Monitoring of plankton diversity in Dianchi Lake by environmental DNA technology

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Abstract. Surveys of zooplankter and phytoplankton through environmental DNA (eDNA) and metagenomic technology across Dianchi Lake and the estuary of inflowing rivers was conducted, to evaluate the microbial diversity of Dianchi Lake and the estuary of four inflowing rivers. Diversity of plankton and the similarity of dominant species results revealed that the dominant eukaryotic phytoplankton in Dianchi Lake was Bacillariophyta. The Shannon diversity index values of phytoplankton and zooplankton ranged from 3.68 to 18.36 and 0.54 to 2.49, respectively. The main genus taxa in the Dianchi Lake were similar with the inflowing river. The distribution map of Shannon diversity index gave overall insight into the variation of plankton diversity. The research proved that the eDNA technology was effective in biodiversity monitoring in aquatic ecosystem, and the inflowing river may impose an adverse influence on the ecosystem health of Dianchi Lake.

Keywords: Dianchi Lake; Environment DNA; Metagenomic technology; Plankton diversity.

1. Introduction

Lake is an important part of ecosystem which provided not only ecological resource but also social and economic supporter for the society [1]. As the accelerating of urbanization processes and industrialization, the threats that the lake ecosystems faced have contributed to long-lasting problems [2]. The decrease in taxonomic diversity resulted by habitat degradation was significant and predictable, and the loss of dominate taxa will cause irreversible adverse effect on the ecosystem functions [3]. The Dianchi Lake basin is the most densely populated and economically developed region in the Yunnan province in China, it located besides Kunyang phosphate rock belt which was the largest reserves in China. Dianchi Lake has been listed as one of the three most serious lakes in China since 1990s, the problems of the CBs, eutrophication, ecological degradation, the increase of the density and biomass of phytoplankton and the decrease of the abundance and biomass of benthic animals and aquatic plants can...
be traced back to 1980s. The pollution controlling of Dianchi Lake has attracted widespread attention, plenty of manpower, material and financial resources has been put in. The water quality has been largely improved, and the focus has shifted from the improvement of conventional water quality to the entire ecosystem function.

One of the main challenges in ecosystem protection and management was to identify the biodiversity and determine the affecting function of biodiversity changes caused by the environment pressure [4]. Plankton diversity was commonly used as bioindicators in lake and river ecosystems, and was a critical monitoring index in the management programs [5, 6]. Traditional plankton monitor used morphological characteristics as species identification parameter, which was time-consuming and laborious [7], the species discrimination accuracy was largely restricted by the qualifications of the researchers [8]. As the development of molecular techniques, advanced met genomics offers a rapid and accurate approach for diversity information of aquatic environments through detection of environmental DNA (eDNA) which is shed into the environment by plants and animals [9, 10].

Herein, we investigated the plankton biodiversity by adopting DNA met genomic examination technology. Through comparing with the results of water quality detection, the response of the relationship between biodiversity and environmental factors were analyzed.

2. Materials and Methods

2.1. Sampling and filed measurements

Water samples were taken at depth of surface (∼5 cm below surface) in 14 study sites of Dianchi Lake and 13 study sites of the estuary of Inflow Rivers (Table 1). Samples were filtered in situ through 0.2 μm Isopore membranes (Millipore, Billerica, MA) after collected and refrigerated, the filters were then stored at −80 °C.

Dissolved oxygen (DO), temperature, conductivity and pH were measured using a Portable multi-parameter water quality analyzer (DZB-712, LEICI) on the spot. Total nitrogen (TN), ammonium nitrogen (NH₄⁺-N), total nitrogen (TN), total phosphorus (TP), BOD and COD were measured in the laboratory [11].

