Annual succession of phytoplankton community in *Apostichopus japonicus* aquaculture cofferdams of two types of sea beds

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Phytoplankton is important to marine ecosystem and sensitive to habitat. As an important part of habitat, different types of sea beds exhibit different nutrient capacities, affecting nutrient distribution in beds and waters. Based on it, we hypothesized that different types of beds might affect the structure and succession of phytoplankton communities and conducted a one-year field investigation in *Apostichopus japonicus* aquaculture cofferdams of muddy and sandy beds. Through field observation and laboratory identification, we tested (1) environmental factors and nutrient concentrations; (2) phytoplankton species and population. It was found that there was no significant difference in phytoplankton species but great significant difference in phytoplankton population between the two cofferdams in the same months, while the number and species of dominant phytoplankton varied greatly in some months. The Shannon-Wiener diversity and Pielou's evenness indexes of the two cofferdams kept a certain gap. These findings augment extensive field-based research addressing the ecological status of sea beds and provide a basic reference for biotechnology maintaining healthy aquaculture.

Keywords: phytoplankton community; *Apostichopus japonicus;* sea beds; cofferdams.

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Introduction

Phytoplankton is an important primary productivity of ocean, important to material cycle and energy transfer of marine ecosystem and is sensitive to habitat [1, 2]. Studies have shown that phytoplankton communities in coast are unstable under the stress of environmental changes, such as nutrient loading and warmer sea temperatures [3-5].

The *Apostichopus japonicus* aquaculture cofferdams are located in the coast where *A. japonicus* naturally distributes and is separated from outer sea by artificial dams made of stones. The water is drained and replenished freely through the stone holes of the dam. They are widely distributed in Shandong and Liaodong Peninsula, China, producing high-quality *A. japonicus* [6].

According to their composition, the beds of cofferdams are mainly divided into two types: sandy beds (particle size 50 – 1,000 μm) and muddy beds (particle size 20 – 50 μm). At the beds of the cofferdams, besides nutrient sedimentations from land sources, there are also massive *A. japonicus*. So, comparing to the natural area, the nutrient concentrations in the beds of the culture cofferdams are high [6, 7].

Different types of beds exhibit different nutrient capacities and affect the distribution of nutrient in beds and overlying waters [8]. Therefore, the nutrient concentrations in water can be affected by beds, resulting influence on phytoplankton communities indirectly. At present, there are few studies on the relationship between different types of beds and the structure and succession of phytoplankton communities. However, previous studies have shown that different types of beds have impacts on benthic diatom communities [9-11]. Based on these, we hypothesized that different types of beds might show different deposition and release rates of nutrient, thus affecting the concentration of nutrient in water and showing influence on the structure and succession of phytoplankton communities.

Therefore, we conducted a one-year field investigation to study the seasonal structure and succession of phytoplankton communities in *A. japonicus* aquaculture cofferdams of different types of beds, aiming to interpret ecological status of sea beds and provide a basic reference for biotechnology maintaining healthy aquaculture.

Materials and Methods

Study area

Two *A. japonicus* culture cofferdams, both covering approximately 5,000 km^2, were constructed in conformation to natural terrain using stones, one cofferdam bed of sand (particle size 50 – 1,000 µm), the other bed of mud (particle size 20 – 50 µm). They were located on the Yellow Sea coast (Figure 1) and water was both drained and replenished through stone holes of the dam with a large water exchange capacity, generally more than 30% per day. The water levels were 2.5 – 3.5 m and stocking density was 30 – 40 kg *A. japonicus*/m^2.

Field experiment

(1) *Measurement of environmental factors and nutrient concentrations*

We selected 5 sampling sites in each cofferdam (Figure 1). The 10th of the first month of each quarter (2018), temperature, pH, salinity, dissolved oxygen, and light intensity were measured on the site 0.5 m beneath the surface at each sampling site using a YSI–6920M multi-parameter water quality monitor (YSI Incorporated, Yellow Springs, Ohio, USA). For each measurement described above, 3 replicates were recorded.

