial differences in community composition.

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References
[1] Xiao L, Zhao Z, Zhang Y, Han X, Zhang J, Gao W 2015 phytoplankton community structure in an Ecology-monitoring area in changli in summer of 2003 *Fisheries Science* 34 89
[2] Mamun A A, Akhtar A, Hassan M, Forruq R M, Warren A 2019 An approach to analyzing environmental drivers of phytoplankton community patterns in coastal waters in the northern Bay of Bengal, Bangladesh Regional Studies in Marine Science 29 100642
[3] Min G, Gao J, Han X, Gao W 2017 Analysis on the Evolution of Ecological Environment in Ecology-monitoring Area of Changli in Recent Ten Years Transactions of Oceanology and Limnology 2017 93
[4] Anthony N B, Dagar L, Becek K, Onu O J 2019 Spatio-temporal dynamics of phytoplankton functional groups in the South China Sea and their relative contributions to marine primary production *Regional Studies in Marine Science* 29 100598
[5] Sun D, Huan Y, Wang S, Qiu Z, Ling Z, Mao Z, He Y 2019 Remote sensing of spatial and temporal patterns of phytoplankton assemblages in the Bohai Sea, Yellow Sea, and east China sea *Water Research* 157 119
[6] Wang Y, Xiang P, Kang J, Ye Y, Lin G, Yang Q Ling M 2017 Environmental controls on spatial variability of summer phytoplankton structure and biomass in the bering sea. *Journal of Sea Research* **131** 1

[7] Marañón E 2015 Cell size as a key determinant of phytoplankton metabolism and community structure. *Annual Review of Marine Science* **7** 241

[8] Marañón E, Pedro C, Mikel L, Remy D T 2015 Resource supply alone explains the variability of marine phytoplankton size structure. *Limnol. Oceanogr* **60** 1848

[9] General Administration of Quality Supervision Inspection and Quarantine of the People's Republic of China 2007 China National Standardization Administration: GB/T 12763.6–2007 specification for oceanographic survey–Part 6: Marine biological survey. Beijing: Standard Press of China.

[10] Shannon, C E, Weaver W 1949 The Mathematical Theory of Communication *University of Illinois Press Urbana IL 1949* 1

[11] General Administration of Quality Supervision Inspection and Quarantine of the People's Republic of China 2008 Specification for offshore environmental monitoring (HJ 442 - 2008) Beijing: Standard Press of China

[12] Xu Y, Liu X, Zhang Q 2009 Studies on species diversity of phytoplankton in the offshore marine areas of the Bohai Bay *Journal of Salt and Chemical Industry* **38** 11

[13] Margalef D R 1968 Perspectives in Ecologicai Theory *Gensesyst* **3** 36

[14] Pielou E C 1969 An Introduction to Mathematical Ecology *New York. Wiley-Inter Science 1969* 1

[15] Luo H, Feng Z, Jin Z, Sun F, Liu Y, Bao H, Duan X, Xu Y, Ma M 2015 Characteristics and variation trend of phytoplankton community in Changli Golden Beach National Ocean Natural Preserve *Journal of Dalian Ocean University* **30** 207

[16] Zhang P, Li D, Liu X, Zhao X, Liu Z 2016 Phytoplankton community composition in the surrounding waters of Changli, Hebei, China *Marine Science Bulletin* **18** 79

[17] Claudinéia A D S, Train S, Cleide R L Phytoplankton assemblages in a Brazilian subtropical cascading reservoir system *Hydrobiologia* **2005** 537

[18] Sun H, Liu X, Sun X, Wang Y, Liu D 2017 Temporal and spatial variations of phytoplankton community and environmental factors in Laizhou bay *Marine Environmental Science* **36** 662

[19] Liao Z, Wang C 2019 Interspecies competition between Chattonella marina and three typical marine diatoms *Marine Environmental Science* **38** 321

[20] Yelda A 2011 Large-scale patterns in summer surface water phytoplankton ( except picophytoplankton) in the Eastern Mediterranean *Estuarine, Coastal and Shelf Science* **91** 551

[21] Gu X, Li K, Pang K, Ma Y, Wang X 2017 Effects of pH on the growth and NH$_4$-N uptake of skeletonema costatum and nitzschia closterium *Marine Pollution Bulletin* **124** 946

[22] Wang Z, Zhao K, Liang L, Du R, Wang R, Liu L 2018 Effects of Temperature, Salinity and Silicate Density on Growth of Skeletonema costatum *Journal of Yantai University ( Natural Science and Engineering Edition)* **31** 275

[23] Zhao H, Liu Y, Song M, Song G, Ding G 2018 Influence of environmental factors on the growth of Skeletonema costatum in the Donggang red-tide-monitoring area of Liaoning *Environment and Development* **30** 25

[24] Jing Y 2019 Don't let the ocean flow back into ‘blue tears’ *China Ocean News*

[25] Tang D, Di B, Wei G 2006 Spatial, seasonal and species variations of harmful algal blooms in the South Yellow Sea and East China Sea *Hydrobiologia* **568** 245

[26] Xu Z 2009 The inter-annual variations in Noctiluca Scintillans abundance and eutrophication in changjiang estuary *Oceanologia Et Limnologia Sinica* **40** 793

[27] Zhou M, Zhu M, Zhang J 2001 Status of humful algal blooms and related research activities in China *Chinese Bulletin of Life Science* **13** 54

[28] Zhou Z, Ma Z, Xue K, Wang N, Guo H, Han D 2002 Study on the Red Tides Caused by
Noctiluca scintillans and Ceratium furca in Liaodong Wan Bay Fisheries Science 21 9

[29] Huang C, Qi Y, Huang Y, Lin X 1997 The population Ecology and Causative Mechanisms of Red Tide of Noctiluca Scintillans in Dapeng Bay, the South China Sea Oceanology and Limnology Sinica 28 245

[30] Qi S, Qin C, Huang S, Zhong C, Sun K 2018 Correlation between patch distribution of Noctiluca Scintillans algae and water environmental factors Journal of Tropical Biology 9 1

[31] Righetti D, Meike V, Nicolas G, Achilleas P, Niklaus E Z 2019 Global pattern of phytoplankton diversity driven by temperature and environmental variability Science advances 5 6253

[32] Mamun A A, Akhtar A, Mustafa K A H, Shafiq I M, Muslem U M, Didarul A M Xu H 2018 Seasonal pattern of zooplankton communities and their environmental response in subtropical maritime channels systems in the bay of bengal, Bangladesh Acta Ecologica Sinica 38 316

[33] Gao Y, Jiang Z, Zeng J, Chen Y, Shou L 2017 Phytoplankton community in relation to environment factors off the north branch of the Yangtze River Estuary Marine Science Bulletin 37 430

[34] Luo X, Shan Y, Yang J 2018 Diatribution of phytoplankton community and its relationship with water environmental in the Yellow River Estuary Periodical of Ocean University of China 48 16

[35] Zhu X, Xu H, Xu X, Ge Y, Chen P, Yang H 2017 Community composition of net-collected phytoplankton and its relation to environmental factors near Lianyungang sea area Journal of Applied Oceanography 36 386

[36] Liang M, Sun L, Jiang Q, Chen Z, Li D, Lu B 2019 Net-phytoplankton community structure characteristics and its relation with environmental factors in coastal waters of Caofeidian Journal of Applied Oceanography 38 252

[37] Sun X 2007 Preliminary Ecological Studies on Phytoplankton in Shacheng Harbour, East China Sea Ocean University of China