### Table 1. Sampling site location

| NO. | longitude | latitude | Name            | type                           |
|-----|-----------|----------|----------------|--------------------------------|
| D1  | 102°45′37.00″ | 24°50′57.59″ | Wulong          | Center area of Dianchi Lake   |
| D2  | 102°44′10.53″ | 24°53′09.15″ | Wangguan        | Northern of Dianchi Lake      |
| D3  | 102°39′48.19″ | 24°56′31.28″ | Haigeng         | Northern of Dianchi Lake      |
| D4  | 102°41′47.40″ | 24°53′07.07″ | Fushan          | Northern of Dianchi Lake      |
| D5  | 102°41′50.28″ | 24°50′15.13″ | Xihua           | Northern of Dianchi Lake      |
| D6  | 102°40′40.41″ | 24°50′08.90″ | Guanyinshan     | Center area of Dianchi Lake   |
| D7  | 102°40′33.43″ | 24°47′58.04″ | Baiyukou        | Center area of Dianchi Lake   |
| D8  | 102°37′12.24″ | 24°45′59.76″ | Haikou          | Center area of Dianchi Lake   |
| D9  | 102°37′12.51″ | 24°42′55.90″ | Hushan          | Southern of Dianchi Lake      |
| D10 | 102°38′39.59″ | 24°41′31.67″ | Dongdahe        | Southern of Dianchi Lake      |
| D11 | 102°40′11.14″ | 24°42′41.73″ | Hebosuo         | Southern of Dianchi Lake      |
| D12 | 102°41′21.31″ | 24°45′13.66″ | Haiyan          | Southern of Dianchi Lake      |
| D13 | 102°42′56.48″ | 24°46′38.38″ | Shati           | Southern of Dianchi Lake      |
| R1  | 102°36′12.47″ | 24°44′09.44″ | Gucheng Lake    | Estuary of inflow rivers      |
| R2  | 102°36′08.22″ | 24°44′05.29″ | Gucheng Lake    | Estuary of inflow rivers      |
| R3  | 102°36′06.51″ | 24°44′04.86″ | Gucheng Lake    | Estuary of inflow rivers      |
| R4  | 102°36′04.26″ | 24°43′57.54″ | Gucheng Lake    | Estuary of inflow rivers      |
| R5  | 102°35′05.84″ | 24°42′32.26″ | Gucheng Lake    | Estuary of inflow rivers      |
| R6  | 102°35′07.75″ | 24°42′34.03″ | Gucheng Lake    | Estuary of inflow rivers      |
| R7  | 102°46′15.64″ | 24°49′31.72″ | Laoyu Lake      | Estuary of inflow rivers      |
| R8  | 102°47′15.10″ | 24°53′01.77″ | Laoyu Lake      | Estuary of inflow rivers      |
| R9  | 102°44′47.62″ | 24°54′45.24″ | Maliao Lake     | Estuary of inflow rivers      |
| R10 | 102°43′27.91″ | 24°55′53.67″ | Baoliao Lake    | Estuary of inflow rivers      |
| R11 | 102°41′51.50″ | 24°57′09.15″ | Daqing Lake     | Estuary of inflow rivers      |
| R12 | 102°41′49.91″ | 24°57′20.22″ | Laoyu Lake      | Estuary of inflow rivers      |
| R13 | 102°41′35.34″ | 24°57′24.06″ | Maliao Lake     | Estuary of inflow rivers      |
2.2. Molecular Analysis
DNA extraction was conducted by using the MO BIO PowerWater DNA Isolation Kit (QIGEN) following the manufacturer’s protocol with minor modifications [12]. 0.22 constant m filter membrane were cut into pieces and put into 5 mL PowerWaterR Bead centrifuge tube with 1 mL lysate preheated at 55 ℃, liquid mixtures were cracked for 10 min, and centrifuged to get the supernatant. Adding 220 μL ethanol, then, the extracted DNA were enriched by HiBindR DNA Mini filter cylinder. After cleaning twice by DNA wash buffer, the DNA concentration was detected by Qubit 2.0. Polymerase chain reaction (PCR) analyses using primers targeted the 3’ end partial sequences of 16S rDNA, 9’ end partial sequences of 18S rDNA, mitochondrial cytochrome oxidase subunit I (COI) and partial sequences of 12S rDNA [13,14]. The strips were detected by 1.5% agarose gel electrophoresis, different samples were mixed in equal quality of 50 ng to constructed the DNA library. Bio-linux 8 system was used to process the obtained gene sequences and remove the low-quality terminal sequences and primer sequences. BLAST comparison was conducted online at NCBI (national center for biotechnology information) genbank. The DNA fragments with very few sequences in the same sample were compared after BLAST was removed. The sample name, picture data and the species obtained after BLAST were compared, analyzed and saved.