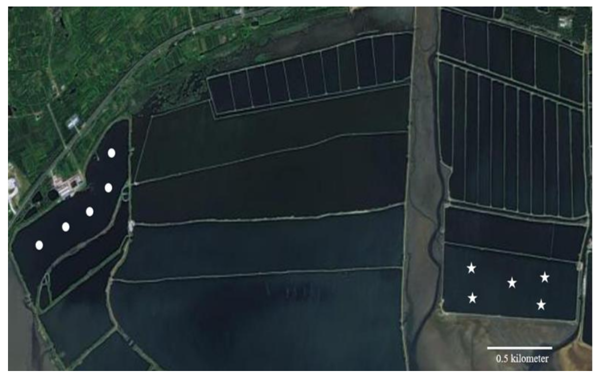

Figure 1. Map of the cofferdams and sampling sites (the left cofferdam is sandy, sample sites are depicted as circles; the right one is muddy, sample sites are depicted as stars).

Meanwhile, we collected water samples at the same place using sterilized polyethylene containers. The samples were refrigerated and transported to the laboratory within 4 h of collection. The concentrations of ammonium nitrogen (NH_4^+-N), total nitrogen (TN), orthophosphate phosphorous (PO_4^{3-}-P), total phosphorus (TP), and silicate silica (SiO_3^{2-}-Si) were determined following the manual "Specifications for Oceanographic Survey" [12].

(2) *Assessment of phytoplankton species composition and abundance*

Sampling sites and collection intervals were the same as mentioned above. Each time, we used a JC-800 water sampler (Juchuang Environmental Corporation, Ltd, Qingdao, Shandong, China) to collect 1 L water at 0.5 m beneath the surface for 3 replicates. Then they were evenly mixed, and 1 L water was reserved. The water samples were fixed immediately with 10 mL Lugol's solution (Sigma-Aldrich, Saint Louis, MO, USA) and transported to laboratory, siphoning off 30 mL of the supernatant after 24-48 hours on standing at room temperature [7].

Quantification of phytoplankton was performed using a Sedgwick rafter counting chamber with an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan) to determine the species of phytoplankton and to quantify each species occurrence [7]. Identification of phytoplankton species followed standard manual "Flora Algarum Marinarum sinicarum" [13].

To assess the phytoplankton community, we used the Margalef species richness index (dMa), the Shannon–Weaver species diversity index (H), and Pielou's species evenness index (J) [14]. The dominance index (Y) was calculated according to formula:

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

where i was the serial number of this species, n_i was the population of species i, N was the total population of all species, and f_i was the frequency of occurrence of species i. If $Y \geq 0.02$, the species was considered as dominant species.

Data analyses
Data were processed using the ANOVA module in the IBM SPSS 17.0 statistics software suite (IBM Corp., Armonk, New York, USA). All statistical analyses were carried out with the significance level set at $P < 0.05$.

Results

The changes of environmental factors year around
The variation of water temperature in two cofferdams was consistent with the seasons with the lowest in January and the highest in July. In the same months, there was no significant difference between two cofferdams ($P > 0.05$) (Table 1). The pH was little affected by seasons and remained stable at about 8. There was no significant difference between the two cofferdams in the same months ($P > 0.05$) (Table 1). Comparing to the muddy bed cofferdam, the sandy bed cofferdam showed a higher salinity in January and slightly lower salinity in other months with the biggest gap in July (Table 1). Dissolved oxygen was higher in October and January, but lower in April and July. Except in July, when the two cofferdams contained the same dissolved oxygen, dissolved oxygen in the muddy bed cofferdam was higher than that in the sandy bed cofferdam in the same months ($P < 0.05$), and the biggest gap occurred in October (Table 1). Light intensity varies seasonally with the lowest in January and the highest in July. In the same months, the light intensity of the muddy bed cofferdam was higher than that of the sandy bed cofferdam ($P < 0.05$), and the biggest gap occurred in April (Table 1).

The changes of nutrient concentrations year around
The concentrations of ammonia nitrogen were the highest in April and the lowest in July. In the same months, the concentrations of ammonia nitrogen in the muddy bed cofferdam were significantly higher than those in the sandy bed cofferdam ($P < 0.05$) (Table 2). Total nitrogen concentrations were the highest in October and the lowest in July. There was little difference between the two cofferdams in April, but in other months the concentrations of total nitrogen in the sandy bed cofferdam were significantly higher than those in the muddy bed cofferdam ($P < 0.05$) (Table 2). Orthophosphate concentrations were the highest in October and the lowest in April. There is little difference between January and July. The muddy bed cofferdam showed higher concentrations of phosphate in January and October and lower ones in April and July than those of the sandy bed cofferdam ($P < 0.05$) (Table 2). Total phosphorus concentrations were the highest in October and lowest in July. The muddy bed cofferdam showed higher concentrations of total phosphate in January and lower ones in the other months than those of the sandy bed cofferdam ($P < 0.05$) (Table 2). The silicate

Table 1. Seasonal environmental factors in the cofferdams (SD).

Month-area	Temperature (℃)	pH	Salinity	Dissolved oxygen (mg/L)	Light intensity (Lux)
Jan-M	5.8 (0.1)	8.11 (0.05)	30.7 (0.5)	8.42 (0.05)	13500 (250)
Jan-S	5.6 (0.1)	8.24 (0.05)	31.6 (0.5)	7.90 (0.05)	12800 (200)
Apr-M	13.4 (0.1)	8.06 (0.05)	30.9 (0.5)	6.34 (0.04)	23800 (250)
Apr-S	13.1 (0.1)	8.05 (0.05)	30.2 (0.5)	6.25 (0.03)	21500 (200)
Jul-M	22.5 (0.1)	8.02 (0.05)	30.5 (0.5)	7.03 (0.07)	37000 (400)
Jul-S	21.7 (0.1)	8.05 (0.05)	29.3 (0.5)	7.03 (0.06)	35900 (350)
Oct-M	19.2 (0.1)	8.01 (0.05)	30.9 (0.5)	8.17 (0.05)	26100 (350)
Oct-S	19.7 (0.1)	8.11 (0.05)	29.8 (0.5)	7.00 (0.07)	24500 (200)

M: Muddy beds. **S**: Sandy beds.

Table 2. Seasonal concentrations of ammonium nitrogen (NH_4^+-N), total nitrogen (TN), orthophosphate (PO_4^{3-}), total phosphorous (TP), and silicate (SiO_3^{2-}-Si) in the cofferdams (SD).

Month-area	NH_4^+-N (μg/L)	TN (μmol/L)	PO_4^{3-}-P (μg/L)	TP (μmol/L)	SiO_3^{2-}-Si (μmol/L)
Jan-M	54.5 (0.5)	25.25 (0.13)	22.1 (0.4)	0.84 (0.03)	33.13 (0.05)
Jan-S	43.8 (0.4)	35.83 (0.17)	21.6 (0.3)	0.74 (0.03)	33.44 (0.04)
Apr-M	76.3 (0.6)	17.53 (0.04)	6.25 (0.2)	1.04 (0.05)	20.09 (0.03)
Apr-S	69.5 (0.6)	17.14 (0.03)	8.13 (0.3)	1.28 (0.04)	21.70 (0.05)
Jul-M	32.5 (0.3)	11.53 (0.03)	20.6 (0.3)	0.45 (0.06)	9.42 (0.04)
Jul-S	26.7 (0.2)	14.66 (0.05)	22.5 (0.4)	0.52 (0.05)	9.25 (0.05)
Oct-M	37.9 (0.4)	49.46 (0.21)	50.1 (0.5)	1.48 (0.03)	29.46(0.07)
Oct-S	20.9 (0.1)	44.09 (0.22)	40.7 (0.6)	1.58 (0.04)	32.00 (0.09)

M: Muddy beds. **S**: Sandy beds.

concentrations were the highest in January and the lowest in July. There was little difference between the two cofferdams in the same months but no significant difference (Table 2).