2.3. Data Analysis and Diversity
Diversity of plankton and degree of dominance were determined by using Shannon diversity index [15]. Identification of the planktons was done following Smith (1950) [16], Desikachary (1959) [17], Kudo (1986) [18] and Pennak (1989) [19].

Shannon-Wiener index was calculated as follows:
\[
Y = \left( \frac{N_i}{N} \right) \times f_i \tag{1}
\]
\[
H = - \sum \left[ \left( \frac{N_i}{N} \right) \times \log_2 \left( \frac{N_i}{N} \right) \right] \tag{2}
\]

In the formula, \(N_i\) referred to the density of one plankton specie, \(N\) referred to the density of all the species of plankton, \(f_i\) is the frequency the specie occurs in observation point. When the value of \(Y\) is above 0.02, the specie or genus was identified as dominant.

Eutrophication evaluation and calculation of water quality parameters were analyzed according to Evaluation of environmental quality of surface water (Trial) [20], using comprehensive nutrition state index (\(TLI(\Sigma)\)) to evaluate eutrophication status of Dianchi Lake. Evaluation classification were as follows: \(TLI(\Sigma) < 30\) means poor nutrition; between 30 and 50 means less nutrition; between 50 and 60 means mild eutrophic; between 60 and 70 means moderate eutrophication; exceed 70 means severe eutrophication [21].

\[
TLI(\Sigma) = \sum_{j=1}^{m} W_j TLI(j) \tag{3}
\]

In the formula, \(TLI\) referred to the comprehensive nutritional status index, \(W_j\) referred to the relevant weight of \(j\) of the nutritional status index of the \(j\) parameter, \(TLI(j)\) represented the nutritional status index of the \(j\) parameter.

With \(Chl\text{-}a\) as the basic parameter, the normalized correlation weight calculation formula of the \(j\) parameter is:
\[
W_j = \frac{r_{ij}^2}{\sum_{j=1}^{M} r_{ij}^2} \tag{4}
\]

The \(r_{ij}\) is the correlation coefficient between the \(j\) parameter and the basic parameter Chlorophyll a (\(Chl\text{-}a\)), the \(r_{ij}\) value of \(Chl\text{-}a\), TP, TN, Transparency (SD) and Permanganate Index (COD\text{mn}) is 1, 0.84, 0.82, -0.83 and 0.83, respectively, and \(M\) is the number of evaluation parameters.

The nutritive state index of each parameter is calculated as follows:
\[
TLI(Chl\text{-}a) = 10(2.5 + 1.086 \ln Chl\text{-}a) \tag{5}
\]
\[
TLI(TP) = 10(9.436 + 1.624 \ln TP) \tag{6}
\]
\[
TLI(TN) = 10(5.453 + 1.694 \ln TN) \tag{7}
\]
\[
TLI(SD) = 10(5.118 - 1.94 \ln SD) \tag{8}
\]
\[
TLI(COD\text{mn}) = 10(0.109 + 2.661 \ln COD\text{mn}) \tag{9}
\]

The unit of each parameter in the calculation formula of nutritional status index was as follows: the unit of \(Chl\text{-}a\) is \(\mu g\cdot L^{-1}\), the unit of \(SD\) is \(M\), and the unit of other indicator unit is \(mg\cdot L^{-1}\).
The spatial distribution maps were plotted by software Surfer 12.2.705 (Golden software, Inc.) with Ordinary Kriging method.