Structure and succession of the phytoplankton community

We identified a total of 34 species from water samples including 28 species (13 genera) of *Bacillariophyta*, 2 species (1 genus) of *Chlorophyta*, 3 species (3 genera) of *Pyrrophyta*, and 1 species (1 genus) of *Chrysophyta* (Table 3). Phytoplankton assemblages were mostly composed of *Bacillariophytes* in the two cofferdams (Figure 2).

Species abundances of phytoplankton were the least in January, peaking the most in April and July, and decreased a bit in October. There was no significant difference in numbers of species between the two cofferdams in the same months (Figure 2). For *Bacillariophyta*, the variation of its species abundances reflected that of entire phytoplankton community. Species abundances of *Bacillariophyta* were the least in January and little changed in the other months. Similarly, species abundances of *Chlorophyta* remained stable except in January when the number of its species were lower. The species of *Pyrrophyta* were fewer in October and January and more in April and July. The *Chrysophyta* only appeared in July.

Table 3. Phytoplankton species in the *Apostichopus japonicus* aquaculture cofferdams.

Species	Species
Bacillariophyta	*Pleurosigma*
Navicula sp.	*Pleurosigma affine*
Melosira sulcata	*Dictyocha fibula*
Coscinodiscus sp.	*Dactylosen mediterrance*
Coscinodiscus.radiatus	*Ditylum brightwellii*
Coscinodiscus asteromphalus	*Diploneis*
Coscinodiscus lineatus	*Biddulphia sinensis*
Chaetoceros sp.	*Thalassiothrix frauenfeldii*
Chaetoceros brevis	*Rhizosolenia*
Chaetoceros lorenzianus	*Rhizosolenia alataf.Indica*
Chaetoceros muelleri	**Chlorophyta**
Chaetoceros lauderi	*Platymonas* sp.
Chaetoceros castracanei	*Platymonas subcordiformis*
Nitzschia sp.	**Pyrrophyta**
Nitzschia longissimi	*Prorocentrum micall*
Nitzschia longissimi var.reversa	*Ceratium furca*
Nitzschia paradoxa	*Noctiluca scintillans*
Nitzschia delicatissima	**Chrysophyta**
Nitzschia pungens	*Isochrysis* sp.

The population of phytoplankton was the lowest in January, then increased gradually, reached the highest in July, and decreased a bit in October ($P< 0.05$) (Figure 3). In January and April, phytoplankton populations in the muddy bed cofferdam were lower than those in the sandy bed cofferdam and reversed in July and October. The trend of *Bacillariophyta* population was similar to that of entire phytoplankton population.

In the case of *Chlorophyta*, its population showed little difference in the two cofferdams in January and April. In July and October, its populations in the sandy bed cofferdam were significantly higher than those in the muddy bed cofferdam ($P < 0.05$) (Figure 3). Especially in October, *Chlorophyta* population in the sandy bed cofferdam was nearly twice that in the muddy bed cofferdam.

The population of *Pyrrophyta* was the highest in April and the lowest in October, maintaining a medium level in January and July. Except in January, when *Pyrrophyta* population in the muddy bed cofferdam was more than twice that in the sandy bed cofferdam, the population of *Pyrrophyta* in the two cofferdams showed little difference (Figure 3). The *Chrysophyta* only appeared in July when its population in the sandy bed cofferdam was more than twice that in the muddy bed cofferdam (Figure 3).

The numbers of dominant species in two cofferdams were relatively stable in January and April, and there was no difference between the two cofferdams (Table 4). The dominant species decreased significantly in July, especially in the muddy bed cofferdam, and increased a little in October, but still lower than those in January and April (Table 4). In general, there was no significant difference in the numbers of dominant species between the two cofferdams in the same months except July when the gap was 3. The dominant species in the two cofferdams were quite different in January and July, and very similar in April and October. Except for *Ceratium furca*, which belonged in *Pyrrophyta* and was the 3rd dominant species in the muddy bed cofferdam in January, dominant species in the two cofferdams were all *Bacillariophytes*.

The changes of indexes of the phytoplankton community

Three indexes were applied in this study. Margalef index reflects the species richness of the phytoplankton community. Pielou's evenness index represents the degree of distribution for each species, a measure of species homogeneity. Shannon-Wiener diversity index reflects species diversity of a community based on species number. The tendency of the three indexes in the two cofferdams was similar. Taking the Shannon-Weaver diversity indexes as an example, the indexes were the lowest in January, then increased gradually reaching the peak in July, and then decreased a bit in October but still higher than those in January. The Shannon-Weaver species diversity index of the muddy bed

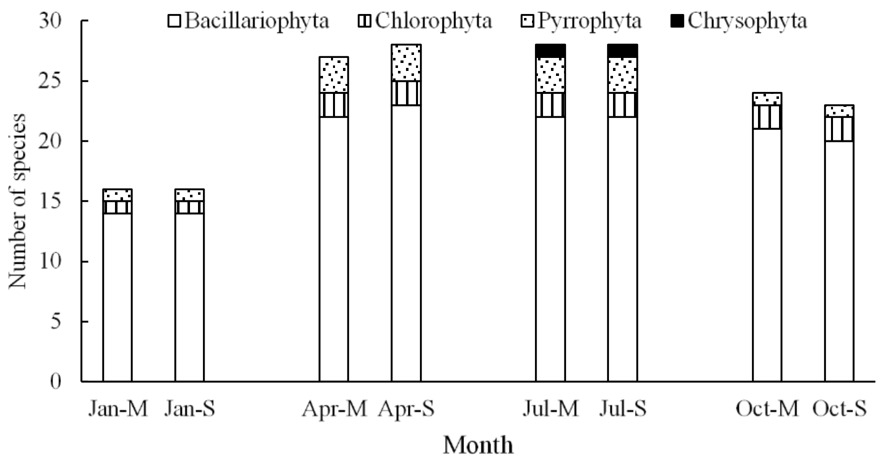

Figure 2. Number of species in 4 genera of phytoplankton found in the cofferdams. **M**: Muddy beds. **S**: Sandy beds.

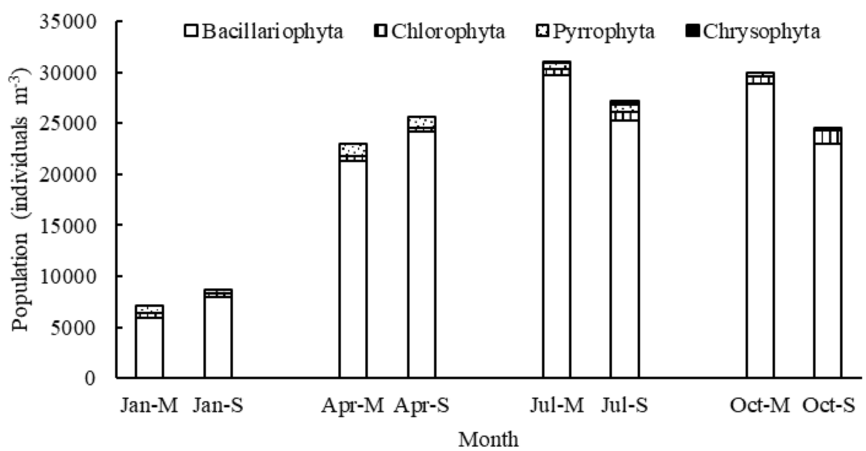

Figure 3. Phytoplankton populations in the cofferdams. **M**: Muddy beds. **S**: Sandy beds.

Table 4. The seasonally dominant species of phytoplankton in the cofferdams.