3. Results and Discussion

3.1. Water quality

The index of water quality was analyzed, and the average value of each index in the sampling site was as follows: SD was 0.46 m, pH value was 9.03, DO was 12.95 mg·L⁻¹, TN was 1.22 mg·L⁻¹, total TP was 0.14 mg·L⁻¹, Chl-a was 0.11 μg·L⁻¹. The comprehensive nutritional status index TLI (Σ) was calculated to be 77.27, which indicated severe eutrophication of the water body.

3.2. Phytoplankton

Distribution characteristics of phytoplankton in different regions were analyzed. There were 74 genera covered by phytoplankton detected in Dianchi Lake, among which 69 genera were the most abundant in south Dianchi Lake, 54 genera in north Dianchi Lake and 57 genera in south Dianchi Lake. Compared with Dianchi Lake, the phytoplankton richness index in most of the estuary of the inflowing rivers was higher than in the middle of the lake, 93 genera of phytoplankton were detected in Gucheng Lake, and 73, 70 and 67 genera were detected in Laoyu River, Baoxiang River and Maliao River, respectively. The phytoplankton species in Daqing River were at the least with 54 genera.

The dominant eukaryotic phytoplankton in Dianchi Lake was Bacillatiophyta, followed by Chlorophyta, and the dominant genus taxa were Codonopsis and Cryptophyta. The dominant group in most of the estuary of inflowing rivers was green algae. Shannon diversity index values of phytoplankton for each sample point ranged from 3.68 to 18.36, the index values of different regions ranged in a descending order were: estuary of the inflowing river > southern part of Dianchi Lake > northern part of Dianchi Lake > center of Dianchi Lake (Table 2). The distribution of diversity index was visually represented in the Kriging map (Figure 2). The results indicated that different topographical and geographical environments and human disturbance could impact the phytoplankton community composition in the water body.

Table 2. The diversity index of phytoplankton in different sampling region

| Region                  | Mean | Minimum | Maximum | Std. | Variable coefficient |
|-------------------------|------|---------|---------|------|----------------------|
| Average                 | 9.54 | 3.68    | 18.36   | 4.04 | 0.42                 |
| Center                  | 5.41 | 3.68    | 7.64    | 1.47 | 0.27                 |
| Northern                | 6.88 | 5.84    | 7.40    | 0.74 | 0.11                 |
| Southern                | 9.18 | 6.93    | 11.14   | 1.68 | 0.18                 |
| Estuary of the inflow river| 12.09| 5.22    | 18.36   | 4.12 | 0.34                 |

Figure 1. Diversity distribution character of phytoplankton (a) and zooplankton (b)
3.3. Zooplankton

A total of 40 species of zooplankton were detected in Dianchi Lake, and there were barely distinctions among the different area of Dianchi Lake in the number of species, and the numbers in the estuary of Inflow Rivers ranged from largest to smallest was Gucheng River, Maliao River, Laoyu River and Baoxiang River. The zooplankton species in Dianchi Lake were larger than in the estuary of Inflow Rivers in the whole.

Distribution of zooplankton species in different regions were showed in Table 3. The number of species of zooplankton detected in the estuary of inflowing rivers was 44. Among them, 34 species were detected in the Gucheng River, 26 species were detected in the Maliao River, 21 species in Laoyu River, 21 species were detected in the Daqing River and 17 species in Baoxiang River. Daphnia magna was only detected in Gucheng River, P. inopinus and Ilyocryptus agilis were only detected in Laoyu River, Synchaeta was only detected in Maliao River, Cyclops sp. was only detected in Baxiang River.