Month-area	number	first	second	third
Jan-M	16	*Chaetoceros brevis*	*Navicula sp.*	*Ceratium furca*
Jan-S	16	*Coscinodiscus sp.*	*Chaetoceros muelleri*	*Ditylum brightwellii*
Apr-M	15	*Nitzschia sp.*	*Coscinodiscus asteromphalus*	*Chaetoceros sp.*
Apr-S	16	*Nitzschia sp.*	*Thalassiothrix frauenfeldii*	*Chaetoceros sp.*
Jul-M	9	*Thalassiothrix frauenfeldii*	*Nitzschia sp.*	*Coscinodiscus asteromphalus*
Jul-S	12	*Coscinodiscus lineatus*	*Pleurosigma sp.*	*Coscinodiscus asteromphalus*
Oct-M	13	*Coscinodiscus sp.*	*Thalassiothrix frauenfeldii*	*Coscinodiscus lineatus*
Oct-S	15	*Coscinodiscus sp.*	*Thalassiothrix frauenfeldii*	*Coscinodiscus asteromphalus*

M: Muddy beds. **S**: Sandy beds.

cofferdam was higher than that of the sandy bed cofferdam in January, and lower than that of the sandy bed cofferdam in the following months. The gap between the Shannon-Weaver species diversity indexes of the two cofferdams in the same months almost remained at 0.2 (Figure 4). The variation of Pielou's evenness indexes of the two cofferdams was practically identical to that of the Shannon-Weaver diversity indexes, fluctuating in 0.72 - 0.84 (Figure 5). The Margalef richness indexes of the two cofferdams remained almost the same in the same months (Figure 6).

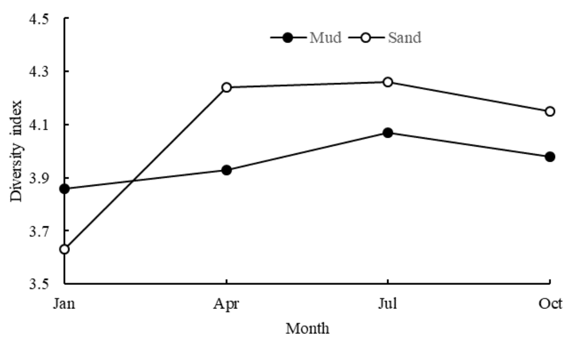

Figure 4. The Shannon–Weaver diversity indexes of phytoplankton in the cofferdams.

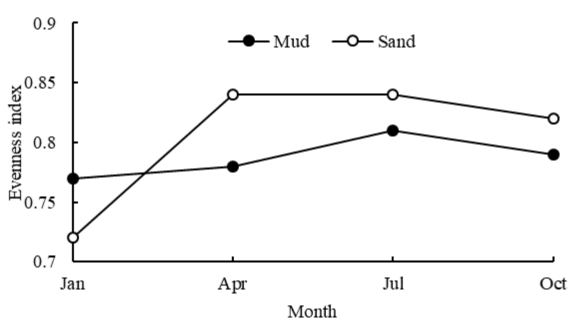

Figure 5. Pielou's evenness indexes of phytoplankton in the cofferdams.

Discussion

Phytoplankton is sensitive to environment such as climate change, water quality, and nutrient concentrations [1-3]. Phytoplankton communities in the two cofferdams changed significantly with the seasons. The species abundances, populations, and indexes of diversity, evenness and richness exhibited strong seasonal patterns showing minimal values in January, intermediate values in April and October, and maximal values in July. The structure of phytoplankton communities in the two cofferdams differed in the same months.

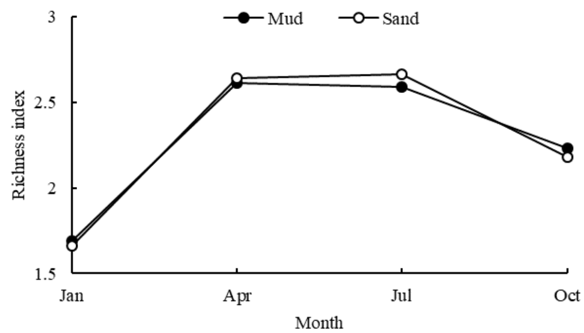

Figure 6. The Margalef richness indexes of phytoplankton in the cofferdams.