### Table 3. Distribution of zooplankton species in different regions

| Region                  | Number of species | Proportion | Number of endemic species | Proportion |
|-------------------------|-------------------|------------|---------------------------|------------|
| Dianchi Lake            |                   |            |                           |            |
| Northern                | 35                | 66.04%     | 0                         | 0          |
| Center area             | 34                | 64.15%     | 0                         | 0          |
| Southern                | 35                | 66.04%     | 0                         | 0          |
| Average                 | 34.67             | 65.41%     | 0                         | 0          |
| Gucheng River           | 34                | 64.15%     | 1                         | 1.89%      |
| Laoyu River             | 22                | 41.51%     | 2                         | 3.77%      |
| Maliao River            | 27                | 50.94%     | 1                         | 1.89%      |
| Baoxiang River          | 17                | 32.08%     | 1                         | 1.89%      |
| Daqing River            | 21                | 39.62%     | 0                         | 0          |
| Average                 | 24.2              | 45.66%     | 1                         | 1.89%      |

Estuary of inflowing rivers

Forty species of zooplankton were detected in Dianchi Lake, and the abundance of zooplankton was basically the same in the three areas of Dianchi Lake, the species in southern part, center part and northern part were 35, 34 and 35, respectively. There were three endemic species, including Conochiloides, Asplanchna and Cyclop sp. Dominant zooplankton genus taxa in different region were showed in Table 4.

### Table 4. Dominant zooplankton genus taxa in different regions

| Region                  | Dominant genus taxa                                            |
|-------------------------|----------------------------------------------------------------|
| Dianchi Lake            | Macrothricidae sp.2, Macrothricidae sp.2, Bosmina fatalis, Daphnia galeata, Polyarthra sp. |
| Gucheng river           | Polyarthra sp., Macrothricidae sp.2, Macrothricidae sp.2, Brachionus calyciflorus |
| Laoyu river             | Macrothricidae sp.2, Daphnia galeata, Polyarthra sp.            |
| Maliao river            | Daphnia galeata, Calanus sinicus sp.                            |
| Baoxiang river          | Macrothricidae sp.2, Daphnia galeata, Simocephalus vetulus     |
| Daqing river            | Daphnia galeata, Calanus sinicus sp.                            |

The Shannon diversity index of zooplankton were calculated (Table 5), the range of the index values for each sample point were 0.54 to 2.49, the index values of different regions ranged in a descending order were: center of Dianchi Lake > north area of Dianchi Lake > south area of Dianchi Lake > estuary of the inflow river, the spatial distribution were illustrated in the Kriging map (Figure 2).

The results indicated that different topographical and geographical environments and human disturbance could impact the phytoplankton community composition in the water body.
Table 5. The diversity index of zooplankton in different sampling region

| Region                | Mean | Minimum | Maximum | Std. | Variable coefficient |
|-----------------------|------|---------|---------|------|-----------------------|
| Average               | 1.83 | 0.54    | 2.49    | 0.47 | 0.26                  |
| Center                | 2.16 | 1.80    | 2.49    | 0.26 | 0.12                  |
| Northern              | 2.09 | 1.96    | 2.19    | 0.10 | 0.05                  |
| Southern              | 2.07 | 1.92    | 2.40    | 0.19 | 0.09                  |
| Estuary of the inflow river | 1.53 | 0.54    | 2.34    | 0.51 | 0.33                  |

4. Conclusion

The phytoplankton diversity was accurately estimated by joint using of eDNA and metagenomic examination technology, which proved that the eDNA technology was an effective method in determination of biodiversity in Dianchi Lake.

The water quality analyzed indicated that the Dianchi Lake was severe eutrophication. The investigation results showed that 74 genera of phytoplankton were detected in Dianchi Lake, Bacillariophyta was the dominant genera both in the Dianchi Lake and the estuary of inflow rivers. The phytoplankton diversity in the estuary of inflowing river was higher than in the Dianchi Lake.

A total of 40 and 44 species of zooplankton was detected in Dianchi Lake and in the estuary of the inflowing rivers, respectively. Contrarily to the results of phytoplankton diversity, the average specie number of Dianchi Lake was higher than in the estuary of Inflow River, and the dominant genus taxa were similar in the two regions.

These results suggested that the pollution source of eutrophication could be derived from the rivers entering the lake, which also offers proposals for the overall management of the Dianchi Lake basin that more significant attention should be gathered in the pollution controlling of the inflowing river.

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