Our results showed that there were no significant differences in temperature, pH, and salinity, but significant differences in dissolved oxygen and light intensity between the two cofferdams, which indicated that different types of beds might show certain effects on environmental factors. Previous studies have shown that dissolved oxygen and light have effects on the structure and succession of phytoplankton [2, 4, 5]. Therefore, these differences might affect phytoplankton community.

Concentrations and structures of nutrient also have important impacts on phytoplankton community [2, 15]. Offshore, usually close to economically developed areas, are severely affected by land-based inputs from human activities, which deposited in beds through adsorption, complexation, sedimentation, and so on, making sea beds an important reservoir of nutrient. The behaviors of nutrient in beds releasing to overlying water are mainly powered by concentration difference and dynamic disturbance such as wind and wave [16]. At the beds of aquaculture cofferdams, besides common sources of nutrient, there are also massive excreta of *A. japonicus* causing elevated nutrient concentrations [17]. In addition, *A. japonicus*, a kind of benthic animal, contributing greatly to biological disturbance, also helped the nutrient releasing [6]. So, we inferred that nutrient concentrations in cofferdam water is more vulnerable to be influenced by bed types than natural sea.

Composed of inhomogeneous sandy and muddy particles, sea bed is a multi-stage dispersed granular system. The sizes, heterogeneity, loose degree, and stability of particles vary greatly. So, different beds have different saturation and composition of nitrogen and phosphorus. The smaller the particle size, the larger the specific surface area, the greater the ability of adsorbing nutrient. The larger the porosity of beds, the looser the texture, the easier to flow, the better the suspension, and the easier to circulate nutrient with overlying water [8, 18]. The components of beds played an important role in the adsorption of ammonia nitrogen [19]. The adsorption and desorption of phosphate increased exponentially with the decrease of particle sizes [20]. In addition, dissolved oxygen and temperature also affected the activities of enzymes related to nitrogen and phosphorus metabolism in beds [21].

Our results showed that, except for silicate, concentrations of ammonia nitrogen, total nitrogen, orthophosphate, and total phosphorus of the two cofferdams exhibited various differences in each month. In general, nutrient concentrations of the muddy bed cofferdam were higher than those of the sandy bed cofferdam, and the variation range of orthophosphate was the largest in different months. Daniel *et al*. found that the effect of bed's type on phosphorus concentration might be greater than that on nitrogen, which is similar to our results [22]. It could be inferred from these researches that different types of beds might affect the structure and concentrations of nutrient in water, possibly leading indirect influences on phytoplankton.

To date, little research about the effects of different types of beds on phytoplankton has been reported. However, studies have shown that different types of beds have important effects on the formation of benthic diatom community structure [23-25]. Xiang *et al*. investigated 23 sites of Hunhe River to analyze the effect of different types of ground substance on diatom community and their results indicated that the diversity of diatom would be higher when the sediment contained large boulders and pebbles, but lower for the sediment with silt and fine sand [11].

According to our results of field investigation, the structure and succession of phytoplankton communities in the two cofferdams of different types of beds exhibited difference. We will further verify the conclusion under controlled laboratory conditions. To understand the mechanism, we'll proceed to analyze the characteristics of nitrogen and phosphorus releasing from different types of beds and the effects of environmental factors such as temperature, light intensity, and dissolved oxygen on nitrogen and phosphorus releasing from different types of beds and the activities of related enzymes.

Conclusion

Our fieldwork results showed that there was no significant difference in phytoplankton species but great significant difference in phytoplankton population between the two cofferdams in the same months. The number and species of dominant phytoplankton varied greatly in some months. The diversity and evenness indexes of the two cofferdams kept a certain gap. There are similarities and differentiae of the structure and succession of phytoplankton communities between the two cofferdams of different types of beds.

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